Antipsychotic compounds differentially modulate high-frequency oscillations in the rat nucleus accumbens: a comparison of first- and second-generation drugs

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Abstract

Improved understanding of the actions of antipsychotic compounds is critical for a better treatment of schizophrenia. Abnormal oscillatory activity has been found in schizophrenia and in rat models of the disease. N-Methyl-D-aspartic acid receptor (NMDAR) antagonists, used to model certain features of schizophrenia, increase the frequency and power of high-frequency oscillations (HFO, 130–180 Hz) in the rat nucleus accumbens, a brain region implicated in schizophrenia pathology. Antipsychotics can be classified as first- and second-generation drugs, the latter often reported to have wider benefit in humans and experimental models. This prompted the authors to examine the pre- and post-treatment effects of clozapine, risperidone (second-generation drugs) and sulpiride and haloperidol (first-generation drugs) on ketamine and MK801-enhanced accumbal HFO. Both NMDAR antagonists increased HFO frequency. In contrast, clozapine and risperidone markedly and dose-dependently reduced the frequency of spontaneous and NMDAR-antagonist-enhanced HFO, whilst a moderate effect was found for sulpiride and a much weaker effect for haloperidol. Unexpectedly, we found reductions in HFO frequency were associated with an increase in its power. These findings indicate that modulation of accumbal HFO frequency may be a fundamental effect produced by antipsychotic compounds. Of the drugs investigated, first- and second-generation compounds could be dissociated by their potency on this measure. This effect may partially explain the differences in the clinical profile of these drugs.

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Introduction

N-Methyl-D-aspartic acid receptor (NMDAR) antagonists are known to produce psychotomimetic effects in healthy humans, which resemble some of the core positive, negative and cognitive symptoms of schizophrenia (Olney et al. 1999). In rodents, NMDAR antagonists produce hyperlocomotion and cognitive and social impairments, which can be reversed, at least partially, by pretreatment with antipsychotic drugs (APD; Neill et al. 2010; Sams-Dodd, 1999; Tiedtke et al. 1990). Second-generation APD, such as clozapine, appear more effective at reducing the cognitive impairments compared to classical APD, such as haloperidol (Abdul-Monim et al. 2003; Bakshi et al. 1994; Grayson et al. 2007; Weiner & Arad, 2009). First-generation antipsychotics (FGA) are thought to achieve their effects by blockade of dopamine D2 receptors, whereas the second-generation antipsychotics (SGA) are known to have potent antagonist properties at several other receptor families, perhaps most notably serotonergic 5-HT2A receptors (Meltzer et al. 1989). However, the precise mechanisms by which APD are able to achieve their effects are not fully understood.

Oscillatory activity recorded in local field potentials (LFP) represents the sum of synchronized changes in the membrane potential of neurons recorded at the electrode vicinity. Abnormal oscillatory activity has been found in schizophrenia (Uhlhaas & Singer, 2010) and in rat models of the disease; for example, NMDAR antagonists can increase the power of γ in the rat hippocampus (Ma & Leung, 2000) and neocortex (Pinault, 2008) and δ power in the hippocampus and thalamus (Zhang et al. 2012). We have shown previously that spontaneous high-frequency oscillations [HFO] 130–180 Hz] can be recorded in the nucleus accumbens (NAc). These oscillations are state-dependent and enhanced substantially by NMDAR...
antagonists (Hunt et al. 2006, 2009). At subanaesthetic doses, NMDAR antagonists dose-dependently increase HFO power, which correlates with locomotor activity. HFO also markedly increase in amplitude during emergence from ketamine anaesthesia (Hunt et al. 2006). Others have also recorded increases in HFO power after NMDAR antagonist injection in other brain regions (Hakami et al. 2009; Nicolas et al. 2011) and an exacerbation of HFO power, in electroencephalogram (EEG) recordings, after injection in the methylazoxymethanol developmental rat model of schizophrenia (Phillips et al. 2012).

Recently, APD have been shown to reduce NMDAR antagonist-induced alterations of γ and δ (Jones et al. 2012; Santana et al. 2011); however, the effects of systemic injection of APD on HFO have not been reported. This prompted us to investigate the effects of four commonly used APD on LFP oscillations in the NAc. The drugs chosen were haloperidol, a D1/D2 receptor antagonist, sulpiride, a selective D2/D3 receptor antagonist, and clozapine and risperidone, SGA, which, in addition to dopamine receptor blockade, possess significant activity at serotonergic receptors.

Here, we show that APD differentially modulate spontaneous and NMDAR-antagonist-enhanced HFO in the NAc. APD produced a reduction in the frequency of HFO and a parallel increase in power. Effects were most robust for clozapine and risperidone and appear to be modulated by extra-accumbal brain regions since local infusion of APD to the NAc did not mimic the effects observed after systemic injection.

