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Title:

NMDA receptor hypofunction phase-couples independent gamma oscillations in the rat visual cortex.

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Running title:

Hallucinogenic gamma oscillation phase-coupling

Abstract

Hallucinations, a hallmark of psychosis, can be induced by the psychotomimetic NMDA receptor antagonists ketamine and phencyclidine and are associated with hypersynchronization in the gamma frequency band, but it is unknown how reduced interneuron activation, associated with NMDA receptor hypofunction can cause hypersynchronisation or distorted perception. Low-frequency gamma oscillations (LF γ) and high-frequency gamma oscillations (HF γ) serve different aspects of perception. Here we test whether ketamine and phencyclidine are able to affect the interactions between HF γ and LF γ in the rat visual cortex *in vitro*.

In slices of the rat visual cortex kainate and carbachol induced LF γ (~34 Hz at 32 °C) in layer V and HF γ (~54 Hz) in layer III of the same cortical column. In control HF γ and LF γ were independent and pyramidal neurons recorded in layer III were entrained by HF γ , but not by LF γ . Sub-anaesthetic concentrations of ketamine selectively decelerated HF γ by 22 Hz (EC_{50} = 2.7 μ M), to match the frequency of LF γ in layer V. This caused phase-coupling of the two gamma oscillations, increased spatial coherence in layer III and entrained the firing of layer III pyramidal neurons by LF γ in layer V. Phencyclidine similarly decelerated HF γ by 22 Hz (EC_{50} = 0.16 μ M), causing cross-layer phase-coupling of gamma oscillations. Selective NMDA receptor antagonism, selective NR2B subunit-containing receptor antagonism and reduced *D*-serine levels caused a similar selective deceleration of HF γ , whereas increasing NMDA receptor activation through exogenous NMDA, *D*-serine or mGluR group 1 agonism selectively accelerated HF γ .

The NMDA-receptor hypofunction-induced phase-coupling of the normally independent gamma-generating networks is likely to cause abnormal cross-layer interactions, which may

distort perceptions due to aberrant matching of top-down information with bottom-up information. If decelerated HF γ and subsequent cross-layer synchronisation also underlies pathological psychosis, acceleration of HF γ could be the target for improved antipsychotic therapy.

Keywords

Gamma oscillation, ketamine, phencyclidine, NMDA receptor, hallucinogenic, psychotomimetic

Introduction

Hallucinations are perceptions in the absence of a stimulus. Where hallucinations are often auditory in schizophrenia, hallucinations associated with neurodegenerative dementias like Lewy body Dementia, and Parkinson's disease with dementia are normally visual (Metzler-Baddeley 2007). Hallucinations, and distortions of visual perception (Muetzelfeldt et al. 2008) are also a desired action of dissociative drugs like the N-methyl-D-aspartic acid (NMDA) receptor antagonists ketamine and phencyclidine (PCP). Because a single sub-anaesthetic dose of ketamine or PCP is psychotomimetic (Farber 2003) and agents that enhance NMDA receptor function are antipsychotic (Kantrowitz et al. 2010; Schlumberger et al. 2010; Marek et al. 2010), NMDA receptor hypofunction is regarded a shared process in both psychotic pathologies and

substance abuse drugs (Coyle 2006). Understanding the mechanism by which NMDA receptor hypofunction causes aberrant perceptions may lead to better antipsychotic treatment.

Perceptions may arise from matching of data-driven (bottom-up) information processing in the sensory cortices with (top-down) information driven by previous experiences stored in higher cortical areas. This process requires neuronal synchronization with millisecond-precision provided by network oscillations at gamma frequencies (30-120 Hz) (Herrmann et al. 2004; Engel et al. 2001). Reduced NMDA receptor-mediated activation of interneurons in cortical networks leads to pyramidal neuron hyperexcitability (Homayoun and Moghaddam 2007), which was hypothesized to cause aberrant perceptions by disruption of gamma synchronization (Marek et al. 2010). However, psychosis and hallucinations have rather been associated with gamma hypersynchronization (Hakami et al. 2009; Spencer et al. 2009; Flynn et al. 2008; Spencer et al. 2009; Herrmann and Demiralp 2005).

In the neocortex high-frequency gamma oscillations ($\text{HF}\gamma$; > 70 Hz) coexist with low-frequency gamma oscillations ($\text{LF}\gamma$; < 70 Hz) and the two can operate independently and serve different aspects of information processing (Wyart and Tallon-Baudry 2008; Vidal et al. 2006) (Crone et al. 2006). Recently we demonstrated that $\text{HF}\gamma$ and $\text{LF}\gamma$ can coexist in the same column of the visual cortex *in vitro* and operate independently (Oke et al. 2010). Interestingly the NMDA receptor antagonist APV selectively reduced the dominant frequency of $\text{HF}\gamma$ so that it approached the frequency of the unaltered $\text{LF}\gamma$ (Oke et al. 2010).

We therefore propose that NMDA receptor hypofunction causes phase-coupling of two normally phase-independent gamma generating networks, causing hypersynchrony and a distortion of perception due to aberrant matching of top-down information with bottom-up information. Here we test the effect of NMDA receptor hypofunction on $\text{HF}\gamma$ and $\text{LF}\gamma$ recorded

in the rat visual cortex *in vitro* and demonstrate that NMDA receptor hypofunction phase-couples the two gamma generating networks.

Materials and methods

Preparation

Male adult Sprague Dawley rats (200-300 g, Charles-River, Margate, UK) were anaesthetized with by intraperitoneal injection of a ketamine (75 mg.kg^{-1}) / medetomidine (1 mg.kg^{-1}) mixture and killed by cardiac perfusion with a chilled sucrose-based solution containing 189 mM sucrose, 2.5 mM KCl, 26 mM NaHCO_3 , 1.2 mM NaH_2PO_4 , 0.1 mM CaCl_2 , 5 mM MgCl_2 , 10 mM *D*-glucose; pH 7.4. All procedures conformed to the UK Animals (Scientific Procedures) Act 1986. The brain was removed and coronal slices (400 μm thick) were cut around 7-6 mm caudal from bregma. The dorsal neocortex containing the visual cortex areas V1 and V2 was isolated from the rest of the slice by scalpel cuts in chilled sucrose-based solution. Slices were either immediately placed at the interface between artificial cerebrospinal fluid (aCSF, at 7 ml/min) and moist carbogen (95% O_2 and 5 % CO_2 at 300 ml/min) at 32 °C, or stored in a static interface type chamber at 23 °C for later use. aCSF contained 125 mM NaCl, 3 mM KCl, 26 mM NaHCO_3 , 1.25 mM NaH_2PO_4 , 2 mM CaCl_2 , 1 mM MgCl_2 , 10 mM *D*-glucose; pH was set at 7.4. Slices were allowed to recover for one hour before recordings started.

Electrophysiological recordings

Gamma oscillations were evoked by adding kainate (400 nM) and the muscarinic acetylcholine receptor agonist carbachol (20 μ M) (Buhl et al. 1998; Oke et al. 2010), and left to develop for one hour before oscillation characteristics were further explored.

