Neurobiology of Disease

PSD-95 Is Essential for Hallucinogen and Atypical Antipsychotic Drug Actions at Serotonin Receptors

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Here, we report that postsynaptic density protein of 95 kDa (PSD-95), a postsynaptic density scaffolding protein, classically conceptualized as being essential for the regulation of ionotropic glutamatergic signaling at the postsynaptic membrane, plays an unanticipated and essential role in mediating the actions of hallucinogens and atypical antipsychotic drugs at 5-HT2A and 5-HT2C serotonin G-protein-coupled receptors. We show that PSD-95 is crucial for normal 5-HT2A and 5-HT2C expression in vivo and that PSD-95 maintains normal receptor expression by promoting apical dendritic targeting and stabilizing receptor turnover in vivo. Significantly, 5-HT2A- and 5-HT2C-mediated downstream signaling is impaired in PSD-95null mice, and the 5-HT2A-mediated head-twitch response is abnormal. Furthermore, the ability of 5-HT2A inverse agonists to normalize behavioral changes induced by glutamate receptor antagonists is abolished in the absence of PSD-95 in vivo. These results demonstrate that PSD-95, in addition to the well-known role it plays in scaffolding macromolecular glutamatergic signaling complexes, profoundly modulates metabotropic 5-HT2A and 5-HT2C receptor function.

Introduction

Known hallucinogens include lysergic acid diethylamide (LSD)-like hallucinogens such as mescaline, LSD, psilocin, and N,N-dimethyltryptamine (Nichols, 2004) and non-LSD-like hallucinogens such as salvinorin A (Roth et al., 2002). 5-HT2A serotonin receptors, which represent the main site of action of the LSD-type hallucinogens (Glennon et al., 1984), are most heavily expressed in the apical dendrites and soma of pyramidal neurons in cortical layers II, III, V, and VI (Willins et al., 1997; Jakab and Goldman-Rakic, 1998), and knockout and tissue-specific rescue studies indicate that cortical 5-HT2A receptors are the main site of action of hallucinogens (González-Maeso et al., 2007). Moreover, the 5-HT2A inverse agonist property of atypical antipsychotic drugs is thought to be an essential feature of their therapeutic actions (Meltzer et al., 1989; Roth et al., 2004a; Gray and Roth, 2007).

The closely related 5-HT2C serotonin receptors are located primarily in choroid plexus, striatum, and hippocampus (Molinaux et al., 1989; Clemett et al., 2000; López-Giménez et al., 2002). 5-HT2C receptors are unique among G-protein-coupled receptors (GPCRs) in that they are post-transcriptionally edited (Burns et al., 1997), a process which affects constitutive activity (Niswender et al., 1999) and the efficiency of G-protein coupling in a functionally selective manner (Price and Sanders-Bush, 2000; Berg et al., 2001; Urban et al., 2007). A number of drugs targeting 5-HT2C receptors have been shown to be efficacious in animal models of schizophrenia, obsessive– compulsive disorder (OCD), depression, and obesity (Dunlop et al., 2005, 2006; Sard et al., 2005; Gray and Roth, 2007; Marquis et al., 2007).

Previous studies by our lab and others have demonstrated that the 5-HT2A and 5-HT2C receptors, which are essential for the actions of atypical antipsychotic drugs and LSD-like hallucinogens (Roth et al., 2004a; Gonzalez-Maeso et al., 2007; Berger et al., 2009), can interact in vitro via a canonical type I postsynaptic density protein of 95 kDa (PSD-95)/Dlg/ZO-1 (PDZ)-binding motif (Bécamel et al., 2002, 2004; Xia et al., 2003a) with PSD-95, a PDZ domain-containing scaffolding protein (Chen et al., 2000; Kim et al., 2006; Nicoll et al., 2006) that is an essential regulator of ionotropic glutamatergic neuronal signaling (Migaud et al., 1998; Sheng and Kim, 2002; Ehrlich and Malinow, 2004; Schluter et al., 2006; Xu et al., 2008). These in vitro data suggest that PSD-95...
Genetic deletion of PSD-95 results in a selective loss of 5-HT2A and 5-HT2C receptors. In this study, we show that genetic deletion of PSD-95 leads to profound membrane and regulating their trafficking and function. In this study, we show that genetic deletion of PSD-95 leads to profound membrane and regulating their trafficking and function. We also show that these 5-HT receptor–PSD-95 interactions are essential for the normal function of the 5-HT2A and 5-HT2C receptors, including their abilities to mediate hallucinogen and atypical antipsychotic drug actions in vivo. Our findings provide new insights into the precise subcellular site(s) of action of hallucinogens and atypical antipsychotic drugs, as well as implicate PSD-95 as an important regulator of metabolotropic 5-HT2A and 5-HT2C receptor function.

Materials and Methods

Mice. A detailed description of how the PSD-95null mice were generated will be reported in a future publication (M. I. Arbuckle, N. H. Komiyama, L. H. Forsty, M. Bence, J. A. Ainge, E. R. Wood, H. J. Carlisle, T. J. O’Dell, and S. G. N. Grant, unpublished observations). Briefly, PSD-95null mice were made by deleting the guanylate kinase (GK) domain of the protein. This results in an almost complete absence of PSD-95 mRNA (~5.7% of wild-type levels, as assessed by gene microarray). Previous studies with these mice using two different antibodies raised against epitopes N terminal to the GK domain of PSD-95 detected no PSD-95 protein whatsoever in the mutant mice (Yao et al., 2004). Our immunocchemical studies confirm these findings (Fig. 1A). All experiments were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University or the University of North Carolina, Chapel Hill. Mice were housed under standard conditions—12 h light/dark cycle and food and water ad libitum.

