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ORIGINAL INVESTIGATION

Antagonism at serotonin 5-HT_{2A} receptors modulates functional activity of frontohippocampal circuit

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Abstract
Rationale Several second-generation antipsychotics are characterised by a significant antagonistic effect at serotonin 5-HT_{2A} receptors (5-HT_{2A}R), a feature that has been associated with lower incidence of extra-pyramidal symptoms and a putative amelioration of positive and negative symptoms experienced by schizophrenic patients. However, the neurofunctional substrate of 5-HT_{2A} antagonism and its exact contribution to the complex pharmacological profile of these drugs remain to be elucidated.
Objectives Here, we used pharmacological magnetic resonance imaging to map the modulatory effects of the selective 5-HT_{2A}R antagonist Ml00907 on the spatiotemporal patterns of brain activity elicited by acute phencyclidine (PCP) challenge in the rat. PCP is a non-competitive NMDA receptor antagonist that induces dysregulation of corticolimbic glutamatergic neurotransmission and produces cognitive impairment and psychotic-like symptoms reminiscent of those observed in schizophrenia.
Results Pre-administration of M100907 produced focal and region-dependent attenuation of PCP-induced response in frontoseptohippocampal areas. As early studies highlighted a permissive role of 5-HT_{2A}R on frontal dopamine release, the role of post-synaptic dopamine D_1 receptors on PCP-induced response was examined by using the potent antagonist SCH23390. Interestingly, SCH23390 did not affect PCP's response in any of the regions examined. This finding rules out a significant contribution of dopamine in the functional changes mapped and, indirectly, the inhibitory effect of M100907, in favour of a glutamatergic origin.
Conclusions Our data expand recent evidence suggesting a key role of 5-HT_{2A}R in modulating glutamate-mediated cognitive performance in the prefrontal cortex and highlight the whole frontoseptohippocampal circuit as a key functional substrate of 5-HT_{2A}R antagonism in normal and disease states.

Keywords fMRI · Phencyclidine · M100907 · phMRI · Schizophrenia · Cognition

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Introduction

Schizophrenia is a disabling psychiatric disorder characterised by complex and severe symptoms, including psychosis, hallucinations, cognitive deficits and mood alterations. Whilst the first antipsychotic agents targeted selectively the dopamine system through dopamine D_2 receptors, second-generation antipsychotics (SGA; e.g. clozapine) are characterised by a multifaceted pharmacological profile, including multiple antagonist or inverse agonist properties at several neuroreceptor systems including serotonin, noradrenaline and histamine (Seeman 2002). This complexity makes it difficult to unravel the role and pharmacological contribution of individual target receptors, and despite almost two decades of active research since the identification of the first atypical antipsychotic clozapine, the precise mechanism responsible for the therapeutic effect of these molecules remains elusive.

The observation that several SGA present relatively low dopamine D_2 receptors affinity but high affinity for serotonin (5-HT) receptors has stimulated great interest in the neurophysiological role of this neurotransmitter in schizophrenia (Meltzer 1996). In particular, it has been suggested that the relatively high affinity of clozapine for the 5-HT_{2A} receptor (5-HT_{2A}R) may contribute to its reduced side effect liability and to its greater efficacy in therapy-resistant schizophrenia (Tandon and Fleischhacker 2005; Ichikawa and Meltzer 1999; Meltzer et al. 1989).

Pre-clinical experimental evidence indicates the possibility of a significant role for $5HT_{2A}$ receptors in modulating specific effects of SGA. Early studies showed that systemic or local administration of selective 5-HT_{2A}R antagonists in the rat medial prefrontal cortex stimulates dopamine efflux (Schmidt and Fadayel 1995). This finding has led to the hypothesis of a permissive role of 5-HT_{2A}R on frontal dopamine release as a contributory factor for a potentially superior cognitive effect of novel generation antipsychotics over classic dopamine D2 receptor antagonists (Kuroki et al. 1999; Ichikawa and Meltzer 1999). This hypothesis, however, has not been consistently confirmed in clinical studies, and the benefit exerted by SGA medications on cognitive performance remains questionable (Davidson et al. 1999). Recent studies have revealed an additional contribution of 5-HT_{2A}R as modulators of glutamatergic neurotransmission in frontocortical areas (Scruggs et al. 2000, 2003; Zhai et al. 2002), an effect that seems to be relevant for the control of attentional and cognitive performance of rat prefrontal cortex (Carli et al. 2005; Mirjana et al. 2004) and could exert a direct anti-psychotic effect in disease states involving hyper-glutamatergic neurotransmission (Coyle 2006). However, most of the studies that investigated the neurobehavioural correlates of frontal 5-HT_{2A}R antagonism have employed local impairment of NMDA and/or 5-HT_{2A}R activity through in situ administration of pharmacological agents (Mirjana et al. 2004; Martin-Ruiz et al. 2001; Ceglia et al. 2004). Whilst this approach is valuable in linking discrete receptor populations with the cellular determinants of behaviour, its pharmacological significance is severely limited by the local nature of the manipulations employed, which neglects potentially important afferent and efferent contributions of a complex receptor system like 5-HT_{2A}. Moreover, the wide distribution of 5-HT_{2A}R in the brain (Hoyer et al. 1986) and its pre- and post-synaptic location at different neuronal sub-types (Meltzer et al. 2003) make it difficult to predict the overall functional effect and exact neuronal substrates of $5HT_{2A}$R antagonism in the living brain. As most of the pre-clinical research so far has focused on the role of the receptor in frontal areas, the function and possible contributions of the wide extra-frontal 5-HT_{2A}R pool has remained virtually unexplored.

