

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

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Abstract

A single non-anaesthetic dose of ketamine, a non-competitive NMDA receptor (NMDAR) antagonist with hallucinogenic properties, induces cognitive impairment and psychosis, and aggravates schizophrenia symptoms in patients. In conscious rats an equivalent dose of ketamine induces key features of animal models of acute psychosis, including hyperlocomotor activity, deficits in prepulse inhibition and gating of auditory evoked potentials, and concomitantly increases the power of ongoing spontaneously occurring gamma (30–80 Hz) oscillations in the neocortex. This study investigated whether NMDAR antagonist-induced aberrant gamma oscillations could be modulated by acute treatment with typical and atypical antipsychotic drugs. Extradural electrodes were surgically implanted into the skull of adult male Wistar rats. After recovery, rats were subcutaneously administered either clozapine (1–5 mg/kg, $n=7$), haloperidol (0.05–0.25 mg/kg; $n=8$), LY379268 (a preclinical agonist at mGluR_{2/3} receptors: 0.3–3 mg/kg; $n=5$) or the appropriate vehicles, and 30 min later received ketamine (5 mg/kg s.c.). Quantitative measures of EEG gamma power and locomotor activity were assessed throughout the experiment. All three drugs significantly reduced the power of baseline EEG gamma oscillations by 30–50%, an effect most prominent after LY379268, and all inhibited ketamine-induced hyperlocomotor activity. However, only pretreatment with LY379268 attenuated trough-to-peak ketamine-induced gamma hyperactivity. These results demonstrate that typical and atypical antipsychotic drugs acutely reduce cortical gamma oscillations, an effect that may be related to their clinical efficacy.

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Introduction

High-frequency neuronal network oscillations in the brain, including those in the gamma band (30–80 Hz), represent collections of neurons firing in synchrony

linking different brain regions or local circuits (Buzsaki & Draguhn, 2004). The functional role of high-frequency gamma oscillations has been linked to a diverse range of higher-order brain function, including cognition (Engel *et al.* 2001), sensory binding of information (Joliot *et al.* 1994), working-memory tasks (Howard *et al.* 2003; Tallon-Baudry *et al.* 1998) and sensory perception (Gross *et al.* 2007). Schizophrenia, a complex heterogeneous psychiatric disorder, is characterized by disturbances in many of such higher-order brain functions, including perception

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and distortions of reality. The proposed role of these gamma oscillations in conscious integration, sensory perception and cognition has led to the conceptualization that abnormalities in gamma frequency activity may underlie many aspects of schizophrenia symptomatology (Lee *et al.* 2003*b*), and indeed the pathophysiology of the disease (Herrmann & Demiralp, 2005; Lee *et al.* 2003*a*).

Ketamine and other NMDA receptor (NMDAR) antagonists at subanaesthetic doses induce perceptual distortions and visual hallucinations in healthy humans, and exacerbate psychotic symptoms in schizophrenia patients (Krystal *et al.* 1994; Lahti *et al.* 1995; Malhotra *et al.* 1997). These observations informed the NMDAR hypofunction hypothesis of schizophrenia, whereby reduced functionality at this receptor in some way manifests as schizophrenia-like symptoms. We (Hakami *et al.* 2009; Pinault, 2008) and others (Ehrlichman *et al.* 2009; Lazarewicz *et al.* 2010; Ma & Leung, 2007) previously demonstrated that, in rodents, NMDAR antagonists dose-dependently increase the power of ongoing gamma frequency oscillations. We also demonstrated that this effect is correlated with the hyperlocomotor response induced by these compounds (Hakami *et al.* 2009), a commonly employed model of acute psychosis (van den Buuse *et al.* 2005). These correlative findings linking drug-induced hyperlocomotor activity to increases in gamma power in rats, and the clinical observations implicating gamma power abnormalities to psychotic symptoms in schizophrenia patients (Baldeweg *et al.* 1998; Gordon *et al.* 2001; Spencer *et al.* 2008*b*, 2009), suggest that NMDAR antagonist-induced increases in gamma power may represent an electrophysiological correlate of acute psychosis (Lee *et al.* 2003*a*).

The current study aimed to test the hypothesis that typical and atypical antipsychotic medications, both clinical and preclinical, acutely inhibit the increase in ongoing gamma power induced by the NMDAR antagonist ketamine, thereby presenting this as a predictive marker of antipsychotic activity. Our experimental design also allowed assessment of the effects of the antipsychotics alone on gamma power. Three compounds were chosen to test these hypotheses, each possessing a different spectrum of receptor binding and therapeutic mechanism of action, to gauge the effect of different classes of antipsychotic drugs: (i) haloperidol, a typical neuroleptic agent with potent antagonist activity at dopamine D₂ receptors (Miyamoto *et al.* 2005); (ii) clozapine, the classic atypical antipsychotic with a diverse pharmacological profile including antagonism at 5-HT

receptors (particularly 5-HT_{2A} receptors), muscarinic and histamine receptors, which also interacts with the glycine site of NMDARs (Schwieler *et al.* 2008), but with weaker binding affinity for dopamine D₂ receptors (Miyamoto *et al.* 2005); and (iii) LY379268, a preclinical drug which is an agonist at metabotropic (mGluR_{2/3}) glutamate receptors, and also dopamine D_{2/3} receptors (Seeman & Guan, 2009), and which possesses efficacy in animal models of acute psychosis (Cartmell *et al.* 2000; Imre, 2007). The primary electrophysiological outcome was baseline, or resting-state, gamma power as measured before and after drug administration, in keeping with our previous studies. This is in contrast with evoked or induced gamma responses, such as those measured following an auditory stimulus (e.g. Uhlhaas & Singer, 2010). Ongoing gamma power is an important, but commonly overlooked, measurement, since many studies examining evoked/induced gamma responses incorporate some form of 'baseline' correction.