Field potentials were recorded with aCSF-filled glass pipette recording electrodes (4-5 M Ω), amplified with Neurolog NL104 AC-coupled amplifiers (Digitimer, Welwyn Garden City, UK), band-pass filtered at 1-200 Hz with Neurolog NL125 filters (Digitimer), and any noise locked to the mains supply (50 Hz) was removed with Humbug noise eliminators (Digitimer). The signal was then digitized and sampled at 2 kHz using a CED-1401 (Cambridge Electronic Design, Cambridge, UK) and Spike-2 software (Cambridge Electronic Design). Slices were scanned for sites where high-frequency gamma oscillations and low-frequency gamma oscillations were found in the same column, which was usually in the medial part of the secondary visual cortex.

Intracellular current-clamp recordings were made using sharp (60-80 M Ω) pipettes, filled with 3 M potassium methylsulphate. Using a MX1641 motorized microdrive (Siskyou, Grants Pass, OR, USA) the electrode was driven (at 5 μ m steps) into the tissue parallel to the layers up to a depth of 250 μ m in search for neurons. The membrane potential was amplified using an Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA, USA), low-pass filtered at 2 kHz and sampled at 10 kHz. Impaled cells were first inspected and accepted for recording if the input resistance was greater than 30 M Ω , the resting membrane potential was stable and more polarized than -55 mV in the presence of kainate and carbachol, and action potentials were overshooting.

Drugs were applied at about two hours after application of kainate and carbachol when the oscillation power and frequency were stable. Drug-induced changes after 25 minutes were compared with baseline values. Drugs were used diluted from the following frozen stock solutions. Ketamine; 50 mM in H₂O, PCP; 2 mM in H₂O (5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801); 10 mM in H₂O, ZnCl₂; 30 μM in H₂O, (aR,bS)-a-(4-Hydroxyphenyl)-b-methyl-4-(phenylmethyl)-1-piperidinepropanol maleate (Ro 25-6981); 10 mM in H₂O, (S)-3,5-Dihydroxyphenylglycine (DHPG); 10 mM in H₂O. D-serine was directly added to the aCSF. The D-serine metabolising enzyme D-amino acid oxidase (DAAO); 800 U/ml was derived from *rhodotorula gracilis* and kindly donated by Professor Dale (University of Warwick). Ketamine, Ro 25-6981 and MK-801 were purchased from Tocris (Bristol, UK). All other drugs and aCSF salts were purchased from Sigma (Poole, UK). Ketamine and PCP were washed out for 30 minutes and if the drug effect was reversible a second higher concentration of the same drug was applied.

Data analysis

The oscillation power was calculated from 60 s recording epochs by fast Fourier transforms (1 Hz bin size, Hanning window), using Spike-2 software. The gamma frequency band was set at 20-80 Hz for recording at 32 °C, taking into account the temperature dependence of gamma oscillations observed in the visual cortex (Oke et al. 2010). The dominant oscillation frequency was determined as the frequency of peak power in the power spectrum smoothed by a 5-point simple moving average. To take into account possible changes in dominant oscillation frequency

the power was averaged over -5 Hz to 5 Hz of the dominant frequency. Waveform cross-correlograms were calculated over 60 s digitally high-pass filtered (FIR at 10 Hz) epochs.

To obtain membrane waveform averages field potential recordings were band-pass filtered (with a phase-true FIR filter) from 10 to 200 Hz. The maximum amplitude for gamma cycles was determined for this signal so that on average one cycle per second exceeded this value. Gamma cycles of medium (0.4-0.6 times the maximum) amplitude were marked at their trough minimum and waveform averages of gamma cycles (> 300 cycles, time-zeroed at the sorted marks) were then calculated from the 10 Hz high-pass (FIR) filtered membrane potential.

To determine the relationship between different events peri-event interval distributions (between -50 ms and 50 ms) were calculated from the timings of the different events and a distribution deviating significantly from a uniform distribution was considered to indicate a relationship between the timing of the two events. To determine the phase relationship between oscillations in recordings from different layers, peri-event intervals were calculated using MATLAB software (The MathWorks, Natick, USA). Simultaneous 100 s recordings were band-pass filtered (with a phase-true FIR filter) at ± 5 Hz of the dominant frequency of LF γ (determined in layer V) and HF γ (determined in layer III). A Hilbert transform provided a discrete-time analytic signal that was converted to phase angles in radians. From the Hilbert-peak times from both recordings peri-event interval distributions were calculated.

Statistics

Data are expressed as means \pm standard error of the mean (SEM), n indicates the number of slices or cells tested. The effect of drugs was tested by unpaired comparison with matched controls, using a two-tailed Student's t -test with and alpha level of 0.05. Deviation from a uniform distribution was determined with the Kolmogorov-Smirnov (K-S) Z test with and alpha level of 0.05.

Results

High-frequency gamma oscillations coexists with low-frequency gamma oscillations

We induced rhythmic field potential oscillations in cortex slices by bath application of kainate (400 nM) and carbachol (20 μ M) that substitute for the glutamatergic and cholinergic inputs lost due to the slicing (Oke et al. 2010; Buhl et al. 1998). Oscillations had substantial power in the gamma frequency range (example in Figs. 1Ai/Bi), which was set at 20-80 Hz for recording at 32 °C, taking into account the temperature dependence of gamma oscillations (Oke et al. 2010). After one hour the visual cortex in each slice was scanned along layer IV to select a cortical column with oscillatory activity with power peaks in both the low-frequency gamma oscillation (LF γ) frequency band (20-45 Hz) and the high-frequency gamma oscillation (HF γ) frequency band (46-80 Hz) range (Oke et al. 2010), which was usually found in area V2M (Figure S1). Simultaneous field potential recordings were then made from layer III (0.5 mm from pial surface) and from layer V (1.2 mm from pial surface, Figure S1) of the selected column. The oscillatory activity in layer III (example in Figure 1Ai, grey trace) was invariably faster than the

oscillatory activity in layer V (example in Figure 1Ai). The power spectrum of the recording from layer III peaked at frequencies in the HF γ range (Table 1, example in Figure 1Bi, grey line), whereas the power spectrum of the recording from layer V peaked at frequencies in the LF γ range (Table 1, example in Figure 1Bi, black line).

Ketamine selectively decelerates HF γ

Application of the psychotomimetic drug ketamine (10 μ M) reduced the dominant frequency of HF γ in layer III, without affecting the dominant frequency of LF γ in layer V (Table 1, example in Figure 1Aii and 1Bii). We determined the change in dominant frequency of HF γ in layer III and LF γ in layer V at 25 minutes after application of different ketamine concentrations and compared it with that after 25 minutes of continued perfusion with control aCSF (Table 1). Ketamine caused a significant reduction in dominant frequency of HF γ at concentrations of 1 μ M and up (Figure 1C). The ketamine-induced deceleration of HF γ in layer III, defined as the ketamine-induced reduction in dominant frequency minus the change with control aCSF, as function of ketamine concentration. (Figure 1C, grey symbols) was fitted with a sigmoid function of the form: $\text{effect} = \text{maximum effect} / (1 + ([\text{drug}] / \text{EC}_{50})^h)$, with a maximum effect of 22.1 ± 0.7 Hz, a concentration of half maximum effect (EC_{50}) of 2.7 ± 0.3 μ M and a slope (h) of 1.1 ± 0.1 . This EC_{50} is well within the range of concentrations obtained with sub-anaesthetic doses used recreationally (shaded area in Figure 1C). The ketamine-induced deceleration of LF γ in layer V was only significant at very high concentrations (Figure 1C, black symbols). To allow for the frequency shift, the effect of ketamine on dominant gamma oscillation power was assessed by taking the average power over +5 Hz and -5 Hz from the dominant frequency. The

dominant HF γ power in layer III increased at lower concentrations and decreased at higher concentrations of ketamine (Figure 1D, grey symbols). The increase in dominant LF γ power in layer V at concentrations from 10 μ M (Figure 1D, black symbols) may well reflect the contribution of decelerated HF γ in layer III. Effects of ketamine were completely reversible up to a concentration of 3 μ M (not shown).