Method

Surgery and electrode implantation

Forty-one male Wistar rats (270–300 g) were used in this study. Rats were anaesthetized with isofluorane and mounted in a stereotaxic frame with blunt ear bars. Electrodes made from twisted stainless steel wire (100 μm), insulated except at the tip, were implanted in the NAc according to coordinates of the stereotaxic atlas (Paxinos & Watson, 1986; anterior-posterior 1.6, mediolateral 0.8, dorsoventral 7 mm). A silver wire was used as ground/reference electrode connected to a screw in the occipital bone. Electrodes and screws were secured firmly in place with dental cement.

Implantation for local injection

Male Wistar rats (270–300 g) were anaesthetized with isofluorane and stainless steel electrodes (100 μm) were implanted unilaterally in the right NAc. Twenty-two-gauge stainless steel guides (Bilaney, Germany) were implanted bilaterally in the NAc. Dummy cannulae were screwed in place at the end of surgery and removed later for infusion of drug. After the experiment, the animals were killed with an overdose of pentobarbital anaesthesia and the brains dissected. The location of the cannulae and electrodes tips were determined on 40 μm Cresyl Violet-stained sections. All experiments were conducted in accordance with the European community guidelines on the care and use of laboratory animals (86/609/EEC) and were also approved by a local ethics committee.

Data acquisition

One week after surgery rats were habituated for 30 min over 2 d to the recording chamber (35 cm wide, 35 cm long, 42 cm high), in which horizontal locomotor activity (LMA) of the animal could be assessed by photocell beam breaks (Columbus Instruments, USA). During the experimental session LFP were recorded through a junction gate field-effect transistor preamplifier connected to a socket on the head of the animal. Cables were relayed at the top of the box by a multi-channel rotating commutator, allowing free movement of the rat inside the recording chamber. The signal was amplified ×1000, filtered 0.1–1 kHz (A-M Systems, USA), digitized at 4 kHz (Micro 1401; Cambridge Electronic Design, UK) and stored on a PC for offline analysis. LMA detected using a photocell beam-break counter was recorded simultaneously with LFP.

Ketamine study

Rats were divided randomly into four groups. Baseline LFP and LMA were recorded for 20 min, followed by i.p. injection of antipsychotic drug or vehicle [clozapine (gift from Professor J. Feldon): 1, 5, 15 mg/kg (n = 6), risperidone (Tocris, UK) 0.1, 1.0, 3.0 mg/kg (n = 5), sulpiride (Tocris, UK): 20, 60, 100 mg/kg (n = 6) and haloperidol (Sigma, Poland): 0.1, 0.5, 1.5 mg/kg (n = 6)]. This was followed 30 min later by injection (i.p.) of 25 mg/kg ketamine (Sigma, Poland). This dose was chosen since we had found previously that 25 mg/kg ketamine induces reliable increases in both LMA and HFO (Hunt et al. 2006). Rats were injected in a pseudorandomized order, whereby each rat was pretreated with different concentrations of a particular antipsychotic drug/vehicle, according to the Latin square design. The wash-out time between experiments was at least 3 d, to minimize possible carry-over effects of the previous drug administration.

MK801 study

Rats were baselined for 20 min and then injected (i.p.) with 0.1 mg/kg MK801 (Tocris; n = 6) or saline (n = 6) followed, 30 min later, by injection of antipsychotic (15 mg/kg clozapine, 3 mg/kg risperidone, 100 mg/kg sulpiride, 1.5 mg/kg haloperidol or vehicle). Rats were injected with antipsychotic drug in a pseudorandomized order so that each rat received injection of all the antipsychotics or vehicle. All antipsychotic drugs were dissolved in a small volume of acetic acid and made up to
the required volume using sterile saline. Ketamine and MK801 were dissolved in sterile saline.

**Microinjection**

Following a baseline recording session of approximately 20 min, rats \( (n=6) \) were removed from the recording chamber, gently restrained and the dummy cannula removed. An infusion cannula 28-gauge (Bilaney), which extended 1 mm below the tip of the guide, was locked in place inside the guide, enabling infusion at a constant depth. The infusion cannula was left in place for 60 s. This was followed by infusion of 1, 10 \( \mu \)g clozapine or vehicle in a volume of 1 \( \mu l \) for 120 s. The cannula was left in place for a further 60 s and then removed.

**Power spectra**

Mean power spectra of the raw LFP were calculated from successive 60-s data-blocks using a fast Fourier transform of 4096 points with Spike 2 software (CED, UK). Dominant frequency and the corresponding power of dominant frequency between 100 and 180 Hz were measured in bins of 60 s. We used a wider-frequency band (100–180 Hz) in the present study since we found unexpectedly that clozapine and risperidone reduced the frequency of HFO to below our standard 130 Hz cut-off. For our primary analysis of power we took the power of dominant frequency, rather than total power of frequency band, since we were interested in how the specific HFO band changes after injection. Also, the 100–180 Hz band yields slightly misleading total power since it also incorporates the tail-end of the high-\( \gamma \) power (around 90 Hz in the NAc) in the spectral analysis.