Methods

Animals

Male Wistar rats (total $n = 26$, 250–350 g) were bred and group-housed (2–3 per cage) in the Biological Research Facility of the Department of Medicine, Royal Melbourne Hospital, The University of Melbourne. The facility was kept on a 12-h light/dark cycle (lights on at 06:00 hours), with food (standard rat chow) and water available *ad libitum*. At all times, care was taken to minimize pain and discomfort of the animals, and all experimental procedures were approved by the University of Melbourne Animal Ethics Committee (no. 0701821).

EEG recording electrode implantation

Animals were anaesthetized by inhalation of isoflurane in equal parts of medical air and oxygen (5% induction, 1.5–2.5% maintenance) and positioned in a stereotaxic frame as described previously (Hakami *et al.* 2009; Pinault, 2008). Briefly, a single midline incision was made over the scalp and six holes were drilled through the skull for stereotaxic implantation (Paxinos & Watson, 2005) of recording brass electrodes [2 mm anterior and 2 mm lateral to bregma bilaterally (active electrodes); 2 mm posterior and 2 mm lateral to bregma bilaterally (ground electrodes); and 2 mm posterior and 2 mm lateral to lambda bilaterally (reference electrodes)]. Brass electrodes were then screwed into the skull without breaching the dura, and

dental cement applied to the skull to fix the electrodes in place. After recovery from anaesthesia, animals were placed in separate cages for at least 7 d prior to further experimentation.

Assessment of drug effects on EEG gamma power and locomotor activity

On the day of experimentation, rats were brought into the Behavioural Testing Facility in the Department of Medicine at least 30 min prior to the start of the study to allow habituation to the environment. Rats were then individually placed into an open arena (1 m diameter) with each recording electrode attached to a cable suspended from the ceiling to facilitate continuous recording of the EEG. Each rat was allowed to explore the arena for 30 min, at which time they were injected subcutaneously (s.c.) with either haloperidol (0.025–0.25 mg/kg s.c.; $n=8$), clozapine (1–5 mg/kg s.c., $n=7$), LY379268 (0.3–3 mg/kg s.c.; $n=5$) or the appropriate vehicles. Following a further 30 min, rats were then injected with ketamine (5 mg/kg s.c.) or vehicle (0.9% saline) and returned to the arena for a further 60 min of EEG recording. This procedure was repeated on subsequent days (with at least 2 d between trials) with a different dose of antipsychotic until each rat had received each dose of its assigned antipsychotic. During the 120-min recording period, the animal's locomotor activity was video-tracked and objectively assessed using Ethovision software (Noldus[®], The Netherlands). Total distance travelled was calculated for every 2-min interval.

A subsequent study was performed to assess the effects of a single administration of antipsychotic over a 24-h period. These studies were performed in the home cages of the animals in the Biological Research Facility of the Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, and were started between 12:00 and 16:00 hours. Animals ($n=6$) were connected to the EEG cables, and allowed a 30-min habituation period prior to being injected with either haloperidol (0.25 mg/kg s.c.), clozapine (5 mg/kg s.c.), LY379268 (3 mg/kg s.c.) or vehicle (0.9% saline) in a randomized fashion, with at least 2 d between experiments. Animals were left undisturbed for a further 24 h of EEG recording following drug treatment.

EEG data acquisition processing

The EEG recordings from each hemisphere were acquired using a MacLab amplifier and A-D converter,

and Chart v. 3.5 software (AD Instruments, Australia) with a bandpass of 1–1000 Hz. The line power 50 Hz noise was eliminated from the signal using selective eliminators (Humbugs; Digitimer, UK). The gamma frequency activity on the EEG recordings was analysed using NeuroScan[®] software (Compumedics, Australia) as previously described (Hakami *et al.* 2009). Briefly, using fast Fourier transformations (FFT), average power in the gamma frequency band (30–80 Hz) was determined for each 2.05-s epoch for the entire recording period, and average power was then calculated for each 2-min interval. For both gamma power and locomotor activity data, the 15 values obtained during the 30-min baseline pre-treatment period were averaged for each individual animal, and all recorded values then expressed as a percent change of this baseline response. For the 24-h recordings, the average power was calculated for each 5 min for the first 3.5 h, and then for each 30 min thereafter, using the same 2.05-s epoch determination as above, this time for all power bands, including gamma (30–80 Hz), delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–30 Hz) frequency oscillations. The first six values, representing the 30-min baseline, were averaged for each individual animal, and all recorded values then expressed as a percent change of this baseline response, also as above.

Drugs and vehicles

Isoflurane was purchased from Abbott Pharmaceuticals (USA). Clozapine (Sigma, USA) was dissolved using pure acetic acid and then diluted with a 10% acetic acid solution (neutralized to pH 6.0) using 10 M NaOH. LY379268 (Tocris, UK) was diluted in sterile water. Haloperidol (Sigma, USA) and ketamine (Parnell Laboratories, Australia) were diluted in 0.9% sterile saline.