Non-invasive neuroimaging techniques such as pharmacological magnetic resonance imaging (phMRI) simultaneously integrate multiple functional contributions from widely distributed receptor populations, providing a spatially resolved description of pharmacological activity that is not straightforwardly related to receptor distribution and density (Jenkins et al. 2003; Gozzi et al. 2006; Honey and Bullmore 2004). In an attempt to identify the circuits modulated by 5-HT_{2A}R antagonism in the living brain, we used a rat phMRI protocol to map the modulatory effect of the selective 5-HT_{2A}R antagonist M100907 (Kehne et al. 1996) on the spatiotemporal pattern of response to an acute challenge with the *N*-methyl-D-aspartic acid receptor (NMDAR) antagonist phencyclidine (PCP). NMDAR antagonists like ketamine and PCP induce perceptual abnormalities, psychosis-like symptoms and mood changes in healthy humans and patients with schizophrenia (Malhotra et al. 1997; Adler et al. 1999; Allen and Young 1978), a finding that has led to the hypothesis that a decreased NMDAR function may be a pre-disposing or even causative factor in schizophrenia (Kristiansen et al. 2007; Krystal et al. 2002). The behavioural and functional effects of NMDAR antagonists are thought to arise primarily from a dose-dependent disinhibition of thalamo-cortical glutamatergic neurotransmission (Greene 2001; Large 2007), an event that cascades to involve several neurotransmitter systems including serotonin and dopamine (Greene 2001; Large 2007; Moghaddam et al. 1997). Recent neuroimaging studies have demonstrated the ability of NMDAR antagonists to elicit focal corticolimbothalamic activation in pre-clinical species (Gozzi et al. 2008b; Littlewood et al. 2006) and humans (Langsjo et al. 2003; Deakin et al. 2008), an effect totally suppressed by agents that modulate glutamate neurotransmission and regionally attenuated by atypical antipsychotics like clozapine (Gozzi et al. 2008a, b). In the present study, we explored the modulatory effect of the selective 5-HT_{2A}R antagonist M100907 on the functional response to PCP as a means to identify and spatially resolve the circuital substrate of 5-HT_{2A}R antagonism in the living brain. This approach allowed us to identify a focal and region-dependent attenuation of PCP-induced response by M100907 in frontoseptohippocampal areas.

Moreover, in an attempt to elucidate the neurochemical determinants of the changes mapped, we examined the role of dopamine D_1 antagonism on the pattern of activation of PCP using the potent antagonist SCH22390 (Neisewander et al. 1998). Dopamine D_1 receptors are crucially involved in the control of cognitive functions processed at a prefrontal level (Robbins 2005). Since M100907 has been reported to stimulate dopamine release in frontal areas (Schmidt and Fadayel 1995), the effect of this drug may involve post-synaptic activation of D_1 dopamine receptors. Given the prevalent role of dopamine D_1 receptors in mediating the fMRI response to dopamine-releasing agents (reviewed by Knutson and Gibbs 2007), by assessing the effect SCH22390, we sought to determine whether the functional response to PCP in our model presents significant contributions of dopaminergic nature. When considered with previous evidence of a negligible role of dopamine D_2 receptors in the same experimental setup (Gozzi et al. 2008b), a lack of modulatory effect by SCH233990 would strongly argue against a predominant contribution of dopamine in the functional response to PCP mapped and, in turn, in the inhibitory effect of M100907. In the light of the established facilitatory role of 5-HT_{2A}R on pyramidal glutamate neurotransmission (Scruggs et al. 2000, 2003; Zhai et al. 2002), this finding would thus provide important indirect evidence supporting a glutamatergic origin of the effects mapped with M100907.

Materials and methods

Animal preparation

The studies were performed on male Sprague–Dawley rats (250–350 g, Charles River, Como, Italy). Animal preparation/monitoring and MRI acquisition have been previously described in greater detail (Gozzi et al. 2008b). Briefly, rats were anaesthetised with 3% halothane, tracheotomised and artificially ventilated with a mechanical respirator. The left femoral artery and vein were cannulated and animal paralysed with a 0.25-mg/kg i.v. bolus of D-tubocurarine followed by a continuous infusion of 0.25 mg/kg/h through the artery. After surgery, halothane level was set to 0.8%. Arterial blood samples (0.5 ml) were taken immediately prior to and at the end of the fMRI time series acquisition, and p_aCO_2 and p_aO_2 were measured using a blood gas analyser (Table SI). No statistically significant difference in mean pre- and post-acquisition p_aCO_2 values for each pair of PCP-challenged groups was found ($p>0.33$, all groups; ANOVA, followed by Fisher's least significant difference (LSD) test for multiple comparisons). The body temperature of all subjects was maintained within physiological range (37±0.8°C) throughout the experiment by using a water heating system. Mean arterial blood pressure (MABP) was monitored continually through a transducer placed in the femoral artery.

rCBV measurement

MRI acquisition parameters have been previously described in greater detail (Gozzi et al. 2008a). Images were acquired using a Bruker Avance 4.7-T system. The MR acquisition for each subject comprised T_2-weighted anatomical images using the rapid acquisition relaxation enhanced (RARE) sequence (Hennig et al. 1986; TR=5,000 ms, TE_{eff}=76 ms, RARE factor 8, FOV 40 mm, 256×256 matrix, 16 contiguous 1 mm slices) followed by a time series acquisition with same spatial coverage (TR_{eff}=2,700 ms, TE_{eff}=110 ms, RARE factor 32, 128×128 matrix, NA=2, dt=40).

Total MRI time-series acquisition time was 77 min (110 repetitions) for all groups. Following six reference images, 2.67 ml/kg of the blood pool contrast agent Endorem (Guerbet, France) was injected so that subsequent signal changes would reflect alterations in relative cerebral blood volume (rCBV; Mandeville et al. 1998).

Compounds, doses and experimental design

In order to allow for a better randomisation and keep the study manageable, drugs were tested in two separate studies. PCP challenge was administered 30 or 20 min after i.p. or s.c. pre-treatment (see below), and MRI data were acquired over a period of 30 min following the administration of the PCP challenge. Male SD rats were randomly assigned to one of the groups below.

M100907 1.5 mg/kg study

1. Intraperitoneal pre-treatment with vehicle (water 1 ml/kg) followed by intravenous challenge with PCP (0.5 mg/kg, 1 ml/rat) 30 min later (n=5)
2. Intraperitoneal pre-treatment with M100907 (1.5 mg/kg) and intravenous challenge with PCP (0.5 mg/kg, 1 ml/rat) 30 min later (n=6)

M100907 0.5 mg/kg study

3. Intraperitoneal pre-treatment with vehicle (saline, 1 ml/kg), followed by intravenous challenge with PCP (0.5 mg/kg, 1 ml/rat) 30 min later (n=8)
4. Intraperitoneal pre-treatment with M100907 (0.5 mg/kg) followed by intravenous administration of PCP (0.5 mg/kg, 1 ml/rat) 30 min later (n=6)