**Statistical analysis**

Time-courses of HFO dominant frequency and power of dominant frequency, as well as LMA, were analysed by repeated-measures analysis of variance (RM-ANOVA). Where significant this was followed by Bonferroni’s post hoc test to find differences between the groups. There was no significant difference in the baseline power of HFO between the experimental groups and doses for rats receiving systemic injection of APD (group \( \times \) time, \( F_{22,2914}^{2141}=0.97, \ p=0.66 \) and group \( F_{22,2914}=0.9, \ p=0.61 \). Therefore, for clarity we expressed changes in the power of HFO as fold change with regard to baseline. Occasionally, there were missing data (i.e. due to artefacts in the LFP signal) and the corresponding time-points were excluded from the RM-ANOVA. One-way ANOVA was used to calculate effect of different doses of APD post injection of ketamine (15 min average) with regard to values at the end of APD pretreatment. One-way ANOVA was also used to calculate changes in number of beam-breaks in the MK801/saline study. Pearson’s (\( r \)) correlation was used to determine the relationship between changes in frequency and power. Data are presented as mean and S.E.M. Differences were considered statistically significant if \( p<0.05 \).

**Results**

**Clozapine, risperidone and haloperidol, but not sulpiride, dose-dependently reduce ketamine-induced hyperlocomotion**

Complete time-courses showing the effects of the antipsychotic drugs clozapine, risperidone, sulpiride and haloperidol on ketamine-induced motor activity are shown in Fig. 1a–d. RM-ANOVA revealed dose \( \times \) time effects for clozapine \( (F_{10,2160}^{321}=7.26, \ p<0.0001) \), risperidone \( (F_{10,2160}^{321}=3.065, \ p<0.0001) \), haloperidol \( (F_{3,2160}^{321}=2.959, \ p<0.0001) \) and sulpiride \( (F_{10,2160}=1.181, \ p=0.0193) \). Bonferroni’s post hoc analyses revealed clear dose-dependent reductions in the LMA for all antipsychotic compounds tested, with the exception of sulpiride, where only the highest dose (100 mg/kg) was associated with a reduction in LMA compared to vehicle and the 20 mg/kg dose \( (p<0.001, \ for both) \). In control rats, ketamine-induced hyperlocomotion typically lasted around 15 min. One-way RM-ANOVA of the first 15 min after ketamine injection revealed effects of dose for clozapine \( (F_{3,15}=14.15, \ p=0.0003) \), risperidone \( (F_{3,15}=6.196, \ p=0.0143) \) and haloperidol \( (F_{3,15}=8.957, \ p=0.0022) \) pretreatment but not sulpiride \( (F_{3,15}=1.12, \ p=0.21) \), confirming the relatively weaker effects of sulpiride on ketamine-induced hyperlocomotion.

**Pretreatment with clozapine, risperidone and sulpiride, but not haloperidol, dose-dependently modulates spontaneous and ketamine-enhanced HFO**

Consistent with our previous studies, injection of ketamine produced an increase in the frequency and power of spontaneous HFO. Time-courses showing the effect of antipsychotics on the dominant frequency of spontaneous and ketamine-enhanced HFO activity are shown in Fig. 2a–d. With regard to dominant frequency, RM-ANOVA for the whole time-course revealed significant dose \( \times \) time effects for all antipsychotics tested [clozapine \( (F_{22,2141}=12.46, \ p<0.0001) \), risperidone \( (F_{22,2141}=4.624, \ p<0.0001) \), sulpiride \( (F_{22,2141}=1.679, \ p<0.0001) \) and haloperidol \( (F_{22,2141}=2.192, \ p<0.0001) \)]. Dose effects were significant for clozapine \( (F_{3,321}=19.19, \ p<0.0001) \) and risperidone \( (F_{3,321}=5.09, \ p=0.0397) \) but not for haloperidol \( (F_{3,321}=1.089, \ p=0.3768) \) or sulpiride \( (F_{3,321}=1.266, \ p=0.3129) \). Post hoc analysis showed significant reductions in HFO frequency for clozapine 5 mg/kg and 15 mg/kg \( (p<0.001, \ for both) \) and for risperidone 1 mg/kg \( (p<0.05) \) and 3 mg/kg \( (p<0.001) \), with regard to the vehicle. A small but significant increase in frequency was found for 0.5 mg/kg haloperidol vs. vehicle \( (p<0.05) \), but this was found for only two time-points and after the
Fig. 1. Effect of antipsychotic pretreatment on ketamine-enhanced motor activity. Time-courses showing the effect of (a) clozapine (1–15 mg/kg), (b) risperidone (0.1–3.0 mg/kg), (c) sulpiride (20–100 mg/kg) and (d) haloperidol (0.1–1.5 mg/kg) pretreatment on spontaneous and ketamine-enhanced locomotor activity (n = 5–6 per group). First dotted line indicates injection of antipsychotic drug (APD) or vehicle (Veh) and the second line indicates injection of ketamine. Values are the mean ± S.E.M. number of beam breaks. Horizontal bars/dots above the time-courses indicate significant differences with regard to Veh, at least p < 0.05.