Deceleration of HF γ causes hypersynchrony

Under control conditions HF γ in layer III was independent of LF γ in layer V (Oke et al. 2010). In the presence of ketamine the difference between the dominant frequency of LF γ in layer V and that of the decelerated HF γ in layer III became so small that there was a possibility that the two gamma oscillation generating networks could become phase-locked. We tested the prediction that ketamine would increase the phase-link between the two oscillators, by determining the phase relationship between HF γ in layer III and LF γ in layer V from the peri-event intervals, calculated from a Hilbert transform of band-pass (\pm 5 Hz around dominant frequency) filtered recordings. Before ketamine, the peri-event interval distribution was not different from uniform in 6 out of 7 slices (K-S test $P < 0.05$, example in Figure 2A*i*), indicating the absence of a relationship between the phase of HF γ in layer III and the phase of LF γ in layer V. After ketamine (10 μ M) the peaks in the inter-event distribution showed a significant relationship between the oscillation phase in layer III and that in layer V (example in Figure 2A*ii*). The K-S test Z increased from 1.11 ± 0.11 in control to 4.23 ± 0.21 in ketamine (Paired Student's t -test $P < 0.001$, $n = 7$, Figure 2A*iii*). On average layer III gamma troughs were 4.3 ± 0.5 ms ahead of layer V gamma troughs. This indicates that, whereas in control the two oscillators

operate independently, the ketamine-induced deceleration of HF γ in layer III causes phase-coupling of the gamma generating networks in layer III and layer V.

Under control conditions LF γ in layer V is spatially coherent, unlike HF γ in layer III (Oke et al. 2010). To test whether the phase-coupling of the decelerated HF γ in layer III to LF γ would increase the spatial coherence of the gamma oscillation in layer III, we determined the cross correlation maximum between two electrodes placed 500 μm apart in layer III of the same slice. Cross correlation was small in control (example in Figure 2Bi), but increased in the presence of ketamine (10 μM , example in Figure 2Bii). For five slices the cross correlation in layer III increased from 0.22 ± 0.02 in control to 0.50 ± 0.05 in ketamine (paired Student's t -test $P < 0.01$, Figure 2Biii). For the same slices the cross correlation maximum between two electrodes placed 500 μm apart in layer V was not affected by ketamine (0.54 ± 0.05 in control versus 0.58 ± 0.06 in ketamine, *n.s.*). This suggests that the increase in spatial coherence of gamma oscillations in layer III is due to the phase coupling of the decelerated HF γ in layer III to LF γ in layer V.

Ketamine causes entrainment of layer III pyramidal neurons by LF γ

Like other cortical gamma oscillations (Buhl et al. 1998; Cunningham et al. 2003), HF γ in layer III is dependent on recurrent inhibition (Oke et al. 2010) and layer III pyramidal neuron firing is entrained by rhythmic IPSCs and EPSCs phase-locked to HF γ in layer III (unpublished observations MV). To test whether ketamine affects the relationship between the activity of layer III pyramidal neurons and the gamma oscillations in the different layers, we recorded cells in layer III under sharp electrode current-clamp conditions. Six cells identified as pyramidal neurons (see Figure S2) were recorded before and after ketamine (10 μM).

To test the relationship between action potential firing times and gamma oscillation phase, neurons were made to fire at ~ 2 Hz by manually adjusting the holding current. The distribution of intervals between action potential peaks (500 per neuron) and HF γ troughs in layer III (example in Figure 3A*i*) was tested for uniformity. For 5 out of 6 neurons the spike timing distribution showed a significant relationship (K-S test for uniformity, $P < 0.05$) between firing and HF γ troughs in layer III, with the peak firing probability in these neurons 4.3 ± 0.7 ms ahead of the HF γ trough. In contrast to the entrainment of firing by HF γ , the distribution of intervals between action potential peaks and LF γ troughs in layer V showed no entrainment of the same six layer III pyramidal neurons by LF γ in layer V (example in Figure 3B*i*).

In the presence of ketamine (10 μ M) the firing of the same layer III pyramidal neurons was still entrained by the now decelerated HF γ in layer III, but the relation was less tight (example in Figure 3A*ii*). The K-S test Z was reduced from 2.96 ± 0.26 in control to 2.47 ± 0.31 in ketamine (paired Student's t -test $P < 0.05$, $n = 6$, Figure 3A*iii*). However, for 4 out of 6 layer III pyramidal neurons the firing was now entrained by LF γ in layer V (example in Figure 3B*ii*), with the peak firing probability in these neurons 0.5 ± 1.0 ms after the HF γ trough. The K-S test Z increased from 0.95 ± 0.14 in control to 1.87 ± 0.19 in ketamine (paired Student's t -test $P < 0.01$, $n = 6$, Figure 3B*iii*). These observations demonstrate that layer III pyramidal neurons that under normal conditions ignore the LF γ in layer III, become entrained by it in the presence of ketamine.

Ketamine increases rhythmic synaptic inputs.

All pyramidal neurons recorded received a constant barrage of IPSPs and EPSP. To determine how synaptic inputs are related to the different gamma oscillations, we held the same neurons

manually just below firing threshold and constructed the membrane potential waveform averages, time-zeroed at the trough of medium-amplitude HF γ gamma cycles in layer III (example in Figure 3Ci). The membrane potential gamma cycle had its peak amplitude 3.4 ± 0.6 ms ahead of the HF γ trough in layer III. The waveform averages of the membrane potential time-zeroed to the LF γ troughs in layer V (example in Figure 3Di) show that the membrane oscillation was only weakly coupled to LF γ in layer V, peaking 1.3 ± 0.8 ms ahead of the LF γ trough.

In the presence of ketamine the membrane potential waveform average time-zeroed at the decelerated HF γ troughs in layer III was larger (example in Figure 3Cii) and peaked later (1.0 ± 0.7 ms, paired Student's *t*-test $P < 0.01$, $n = 6$). The maximum (peak-to-trough) amplitude was 0.23 ± 0.06 mV in ketamine versus 0.18 ± 0.04 mV in control (paired Student's *t*-test $P < 0.05$, $n = 6$, Figure 3Ciii). Ketamine strongly increased the maximum amplitude of the membrane potential waveform average for LF γ troughs in layer V (example in Figure 3Dii), without affecting the peak time (0.4 ± 0.7 ms after the LF γ trough, *n.s.*). The maximum amplitude was 0.26 ± 0.06 mV versus 0.10 ± 0.03 mV in control (paired Student's *t*-test $P < 0.01$, $n = 6$, Figure 3Diii). These observations demonstrate that ketamine increases the LF γ -related rhythmic synaptic inputs to layer III pyramidal neurons.