Immunocchemistry. The following antibodies and dilutions were used: mouse anti-5-HT2A (PharMingen/BD Biosciences), 1:500 (sections), 1:1000 (neurons); mouse anti-PSD-95 (Upstate Biotechnology), 1:1000; mouse anti-5-HT2C (Santa Cruz Biotechnology), 1:500; rabbit anti-microtubule-associated protein 2 (MAP2; Millipore Bioscience Research Reagents), 1:1000; rabbit anti-green fluorescent protein (GFP) A11122 (Invitrogen), 1:1000; rabbit anti-c-fos PC38 (Calbiochem), 1:1000; Alexa Fluor 488 goat anti-mouse or goat anti-rabbit, and Alexa Fluor 594 goat anti-mouse or goat anti-rabbit (Invitrogen), 1:200. For immunocchemistry on brain tissue sections, PSD-95null and PSD-95wildtype mice were perfused with 4% paraformaldehyde in 1× PBS and their brains harvested and placed overnight in 4% paraformaldehyde in 1× PBS at 4°C. Over the next night, brains were placed in 30% sucrose in 1× PBS until they sank, then frozen on dry ice and stored at ~80°C. Sections were either free-floating in 1× PBS (one or two sections per well in a 24-well plate) or thaw-mounted onto coated microscope slides, and they were then permeabilized with 0.3% Triton in 1× PBS for 15–20 min. For immunocchemistry on cultured cortical neurons, 4 d in vitro (DIV) neurons were washed twice with 1× PBS, fixed in 4% paraformaldehyde in 1× PBS for 30 min, then washed twice more with 1× PBS before permeabilizing. Blocking was performed using 5% milk in 1× PBS for 1–2 h. Primary antibodies were incubated in 5% milk in 1× PBS at room temperature for 2 h or overnight at 4°C while shaking. Secondary antibodies were incubated in 5% milk in 1× PBS at room temperature for 1 h in the dark, while shaking. Sections were washed three times in 1× PBS (10 min for each wash). Free-floating sections and neuronal coverslips were transferred to a microscope slide and mounted for fluorescence microscopic visualization.

Saturation radioligand binding. For saturation binding assays, brain regions were microdissected and frozen on dry ice, then stored at ~80°C. A Tissue Tearor (BioSpec Products) was used to homogenize tissue (10 s, 15,000 rpm) in 2 ml of standard binding buffer (SBB; 50 mM TrisHCl, pH 7.4, 0.1% nonidet P-40, 100 mM NaCl, 1 mM EDTA, 10 μM leupeptin, 2 μM pepstatin, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml aprotinin). The homogenate was centrifuged at 15,000 rpm for 1 h, at which time the supernatant was aspirated and the pellet was washed two more times with 1 ml of SBB. The final supernatant was used to prepare the binding assays.

Figure 1. Genetic deletion of PSD-95 results in a selective loss of 5-HT2A and 5-HT2C receptors. A, 5-HT2A and PSD-95 null double-label immunocchemistry in medial prefrontal cortex of PSD-95null and PSD-95wildtype mice shows a large reduction in 5-HT2A receptor expression in null mice (N = 3 littermate pairs). B, 5-HT2C immunocchemistry in PSD-95null and PSD-95wildtype striatum and hippocampus reveals that 5-HT2C receptor expression is almost completely abolished in the absence of PSD-95 in both striatum and hippocampus (N = 3 littermate pairs). C, Comparison of Bmax estimates for the 5-HT2A receptor (N = 4 littermate pairs) and the 5-HT2C receptor (N = 5 littermate pairs) in PSD-95null and PSD-95wildtype cortices. Bmax estimates were obtained by performing [3H]ketanserin (5-HT2A) and [3H]-WAY100635 (5-HT1A) saturation binding on microdissected and homogenized cortical tissue. Quantitation showed an ~40% reduction in 5-HT2A expression and no change in 5-HT2C expression in the cortices of PSD-95null mice. D, Comparison of Bmax estimates for the 5-HT2A receptor (N = 3; tissue from 3 animals was pooled for each measurement, for a total of 9 animals; all littermate pairs) and 5-HT2C receptor (N = 6; littermate pairs) in PSD-95null and PSD-95wildtype hippocampi. Bmax estimates were obtained by performing [3H]mesulergine saturation binding in the presence of 100 nM spiperone to block the vast majority of 5-HT2A receptors (5-HT2C) and [3H]-WAY100635 saturation binding (5-HT1A). Quantitation showed an almost 70% reduction in 5-HT2A expression and no change in 5-HT2C expression in hippocampus in the absence of PSD-95. All saturation binding was analyzed using nonlinear least squares fitting.
7.4; 10 mM MgCl₂, 0.1 mM EDTA). Homogenized tissue was spun for 10 min at 26,000 × g (4°C) and the SBB removed. The pellet was resuspended in 1 ml of SBB and transferred to a 1.7 ml Eppendorf tube, then spun at top speed in a microcentrifuge for 5 min at 4°C. The SBB was removed, and the pellet was either used immediately for binding or stored at −80°C until use. Saturation binding assays were performed with the homogenized brain tissue and [³H]-ketanserin (5-HT₂A, cortex; [³H]-mesulergine plus 100 nM spiperone (5-HT₄, hippocampus); or [³H]-WAY100635 (5-HT₂C, cortex, hippocampus), then incubated in SBB for 1.5 h. The following [³H] concentrations were used: 8 nM, 6 nM, 4 nM, 2 nM, 1.5 nM, 1.0 nM, 0.5 nM, 0.25 nM (5-HT₂A and 5-HT₂C), or 2 nM, 1.2 nM, 0.8 nM, 0.4 nM, 0.2 nM, 0.1 nM, 0.05 nM, 0.025 nM (5-HT₁A), all in duplicate for total and nonspecific (four reactions at each concentration for each brain sample in which receptor was measured). Nonspecific binding was determined by incubating the reactions with 8 μM ritanserin (5-HT₂A and 5-HT₂C) or 10 μM 5-HT (5-HT₁A). For 5-HT₂C measurements, three samples were pooled for each assay. Bradford protein assays were performed to normalize Bₘₐₓ determinations to the amount of protein in each assay. Reactions were vacuumed through glass filters (3× ice-cold 50 mM Tris, pH 7.4; pH 6.9 at room temperature) and counted by liquid scintillation using a Perkin-Elmer Tri-Carb 2800TR. Microsoft Excel and Graphpad Prism were used for all data analysis.

EEDQ, EEDQ (N-ethoxycarbonyl-1,2-ethylenedithioquinoline; dissolve EEDQ in 100% ethanol, dilute 1:3 in saline) was injected intraperitoneally at a dose of 10 mg/kg. Mice were killed at 1, 2, 3, 5, 7, and 13 d after EEDQ treatment, and the 5-HT₂A, Bₘₐₓ was measured by saturation binding. Receptor production was assumed to be a zero-order process and receptor trafficking a first-order process (Pinto and Battaglia, 1994). Thus, the equation derived to model receptor recovery was

\[ \frac{B_{\text{max}}}{B_{\text{max},0}} = \left(1 - e^{-kt}\right) \]

where \(B_{\text{max},0}\) is the amount of receptor at time \(t\), \(B_{\text{max}}\) is the steady state \(B_{\text{max}}\) after the receptors have recovered, \(k\) is the rate constant for receptor turnover (inverse days, or d⁻¹), and \(t\) is the time at which \(B_{\text{max},0}\) was measured in days. This model was fit by nonlinear least squares regression to a plot of the average \(B_{\text{max}}\) value at each time point.

