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Chronic methamphetamine self-administration dysregulates 5-HT_{2A} and mGlu₂ receptor expression in the rat prefrontal and perirhinal cortex: comparison to chronic phencyclidine and MK-801.

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Abstract

Chronic methamphetamine (meth) abuse often turns into a compulsive drug-taking disorder accompanied by persistent cognitive deficits and re-occurring psychosis. Possible common neurobiological substrates underlying meth-induced deficits and schizophrenia remain poorly understood. Serotonin 2A (5-HT_{2A}) and metabotropic glutamate 2 (mGlu₂) receptors coregulate psychosis-like behaviors and cognitive function in animals. Therefore, in the present study we examined the effects of chronic exposure to three different drugs known to produce persistent deficits in sensorimotor gating and cognition [meth, phencyclidine (PCP) and MK-801] on the expression of 5-HT_{2A} and mGlu₂ within the rat medial prefrontal cortex (PFC), dorsal hippocampus (dHPC) and perirhinal cortex (PRh). Adult male rats underwent 14 days of: (a) meth self-administration (6 hr/day), (b) phencyclidine (PCP; 5 mg/kg, twice/day) administration, or (c) MK-801 (0.3 mg/kg, twice/day) administration. Seven days after the discontinuation of drug administration, tissues of interest were collected for protein expression analysis. We found that despite different pharmacological mechanism of action, chronic meth, PCP, and MK-801 similarly

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Author contributions

MS, VBV and JS designed the study; MS, VBV and JS performed the behavioral experiments; MZ performed the 5-HT_{2A} receptor deglycosylation study; MS and PH analyzed the protein data and wrote the initial version of the manuscript with subsequent contribution from all other authors. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

dysregulated 5-HT_{2A} and mGlu₂, as indicated by an increase in the 5-HT_{2A}/mGlu₂ expression ratio in the mPFC (all three tested drugs), PRh (meth and PCP), and dHPC (MK-801 only). Complementary changes in G-protein expression (increase in G α_q and decrease in G α_i were also observed in the mPFC of meth animals. Finally, we found that 5-HT_{2A}/mGlu₂ cooperation can be mediated in part by the formation of the receptor heteromer in some, but not all cortical regions. In summary, these data suggest that a shift towards increased availability (and G-protein coupling) of cortical 5-HT_{2A} vs. mGlu₂ receptors may represent a common neurobiological mechanism underlying the emergence of psychosis and cognitive deficits observed in subjects with meth use disorder and schizophrenia.

Keywords

Methamphetamine; Phencyclidine; MK-801; Heterocomplex; mGlu₂; 5-HT_{2A}

1. Introduction

Methamphetamine (meth) is the most commonly abused synthetic drug worldwide (United Nations Office on Drugs and Crime, 2016). A significant proportion of meth users develop a pattern of uncontrollable, compulsive drug use that can be classified as meth use disorder (MUD, Paulus et al., 2017). The treatment and recovery of individuals with MUD is complicated by high rates of relapse, and persistent meth-induced psychopathologies. In this respect, memory impairments are among the most pronounced and persistent cognitive complications in MUD (Scott et al., 2007). A number of clinical studies reported deficits in working and episodic memory that can persist weeks or months into the meth-free abstinence (Bechara and Martin, 2004; Gonzalez et al., 2007; Morgan et al., 2012; Simon et al., 2004; Woods et al., 2005). Another pervasive complication in MUD that affects almost 40% of meth users is the emergence of psychotic symptoms (Grant et al., 2012). Psychotic episodes can be triggered acutely by recent meth use, or spontaneously re-appear even after prolonged periods of abstinence (Glasner-Edwards and Mooney, 2014; McKetin et al., 2006; Zweben et al., 2004). While meth can precipitate psychotic symptoms in individuals with no history of a primary psychotic disorder, it can also exacerbate psychotic symptoms in schizophrenia patients, hinting at potential commonalities in the underlying neurobiology of these two conditions (Chen et al., 2003; McKetin et al., 2006). Progress in identifying neurobiological substrates underlying cognitive complications and psychosis in MUD is critical, as they can collectively contribute to poor treatment outcomes in MUD (Paulus et al., 2017).

A well-accepted animal model that recapitulates many complications of MUD is the self-administration paradigm, in which animals are allowed to have extended access to self-administered meth for a period of two or more weeks. Specifically, studies by our lab and others have shown that extended-access meth self-administration results in: escalated meth use (Rogers et al., 2008), increased reinstatement of meth-seeking (Rogers et al., 2008; Schwendt et al., 2009), reduced cognitive flexibility (Cox et al., 2016; Parsegian et al., 2011), as well as impaired episodic (object recognition) memory (Reichel et al., 2012a; Schwendt et al., 2011) and working memory (Recinto et al., 2012). Extended access to self-

administered meth also produces deficits in sensorimotor gating akin to deficits observed in schizophrenia patients (Hadamitzky et al., 2011), or across various animal models of schizophrenia (Braff, 1990).

Both cognitive impairments and psychotic symptoms produced by chronic meth are thought to arise from the dysregulation of several neurotransmitter systems within the frontostriatal circuit. Targeting aberrant dopamine transmission using D2 receptor antagonists is a standard-approach therapy of schizophrenia and more recently, also meth psychosis (Samiei et al., 2016; Verachai et al., 2014). However, D2 receptor antagonists do not typically improve cognitive dysfunction, and in some cases can even impair learning and memory performance in humans (Lustig and Meck, 2005; Saeedi et al., 2006; Xu, 2017) and animals (Abdel-Salam et al., 2012; Hou et al., 2006; Hutchings et al., 2013). Alternatively, recent evidence suggests that simultaneous targeting of serotonin and glutamate neurotransmission, namely 5-HT2A and mGlu2(3) receptors, might produce an anti-psychotic effect comparable to that of D2 receptor antagonists (González-Maeso et al., 2008; Moreno et al., 2016). The validity of this approach is based on findings that demonstrate the: *a*) possible association of mGlu2(3) and 5-HT2A gene polymorphisms with schizophrenia and/or meth-induced psychosis (e.g. Harrison et al., 2008; Tsunoka et al., 2010), *b*) synergistic antipsychotic properties of 5-HT2A antagonists and mGlu2/3 receptor agonists, *c*) co-localization of both receptors across several cortical brain regions, and *d*) close physical (heteroreceptor complex) and functional (signaling cross-talk) interactions between 5-HT2A and mGlu2 receptors (for review see: Wischhof and Koch, 2016). Moreover, targeting 5-HT2A and/or mGlu2 receptors has been shown to improve cognitive function across a variety of psychopathologies, including MUD and schizophrenia (Wischhof and Koch, 2016; Zhang and Stackman, 2015). As such, enhancing mGlu2 receptor function, or inhibiting 5-HT2A receptor activity ameliorated cognitive deficits in pharmacological models of schizophrenia based on NMDA receptor inhibition by drugs like MK-801 (also known as dizocilpine), or phencyclidine (PCP; Griebel et al., 2016; Snigdha et al., 2010). Finally, our data demonstrate that administration of mGlu2 positive allosteric modulator (PAM) reverses object recognition memory deficits in rats with a history of chronic meth self-administration (Schwendt et al., 2011).