PCP decelerates HF γ and causes hypersynchrony

The psychotomimetic drug PCP has a similar NMDA receptor-antagonistic action to ketamine and we predicted that PCP would have a ketamine-like effect on gamma oscillations in the visual cortex. Like with ketamine, application of 1 μ M PCP reversibly decelerated HF γ in layer III, but did not affect the dominant frequency of LF γ in layer V (Table 1, example in Figure

S3A/B). Like ketamine, 1 μM PCP phase-locked the gamma-generating networks in layer III and layer V that were independent in control (Figure S3C). The deceleration of HF γ in layer III as function of PCP concentration (Figure S3D) was fitted with a sigmoid function with a maximum effect of 21.7 ± 0.9 Hz, an EC_{50} of 0.16 ± 0.02 μM and a slope of 1.7 ± 0.3 . PCP suppressed the dominant HF γ power at higher concentrations and increased the dominant LF γ power at intermediate concentrations (Figure S3E), possibly due to the contribution from the decelerated HF γ in layer III. At concentrations obtained with doses used to induce hallucinations PCP has the same network effects as ketamine.

NMDA receptor modulating drug effects

Ketamine and PCP are not selective NMDA receptor antagonists and may exert their effect through inhibition of I_h (Tanabe 2007) and/or dopamine D_2 receptor stimulation (Seeman et al. 2009). Therefore we tested the effect of the selective non-competitive NMDA receptor antagonist MK-801. MK-801 (10 μM) decelerated HF γ , but left the dominant frequency of LF γ unchanged (Table 1). MK-801 increased the dominant power of both HF γ and LF γ (Table 1). NMDA receptor activation also requires occupation of the glycine site by its endogenous ligand *D*-serine (Oliet and Mothet 2009). We reduced endogenous *D*-serine levels by increasing *D*-serine metabolism by applying the enzyme DAAO. DAAO (0.17 U/ml, 60 minutes) selectively decelerated HF γ (Table 1, example in Figure 4A). These observations, together with the increase in HF γ power observed with the competitive NMDA receptor antagonist APV (Oke et al. 2010), confirm that the HF γ -decelerating effect of ketamine and PCP is mediated through NMDA receptor antagonism.

In order to determine which NMDA receptor type is responsible we tested the effect of Ro 25-6981 (10 μM), a selective antagonist of NR2B subunit-containing NMDA receptors (Fischer et al. 1997) and of nanomolar zinc, which selectively blocks NR2A subunit-containing NMDA receptors (Paoletti et al. 1997). Ro 25-6981 selectively decelerated HF γ , increased dominant LF γ power and reduced dominant HF γ power (Table 1, example in Figure 4B). However, zinc (30 nM ZnCl₂) had no effect on the dominant frequency of HF γ or LF γ or on their dominant gamma power (Table 1). These observations indicate that the deceleration of HF γ by NMDA receptor antagonists is mediated by antagonism of NR2B subunit-containing NMDA receptors.

Based on the observation that NMDA receptor hypofunction can cause phase-coupling of the gamma oscillators by selectively decelerating HF γ , we predicted that the opposite effect would be obtained by increasing NMDA receptor activation. To test this we applied NMDA directly (Mann and Mody 2010) to slices with HF γ and LF γ . NMDA (3 μM) selectively accelerated HF γ and suppressed the gamma power of both HF γ and LF γ (Table 1). NMDA receptor activity depends on the activation of the glycine site of the receptor. Increasing glycine site occupation by applying a high concentration of *D*-serine (100 μM) selectively accelerated HF γ without affecting oscillation power (Table 1, example in Figure 4C). NMDA receptor activation can also be increased by activation of metabotropic glutamate group I receptors (Pisani et al. 1997). In line with this the metabotropic glutamate group I receptor agonist DHPG (10 μM) selectively accelerated HF γ , without affecting the oscillation power (Table 1, example in Figure 4D). These observations confirm that, whatever the method, increasing NMDA receptor activity selectively accelerates HF γ .

Discussion

We investigated the effect of psychotomimetic NMDA receptor antagonists ketamine and PCP and demonstrated that in the visual cortex NMDA receptor hypofunction causes phase-coupling of both HF γ in layer III and layer III pyramidal neuron activity to LF γ in layer V, by selectively decelerating HF γ . This effect is mediated through *D*-serine-dependent NR2B subunit-containing receptors, and can be reversed by increasing NMDA receptor activity.

Ketamine and PCP selectively decelerate HF γ in layer III with an EC₅₀ within the sub-anaesthetic concentration range. At sub-anaesthetic doses ketamine strongly increases cortical LF γ power in the behaving rat (Hakami et al. 2009) and in healthy humans (Hong et al. 2010). This increase can be explained by the increase in HF γ power in layer III, and by the deceleration and increased spatial coherence of HF γ . Reducing the NMDA receptor-mediated drive of cortical interneurons increases pyramidal neuron firing rate (Homayoun and Moghaddam 2007) which, according to computational modelling, would increase in cortical gamma power (Spencer 2009). Because the spatial coherence of HF γ is relatively low in control conditions (Oke et al. 2010), recordings taken from the cortical surface are likely to miss the HF γ that can be recorded with indwelling electrodes and magnetoencephalography (Tallon-Baudry 2003). The deceleration of HF γ and subsequent phase-coupling to the spatially more-coherent LF γ will increase the spatial coherence of HF γ and the rhythmic IPSC amplitude in layer III pyramidal neurons, and hence will increase the power of the oscillation recorded with surface electrodes (illustrated in Figure 5).

Why does NMDA receptor modulation selectively affect HF γ frequency? The relatively high frequency of HF γ could be caused by 1) faster IPSC decay kinetics (Whittington et al. 1995; Heistek et al. 2010), 2) smaller IPSC amplitude (Atallah and Scanziani 2009), but see (Oke et al. 2010), 3) increased interneuron firing rate due to increased NMDA receptor-mediated depolarization and/or reduced tonic GABA_Aergic inhibition of interneurons (Mann and Mody 2010), or 4) the selective NMDA receptor-mediated activation of fast-firing parvalbumin-expressing interneurons (Middleton et al. 2008). The consistent, bi-directional effects of modulating NMDA receptor activation on HF γ frequency suggest that NMDA receptor hypofunction decelerates HF γ by reduced activation of fast-firing, probably parvalbumin-expressing interneurons in the superficial layers that drive HF γ . Interneurons involved in LF γ in layer V may not be dependent on NMDA receptor-mediated activation. Indeed, GABA_Aergic IPSCs in layer V neurons are NMDA receptor activation-independent (Ling and Benardo 1995). Because NMDA receptor-mediated modulation of interneuron activity can be presynaptic, NMDA receptor antagonists may selectively reduce glutamate release to interneurons involved in HF γ . Indeed, NR2B-containing (and not NR2A-containing) NMDA auto-receptors modulate glutamate release in visual cortex and are located in layer II/III (Li et al. 2009). This is consistent with the differential effects of zinc and Ro 25-6981 in decelerating HF γ .

Psychosis and hallucinations have been associated with increased cortical LF γ (Flynn et al. 2008; Spencer et al. 2009; Becker et al. 2009) and the gestalt-induced gamma oscillation is decelerated in the visual cortex of schizophrenics (Spencer et al. 2004). Because NMDA receptor hypofunction is a common factor in the action of substance abuse drugs and schizophrenia-associated psychosis (Coyle 2006; Farber 2003), the latter could be due to an increased

propensity of a cortical network to hypersynchronize (Spencer et al. 2009), due to network changes that can decelerate HF γ .