Quantitative reverse transcription-PCR. Trizol (Invitrogen) was used to extract RNA from microdissected cortical tissue. RNA (10 μg) was treated with DNase (DNA-free; Ambion), and 2 μg of the DNase-treated RNA was added to a reverse transcription (RT) reaction which was performed using the Superscript III RNase H Reverse Transcriptase kit (Invitrogen) for quantitation. All steps were performed according to the manufacturer’s instructions.

Microdissection. RNA was extracted from microdissected cortical tissue using Trizol (Invitrogen). The gene chip assay was performed by the Gene Expression and Genotyping Core Facility at the Case Comprehensive Cancer Center using the Affymetrix Genechip Mouse Genome U74Av2 25K. Clones were miniprepped and sequenced to determine the extent of editing for each transcript.

Cortical neuronal cultures. Cortical neurons were prepared from postnatal (P) day 0.5 mouse pups as described previously (Ahlemeyer and Baumgart-Vogt, 2005). Briefly, cortex was microdissected in Mg²⁺/Ca²⁺-free HBSS under a dissecting microscope and incubated at 37°C for 20 min in neurobasal medium containing 0.1% papain and 0.02% BSA. The supernatant was removed and the tissue was then mechanically triturated in neurobasal medium. The supernatant was transferred to a new sterile Eppendorf, leaving the aggregates, and spun down at 200 × g for 10 min. The supernatant was discarded and the pellet resuspended in pre-equilibrated (to 37°C and 5% CO₂) neurobasal medium containing B27 supplement, antibiotics, and 0.5 mM glutamine, and plated on coverslips coated with low molecular weight poly-l-lysine. Immunohistochemical experiments were performed at 4–5 DIV.

Lentiviral preparation. PSD-95 was cloned into the Flip, ubiquitin promoter, GFP, and woodchuck hepatitis virus response element (FUGW) lentiviral vector (Lois et al., 2002) by ligating a Bcl–I digested PSD-95 PCR fragment into the BanHI site 5’ to the GFP (forward primer: 5’-AAA TGA TCA ATG GAC TGT CTC TCT GTG ATA GTG ACA ACC-3’; reverse primer: 5’-AAA TGA TCA GAG TAC TCT CTC TCG GGC TGG GAC CCA-3’). Site-directed mutagenesis was performed to mutate away the stop site that results from the Bcl–BanHI ligation at the 3’ end of PSD-95 and shift the reading frame so that PSD-95 is in frame with GFP (sense primer: 5’-GCC CGA GAG AGA CTC TTA TTC CCC CCG GTG GGA TTG CCG GT-3’; antisense primer: 5’-AGC GGT ACC CCC GGG GAA AAT AAG AGT CTC TCT CCG GC-3’). Fugene6 (50 μl Fugene6, 10 μg total DNA per 10 cm plate) was used to cotransfect HEK293T cells with three plasmids (FUGWΔ8.9 HIV-1/TVSVG (vesicular stomatitis viral glycoprotein) in a ratio of 3.3:2.5:1. Lentivirus-containing media was collected 48 h later and filtered through a 0.45 μm filter to remove cellular debris. Lentivirus was aliquoted and frozen at −80°C until use. Cortical neurons were infected with 20–50 μl GFP or PSD-95 lentivirus at 2 DIV. Immunohistochemistry was performed at 5 DIV.

MK-2212-induced c-fos in hippocampus. Mice were injected intraperitoneally with 5 mg/kg MK-2212 in 0.9% sterile NaCl or vehicle. Forty-five minutes later, they were perfused with 4% paraformaldehyde. Frozen sections (Bregma −1.34 mm to Bregma −2.7 mm) were thaw mounted onto frosted slides and then used for immunohistochemistry and subsequent c-fos quantitation.

DOI-induced head-twitch. Mice were injected intraperitoneally with 5 mg/kg of DOI [(2,5-dimethoxy-4-iodophenyl)-2-aminopropano]. The number of head-twitches was counted and recorded in 5 min bins for the half hour period immediately after injection. A subset of the 5 mg/kg injections (N = 7) were counted by two observers, one of whom was blinded to the genotype. A comparison of the results produced by the two different observers was not significantly different (data not shown). All the other head-twitch experiments were performed by one blinded observer.

8-OH-DPAT-induced hypothermia. Rectal temperature was measured using the TH-5 Thermalert Monitoring Thermometer (Physitemp Instruments) equipped with a RET-3 probe. The probe was sterilized with 70% ethanol and covered with baseline before measuring each mouse’s temperature. Mice were then injected intraperitoneally with 5 mg/kg 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetrailin], and rectal temperature was measured 20 min later.

Western blot. DOI or vehicle was injected intraperitoneally, with light restraint to minimize stress effects, and then mice were killed 15 min later by cervical dislocation. Microdissection was performed on ice as quickly as possible. Tissue was homogenized in 400 μl of SBB plus protease and phosphatase inhibitors and 5% glycerol. Tissue was spun for 10 min at 20–25,000 × g. The supernatant, which contains the proteins of interest, was collected and a protein assay performed for quantitation. SDS was added to 25 μg of protein boiled for 5 min to denature and then used for Western blots. The following antibodies were used for Western blot, all at 1:1000 dilution: rabbit polyclonal p-ERK1/2 (9101L; Cell Signaling Technology), rabbit polyclonal ERK1/2 (9102L; Cell Signaling Technology), p-GSK3β (9331; Cell Signaling Technology), and rabbit monoclonal GSK3β (9315; Cell Signaling Technology).

