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RESEARCH PAPER

First and second generation antipsychotics influence hippocampal gamma oscillations by interactions with 5-HT_3 and D_3 receptors

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BACKGROUND AND PURPOSE

Disturbed cortical gamma band oscillations (30–80 Hz) have been observed in schizophrenia: positive symptoms of the disease correlate with an increase in gamma oscillation power, whereas negative symptoms are associated with a decrease.

EXPERIMENTAL APPROACH

Here we investigated the effects of first and second generation antipsychotics (FGAs and SGAs, respectively) on gamma oscillations. The FGAs haloperidol, flupenthixol, chlorpromazine, chlorprothixene and the SGAs clozapine, risperidone, ziprasidone, amisulpride were applied on gamma oscillations induced by acetylcholine and physostigmine in the CA3 region of rat hippocampal slices.

KEY RESULTS

Antipsychotics inhibited the power of gamma oscillations and increased the bandwidth of the gamma band. Haloperidol and clozapine had the highest inhibitory effects. To determine which receptor is responsible for the alterations in gamma oscillations, the effects of the antipsychotics were plotted against their pK_i values for 19 receptors and analysed for correlation. Our results indicated that 5-HT_3 receptors have an enhancing effect on gamma oscillations whereas dopamine D_3 receptors inhibit them. To test this prediction, m-chlorophenylbiguanide, PD 128907 and CP 809101, selective agonists at 5-HT_3, D_3 and 5-HT_{2C} receptors were applied and revealed that 5-HT_3 receptors indeed enhanced the gamma power whereas D_3 receptors reduced it. As predicted, 5-HT_{2C} receptors had no effects on gamma oscillations.

CONCLUSION AND IMPLICATIONS

Our data suggest that antipsychotics alter hippocampal gamma oscillations by interacting with 5-HT_3 and dopamine D_3 receptors. Moreover, a correlation of receptor affinities with the biological effects can be used to predict targets for the pharmacological effects of multi-target drugs.

Abbreviations

ACh, acetylcholine; CCK, cholecystokinin; FGA, first generation antipsychotic; mCPBG, m-chlorophenylbiguanide hydrochloride; Physo, physostigmine; SGA, second generation antipsychotic

Introduction

Neural network oscillations in the gamma band (30–80 Hz) mediate the synchronization and functional coordination of distant cortical areas enabling information transfer (Womelsdorf *et al.*, 2007; Fries, 2009; Gregoriou *et al.*, 2009). They have been implicated in a range of higher-order brain functions such as sensory processing, working memory and attention (Jensen *et al.*, 2007), and are supposed to be generated by the synchronous firing of perisomatic parvalbumin containing fast-spiking basket cells (Bartos *et al.*, 2007; Gulyás *et al.*, 2010).

An increasing number of studies demonstrate that gamma oscillations are disturbed in schizophrenia patients (Kissler *et al.*, 2000; Minzenberg *et al.*, 2010). Schizophrenia is characterized by dysfunctions in many of higher brain functions known to be linked to gamma oscillations. This has led to the concept that disturbances in gamma band network activity may be involved in the pathophysiology of the disease (Lee *et al.*, 2003). It has been observed that negative symptoms such as social and emotional withdrawal correlate with a decrease in gamma oscillations, whereas positive symptoms, such as hallucinations, seem to be associated with an increase in the gamma power (Herrmann and Demiralp, 2005; Lee *et al.*, 2010; Mulert *et al.*, 2011). In schizophrenia patients, parvalbumin-containing interneurons in the prefrontal cortex have markedly reduced levels of the 67 kDa isoform of GAD67 (Hashimoto *et al.*, 2003), which is essential for GABA synthesis in the cortex. Parvalbumin-positive fast-spiking cells thus seem to be functionally impaired in schizophrenia and may be critically involved in the disturbances of gamma network activity related to the typical symptoms of the disease (Gandal *et al.*, 2012).

Both first and second generation antipsychotics (FGAs and SGAs, respectively) have a highly complex pharmacology with considerable affinity for a variety of receptors (e.g. 5-HT, dopamine and adrenoceptors) (Roth *et al.*, 2004) found on principal cells and/or interneurons playing a crucial role in the generation of gamma oscillations. Therefore, the aim of the present study was to investigate the effects of FGAs and SGAs on gamma oscillations. The oscillations were induced in acute hippocampal slices by bath application of acetylcholine (ACh), mimicking cholinergic input from the septum (Fischer *et al.*, 2002). These cholinergic oscillations share many characteristics with *in vivo* intrahippocampal gamma oscillations (Fisahn *et al.*, 1998; Csicsvari *et al.*, 2003) making them an appropriate *in vitro* model for this network activity (Traub *et al.*, 2004; Hájos and Paulsen, 2009).

Methods

Animals

Female Wistar rats (*Rattus norvegicus* f. domestica), aged 5–7 weeks (150–180 g), were kept under 12 h light/dark conditions and given food and water *ad libitum*. All animal procedures were conducted in accordance with the guidelines of the European Communities Council and the institutional guidelines approved by the Berlin Animal Ethics Committee (Landesamt für Gesundheit und Soziales Berlin, T0096/02). All studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Slice preparation

Preparation of acute brain slices and recordings were done as described previously (Schulz *et al.*, 2012). In brief, rats were anaesthetized with 2% isoflurane added to a 70:30 mix of N_2O and O_2 and then decapitated. Their brains were rapidly removed and washed with ice-cold artificial (A) CSF containing (in mM): NaCl, 129; KCl, 3; NaH_2PO_4, 1.25; $NaHCO_3$, 21; $CaCl_2$, 1.6; $MgSO_4$, 1.8; D-glucose, 10, saturated with carbogen (95% O_2/5% CO_2). The brain was cut into 400 µm thick horizontal hippocampal slices with a vibratome (DSK microslicer DTK-1000; Dosaka, Kyoto, Japan). Slices were immediately transferred to an interface-type recording chamber perfused with warm and carbogenated ACSF (36°C, flow rate 1.6–1.7 mL·min^{-1}, pH 7.4). Slices were left for recovery for at least 2 h before the experiments were commenced. Several slices of each brain were used for experiments, but not more than one slice per hemisphere for each drug.

Extracellular recordings

Extracellular field potentials were recorded from stratum pyramidale of area CA3b with glass pipettes filled with ACSF (resistance < 3 MΩ) and placed 80–120 µm below the cut surface of the slice. Recordings were amplified by a custom-made amplifier, low-pass filtered at 1 kHz and sampled at 5 kHz by a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). Gamma oscillations were induced by bath perfusion of 10 µM ACh and 2 µM physostigmine (Physo) and stabilized after ~90 min. Drugs were applied 100 min after the application of ACh/Physo for a period of 60 min. If the gamma oscillation did not stabilize (i.e. if the peak power varied more than 10% within minutes 90–100), the slice was excluded from analysis (34 of 145 slices).