SCH23390 0.5 mg/kg study

5. Subcutaneous pre-treatment with vehicle (water, 1 ml/kg) followed by intravenous challenge with PCP (0.5 mg/kg, 1 ml/rat) 20 min later (n=6)
6. Subcutaneous pre-treatment with SCH23390 (0.1 mg/kg; 1 ml/kg) followed by intravenous challenge with PCP (0.5 mg/kg, 1 ml/rat) 20 min later (n=8)
7. Intraperitoneal pre-treatment with water (1 ml/kg) followed by intravenous challenge with saline (1 ml/rat) 30 min later (n=6). This group served as reference rCBV baseline for PCP in all studies

Phencyclidine hydrochloride was purchased from Tocris (Bristol, UK). M100907 was synthesised by the GSK department of Medicinal Chemistry. All compounds were dissolved in saline and injected at a rate of 1 ml/min. The doses chosen for the different drugs were based on previously published in vivo studies. PCP was tested at a sub-anaesthetic dose (0.5 mg/kg i.v.) that produces robust corticolimbothalamic activation in halothane-anesthetised rat (Gozzi et al. 2008c). The same dose of PCP has also been reported to elicit robust behavioural and metabolic (2-deoxyglucose) effects in conscious and freely-moving rats (Weissman et al. 1987; Gozzi et al. 2008b).

The doses of M100907 used in the present study showed robust effects in multiple behavioural readouts in rodents (reviewed by Kehne et al. 1996). The compound exhibits high potency and excellent selectivity (>100-fold separation at 26 receptors) and has been shown to be devoid of ex vivo receptor binding at alpha1-adrenetrgic or D_2-dopamine receptor at doses 7-fold higher than the maximal dose tested in our experiments (Kehne et al. 1996). SCH23390 is a potent dopamine D_1 antagonist (Andersen et al. 1992). The pre-treatment regimen used with SCH23390 has been reported to produce rapid and sustained exposure in the rat brain (Hietala et al. 1992). The same dose of SCH223390 tested produced robust in vivo antagonism of acute and chronic effect of dopaminergic agents in numerous rat behavioural paradigms (Molloy and Waddington 1984; Garris et al. 1994; Wolf and Xue 1999; Zahrt et al. 1997) whilst minimising the cataleptic and cognitive-impairing effects reported at higher doses (Wadenberg 1992).

Data analysis

rCBV time series image data for each experiment were analysed within the framework of the general linear model as described in greater detail elsewhere (Worsley et al. 1992; Schwarz et al. 2006b). The maps thus obtained were used to guide the selection of activated/deactivated regions for subsequent volume of interest (VOI)-based quantification and comparison of efficacy of pre-treatments.

Signal intensity changes in the time series were converted into fractional rCBV on a pixel-wise basis, using a constrained exponential model of the gradual elimination of contrast agent from the blood pool (Schwarz et al. 2003, 2006b). Individual subjects in each study were spatially normalised by a 9 degree-of-freedom affine transformation mapping their T_2-weighted anatomical images to a stereotaxic rat brain MRI template set (Schwarz et al. 2006a) and applying the resulting transformation matrix to the accompanying rCBV time series. rCBV time series for the PCP or vehicle challenge were calculated covering 8 min (12 time points) pre-challenge baseline and 25 min (38 time points) post-challenge window, normalised to a common injection time point. Image-based time series analysis was carried out using FMRI Expert Analysis Tool Version 5.63, part of FMRIB's Software Library (www.fmrib.ox.ac.uk/fsl) with 0.8 mm spatial smoothing (≈2.5×in-plane voxel dimension) and using a model function identified by Wavelet Cluster Analysis across all animals in the cohort, capturing the temporal profile of the signal change induced by PCP challenge (Whitcher et al. 2005; Schwarz et al. 2006b). As no substantial differences in the temporal profile of PCP-induced changes were observed across PCP-challenged groups (see "Results" section), a common regressor was used (Supplementary Figure 1). Consistent with previous studies, PCP did not produce any significant short-lived or negative signal changes in any of the regions analysed (Gozzi et al. 2008a, b).

The design matrix also included the temporal derivative of this regressor and a linear ramp (both orthogonalised to the regressor of interest) with the aim to capture additional variance due to slight deviations in individual subjects or brain regions from the signal model time course as described in more detail in Schwarz et al. (2006b). The coefficients of the model function thus provided a map of rCBV response amplitude for each injection in each subject. Higher-level group comparisons were carried out using FMRIB's Local Analysis of Mixed Effects; Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>2.3 and a corrected cluster significance threshold of p=0.01 (Worsley et al. 1992; Friston et al. 1994). Volumetric three-dimensional reconstructions of activation maps were generated using custom in-house software written in IDL (Research Systems Inc., Boulder, CO, USA).

VOI time courses for the PCP challenge were extracted from unsmoothed rCBV time series data using a 3D digital reconstruction of a rat brain atlas (Paxinos and Watson 1998) co-registered with the MRI template (Schwarz et al. 2006a), using custom in-house software written in IDL (Research Systems Inc., Boulder, CO, USA). A list of the VOIs examined and their anatomical definitions can be found in (Gozzi et al. 2008b). For each VOI time course, the average rCBV over a 16-min time window covering the peak response to PCP (4–20 min post-injection) was used as a summary statistic of the relative change. The effect of pre-treatment on the magnitude of average rCBV in different VOIs was assessed by a one-way ANOVA followed by Fisher's LSD test for multiple comparisons.

VOI time courses pre- and post-M100907 administration were also examined to assess potential effects of pre-treatment per se on basal CBV. To this end, rCBV time courses were also calculated for the pre-treatment over a time window covering 6 min (8 time points) pre-injection baseline and 22 min (32 time points, groups 1–4 and 7) or 17 min (24 time points, groups 5 and 6) post-injection window normalised to a common injection time point. VOI time courses were extracted from unsmoothed rCBV time series in the same regions examined for the PCP challenge.

Administration of vehicle, SCH23390 or PCP was accompanied by small and transient alterations of MABP. M100907 produced a sustained decrease in MABP that lasted throughout PCP's pre-injection time window (mean MABP≈65 mmHg). In all cases, peak magnitude of the MABP observed was within the cerebral blood flow autoregulation range measured under the same anaesthetic conditions used in the present study (Gozzi et al. 2007). As shown by us and other groups, positive or negative pharmacologically evoked MABP changes within the autoregulation range mentioned above do not result in significant central rCBV response when spin-echo MRI sequences are used (Zaharchuk et al. 1999; Gozzi et al. 2006).

