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Acute administration of typical and atypical antipsychotics reduce 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 abnormal behaviour, hyperlocomotion, deficits in prepulse inhibition to an acoustic startle response and gating of auditory evoked potentials, and concomitantly increases the power of spontaneously occurring gamma 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. Adult male Wistar rats that has been implanted with extradural electrodes were placed in an arena for 30 minutes (baseline) and then subcutaneously administered either clozapine (1-5mg/kg, n=7), haloperidol (0.05 – 0.25mg/kg; n=8), LY379268 (a preclinical agonist at mGluR_{2/3} receptors: 0.3 – 3mg/kg; n=5) or their vehicles alone, and 30 minutes later received ketamine (5mg/kg sc). 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.

Keywords: antipsychotic, gamma power, ketamine, schizophrenia, EEG

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 and 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) sensory perception (Gross et al., 2007). Schizophrenia, a complex heterogeneous psychiatric disorder, is characterised by disturbances in many of such higher order brain functions, including perception 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., 2003b), and indeed the pathophysiology of the disease (Herrmann and Demiralp, 2005; Lee et al., 2003a).

Ketamine and other NMDA receptor (NMDAr) antagonists at subanaesthetic doses induce perceptual distortions and visual hallucinations in healthy humans, and exacerbate psychotic symptoms in schizophrenic 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 schizophrenic-like symptoms. We (Hakami et al., 2009; Pinault, 2008) and others (Ehrlichman et al., 2009; Lazarewicz et al., 2009; Ma and 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 schizophrenic patients (Baldeweg et al., 1998; Gordon et al., 2001; Spencer et al., 2009; Spencer et al., 2008b), suggest that NMDAr antagonist-induced increases in gamma power may represent an electrophysiological correlate of acute psychosis (Lee et al., 2003a).

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, and also interacts with the glycine site of NMDA receptors (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 and dopamine D_{2/3} receptors (Seeman and Guan, 2009), and possesses efficacy in animal models of 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 power, such as those measured in response to an auditory stimulus (Uhlhaas and 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; 250-350g) 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 hour light/dark cycle, with lights on at 6am, and food (standard rat chow) and water available to animals *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 (#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 and Watson, 2005) of recording brass electrodes (2mm anterior and 2mm lateral to bregma bilaterally (active electrodes); 2mm posterior and 2mm lateral to bregma bilaterally (ground electrodes); and 2mm posterior and 2mm 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 days 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 minutes prior to the start of the study to allow for 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 minutes, at which time they were injected subcutaneously (sc) with either haloperidol (0.025 – 0.25 mg/kg; n=8), clozapine (1-5 mg/kg sc, n=7), LY379268 (0.3 – 3 mg/kg sc; n=5) or the appropriate vehicles. Following a further 30 minutes, rats were then injected with ketamine (5mg/kg sc) or vehicle (0.9% saline) and returned to the arena for a further 60 minutes EEG recording. This procedure was repeated on subsequent days (with at least 2 days in between trials) with a different dose of antipsychotic until each rat had received each dose of its assigned antipsychotic. During the 120 minute recording period, the animal’s locomotor activity was video-tracked and objectively assessed using

Ethovision Software (Noldus®). Total distance traveled was calculated for every 2 minute interval.

A subsequent study was performed to assess the effects of a single administration of antipsychotic over a 24 hour 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 noon and 4pm. Animals (n=6) were connected to the EEG cables, and allowed a 30 minute habituation period prior to being injected with either haloperidol (0.25 mg/kg sc), clozapine (5 mg/kg sc), LY379268 (3 mg/kg sc) or vehicle (0.9% saline) in a randomised fashion, with at least 2 days in between experiments. Animals were left undisturbed for a further 24 hours 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 (ADInstruments, Bella Vista, NSW, Australia) with a bandpass of 1-1000 Hz. The line power 50 Hz noise was eliminated from the signal using selective eliminators (Humbugs; Digitimer, Letchworth Garden City, UK). The gamma frequency activity on the EEG recordings were analyzed using NEUROSCAN® software (Compumedics, Melbourne, 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 sec 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 hour recordings, the average power was calculated for each 5 minutes for the first 3.5 hours, and then for each 30 minutes thereafter, using the same 2.05 sec epoch determination as above, this time for all power bands, including gamma (30-80Hz), delta (1-4Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30Hz) frequency oscillations. The first 6 values, representing the 30 minute 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 (IL, USA). Clozapine (Sigma, St Louis, USA) was dissolved using pure acetic acid and then diluted with a 10% acetic acid solution that was neutralised to a pH of 6.0 using 10M NaOH. LY379268 (TOCRIS, Bristol, UK) was diluted in sterile water. Haloperidol (Sigma, St Louis, 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 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 minute 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 6-10 mins 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 here that both the trough and the peak values of each recording comprised of the averages of 119 x 2.05 sec 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®, 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 to decrease the power of spontaneous gamma power 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 insert) 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 insert). 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 insert), 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 minutes post-injection. Therefore, a subsequent set of studies were performed to investigate these effects over a 24 hr period. In these studies, the statistically significant reductions in gamma power induced of 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 hr following 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 (1-4Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30Hz). 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.