Prepulse inhibition. All prepulse inhibition (PPI) experiments were performed at the Mouse Behavioral Phenotyping Laboratory Core Facility in the Neurodevelopmental Disorders Research Center at the University of North Carolina, Chapel Hill using the SR-Lab (San Diego Instruments). Briefly, mice were placed in a small, Plexiglas cylinder housed within a large sound-proofed chamber. The cylinder is seated on a piezo-electric transducer which quantifies movement-induced vibrations. The SR-Lab chamber also contains a light, fan, and loudspeaker for acoustic stimuli. Calibration of 70 dB background sound levels and prepulse acoustic stimuli was performed with a digital sound level meter (San
Diabetes Instruments). Each session consisted of a 5 min habituation period followed by 42 trials of seven types—no stimulation, 120 dB acoustic stimulus (AS50), and five different prepulse stimuli ranging from 4 dB over back ground (PP74) to 20 dB over background (PP90). The trial types were performed in six sets of seven, with the trial type order in each set randomized. Intertrial intervals were 10–20 s, with an average interval of 15 s. The AS50 was 40 ms long, whereas the prepulse stimulus was 20 ms long and occurred 100 ms before the onset of the startle stimulus. The sample window for measuring startle amplitude was 65 ms. The formula used to calculate percentage PPI was: ((AS50 − startle after prepulse)/AS50) × 100. Mice were injected with vehicle, phencyclidine (PCP), or antipsychotic and PCP before being placed in the PPI chamber. When treated with vehicle or PCP, mice were immediately placed in the chamber. When treating with antipsychotic, mice were injected with antipsychotic 15 min before injecting PCP, after which mice were immediately placed in the chamber. There was a 1 week washout period between treatments.

PCP-induced hyperlocomotion. Locomotion was measured in a 1 h session in an open field chamber (40 × 40 × 30 cm) crossed by a grid of photobeams (VersaMax system; AccuScan Instruments). The mice were injected with vehicle or SR46349B and placed in the chamber for a 20 min acclimation period, and then they were injected with vehicle or PCP, and locomotion was measured for 1 h. Mice were treated with vehicle, 6.0 mg/kg PCP, or 1.0 mg/kg SR46349B plus 6.0 mg/kg PCP. Mice were allowed a 1 week washout period between treatments. The number of photobeam breaks was counted during the 60 min trial in 5 min bins, and a total distance traveled in centimeters was calculated from the beam break counts.

Results

PSD-95 is essential for maintaining normal 5-HT2A and 5-HT2C receptor expression in vivo

Previous studies demonstrated that PSD-95 interacts with 5-HT2A (Xia et al., 2003a,b; Bécamel et al., 2004) and 5-HT2C (Bécamel et al., 2002) receptors in vitro and in vivo. Additionally, ectopic expression of PSD-95 inhibits the agonist-mediated internalization of the 5-HT2A receptor (Xia et al., 2003a) and promotes desensitization of 5-HT2C receptors (Gavarini et al., 2006) in vitro. Eliminating the type I PDZ ligand motif abrogates both PSD-95 binding and functional activity in vitro (Xia et al., 2003a,b). What, if any, effect PSD-95 might have in vivo is unknown, although we predicted that PSD-95 is responsible for proper targeting and synaptic membrane stabilization of 5-HT2A and 5-HT2C serotonin receptors.

To test this prediction, we examined 5-HT2A and 5-HT2C receptor expression in PSD-95<sup>wildtype</sup> and PSD-95<sup>null</sup> mice. As seen in Figure 1A, PSD-95<sup>null</sup> mice exhibit very little apical dendritic immunofluorescence compared with PSD-95<sup>wildtype</sup> littermate controls. We also performed saturation binding experiments with [3H]-ketanserin on microdissected cortices to obtain a quantitative estimate of 5-HT2A receptor levels in null mice (Fig. 1C). We determined that, consistent with our immunohistochemical findings, PSD-95<sup>null</sup> mice exhibit a significant reduction in 5-HT2A receptor expression.

As shown in Figure 1B, PSD-95<sup>null</sup> animals displayed even larger decrements of striatal and hippocampal 5-HT2C receptors as assessed by a 5-HT2C-selective antibody. Saturation binding isotherms using [3H]-mesulergine under conditions which selectively label 5-HT2C receptors (see Materials and Methods) demonstrated a 72% reduction in 5-HT2C receptor expression levels in the hippocampus (Fig. 1D).

PSD-95 regulates serotonin receptor turnover

We next examined several potential mechanisms which might account for the PSD-95-mediated modulation of 5-HT2A and 5-HT2C receptor expression. These included the following: (1) nonspecific effects on the serotonin system; (2) PSD-95-mediated regulation of 5-HT receptor transcription and/or a generalized disruption of the machinery essential for neuronal regulation of receptors; (3) PSD-95-mediated alterations in serotonin receptor mRNA editing; and (4) alterations in serotonin receptor turnover. Each of these possibilities will be dealt with in turn.

We first examined the possibility that genetic deletion of PSD-95 leads to generalized serotonergic system dysfunction leading to a reduction in serotonin receptor levels. We examined this first possibility by measuring the expression of a related 5-HT receptor which is also highly expressed in cortex and hippocampus but lacks a PDZ-ligand motif—the 5-HT2A<sub>1A</sub> receptor. As our saturation binding experiments using [3H]-WAY100635 indicate, 5-HT2A<sub>1A</sub> expression levels were unchanged in PSD-95<sup>null</sup> mice in both cortex (Fig. 1C) and hippocampus (Fig. 1D). These results indicate that genetic deletion of PSD-95 does not lead to a generalized alteration in the serotonergic system.

To examine the unlikely possibility that deleting PSD-95 leads to an alteration in 5-HT receptor gene transcription, we performed quantitative RT-PCR to measure 5-HT2A and 5-HT2C receptor mRNA levels. We found that 5-HT2A mRNA levels are unchanged in cortex (Fig. 2A), and 5-HT2C mRNA levels are unchanged in hippocampus (Fig. 2A). To further assess the role of PSD-95 in modulating mRNA levels more broadly, or the possibility that compensatory changes in global gene expression occur in null animals and that these compensatory changes cause the observed phenotypes, we performed whole-genome microarray analysis on cDNA prepared from PSD-95<sup>wildtype</sup> and PSD-95<sup>null</sup> cortices.

Overall, there were few differences in transcript levels, and only 28 genes (27 genes decreased, 1 gene increased) appear to be modulated greater than twofold in the absence of PSD-95—none of which are GPCRs or are expected to modulate the expression of 5-HT receptors (supplemental Table 1, available at www.jneurosci.org as supplemental material). Thus, the whole genome microarray data are more consistent with a role for PSD-95 in post-transcriptional/post-translational regulation of 5-HT2A and 5-HT2C receptors.