Firstly, parvalbumin-expressing fast-firing interneurons may be functionally impaired. In the visual cortex of schizophrenics parvalbumin-immunoreactivity is selectively reduced (Hashimoto et al. 2008). GABA synthesis is reduced in parvalbumin-expressing cortical interneurons (Hashimoto et al. 2003), possibly due to reduced activity of TrkB receptors, that are normally predominantly expressed on parvalbumin-expressing interneurons (Lewis et al. 2005), but are reduced in schizophrenics (Hashimoto et al. 2005). Increased activation of the remaining TrkB receptors may therefore have antipsychotic potential.

Secondly, the NMDA receptor-dependent activation of interneurons may be reduced. Although the total cellular expression of NR2 proteins is not consistently altered in the cortex of schizophrenics, the trafficking of NR2B subunit-containing NMDA receptors to their pre- and postsynaptic locations is impaired (Kristiansen et al. 2010). In the chronic low-dose MK-801-treated rat model the parvalbumin-expressing interneurons have a reduced NMDA receptor expression (Xi et al. 2009). As increased activation of NR2B subunit-containing NMDA receptors accelerated HF γ , selective facilitation of these receptors may therefore have antipsychotic potential.

NMDA receptor activation requires co-activation of the glycine site by its endogenous ligand *D*-serine (Oliet and Mothet 2009). Schizophrenia has been associated with aberrant NMDA receptor *D*-serine/glycine site function (Labrie and Roder 2010) and increasing *D*-serine levels has an antipsychotic-like profile (Kantrowitz et al. 2010; Marek et al. 2010). Interestingly, in the rat visual cortex glycine site agonists facilitate glutamate release in layer III by acting on presynaptic NR2B subunit-containing NMDA auto-receptors (Li et al. 2008). The deceleration of

HF γ with reducing endogenous *D*-serine levels and the acceleration with exogenous *D*-serine confirm that the deceleration of HF γ is involved in the psychotic action of NMDA receptor hypofunction.

NMDA receptor activation is facilitated by metabotropic glutamate receptor group 1 activation (Pisani et al. 1997) through mGluR5 receptors. Although the mGluR5 expression is not altered in the cortex of schizophrenics (Gupta et al. 2005), increasing mGluR5 receptor activity has promising antipsychotic effects (Schlumberger et al. 2010), possibly due to the acceleration of HF γ observed with DHPG.

Thirdly, non-NMDA receptor-dependent activation of fast-spiking interneurons may be impaired. Metabotropic glutamate receptor GluR2-selective agonists selectively activate fast-spiking interneurons in the cortex (Sun et al. 2009) and have an antipsychotic action (Marek et al. 2010). The expression of kainate receptor subunit GluR5 (not mGluR6) mRNA was reduced in cortical interneurons of schizophrenics (Woo et al. 2007). Because the GluR5 subunit-selective kainate receptor agonist ATPA accelerated HF γ frequency (Oke et al. 2010), selective GluR5 potentiators may have an antipsychotic potential (Marek et al. 2010).

At a cellular level the ketamine-induced deceleration of HF γ caused the firing of layer III pyramidal neurons to be entrained by LF γ in layer V. Whereas in control conditions the firing of pyramidal neurons in the superficial layers is independent of that in the deep layers, NMDA receptor hypofunction aligns the firing of the two populations, increasing the interactions between the layers (illustrated in Figure 5). Because the decelerated HF γ in layer III phase leads LF γ in layer V, as in the somatosensory cortex under control conditions (Buhl et al. 1998), the EPSPs from layer III pyramidal neurons may arrive just before the IPSCs in layer V pyramidal

neurons. This is likely to increase the information transfer between the layers during gamma oscillations. In addition it may increase the synaptic strength of layer III to layer V pyramidal neuron connections through Hebbian plasticity and change information transfer long-term. In addition the phase-coupling of neuronal firing across layers is likely to increase burst firing in layer V pyramidal neurons that stretch through all layers (Larkum et al. 1999; Larkum et al. 2004).

Functionally it is tempting to speculate how hallucinations can arise from the increased coupling of processes in the superficial layers and the deep layers. Co-existence of HF γ and LF γ is found throughout the cortex, including the auditory cortex and prefrontal cortex (Crone et al. 2006). In the visual cortex HF γ and LF γ are associated with different aspects of visual information processing (Wyart and Tallon-Baudry 2008; Vidal et al. 2006; Chaumon et al. 2009) and relate to the degree of top-down or bottom-up processing (Tallon-Baudry, personal communication). Conscious perception of complex visual stimuli may need the matching of stimulus-driven bottom-up information with memory-based top-down predictions (Wyart and Tallon-Baudry 2008; Herrmann et al. 2004; Engel et al. 2001). Top-down processes may modulate the temporal structure of bottom-up processes (Engel et al. 2001), and/or boost the response of layer V neurons when bottom-up and top-down information arrives simultaneously at basal and apical dendrites respectively (Siegel and Konig 2003; Larkum et al. 2004). The phase-coupling induced by NMDA receptor hypofunction may therefore cause erroneous matching and hence distortion of perception of bottom-up information, or even perception without it during hallucinations. *In vivo* experiments are needed to test the relationship between increased phase-coupling of HF γ and LF γ and psychosis.

The negative symptoms in schizophrenics are associated with gamma band hyposynchronisation (Spencer et al. 2009; Herrmann and Demiralp 2005), which poses the question how gamma band hypersynchronisation can arise from the same distorted network? Computational modelling has demonstrated that different kinds of network abnormalities associated with schizophrenia can produce gamma oscillation deficits as well as increases (Spencer 2009). We propose that even in a network with reduced LF γ , the coincidence of several of the modulatory factors that each can cause selective deceleration of HF γ in the superficial layers, may cause a temporary cross-layer gamma band hypersynchronization and consequent distortion of perception, due to aberrant matching of top-down information with bottom-up information. Our study suggests that a combination of therapies to counteract several of these modulating factors may provide the most effective antipsychotic therapy.

Disclosure/Conflict of Interest

The authors declare that, except for income received from the primary employer, or stipends unrelated to pharmaceutical corporations, no financial support or compensation has been received from any individual or corporation over the last three years of research and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Acknowledgments

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Table 1 Pharmacological modulation of HF γ and LF γ

		LF γ in layer V		HF γ in layer III	
		power	frequency	power	frequency
Control values		<i>n</i> (μV^2)	(Hz)	<i>n</i> (μV^2)	(Hz)
		55 26 \pm 3	34.3 \pm 0.4	55 33 \pm 4	54.1 \pm 0.6
Changes		<i>n</i> (%)	Δ (Hz)	<i>n</i> (%)	Δ (Hz)
Control		8 101 \pm 6	-0.5 \pm 0.7	8 95 \pm 6	-0.7 \pm 1.0
Ketamine	10 μM	7 131 \pm 29	-2.0 \pm 0.8	7 112 \pm 19	-18.8 \pm 3.2 **
PCP	1 μM	6 99 \pm 25	-1.9 \pm 0.9	6 61 \pm 12 *	-22.2 \pm 2.8 **
MK-801	10 μM	5 163 \pm 21 *	-1.4 \pm 0.6	5 127 \pm 13*	-18.2 \pm 2.6 **
DAAO	0.17 U/ml	6 112 \pm 6	0.4 \pm 0.8	6 71 \pm 8 *	-10.4 \pm 1.4 **
Ro 25-6981	10 μM	4 161 \pm 18 **	-0.8 \pm 0.5	4 64 \pm 9 **	-20.1 \pm 2.2 **
ZnCl ₂	30 nM	5 106 \pm 3	-1.1 \pm 1.2	5 114 \pm 6	-3.1 \pm 1.2
NMDA	3 μM	5 83 \pm 6 *	0.6 \pm 1.0	5 73 \pm 7 *	4.2 \pm 0.6 **
DHPG	10 μM	4 81 \pm 13	1.1 \pm 0.8	4 129 \pm 14*	5.7 \pm 1.6 **
D-serine	100 μM	5 93 \pm 8	-0.3 \pm 0.7	5 112 \pm 7	3.7 \pm 0.7 **

