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Identification of PSD-95 as a Regulator of Dopamine-Mediated Synaptic and Behavioral Plasticity

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Summary

To identify the molecular mechanisms underlying psychostimulant-elicited plasticity in the brain reward system, we undertook a phenotype-driven approach using genome-wide microarray profiling of striatal transcripts from three genetic and one pharmacological mouse models of psychostimulant or dopamine supersensitivity. A small set of co-affected genes was identified. One of these genes encoding the synaptic scaffolding protein PSD-95 is downregulated in the striatum of all three mutants and in chronically, but not acutely, cocaine-treated mice. At the synaptic level, enhanced long-term potentiation (LTP) of the fronto-cortico-accumbal glutamatergic synapses correlates with PSD-95 reduction in every case. Finally, targeted deletion of PSD-95 in an independent line of mice enhances LTP, augments the acute locomotor-stimulating effects of cocaine, but leads to no further behavioral plasticity in response to chronic cocaine. Our findings uncover a previously unappreciated role of PSD-95 in psychostimulant action and identify a molecular and cellular mechanism shared between drug-related plasticity and learning.

Introduction

Alterations of the brain reward system following chronic exposure to addictive drugs underlie the neural mechanisms related to addiction (Koob and Le Moal, 1997; Berke and Hyman, 2000; Laakso et al., 2002; Wise, 2002). A compelling model of behavioral plasticity thought to underlie certain aspects of addiction is the progressive and enduring escalation in psychomotor responses following repeated psychostimulant exposures, known as "behavioral sensitization" or "reverse tolerance" (Woolverton and Johnson, 1992; Robinson and Berridge, 1993; White and Kalivas, 1998; Nestler, 2001). This phenomenon can be elicited by a variety of agents with distinct profiles of molecular action and can be generalized across many drugs of abuse. Psychostimulants act directly on monoamine systems; for example, cocaine blocks the activity of transporters for dopamine (DA), norepinephrine (NE), and serotonin (5-HT), resulting in elevated extracellular levels of monoamines (Amara and Kuhar, 1993). Adaptive changes within the striatum/nucleus accumbens and two of its prominent converging afferents, the midbrain dopaminergic and the cortical glutamatergic inputs, are believed to contribute to the behavioral plasticity (Wolf, 1998; Vanderschuren and Kalivas, 2000; Nestler, 2001; Gerdeman et al., 2003; Tzschentke and Schmidt, 2003).

After chronic psychostimulant administration, striatal dopamine release from presynaptic terminals may be increased, suggesting hyperresponsiveness of the mesolimbic dopamine pathway (Woolverton and Johnson, 1992). However, sensitization to psychostimulants can occur without increased dopamine release, as evidenced by similar sensitizing effects of direct DA agonists (Wise and Leeb, 1993). Furthermore, behavioral supersensitivity can also be produced by DA receptor antagonists (Burt et al., 1977), by genetic or pharmacological depletion of DA (Creese et al., 1977; Rubinstein et al., 1988; Kim et al., 2000), or by chronic antidepressant treatment (Spyraki and Fibiger, 1981; D'Aquila et al., 2000). Enhanced sensitivity of postsynaptic dopamine receptors mediated by components of GPCR signaling (Gainetdinov et al., 2003; Rahman et al., 2003) and adaptations of further downstream mechanisms related to transcription, intracellular signaling pathways, and neuronal structures in the striatum are believed to be primarily responsible for these behavioral manifestations (reviewed in Berke and Hyman, 2000; Nestler, 2001). Molecular manipulation of some adaptations per se in rodents effectively recapitulates or attenuates the behavioral responses to drug challenges (Carlezon et al., 1998; Kelz et al., 1999; Bibb et al., 2001), supporting the notion that an enhancement in dopamine transmission is sufficient but not necessary for drug-dependent psychomotor plasticity. Finally, psychostimulant administration leads to long-lasting modifications of cortical glutamatergic efferent to the mesolimbic dopamine system, implicating glutamatergic mechanisms in the induction and maintenance of behavioral sensitization (White et al., 1995; Thomas et al., 2001; Ungless et al., 2001). While each of the above mechanisms may contribute to certain aspects of behavioral supersensitivity, common underlying molecular and cellular mechanisms may exist that have yet to be identified.