While the pharmacological and gene-linkage studies suggest that the mGlu2 and 5-HT2A receptors play a role in manifestation of common MUD and schizophrenia symptoms, (co)regulation of these receptors in either psychopathology is inadequately understood. Postmortem analysis of brain tissue from schizophrenia patients provided contradictory evidence regarding the changes in mGlu2(3) and/or 5-HT2A expression in the forebrain (Ghose et al., 2008; González-Maeso et al., 2008; Muguruza et al., 2016; Rasmussen et al., 2010), while changes in either receptor expression have not been evaluated in MUD. Only a single study analyzed co-regulation of mGlu2 and 5-HT2A following repeated non-contingent administration of meth in mice, showing increased 5-HT2A and decreased mGlu2 receptor expression in the mPFC (Chiu et al., 2014). Despite similarities in the clinical manifestation of MUD and schizophrenia, to this date no studies have concurrently evaluated mGlu2 and 5-HT2A receptor expression in commonly used animal models of these two psychopathologies. The current study aimed to address this knowledge gap by *first*, validating the tools (antibodies) suitable for the detection of mGlu2 and 5-HT2A

proteins in rodent brain tissue. *Second*, we evaluated the possibility that functional cross-talk occurs via direct physical interaction between these two receptors in the rat brain tissue, and *third*, we analyzed protein expression of said receptors and their partner G-proteins following the chronic exposure to meth or NMDA receptor antagonists (MK-801 and PCP) within the rat brain regions implicated in psychosis-like behavior (Lewis and Sweet, 2009) and episodic (object recognition) memory, as characterized by: (Warburton and Brown, 2015, 2010). *Finally*, we focused on the analysis of possible mGlu2-5-HT2A co-regulation at a withdrawal time (7 days), when many of the post-meth, -PCP and -MK-801 behavioral deficits have been previously observed by our laboratory, as well as others (see above).

2. Materials and Methods

2.1. Animals

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA, USA) and male Wistar rats (Velaz, Kolec u Kladna, Czech Republic) weighing 250-300 g at the time of delivery were individually housed in a temperature- and humidity-controlled vivarium on a reversed 12 h light-dark cycle. Rats received *ad libitum* water and standard rat chow (Harlan, Indianapolis, IN, USA (Long-Evans) or Velaz, Czech Republic (Wistar) unless noted otherwise. Rats that were assigned to the self-administration study received 25 g of chow daily (to maintain their weight at ~85 % of the free-feeding weight). After the completion of the self-administration regimen, rats were switched to *ad libitum* food access. Mouse cortical tissue used for the validation of 5-HT2A and mGlu2 antibodies was obtained from adult male mice with the following background: 129S6/Sv mice (control tissue), *Htr2a*^{-/-} and *GRM2*^{-/-} mice backcrossed for at least 10 generations onto the 129S6/Sv background (5-HT2A and mGlu2 knock-out tissue respectively). For more information see: (González-Maeso et al., 2003; Moreno et al., 2011). Adult male C57BL/6 mice were used to prepare cortical lysates for the 5-HT2A deglycosylation experiment.

All animal procedures were approved by the Institutional Animal Care and Use Committees of the Medical University of South Carolina, or the Expert Committee for Protection of Experimental Animals of the Prague Psychiatric Center and performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) or with the Guidelines of the European Union Council (86/609/EU).

2.2. Drugs

(+)-Methamphetamine hydrochloride (meth) was obtained from Sigma Aldrich (St. Louis, MO, USA), dissolved in saline (0.9% NaCl) to 0.4 mg/ml concentration and used for intravenous self-administration. MK-801 ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H* dibenzo [*a,d*] cyclohepten-5,10-imine hydrogen maleate; Sigma-Aldrich, Czech Republic), was dissolved in saline and administered intraperitoneally (IP) at 0.3 mg/kg dose (1 ml/kg b.w.). Phencyclidine (1-(1-Phenylcyclohexyl) piperidine; Sigma-Aldrich, Czech Republic) was dissolved in saline and administered IP at 5 mg/kg dose (1 ml/kg b.w.). The control animals self-administered saline (meth rat controls) or received equivalent volumes of IP saline (MK-801 and PCP controls). The doses of each drug were based on previously published

studies (Jentsch et al., 1997; Li et al., 2011; Schwendt et al., 2012; Stefani and Moghaddam, 2002; Vales et al., 2006).

2.3. Immunohistochemistry

For the immunohistochemical analysis of 5-HT_{2A} receptor expression, rats were anesthetized and transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS) (in mM: NaCl 137, KCl 2.7, Na₂HPO₄ 4.3, KH₂PO₄ 1.47, pH 7.4). Brains were removed and post-fixed for 4 h and saturated in 30% sucrose/PBS solution. After embedding and freezing, 30µm sections (spanning the rat prefrontal cortex) were cut on a freezing microtome and stored in PBS. Next, the sections were washed in PBS, permeabilized in 0.3% Triton-X-100/1× PBS and blocked in 0.3% Triton-X-100/1× PBS/10% normal goat serum (NGS)/2% bovine serum albumin (BSA) for 2 hours. To detect 5-HT_{2A} receptor signal, PFC sections were incubated with affinity purified rabbit anti-5-HT_{2A} receptor antibody (#24288, ImmunoStar, Hudson, WI) diluted to 1:500 in 0.3% Triton-X-100/1× PBS/2% NGS/2% BSA as previously described (Brownfield et al., 1998). After washing in PBS, sections were incubated with a secondary anti-rabbit antibody conjugated to Alexa Fluor 594 (Life Technologies, Carlsbad, CA), washed again, mounted onto Superfrost slides (Thermo-Fisher, Waltham, MA) and coverslipped with an antifade medium (ProLongTM Gold, Life Technologies, Carlsbad, CA). The staining was visualized and photographed using a BX-51 Olympus microscope equipped with a high-resolution digital camera.