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 minute pre-treatment window, compared with vehicle treatment (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 sc) induced an acute increase in gamma power which peaked 8-12 minutes 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 – 0.25mg/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 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), demonstrating a clear differential effect compared with the other antipsychotics.

Pretreatment with antipsychotics inhibits the ketamine-induced hyperlocomotor response.

Ketamine (5 mg/kg) induced an acute hyperlocomotor response which peaked 6-8 minutes after injection and persisted for up to 30 minutes (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 mg/kg 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: $F_{(1, 5)}=10.60$, $p=0.012$).

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 suppresses this effect, in direct contrast to clozapine and haloperidol which were unable to modify the ‘trough-to-peak’ response of ketamine. LY379268, which is a preclinical compound which is being evaluated as a potentially novel treatment for patients with schizophrenia, exerted its effect in a clear and powerful dose dependent manner. We speculate that this differential effect to that of the two compounds in current clinical use as anti-psychotic drugs, 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, reduced, at least to some extent, the peak in EEG gamma power induced by ketamine administration. 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 hours. 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 NMDA_R 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., 2009; Ma and 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 schizophrenic patients have reported *decreases* in EEG gamma power responses to evoked stimuli (eg: (Ford et al., 2008; Kwon et al., 1999; Spencer et al., 2003; Spencer et al., 2008a). However, most of these studies group do not take into account the influence of antipsychotic medication (see (Hong et al., 2004)). When examining subsets of schizophrenia patients, studies of evoked and induced gamma power responses describe positive correlations between hallucinatory symptoms and gamma power responses (Gordon et al., 2001; Spencer et al., 2009; Spencer et al., 2008b), 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 and 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 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 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 hours 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 timepoint.

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 and 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 and Adams, 1998), providing a negative feedback loop on pyramidal cell firing. MK801, which like ketamine, is another NMDAR antagonist which we have previously demonstrated 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 et al., 2005; Homayoun and Moghaddam, 2008). 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, it is further confounding the understanding of the mechanism of this effect is a previous study that reported that subchronic pretreatment with the related compound, LY354740 which is 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

mGluR₅ receptors and NMDA receptors (Awad et al., 2000; Lecourtier et al., 2007), further studies are required to exact the precise cellular mechanisms linking mGluR_{2/3} and NMDA receptors.

The current study also supports the findings of our previous research (Hakami et al., 2009; Pinault, 2008) that, although NMDA 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 and 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 for its effect on gamma power. It is not clear what ramifications this would have clinically, but beta power also has been strongly linked to cognitive processing (Uhlhaas and Singer, 2010).

In conclusion, the data from this 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 currently used in the pre-clinical anti-psychotic drug development, including drug-induced hyperlocomotor activity.

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Disclosures/Conflicts of Interest:

All authors declare no conflicts of interest, financial or otherwise, associated with this work.