Drugs

All drugs were purchased from Sigma-Aldrich, Taufkirchen, Germany (ACh, chlorpromazine, chlorprothixene, clozapine, haloperidol, risperidone) or Tocris, Bristol, UK [amisulpride, CP 809101, flupenthixol, m-chlorophenylbiguanide hydrochloride (mCPBG), PD 128907, Physo, ziprasidone] and dissolved in ACSF except amisulpride, clozapine, risperidone and ziprasidone, which were first dissolved in DMSO and then further diluted in ACSF with a final DMSO concentration of 0.2‰ (v v^{-1}). Therapeutic plasma concentrations of antipsychotics were found to be in the nanomolar range (0.05–1 µM) (Dahl, 1986; Mauri *et al.*, 2007). In the present study, higher concentrations (10–30 µM) were chosen depending on the therapeutic concentrations to assure the saturation of the receptors and to obtain faster tissue penetration.

Correlation of pK_i values with effects on gamma oscillations

K_i (dissociation constant) values for each antipsychotic agent were generously provided by the National Institutes of Mental Health's Psychoactive Drug Screening Program (PDSP) Contract # HHSN-271-2008-00025-C (NIMH PDSP) (http://

pdsp.med.unc.edu/). Rat K_i values from the database were converted in pK_i (negative logarithm of K_i) and the mean pK_i was used for the correlation analysis (i.e. the geometric mean of the K_i values). In the few cases where no rat data were available (e.g. most chlorprothixene pK_i values, 5-HT$_{1D}$ and some muscarinic receptor data), K_i values for the human receptor were taken. H_3 receptor K_i values were taken in two cases (ziprasidone and haloperidol) from guinea pig and the 5-HT$_3$ receptor K_i value for flupenthixol from the mouse. The normalized effects of antipsychotics on power and bandwidth were plotted against the (averaged) pK_i values and analysed for correlation using a simple linear regression analysis. The r^2 values and the slope of the regression line were determined for each receptor. Positive slope of the regression line for a receptor indicates gamma-enhancing effects of the receptor (enhancement of peak gamma power, decrease of gamma bandwidth) whereas a negative slope for a receptor indicates gamma-inhibiting effects (inhibition of peak gamma power, increase of gamma bandwidth). For this analysis, it was assumed that antipsychotics are competitive antagonists at the receptors analysed.

Data analysis and statistics

Using a custom-made script for the Spike2 software (Cambridge Electronic Design), power spectra were calculated for 2 min periods throughout the recording, of which peak power, integral power (from 20 to 80 Hz), peak frequency and peak bandwidth (at 50% of maximum peak power) were determined. Because of the large variability in absolute power of the oscillations, it was normalized to a 10 min period before drug application or the corresponding time in control experiments, where slices received only ACh/Physo during the whole recording. The magnitude of absolute gamma power before drug application did not correlate with the normalized effects of drugs on gamma power in any measurements. Data are presented as mean ± SEM. Statistical comparisons between the drug-induced changes and the time-matched control experiments were made using one-way ANOVA with Fisher's least significant difference *post hoc* test. In order to judge the significance of Pearson's correlation coefficients (r), Student's t-test was used after using the formula:

$$t = \frac{r}{\sqrt{\frac{1-r^2}{N-2}}}$$

to transform the sampling distribution of r to Student's t distribution. N is the size of the sample on which r is based. To test whether the slope of the linear regression fit between the pK_i values and the effects on gamma oscillation is significantly different from 0, we used an F-test of the overall fit, followed by Student's t-test of individual parameters. Significance level was set at $P < 0.05$.

Nomenclature

The nomenclature of all molecular targets (receptors, ion channels, etc.) cited in this work conforms to the British Journal of Pharmacology's Guide to Receptors and Channels (Alexander *et al.*, 2011).

Results

Effects of high-potency FGAs on gamma oscillations

Gamma oscillations were induced in the hippocampus by bath application of ACh (10 μM) and Physo (2 μM). Measurement of field potentials in the stratum pyramidale of area CA3 revealed an average frequency and peak power of 32.4 ± 0.5 Hz and 2198 ± 385 μV^2 respectively (n = 111). Peak and integrated power, bandwidth, and frequency of gamma oscillations were significantly altered by the drugs applied as confirmed by ANOVA (peak power: $F_{0.05(1),12,98} = 7.127$, $P < 0.001$; integrated power: $F_{0.05(1),12,98} = 7.447$, $P < 0.001$; bandwidth: $F_{0.05(1),12,98} = 2.897$, $P = 0.002$; peak frequency: $F_{0.05(1),12,98} = 2.55$, $P = 0.006$).

Haloperidol (10 μM), the prototype high-potency FGA, inhibited the power of peak gamma oscillations to 56.7 ± 10.2% (n = 8, $P < 0.05$ compared with control, Figure 1A, E). The integrated gamma power and the peak frequency of the oscillations were also inhibited to 78.1 ± 12.5% and 96.2 ± 3.4%, respectively; $P < 0.05$; Figure 1A, F). We also investigated the change in the bandwidth of the oscillations. A narrow gamma band in the power spectrum indicates a high temporal coherence and regular oscillations, whereas a wide gamma band means low coherence and relatively irregular oscillations. Haloperidol increased the bandwidth to 174.2 ± 18.7% ($P < 0.05$, Figure 1A, G), indicating reduced temporal coherence. Thus, haloperidol not only reduced the power of the oscillations but also their regularity.

Application of flupenthixol (30 μM), another high-potency FGA, did not significantly affect gamma oscillations (peak power: 76.4 ± 6.3% of control; integrated power: 108.7 ± 9.8%; bandwidth: 141.5 ± 21.2%; frequency: 107.7 ± 1.7%; n = 9, $P > 0.05$; Figure 1B–G).

Effects of low-potency FGAs on gamma oscillations

We next applied the low-potency FGA chlorprothixene (10 μM) and found that it also did not significantly change either the peak and integrated gamma power, the bandwidth or peak frequency of gamma oscillations (86.9 ± 16.2%, 86.0 ± 13.7%, 127.2 ± 14.7% and 102.6 ± 2.9% of control, respectively; n = 7, $P > 0.05$; Figure 1C, E–G).

Similarly, application of chlorpromazine (10 μM), another low-potency FGA, also did not significantly affect the parameters of gamma oscillations (peak power: 123.4 ± 13.7% of control; integrated power: 123.3 ± 13.3%; bandwidth: 107.6 ± 5.1%; peak frequency: 105.3 ± 5.2%; n = 9, $P > 0.05$, Figure 1D–G).

Effects of SGAs on gamma oscillations

Application of clozapine (30 μM) reduced the peak power and the integrated gamma power to 32.6 ± 8.0% and 40.3 ± 7.6%, respectively (n = 10, $P < 0.05$, Figure 2A, E), but did not change the peak frequency of gamma oscillations (112.9 ± 4.4%, $P > 0.05$, Figure 2A, F). Clozapine also increased the bandwidth of the oscillations to 176.8 ± 32.3% ($P < 0.05$, Figure 2A, G), indicating that it reduces both the power and the coherence of gamma oscillations.