2.4. Protein deglycosylation and analysis

Mouse (C57BL/6) cortical lysate (containing 80 µg of protein) was treated with 1U of Peptide-N-glycosidase F (PNGase F; Roche, Mannheim, Germany) in incubation buffer (39 mM sodium phosphate buffer pH 7.2, 8 mM EDTA, 0.8% NP-40, 0.1% SDS, 1% 2-mercaptoethanol) containing 1 mM PMSF, 7.5 µg/ml aprotinin, 38 mM NaF and protease (cOmplete) and phosphatase (PhosSTOP) inhibitor cocktails (Roche Applied Science, Mannheim, Germany) at 37°C for 3 h. Mouse cortical lysate (80 µg of protein) without PNGaseF was used as a control. The mouse samples and, for a comparison, the lysate from the rat cerebral cortex (40 µg of the protein) were mixed with protein loading buffer (Roti-Load 1, Carl Roth, Karlsruhe, Germany), quickly chilled on ice and immediately separated on a 10% SDS–polyacrylamide gel and transferred onto a PVDF membrane. Next, the membrane was blocked for 1h at room temperature with Odyssey blocking buffer (LI-COR Biotechnology, Bad Homburg, Germany) and incubated for 21h at 4°C with a solution of cold rabbit anti-5-HT_{2A} receptor antibody (#24288; ImmunoStar, Hudson, WI, USA) diluted at 1:500 in Tris-buffered saline solution pH 7.5/0.05% Tween 20 (TBS-T). After washing with TBS-T, the membrane was incubated for 1h at room temperature with secondary donkey anti-rabbit antibody (800CW; LI-COR Biotechnology) diluted at 1:10000 in TBS-T. After washing with TBS-T, the membrane was washed for 10 min at room temperature with TBS and then the detection was performed using Odyssey imaging system (Odyssey CLx; LI-COR Biotechnology).

2.5. Co-immunoprecipitation and immunoblotting

For the co-immunoprecipitation, rats were euthanized by rapid decapitation. Brains were extracted and 2 mm-thick forebrain coronal slices were harvested using a chilled rat brain matrix (ASI instruments, Warren, MI, USA). Next, mPFC, dHPC and PRh were hand-dissected from these acute brain slices (Figure 1) and cut into 250µm thick sections using McIlwain tissue chopper (Ted Pella, Redding, CA, USA). Tissue proteins were immediately crosslinked by incubating the sections with 2.5mM dithiobis-(succinimidyl propionate) (or DSP) in PBS for 2hrs. Crosslinking conditions, such as incubation time and DSP concentration, were based on the manufacturer's recommendations (#22585, Thermo-Fisher) and series of pilot slice experiments (data not shown). Cross-linking reaction was terminated with 50mM Tris-HCl buffer, pH = 7.5. Tissue sections were transferred to a RIPA lysis buffer (Thermo-Fisher) containing protease (cOmplete Mini; Roche Applied Science, Mannheim, Germany) and phosphatase inhibitors (Halt, Thermo-Fisher) and rapidly homogenized by sonication. Following the solubilization in ice-cold RIPA buffer, insoluble debris was removed by centrifugation and protein concentration in each sample was determined using the BCA assay. Equivalent amount of the lysate protein (500 µg) was used for immunoprecipitation experiments with either rabbit polyclonal anti-mGlu2 antibody (#07-261-I; EMD Millipore, Billerica, MA, USA), or with rabbit anti-PLCα.1 antibody (#sc-9050, Santa Cruz Biotechnology; negative control). Antibody-protein complexes were captured with protein-G magnetic beads (SureBeads; Bio-Rad Laboratories, Hercules, CA, USA) according to manufacturer's instructions. Bound proteins were then stripped off the beads by heating in the high-stringency elution buffer (Laemmli sample buffer; Bio-Rad). Immunoprecipitated proteins were analyzed by immunoblotting as described below.

For immunoblotting analysis, total protein was extracted from the mouse frontal cortex, or rat mPFC, dHPC and PRh tissues as previously described (Schwendt et al., 2006) with few modifications. Protein samples were lysed in RIPA buffer and analyzed under low stringency conditions (heat < 37 °C, dithiothreitol < 20mM) to preserve native receptor oligomers (Copani et al., 2000). Equal protein amounts (15-25 µg) were separated by SDS-PAGE (4–15% polyacrylamide) and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked for 1 h in 5% milk/Tris-buffered saline and probed overnight at 4 °C with a primary antibody diluted in 5% milk/Tris-buffered saline with 0.1% Tween 20. The following primary 5-HT_{2A} antibodies were used: goat anti-5-HT_{2A} (1:500, #sc-15074), goat anti-5-HT_{2A} (1:500, #sc-15073), goat anti-5-HT_{2A} (1:500, #sc-32538) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); rabbit anti-5-HT_{2A} receptor (1:2500, #Ab16028), rabbit anti-5-HT_{2A} receptor (1:500, #Ab173545) (Abcam Biochemical, Cambridge, MA, USA); rabbit anti-5-HT_{2A} (1:500, #LS-C159876; LifeSpan BioSciences, Seattle, WA, USA) and rabbit anti-5-HT_{2A} receptor (1:500, #24288; ImmunoStar, Hudson, WI, USA). For mGlu2 detection, rabbit anti-mGlu2 (1:1500, #07-261-I; EMD Millipore, Billerica, MA, USA) was used. Tissue G-proteins were detected using the following antibodies: mouse anti-Gα_i (1:10000, #26003; NewEast Biosciences, King of Prussia, PA, USA), rabbit anti-Gα_o (#sc-387; 1:25000; Santa Cruz Biotechnology), rabbit anti-Gα_{q/11α} (1:5000, #AB1643; EMD Millipore (Billerica, MA, USA). After the incubation with an appropriate HRP-conjugated secondary antisera (Jackson Immuno Research, West Grove, PA), immunoreactive bands on the membranes were detected by ECL+ chemiluminescence

reagents on a high performance chemiluminescence film (GE Healthcare, Piscataway, NJ). Equal loading and transfer of proteins were confirmed by stripping and reprobing of the same membranes for the housekeeping protein calnexin (1:20,000, #ADI-SPA-860, Enzo Life Sciences, Farmingdale, NY, USA). The integrated band density of each protein sample was measured using Gel-Pro Analyzer software (Media Cybernetics, Rockville, MD, USA).