Reference list:

- Awad, H, Hubert, GW, Smith, Y, Levey, AI, Conn, PJ** (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci*, 20(21): 7871-7879.
- Baldessarini, RJ, Centorrino, F, Flood, JG, Volpicelli, SA, Huston-Lyons, D, Cohen, BM** (1993) Tissue concentrations of clozapine and its metabolites in the rat. *Neuropsychopharmacology*, 9(2): 117-124.
- Baldeweg, T, Spence, S, Hirsch, SR, Gruzelier, J** (1998) Gamma-band electroencephalographic oscillations in a patient with somatic hallucinations. *Lancet*, 352(9128): 620-621.
- Bartos, M, Vida, I, Jonas, P** (2007) Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci*, 8(1): 45-56.
- Buzsaki, G, Draguhn, A** (2004) Neuronal oscillations in cortical networks. *Science*, 304(5679): 1926-1929.
- Campbell, A, Herschel, M, Cohen, BM, Baldessarini, RJ** (1980) Tissue levels of haloperidol by radioreceptor assay and behavioral effects of haloperidol in the rat. *Life Sci*, 27(8): 633-640.
- 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(4): 423-429.
- 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(2): 705-712.
- Engel, AK, Fries, P, Singer, W** (2001) Dynamic predictions: oscillations and synchrony in top-down processing. *Nat Rev Neurosci*, 2(10): 704-716.
- Fisahn, A, Pike, FG, Buhl, EH, Paulsen, O** (1998) Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature*, 394(6689): 186-189.
- 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(1-3): 262-271.
- Ford, JM, Roach, BJ, Faustman, WO, MATHALON, DH** (2008) Out-of-synch and out-of-sorts: dysfunction of motor-sensory communication in schizophrenia. *Biol Psychiatry*, 63(8): 736-743.
- Fuchs, EC, Zivkovic, AR, Cunningham, MO, Middleton, S, Lebeau, FE, Bannerman, DM, Rozov, A, Whittington, MA, Traub, RD, Rawlins, JN, Monyer, H** (2007) Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron*, 53(4): 591-604.
- Gordon, E, Williams, LM, Haig, AR, Bahramali, H, Wright, J, Meares, R** (2001) Symptom profile and 'gamma' processing in schizophrenia. *Cognitive Neuropsychiatry*, 6(1): 2001.
- Gross, J, Schnitzler, A, Timmermann, L, Ploner, M** (2007) Gamma oscillations in human primary somatosensory cortex reflect pain perception. *PLoS Biol*, 5(5): e133.

- Hakami, T, Jones, NC, Tolmacheva, EA, Gaudias, J, Chaumont, J, Salzberg, M, O'Brien, TJ, Pinault, D** (2009) NMDA Receptor Hypofunction Induces Generalized and Persistent Aberrant γ Oscillations Independent of Hyperlocomotion and Conscious Sensorimotor Processing.
- Herrmann, CS, Demiralp, T** (2005) Human EEG gamma oscillations in neuropsychiatric disorders. *Clin Neurophysiol*, 116(12): 2719-2733.
- Homayoun, H, Jackson, ME, Moghaddam, B** (2005) Activation of metabotropic glutamate 2/3 receptors reverses the effects of NMDA receptor hypofunction on prefrontal cortex unit activity in awake rats. *J Neurophysiol*, 93(4): 1989-2001.
- Homayoun, H, Moghaddam, B** (2008) Orbitofrontal cortex neurons as a common target for classic and glutamatergic antipsychotic drugs. *Proc Natl Acad Sci U S A*, 105(46): 18041-18046.
- Hong, LE, Summerfelt, A, Buchanan, RW, O'Donnell, P, Thaker, GK, Weiler, MA, Lahti, AC** (2010) Gamma and delta neural oscillations and association with clinical symptoms under subanesthetic ketamine. *Neuropsychopharmacology*, 35(3): 632-640.
- Hong, LE, Summerfelt, A, McMahon, R, Adami, H, Francis, G, Elliott, A, Buchanan, RW, Thaker, GK** (2004) Evoked gamma band synchronization and the liability for schizophrenia. *Schizophr Res*, 70(2-3): 293-302.
- Howard, MW, Rizzuto, DS, Caplan, JB, Madsen, JR, Lisman, J, Aschenbrenner-Scheibe, R, Schulze-Bonhage, A, Kahana, MJ** (2003) Gamma oscillations correlate with working memory load in humans. *Cereb Cortex*, 13(12): 1369-1374.
- Imre, G** (2007) The preclinical properties of a novel group II metabotropic glutamate receptor agonist LY379268. *CNS Drug Rev*, 13(4): 444-464.
- Imre, G, Fokkema, DS, Den Boer, JA, Ter Horst, GJ** (2006a) Dose-response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity. *Brain Res Bull*, 69(3): 338-345.
- Imre, G, Fokkema, DS, Ter Horst, GJ** (2006b) Subchronic administration of LY354740 does not modify ketamine-evoked behavior and neuronal activity in rats. *Eur J Pharmacol*, 544(1-3): 77-81.
- Jackson, ME, Homayoun, H, Moghaddam, B** (2004) NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. *Proc Natl Acad Sci U S A*, 101(22): 8467-8472.
- Joliot, M, Ribary, U, Llinas, R** (1994) Human oscillatory brain activity near 40 Hz coexists with cognitive temporal binding. *Proc Natl Acad Sci U S A*, 91(24): 11748-11751.
- Jones, NC, Martin, S, Megatia, I, Hakami, T, Salzberg, MR, Pinault, D, Morris, MJ, O'Brien, TJ, van den Buuse, M** (2010) A genetic epilepsy rat model displays endophenotypes of psychosis. *Neurobiol Dis*, 39: 116-125.
- Krystal, JH, Karper, LP, Seibyl, JP, Freeman, GK, Delaney, R, Bremner, JD, Heninger, GR, Bowers, MB, Jr., Charney, DS** (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry*, 51(3): 199-214.
- 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(11): 1001-1005.