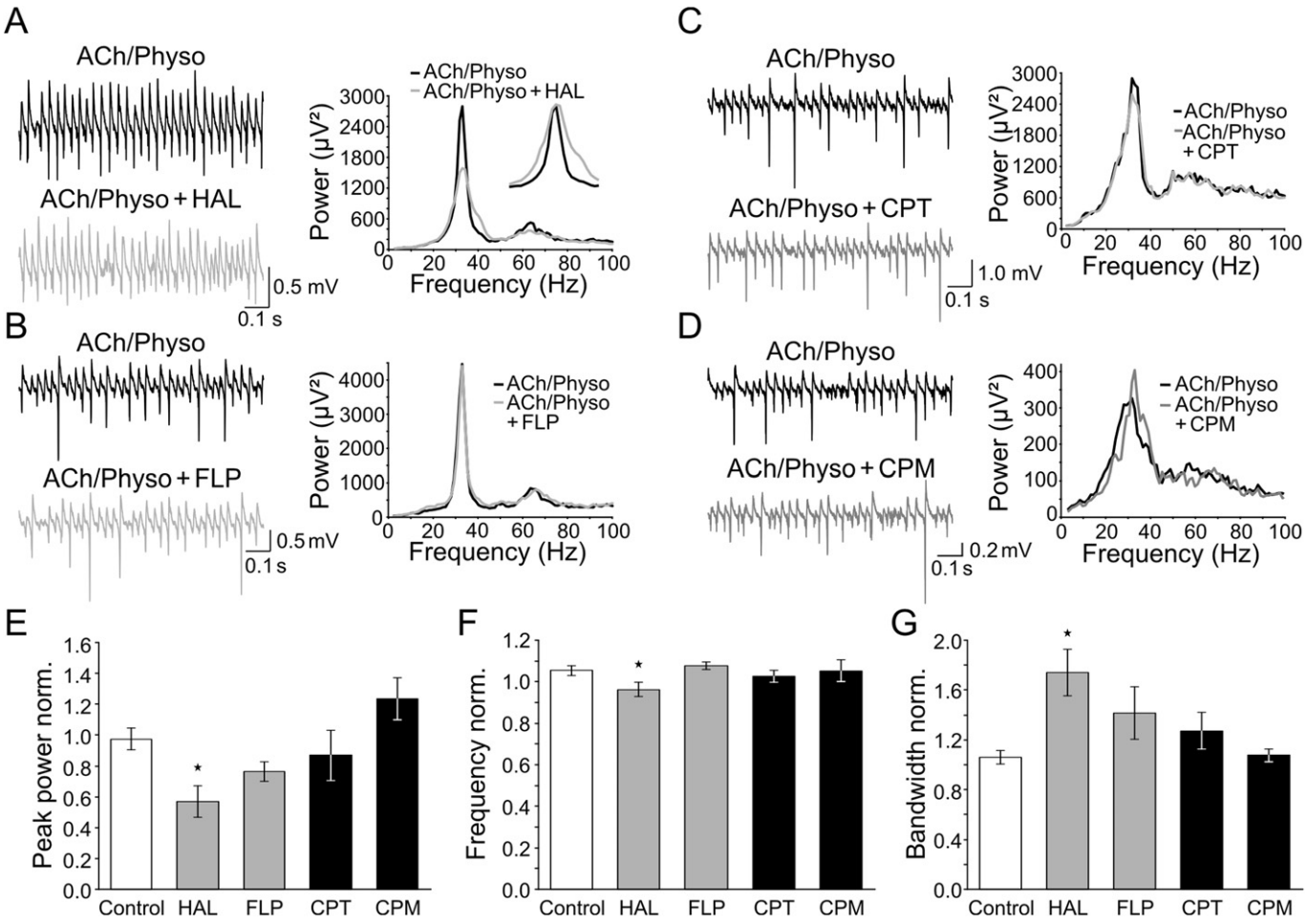

Figure 1

High- and low-potency first generation antipsychotics (FGAs) differentially alter ACh-induced gamma oscillations. (A–D) Left: field recordings of ACh-induced gamma oscillations before and during the application of the high-potency FGAs haloperidol (HAL, A) and flupenthixol (FLP, B), and the low-potency FGAs chlorprothixene (CPT, C) and chlorpromazine (CPM, D). Right: corresponding power spectra of the oscillations directly before (black) and 60 min after the application of the particular FGA (grey). Insets show the same power spectra rescaled for the peak to better illustrate the effects of HAL and FLP on bandwidth. (E–G) Quantification of the experiments shown in (A–D). Columns represent the change in the peak power (E), peak frequency (F) and peak bandwidth (G) of ACh-induced gamma oscillations normalized to the baseline directly before the wash-in of the high-potency (grey) and low-potency (black) FGAs (n = 7–8). * $P < 0.05$ compared with time-matched controls (white, n = 9) (ANOVA with Fisher's least significant difference *post hoc* test).

The SGA risperidone (10 μM) inhibited the peak power of gamma oscillations to 66.1 ± 10.8% (n = 9, $P < 0.05$, Figure 2B, E) whereas it did not significantly change the integrated gamma power, bandwidth and peak frequency of gamma oscillations (87.9 ± 10.5%, 138.4 ± 11.3% and 105.9 ± 1.4% of control, respectively, $P > 0.05$, Figure 2B, F, G), suggesting that it reduces the power of oscillations without affecting their coherence.

Ziprasidone (30 μM) and amisulpride (10 μM) did not significantly influence gamma oscillations (ziprasidone: peak power: 92.0 ± 5.3%; integrated power: 110.7 ± 8.5%; bandwidth: 137.9 ± 13.2%; peak frequency: 107.1 ± 2.0%; n = 11, $P > 0.05$; amisulpride: peak power: 90.7 ± 13.7%; integrated power: 96.1 ± 12.4%; bandwidth: 126.5 ± 14.0%; peak frequency: 101.9 ± 1.8%; n = 11, $P > 0.05$; Figure 2C–G).