2.6. Chronic methamphetamine self-administration and withdrawal.

Fifteen Long-Evans rats were first surgically implanted with jugular vein catheters as described previously (Knackstedt et al., 2014). Following at least 5 days of recovery from surgery, rats were randomly assigned to either meth or yoked-saline groups and underwent meth self-administration. Self-administration procedures were conducted in standard operant conditioning chambers (30×20×20 cm, Med Associates, East Fairfield, VT) housed inside sound-attenuating cubicles containing a fan. The chambers were equipped with a house light, two retractable levers, two stimulus lights, and a tone generator. As shown in Figure 3A, animals were first trained to self-administer meth (Sigma, St. Louis, MO) in 7 daily 1 hr-long sessions, followed by 14 days of extended (6-hr) access to intravenous (IV) meth. During each session, a response on the active lever resulted in activation of the pump for a 2-sec meth infusion (20 µg/50 µl bolus infusion) and presentation of a 5-sec tone (78 dB, 4.5 kHz) and a white stimulus light over the active lever, followed by a 20-sec time out. Yoked controls received a 50 µl bolus of saline when the matched subject received the meth infusion. During self-administration, catheter patency was periodically verified with IV methohexital sodium (10 mg/ml dissolved in 0.9% physiological saline; Eli Lilly, Indianapolis, IN, USA). Out of the 15 rats that were implanted with jugular vein catheters, three were removed from the study during self-administration due to catheter patency issues, or other health complications. Following self-administration, rats underwent 7 days of withdrawal in their home cage with daily handling. This experimental design was based on our previous studies indicating that meth-induced behavioral and neurochemical changes can be detected at this exact withdrawal interval (Reichel et al., 2012b; Scofield et al., 2015).

2.7. Chronic phencyclidine, MK-801 administration and withdrawal.

Thirty-two Wistar rats were given IP injections (1.0 ml/kg) of saline, PCP (5 mg/kg) or MK-801 (0.3 mg/kg) twice a day (approximately 08:30 and 16:30 h) for a period of 14 days, followed by 7 days of withdrawal. At the end of the withdrawal period, animals were euthanized and brain tissues collected. Two brains were lost during the storage and shipping therefore they could not be included in the tissue analysis. Doses of PCP and MK-801 used in this study were selected on the basis of their comparable NMDA receptor inhibitory potency (Boireau et al., 1996). The drug administration regimen was designed to correspond with the length of extended meth access and was also based on the previously published studies (for review see: Mouri et al., 2007). The length of the withdrawal period was chosen to correspond with meth experiment and to mimic the time-course of PCP- and MK-801-induced behavioral deficits (Li et al., 2011; Santini et al., 2013; Snigdha et al., 2010). Non-contingent administration of PCP and MK-801 was utilized, as achieving stable self-administration rates of PCP or MK-801 has proven to be difficult in rodents [e.g. (Marquis et al., 1989)].

2.8. Statistical analysis

A Student's *t*-test was used to compare meth intake (mg/kg) averaged over the first three (d1-d3) vs. the final three (d12-d14) days of extended access. Immunoblotting data, represented by integrated density of individual bands, were normalized to the density of calnexin immunoreactivity within the same sample, analyzed by a Student's *t*-test, and expressed as the percentage of the saline-treated animals. GraphPad Prism v7.0 software (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis of all data. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Validation of antibodies for the detection of 5-HT_{2A} receptors in rodent brain tissue.

Since the specificity of several antibodies used for 5-HT_{2A} receptor immunodetection have been recently challenged (e.g. Weber and Andrade, 2010), we decided first to validate the most frequently used commercial anti-5-HT_{2A} antibodies. To unambiguously determine antibody specificity, immunoreactivity of all antibodies was tested in cortical lysates obtained from rats, wild type and knockout (KO) mice (see Materials and Methods section for the complete list of antibodies tested). As shown in Figure 2A, only one anti-5-HT_{2A} receptor antibody (Imunostar, cat. no. 24288) detected a band (~75 kDa) in rat and mouse cortical lysates that was not present in the lysates from *5-HT_{2A} receptor* KO mice. This band is heavier than the predicted molecular size of the rat 5-HT_{2A} receptor (~52 kDa), because it corresponds to an N-glycosylated form of the receptor (Maginnis et al., 2010). To confirm this, we treated mouse cortical lysate with PNGase F (enzyme that cleaves N-linked glycans from proteins) and observed a corresponding shift in the electrophoretic mobility of 5-HT_{2A} receptor from ~75 to ~52 kDa (Figure 2B). We have further validated 5-HT_{2A} (24288) antibody by preadsorption with its respective immunizing peptide.

Immunohistochemical analysis of the rat mPFC using this antibody revealed laminar staining [corresponding to a typical distribution of 5-HT_{2A} receptors in this brain region (Hamada et al., 1998; Weber and Andrade, 2010)] that was completely absent in tissues incubated with 5-HT_{2A} (24288) antiserum preadsorbed with its immunogenic peptide (Figure 2C). In a separate immunoblotting experiment, we observed a loss of the 75 kDa 5-HT_{2A} band in cortical lysates that were incubated with the preadsorbed 5-HT_{2A} (#24288) antibody (Figure 2C).

3.2. Co-immunoprecipitation of mGlu2 and 5-HT_{2A} receptors in the rat brain.

Recent evidence suggests that behavioral and functional interaction between mGlu2 and 5-HT_{2A} is mediated in part by a direct physical interaction between these two receptors, as detected in the mouse and human cortical tissue using co-immunoprecipitation (Fribourg et al., 2011; González-Maeso et al., 2008). Here we analyzed the presence of 5-HT_{2A} signal in a protein fraction prepared from the three preselected brain regions (mPFC, dHPC and PRh) and immunoprecipitated with anti-mGlu2 antibody. Specificity of the mGlu2 antibody was first validated in cortical samples from mGlu2 KO mice. Figure 3A shows that under low stringent conditions, mGlu2 receptors in the cortex can be detected almost exclusively as dimers (~220 kDa) that are thought to represent the active form of the receptor (El Moustaine et al., 2012). mGlu2 dimer was not detected in the mGlu2 KO samples,

confirming the specificity of the mGlu2 antibody (Figure 3A, also see: Sanger et al., 2013; Wood et al., 2017). In the co-immunoprecipitation experiment, mGlu2/5-HT2A receptor interaction was detected in the PRh samples, but not in the samples prepared from the mPFC (both, Figure 3B) or dHPC (data not shown). A lack of 5-HT2A signal in PRh samples precipitated with an unrelated antibody (IP-*Ab; Figure 3B) confirms the specificity of the mGlu2/5-HT2A receptor complex detection.