Recordings were made from layer III and layer V of slices with LF γ and HF γ . The drug-induced change of dominant gamma power (average over -5 Hz to +5 Hz of the dominant frequency, expressed as % of pre-drug power), and the drug-induced shift in dominant frequency (in Hz) of LF γ in layer V and HF γ in layer III is given as mean \pm SEM for *n* slices. Drug effects are compared with the change after 25 minutes in control solution. Statistical significant differences (unpaired Student's *t*-test) are indicated as * for $P < 0.05$ and as ** for $P < 0.01$.

Titles and legends to the figures

Figure 1. Ketamine decelerates selectively HF γ . **A.** Example of oscillatory activity simultaneously recorded in layer III (grey line) and layer V (black line) of a cortical column in area V2M, before (*i*) and 20 minutes after bath application of 10 μ M ketamine (*ii*). Scale bars: 50 ms and 50 μ V. **B.** Power spectrum of one-minute recordings from layer III and layer V of the slice in (A), before (*i*) and after ketamine (*ii*). Ketamine reduces the frequency of HF γ in layer III, without affecting LF γ in layer V. **C.** Concentration-effect relationship of the deceleration of the dominant oscillation frequency for HF γ in layer III (grey symbols) and LF γ in layer V (black symbols). Data are average and SEM. of 3-8 observations per concentration. For each concentration the ketamine-induced change in dominant frequency was compared with the change in control solution, with * indicating Student's *t*-test $P < 0.05$; **, $P < 0.01$ and *, $P < 0.001$. The deceleration as function of ketamine concentration is fitted with a sigmoid function for HF γ and with a smooth function for LF γ . Shaded area indicates concentrations reached with psycho-toxic ketamine doses in humans. **D.** Dominant gamma power (average from -5 Hz to +5 Hz from the dominant gamma oscillation frequency) normalized to control values as function of ketamine concentration for the same experiments as in (C). Details as in C. Data are fitted with smooth functions.

Figure 2. Ketamine increases phase-coupling. **A.** Peri-event interval distribution of the interval between phase peaks of the Hilbert-transform of HF γ in layer III and those of LF γ in layer V, before (*i*) and after 10 μ M ketamine (*ii*) for the same slice as in Figure 1A/B. *iii.* Z value of the Kolmogorov-Smirnov test for uniform distribution for control and ketamine. Data are

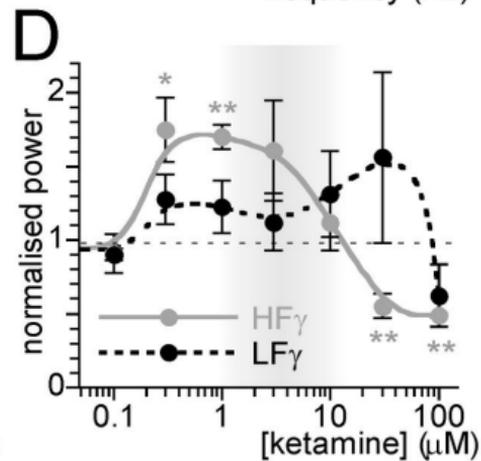
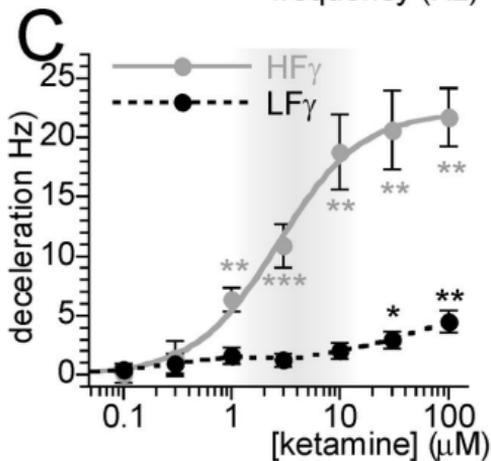
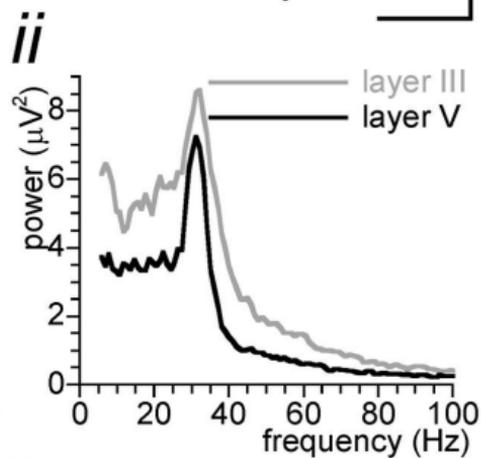
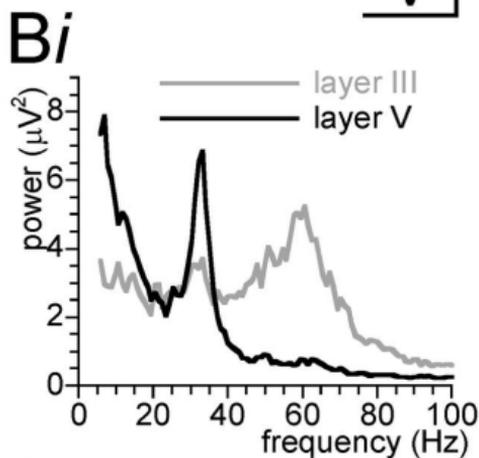
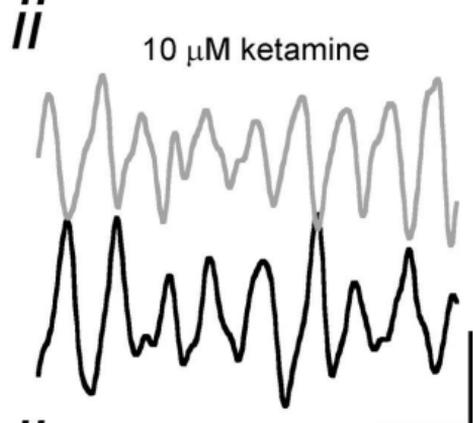
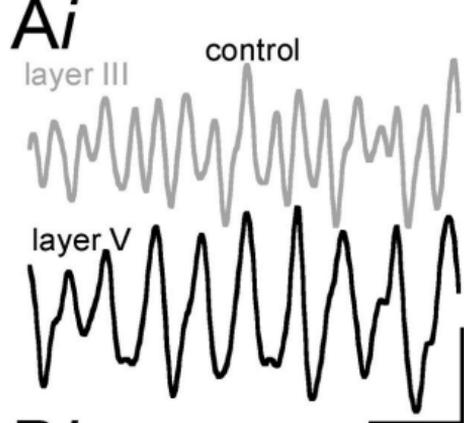
mean \pm SEM for $n=7$. Ketamine phase-locks the oscillation in layer III to the oscillation in layer V. Ketamine phase-locks the oscillation in layer III and the oscillation in layer V. **B.** Simultaneous recordings from layer III at locations 500 μm apart (top panel) and waveform cross-correlogram (bottom panel), before (*i*) and after 10 μM ketamine (*ii*). *iii.* Cross correlation maximum in control and ketamine. Data are mean \pm SEM for $n=5$. Ketamine increases maximum cross-correlation between layer III sites.