- Lahti, AC, Koffel, B, LaPorte, D, Tamminga, CA** (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology*, 13(1): 9-19.
- Lazarewicz, MT, Ehrlichman, RS, Maxwell, CR, Gandal, MJ, Finkel, LH, Siegel, SJ** (2009) Ketamine Modulates Theta and Gamma Oscillations. *J Cogn Neurosci*.
- Lecourtier, L, Homayoun, H, Tamagnan, G, Moghaddam, B** (2007) Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-Methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol Psychiatry*, 62(7): 739-746.
- 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(1): 57-78.
- Lee, KH, Williams, LM, Haig, A, Gordon, E** (2003b) "Gamma (40 Hz) phase synchronicity" and symptom dimensions in schizophrenia. *Cogn Neuropsychiatry*, 8(1): 57-71.
- Lisman, JE, Coyle, JT, Green, RW, Javitt, DC, Benes, FM, Heckers, S, Grace, AA** (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci*, 31(5): 234-242.
- 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(4): 961-974.
- Malhotra, AK, Pinals, DA, Adler, CM, Elman, I, Clifton, A, Pickar, D, Breier, A** (1997) Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology*, 17(3): 141-150.
- Miyamoto, S, Duncan, GE, Marx, CE, Lieberman, JA** (2005) Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry*, 10(1): 79-104.
- Moghaddam, B, Adams, B, Verma, A, Daly, D** (1997) Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci*, 17(8): 2921-2927.
- Moghaddam, B, Adams, BW** (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science*, 281(5381): 1349-1352.
- Monn, JA, Valli, MJ, Massey, SM, Hansen, MM, Kress, TJ, Wepsiec, JP, Harkness, AR, Grutsch, JL, Jr., Wright, RA, Johnson, BG, Andis, SL, Kingston, A, Tomlinson, R, Lewis, R, Griffey, KR, Tizzano, JP, Schoepp, DD** (1999) Synthesis, pharmacological characterization, and molecular modeling of heterobicyclic amino acids related to (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): identification of two new potent, selective, and systemically active agonists for group II metabotropic glutamate receptors. *J Med Chem*, 42(6): 1027-1040.
- Paxinos, G, Watson, C** (2005) *The Rat Brain in Stereotaxic Coordinates*. Elsevier Academic Press.
- 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(8): 730-735.