Results

Vehicle-pre-treated animals (groups 1 and 3 and 5) showed a robust and sustained rCBV response to PCP in several corticolimbothalamic structures (Figs. 1, 2, 3, 4 and 5), consistent with previous observations (Gozzi et al. 2008b, c). Statistically significant activation was observed in limbic cortical regions with extension into the motor, visual, parietal- and temporal association and rhinal cortices. Additional foci of subcortical activation were observed in the medial and lateral habenula, amygdala, anterodorsal, dorsolateral and ventromedial thalamus, posterodorsal, anterodorsal and ventral and posterior hippocampus, the striatum and the nucleus accumbens. The overall temporal profile of PCP-induced activation was comparable in all the activated regions (Fig. 4; Supplementary Figures 2 and 3). Despite differences in the peak magnitude of PCP response across studies, the spatial distribution and relative amplitude of the regional response to PCP were very consistent and conserved across the different control groups (groups 1, 3 and 5; Figs. 3 and 5).

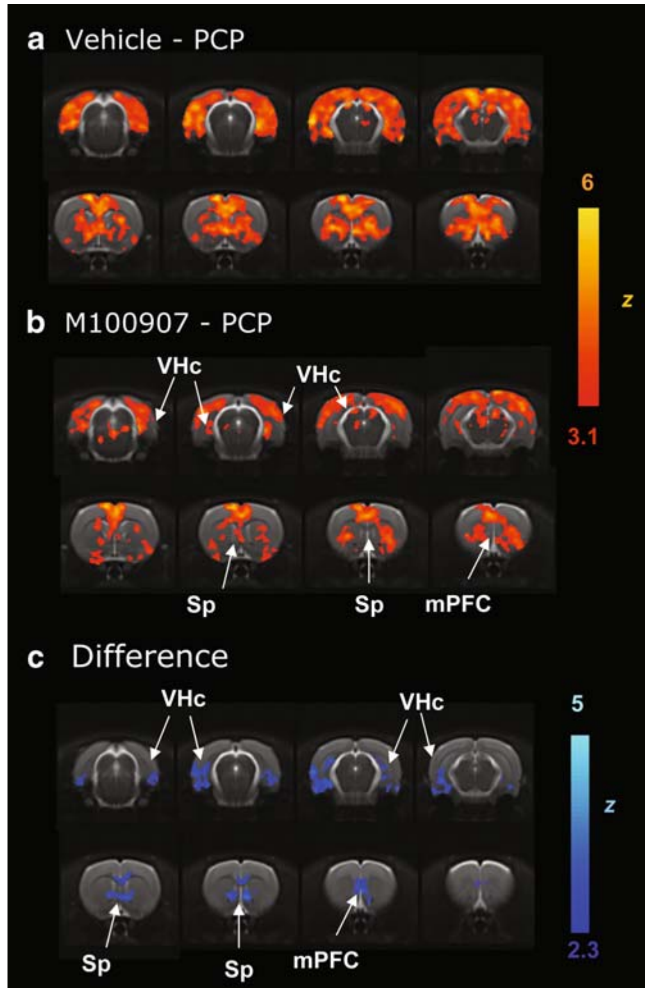

Fig. 1 **a** Anatomical distribution of the rCBV response following acute challenge with PCP (0.5 mg/kg i.v., group 1) with respect to baseline (vehicle–vehicle, group 5). **b** Anatomical distribution of the rCBV response following acute challenge with PCP (0.5 mg/kg i.v., group 1) in animals pre-treated with M100907 with respect to baseline (vehicle–vehicle, group 5). *Orange/yellow* indicates increased rCBV versus baseline (vehicle–vehicle). **c** Map of the regions showing an attenuated PCP response in animals pre-treated with M100907 (1.5 mg/kg i.p., group 1 vs. group 2). *Blue* indicates decreased rCBV versus baseline. *Z* statistics threshold levels are reported beside each map. Maps were cluster-corrected using a $p=0.01$ significance level. *mPFC* medial prefrontal cortex, *Sp* septum, *VHc* ventral hippocampus

Pre-administration of M100907 (1.5 mg/kg i.p.) produced region-dependent and sustained attenuation of PCP-induced rCBV response (Figs. 1, 2, 3 and 4). Foci of significant inhibition were observed in the medial prefrontal cortex, diagonal band, septal nuclei and in ventral hippocampal and peri-hippocampal areas, including the rhinal cortex ($p<0.05$, ANOVA; Figs. 1, 2, 3 and 4). Three-dimensional reconstruction of the areas of attenuation highlighted the involvement of contiguous septofrontal and hippocampal structures (Fig. 2). No areas of increased response to PCP were observed. The lower dose of M100907 (0.5 mg/kg i.p.) did not produce statistically significant attenuation of PCP

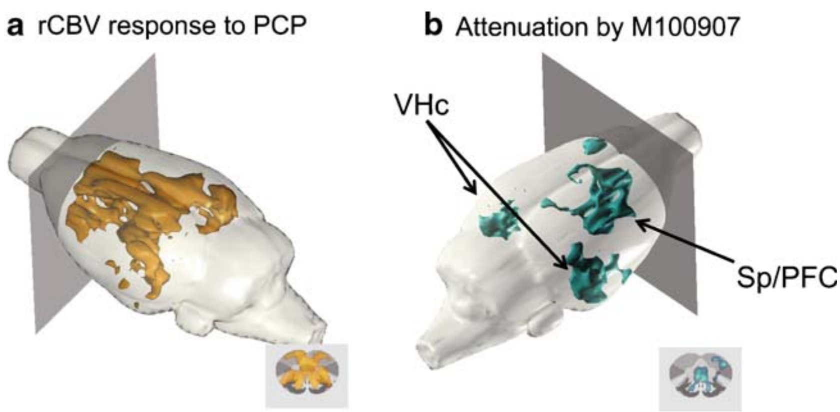

Fig. 2 **a** Volumetric reconstruction of the pattern of rCBV activation produced by acute challenge with PCP with respect to vehicle and **b** attenuating effect of pre-treatment with the selective 5-HT_{2A} antagonist M100907 (1.5 mg/kg i.p.) in frontoseptohippocampal regions. *PFC* medial prefrontal cortex, *VHc* ventral hippocampus, *Sp* septum

response in any of the regions examined ($p>0.28$ all regions; Fig. 3), although a trend was evident in the medial prefrontal cortex ($p<0.09$). This effect was best seen on rCBV time courses (Supplementary Figure 2). Pre-administration of SCH23390 (0.1 mg/kg i.p.) did not produce any significant alteration of PCP response in any of the regions examined ($p>0.24$, all regions; Fig. 5; Supplementary Figure 3).