Statistical analyses

For both the gamma power and locomotor activity data, the values obtained during the 30-min pre-injection period were averaged for each individual animal, and all recorded values then expressed as a percent change from this baseline value. Two statistical analyses were designed for this study to answer the two separate study aims. The first determined the effect of exposure to antipsychotics on spontaneous ongoing EEG gamma power and locomotor activity. This was statistically assessed separately for each antipsychotic compound using two-way ANOVA

Table 1. Descriptive summary of effects of antipsychotics on gamma power and locomotor activity. Upper panel describes effects of antipsychotics on baseline and ketamine-induced changes in gamma power; lower panel describes effects of drugs on locomotor activity. Percentage change of ketamine-induced effects (middle column) were calculated 6–8 min after ketamine injection at the peak of the ketamine response

	Baseline gamma power	Ketamine-induced gamma hyperactivity	Trough-to-peak ketamine-induced gamma hyperactivity
Haloperidol	32% ↓, dose-dependent	16% ↓, not dose-dependent	No effect
Clozapine	26% ↓, not dose-dependent	12% ↓, not dose-dependent, n.s.	No effect
LY379268	59% ↓, dose-dependent	100% ↓, dose-dependent	70% ↓, dose-dependent
	Baseline locomotion	Ketamine-induced locomotor hyperactivity	Trough-to-peak ketamine-induced locomotor hyperactivity
Haloperidol	No effect	95% ↓, dose-dependent	100% ↓, dose-dependent
Clozapine	No effect	84% ↓, dose-dependent, n.s.	67% ↓, dose-dependent
LY379268	No effect	100% ↓, not dose-dependent ^a	94% ↓, dose-dependent

n.s., Non-significant effect.

^a In this instance, 100% inhibition of response was observed with the lowest dose of LY379268, therefore no dose dependence was attained.

with repeated measures (time after injection), with Bonferroni's *post-hoc* analysis when appropriate. The second analysis was designed to assess the effect of antipsychotic drugs on ketamine-induced changes in gamma power and locomotor activity. This was also performed using two-way ANOVA with repeated measures (time after injection). However, since the antipsychotic drugs influenced the baseline gamma power, the trough-to-peak rises of both gamma power and locomotor activity following ketamine administration were compared using a one-way ANOVA with repeated measures (dose of drug). Trough values corresponded to the 2-min interval immediately prior to ketamine injection (i.e. $t=58$ min after trial initiation). Peak values were calculated as the point of greatest gamma power or locomotor response following ketamine injection, and were calculated for each individual animal and each drug dose. In ~88% of all cases, the peak response occurred between 6 and 10 min after ketamine injection. The trough-to-peak values were then determined by subtracting the value of the trough from the peak for each individual animal, and then averaged per group. It should be noted that both the trough and the peak values of each recording comprised of the averages of 119×2.05 -s epochs, ensuring that sporadic experimental noise of a single epoch did not overly affect the trough or peak values of the group mean. Statistical comparisons were performed using GraphPad Prism[®] (USA), and statistical significance was set at $p < 0.05$ for all tests.

Results

The antipsychotics haloperidol, clozapine and LY379268 reduce spontaneous ongoing cortical gamma power

All three antipsychotics tested had a clear and rapid effect in decreasing the power of spontaneous gamma oscillations in the EEG, compared to vehicle treatments. Haloperidol significantly reduced gamma power in a time- and dose-dependent manner ($F_{1,8}=6.94$, $p=0.001$; Fig. 1a inset) with a maximal reduction to 68% of control levels. Clozapine also rapidly reduced ongoing gamma power, with all three doses achieving this to the same extent ($F_{1,8}=11.63$, $p < 0.001$; Fig. 2a inset). The maximal reduction induced by clozapine was to 74% of control. LY379268 also induced an abrupt and dose-dependent drop in ongoing gamma power ($F_{1,5}=39.35$, $p < 0.001$; Fig. 3a inset), with the highest dose (3 mg/kg) reducing power to 41% of control. The experimental design of these studies only allowed assessment of the effects of antipsychotic alone for 30-min post-injection. Therefore, a subsequent set of studies were performed to investigate these effects over a 24-h period. In these studies, the statistically significant reductions in gamma power induced by all antipsychotics were replicated [haloperidol (reduced to 61% of control): $F_{1,6}=16.39$, $p=0.003$; clozapine (66%): $F_{1,6}=19.86$, $p=0.001$; LY379268 (36%): $F_{1,6}=180.4$, $p < 0.001$; Fig. 4]. Furthermore, the effects of all antipsychotics were still significantly reduced for at least 12 h fol-