Fig. 2. Effect of antipsychotic pretreatment on ketamine-induced increases in the frequency of high-frequency oscillations (HFO) in the nucleus accumbens. Time-courses showing the effect of (a) clozapine (1–15 mg/kg), (b) risperidone (0.1–3.0 mg/kg), (c) sulpiride (20–100 mg/kg) and (d) haloperidol (0.1–1.5 mg/kg) pretreatment on ketamine-enhanced HFO frequency (n = 5–6 per group). The first dotted line indicates injection of antipsychotic drug (APD) or vehicle (Veh), the second line indicates injection of ketamine. Values are the mean ± S.E.M. of HFO frequency. Horizontal bars/dots above the time-courses indicate significant differences with regard to vehicle, at least p < 0.05.
initial ketamine effect had subsided. No significant differences were found for the sulpiride group. Pretreatment of antipsychotic alone, reduced the dominant frequency of spontaneous HFO; clozapine ($F_{84,560}=2.41, p<0.0001$), risperidone ($F_{84,448}=1.755, p=0.0002$) and sulpiride ($F_{84,448}=1.822, p<0.0001$) with the exception of haloperidol ($F_{84,560}=0.9225, p=0.667$). To control for this reduction, we calculated the change in frequency at the end of the pretreatment compared to the first 15 min after injection of ketamine. One-way ANOVA confirmed that clozapine ($F_{3,321}=30.84, p<0.0001$) and risperidone ($F_{3,321}=9.09, p=0.002$) affected the ketamine-induced increase in frequency, in a dose-dependent manner.

We analysed the power of dominant frequency and expressed the data as fold change from baseline values. RM-ANOVA for the complete time-course of changes in power of dominant frequency revealed significant dose×time interactions for clozapine ($F_{3,321,3146}=1.47, p<0.0001$), risperidone ($F_{3,321,3146}=2.137, p<0.0001$), sulpiride ($F_{3,321,2140}=1.962, p<0.0001$) but not haloperidol ($F_{3,321,2140}=0.8181, p=0.989$). As shown in Fig. 3a−d, the presence of these antipsychotics, with the exception of haloperidol, increased the duration of ketamine-induced increases in HFO power. With regard to vehicle, significant increases in power were found for 1, 5 mg/kg clozapine ($p<0.001$) and 15 mg/kg ($p<0.05$), all doses risperidone ($p<0.001$) and 100 mg/kg sulpiride ($p<0.001$). During pretreatment alone we found dose×time interactions for clozapine ($F_{4,540}=3.185, p<0.0001$), risperidone ($F_{4,448}=1.755, p=0.0002$), sulpiride ($F_{4,568}=2.569, p<0.0001$) and haloperidol ($F_{4,540}=1.39, p=0.019$). To control for effects on baseline HFO power, we calculated the fold-change post ketamine (15 min average) relative to the end of pretreatment values. One-way RM-ANOVA revealed a significant effect of dose for clozapine ($F_{4,321}=4.5, p=0.02$), which post hoc analysis revealed was caused by a significant difference between 1 and 15 mg/kg doses. No significant effects of dose were found for the other antipsychotics.

Clozapine, risperidone and sulpiride, but not haloperidol, modulate spontaneous and MK801-enhanced HFO

We next examined the effect of post-treatment of antipsychotics on HFO enhanced by injection of 0.1 mg/kg MK801. We have shown previously that this dose reliably enhances the power and frequency of HFO for at least 1 h (whilst the 25 mg/kg ketamine effect typically lasts for only 15−20 min; Hunt et al. 2006). In addition, the experiments described above demonstrated modulation of spontaneous HFO during the antipsychotic pretreatment stage; however, pretreatment lasted only 30 min. Therefore, we conducted an additional experiment specifically designed to confirm the effect of

![Image](https://example.com/image1.png)

**Fig. 3.** Effect of antipsychotic pretreatment on ketamine-induced increases of high-frequency oscillations (HFO) power in the nucleus accumbens. Time-courses showing the effect of (a) clozapine (1−15 mg/kg), (b) risperidone (0.1−3.0 mg/kg), (c) sulpiride (20−100 mg/kg) and (d) haloperidol (0.1−1.5 mg/kg) pretreatment on ketamine-enhanced HFO power ($n=5−6$ per group). The first dotted line indicates injection of antipsychotic drug (APD)/vehicle (Veh), the second line indicates injection of ketamine. Values are the mean ± S.E.M. of HFO power. Horizontal bars/dots above the time-courses indicate significant differences with regard to vehicle, at least $p<0.05$. 
antipsychotic injection alone on the power and frequency of spontaneous HFO.