3.3. Extended access to self-administered meth: behavior and protein expression changes.

Figure 4A depicts the timeline of the meth self-administration experiment. Rats assigned to the meth group rapidly acquired self-administration and displayed a clear discrimination between active and passive lever responses throughout the whole study (300 % and 900 % active lever discrimination during the limited and extended access period respectively; Figure 4B). Yoked-saline rats indiscriminately displayed low levels of responding on both levers. During the 14 days of extended access to meth, escalation of meth intake was observed (Figure 4C). Specifically, the average daily meth intake during the last three days (days 12-14) of extended access to meth was significantly higher than on the first three days (days 1-3; $t(4) = 2.93$, $p < 0.05$). Cumulative meth intake ranged from 52.4–76.3 mg/kg and was comparable to meth intake observed in our previous studies (Reichel et al., 2012b; Schwendt et al., 2012). There was no difference in weight gain between yoked-saline and meth groups (data not shown).

Next, we analyzed protein levels of mGlu2 and 5-HT2A receptors (and their cognate G-proteins) in the mPFC, dHPC and PRh following 7 days of withdrawal (Figure 5). This interval was based on the previous studies showing that extended access to self-administered meth and 7 days of withdrawal alters synaptic plasticity and mGlu expression in the PRh (Reichel et al., 2011; Scofield et al., 2015), produces PRh/dHPC-dependent memory deficits (Reichel et al., 2011), dysregulates 5-HT levels (Brennan et al., 2010) and glutamate neurophysiology in the PFC (Mishra et al., 2016). As the functional (and behavioral) outcome of the 5-HT2A and mGlu2 interaction depends on the relative amount of each receptor (Baki et al., 2013), the 5-HT2A/mGlu2 protein expression ratio was also calculated. Extended access to meth upregulated 5-HT2A protein levels in the mPFC [$t(9) = 2.96$, $p < 0.05$] and the PRh [$t(9) = 2.61$, $p < 0.05$] compared to yoked-saline rats. While mGlu2 expression was not altered by meth, the 5-HT2A/mGlu2 expression ratio was increased in both the mPFC [$t(9) = 3.41$, $p < 0.01$] and PRh [$t(9) = 2.91$, $p < 0.05$] regions of the cortex of meth rats. With regard to G-proteins, only changes in $G\alpha_q$ protein subunit were detected in this experiment. Extended access to self-administered meth increased $G\alpha_q$ protein levels in the mPFC [$t(9) = 2.46$, $p < 0.05$], while reducing it in the PRh region [$t(9) = 3.41$, $p < 0.01$].

3.4. Chronic PCP administration and protein expression changes.

Figure 6A depicts the timeline of the PCP experiment. Chronic PCP administration (5mg/kg IP, twice a day for 14 days) did not affect body weight gain when compared to the saline group (data not shown). Seven days after the discontinuation of PCP administration, the 5-HT2A/mGlu2 expression ratio was increased in the mPFC [$t(14) = 2.31$, $p < 0.05$] and the PRh [$t(14) = 4.66$, $p < 0.001$] regions, but not in the dHPC. PCP-induced changes in

individual proteins were limited to a decrease in mGlu2 levels in the PRh [$t(14) = 4.15$, $p < 0.001$], with no other proteins altered in any of the brain regions analyzed (Figure 6 and Table 1).

3.5. Chronic MK-801 administration and protein expression changes.

Figure 7A depicts the timeline of the MK-801 experiment. Chronic MK-801 administration (0.3 mg/kg IP, twice a day for 14 days) did not affect body weight when compared to the saline group (data not shown). Seven days after the discontinuation of MK-801 administration, the 5-HT2A/mGlu2 expression ratio was increased in the mPFC [$t(14) = 2.25$, $p < 0.05$] and dHPC [$t(14) = 2.54$, $p < 0.05$], but not in the PRh. No changes in the levels of G-protein subunits measured were detected in any brain region analyzed (Table 2).

4. Discussion

The present study characterized co-regulation of 5-HT2A and mGlu2 receptor expression within the rat mPFC, dHPC and PRh cortex following a chronic exposure to drugs known to induce psychotic symptoms and cognitive impairment in humans. Two weeks of extended access to self-administered meth (or administration of PCP or MK-801 over the same period) increased the ratio of 5-HT2A/mGlu2 protein expression in the mPFC. Chronic meth and PCP also increased the 5-HT2A/mGlu2 expression ratio in the PRh, while MK-801 produced the similar change in the dHPC. The rationale behind the parallel analysis of 5-HT2A and mGlu2 receptor expression is based on the evidence of close functional cooperation between these two receptors in triggering psychosis or maintaining cognitive function. This cooperation could be mediated in part by a formation of the 5-HT2A-mGlu2 heterocomplex in the PRh. Given the known psychosis-like effects of 5-HT2A receptor activation and cognitive deficits associated with the loss of mGlu2 function, an increase in the 5-HT2A/mGlu2 expression ratio offers a novel neural mechanism for targeting the pro-psychotic and cognitive-impairing effects of chronic meth, PCP or MK-801 exposure.

Recent reports suggest that alarmingly low reproducibility of published protein analysis data is related to poor specificity of thousands of commercially-available antibodies, often caused by the lack of thorough antibody validation (Berglund et al., 2008; Bradbury and Plückthun, 2015). Since 5-HT2A receptor antibodies have been plagued by specificity issues, a critical part of this study was to identify antibody/antibodies suitable for the detection of this receptor in the rat brain tissue. To that end, we conducted a comparative validation of six commercially-available 5-HT2A antibodies (Figure 2). Surprisingly, only one of the tested antibodies (anti-5-HT2A #24288, currently distributed by Immunostar), was found to be specific. First, this antibody detected a single band corresponding to the molecular weight of the mature (glycosylated) rat 5-HT2A receptors that was not present in cortical samples obtained from the *5-HT2A receptor* KO mice. Next, enzymatic deglycosylation increased electrophoretic mobility of the 5-HT2A receptors to yield a band with a molecular weight corresponding to the known rat 5-HT2A receptor amino-acid sequence. And finally, this antibody produced a characteristic laminar immunostaining signal previously seen in the mPFC of transgenic animals expressing a fluorescent marker under the control of the 5-HT2A receptor promoter (Weber and Andrade, 2010).