Interestingly, 6 of the 28 genes, out of ~45,000 transcripts on the microarray, have previously been reported to be induced after hallucinogen administration (Table 1) (Nichols and Sanders-Bush, 2002; González-Maeso et al., 2003). In one study of transcripts induced by 5-HT2A agonists, only 3 of 13 transcripts shown to be changed by agonist administration were specific to hallucinogenic agonists (González-Maeso et al., 2003). Two of these three genes, egr2 and per1, are downregulated in the absence of PSD-95 according to our microarray data, which is consistent with a possible role of PSD-95 in mediating some 5-HT2A signaling pathways, particularly those related to hallucinogen actions.

The 5-HT2C receptor undergoes mRNA editing which modulates its constitutive activity, G-protein coupling efficiency, and expression (Niswender et al., 1999; Price and Sanders-Bush, 2000). It is, therefore, conceivable that changes in 5-HT2C receptor expression are secondary to altered editing of 5-HT2C mRNAs. To examine this possibility, we examined RNA editing at all possible sites in PSD-95<sup>wildtype</sup> and PSD-95<sup>null</sup> hippocampal tissue, and we found that there is no change in the frequency of editing at any of the five sites (Fig. 2B). Furthermore, there is no significant change in the proportions of 14 of the 15 different isoforms detected in the PSD-95<sup>null</sup> mice compared with PSD-95<sup>wildtype</sup> mice (Fig. 2C,D). An increase in PSD-95<sup>null</sup> mice of 1 isoform out 15, the valine-serine-isoleucine (VSI) isoform, is in-
consistent with a role for mRNA editing in downregulating 5-HT2C receptors in PSD-95
null mice. These findings indicate that neither transcriptional nor post-
transcriptional mechanisms (i.e., RNA editing) can account for the large effect that genetic deletion of PSD-95 has on the expression of 5-HT2A and 5-HT2C receptors.

Our data clearly point to the fourth prediction that PSD-95 is exerting its effect on the 5-HT2A and 5-HT2C receptors by regulating their turnover. Implicit in our hypothesis is that in the absence of PSD-
95, 5-HT2A receptors will have greater access to intracellular trafficking machinery, or will enter alternative trafficking pathways, leading to higher rates of receptor turnover. To assess the rates of receptor turnover in PSD-95
vidtype and PSD-95
null animals, we took advantage of the properties of EEDQ, which binds irre-
versibly to 5-HT2A receptors (surface and intracellular), occluding them from recog-
nition by their ligands after EEDQ treatment. By treating mice with EEDQ and modeling the rate of receptor recovery over time, one can measure the rate of 5-HT2A receptor turnover in vivo (Pinto and Battaglia, 1994).

For these studies, we injected mice once with EEDQ (10 mg/kg), a dose that achieves ~90% irreversible blockade of 5-HT2A receptors, and performed saturation binding experiments at different time points after EEDQ treatment to measure the recovery rate of 5-HT2A receptors. If 5-HT2A receptors in null mice have a higher rate of turnover, then the rate constant, the frequency expressed as a fraction of the total, should be higher in these mice. Consistent with this prediction, the modeled receptor recovery in PSD-95
vidtype and PSD-95
null mice (Fig. 3 A, B) showed that the rate constant, k (d⁻¹), was substantially higher in null mice. These findings indicate that genetic deletion of PSD-95 accelerates 5-HT2A receptor turnover in vivo. Attempts to perform similar studies with 5-HT2C receptors were unsuccessful because of the exceedingly low levels of 5-HT2C receptors expressed in PSD-95
null mice.

PSD-95 is required for the polarized sorting of 5-HT2A receptors to pyramidal neuron apical dendrites

Another important aspect of our hypothesis focuses on 5-HT2A receptors and the prediction that PSD-95 is crucial for proper targeting to the apical dendrites. Previous studies showed that mutating the PDZ ligand motif prevents dendritic targeting of the 5-HT2A receptor in vitro (Xia et al., 2003b). To determine if PSD-95 is one of the PDZ-domain proteins responsible for the preferential dendritic targeting of 5-HT2A receptors, we examined the ability of 5-HT2A receptors to be sorted to neuronal dendrites in cortical neurons prepared from PSD-95
vidtype and PSD-95
null mice.

For these studies, we performed confocal immunofluorescent studies of mouse cortical neurons for 5-HT2A receptors and the dendritic marker MAP2 (Caceres et al., 1984). As Figure 4, A and B, illustrates, neurons prepared from PSD-95
null animals exhibit significantly lower 5-HT2A receptor expression in both the neuronal soma and dendrites—a finding consistent with our in vivo data. To examine the impact of PSD-95 on dendritic trafficking, we also calculated a 5-HT2A receptor cell body/dendrite expression (CB/D) ratio. If dendritic targeting is impaired in PSD-95
null neurons, we predicted that the CB/D ratio should be higher in

Table 1. Genes of interest affected in PSD-95 knock-out mice

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>Alternate names</th>
<th>Downregulation as percentage of wild type</th>
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<tbody>
<tr>
<td>Arcb</td>
<td>3.1</td>
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<tr>
<td>eg2a,b</td>
<td>krox20; nfg1b; zfp-25; zfp-6</td>
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<td>per</td>
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Six transcripts that are downregulated in PSD-95
null mice have been reported to be induced after hallucinogenic drug administration in mice. Most are immediate early genes, which is consistent with a role for PSD-95 in regulating 5-HT2A signaling.

*González-Mariscal et al., 2003; Yuen et al., 2003.


The gene previously reported to be upregulated after hallucinogenic administration is amid3, a closely related isoform that differs only in the 5 untranslated region and a few amino acids at the C terminus.
PSD-95 is required for 5-HT$_{2A}$ signaling in vivo

Having provided strong evidence that PSD-95 profoundly regulates the expression of 5-HT$_{2A}$ receptors, we next examined the consequences of knocking out PSD-95 on 5-HT$_{2A}$ function in vivo. It is well established that c-fos is an immediate early gene (IEG) which is transcribed after GPCR activation (Lo and Wong, 2006) and which is useful as a general marker of neuronal activity (Chaudhuri, 1997). To examine the consequences of genetic deletion of PSD-95 on signaling downstream of the 5-HT$_{2A}$ receptor and on neural activity, we treated mice with MK-212, a 5-HT$_{2A}$-selective agonist (Thomsen et al., 2008), and assessed its effect on re-expression of PSD-95 on 5-HT$_{2A}$ expression and targeting of PSD-95 on signaling downstream of the 5-HT$_{2C}$ receptor.