**MK801-enhanced HFO**

Time-courses showing the effect of different antipsychotics on the frequency and power of MK801-enhanced HFO are shown in Figs 4a and 5a, respectively. Clozapine and risperidone were most effective at reducing HFO frequency and at the end of the experiment had reduced the dominant frequency by 35.2 ± 3.6 Hz and 31.4 ± 3.5 Hz, respectively. RM-ANOVA revealed a drug × time effect (F_{4,2517} = 8.224, p < 0.0001) and drug alone (F_{1,2517} = 5.536, p = 0.0025). Post hoc analysis revealed reductions in frequency with regard to vehicle for clozapine, risperidone and sulpiride (p < 0.001, for all) but not for haloperidol. Clozapine and risperidone, but not sulpiride, reduced HFO frequency compared to haloperidol (p < 0.001). Clozapine also reduced the frequency compared to sulpiride (p < 0.05). No other differences were found. RM-ANOVA also revealed a significant effect for HFO power (drug × time; F_{4,2517} = 2.291, p < 0.0001), but due to the large variance no effect was found for drug alone (F_{1,2517} = 2.014, p = 0.123). Bonferroni’s post hoc test revealed an increase in power with regard to vehicle for risperidone and sulpiride (p < 0.001), but not for clozapine or haloperidol.

### Spontaneous HFO

The effects of antipsychotic compounds on the frequency and power of spontaneous HFO are shown in Figs 4b and 5b, respectively, and largely agree with our findings from the pretreatment study. For dominant frequency RM-ANOVA revealed a drug effect (F_{4,2500} = 3.411,
and drug × time interaction ($F_{400,2500} = 3.693$, $p < 0.0001$). Post hoc analysis revealed reductions in frequency with regard to vehicle for clozapine, risperidone ($p < 0.001$, for both) but not for haloperidol or sulpiride. Clozapine vs. sulpiride was also significant ($p < 0.05$).

RM-ANOVA for the changes in HFO power revealed an effect of drug ($F_{4,2500} = 5.419$, $p = 0.0028$) and drug × time interaction ($F_{400,2500} = 2.486$, $p < 0.0001$). An increase in HFO power was found for clozapine, risperidone ($p < 0.001$, for both) and sulpiride ($p < 0.05$) with regard to vehicle. Clozapine and risperidone were also significantly different compared to haloperidol ($p < 0.001$).

Locomotor activity

In the presence of MK801, analysis of mean LMA for 60 min post injection of APD revealed a significant effect ($F_{4,59} = 19.56$, $p < 0.0001$, one-way RM-ANOVA). A significant effect was also found in the presence of saline ($F_{4,2500} = 26.20$, $p < 0.0001$). Bonferroni post hoc showed clozapine ($p < 0.01$ presence of MK801, $p < 0.001$ presence of saline), risperidone and haloperidol ($p < 0.001$, for both) reduced LMA compared to vehicle. In both instances, sulpiride was not significantly different from vehicle (Supplementary Fig. S1).

Relationship between HFO power and frequency

After NMDAR antagonist injection frequency and power positively correlated for all rats 24/24 injected with ketamine ($p < 0.01$, Pearson’s correlation) and 5/6 rats injected with MK801 ($p < 0.001$). In contrast, after injection of APD changes in frequency and power of spontaneous HFO negatively correlated for the doses 15 mg/kg clozapine ($p < 0.001$, 12/12 rats), 3.0 mg/kg risperidone ($p < 0.05$, 7/11 rats), 100 mg/kg sulpiride ($p < 0.01$, 7/12 rats) and 1.5 mg/kg haloperidol ($p < 0.05$, 3/12 rats). In the presence of the NMDAR antagonist, MK801, the power and frequency of HFO negatively correlated after injection of clozapine ($p < 0.01$, 5/6 rats), risperidone, ($p < 0.001$, 2/6 rats), sulpiride ($p < 0.05$, 3/6 rats) and haloperidol ($p < 0.05$, 5/6 rats).