Correlation between pK_i values and changes in peak gamma power

To determine which receptors might be responsible for the modulation of gamma oscillations by the antipsychotics, we plotted the normalized changes in gamma peak power against the pK_i values of the individual antipsychotics for 19 different receptors and analysed for correlation. The pK_i values were from the NIMH PDSP K_i database as described in Methods. Figure 3C, D shows the r^2 values and the slope of the regression for 19 receptors. A positive slope (e.g. for the 5-HT_3 receptor), that is, a positive correlation between the pK_i values and the normalized inhibition of peak power, indicates that drugs with high pK_i values (high affinity) inhibited the power more effectively. This means that activation of the

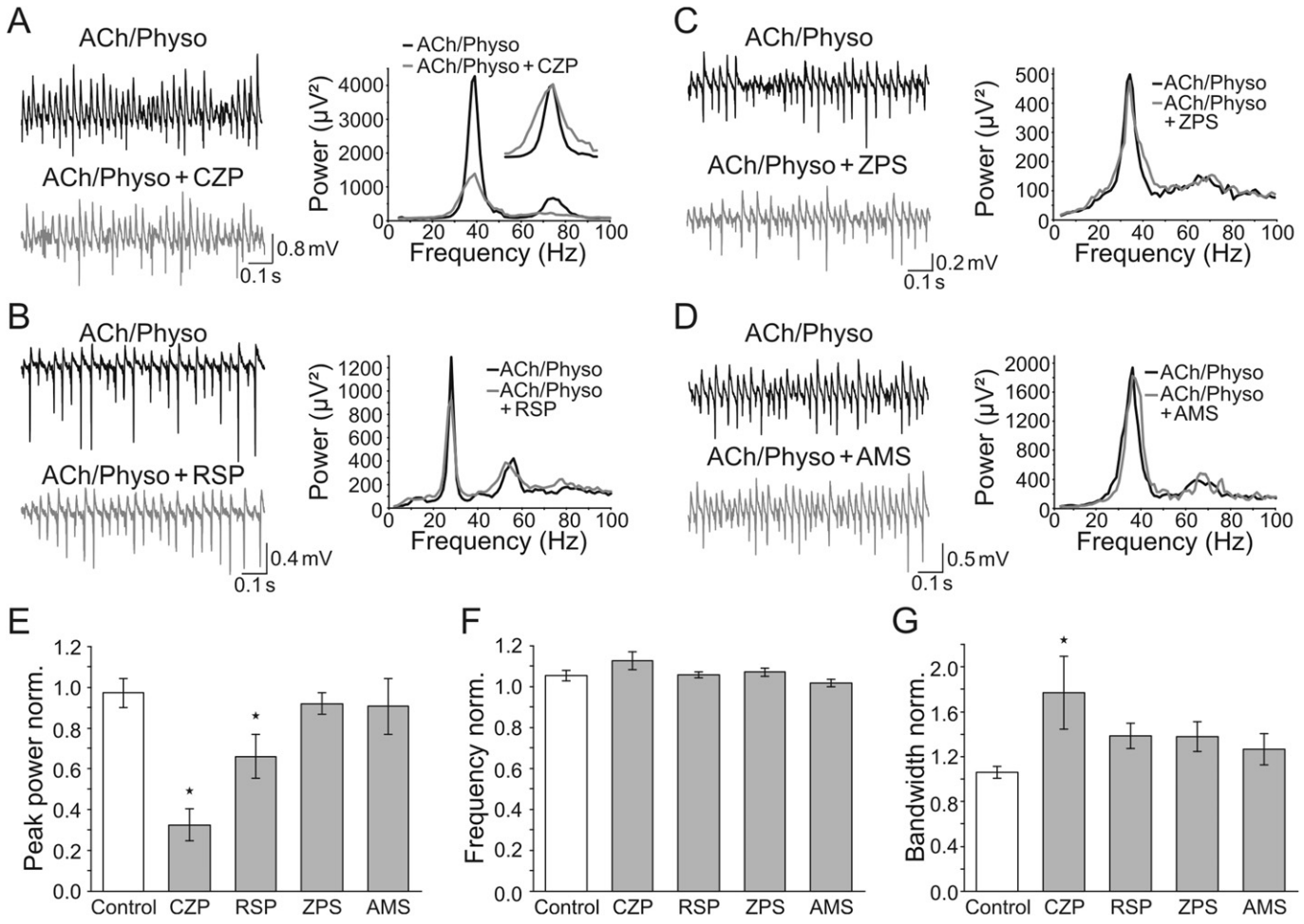

Figure 2

Second generation antipsychotics (SGAs) alter ACh-induced gamma oscillations with different efficacies. (A–D) Left: field recordings of ACh-induced gamma oscillations before and during the application of the SGAs clozapine (CZP, A), risperidone (RSP, B), ziprasidone (ZPS, C) and amisulpride (AMS, D). Right: corresponding power spectra of the oscillations directly before (black) and 60 min after the application of the particular SGA (grey). Inset in (A) shows the same power spectra rescaled for the peak to better illustrate the effects of CZP on bandwidth. (E–G) Quantification of the experiments shown in A–D. Bars represent the change of the peak power (E), peak frequency (F) and peak bandwidth (G) of ACh-induced gamma oscillations normalized to the baseline directly before the wash-in of the SGAs (grey, $n = 7$–10). * $P < 0.05$ compared with time-matched controls (white) (ANOVA with Fisher's least significant difference *post hoc* test).

receptor may enhance power and blockade of the receptor may be responsible for the inhibitory effects on gamma oscillations. In contrast, a negative slope (e.g. for the D_3 receptor) indicates that there was a negative correlation between the pK_i values and the normalized inhibition of peak power values. The negative slope indicates that drugs with low pK_i values (low affinity) inhibited the power more effectively, meaning that the receptor was endogenously activated during gamma oscillations and inhibited the power. The more effectively a drug inhibits the receptor, the less is the endogenous blockade of the power.

As seen on Figure 3C, D, all five muscarinic receptors were found to positively correlate with the power, as expected, because the oscillations were induced by ACh. However, these correlations were found to be not significant ($r^2 = 0.13$–0.28, slope = 0.06–0.10; $P > 0.05$). A significant positive correlation was found for the 5-HT_3 receptor ($r^2 = 0.68$, slope = 0.25; $P < 0.05$, Figure 3B–D), indicating that these receptors enhance gamma power. In contrast to this, the dopamine D_3 receptor was found to negatively correlate with the gamma power ($r^2 = 0.74$, slope = –0.28; $P < 0.05$, Figure 3A, C, D), suggesting that these receptors reduce peak power. The D_2 receptor also showed a negative correlation with the gamma power but without statistical significance ($r^2 = 0.30$, slope = –0.17; $P > 0.05$; Figure 3C, D).