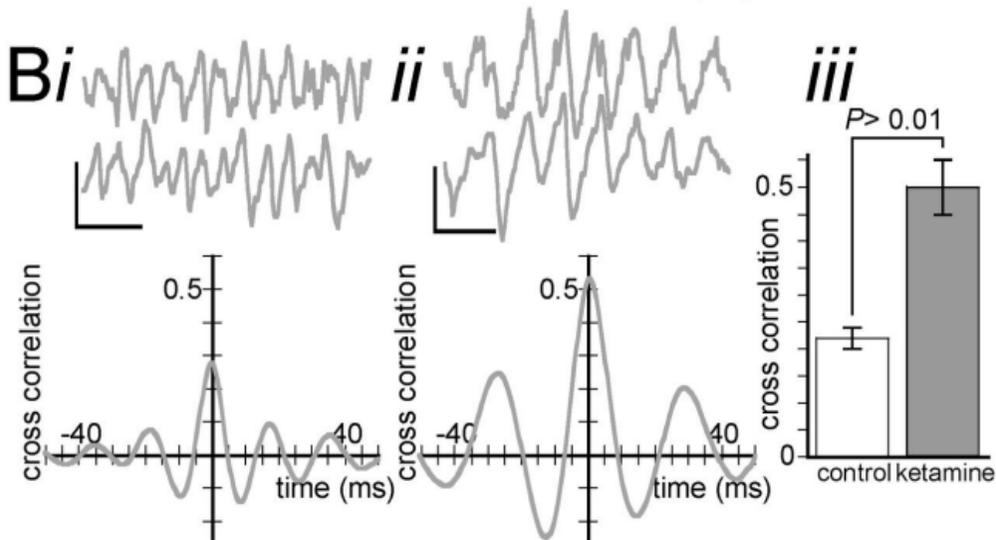
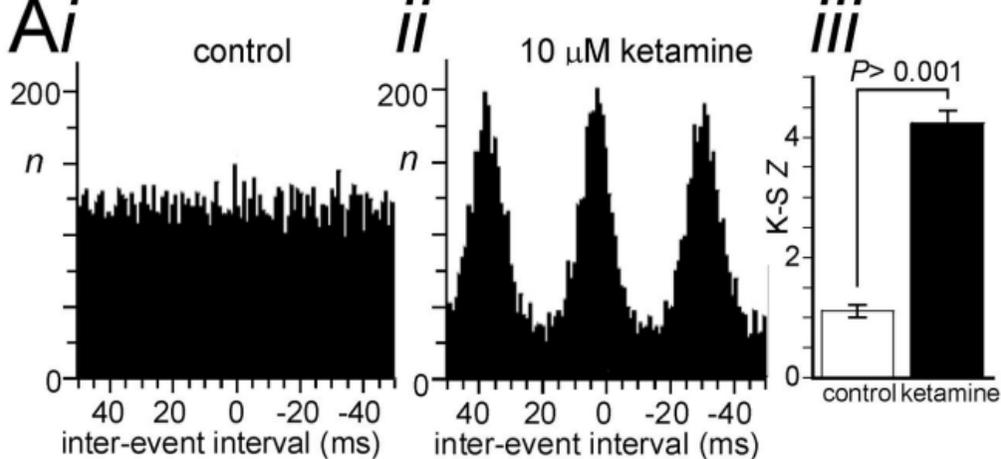
Figure 3. Ketamine effect on layer III pyramidal neurons. **A.** Peri-event interval distribution of action potential peak times relative to HF γ troughs in layer III, before (*i*) and after 10 μM ketamine (*ii*). *iii.* Z value of the Kolmogorov-Smirnov test for uniform distribution in control and ketamine. Data are mean \pm SEM for $n=6$. **B.** Same as in (A), but relative to LF γ troughs in layer V. **C.** Membrane potential (held just below firing threshold) waveform average phase-zeroed to the HF γ troughs in layer III, before (*i*) and after ketamine (*ii*). Scale bars: 20 μV , 20 ms. *iii.* Maximum peak-to-trough amplitude of the postsynaptic potential (PSP) waveform average in control and ketamine. Data give mean \pm SEM for $n=6$. **D.** Same as in (C), but phase-zeroed to the LF γ troughs in layer V. Ketamine entrains layer III pyramidal neurons to LF γ troughs in layer V and increases LF γ -related rhythmic synaptic inputs.

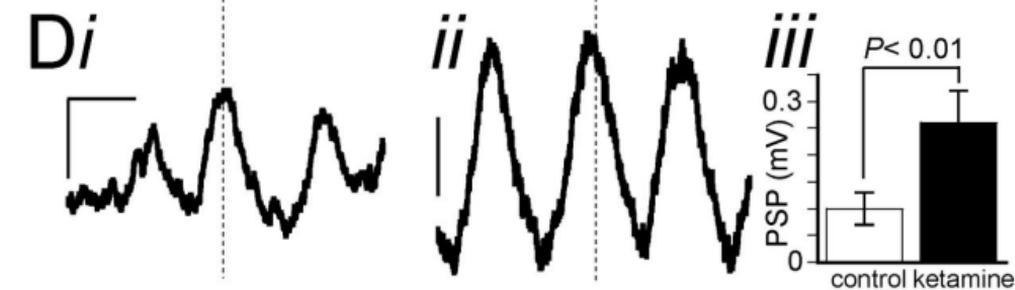
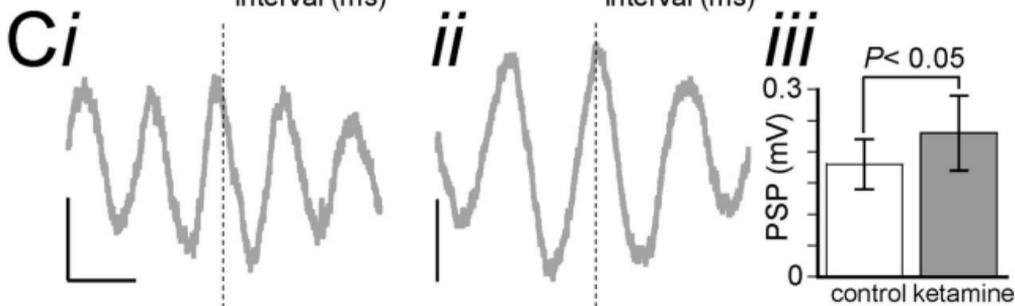
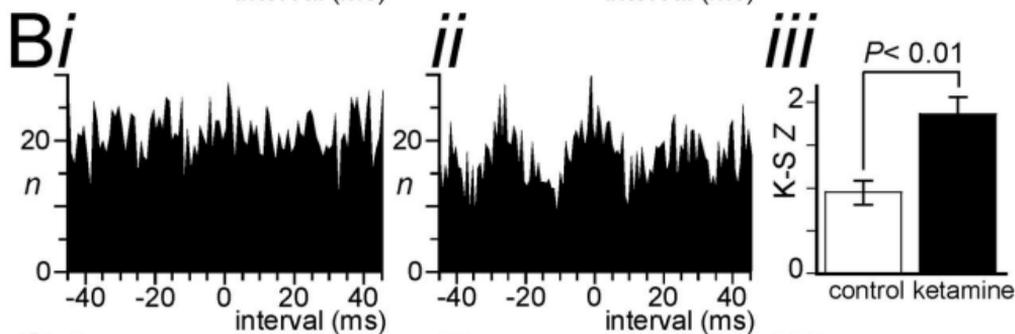
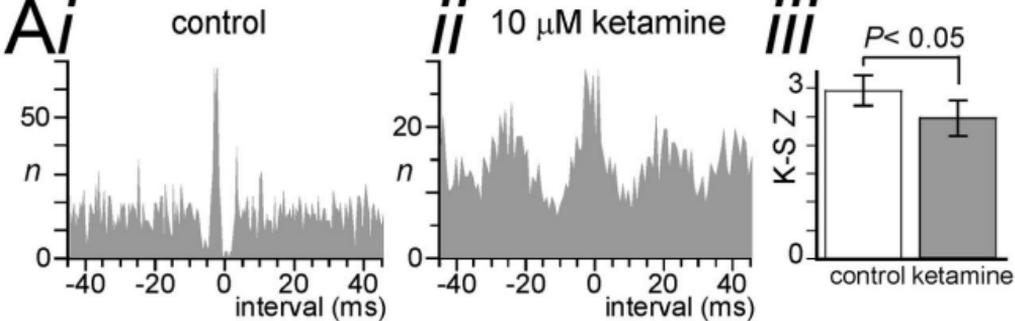
Figure 4. Effect of NMDA receptor modulation on HF γ . **A.** Power spectrum of a one-minute recording from layer III before (grey line) and after reducing endogenous *D*-serine levels with DAAO (0.17 U/ml, 60 minutes, black line). **B.** Effect of the NR2B subunit-containing receptor antagonist Ro 25-6981 (10 μM , 25 minutes). Details as in (A). **C.** Effect of application of *D*-