Local infusion of clozapine to the NAc does not modify HFO frequency but reduces power

We examined the effect of bilateral local infusion of clozapine (1, 10 μg) or vehicle to the NAc. Time-courses showing the frequency and power are shown in Fig. 6. In contrast to systemic injection, RM-ANOVA revealed that local infusion did not influence the frequency of HFO recorded in the NAc (dose × time, $F_{114,855} = 1.03$, $p = 0.397$). A dose × time effect was found for HFO power ($F_{114,855} = 1.69$, $p < 0.0001$), which post hoc analysis showed was due to a reduction in HFO power after 10 μg vs. vehicle ($p < 0.05$).

Discussion

Acute systemic administration of APD produced reductions in the frequency of NMDAR-enhanced and spontaneous HFO recorded in the rat NAc. We also found APD produced an increase, in the power of HFO. These changes are most likely due to modified activity of afferent brain regions, since local infusion of clozapine
to the NAc did not replicate the effect found after systemic injection of the drug.

First- and second-generation APD differentially modulate HFO in the NAc

Although all the APD opposed effects produced by NMDAR antagonists on HFO frequency, we observed marked differences in their efficacy. Profound reductions were observed with clozapine and risperidone: SGA, which reduced HFO frequency well below baseline values. Weak effects were found for haloperidol, an FGA with affinity for D$_2$ receptors. An intermediate effect was found using sulpiride, also a first-generation APD, that preferentially targets mesolimbic D$_2$/D$_3$ receptors. Conventionally, sulpiride is considered an FGA, due to its high selectivity at dopamine receptors. However, clinical studies showed that this drug produces relatively mild extrapyramidal side-effects, a profile more consistent with SGA (or atypicals; Mielke et al. 1977; Rao et al. 1981). This has led many investigators to refer to sulpiride as an atypical antipsychotic drug. The differences we observed between the APD, therefore, may best be explained by drug-class and suggest that examination of changes in HFO may provide a useful framework for studying the underlying mechanisms of atypical compounds.

For the ketamine and MK801 studies, haloperidol, clozapine and risperidone produced comparable reductions in motor activity, but differed markedly in their effects on HFO. Sulpiride failed to produce comparable reductions in locomotor activity in the presence of ketamine or MK801, but did influence HFO power and frequency. The weaker effect of sulpiride or other D$_2$ antagonists on NMDAR antagonist-induced hyperlocomotion is in line with some studies (Freed et al. 1984; Martin et al. 1994) but not others (Adriani et al. 1998; Sams-Dodd, 1998). Nonetheless, our findings suggest that reduced locomotor activity is not a prerequisite for the effects APD produce on HFO frequency or power, a finding consistent with our previous dopamine antagonist infusion study (Matulewicz et al. 2010).

APD-induced reductions in HFO frequency were often associated with increases in HFO power. This was the case for HFO recorded in animals injected with APD either alone or in the presence of NMDAR antagonists. This contrasted with effects observed for control experiments, where rats received ketamine or MK801 alone, and the correlation between HFO frequency and power in almost every instant was positive. Changes in HFO power and frequency therefore seem to be dependent, at least in part, on each other, but respond differently depending on whether a psychotomimetic or antipsychotic drug is administered. Notwithstanding this, although APD produced much smaller increases in HFO power, compared to NMDAR antagonists, the finding that APD alone can increase HFO power might at first sight appear paradoxical. Although both types of compound increase the power of HFO, indicating greater synchronization or involvement of a greater number of active neurons, the HFO that result in the end are not identical. Indeed, the primary effects on HFO frequency of NMDAR antagonists and APD are in opposition, and up to 40 Hz separates the dominant frequencies in these conditions. Thus, examining changes in HFO both the frequency and power must be analysed in parallel.

In our study, we used a Latin square design, thus all animals received each drug/dose and vehicle. Because the half-life of the APD drugs used was relatively short, i.e. 1–2 h in rats, and we used a 3-d washout between experiments, it is unlikely that much of the drug carried over to the subsequent experiment. Additionally, considering that we observed a similar profile of APD drug effects in the saline-pretreated animals (Figs 4b and 5b) as we observed in the ketamine and MK801 studies, it seems unlikely that repeated injection of NMDAR antagonist contributed to the fundamental APD effects we report here.

Possible mechanism

At least some of the neurons of the NAc seem to form the basic ‘generator’ of the HFO we record and, hence, if larger clusters of cells become synchronized, increases in power become evident. This has been supported by our bipolar LFP recordings, current source density analyses (Hunt et al. 2010) and, more recently, our studies showing that intra-accumbal infusion of tetrodotoxin reduces the power of HFO (Hunt et al., unpublished observations). However, clozapine, the APD that demonstrated the greatest efficacy on the HFO band in the systemic studies when applied locally, failed to reproduce the effects observed after systemic injection. In fact, in contrast to systemic injection we found that local infusion produced a small but significant decrease in HFO power. This finding, if confirmed in other experiments, would also suggest that the frequency of HFO and power can be controlled by separate mechanisms.