These results are in agreement with previous studies (Campbell and Merchant, 2003). This reduction did not appear to be related to alterations in hippocampal morphology or volume, as cresyl violet staining does not reveal any gross abnormalities in the absence of PSD-95 (supplemental Fig. S2, available at www.jneurosci.org as supplemental material). Furthermore, rodents (Nichols, 2004), head-twitch behavior has been shown to be the most specific for hallucinogenic action in that nonhallucinogenic 5-HT$_{2A}$ agonists such as lisuride do not induce the behavior (González-Maeso et al., 2007). PSD-95$_{wildtype}$ and PSD-95$_{null}$ mice were injected with a 5 mg/kg dose of the prototypical 5-HT$_{2A}$ hallucinogen 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine hydrochloride (Imamura et al., 2002). We found that there was a large and significant decrease in DOI-induced head-twitch in PSD-95$_{null}$ animals compared with PSD-95$_{wildtype}$ animals (Fig. 7A). Head twitches are virtually nonexistent in both PSD-95$_{wildtype}$ and PSD-95$_{null}$ animals after saline treatment (0–2 per 30 min; data not shown). In contrast, 8-OH-DPAT, which is known to induce hypothermia via agonist action at 5-HT$_{1A}$ receptors in the CNS (Martin et al., 1992), leads to the same decrease in temperature in both PSD-95$_{wildtype}$ and PSD-95$_{null}$ mice (Fig. 7A). Together, the findings suggest that PSD-95 selectively affects behaviors mediated by 5-HT$_{2A}$ receptors.

Given the reduction in DOI-induced head-twitch seen in the absence of PSD-95 in vivo, we also predicted that 5-HT$_{2A}$-mediated signaling would be reduced or absent in PSD-95$_{null}$ mice. The 5-HT$_{2A}$ receptor has been shown to signal through a large number of canonical (phospholipase Cβ) and noncanonical (phospholipase A$_2$, phospholipase D, etc.) pathways (Nichols, 2004). The evidence thus far suggests that hallucinogenic action is not correlated with canonical signaling pathways, since both hallucinogenic and nonhallucinogenic agonists at the 5-HT$_{2A}$ receptor activate those pathways with similar potencies (Nichols, 2004). 5-HT$_{2A}$ agonists have been shown to activate ERK1/2 via a number of different mechanisms (Hershenson et al., 1995; Greene et al., 2000; Quinn et al., 2002; Gööz et al., 2006), and 5-HT$_{2A}$ agonists also lead to Akt activation (i.e., Akt phosphorylation) (Johnson-Farley et al., 2005). Phosphorylation of Akt leads to phosphorylation of GSK3β, which renders that protein inactive (Beaulieu et al., 2008). Accordingly, we predicted that the induction of phospho-ERK1/2 (pERK1/2) and phospho-GSK3β (p-GSK3β) after treatment with DOI (5 mg/kg) would be reduced or absent in PSD-95$_{null}$ mice (Li et al., 2004; Schmid et al., 2008). Consistent with our prediction, we found that DOI was unable to induce pERK1/2 or pGSK3β in PSD-95$_{null}$ mice (Fig. 7B–D). Thus, our evidence suggests that PSD-95 plays an important role in mediating 5-HT$_{2A}$ downstream signaling, and its absence results in signaling and behavioral abnormalities.

**Figure 3.** Genetic deletion of PSD-95 leads to an accelerated turnover of 5-HT$_{2A}$ receptor protein. A and B represent fitted curves modeling 5-HT$_{2A}$ receptor turnover in PSD-95$_{wildtype}$ and PSD-95$_{null}$ mice, respectively (N = 3–4 littermate pairs at each data point). Visual inspection shows that steady-state levels for the 5-HT$_{2A}$ receptor are reached sooner in the absence of PSD-95, suggesting accelerated turnover. The higher k in PSD-95$_{null}$ cortex indicates a higher rate of receptor turnover in the absence of PSD-95. Rate constant, k, is a nonleast squares fitted parameter of an equation modeling receptor recovery (for details, see Materials and Methods). ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001; one-tailed unpaired t test.
Deletion of PSD-95 renders atypical antipsychotics ineffective

It has been recently demonstrated that synaptic and behavioral measures of dopamine-mediated synaptic plasticity are also altered by genetic deletion of PSD-95 (Yao et al., 2004). We thus hypothesized that the prototypical, gold standard atypical antipsychotic drug clozapine, whose actions are mediated via inverse agonism at 5-HT2A and 5-HT2C receptors (Meltzer et al., 1989; Rauser et al., 2001) and by weak D2/D3/D4-dopamine antagonism (Roth et al., 2004b), would have an altered activity in PSD-95null mice. In this regard, the PCP-induced disruption of PPI is a well accepted pharmacological model of schizophrenia (Geyer et al., 2001; Linn and Javitt, 2001). Importantly, clozapine preferentially normalizes PCP-induced disruption of PPI in both rodents and monkeys, whereas typical antipsychotics like haloperidol have little to no effect (Geyer et al., 2001; Linn et al., 2003). As all the published evidence suggests that 5-HT2A receptors are important in mediating clozapine’s reversal of the PCP-induced disruption of PPI (Yamada et al., 1999), we predicted that clozapine would exhibit an altered ability to inhibit PCP-induced disruption of PPI in PSD-95null mice.

To test this prediction, we injected littermate pairs of PSD-95wildtype and PSD-95null mice with vehicle, PCP, or clozapine plus PCP, followed by PPI assessment. PCP significantly disrupted PPI at all prepulse levels in PSD-95wildtype mice and at two of the four prepulse levels in PSD-95null mice (Fig. 8A; supplemental Fig. S3, available at www.jneurosci.org as supplemental material). Clozapine normalized the PCP-induced deficit of PPI in PSD-95wildtype mice while having no significant effect in PSD-95null mice. As a control, we also measured startle response and found no significant effect of genotype on startle response (AS50) with and without drug treatments (supplemental Fig. S3, available at www.jneurosci.org as supplemental material), and clozapine treatment alone had no effect on PPI compared with vehicle-treated mice (Fig. 8B). Thus, genetic deletion of PSD-95 abolishes the antipsychotic-like actions of clozapine.