Most SGAs are effective 5-HT_{2A} receptor antagonists with much weaker D_2 receptor blocking activity. The difference in pK_i values between the 5-HT_{2A} and D_2 receptors (5-HT_{2A} – D_2) has been found to be higher in most SGAs than in FGAs, suggesting that a higher 5-HT_{2A} receptor affinity over D_2 receptors is characteristic of SGAs (Kuroki *et al.*, 2008). Therefore, we also investigated whether the pK_i difference 5-HT_{2A} – D_2 correlates with the effects on peak power and found that they were not significantly correlated ($r^2 = 0.11$, slope = 0.05;

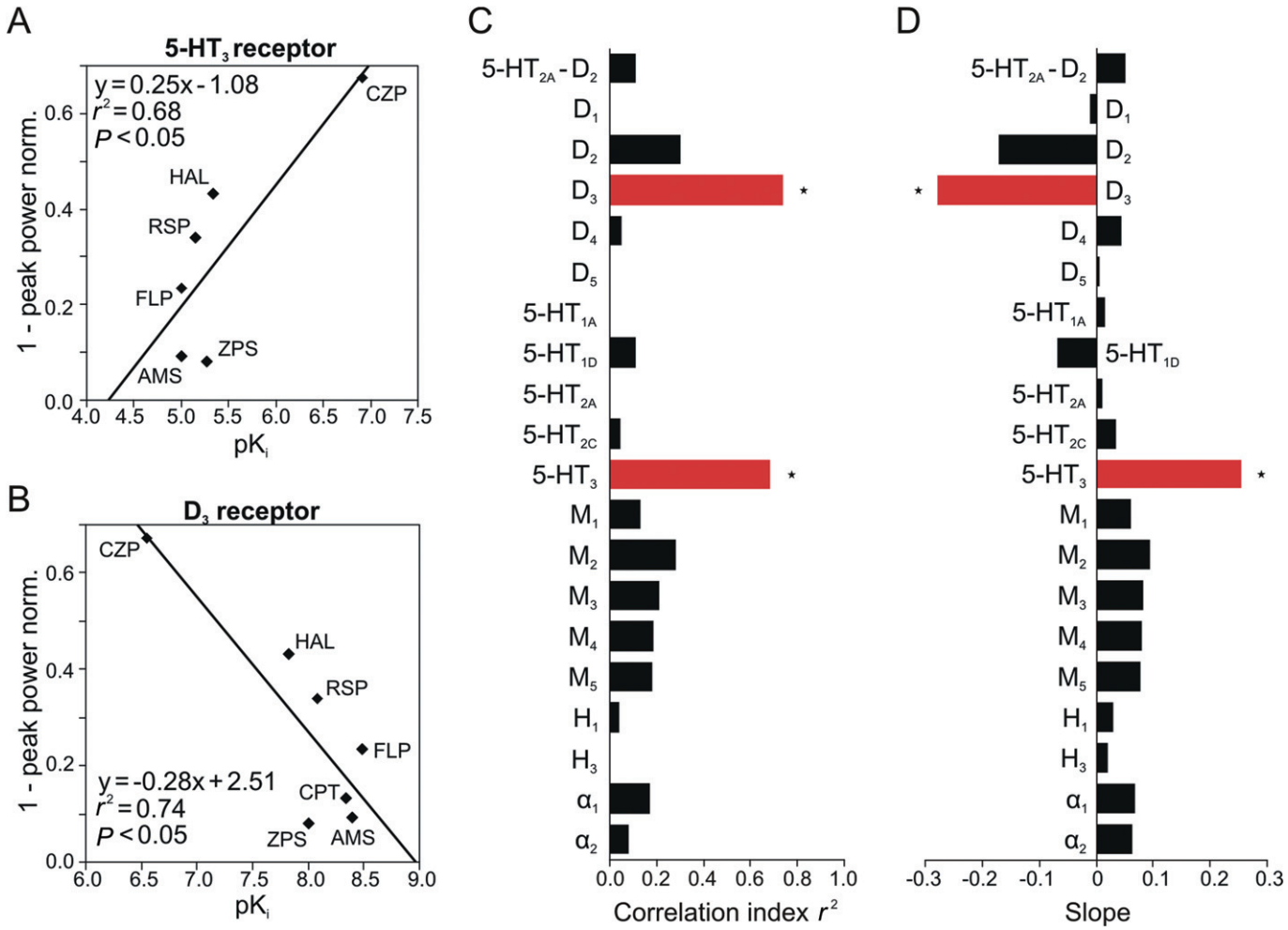

Figure 3

Correlations of the drug-induced inhibition of ACh-induced gamma power with the drug-receptor pK_i values. The normalized inhibition of peak power (1 – peak power norm.) of ACh-induced gamma oscillations by the antipsychotics amisulpride (AMS), chlorprothixene (CPT), clozapine (CZP), flupenthixol (FLP), haloperidol (HAL), risperidone (RSP) and ziprasidone (ZPS) was plotted against the pK_i values of these drugs for 19 receptors, among them the 5-HT_3 (A) and the D_3 receptor (B), and analysed for correlation. Chlorpromazine was excluded from the analysis because it was the only antipsychotic drug that enhanced the peak power. (C) The correlation index r^2 is shown for each receptor. (D) The slope of the regression line is shown for each receptor. 5-HT_{2A}-D_2 indicates the difference in pK_i values between the 5-HT_{2A} and D_2 receptors. Bars to the left indicate a negative correlation, bars to the right a positive correlation. * $P < 0.05$ (C, D).

$P > 0.05$, Figure 3C, D), indicating that a difference in the affinities for 5-HT_{2A} and D_2 receptors is not responsible for the modulation of the power of gamma oscillations.

Correlation between pK_i values and bandwidth

Next we plotted the normalized changes in gamma bandwidth induced by the individual antipsychotics against the pK_i values and analysed for correlation. Analogous to the correlation with the reduction in peak power, the slope was negative when there was a negative correlation between the pK_i value and the normalized increase of bandwidth [drugs with lower pK_i values (lower affinity) increased the bandwidth more effectively], meaning that activation of the receptor potentially inhibits gamma oscillations by increasing the bandwidth. A positive slope indicates a positive correlation between the pK_i value and the normalized increase of bandwidth (drugs with higher pK_i values increased the bandwidth more effectively), meaning that the receptor may narrow the gamma band and thus increase the coherence of gamma oscillations.

Fewer receptors correlated with the changes in bandwidth than with the reduction in peak power (Figure 4C, D). The D_3 receptor was the only one that significantly correlated with the gamma bandwidth ($r^2 = 0.64$, slope = –0.29; $P < 0.05$, Figure 4A, C, D), suggesting that activation of these receptors may worsen the coherence of gamma oscillations. There was also a negative relationship between the D_2 receptor and the gamma bandwidth; however, it did not reach statistical significance ($r^2 = 0.15$, slope = –0.14; $P > 0.05$). All the other receptors showed no correlation with the bandwidth (e.g. the 5-HT_3 receptor: $r^2 = 0.15$, slope = 0.14; $P > 0.05$, Figure 4B–D).