The intra-NAc infusion studies indicated that the effects of clozapine on HFO most probably originate in extra-accumbal areas. We cannot exclude the possibility that changes may have been observed at higher doses, but this seems unlikely, since even low doses of systemically administered clozapine produced effects on HFO. This is in accordance with our earlier study, where we showed that systemic, but not local, infusion of lamotrigine reduced the frequency of HFO (Hunt et al. 2008). Similarly, local infusion of the APD raclopride to the NAc did not influence power or frequency of HFO (Matulewicz et al. 2010). It therefore seems likely that regions afferent to the NAc can modulate the frequency and power of HFO. Electrophysiological and microscopic
studies have shown that the NAc receives excitatory input from the cortical areas, hippocampus and amygdala that can converge on single accumbal units (Callaway et al. 1991; French & Totterdell, 2002, 2003; O’Donnell & Grace, 1995). Homayoun & Moghaddam (2007) demonstrated that clozapine, but not haloperidol, exerts a state-dependent effect on tonic firing of prefrontal cortex (PFC) neurons, that is to say, that it stimulates firing of neurons with low baseline activity while reducing the rate of neurons with a high baseline firing rate. Strikingly, the reported kinetics of clozapine-induced changes in firing rate of PFC neurons are comparable with the changes we recorded in NAc HFO. These authors also demonstrated that clozapine, but not haloperidol, reversed the stimulatory effects caused by MK801 on PFC neuronal firing (Homayoun & Moghaddam, 2007). Further studies are warranted to determine how the activity of afferent regions may influence HFO recorded in the NAc.

A central feature of both first- and second-generation APD is their ability to block D$_2$ receptors, which correlates with APD efficacy in humans (Seeman, 2002; Seeman et al. 1975). Given that haloperidol and sulpiride are both potent D$_2$ antagonists, it is unlikely that blockade of this receptor alone underlies the effect we observed. Clozapine has weak antagonistic actions at D$_2$ dopamine receptors, whereas risperidone is more potent at this receptor. Both drugs interact with many other receptors, including actions at 5-HT$_2A$, 5-HT$_3C$, 5-HT$_7$ as well as histamine H$_1$ and $\alpha$-1 and -2 adrenergic receptors (Kinon & Lieberman, 1996; Meltzer et al. 1989; Miyamoto et al. 2005). 5-HT$_2A$ receptors have received increasing attention for their potential involvement in the clinical efficacy of atypical APD (Kuroki et al. 2008; Meltzer & Huang, 2008). However, the selective 5-HT$_2A$ receptor antagonist, MDL11, 939 does not reduce the frequency of HFO (Goda et al., unpublished observations), indicating that 5-HT$_2A$ receptor blockade alone is not responsible for effects on HFO. We cannot exclude the possibility that APD produce their effects through combined blockade of D$_2$ and 5-HT$_2A$ receptors, which appears important for the clinical effects of atypicals (Kuroki et al. 2008). Alternatively, the common effects these drugs have on other receptors alone or in combination may underlie the effect we observed.

**Significance of HFO in the NAc**

In the rat NAc, the dominant increase in power spectra after injection of NMDAR antagonists occurs in the HFO band (Hunt et al. 2010, 2011; see Fig. 7). This was one of the reasons [together with the wealth of experimental and theoretical studies implicating the NAc in the pathophysiology of schizophrenia (see Grace, 2000)] why we chose to focus on the HFO band. Increases in HFO power following NMDAR antagonist injection have also been reported in several cortical and subcortical regions (Hakami et al. 2009; Hunt et al. 2011; Nicolas et al. 2011, fig. 7).

![Fig. 7. Sonograms showing the effect of clozapine or vehicle on MK801-enhanced high-frequency oscillations (HFO). Rats were recorded for 20 min prior to i.p. injection of MK801. Then, 30 min later the rat received an i.p. injection of clozapine (Cloz; 15 mg/kg) or vehicle (a). Complete time-course showing the effect of Cloz pretreatment on ketamine-enhanced HFO (b). Rats were recorded for 20 min and then injected with Cloz (15 mg/kg) or vehicle, this was followed by ketamine i.p. 30 min later. The HFO band is indicated by an arrow.](image)
Phillips et al. (2012), but at least in our studies these tend to be smaller in amplitude than those recorded in the NAc (Hunt et al. 2011). It is unlikely that changes in HFO are merely an epiphenomenon, relating to changes that occur in other frequency bands, such as increases in the power of hippocampal and cortical $\gamma$ (Ma & Leung, 2000; Pinault, 2008). First, substantial increases in HFO power can be observed in the absence of any clear change in $\gamma$ and $\delta$ (Hunt et al. 2010). Here, we found marked effects on HFO frequency (increases after NMDAR antagonist and decreases after antipsychotics) without any obvious change in the frequency of other bands (see Fig. 7).