Although the actions of clozapine in NMDA-antagonist-based psychosis models such as PCP-induced disruption of PPI are known to be 5-HT2A-mediated, clozapine is nonetheless a pharmacologically “dirty” drug, with a high affinity for a number of other receptors (Roth et al., 2004a). To more firmly establish that 5-HT2A dysfunction is responsible for the abnormal antipsychotic-like efficacy seen in the aforementioned clozapine experiment, we used two selective antagonists of the 5-HT2A receptor, M100907 and SR46349B, which have been shown to be effective in NMDA antagonist-based animal models of psychosis (Varty et al., 1999) and in clinical studies of schizophrenic patients (Meltzer et al., 2004). If the impaired efficacy of clozapine is because of 5-HT2A dysfunction, then 5-HT2A antagonists should be ineffective as antipsychotics in multiple psychosis models. In this experiment, PCP significantly disrupted PPI at 4 and 8 dB in both PSD-95wildtype and PSD-95null mice (Fig. 8C). As predicted, pretreatment with M100907 (0.5 mg/kg) or SR46349B (1 mg/kg) normalized PCP-induced disruption of PPI in PSD-95wildtype mice only, having no effect in PSD-95null mice (Fig. 8C). To provide further evidence that antipsychotic-like efficacy medi-
Reintroduction of PSD-95 rescues 5-HT<sub>2A</sub> receptor expression and trafficking deficits in PSD-95<sup>−/−</sup> cortical neurons in vitro. 

**A**

Representative images of double-label immunocytochemistry performed on P0.5 cortical neurons of PSD-95<sup>−/−</sup> mice infected with either GFP lentivirus (top 2 rows of panels) or PSD-95–GFP lentivirus (bottom 2 rows of panels). PSD-95<sup>−/−</sup> neurons from each animal were plated in two wells, one for GFP lentiviral infection and the other for PSD-95–GFP lentiviral infection. The yellow arrows highlight dendritic 5-HT<sub>2A</sub> receptor expression in an infected neuron. White arrows highlight 5-HT<sub>2A</sub> receptor expression in an uninfected neuron. GFP-infected neurons display low overall 5-HT<sub>2A</sub> expression and low dendritic targeting. In contrast, PSD-95–GFP-infected neurons display a dramatic increase in overall 5-HT<sub>2A</sub> receptor expression and substantially more receptor appears to be targeted to the dendritic compartment, both compared with control GFP-infected neurons and compared with uninfected neurons in the same field. 

**B**

Comparison of 5-HT<sub>2A</sub> receptor expression in cell bodies and dendrites and the CB/D ratio in GFP- and PSD-95–GFP-infected PSD-95<sup>−/−</sup> neurons. Expression is normalized to GFP or PSD-95–GFP. PSD-95–GFP leads to substantial rescue of 5-HT<sub>2A</sub> receptor expression compared with GFP-infected control neurons. PSD-95–GFP addback leads to a significant reduction in the CB/D ratio compared with GFP-expressing control neurons, suggesting that PSD-95–GFP is rescuing dendritic targeting of 5-HT<sub>2A</sub> receptor expression. N = 3 animals for each animal/lentivirus, and 10 infected neurons from each animal/lentivirus were measured (60 neurons total). Data are presented as the mean ± the SEM; *p < 0.05, **p < 0.01, ***p < 0.001; one-tailed paired t test.

Figure 5.

Discussion

The main finding of this study is that PSD-95 is essential for serotonin receptor function and hallucigen and atypical antipsychotic actions in vivo. These findings suggest that in addition to its well-known modulatory effect on ionotropic glutamatergic signaling, PSD-95 is required for normal metabolotropic serotonin receptor function. We show that PSD-95, a modular PDZ domain-containing protein which scaffolds a wide range of proteins at postsynaptic clusters, is an important regulatory partner for both 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors in vivo. In the absence of PSD-95, the 5-HT<sub>2A</sub> receptor has accelerated receptor turnover kinetics and decreased targeting to the appropriate dendritic compartment, leading to a decrease in total receptor expression and a concomitant decrease in 5-HT<sub>2A</sub>-mediated signaling (p-ERK1/2 and p-GSK3β) and behaviors (e.g., head-twitch response). In the absence of PSD-95, the 5-HT<sub>2C</sub> receptor exhibits a larger decrease in receptor protein expression and downstream signaling. Finally, we show that, in the absence of PSD-95, the prototypical atypical antipsychotic drug clozapine, as well as the selective 5-HT<sub>2A</sub> antagonists M100907 and SR46349B, are unable to mediate their therapeutic effects in animal models of psychosis.

5-HT<sub>2C</sub> receptors, PSD-95, and psychiatric disease

There is considerable evidence that 5-HT<sub>2C</sub> serotonin receptors regulate hippocampal function. Genetic evidence from 5-HT<sub>2C</sub> knockout mice shows that long-term potentiation (LTP) is impaired in the dentate gyrus in the absence of 5-HT<sub>2C</sub> receptors (Tecott et al., 1998). 5-HT<sub>2C</sub> knockout mice also exhibit deficits in behaviors thought to be mediated by the dentate gyrus (Tecott et al., 1998) and are more susceptible to spontaneous and audiogenic seizures (Tecott et al., 1995), which are known to involve limbic recruitment.

Despite this evidence that 5-HT<sub>2C</sub> receptors modulate neuronal function, nothing is known regarding their targeting and regulation by 5-HT<sub>2A</sub> receptors is impaired in the absence of PSD-95, we examined the effect of SR46349B (1 mg/kg) in another widely used animal model of psychosis, PCP-induced hyperlocomotion, which is also normalized by atypical antipsychotics such as clozapine (Gleason and Shannon, 1997; Geyer and Ellenbroek, 1995), which are expected, SR46349B (1 mg/kg) normalizes PCP-induced hyperlocomotion in PSD-95<sup>wildtype</sup> but not PSD-95<sup>−/−</sup> mice (Fig. 8D). Thus, our findings are very consistent in showing that the genetic deletion of PSD-95 leads to 5-HT<sub>2A</sub> receptor dysfunction which in turn prevents atypical antipsychotics from being therapeutically efficacious in animal models of psychosis.
ulation of neuronal function. Our data concerning the 5-HT\textsubscript{2C} receptor’s interaction with, and regulation by, PSD-95 suggests that the 5-HT\textsubscript{2C} receptor is present at PSD-95-enriched neuronal domains where it would be well placed to influence neuronal excitability and synaptic properties and, therefore, brain electrical activity. Although our studies focused on 5-HT\textsubscript{2C} function in the hippocampus, the potential relevance of our findings to 5-HT\textsubscript{2C} function in other brain regions is also of interest. The 5-HT\textsubscript{2C} receptor has shown promise as a target in the treatment of a number of psychiatric disorders, including in particular schizophrenia and obesity, although it has been proposed that 5-HT\textsubscript{2C} receptors may also play a role in the etiology and treatment of OCD and depression (Dunlop et al., 2005, 2006; Sard et al., 2005; Gray and Roth, 2007; Marquis et al., 2007). Thus, PSD-95 may play a role in regulating 5-HT\textsubscript{2C} function in various disease states, although further study will be needed to investigate this intriguing possibility.

