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

Atheer I. Abbas,* Prem N. Yadav,* Wei-Dong Yao,†,‡,§,∥,¶,†† Margaret I. Arbuckle,¶ Seth G. N. Grant,¶ Marc G. Caron,‖,§,∥,‡,¶,†† and Bryan L. Roth*†,‡,§,∥,¶,††

1Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, Departments of 2Pharmacology, 3Medicinal Chemistry, and 4Psychiatry, 5Lineberger Cancer Center, and 6National Institute of Mental Health Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, North Carolina 27599, Departments of 7Cell Biology, 8Medicine, and 9Neurobiology, Duke University Medical Center, Durham, North Carolina 27710, 10Department of Psychiatry, Division of Neurosciences, New England Primate Research Center, Harvard Medical School, Boston, Massachusetts 02115, 11Division of Neuroscience, University of Edinburgh, Edinburgh EH8 9JZ, United Kingdom, and 12Genes to Cognition Programme, Wellcome Trust Sanger Institute, Cambridge CB10 1SA, United Kingdom

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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-HT$_{2A}$ and 5-HT$_{2C}$ serotonin G-protein-coupled receptors. We show that PSD-95 is crucial for normal 5-HT$_{2A}$ and 5-HT$_{2C}$ expression in vivo and that PSD-95 maintains normal receptor expression by promoting apical dendritic targeting and stabilizing receptor turnover in vivo. Significantly, 5-HT$_{2A}$- and 5-HT$_{2C}$-mediated downstream signaling is impaired in PSD-95null mice, and the 5-HT$_{2A}$-mediated head-twitch response is abnormal. Furthermore, the ability of 5-HT$_{2A}$ 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 macro-molecular glutamatergic signaling complexes, profoundly modulates metabotropic 5-HT$_{2A}$ and 5-HT$_{2C}$ 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-HT$_{2A}$ 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-HT$_{2A}$ receptors are the main site of action of hallucinogens (González-Maeso et al., 2007). Moreover, the 5-HT$_{2A}$ 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-HT$_{2C}$ serotonin receptors are located primarily in choroid plexus, striatum, and hippocampus (Molina- eaux et al., 1989; Clemett et al., 2000; López-Gimenez et al., 2002). 5-HT$_{2C}$ 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-HT$_{2C}$ 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-HT$_{2A}$ and 5-HT$_{2C}$ 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)/DiGiZO-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., 2006). These in vitro data suggest that PSD-95

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alterations in 5-HT2A and 5-HT2C receptor targeting, expression, study, we show that genetic deletion of PSD-95 leads to profound membrane and regulating their trafficking and function. In this

pairs) and the 5-HT1A receptor (5-HT1A). Quanti-

nM spiperone to block the vast majority of 5-HT2A receptors (5-HT2C) and [3H]-WAY100635 saturation binding (5-HT1A). Quanti-

tings provide new insights into the precise subcellular site(s) of interactions are essential for the normal function of the 5-HT2A and signaling. We also show that these 5-HT receptor–PSD-95

max 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). Quantita-

tion showed an almost 70% reduction in 5-HT2A expression and no change in 5-HT1A expression in hippocampus in the absence of PSD-95. All saturation binding was analyzed using nonlinear least squares fitting. B_{max} estimates were obtained as means ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001; one-tailed unpaired t test.

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 mRNAs (~5.7% of wild-type levels, as assayed 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 immunochemical 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.

Immunochemistry. The following antibodies and dilutions were used: mouse anti-5-HT1A (PharMingen/BD Biosciences), 1:500 (sections), 1:1000 (neurons); mouse anti-PSD-95 (Upstate Biotechnology), 1:1000; mouse anti-

5-HT2C D-12 (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 immunochemistry 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 immunochemistry 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. Sections were then washed three times in 1× PBS (10 min for each wash). 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 overslips 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

could play a critical role in regulating 5-HT2A and 5-HT2C recep-
tors in neurons in vivo, tethering these receptors to the plasma membrane and regulating their trafficking and function. In this study, we show that genetic deletion of PSD-95 leads to profound alterations in 5-HT2A and 5-HT2C receptor targeting, expression, and signaling. 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 metabotropic 5-HT2A and 5-HT2C receptor function.
Operating Software, version 1.4.0.036. 