Similarly, in a previous study we showed, using simultaneous accumbal and hippocampal recordings, that ketamine-induced increases in accumbal HFO power are more substantial than increases in hippocampal $\gamma$ power (Hunt et al. 2011). Outside the NAc, antipsychotics have been shown to reduce the power of cortical $\gamma$ (Jones et al. 2012), indicating that HFO in the NAc cannot be a second harmonic of $\gamma$ arising from the cortex. In clinical studies, many investigators have focused on $\gamma$ oscillations and reported deficits in evoked $\gamma$ oscillations in schizophrenic patients (Lee et al. 2003). Recently, several studies have shown that baseline $\gamma$ power may increase in some cortical areas when compared to healthy subjects (Spencer, 2011; Venables et al. 2009). Also, in healthy humans, acute administration of ketamine increases the power of auditory-evoked 40–85 Hz $\gamma$, but not HFO (Hong et al. 2010). Clinical EEG studies do not typically report findings in the HFO range we are reporting, rather focusing on changes in $\gamma$ activity 30–80 Hz with regard to high frequencies. This may be for technical reasons, where frequencies >100 Hz (unless they are large in amplitude and generated cortically) may be hard to detect due to background noise. Also, many EEG studies have used a cut-off at 100 Hz. High frequencies, in the range we are reporting, have been recorded in the human NAc, from patients implanted with deep-brain electrodes (Cohen et al. 2009). However, it is unknown whether and to what extent these are changed in schizophrenia or by NMDAR antagonists.

Antipsychotics and NMDAR antagonists induce fundamentally opposed effects on the frequency of spontaneous HFO. The increases in HFO frequency coupled with increased power might be related to the psychotomimetic effects produced by these compounds, indicating a pathological range of frequencies. In contrast, antipsychotics, in particular SGA, reduce HFO frequency, inducing a transition away from the ‘pathological range’ to lower frequencies. In rats, SGA are far more effective than FGA at reversing the cognitive and sensorimotor impairments produced by NMDAR antagonists (Abdul-Monim et al. 2003; Idris et al. 2005; Neill et al. 2010; Paine & Carlezon, 2009). Therefore, we may speculate that the effects we observed on accumbal HFO are related to differences in the behavioural profile of the FGA and SGA tested. It is worth noting that we previously reported that lamotrigine, a compound that can reverse some of the behavioural effects produced by NMDAR antagonists in humans (Anand et al. 2000), dose-dependently reduced the frequency of HFO after ketamine (Hunt et al. 2008).

Notably, instead of returning the HFO frequency to the baseline value, the SGA decreased the frequency well below it, with more moderate reductions found for sulpiride. This effect was dose-dependent, demonstrating that too high a dose can cause an excessive response to the drug. Considering that these drugs also increase the power of HFO, it might be expected that this ‘slower’ form of HFO, which is different to baseline conditions, may have behavioural consequences. In rats, some deficits in cognitive function have been noted after acute administration of antipsychotics. For example, reduced performance in the five-choice serial reaction time task at baseline and after acute PCP injection was found for clozapine and risperidone, but not for haloperidol (Amitai et al. 2007). Sulpiride also produced an impairment in normal rats in this task (Passetti et al. 2003). Clozapine can also worsen prepulse inhibition and reversal learning deficits in rats given subchronic NMDAR antagonists (Abdul-Monim et al. 2006; Schwabe et al. 2005).

In humans, the emergence or exacerbation of obsessive-compulsive symptoms following treatment of atypicals, such as clozapine and risperidone, is a widely reported psychiatric side-effect in schizophrenic and bipolar patients (Baker et al. 1992; Lykouras et al. 2003; Sa et al. 2009). These side-effects occur far less frequently with typical APD, which, in our experiments (at least at the doses we used), did not influence the HFO frequency as much as atypical APD. Experimental and theoretical considerations have implicated the NAc in the pathogenesis of obsessive-compulsive disorder (Sturm et al. 2003). The reduction in frequency may represent one way whereby APD attempt to bring the frequency of NMDAR-enhanced HFO to the normal healthy condition. However, at higher doses, APD reduce HFO frequency well below their normal range, which may be associated with some of their neuropsychiatric side-effects.

Conclusions

Improved understanding of the mechanisms that underlie the effects of new-generation APD, such as clozapine, is important for novel targets and ultimately better treatment. These findings show that modulation of accumbal HFO is a useful indicator of drugs with antipsychotic properties, more specifically new-generation compounds. These findings are in line with data demonstrating the importance of the NAc in the actions of APD and provide a useful framework for the development of improved high-throughput screening of potentially useful novel compounds.
Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1168/journal.cri.2016.9.6.1019.

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Statement of Interest

None.

References