Administration of M100907 per se (0.5 or 1.5 mg/kg i.p.) produced small (2–8%) and short-lived (4–9 min) rCBV increases in various brain regions, including the medial prefrontal cortex and ventral hippocampus (Supplementary Figures 4 and 5). At the time of PCP challenge, no apparent basal rCBV alteration with respect to vehicle was present in any of the regions examined. Intraperitoneal administration of SCH23390 did not produce visible alteration of basal rCBV with respect to vehicle in any of the regions examined (Supplementary Figure 6).

Discussion

In the present study, we show that selective antagonism of 5-HT_{2A}R induces focal attenuation of PCP-induced activation in frontoseptohippocampal areas of the rat brain. Our results extend previous findings of a role of 5-HT_{2A}R in modulating frontocortical activity (Ceglia et al. 2004; Mirjana et al. 2004) by highlighting the additional involvement of septal and ventral–hippocampal structures

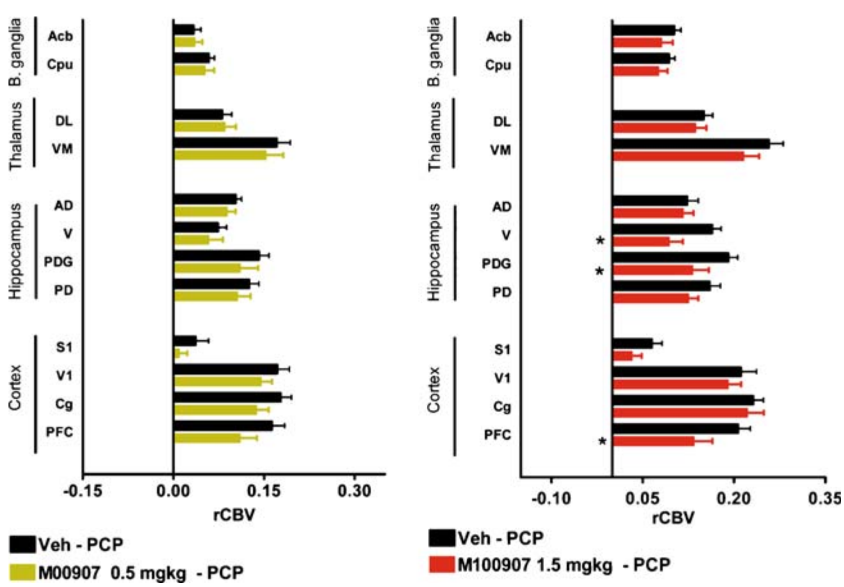

Fig. 3 Magnitude of mean rCBV response ($AUC_{4–20\ min}$) to PCP in representative regions of interest. *Left* Veh-PCP (group 3); Veh-PCP (group 4). *Right* Veh-PCP (group 1), M100907 1.5 mg/kg–PCP (group 2); *$p<0.05$ versus Veh-PCP (group 1), ANOVA followed by Fisher LSD test for multiple comparison. *Acb* nucleus accumbens, *Cpu* caudate putamen, *DL* dorsolateral thalamus, *VM* ventromedial thalamus, *AD* anterodorsal hippocampus, *V* ventral hippocampus, *PDG* posterior dentate gyrus, *PD* posterodorsal hippocampus, *S1* primary somatosensory cortex, *V1* primary visual cortex, *Cg* cingulate cortex, *PFC* medial prefrontal cortex

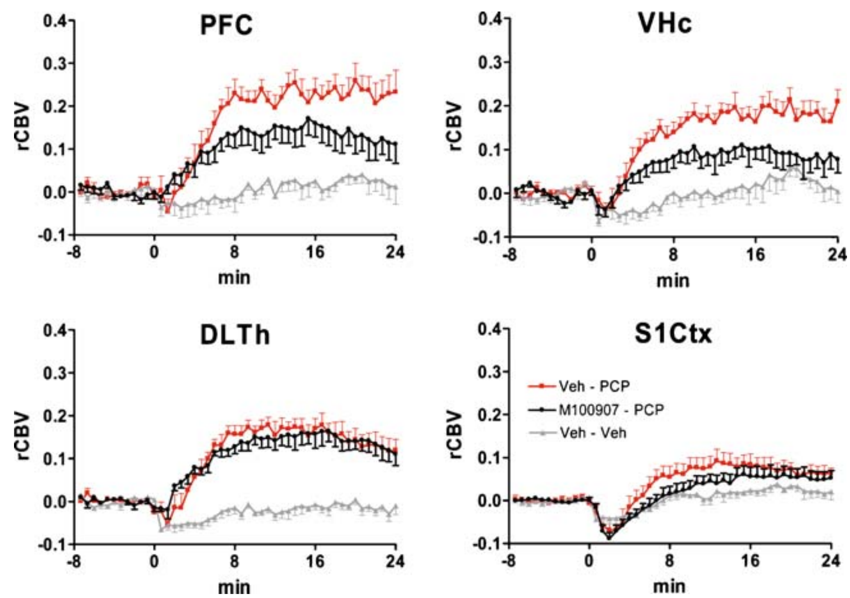

Fig. 4 rCBV time course following PCP injection in representative brain structures. PCP was administered at time 0. Baseline data were obtained in animals pre-treated and challenged with vehicle (saline, group 5). Data are plotted as mean ± SEM within each group. Veh-PCP: group 1, M100907 (1.5 mg/kg i.p.)–PCP: group 2, Veh-Veh: group 7. *PFC* medial prefrontal cortex, *VHc* ventral hippocampus, *DLTh* dorsolateral thalamus, *S1Ctx* primary somatosensory cortex

as integrated substrate of the action of 5-HT$_{2A}$R antagonism in the living brain. This finding is of particular interest in the light of clinical evidence suggesting a correlation between frontohippocampal hyperactivity and cognitive