Nome 430 2.0 Array. Data were analyzed using the Affymetrix Genechip by the Gene Expression and Genotyping Core Facility at the Case Com-
tical tissue using Trizol (Invitrogen). The gene chip assay was performed permix (Bio-Rad) was used in a 7300 RT-PCR system (Applied 

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 mE glutamine, and plated on coverslips coated with low molecular weight poly-L-lysine. Immunohistochemical ex-

periments were performed at 4–5 DIV. 

Lentiviral preparation. PSD-95 was cloned into the Flip, ubiquitin pro-

meter, GFP, and woodchuck hepatitis virus response element (FUGW) lentiviral vector (Lois et al., 2002) by ligating a Bcl–Bcl-HIV-PSD-95 PCR fragment into the BamHI site 5′ to the GFP (forward primer: 5′-AAA TGA TCA ATG GAC TGT CTC TGT ATA GTG ACA ACC-3′; reverse primer: 5′-AAA TGA TCA GAG TCT CTC TCG GGC TGG GAC CCA-3′). Site-directed mutagenesis was performed to mutate away the stop site that results from the Bcl–Bcl-HIV ligation at the 3′ end of PSD-95 and shift the reading frame so that PSD-95 is in frame with GFP (sense prim-

er: 5′-GCC CGA AGA AGA CTC TTA TTT CCC CCG GGA GTG GT-3′; antisense primer: 5′-ACC GGT ACC CCC GGG GGA AAT AAG AGT CTC TCT CGG GC-3′). Fugene6 (50 μL Fugene6, 10 μg total DNA per 10 cm plate) was used to cotransfect HEK293T cells with three plas-
mids [FUGW/ΔB.9 HIV-1/VSVC (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 GFP lentivirus at 2 DIV. Immunochemistry was performed at 5 DIV. 

MK-212 induced c-fos in hippocampus. Mice were injected intraperi-
toneally with 5 mg/kg MK-212 in 0.9% sterile NaCl 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-aminopropane]. 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 In-

struments) 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)tetrain], and rectal 
temperature was measured every 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% glyceral. 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 a 1:1000 dilution: rabbit polyclonal p-ERK1/2 (9101L; Cell Signaling 

Technology), rabbit polyclonal EK1/2 (9102L; Cell Signaling Technol-

ogy), p-GSK3β (9331; Cell Signaling Technology), and rabbit monoclo-
nal 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 Instru-

ments). Briefly, mice were placed in a small, Plexiglas cylinder housed 

within a large sound-proof chamber. The chamber 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
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-95wildtype and PSD-95null mice. As seen in Figure 1A, PSD-95null mice exhibit very little apical dendritic immunofluorescence compared with PSD-95wildtype 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-95null mice exhibit a significant reduction in 5-HT2A receptor expression.

As shown in Figure 1B, PSD-95null 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-HT1A receptor. As our saturation binding experiments using [3H]-WAY100635 indicate, 5-HT1A expression levels were unchanged in PSD-95null 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-95wildtype and PSD-95null 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 hallucinogenic actions.