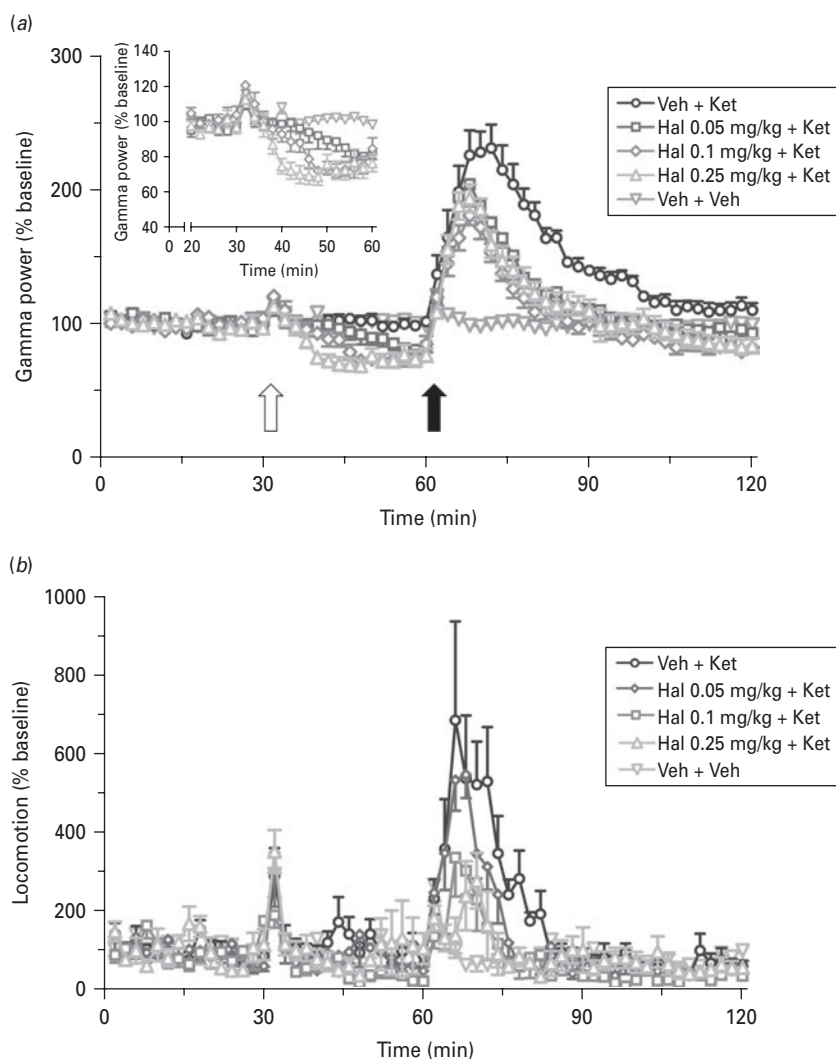


Fig. 1. Effects of haloperidol on ketamine-induced elevations in gamma power and locomotor activity in rats. (a) Haloperidol (0.05–0.25 mg/kg s.c.) dose-dependently depressed ongoing gamma power by up to 30% during the 30-min pretreatment phase of the recordings, compared to vehicle treatment. (a) Haloperidol significantly but incompletely reduced the rise in gamma power induced by ketamine (5 mg/kg s.c.), compared to vehicle pretreatment. This effect was not dose-dependent. (b) Pretreatment with haloperidol dose-dependently suppressed ketamine-induced hyperlocomotor activity, compared to vehicle pretreatment. Data represent mean \pm S.E.M. ($n = 8$). Open arrow indicates timing of haloperidol administration, and closed arrow indicates ketamine administration.

lowing administration compared to vehicle (using Bonferroni's *post-hoc* analyses).

We also examined the acute impact of antipsychotics in other ongoing EEG activities, which could be distinguished from their frequency bands, including delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–30 Hz). No significant change in delta or theta was observed after treatment with any of the tested drugs ($p > 0.05$ for all drugs and all powers, compared to vehicle). However, the power of both alpha ($F_{1,6} = 11.58, p = 0.007$) and beta ($F_{1,6} = 24.05, p < 0.001$) was significantly reduced by LY379268. The other drugs did

not significantly alter these bands either ($p > 0.05$ for all analyses), suggesting that their action specifically targeted spontaneously occurring gamma oscillations. This data is located in the Supplementary Figures (online).

Although some heavy sedation was seen following administration of the highest doses of clozapine (5 mg/kg) and LY379268 (3 mg/kg), and ataxia without sedation with the highest dose of haloperidol (0.25 mg/kg), these effects did not result in any difference in total locomotor activity during the 30-min pre-treatment window, compared to vehicle treatment