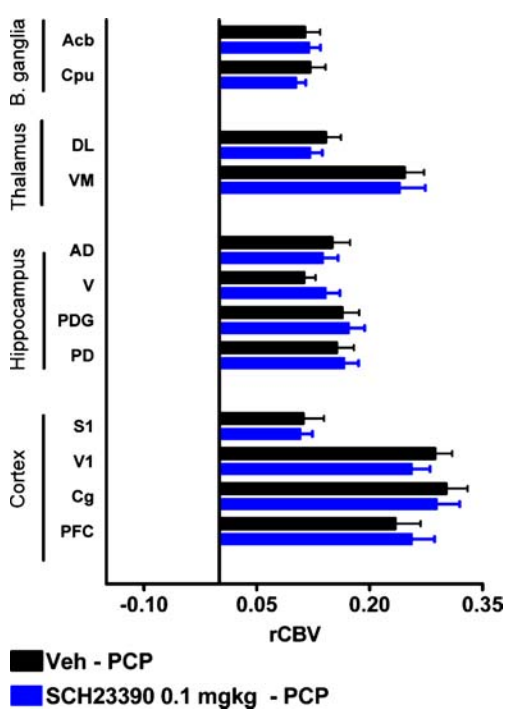

Fig. 5 Magnitude of mean rCBV response (AUC$_{4–20\ min}$) to PCP in representative regions of interest. Veh-PCP (group 5), SCH23390 0.1 mg/kg (group 6). *Acb* nucleus accumbens, *Cpu* caudate putamen, *DL* dorsolateral thalamus, *VM* ventromedial thalamus, *AD* anterodorsal hippocampus, *V* ventral hippocampus, *PDG* posterior dentate gyrus, *PD* posterodorsal hippocampus, *S1* primary somatosensory cortex, *V1* primary visual cortex, *Cg* cingulate cortex, *PFC* medial prefrontal cortex

and perceptual alterations observed in unmedicated schizophrenia patients (Silbersweig et al. 1995; Liddle et al. 2000; Parellada et al. 1994; Ngan et al. 2002; Soyka et al. 2005; Medoff et al. 2001)

The observation that 5-HT$_{2A}$R antagonism affects brain activity in frontohippocampal areas is consistent with previous pre-clinical research. 5-HT$_{2A}$ receptor density in these regions is high (Cornea-Hebert et al. 1999), and immunofluorescence studies have demonstrated marked 5-HT$_{2A}$R immunoreactivity in GABAergic and cholinergic septohippocampal terminals, as well as in pyramidal and granule cells of the hippocampus (Luttgen et al. 2004). These findings suggest that 5-HT$_{2A}$R can regulate hippocampal activity both via local pre-synaptic mechanisms and upstream modulation of septal outputs. In agreement with this, electrophysiology studies showed that M100907 and atypical antipsychotic can potently inhibit the excitatory action of serotonin on various septohippocampal neuronal populations (Alreja 1996; Liu and Alreja 1997; Piguet and Galvan 1994; Shen and Andrade 1998). However, the effect does not trivially reflect 5-HT$_{2A}$R receptor distribution. Indeed, high 5-HT$_{2A}$R density has been reported in large brain structures such as basal ganglia, thalamus and neocortex (Cornea-Hebert et al. 1999) which did not show significant modulation by M100907. This finding is of interest, as it highlights a discrete circuit whose activity is focally modulated by a widely distributed receptor population, and underscores the possibility to use functional neuroimaging methods to describe specific psychopharmacological contributions in terms of modulation of focal neural circuits.

The functional imaging technique used for this study does not provide direct information on the specific cellular or neurochemical determinants of the modulatory action of

M100907. However, multiple lines of evidence support a glutamatergic origin of the effect mapped. Firstly, 5-HT_{2A}R can positively modulate glutamatergic neurotransmission in frontocortical areas (Ceglia et al. 2004; Scruggs et al. 2000; Aghajanian and Marek 1997), through blockade of 5-HT_{2A} pre-synaptic heteroreceptors (Aghajanian and Marek 2000; Martin-Ruiz et al. 2001). Consistent with this hypothesis, NMDAR antagonists have been shown to induce a dose- and use-dependent hyper-glutamatergic state through de-regulation of pyramidal glutamatergic activity by selectively impairing recurrent feedback from GABAergic inter-neurons (Gozzi et al. 2008a; Greene 2001; Homayoun and Moghaddam 2007). Secondly, compounds that modulate pyramidal glutamate release have been shown to markedly attenuate the functional and behavioural cascade triggered by NMDAR antagonism (Gozzi et al. 2008a, b; Cartmell et al. 1999). In agreement with this, Ceglia et al. (2004) reported the ability of M100907 to prevent the increase in frontocortical glutamate induced by the NMDAR antagonist 3-(*R*)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP), an effect that also produced an improvement of CPP-induced impairment in attentional performance. Conversely, little or no inhibitory effect has been observed with drugs that target neurotransmitter systems secondarily activated by the effect of NMDAR antagonism such as dopamine D_2 antagonists (Idris et al. 2005; Gozzi et al. 2008b; Large 2007).

Alternatively, since M100907 has been reported to stimulate dopamine release in frontal areas (Schmidt and Fadayel 1995), the inhibitory effect observed could reflect post-synaptic activation of D_1 dopamine receptors. However, this effect, however, cannot be straightforwardly investigated by using dopamine-mimetic drugs, as these compounds produce robust and widespread haemodynamic alterations that could saturate the subsequent response to a PCP challenge (Choi et al. 2006; Schwarz et al. 2004, 2007). We therefore examined the role of post-synaptic dopamine D_1 receptors on PCP-induced fMRI response examined using a potent D_1 antagonist (SCH23390, 0.1 mg/kg; Andersen et al. 1992). Acute administration of PCP generates disinhibition of corticothalamic glutamatergic neurotransmission, an event that cascades to involve several neurotransmitter systems including serotonin and dopamine (Greene 2001; Large 2007; Moghaddam et al. 1997). Increased dopamine release upon acute administration of NMDAR antagonists has been observed in mesolimbic areas and in frontal regions of the rat (Moghaddam et al. 1997; Javitt et al. 1999). Given the prevalent role of dopamine D_1 receptors in mediating the fMRI response to dopamine-releasing agents (Knutson and Gibbs 2007), by assessing the effect of selective DA antagonists, we sought to determine whether the functional response to PCP in our model presents significant contributions of dopaminergic nature. Interestingly, pre-administration of SCH23390 did not produce any significant alteration of PCP response in any of the regions examined. When considered with previous evidence of a negligible role of dopamine D_2 receptors in the same experimental setup (Gozzi et al. 2008b), this finding strongly argues against a predominant role of dopamine in the functional changes mapped and, in turn, in the inhibitory action observed with M100907. In the light of the established facilitatory role of 5-HT_{2A}R on pyramidal glutamate neurotransmission discussed above, it seems thus likely that that the effect of M100907 reflects a local reduction in glutamatergic neurotransmission. However, whilst this is by far the most plausible neurochemical mechanism, our data do not permit to rule out contributions of PCP-induced serotonergic neurotransmission independent of the neuromodulatory role of the 5-HT_{2A}R on glutamate release