Next, using this 5-HT_{2A} antibody and the validated mGlu₂ antibody (Figure 3; and Sanger et al., 2013; Wood et al., 2017), we sought to determine whether these two receptors physically interact in the rat brain. As described below, the rationale for investigating 5-HT_{2A}-mGlu₂ receptor-receptor interaction is the proposed role of this receptor heterocomplex in psychosis and in mediating the effects of hallucinogens, including 5-HT_{2A} receptor agonists (González-Maeso et al., 2008; Moreno et al., 2011). Previously, 5-HT_{2A}-mGlu₂ heterocomplex was detected in cell lines overexpressing both receptors, as well as in the mouse and human cortical tissue (Fribourg et al., 2011; González-Maeso et al., 2008). Here, we report for the first time that 5-HT_{2A} co-immunoprecipitates with mGlu₂ in the rat brain tissue. Interestingly, under the conditions used in this study, 5-HT_{2A}-mGlu₂ receptor-receptor interaction was only detected in the PRh tissue, but not in the PFC or dHPC. There are several possible explanations for this observation. First, unlike previous studies (Fribourg et al., 2011; González-Maeso et al., 2008), we investigated 5-HT_{2A}-mGlu₂ interaction in the rat, not mouse, or human brain tissue. We utilized a cross-linking approach to stabilize protein-protein interactions, while other studies have not. While in the majority of cases, protein cross-linking increases the likelihood of heterocomplex detection, in some cases, crosslinker can alter antibody-antigen binding hindering protein detection (Corgiat et al., 2014). Secondly, there may be regional differences in the presence of 5-HT_{2A}-mGlu₂ heterocomplexes. Previously, 5-HT_{2A}-mGlu₂ heterocomplexes were reported in a broadly-defined area of the frontal cortex, but were not evaluated specifically in the PFC. Further, our finding does not contradict previously observed functional interactions between cortical 5-HT_{2A} and mGlu₂ receptors, as they might be mediated indirectly, via integration of downstream cellular signaling (Delille et al., 2013). In the HPC, the expression of 5-HT_{2A} receptors is relatively low (Hamada et al., 1998; Wright et al., 1995), also evidenced by the difficulty to detect 5-HT_{2A} by immunoblotting in our experiments, which might have limited our ability to detect heterocomplexes.

Regardless of the exact mechanism mediating the 5-HT_{2A}-mGlu₂ interaction, preclinical and clinical evidence suggest that communication between these two receptors plays a role in pathophysiology of psychosis and cognitive dysfunction (as reviewed by: Wischhof and Koch, 2016). However, almost all the published studies investigated 5-HT_{2A}-mGlu₂ interaction in relation to schizophrenia, but not MUD. This is a critical knowledge gap, as meth-dependent subjects frequently develop psychotic symptoms and cognitive deficits (such as decline in memory function), akin to schizophrenia (Grant et al., 2012).

Surprisingly, there is a relative paucity of information on: what is the identity of potential common neural substrates (e.g. genes, proteins), and how these substrates are dysregulated in MUD vs. schizophrenia patients or in animal models of either disorder. While two previous studies analyzed the profile of cortical gene expression after acute meth or PCP administration (Ouchi et al., 2005, 2004), no published studies compared the expression of candidate susceptibility proteins such as 5-HT_{2A} and mGlu₂ in validated animal models of MUD, and/or schizophrenia. Therefore, the main (and novel) finding of the current study is that chronic meth, PCP or MK-801 produce analogous increases in the 5-HT_{2A}/mGlu₂ protein expression ratio in the PFC (and to a lesser extent in the PRh). In the PFC and PRh of meth animals, altered 5-HT_{2A}/mGlu₂ expression ratio was primarily driven by an increase in 5-HT_{2A} receptors themselves. Similarly, Chiu et al. (2014) reported that non-

contingent meth administration upregulates 5-HT_{2A} receptor expression in the mouse frontal cortex, as well as cellular and behavioral responses to 5-HT_{2A} receptor agonists. It is possible that the mechanism driving upregulation of cortical 5-HT_{2A} receptor is a compensatory response to a serotonin depletion (Heal et al., 1985), as low serotonin levels have been reported in the frontal cortex of rodents following a chronic meth exposure (Brennan et al., 2010; McFadden et al., 2013) and in the forebrain of abstinent meth abusers (Wilson et al., 1996). It is tempting to speculate that meth-induced upregulation of cortical 5-HT_{2A} receptors contributes to an increased likelihood of psychotic episodes in meth-dependent users (Ujike and Sato, 2004). In addition to the change in receptor numbers, upregulation of their cognate G-proteins might also facilitate receptor function. Since 5-HT_{2A} couples through G α_q proteins (Roth et al., 1998), while mGlu2 uses G $\alpha_{i/o}$ (Kammermeier et al., 2003), increased availability of G α_q (but not G $\alpha_{i/o}$) proteins observed in the PFC and PRh of meth animals (Figure 5) can further facilitate cellular activity of 5-HT_{2A} receptors. Indeed, Chiu and colleagues (2014) observed augmented 5-HT_{2A} activity following the non-contingent repeated meth administration, though G-protein expression was not analyzed in that study.

Unlike meth, PCP and MK-801 are primarily non-competitive NMDA receptor antagonists. Still, acute administration of all three investigated drugs has been shown to increase synaptic levels of dopamine, serotonin and glutamate in the PFC; albeit via distinct pharmacological mechanisms (Adams and Moghaddam, 1998; Amargós-Bosch et al., 2006; Halpin et al., 2014; Hondo et al., 1994; Jentsch et al., 1998; López-Gil et al., 2009; Martin et al., 1998; Schmidt and Fadaye, 1996; Zuo et al., 2006). For example, meth increases serotonin in the PFC via canonical mechanisms involving 5-HT transporters, while acute serotonergic effects of MK-801 and PCP depend on direct or indirect activation of local 5-HT_{2A} receptors (Kapur and Seeman, 2002; López-Gil et al., 2009). However, our findings need to be interpreted within the context of neurochemical consequences produced by the chronic meth, PCP or MK-801 exposure in the PFC. Parsegian et al. reported that chronic meth self-administration (followed by 10-14 days of extinction training) reduced basal glutamate levels in the PFC and increased glutamate efflux during reinstatement of meth-seeking, an observation consistent with a reduced function of mGlu2 receptors in this brain region (Parsegian and See, 2014). However, utilizing the identical meth paradigm we did not observe lasting changes in the overall 5-HT tissue content in the PFC (Schwendt et al., 2009). In a recent study, no significant changes in 5-HT₂ receptor binding in the PFC were detected immediately after the cessation of meth self-administration (McFadden et al., 2018). However, neither of these studies evaluated glutamatergic, serotonergic markers in the PFC after a undisturbed withdrawal period (this study). In comparison to meth, serotonergic and glutamatergic consequences of chronic PCP or MK-801 administration are less understood. Studies using shorter administration regimen and lower doses of PCP (subchronic paradigms) reported blunted glutamate release in the PFC (Amitai et al., 2012), but no observable changes in 5-HT_{2A} receptors in the PFC (Choi et al., 2009; Santini et al., 2013).