The 5-HT3C 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-95wildtype and PSD-95null 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-95null mice compared with PSD-95wildtype mice (Fig. 2C,D). An increase in PSD-95null 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-95null 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 and 5-HT2C 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-95wildtype and PSD-95null animals, we took advantage of the properties of EEDQ, which binds irreversibly to 5-HT2A receptors (surface and intracellular), occluding them from recognition 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, 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-95null 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-95wildtype and PSD-95null 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-95null 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-95null 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>
</tr>
</thead>
<tbody>
<tr>
<td>Arcb</td>
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<td>36.6</td>
</tr>
<tr>
<td>egr2a,b</td>
<td>krox20; ngl1b; zfp-25; zfp-6</td>
<td>38.6</td>
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<tr>
<td>per3</td>
<td>rigui</td>
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<tr>
<td>Jun-B</td>
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<tr>
<td>Nr4a1a</td>
<td>N-10; gfpp; gfrp1; hbr-1; hmr1; np10; tr3; nar77; tis1</td>
<td>50</td>
</tr>
<tr>
<td>Homer1a,b,c</td>
<td></td>
<td>35.3</td>
</tr>
</tbody>
</table>

Six transcripts that are downregulated in PSD-95null mice have been reported to be upregulated 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.

1González-Maeso et al., 2003; Yuen et al., 2003.


3The 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.
these neurons, as impairment of 5-HT\textsubscript{2A} trafficking to dendrites should result in a relative accumulation of receptors in the neuronal cell body. As predicted, Figure 4 B confirms that the CB/D ratio is higher in PSD-95\textsuperscript{null} neurons. If PSD-95 is essential for 5-HT\textsubscript{2A} expression and sorting to the dendrites, reintroduction of PSD-95 should increase receptor expression in both the neuronal soma and dendrites. Furthermore, PSD-95 re-expression should decrease the CB/D ratio, representing an increase in dendritic targeting of receptor. To assess the effect of re-expression of PSD-95 on 5-HT\textsubscript{2A} expression and targeting, we generated PSD-95–GFP lentivirus and a control GFP lentivirus and infected cortical neuronal cultures prepared from PSD-95\textsuperscript{null} animals (Fig. 5A). PSD-95–GFP expression led to an ~2-fold increase in cell body 5-HT\textsubscript{2A} expression and an ~5-fold increase in dendritic 5-HT\textsubscript{2A} expression compared with GFP expression in neurons prepared from the same PSD-95\textsuperscript{null} animals (Fig. 5B). Furthermore, as predicted, the CB/D ratio is greatly decreased in PSD-95\textsuperscript{null} neurons expressing PSD-95–GFP compared with those expressing the control GFP (Fig. 5B).

### PSD-95 is required for 5-HT\textsubscript{2C} signaling in vivo

Having provided strong evidence that PSD-95 profoundly regulates the expression of 5-HT\textsubscript{2C} receptors, we next examined the consequences of knocking out PSD-95 on 5-HT\textsubscript{2C} function in vivo. It is well established that \textit{c-fos} is an immediate early gene (IEG) which is transcribed after GPCR activation (Lo and Wong, 2007). PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{null} mice were injected with a 5 mg/kg dose of the prototypical 5-HT\textsubscript{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\textsuperscript{null} animals compared with PSD-95\textsuperscript{wildtype} animals (Fig. 7A). Head twitches are virtually nonexistent in both PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{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\textsubscript{1A} receptors in the CNS (Martin et al., 1992), leads to the same decrease in temperature in both PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{null} mice (Fig. 7A). Together, the findings suggest that PSD-95 selectively affects behaviors mediated by 5-HT\textsubscript{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\textsubscript{2A}–mediated signaling would be reduced or absent in PSD-95\textsuperscript{null} mice. The 5-HT\textsubscript{2A} receptor has been shown to signal through a large number of canonical (phospholipase C\textsubscript{B}) and noncanonical (phospholipase A\textsubscript{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\textsubscript{2A} receptor activate those pathways with similar potencies (Nichols, 2004). 5-HT\textsubscript{2A} agonists have been shown to activate ERK1/2 via a number of different mechanisms (Herschenson et al., 1995; Greene et al., 2000; Quinn et al., 2002; Gööz et al., 2006), and 5-HT\textsubscript{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 phosphorylation of GSK3β (p-GSK3β) after treatment with DOI (5 mg/kg) would be reduced or absent in PSD-95\textsuperscript{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\textsuperscript{null} mice (Fig. 7B–D). Thus, our evidence suggests that PSD-95 plays an important role in mediating 5-HT\textsubscript{2A} downstream signaling, and its absence results in signaling and behavioral abnormalities.