- Schwieler, L, Linderholm, KR, Nilsson-Todd, LK, Erhardt, S, Engberg, G** (2008) Clozapine interacts with the glycine site of the NMDA receptor: electrophysiological studies of dopamine neurons in the rat ventral tegmental area. *Life Sci*, 83(5-6): 170-175.
- Seeman, P, Guan, HC** (2009) Glutamate agonists for treating schizophrenia have affinity for dopamine D2High and D3 receptors. *Synapse*, 63(8): 705-709.
- Spencer, KM, Nestor, PG, Niznikiewicz, MA, Salisbury, DF, Shenton, ME, McCarley, RW** (2003) Abnormal neural synchrony in schizophrenia. *J Neurosci*, 23(19): 7407-7411.
- Spencer, KM, Niznikiewicz, MA, Nestor, PG, Shenton, ME, McCarley, RW** (2009) Left auditory cortex gamma synchronization and auditory hallucination symptoms in schizophrenia. *BMC Neurosci*, 10: 85.
- Spencer, KM, Niznikiewicz, MA, Shenton, ME, McCarley, RW** (2008a) Sensory-evoked gamma oscillations in chronic schizophrenia. *Biol Psychiatry*, 63(8): 744-747.
- Spencer, KM, Salisbury, DF, Shenton, ME, McCarley, RW** (2008b) Gamma-band auditory steady-state responses are impaired in first episode psychosis. *Biol Psychiatry*, 64(5): 369-375.
- Tallon-Baudry, C, Bertrand, O, Peronnet, F, Pernier, J** (1998) Induced gamma-band activity during the delay of a visual short-term memory task in humans. *J Neurosci*, 18(11): 4244-4254.
- Turetsky, BI, Siegel, SJ (2007) Persistent auditory-evoked gamma band oscillations in schizophrenia. *American College of Neuropsychopharmacology*. Boca Raton, FL, USA.
- Uhlhaas, PJ, Singer, W** (2010) Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci*, 11(2): 100-113.
- van den Buuse, M, Garner, B, Gogos, A, Kusljic, S** (2005) Importance of animal models in schizophrenia research. *Aust N Z J Psychiatry*, 39(7): 550-557.

Figure and table:

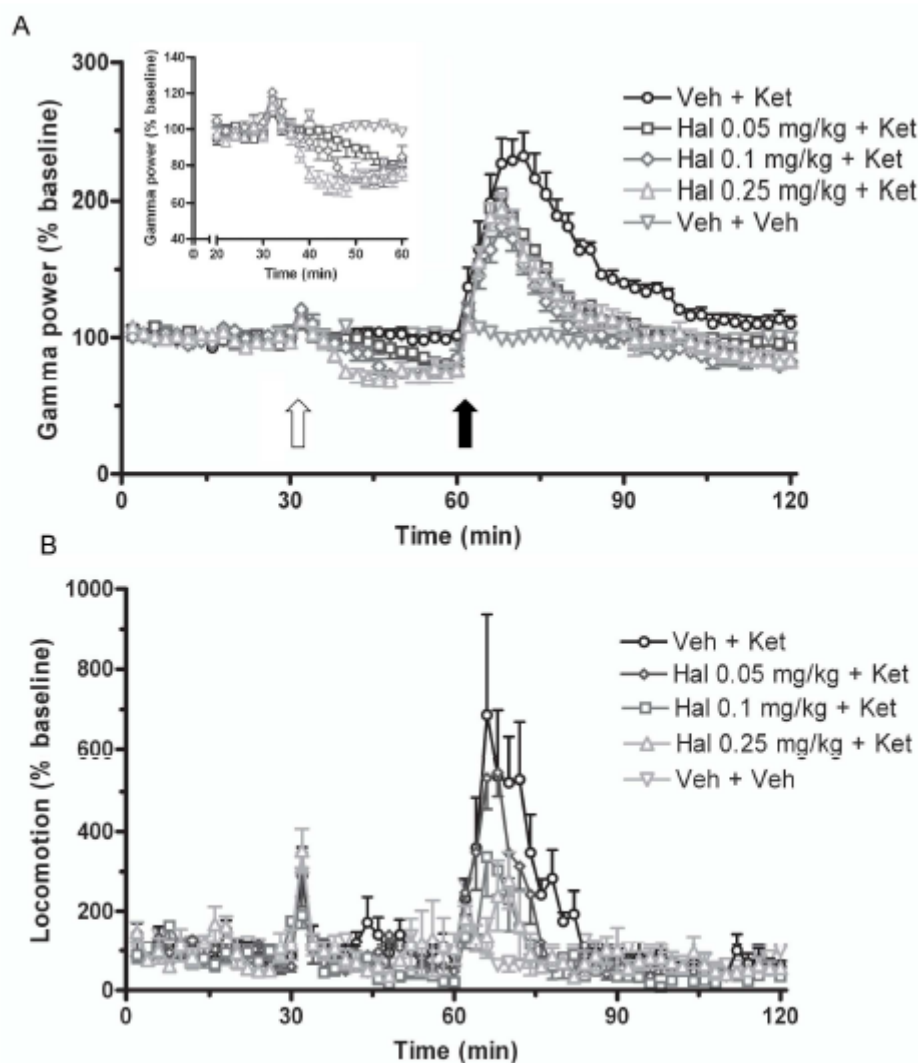


Figure 1

Figure 1. Effects of haloperidol on ketamine-induced elevations in gamma power and locomotor activity in rats. **(A - insert)** Haloperidol (0.05 – 0.25 mg/kg sc) 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 (5mg/kg sc), compared with vehicle pretreatment. This effect was not dose-dependent. **(B)** Pretreatment with haloperidol dose-dependently suppressed ketamine induced hyperlocomotor activity, compared with vehicle pretreatment. Data represent mean \pm S.E.M.; n=8. Open arrow indicates timing of haloperidol administration, and closed arrow indicates ketamine administration.

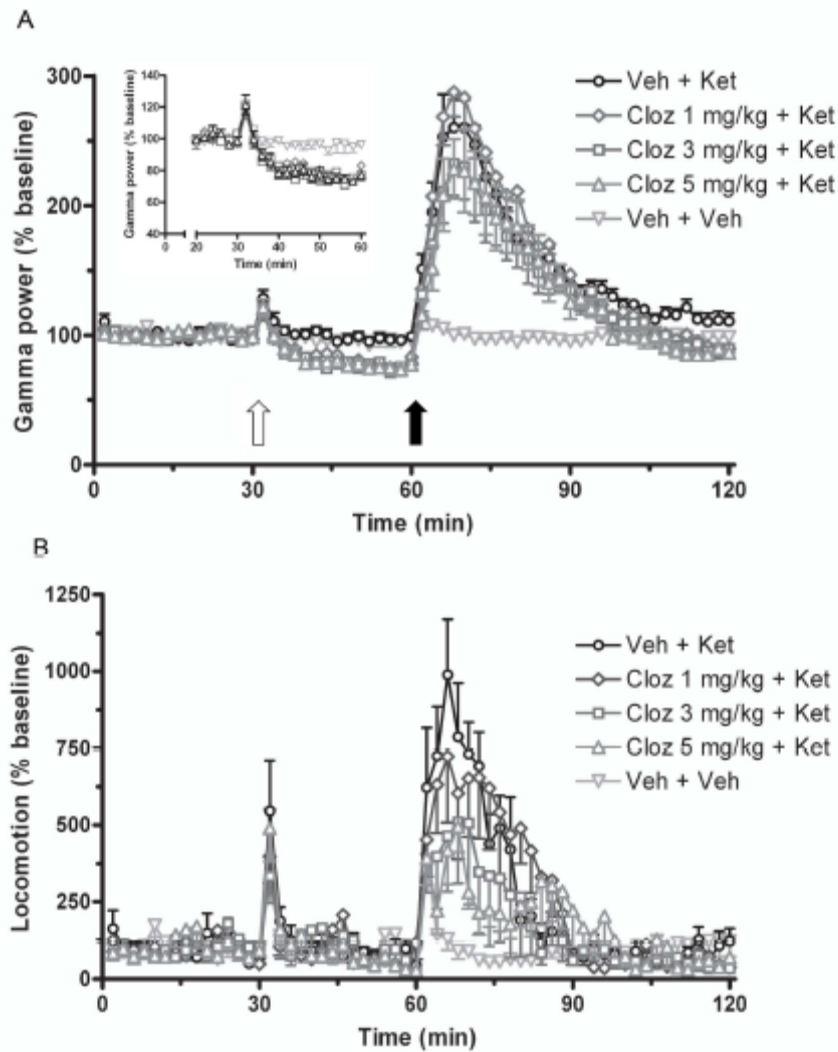


Figure 2

Figure 2. Effects of clozapine on ketamine-induced elevations in gamma power and locomotor activity in rats. **(A - insert)** 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 (5mg/kg sc), compared with vehicle pretreatment. **(B)** Pretreatment with clozapine (1 - 5 mg/kg sc) induced a dose-dependent but incomplete suppression of ketamine (5 mg/kg sc) induced hyperlocomotor activity compared with vehicle pretreatment. Data represent mean \pm S.E.M.; n=7. Open arrow indicates timing of clozapine administration, and closed arrow indicates ketamine administration.