In the present study, chronic meth, PCP, and MK-801 administration all increased the 5-HT_{2A}/mGlu2 ratio in the PFC, with an additional increase detected in the PRh of PCP-treated animals and in the dHPC of animals treated with MK-801. In comparison to chronic

meth, increases in 5-HT2A/mGlu2 ratios were driven by more subtle changes in 5-HT2A and mGlu2 receptor protein expression. Despite this, we argue that the receptor ratio (as calculated in this study) is not an artificial construct, rather it reflects the balance between 5-HT2A and mGlu2 receptor signaling (as described below), and is predictive of the psychoactive properties of their respective receptor ligands (Baki et al., 2013; Fribourg et al., 2011). For example, Santini and colleagues (2013) reported that in mice, chronic PCP treatment increases behavioral and cellular effects of 5-HT2A agonists, but not the total protein levels of cortical 5-HT2A receptors (Santini et al., 2013). This suggests that a history of PCP administration altered one of the 5-HT2A partner proteins (such as mGlu2) which in turn altered coupling, trafficking, or surface availability of 5-HT2A receptors to produce the observed effect. In the present study, chronic PCP significantly reduced mGlu2 protein levels in the PRh. It is not clear what the cause is for this brain region-selective effect of this drug, though in one previously published study, repeated (14 day-long) PCP administration produced a downregulation of mGlu2 mRNA levels selectively only in some, but not all regions of the rat frontal cortex (Abe et al., 2001). It should be noted that in general, the scale and/or the character of neuroadaptations produced by chronic drug exposure may depend on the contingency of drug delivery. Here we compared the contingent (meth) vs. non-contingent (PCP, MK-801) drug administration. This was due to practical reasons (e.g. difficulty and low rates of PCP or MK-801 self-administration in rodents), as well as our goal to employ drug administration paradigms with well-characterized behavioral outcomes (e.g. object recognition and working memory deficits, sensorimotor gating deficits). Interestingly, we observed a similar increase in PFC 5-HT2A receptors as shown in mice after non-contingent binge meth administration (Chiu et al., 2014), suggesting that contingency of drug delivery did not affect this finding.

A number of gene-association and postmortem studies evaluated the link between 5-HT2A and/or mGlu2 receptors and schizophrenia, or psychosis in general (for review see: Wischhof and Koch, 2016). Unfortunately, discrepancies exist on the direction (increase/decrease) or the presence of 5-HT2A and/or mGlu2 receptor changes. The lack of consensus is likely due to variations in clinical and demographic factors (such as a history of antipsychotic treatment, gender and age). A single study that evaluated postmortem levels of 5-HT2A and mGlu2 receptors in the same population of unmedicated schizophrenia subjects reported that upregulation of 5-HT2A receptors is accompanied by downregulation of mGlu2 receptors in the frontal cortex (González-Maeso et al., 2008).

The majority of available evidence suggests that the interaction between cortical 5-HT2A and mGlu2 receptors has an antagonistic character. At the cellular level, this is evidenced by the suppressive effects of mGlu2 receptor agonists on excitatory postsynaptic potentials (Marek et al., 2000) and immediate early gene activation (Moreno et al., 2011; Zhai et al., 2003) evoked by 5-HT2A receptor agonists in the PFC. At the behavioral level, the frequency of a typical 'head-twitch' response induced in rodents by hallucinogenic 5-HT2A receptor agonists (administered systemically or directly into the PFC) is suppressed by pharmacological activation of mGlu2/3 receptors (Gewirtz and Marek, 2000; Klodzinska et al., 2002; Moreno et al., 2011). Therefore, it could be predicted that upregulation of the 5-HT2A/mGlu2 ratio in the PFC, a common consequence of chronic meth, PCP, and MK-801 exposure observed in the present study, augments 5-HT2A receptor function and/or reduces

inhibitory control of mGlu2 receptor over 5-HT2A receptor activity. To confirm this, future studies will investigate meth/PCP/MK-801-induced changes in 5-HT2A receptor function within the PFC and PRh as assessed by the efficacy of 5-HT2A receptor agonists to induce downstream cellular signaling (tissue PLC activity; (Shi et al., 2006), or to activate immediate early genes (e.g. c-fos or Arc; González-Maeso et al., 2003). Changes in the ability of mGlu2 receptor agonists to suppress 5-HT2A receptor activation will be also evaluated to gain an insight on altered cooperation of 5-HT2A and mGlu2 receptors in the brain regions of interest.

5. Conclusions.

The present study demonstrated that chronic exposure to either meth, PCP, or MK-801 produces complementary changes in 5-HT2A and mGlu2 receptors in the PFC that can be commonly described as an increase in the 5-HT2A/mGlu2 protein expression ratio. Increase in this ratio was also observed in the PRh (meth and PCP) and dHPC (MK-801) tissues. As cortical 5-HT2A and mGlu2 receptors closely cooperate in regulating sensorimotor and cognitive processing, an altered 5-HT2A/mGlu2 ratio could represent a common neurobiological mechanism underlying the emergence of psychosis and cognitive disruption observed with the illicit use of meth or NMDA-receptor antagonists (such as PCP and MK-801). Further, some (though not all) studies suggest that dysregulation of 5-HT2A and mGlu2 receptor expression also occurs in the brains of subjects with schizophrenia diagnosis. Therefore, future studies will need to address not only cellular, but also brain-wide functional and behavioral consequences of disrupted 5-HT2A/mGlu2 balance in translational animal models of MUD and schizophrenia.

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Highlights

- 5-HT_{2A} and mGlu₂ receptors co-immunoprecipitate in the rat cortical tissue.
- Chronic meth increased the 5-HT_{2A}/mGlu₂ expression ratio in the mPFC and PRh
- Chronic PCP and MK-801 also increased the 5-HT_{2A}/mGlu₂ expression ratio in the mPFC
- Chronic meth increased 5-HT_{2A} partner G-protein (G α_q) in the mPFC

Fig. 1.