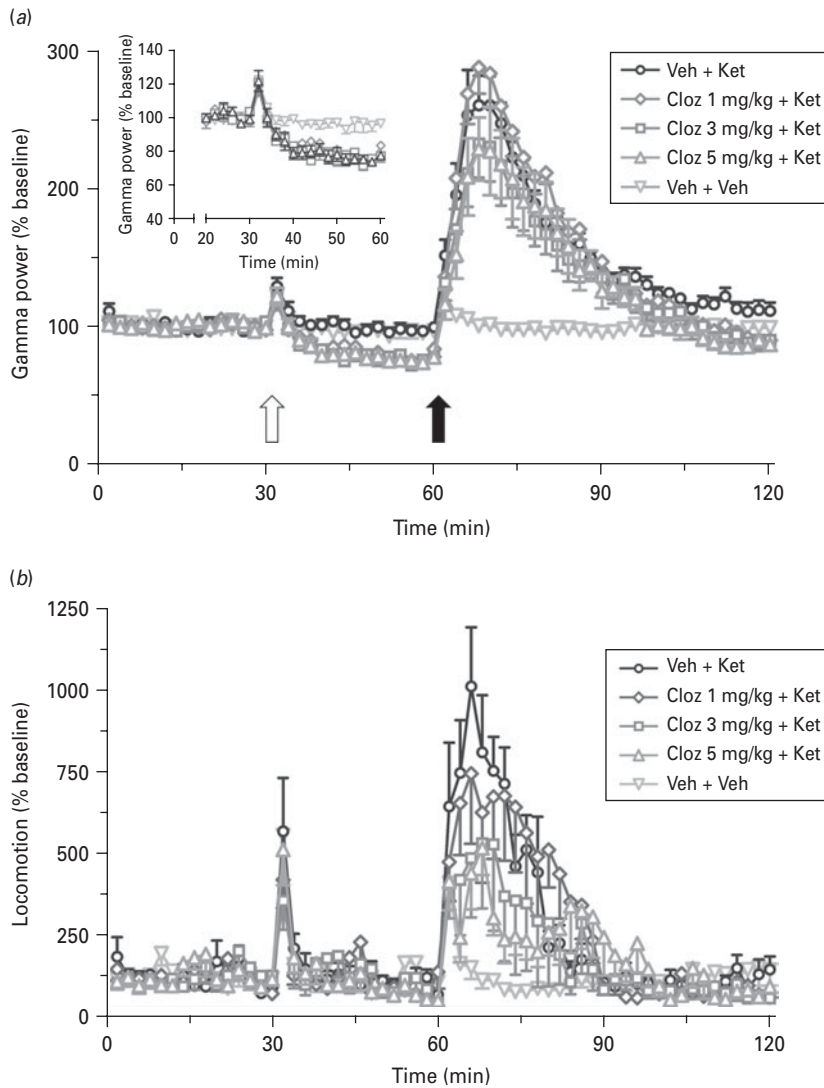


Fig. 2. Effects of clozapine on ketamine-induced elevations in gamma power and locomotor activity in rats. (a, inset) Clozapine depressed ongoing gamma power by up to 25% during the 30-min pretreatment phase of the recordings, compared to vehicle treatment, although this was not dose-dependent for the doses used. (a) Clozapine did not significantly affect the rise in gamma power induced by ketamine (5 mg/kg s.c.), compared with vehicle pretreatment. (b) Pretreatment with clozapine (1–5 mg/kg s.c.) induced a dose-dependent but incomplete suppression of ketamine (5 mg/kg s.c.)-induced hyperlocomotor activity compared to vehicle pretreatment. Data represent mean \pm S.E.M. ($n = 7$). Open arrow indicates timing of clozapine administration, and closed arrow indicates ketamine administration.

(haloperidol: $F_{1,8} = 1.14$, $p = 0.352$; clozapine: $F_{1,7} = 0.13$, $p = 0.940$; LY379268: $F_{1,5} = 3.611$, $p = 0.062$). Locomotor activity was not measured during the long-term recordings performed in home cages.

Pretreatment with LY379268, but not haloperidol or clozapine, reduces the trough-to-peak ketamine-induced increase in gamma power

In keeping with our previous studies (Hakami *et al.* 2009; Pinault, 2008), administration of ketamine

(5 mg/kg s.c.) induced an acute increase in gamma power which peaked 8–12 min after injection (peak response – haloperidol study: $231 \pm 17\%$ compared to baseline; clozapine study: $260 \pm 21\%$; LY379268 study: $252 \pm 39\%$). Pretreatment with haloperidol (0.05–0.25 mg/kg) significantly reduced the ketamine response on gamma power ($F_{1,8} = 13.43$, $p < 0.001$; Fig. 1a). Further, this attenuating effect of haloperidol did not demonstrate dose-dependence. Clozapine pretreatment (1–5 mg/kg) appeared to reduce the gamma power response of ketamine, but this was not