### Conclusion

PSD-95 is essential for hallucinogen actions in vivo

We also predicted that the alterations in 5-HT\textsubscript{2A} expression induced by deleting PSD-95 should lead to a reduction in hallucinogenic actions in vivo. Although a number of animal models have been proposed for studying hallucinogenic action in rodents (Nichols, 2004), head-twitch behavior has been shown to be the most specific for hallucinogenic action in that nonhallucinogenic 5-HT\textsubscript{2A} agonists such as lisuride do not induce the behavior (González-Maeso et al., 2007). 5-HT\textsubscript{2A}– and 5-HT\textsubscript{2C}–mice were injected with a 5 mg/kg dose of the prototypical 5-HT\textsubscript{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\textsuperscript{null} animals compared with PSD-95\textsuperscript{wildtype} animals (Fig. 7A). Head twitches are virtually nonexistent in both PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{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\textsubscript{1A} receptors in the CNS (Martin et al., 1992), leads to the same decrease in temperature in both PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{null} mice (Fig. 7A). Together, the findings suggest that PSD-95 selectively affects behaviors mediated by 5-HT\textsubscript{2A} receptors.

Figure 3. Genetic deletion of PSD-95 leads to an accelerated turnover of 5-HT\textsubscript{2A} receptor protein. A and B represent fitted curves modeling 5-HT\textsubscript{2A} receptor turnover in PSD-95\textsuperscript{wildtype} and PSD-95\textsuperscript{null} mice, respectively (\(N = 3–4\) littermate pairs at each data point). Visual inspection shows that steady-state levels for the 5-HT\textsubscript{2A} receptor are reached sooner in the absence of PSD-95, suggesting accelerated turnover. The higher \(k\) in PSD-95\textsuperscript{null} cortex indicates a higher rate of receptor turnover in the absence of PSD-95. Rate constant, \(k\).
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., 2004a), 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 (ASS0) 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-
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Lentivirus (top 2 rows of panels) or PSD-95–GFP lentivirus (bottom 2 rows of panels). Figures show that PSD-95–GFP is rescuing dendritic targeting of 5-HT2A receptor expression.复数

Representative images of double-label immunochemistry performed on P0.5 cortical neurons of PSD-95null mice infected with either GFP lentivirus (top 2 rows of panels) or PSD-95–GFP lentivirus (bottom 2 rows of panels). PSD-95null 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-HT2A receptor expression in an infected neuron. White arrows highlight 5-HT2A receptor expression in an uninfected neuron. GFP-infected neurons display low overall 5-HT2A expression and low dendritic targeting. In contrast, PSD-95–GFP-infected neurons display a dramatic increase in overall 5-HT2A 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.

Discussion

The main finding of this study is that PSD-95 is essential for serotonin receptor function and hallucinogen 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 metabotropic 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-HT2A and 5-HT2C receptors in vivo. In the absence of PSD-95, the 5-HT2A 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-HT2A-mediated signaling (p-ERK1/2 and p-GSK3β) and behaviors (e.g., head-twitch response). In the absence of PSD-95, the 5-HT2C 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-HT2A antagonists M100907 and SR46349B, are unable to mediate their therapeutic effects in animal models of psychosis.