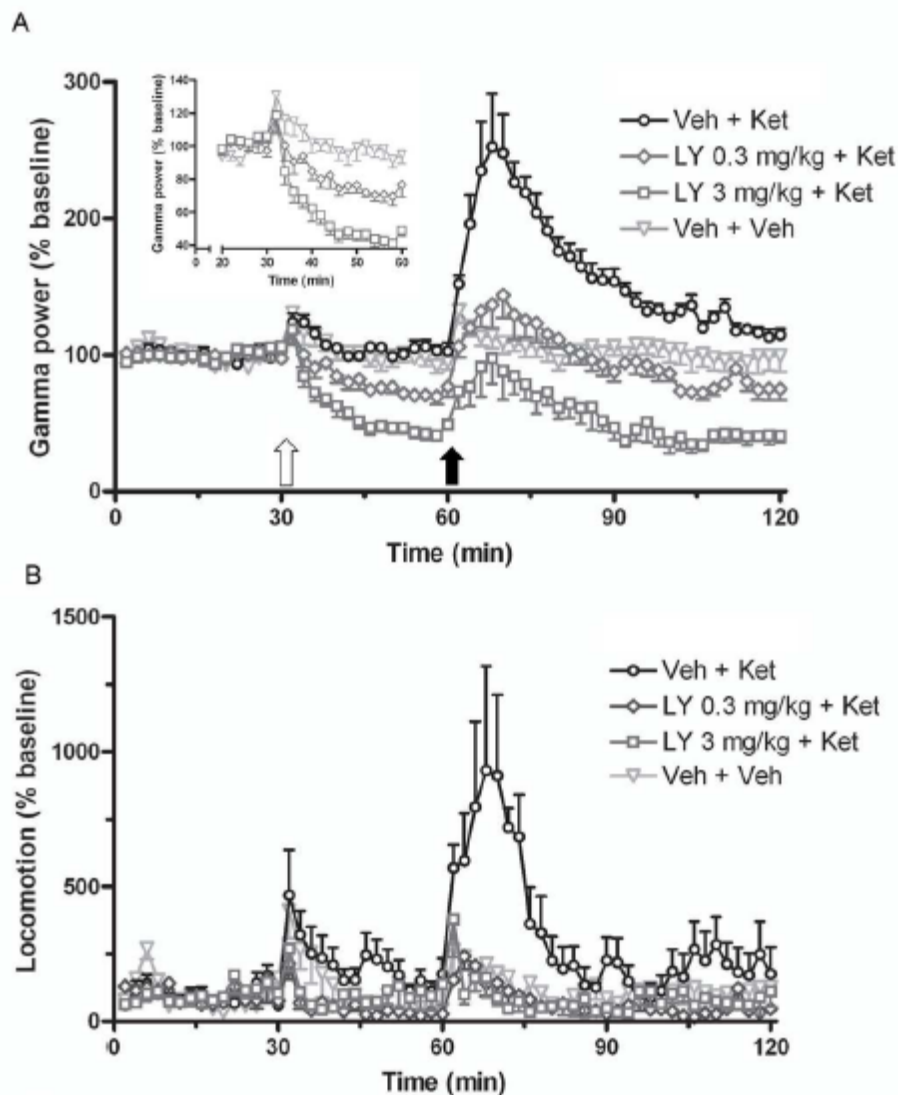


Figure 3

Figure 3. Effects of LY379268 on ketamine-induced elevations in gamma power and locomotor activity in rats. **(A - insert)** 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 (5mg/kg sc), compared with vehicle pretreatment. This effect was dose-dependent. **(B)** Pretreatment with LY379268 (0.3 - 3 mg/kg sc) induced a dose-dependent and complete suppression of ketamine (5 mg/kg sc) induced hyperlocomotor activity compared with vehicle pretreatment. Data represent mean \pm S.E.M.; n=5. Open arrow indicates timing of LY379268 administration, and closed arrow indicates ketamine administration.