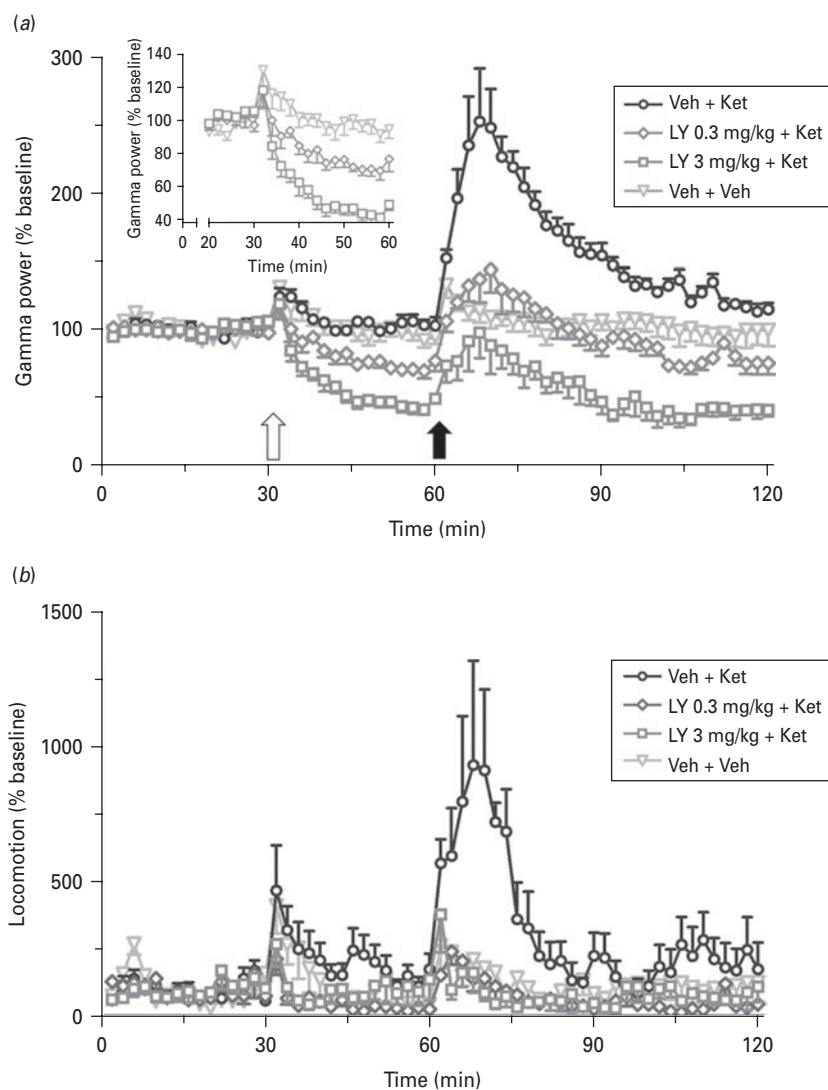


Fig. 3. Effects of LY379268 on ketamine-induced elevations in gamma power and locomotor activity in rats. (a, inset) LY379268 potently and dose-dependently depressed ongoing gamma power by up to 60% during the 30-min pretreatment phase of the recordings, compared to vehicle treatment. (a) LY379268 significantly and completely reduced the rise in gamma power induced by ketamine (5 mg/kg s.c.), compared to vehicle pretreatment. This effect was dose-dependent. (b) Pretreatment with LY379268 (0.3–3 mg/kg s.c.) induced a dose-dependent and complete suppression of ketamine (5 mg/kg s.c.)-induced hyperlocomotor activity compared to vehicle pretreatment. Data represent mean \pm S.E.M. ($n=5$). Open arrow indicates timing of LY379268 administration, and closed arrow indicates ketamine administration.

a dose-dependent effect, and failed to achieve statistical significance ($F_{1,7}=2.18$, $p=0.117$; Fig. 2a). Pretreatment with LY379268 (0.3–3 mg/kg) dramatically and dose-dependently reduced the ketamine-induced increase in gamma power ($F_{1,5}=95.28$, $p<0.001$; Fig. 3a) with the highest dose preventing any rise in gamma power above the original baseline level.

The attenuating effect of the antipsychotics on the ketamine response appeared to be influenced by the

ability of these compounds to reduce the peak power of gamma oscillations themselves. To take this into account, 'trough-to-peak' values were calculated, thereby controlling for the 'antipsychotic alone' effect on gamma power. Neither haloperidol ($F_{1,8}=1.51$, $p=0.241$; Fig. 5a) nor clozapine ($F_{1,7}=1.26$, $p=0.317$; Fig. 5b) had any statistically significant effect on the ketamine-induced trough-to-peak increases in gamma power. However, LY379268 significantly attenuated the effect of ketamine ($F_{1,5}=5.97$, $p=0.026$; Fig. 5c),

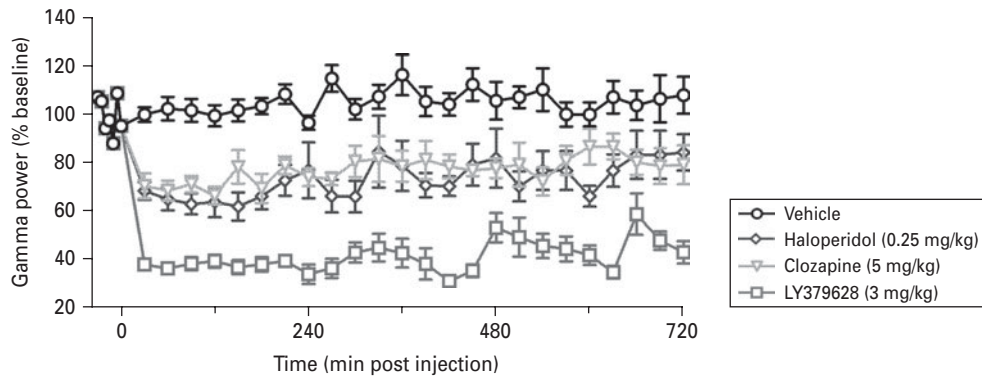


Fig. 4. Extended effects of antipsychotics on ongoing gamma power. When administered at high doses, all three antipsychotics produced a robust and significant decrease in spontaneous ongoing gamma power for 12 h, compared to vehicle. Data represent mean \pm S.E.M. ($n=6$). This effect persisted for at least 24 h.

demonstrating a clear differential effect compared to the other antipsychotics.

Pretreatment with antipsychotics dose-dependently inhibits the ketamine-induced hyperlocomotor response.

Ketamine (5 mg/kg) induced an acute hyperlocomotor response which peaked 6–8 min after injection and persisted for up to 30 min (peak values – haloperidol study: $736 \pm 241\%$ compared to baseline; clozapine study: $989 \pm 182\%$; LY379268 study: $931 \pm 388\%$). Pretreatment with haloperidol [reaching significance ($p < 0.05$) at 0.1 and 0.25 mg/kg] and LY379268 (reaching significance at 0.3 mg/kg, and further suppression with 3 mg/kg) prior to ketamine injection dose-dependently inhibited this hyperlocomotor response (overall two-way ANOVA – haloperidol: $F_{1,8}=4.01$, $p=0.017$; LY379268: $F_{1,5}=6.83$, $p=0.006$), whereas the inhibitory effect of clozapine on locomotor activity did not reach statistical significance ($F_{1,7}=0.677$, $p=0.575$). When comparing trough-to-peak values, all three compounds significantly attenuated locomotor activity induced by ketamine (one-way ANOVA – haloperidol: $F_{1,8}=9.64$, $p < 0.001$; clozapine: $F_{1,7}=3.99$, $p=0.024$; LY379268: $F_{1,5}=10.60$, $p=0.012$). A summary of the effects of antipsychotics on locomotor activity and on gamma power is located in Table 1.

Discussion

The current study describes a differential effect of antipsychotics on the ketamine-induced increase in the power of spontaneously occurring gamma oscillations: LY379268 potently suppressed this effect, in direct contrast to clozapine and haloperidol which were unable to modify the ‘trough-to-peak’ response

of ketamine. LY379268, a preclinical compound, exerted its effect in a clear and powerful dose-dependent manner, and we speculate that this may be due to its agonist effects on mGluR_{2/3} receptors, a property not shared by the other two drugs. All three compounds were, however, able to reduce the peak in EEG gamma power induced by ketamine administration, at least to some extent. An additional, and no less intriguing finding, was that all antipsychotic drugs tested reduced the baseline power of ongoing EEG gamma activity, an effect which is maintained for at least 12 h. Given that this property was similar across all three drugs tested, including two well-established clinical compounds, despite their very different spectrum of pharmacological actions, this may represent an electrophysiological biomarker of clinical antipsychotic efficacy.