Implications of PSD-95 regulation of hallucinogen action

Although it is known that hallucinogens exert their effects via activation of the 5-HT\textsubscript{2A} receptor (Glennon et al., 1983), the signaling processes underlying hallucinogen action are still poorly characterized. Recent evidence suggests that cortical 5-HT\textsubscript{2A} receptors are required for hallucinogen actions, possibly by facilitating corticocortical activity (González-Maeso et al., 2007). Our data are relevant in several respects. First, our findings are consistent with the hypothesis that hallucinogens exert their effects at cortical pyramidal neuron apical dendrites. Apical dendritic activity has been implicated as forming the neural basis for cognition and consciousness (LaBerge, 2006; Laberge and Kasevich, 2007), and it is thought that corticocortical connections, which are primarily composed of synaptic contacts at apical dendrites (Spratling, 2002), are important in generating and shaping the neural activity that underlies consciousness (Tononi and Edelman, 1998). Furthermore, the primary neuro-anatomical site of expression of 5-HT\textsubscript{2A} receptors is the apical dendrites of cortical pyramidal neurons, particularly in layer V pyramidal neurons (Willins et al., 1997; Jakab and Goldman-Rakic, 1998). Finally, a wide range of evidence supports altered glutamatergic signaling in neocortex as playing a key role in mediating the effects of hallucinogens on consciousness (Aghajanian and Marek, 1971).
Importantly, our studies demonstrate that the overall expression and apical dendritic targeting of \(5\text{-HT}_{2A}\) receptors to postsynaptic densities is significantly impaired in cortical neurons prepared from \(\text{PSD-95null}\) mice. Also, DOI is unable to induce p-ERK1/2 and p-GSK3\(\beta\) in \(\text{PSD-95null}\) mice. Not surprisingly, DOI-induced head-twitch behavior, the behavioral correlate of hallucinogen action, is also reduced. Moreover, we found that the reintroduction of PSD-95 into \(\text{PSD-95null}\) neurons rescues both the deficient expression and targeting phenotype. Our data provide a mechanism whereby \(5\text{-HT}_{2A}\) receptors can be targeted to a cortical, postsynaptic site of action and trafficked appropriately once they have arrived. In fact, our studies have provided the first candidate subcellular locus for hallucinogen action, the PSD-95-scaffolded macromolecular signaling complex of cortical neurons. Given the accumulating evidence that hallucinogenic action involves alterations in synaptic activity, our data further suggest the possibility that hallucinogens may exert their actions via PSD-95-mediated interactions with glutamatergic signaling complexes downstream of \(5\text{-HT}_{2A}\) receptor activation.

Atypical antipsychotics are ineffective in the absence of PSD-95

It has been known for some time that PCP, a noncompetitive NMDA receptor antagonist, induces psychotic and “deficit” states that are nearly indistinguishable from the positive and negative symptoms of schizophrenia (Jentsch and Roth, 1999; Olney et al., 1999; Javitt, 2004). Furthermore, clozapine and other drugs with potent \(5\text{-HT}_{2A}\) inverse agonist actions ameliorate PCP-induced PPI deficits (Carlsson et al., 1999; Yamada et al., 1999; Geyer et al., 2001; Linn et al., 2003).

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Finally, genetic deletion of PSD-95 or deletion of one of the PDZ domains results in abnormalities in LTP, a phenotype related to glutamatergic dysfunction (Migaud et al., 1998; Yao et al., 2004). Together with our data, the evidence suggest that one of the key subcellular locations at which the functional interplay between 5-HT2A receptors and glutamatergic signaling takes place is the PSD-95-scaffolded postsynaptic density.

Since, in the absence of PSD-95, glutamatergic signaling is abnormal, and 5-HT2A receptors are mistargeted and mistrafficked, we predicted that there may be abnormalities in the ability of clozapine and selective 5-HT2A antagonists to alleviate PCP-induced psychotic-like behaviors in mice. We found that clozapine, M100907, or SR46349B treatment, which reduced PCP-induced deficits of PPI in PSD-95<sup>wildtype</sup> mice, was completely ineffective in PSD-95<sup>null</sup> mice. The dramatically impaired antipsychotic-like action of the aforementioned atypical antipsychotics in the PSD-95<sup>null</sup> mice is likely attributable to the combined abnormalities in 5-HT2A and 5-HT2C receptor function—both of which have long been thought to be essential for their unique benefits (Roth et al., 2004a). Our studies clearly implicate the 5-HT2A dysfunction that results in the absence of PSD-95 as being responsible for the lack of atypical antipsychotic efficacy in PSD-95<sup>null</sup> mice.

**Conclusions**

In this study, we demonstrate that PSD-95, in addition to its well-known role in scaffolding glutamatergic signaling complexes and facilitating neuronal plasticity, potently regulates neuronal metabotropic serotonin receptor targeting, trafficking, and signaling in vivo. Furthermore, we show that the absence of PSD-95 results in abnormal downstream signaling for both 5-HT<sub>2A</sub> receptors and 5-HT<sub>2C</sub> receptors, both of which are important therapeutic targets for a number of psychiatric diseases. We also show that the 5-HT<sub>2A</sub> dysfunction has profound consequences with regards to the treatment of psychotic-like states in relevant animal models. Our findings demonstrate an unexpectedly profound role for PSD-95 in regulating 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor function and the behavioral responses to drugs acting at these receptors. These results imply that PSD-95 may serve as a scaffold to integrate information between ionotrophic and metabotropic neurotransmission at postsynaptic densities.

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