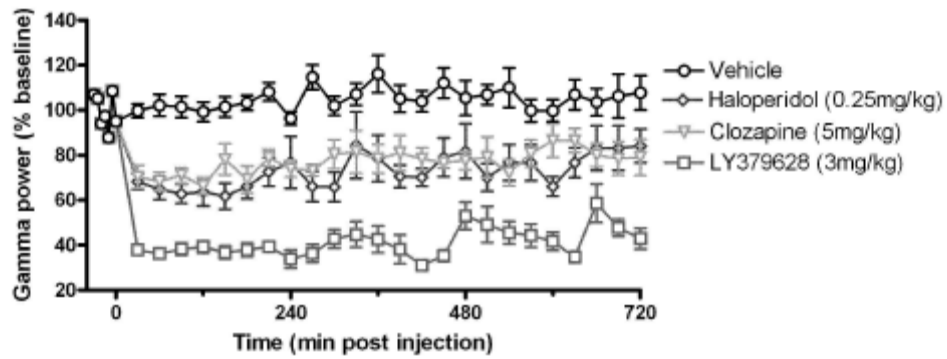


Figure 4

Figure 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 hours, as compared to vehicle. Data represent mean \pm S.E.M.; n=6. This effect persisted for at least 24 hours.

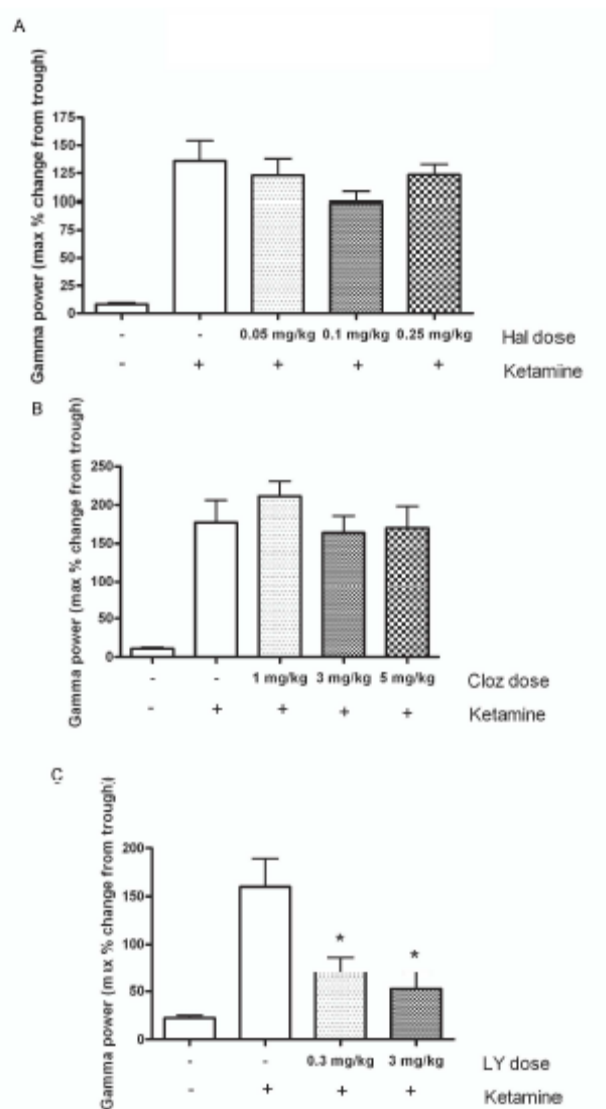


Figure 5

Figure 5. Effects of antipsychotics on ‘peak-to-trough’ gamma power increase induced by ketamine (5 mg/kg sc). Neither haloperidol (Hal) (A) nor clozapine (Cloz) (B) 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 LY379268 (LY) (C) was able to significantly suppress the ‘peak-to-trough’ gamma power rise induced by ketamine (* $p < 0.05$). Data represent mean + S.E.M.; sample sizes as for Figs 1-3.

	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*	94% ↓, dose-dependent

Table 1

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 columns) were calculated 6-8 mins after ketamine injection at the peak of the ketamine response. * in this instance, 100% inhibition of response was observed with the lowest dose of LY379268, so no dose-dependence was attained. n.s. non-significant effect.