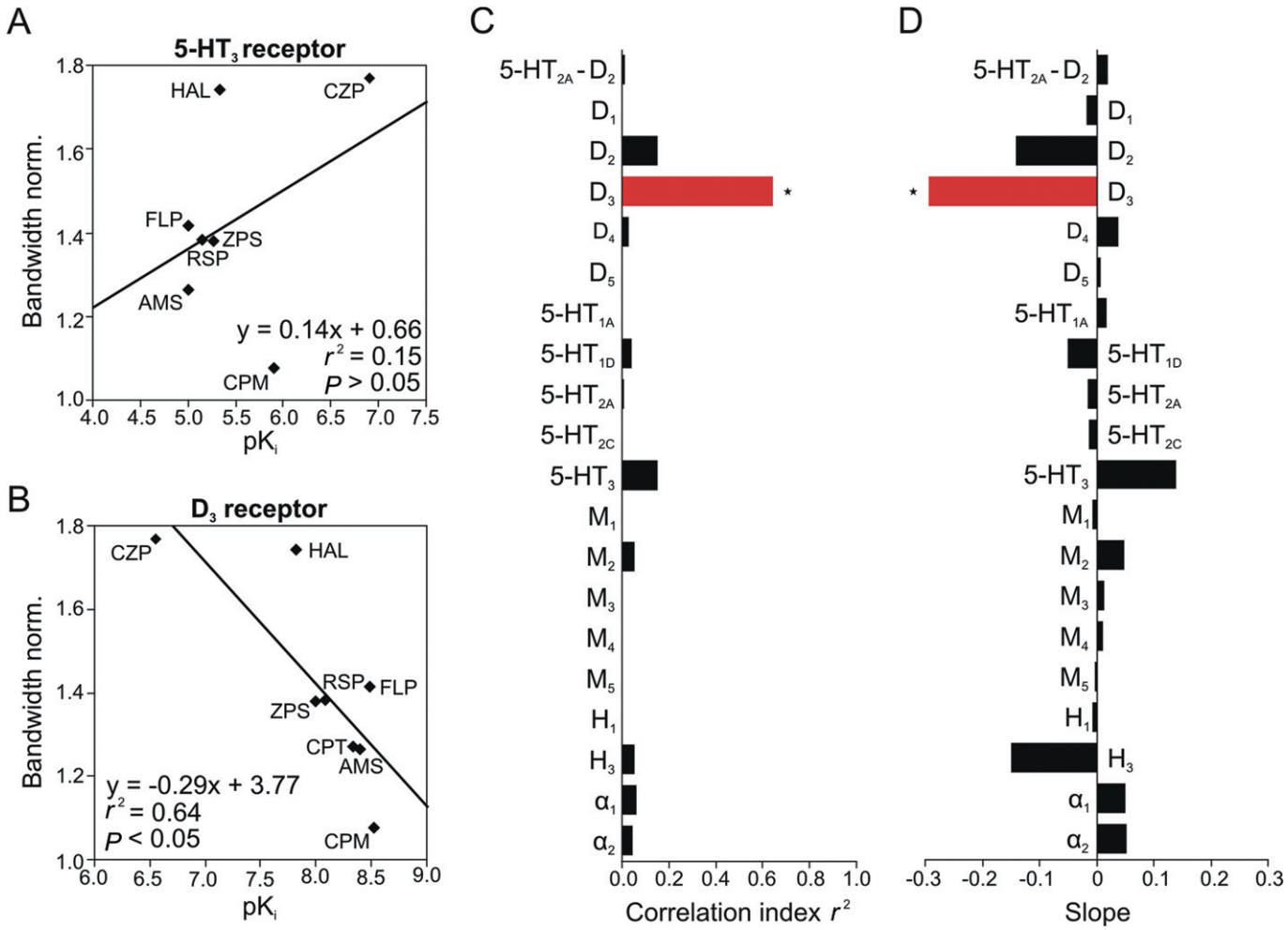

Figure 4

Correlations of the drug-induced increase in peak bandwidth of ACh-induced gamma oscillations with the drug-receptor pK_i values. (A) The normalized increase of gamma bandwidth by the antipsychotics amisulpride (AMS), chlorpromazine (CPM), chlorprothixene (CPT), clozapine (CZP), flupenthixol (FLP), haloperidol (HAL), risperidone (RSP) and ziprasidone (ZPS) was plotted against the pK_i values of these drugs for 19 receptors, among them the 5-HT_3 (A) and the D_3 receptor (B), and analysed for correlation. (C) The correlation index r^2 is shown for each receptor. (D) The slope of the regression line is shown for each receptor. 5-HT_{2A}-D_2 indicates the difference in pK_i values between the 5-HT_{2A} and D_2 receptors. Bars to the left indicate a negative correlation, bars to the right a positive correlation. * $P < 0.05$ (C, D).

In addition, the difference between the affinities for 5-HT_{2A} and D_2 receptors showed a negligible correlation with the bandwidth ($r^2 = 0.01$, slope = 0.02; $P > 0.05$, Figure 4C, D).

Effects of selective agonists of 5-HT_3, D_3 and 5-HT_{2C} receptors on gamma oscillations

To test the predictions of these calculations, we next applied selective agonists for 5-HT_3 and D_3 receptors to investigate their effects on the gamma oscillations, as these were the receptors with significant correlations. In addition, a 5-HT_{2C} receptor agonist was used to test a receptor without any correlation ($R^2_{pos} = 0.04$ for pK_i vs. power and $R^2_{pos} = 0.005$ for pK_i vs. bandwidth, $P > 0.05$; Figures 3C and 4C). Application of the selective agonists significantly altered the peak and integrated power and bandwidth of gamma oscillations as confirmed by ANOVA (peak power: $F_{0.05(1),3,27} = 7.524$, $P = 0.001$; integrated power: $F_{0.05(1),3,27} = 11.868$, $P < 0.001$; bandwidth: $F_{0.05(1),3,27} = 6.296$, $P = 0.002$), whereas the peak frequency was not affected ($F_{0.05(1),3,27} = 2.64$, $P > 0.05$).

The 5-HT_3 receptor agonist mCPBG (30 μM) increased the peak and integrated gamma power to 149.2 ± 15.7% and 160.3 ± 17.7%, respectively ($n = 7$, $P < 0.05$; Figure 5A, D), whereas the peak frequency and the gamma bandwidth did not change (95.6 ± 2.3% and 108.9 ± 8.5% of control, respectively, $P > 0.05$, Figure 5A, E, F). Also the effect of the D_3 receptor agonist was found to be in accordance with our prediction: the D_3 agonist PD 128907 (10 μM) reduced the peak and integrated gamma power to 24.2 ± 8.4% and 36.1 ± 10.7%, respectively ($n = 7$, $P < 0.05$; Figure 5B, D), without affecting the peak gamma frequency (96.4 ± 5.4% of control, $P > 0.05$, Figure 5B, E). As predicted, PD 128907 also increased the bandwidth of the oscillations to 209.1 ± 33.2% of control ($P < 0.05$, Figure 5B, F).