A number of previous studies in rodents examining the effect of psychotomimetic drugs, in particular NMDAR antagonists such as ketamine, have demonstrated increases in the power of ongoing gamma oscillations on the EEG (Ehrlichman *et al.* 2009; Hakami *et al.* 2009; Lazarewicz *et al.* 2010; Ma & Leung, 2007; Pinault, 2008). It is proposed that this abnormal gamma activity represents aberrant diffuse network noise and is a potential electrophysiological correlate of a psychotic-like state. In apparent conflict with this, many clinical studies describing abnormalities in gamma oscillatory power in schizophrenia patients have reported *decreases* in EEG gamma power responses to evoked stimuli (e.g. Ford *et al.* 2008; Kwon *et al.* 1999; Spencer *et al.* 2003, 2008a). However, most of these studies did not take into account the influence of antipsychotic medication (but see Hong *et al.* 2004). When examining subsets of schizophrenia patients, studies of evoked and induced gamma power responses describe positive correlations between

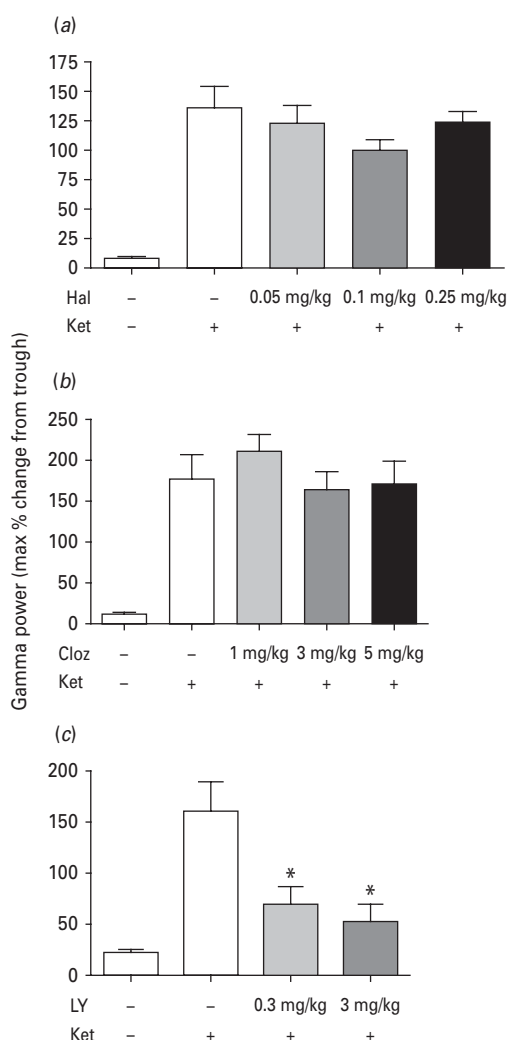


Fig. 5. Effects of antipsychotics on 'peak-to-trough' gamma power increase induced by ketamine (5 mg/kg s.c.). Neither (a) haloperidol (Hal) nor (b) clozapine (Cloz) influenced the net increase (expressed as max % change from trough) of ongoing gamma power induced by ketamine at any pretreatment dose. In contrast, pretreatment with (c) LY379268 (LY) was able to significantly suppress the 'peak-to-trough' gamma power rise induced by ketamine (* $p < 0.05$). Data represent mean \pm S.E.M.; sample sizes as for Figs 1–3.

hallucinatory symptoms and gamma power responses (Gordon *et al.* 2001; Spencer *et al.* 2008b, 2009), suggesting that certain psychotic disease states may be associated with different levels gamma activity and reactivity. Further, a single case report measured ongoing gamma oscillatory power during acute psychotic episodes, and described significantly *elevated* gamma oscillatory power, most prominent at times when the symptoms were most severe (Baldeweg *et al.*

1998) and other reports describe increases in basal ongoing gamma waves in patients with schizophrenia (Turetsky & Siegel, 2007). These reports complement a study of first-episode psychosis patients who displayed an excess of absolute gamma phase synchrony elicited by a selective attention task compared to controls (Flynn *et al.* 2008), and a study describing increases in auditory-evoked gamma band responses in humans following acute ketamine (Hong *et al.* 2010). Collectively, these findings suggest that increased gamma power responses, and indeed ongoing gamma oscillatory power, may be positively associated with psychotic symptoms in schizophrenia. If this were the case, then it may be expected that drugs that reduce psychotic symptoms may reduce the amount of EEG gamma power. This is supported by the results of this study, demonstrating a potent (and in some cases dose-dependent) suppression of the power of the spontaneous background gamma oscillations that was common to all antipsychotic drugs tested.