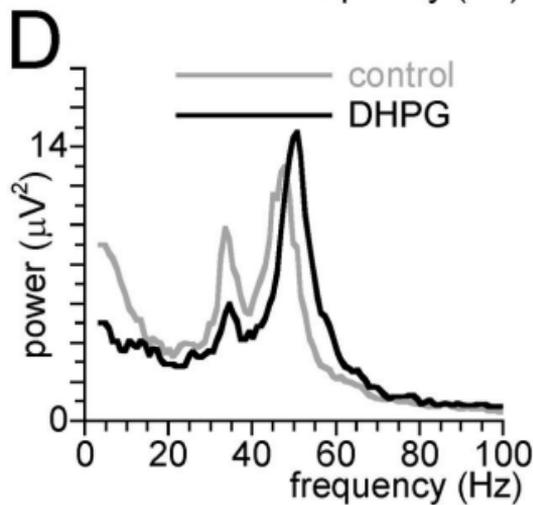
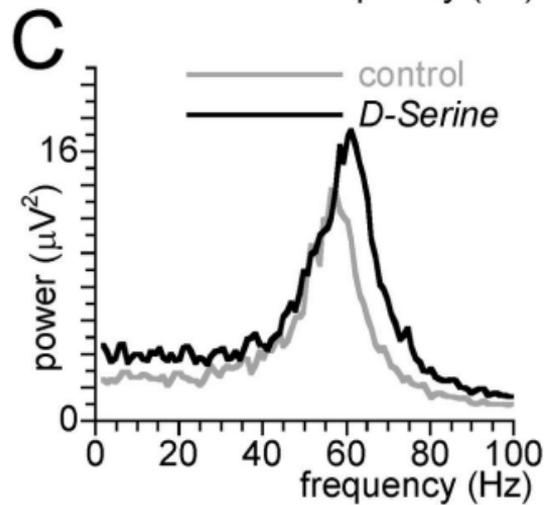
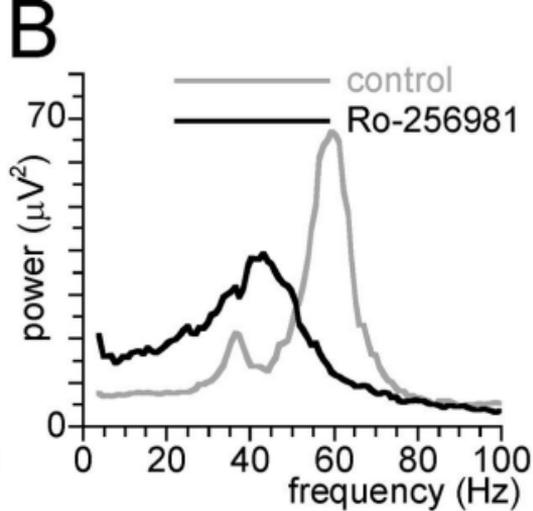
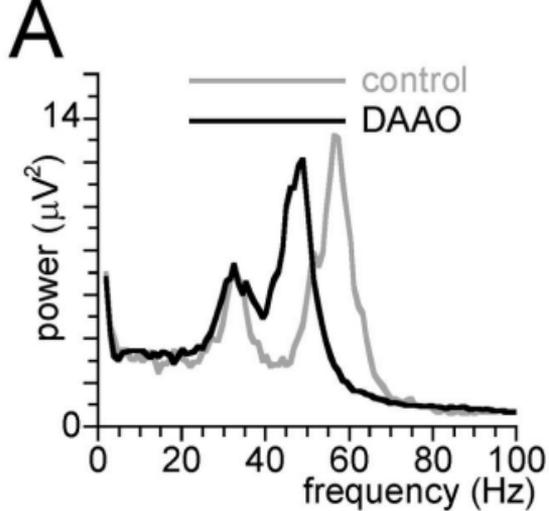
serine (100 μM). Details as in (A). **D.** Effect of facilitating NMDA receptor function by DHPG (10 μM). Details as in (A).

Figure 5. Illustration of the effect of hypersynchrony within and across layers. Recordings of gamma oscillations (modeled by sinusoids) from extracellular electrodes in two positions (500 μm apart, indicated in left panel) of layer III (grey traces) and layer V (bottom traces). In control (left hand traces) oscillations in layer III positions are not phase-locked within the layer or with the oscillation in layer V. After ketamine (right hand traces) the oscillation is phase-locked within and between the layers, increasing the signal picked up from the surface (top traces: summation of sinusoids). Dotted lines indicate times where potential firing times of layer III and layer V pyramidal neurons in position A would be nearly simultaneous, facilitating cross-layer interactions.



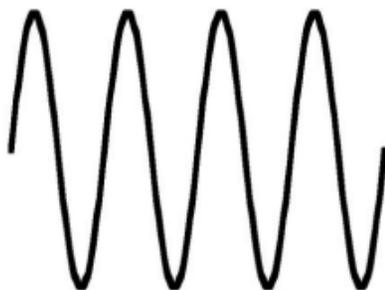
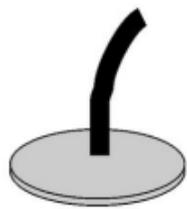






control

ketamine



20 ms