5-HT2C receptors, PSD-95, and psychiatric disease

There is considerable evidence that 5-HT2C serotonin receptors regulate hippocampal function. Genetic evidence from 5-HT2C knockout mice shows that long-term potentiation (LTP) is impaired in the dentate gyrus in the absence of 5-HT2C receptors (Tecott et al., 1998). 5-HT2C knockout mice also exhibit defects 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-HT2C receptors modulate neuronal function, nothing is known regarding their targeting and regulation by 5-HT2A receptors 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 known to involve limbic recruitment. As expected, SR46349B (1 mg/kg) normalizes PCP-induced hyperlocomotion in PSD-95wildtype but not PSD-95null mice (Fig. 8D). Thus, our findings are very consistent in showing that the genetic deletion of PSD-95 leads to 5-HT2A 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<sub>2C</sub> receptor’s interaction with, and regulation by, PSD-95 suggests that the 5-HT<sub>2C</sub> 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<sub>2C</sub> function in the hippocampus, the potential relevance of our findings to 5-HT<sub>2C</sub> function in other brain regions is also of interest. The 5-HT<sub>2C</sub> 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<sub>2C</sub> 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<sub>2C</sub> 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<sub>2A</sub> receptor (Glennon et al., 1983), the signaling processes underlying hallucinogen action are still poorly characterized. Recent evidence suggests that cortical 5-HT<sub>2A</sub> 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<sub>2A</sub> 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, Figure 6. Deletion of PSD-95 attenuates 5-HT<sub>2C</sub> receptor-mediated induction of c-fos. **A**, 5-HT<sub>2C</sub> and c-fos double-label immunohistochemistry in the hippocampus of PSD-95<sup>wildtype</sup> and PSD-95<sup-null</sup> mice after MK-212 treatment (N = 3 littermate pairs). Representative images of CA1 and CA2 are shown. There are fewer c-fos-positive cells in the PSD-95<sup-null</sup> mice treated with MK-212 in both CA1 and CA2. **B**, Higher magnification image of CA1 to examine colocalization of 5-HT<sub>2C</sub> receptors and c-fos. 5-HT<sub>2C</sub> receptor colocalizes with c-fos, suggesting that 5-HT<sub>2C</sub> is inducing this IEG directly, rather than indirectly in surrounding neurons. **C**, Analysis of c-fos induction was performed by counting the number of c-fos-positive cells in CA1, CA2, CA3, and DG. Data are presented as the mean number of c-fos-positive cells ± the SEM. There is a significant reduction in c-fos-positive cells in the absence of PSD-95 in all four regions that were measured. c-fos counts were performed separately in the hippocampus of each hemisphere (2 values for each section analyzed). Every seventh section was analyzed, for a total of six sections per animal. *p < 0.05, **p < 0.01, ***p < 0.001; one-tailed unpaired t test.
Importantly, our studies demonstrate that the overall expression and apical dendritic targeting of 5-HT2A receptors to postsynaptic densities is significantly impaired in cortical neurons prepared from PSD-95null mice. Also, DOI is unable to induce p-ERK1/2 and p-GSK3β in 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 PSD-95null neurons rescues both the deficient expression and targeting phenotype. Our data provide a mechanism whereby 5-HT2A 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-HT2A 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 psychotomimetic 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-HT2A inverse agonist actions ameliorate PCP-induced PPI deficits (Carlsson et al., 1999; Yamada et al., 1999; Geyer et al., 2001; Linn et al., 2003).
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-HT_2A 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-HT_2A receptors are mistargeted and mislocalized, we predicted that there may be abnormalities in the ability of clozapine and selective 5-HT_2A antagonists to alleviate PCP-induced psychotomimetic-like behaviors in mice. We found that clozapine, M100907, or SR46349B treatment, which reduced PCP-induced deficits of PPI in PSD-95null mice, was completely ineffective in PSD-95wildtype mice. The dramatically impaired antipsychotic-like action of the aforementioned atypical antipsychotics in the PSD-95null mice is likely attributable to the combined abnormalities in 5-HT_2A and 5-HT_2C 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-HT_2A dysfunction that results in the absence of PSD-95 as being responsible for the lack of atypical antipsychotic efficacy in PSD-95null 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_2A receptors and 5-HT_2C receptors, both of which are important therapeutically for a number of psychiatric diseases. We also show that the 5-HT_2A 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_2A and 5-HT_2C 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 ionotopic and metabotropic neurotransmission at postsynaptic densities.

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