Although a comprehensive discussion of the role of dopamine in the cascade elicited by NMDAR antagonism is beyond the scope of the manuscript, the lack of effect of SCH22390 is of interest per se as it provides additional evidence of a subsidiary role of this neurotransmitter in mediating the neurobehavioural effects of these drugs, a finding observed by numerous investigators using dopamine D_2 antagonists in different experimental models and readouts (Idris et al. 2005; Gozzi et al. 2008b; Krystal et al. 1999; Linn et al. 2003). Our finding extends these results to the D_1 receptor subtype, suggesting that dopaminergic mechanisms are engaged far downstream in the neurofunctional cascade triggered by psychotogenic doses of NMDAR antagonists.

M100907 produced significant attenuation of PCP only at the highest dose (1.5 mg/kg), although a trend in the mPFC was apparent at the lower dose tested (0.5 mg/kg; Fig. 3). As pharmacodynamic studies reported complete inhibition of behavioural response to serotonergic agents at doses of 0.1 mg/kg (Schreiber et al. 1995; Kehne et al. 1996), the presence of significant attenuation only at the higher dose may call into question putative contributions from other receptor types, namely alpha1-adrenergic, dopamine D_2 or 5-HT_{2c}. However, multiple lines of evidence make this hypothesis very unlikely. Firstly, M100907's affinity for D_2 receptors is >2,500-fold lower than 5-HT_{2A} (Kehne et al. 1996). Consistent with this, the drug failed to reduce apomorphine induced climbing in rats, an index of D_2 receptor antagonism, at a dose as high as 8 mg/kg (Kehne et al. 1996; Sorensen et al. 1993). Although the selectivity at alpha$_1$-adrenergic receptor is slightly lower (>100-fold), the drug did not show significant ex vivo receptor binding at alpha$_1$-adrenergic receptors at doses up to 10 mg/kg (Kehne et al. 1996). Moreover, a dose of 16 mg/kg of M100907 (i.e. 10-fold higher than the effective dose of our study) failed to antagonise the acute cardiovascular effects of the alpha$_1$-adrenergic agonist phenylephrine (Kehne et al. 1996) in a widely used behavioural assay index of alpha$_1$-receptor antagonism (Peroutka et al. 1977). Finally, 1 mg/kg of M100907 did not show significant antagonism of the pre-pulse inhibition-disruptive effect of the potent alpha$_1$-adrenergic agonist cirazoline in two different rat strains (Varty et al. 1999). In the light of these findings, a significant contribution of dopamine D_2 or alpha$_1$-adrenergic receptors appears extremely unlikely.

Secondly, although receptor binding data indicate a >100-fold selectivity over 5-HT$_{2C}$ receptors (Kehne et al. 1996; Palfreyman et al. 1993), in vitro antagonism assays of functional selectivity highlighted a >1,000-fold separation between the two receptors (Kehne et al. 1996). In agreement with this, a number of in vivo studies showed that M100907, at the same or higher doses tested here, did not produce detectable effects in behavioural paradigms sensitive to the action of selective 5-HT$_{2C}$ antagonism (Fletcher et al. 2002; Zaniewska et al. 2007; Hajos et al. 2003), or produced significant effects that were not paralleled by the action of selective 5-HT$_{2C}$ antagonists (Varty et al. 1999). Thirdly, the nature of the behavioural alterations produced by 5-HT$_{2C}$ antagonism in models of NMDAR hypo-function cannot be easily be reconciled with our findings, as several reports showed that 5-HT$_{2C}$ antagonism does not inhibit, but rather exacerbates, the acute effects of NMDAR antagonists (Higgins et al. 2003; Hutson et al. 2000; O'Neill et al. 1999; Wood et al. 2001). These effects have been linked to an increased dopaminergic tone consequent to the blockade of 5- HT$_{2C}$ receptors in several mesocortical areas, including the medial prefrontal cortex (Gobert et al. 2000). However, as discussed above, our data with the dopamine D_1 antagonists SCH23390 argue against a significant contribution of dopaminergic neurotransmission in the functional effect mapped. Moreover, consistent with the hypothesis of an opposing functional role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Ichikawa and Meltzer 1999), electrophysiology studies demonstrated that 5-HT$_{2C}$ antagonism do not decreases, but rather *increases* the activity of septohippocampal circuit (expressed as theta waves recordings), an effect reversed by selective 5-HT$_{2C}$ agonists (Hajos et al. 2003). In agreement with this, selective 5-HT$_{2C}$ *agonists* have been recently shown to be highly efficacious in inhibiting the behavioural effects of NMDAR antagonism (Marquis et al. 2007).When considered together, these data strongly argue against a significant contribution of 5-HT$_{2C}$ or other spurious receptor systems in the inhibitory effect of M100907 observed in this study.