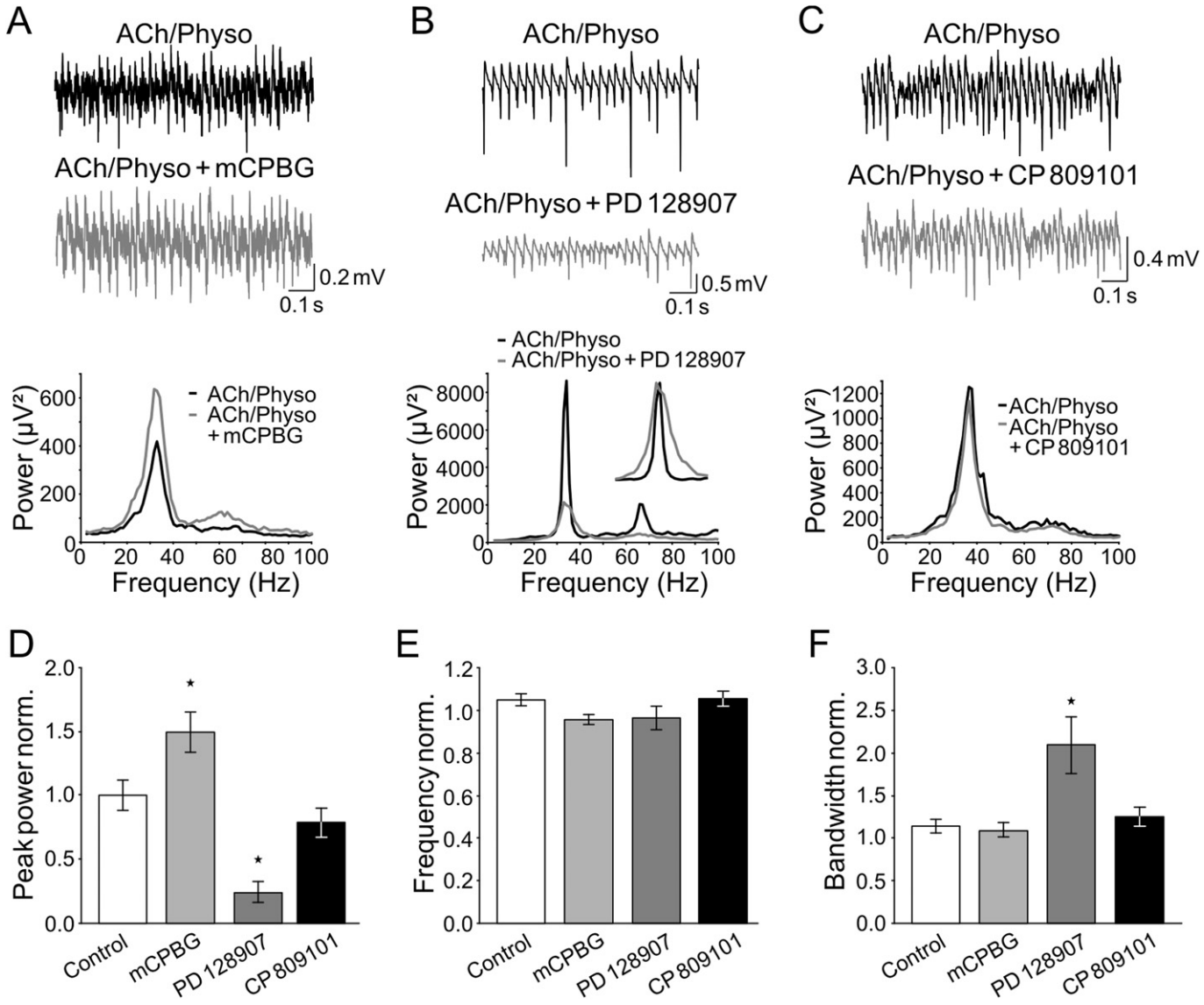

Figure 5

The 5-HT_3 receptor agonist enhances, the dopamine D_3 receptor agonist suppresses, and the 5-HT_{2C} receptor agonist does not influence ACh-induced gamma oscillations. (A–C) Top: field recordings of ACh-induced gamma oscillations before and during the application of the 5-HT_3 receptor agonist mCPBG (A), the D_3 receptor agonist PD 128907 (B) and the 5-HT_{2C} receptor agonist CP 809101 (C). Bottom: corresponding power spectra of the oscillations immediately before (black) and 60 min after the application of the particular agonist (grey). (D–F) Quantification of the experiments shown in (A–C). Columns represent the change of the peak power (D), peak frequency (E) and peak bandwidth (F) of ACh-induced gamma oscillations normalized to the baseline directly before the wash-in of mCPBG (light grey, $n = 7$), PD 128907 (dark grey, $n = 7$) and CP 809101 (black, $n = 7$) respectively. * $P < 0.05$ compared with time-matched controls (white) (ANOVA with Fisher's least significant difference *post hoc* test).

To further test our correlation data, we next applied the selective 5-HT_{2C} agonist CP 809101 (30 μM) and found that it did not change any of the peak and integrated gamma power, frequency or bandwidth of gamma oscillations (78.1 ± 11.4%, 100.0 ± 10.8%, 105.6 ± 3.4% and 124.7 ± 11.3% of control, respectively, $n = 7$, $P > 0.05$; Figure 5C–F). In conclusion, these data confirm the correlation analysis and the prediction for the receptor subtypes involved in the modulation of gamma oscillations and further verify that 5-HT_3 and D_3 receptors have opposing effects on hippocampal gamma oscillations.

Discussion and conclusions

We investigated the effect of antipsychotics on neural gamma oscillation power and coherence in the hippocampus. Haloperidol and clozapine inhibited gamma oscillations

most effectively. FGAs with low efficacy did not reduce gamma oscillations whereas the SGAs clozapine and risperidone did so. The SGAs ziprasidone and amisulpride did not affect gamma network activity. Because the tested compounds are multi-target drugs, to find out which receptor may be responsible for the observed effects we correlated the normalized effects of antipsychotics on power and bandwidth with their receptor pK_i values. Using this approach, we found that 5-HT_3 receptors may have an enhancing effect on gamma oscillations whereas dopamine D_3 receptors may inhibit them. To test our prediction, selective agonists were applied which revealed that selective stimulation of 5-HT_3 receptors indeed increases the power of gamma oscillations and that activation of D_3 receptors reduces it.

Gamma oscillations and schizophrenia

In schizophrenic patients, disturbed gamma oscillations have been observed. Whereas negative symptoms such as social and emotional withdrawal correlate with decreased gamma band activity, during positive symptoms, such as hallucinations, an increase in gamma power has been observed (Herrmann and Demiralp, 2005; Lee *et al.*, 2010; Mulert *et al.*, 2011). FGAs effectively reduce the positive symptoms without significantly affecting the negative ones. The introduction of SGAs in the 1990s was accompanied by promising reports, suggesting that they were able to improve the negative symptoms as well. In fact, it was found that SGAs were not more effective than FGAs, even for negative symptoms (Leucht *et al.*, 2009). Thus, the recovery of patients with negative symptoms has still remained one of the unresolved problems of psychopharmacology (Erhart *et al.*, 2006). We found that both FGAs and SGAs are able to reduce gamma oscillations, which is in accordance with the overall effectiveness of antipsychotics against positive symptoms. The findings that the high-potency FGA haloperidol and the SGA clozapine had the strongest inhibitory effects on gamma oscillations reflect their higher antipsychotic effectiveness in reducing the positive symptoms of schizophrenia (Essali *et al.*, 2009; Asenjo Lobos *et al.*, 2010).