The generation of gamma oscillations is believed to be driven by populations of fast-spiking interneurons, providing feedback inhibition onto pyramidal cells (Bartos *et al.* 2007; Fisahn *et al.* 1998; Fuchs *et al.* 2007). Systemic administration of NMDAR antagonists is thought to block NMDAR-mediated firing of these interneurons, resulting in enhanced firing rates of pyramidal neurons and therefore an elevation of the power of local gamma rhythms (Jackson *et al.* 2004; Moghaddam *et al.* 1997). Gamma frequency oscillations can also be influenced by many other receptor systems, including dopaminergic and cholinergic (Lisman *et al.* 2008), receptor types which are targeted by the antipsychotics used in the current project. Taking into account the distinct mechanisms of action of the drugs tested here, the common property of the antipsychotics to reduce ongoing gamma power is probably not be mediated via a common receptor mechanism, but may represent a more fundamental effect against downstream psychotic mechanisms. In addition, the duration of effects of these compounds (lasting > 12 h following administration), is suggestive of a gene transcription-mediated effect, since brain levels, at least of clozapine and haloperidol (Baldessarini *et al.* 1993; Campbell *et al.* 1980), would be expected to be low/negligible at this time-point.

Although we observed common features of the different antipsychotics tested (i.e. all reduce baseline gamma power and all inhibit the ketamine-induced hyperlocomotor response), only the preclinical mGluR_{2/3} agonist was able to suppress the trough-to-peak effect of ketamine on gamma power. LY379268 has also been shown to effectively block behavioural

effects of NMDAR antagonists, such as disruptive effects on working memory (Moghaddam & Adams, 1998) and hyperlocomotor activity (Imre *et al.* 2006a), effects promoting this as a potential treatment for patients with schizophrenia. The unique ability of this compound to block ketamine-induced increases in gamma EEG power reported here may reflect an effect of this drug more directly on the neuronal mechanisms controlling gamma oscillations in the brain than either clozapine or haloperidol. Group II mGluRs (i.e. mGluR₂ and mGluR₃) are generally located presynaptically and inhibit the release of glutamate from the presynaptic terminal (Moghaddam & Adams, 1998), providing a negative feedback loop on pyramidal cell firing. MK801, another NMDAR antagonist which we have previously shown to increase EEG gamma power (Hakami *et al.* 2009; Pinault, 2008), has also been shown to increase the firing rate of cortical neurons in awake rats (Jackson *et al.* 2004). Activation of mGluR_{2/3} with LY354740 dose-dependently suppresses this effect (Homayoun & Moghaddam, 2008; Homayoun *et al.* 2005). This may well be relevant to the observations in this study showing that a drug that activates mGluR_{2/3} receptor subtypes inhibits the ability of ketamine to increase cortical gamma activity, in contrast to the two other antipsychotic drugs, suggesting a direct cellular interaction between ionotropic (NMDA) and metabotropic (mGluR_{2/3}) receptors. While this observation raises the possibility that the effect of LY379268 to inhibit ketamine-induced elevations in gamma power is mediated via its agonist activity at mGluR_{2/3} receptors, this needs to be confirmed by other studies specifically investigating this speculation. Indeed, further confounding the mechanism of this effect is a previous study reporting that subchronic treatment with the related compound, LY354740, purported to be a more potent agonist of mGluR_{2/3} (Monn *et al.* 1999), was unable to block ketamine-induced hyperlocomotor activity (Imre *et al.* 2006b). Although evidence exists for direct interactions between mGluR₃ receptors and NMDARs (Awad *et al.* 2000; Lecourtier *et al.* 2007), further studies are required to exact the precise cellular mechanisms linking mGluR_{2/3} and NMDARs.

The current study also supports the findings of our previous research (Hakami *et al.* 2009; Pinault, 2008) that, although NMDAR antagonist-induced increases in hyperlocomotor activity and in gamma power often occur together, the hyperlocomotor response does not, in itself, drive the elevations in gamma power. Here we showed that although both haloperidol and clozapine both inhibited the hyperlocomotor response of ketamine, they did not completely reverse the rise in

gamma EEG power. In addition, animals under anaesthesia still demonstrate a gamma power response to ketamine, and other psychotomimetic compounds such as amphetamines which potently elicit a hyperlocomotor response (Jones *et al.* 2010) have minimal effects on cortical or hippocampal gamma power (Ehrlichman *et al.* 2009; Ma & Leung, 2007; Pinault, 2008).

Although all three compounds were able to reduce the amount of ongoing gamma oscillations, only LY379268 also influenced EEG activity in other frequency bands. Both alpha and beta power were significantly reduced by this drug in a similar abrupt and enduring fashion as its effect on gamma power. It is not clear what ramifications this would have in a clinical setting, but beta power also has been strongly linked to cognitive processing (Uhlhaas & Singer, 2010).

In conclusion, the data from our study demonstrate prominent and long-lasting reductions in ongoing gamma EEG power following treatment with a range of antipsychotics, both clinical and preclinical, with differing pharmacological mechanisms of action. The wealth of research investigating gamma power abnormalities in schizophrenia have so far largely neglected to address the effect of antipsychotic medications when assessing disease-induced changes in gamma power, but this should be taken into account in future studies. Indeed, this property may be related to the clinical efficacy against positive symptoms of schizophrenia, since both clozapine and haloperidol were able to reduce the baseline gamma power, and may therefore provide an electrophysiological biomarker of antipsychotic activity. These compounds also mitigated the maximum increase in gamma power in response to acute systemic administration of ketamine, which may also be an important and potentially clinically relevant predictive outcome in its own right. Although the trough-to-peak response of ketamine was not influenced by the currently used antipsychotics, we have not here examined the effects of chronic antipsychotic treatment, nor the effects of other clinically used agents possessing different spectra of clinical effects (i.e. including those efficacious against cognitive deficits). This measure may yet prove to have added value to the predictive models used in the preclinical antipsychotic drug development, including drug-induced hyperlocomotor activity.

Note

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/npn>).

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Statement of Interest

None.

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