Outlines of the rat brain coronal sections adopted from (Paxinos and Watson, 2006) with highlighted locations used for tissue dissection of the medial prefrontal cortex (mPFC), dorsal hippocampus (dHPC) and the perirhinal cortex (PrH). Numbers above each section indicate distance (in mm) from Bregma.

Fig. 2.

Identification of antibodies suitable for the detection of 5-HT2A and mGlu2 receptor proteins in the rat brain tissue. (A) Validation of six different commercially-available antibodies in the frontal cortex of rats (R), wild-type mice (WT) and 5-HT2A knockout mice (KO). Only one antibody (Immunostar #24288) detected a ~75kDa band (◀) corresponding to a mature, glycosylated rat 5-HT2A receptor that was not present in 5-HT2A KO mice. (B) Deglycosylation of the wild type (WT) mouse cortical lysate (lanes 2 and 3) by PNGase F increased electrophoretic mobility of the ~75 kDa (◀) band to yield ~52 kDa (◀) band (detected by Immunostar #24288 5-HT2A receptor antibody) that corresponds to the sequenced-based molecular weight of the rat 5-HT2A receptor. For a comparison, the 5-HT2A receptor detection (Immunostar #24288) in the rat (R) cerebral cortex has been shown (lane 1). (C) Immunostaining of rat cortical sections with 5-HT2A #24288 antibody produced a laminar staining pattern consistent with the known 5-HT2A distribution in the mPFC (as shown under 10× magnification). This signal was not present in the mPFC slices, or in the mPFC tissue homogenates incubated with the 5-HT2A antiserum preadsorbed with its immunogenic peptide.

Fig. 3.

Co-immunoprecipitation of mGlu2 and 5-HT2A in the rat brain. (A) Validation of the mGlu2 (07-261) antibody. Under the low reducing conditions this antibody primarily detected mGlu2 receptor dimer (◄; ~220 kDa) in cortical lysates from rat (R) and wild-type mice (WT) that was absent in mGlu2 knock-out (ko) mice. (B) A band corresponding to mature rat 5-HT2A receptors (◄; ~75 kDa) as detected in the PRh lysates immunoprecipitated with mGlu2 (07-261) antibody. This band was not detected in the mPFC and dHPC (not shown) tissue lysates. mGlu2xx – cross-linked mGlu2 receptors. IgG HC – heavy chain of the denatured mGlu2 antibody used for immunoprecipitation.

Fig. 4.

Chronic meth self-administration. (A) Timeline of the experiment. (B) Mean daily lever responding for meth and yoked-saline animals (a/i – active/inactive lever presses). (C) Mean daily meth intake (mg/kg) during short (1 hr) and extended (6 hr) self-administration sessions. Escalation of meth intake was observed during extended access to meth (average intake on days 12-15 vs. days 1-3; * $p < 0.05$). $n = 5-7$ per group.

Fig. 5.

Analysis of the protein expression after chronic meth self-administration in the (A) mPFC (B) dHPC and (C) PRh. (A,B,C - top insets): Representative immunoblotting results show the expression of 5-HT_{2A}, mGlu₂, Gα_{q/11}, Gα_i and Gα_o proteins in saline and meth animals. Calnexin - control protein. (A, B, C - bar graphs): Quantitative analysis of the individual protein expression and of the calculated 5-HT_{2A}/mGlu₂ protein expression ratio. Data are expressed as the percentage of saline controls, n = 5-7 animals per group. (*p <

0.05, ** $p < 0.01$ vs. yoked-saline controls). 2A/2 ratio - 5-HT_{2A}/mGlu₂ protein expression ratio.

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Fig. 6.

Analysis of protein expression after chronic PCP administration. (A) Timeline of the experiment. (B, C, D): Quantitative analysis of the individual protein expression and of the calculated 5-HT2A/mGlu2 protein expression ratio in the mPFC (B), dHPC (C) and PRh (D). Data are expressed as the percentage of saline controls. $n = 7-8$ animals per group. (* $p < 0.05$, ** $p < 0.01$ vs. saline-treated controls). 2A/2 ratio - 5-HT2A/mGlu2 protein expression ratio.

Fig. 7.

Analysis of protein expression after chronic MK-801 administration. (A) Timeline of the experiment. (B, C, D): Quantitative analysis of the individual protein expression and of the calculated 5-HT2A/mGlu2 protein expression ratio in the mPFC (B), dHPC (C) and PRh (D). Data are expressed as the percentage of saline controls. $n = 7-8$ animals per group. (* $p < 0.05$ vs. saline-treated controls). 2A/2 ratio - 5-HT2A/mGlu2 protein expression ratio.

Table 1:

Protein levels of the selected G-protein subunits following chronic PCP administration.

	PFC		dHPC		PRh	
	Sal	PCP	Sal	PCP	Sal	PCP
Gq	100 ± 12.4	96 ± 12.9	100 ± 4.4	103 ± 4.5	100 ± 7.6	82 ± 5
Gi	100 ± 18.4	108 ± 20.6	100 ± 3.7	105 ± 3.3	100 ± 7.8	101 ± 6.6
Go	100 ± 7.8	106 ± 12.3	100 ± 6.1	105 ± 5.2	100 ± 5.2	102 ± 5

Data represent mean immunoreactivity of each protein normalized to calnexin and expressed as percent of the Sal group ± SEM (n = 8). PFC, prefrontal cortex; dHpc, dorsal hippocampus; PRh, perirhinal cortex; Sal, saline; PCP, phencyclidine.

Table 2:

Protein levels of the selected G-protein subunits following chronic MK-801 administration.

	PFC		dHPC		PRh	
	Sal	MK	Sal	MK	Sal	MK
Gq	100 ± 9.2	112 ± 15.3	100 ± 5.5	95 ± 3.4	100 ± 6	81 ± 7.4
Gi	100 ± 6.2	93 ± 3.3	100 ± 5.6	95 ± 2.8	100 ± 11.2	95 ± 9.9
Go	100 ± 8.3	90 ± 5.1	100 ± 6.6	88 ± 4.1	100 ± 11.4	110 ± 8.1

Data represent mean immunoreactivity of each protein normalized to calnexin and expressed as percent of the Sal group ± SEM (n = 8). PFC, prefrontal cortex; dHpc, dorsal hippocampus; PRh, perirhinal cortex; Sal, saline; MK, dizocilpine.