Based on recent ex vivo receptor occupancy data in the rat frontal cortex, the doses of M100907 used in the present manuscript (1.5 and 0.5 mg/kg i.p.) would be expected to have an estimated receptor occupancy of approximately 100% and 80% at the end 30-min post-PCP time window examined (Knauer et al. 2008). Although the different receptor occupancy alone could explain the lack of response at the low dose, other experimental factors could have contributed to stretch or right-shift the effective dose–efficacy curve. For example, pharmacokinetic studies of M100907 in the rat showed that the compound reaches peak brain concentrations (T_{max}) 32±11 min after its intravenous administration (5 mg/kg; Scott and Heath 1998). Assuming similar parameters following use of intraperitoneal route, the relatively long-time window used to quantify its effect in the present manuscript (30–60 min post-administration) may not be optimally suited to maximise the sensitivity of the measurements. Furthermore, molecular interactions between M100907, PCP and the anaesthetic used (halothane) could also play a significant contribution in vivo. Recent work from (Kapur and Seeman 2002) showed the ability of PCP and ketamine to bind to the high-affinity state of 5-HT$_{2A}$ receptor with micromolar affinity, a value consistent with brain exposure of PCP at the dose used in the present work (Proksch et al. 2000). Moreover, the same authors recently demonstrated that low doses of volatile anaesthetics such as halothane or isoflurane can also bind to (and stimulate) the activity of 5-HT$_{2A}$ receptors (Seeman and Kapur 2003). Thus, simultaneous interactions of PCP and anaesthetic with 5-HT$_{2A}$ receptors may produce significant functional antagonisation or pharmacological displacement of M100907, resulting in the need of higher doses to exert pharmacologically significant effects. Interestingly, a number of studies of 5-HT$_{2A}$ antagonism in PCP models of NMDAR hypo-function showed significant effects only at doses similar to those used in our study (Varty et al. 1999; Habara et al. 2001), whilst studies performed at lower doses do not consistently show effects (Rodefer et al. 2008; Winter et al. 2004; Adams and Moghaddam 2001). This suggests that PCP-5-HT$_{2A}$ receptor interactions might be non-negligible even in absence of anaesthesia. Future experiments using NMDAR antagonist devoid of significant 5-HT$_{2A}$ affinity (i.e. CPP; Lehmann et al. 1987) may be performed to investigate this hypothesis. Nonetheless, it should be emphasised that if these interactions do occur in vivo, they are expected to affect the effectiveness, but not the outcome, of 5-HT$_{2A}$ antagonism in the brain, thus leaving unaltered the functional significance of the effects described in our manuscript.

Whilst the transient state produced by the acute administration of PCP cannot possibly mimic the entire syndrome and course of a multi-factorial disease like schizophrenia, the ability of NMDAR antagonists to produce behavioural effects akin to positive and negative symptoms of the disorder in human volunteers (Krystal et al. 2003; Adler et al. 1999) suggests that the hyper-glutamatergic state produced by these drugs alters neural function in circuits that are relevant for this condition. Importantly, the circuits activated by NMDAR antagonists in rodents and humans as seen with various functional imaging modalities (Deakin et al. 2008; Gozzi et al. 2008c; Langsjo et al. 2003; Vollenweider, personal communication) show a high degree of homology between species and do not appear to be qualitatively affected by the anaesthesia (Gozzi et al. 2008c). Several neuroimaging studies have provided evidence for localised anatomical and functional abnormalities in frontohippocampal areas of schizophrenia patients. Imaging studies of haemodynamic parameters have highlighted increased blood flow and abnormal hippocampal activity at rest and during the performance of memory retrieval tasks (Heckers 2001; Medoff et al. 2001). Similarly, neurometabolic studies in unmedicated schizophrenic patients have highlighted increased tonic frontocortical activity, a feature that has been linked to the sensory flooding, cognitive fragmentation and ego-dissolution seen in both drug-induced and disorder-based psychosis (Parellada et al. 1994; Soyka et al. 2005; Geyer and Vollenweider 2008; Volkow et al. 1986). Thus, the ability of 5-HT_{2A}R antagonism to produce region-selective attenuation of aberrant frontohippocampal states suggests that this pharmacological mechanism might contribute to some of the therapeutic effect of clozapine and other second generation anti-psychotics that possess significant 5-HT_{2A}R affinity (Ichikawa and Meltzer 1999). A few clinical studies have recently addressed the role of selective 5-HT_{2A}R antagonism in schizophrenia patients. In a two multicenter, placebo and haloperidol-controlled studies in USA, M100907 showed statistically significant efficacy on total score versus placebo of positive and negative symptoms (De Paulis 2001; Marder 1999), although the drug was less effective than haloperidol. The effect was not confirmed in a European study involving patients with predominantly negative symptoms, although M100907-treated schizophrenic subjects showed significantly fewer preservative errors in the Wisconsin Card Sorting Test (Roth et al. 2004). A recent placebo-controlled study using a the 5-$HT_{2A}/_{2C}$R antagonist SR46349B produced significant reductions in the positive and negative syndrome scale total and negative scores versus placebo (Meltzer et al. 2004). Likewise, the 5-$HT_{2A}/_{2C}$R antagonist mianserin produced significant improvement in measures of cognitive function (learning, memory and sustained attention) when the drug was tested as add-on therapy in schizophrenic patients (Poyurovsky et al. 2003). Collectively, the limited clinical data available suggest that 5-HT_{2A} antagonism per se may produce mild, but clinically significant antipsychotic effects, involving a moderate improvement of both positive and negative symptoms. This is in agreement with our observation that the 5-HT_{2A}R antagonist M100907, unlike glutamatergic compounds (Gozzi et al. 2008a, b), is unable to entirely suppress the functional cascade produced by PCP in the rat brain, but selectively reduces PCP-induced activation in the frontoseptohippocampal circuit, a key substrate of higher cognitive functions that appears to be tonically hyper-activated in drug-induced and disorder-based psychosis. Consistent findings have been reproduced in pre-clinical behavioural models, where 5-HT_{2A}R receptor antagonists do not consistently antagonise the entire spectrum of behavioural and neurochemical responses produced by NMDAR antagonists in the rat (Large 2007; Adams and Moghaddam 2001) but have been shown to improve frontocortical functions (Mirjana et al. 2004; Winstanley et al. 2003). Of interest, glucose metabolism studies using positron emission tomography highlighted a tight correlation between depression of corticohippocampal activity and antipsychotic action elicited by a single dose of the atypical anti-psychotics risperidone (Liddle et al. 2000). Whilst multiple receptor contributions are likely to contribute to this effect, this finding is important as it suggests that the circuital mechanism identified in our study may be of clinical significance.

In conclusion, we have shown that 5-HT_{2A}R antagonist reduces PCP-induced activation in discrete brain regions, including frontal cortex, septum and ventral–hippocampal areas. These results are consistent with pre-clinical studies highlighting a key role of 5-HT_{2A}R in modulating glutamate-mediated cognitive performance in the rodent prefrontal cortex and extend those findings by highlighting a role of the frontoseptohippocampal circuit as an integrated substrate of the action of $5HT_{2A}$ antagonism in the living brain. Collectively, pre-clinical and clinical research provide converging evidence that 5-HT_{2A}R antagonism can exert a region-selective modulation of frontoseptohippocampal activity that might be of clinical benefit when the circuit is functionally hyperactive.

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All in vivo studies were conducted in accordance with the Italian laws (DL 116, 1992 Ministero della Sanità, Roma). Animal research protocols were also reviewed and consented to by the GSK animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985).