The hypothesized relationship between schizophrenia and altered gamma oscillations is supported by a number of studies in rodents examining the effects of psychotomimetic drugs, such as MK-801 and ketamine, which have been shown to increase the gamma band power on *in vivo* EEGs (Pinault, 2008; Ehrlichman *et al.*, 2009; Hong *et al.*, 2010; Jones *et al.*, 2011; Kocsis, 2012). Moreover, haloperidol and clozapine have been found to reduce EEG gamma power after acute s.c. administration (Jones *et al.*, 2011) and clozapine was demonstrated to suppress synchronized pyramidal network activity in the prefrontal cortex (Gao, 2007). These findings are further supported by our results demonstrating an inhibition of *in vitro* gamma power by the same antipsychotic drugs. Thus, psychotic-like symptoms both in humans and rodent models are associated with an increase in gamma power and administration of high-potency antipsychotics such as clozapine and haloperidol is able to suppress this increase. However, it should be noted that these studies, similar to the present one, investigated the effect of antipsychotics on physiological gamma oscillations but not on pathologically altered gamma synchrony observed in schizophrenia.

Correlation of pK_i values with the gamma-influencing effects

Antipsychotics are known to be drugs interacting with a number of receptors (Roth *et al.*, 2004). To reveal which receptors are involved in the gamma-influencing effects of antipsychotics, we correlated these effects with their pK_i values for different receptors and found that the blockade of 5-HT_3 receptors by antipsychotics may be responsible for their inhibition of gamma oscillations. In contrast, the blockade of D_3 receptors negatively correlated with their inhibition of gamma activity. This indicates that D_3 receptor antagonism may counterbalance the inhibitory effect of antipsychotics via other receptors. Thus, our calculations suggest that activation of 5-HT_3 receptors may have augmenting effects on the gamma power while activation of D_3 receptors might reduce both the power and the coherence of the gamma oscillations. To test these predictions and to reveal the precise effects of these receptors on gamma oscillations, we applied selective 5-HT_3, 5-HT_{2C} and D_3 receptor agonists. In accordance with the correlation data, the 5-HT_3 agonist enhanced, the D_3 agonist inhibited, and the 5-HT_{2C} agonist did not affect the power of gamma oscillations. The bandwidth of the oscillations was only affected by the D_3 agonist, as predicted from the calculations. These data indicate that the correlation of pK_i values for different receptors with the effects of compounds interacting with multiple receptors may provide a valid approach to predict receptors involved in pharmacological effects.

Involvement of 5-HT_3 and D_3 receptors in the alteration of gamma oscillations

Gamma oscillations are driven by a precisely timed perisomatic feedback inhibition onto pyramidal cells (Hájos *et al.*, 2004; Bartos *et al.*, 2007; Gulyás *et al.*, 2010). While parvalbumin-positive fast-spiking interneurons are the most likely generator of gamma oscillations (Gulyás *et al.*, 2010), cholecystokinin (CCK)-containing regular spiking basket cells seem to have a modulatory role on the synchrony of neuronal populations, especially as they are able to excite parvalbumin positive cells by releasing CCK with subsequent postsynaptic activation of CCK_2 receptors. This activation is target specific in that there is no significant depolarization of neighbouring pyramidal cells (Lee *et al.*, 2011). Unlike parvalbumin-positive basket cells, CCK-containing interneurons express a number of neuromodulatory receptors such as 5-HT_3, cannabinoid CB_1 (Katona *et al.*, 1999) and nicotinic α7 receptors (Morales *et al.*, 2008) that enable the network to be modulated by extrinsic inputs and neuromodulatory signalling molecules (Freund, 2003). Indeed, activation of presynaptic CB_1 receptors on CCK-containing cells has been shown to reduce gamma power, possibly due to the suppression of GABA release onto pyramidal neurons (Hájos *et al.*, 2000).

Postsynaptic 5-HT_3 receptors on these cells are also able to selectively modulate CCK-positive basket cells: they were shown to increase their excitability (McMahon and Kauer, 1997) and facilitate the spontaneous release of GABA onto the perisomatic region of pyramidal neurons (Katsurabayashi *et al.*, 2003; Turner *et al.*, 2004). Thus, 5-HT_3 receptors are likely to increase the power of gamma oscillations by activation of the modulatory perisomatic CCK-positive basket cells.

Our finding that antagonism at 5-HT_3 receptors is primarily responsible for the gamma inhibitory effects of antipsychotics is indeed in line with previous findings showing that the 5-HT_3 receptor antagonist ondansetron is an effective adjunctive agent to haloperidol in the therapy for chronic, treatment-resistant schizophrenia (Zhang *et al.*, 2006).

Dopamine D_3 receptor gene polymorphisms are known to be associated with schizophrenia (Jönsson *et al.*, 2003; Talkowski *et al.*, 2006), suggesting the involvement of this receptor in the pathophysiology of the disease. D_3 receptors are expressed in the hippocampus and the mesolimbic system (Bouthenet *et al.*, 1991; Richtand *et al.*, 1995), structures that are involved in the aetiology of schizophrenia. In the CA1 area of the hippocampus, they have been shown to inhibit the amplitude of IPSCs evoked in stratum radiatum possibly by causing endocytosis of $GABA_A$ receptors in the apical dendrites of pyramidal cells (Hammad and Wagner, 2006; Swant *et al.*, 2008). This inhibition of inhibitory inputs onto hippocampal pyramidal cells may represent a possible mechanism by which D_3 receptors are able to suppress the power and coherence of hippocampal gamma oscillations. Our data suggest that D_3 receptor agonists have an antipsychotic effect. However, because schizophrenia is characterized by disturbed (i.e. alternately enhanced *and* diminished) gamma oscillations, the aim of the pharmacotherapy should not be a reduction or increase of gamma oscillation power but a stabilization of the gamma activity under various circumstances (e.g. different extracellular dopamine levels). This could explain why D_3 partial agonists/antagonists seem to have a better overall antipsychotic effect, including an improvement in the negative and cognitive symptoms, than selective antagonists (Joyce and Millan, 2005; Gyertyán *et al.*, 2011; MacDonald and Bartolomé, 2010).

In conclusion, our data indicate that antipsychotics might inhibit neural gamma oscillations in the hippocampus by inhibiting 5-HT_3 receptors on CCK-positive perisomatic interneurons. Moreover, selective activation of these receptors increases the power of oscillations. In contrast, D_3 receptors on pyramidal cells seem to have negative modulatory effects on both the power and regularity of gamma oscillations. Our data also suggest that a correlation of receptor affinities with their biological effects can be used as a reliable approach to predict the targets responsible for the pharmacological effects of multi-target drugs.

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Conflict of interest

None.

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