Several genetically engineered mice targeting monoamine transporters, while differing in their monoamine homeostasis and dynamics, all display heightened sensitivity to either direct or indirect dopamine agonists (Figure 1B). Plasma membrane monoamine transporters control the homeostasis and transmission of monoamines through recapturing the released neurotransmitters, while vesicular monoamine transporters (VMAT) load DA, NE, and 5-HT into synaptic vesicles (Amara and

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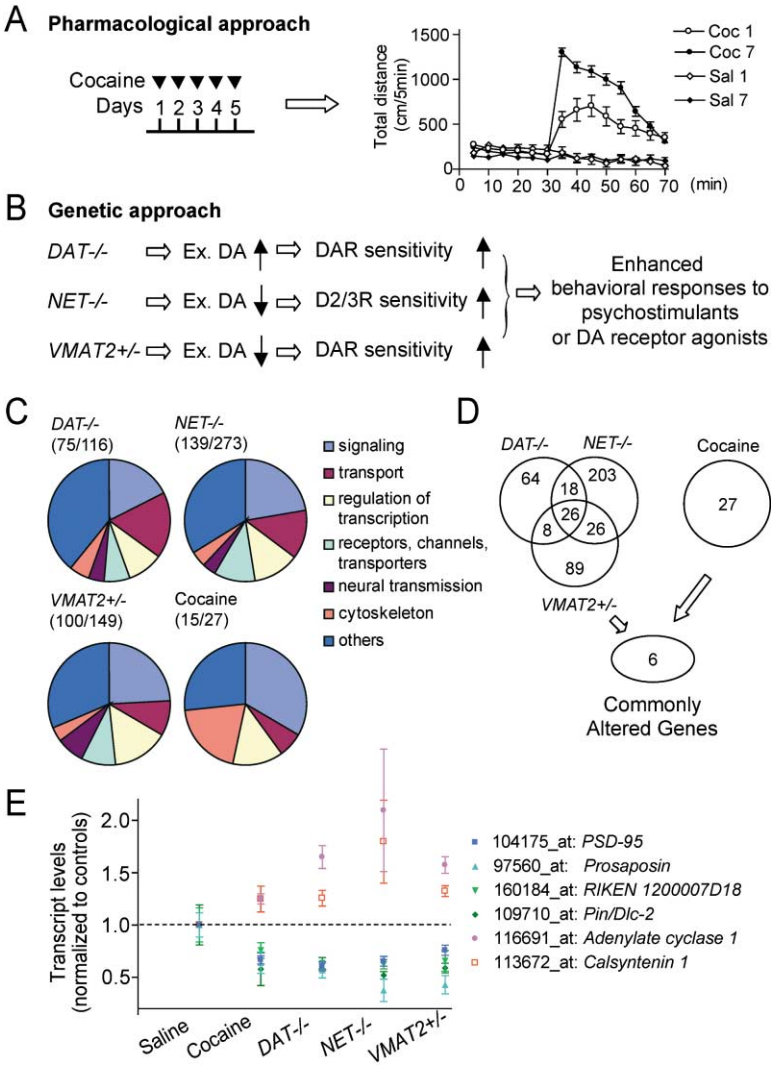

Figure 1. Microarray Experiments

(A) Pharmacological model of sensitization. C57BL/6J wild-type mice were injected daily with cocaine (20 mg/kg, i.p.) or saline for 5 days. Locomotor activity measurements were performed on day 1 and day 7 (n = 8–22 for each group). Saline or cocaine challenges were given at the 30 min mark. Note the significantly enhanced locomotor responses to the same dose of cocaine challenge on day 7 compared to day 1.

(B) Genetic models of behavioral supersensitivity: summary of neurochemical and behavioral pharmacological characteristics. See Introduction for details.

(C) Functional breakdown of microarray-identified genes in the striatum of three transgenic mutants and cocaine-treated mice. Numbers in parentheses represent the number of annotated genes versus total number of genes. The complete gene lists are shown in Supplemental Tables S1–S4 at http://www.neuron.org/cgi/content/full/41/4/625/DC1.

(D) Venn diagram showing the overlapping genes in the striatum of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice. Numbers in the overlapping regions represent commonly regulated genes.

(E) Relative expression levels of the six commonly affected genes identified in (C). Probe set ID and gene descriptions are shown.

Kuhar, 1993). Targeted inactivation of the DA transporter (*DAT*$^{-/-}$) leads to disrupted clearance of DA and 5-fold elevation in extracellular DA concentration (Giros et al., 1996; Gainetdinov et al., 2002). Lack of the major target of psychostimulants in *DAT*$^{-/-}$ prevents assessment of locomotor-stimulating (Gainetdinov et al., 1999b), but not rewarding (Rocha et al., 1998), effects of these drugs. However, responses to direct DA agonists suggest that despite marked downregulation of major DA receptor populations, some postsynaptic responses are in fact increased (Gainetdinov et al., 1999a). NE transporter knockouts (*NET*$^{-/-}$) demonstrate prolonged synaptic NE clearance and elevated extracellular NE but diminished Striatal DA concentration, presumably underlying the postsynaptic D2/D3 DA receptor supersensitivity observed in these mice (Xu et al., 2000). While *VMAT2*$^{-/-}$ mice die shortly after birth, *VMAT2*$^{+/-}$ mice show diminished extracellular DA levels and release and display enhanced sensitivity to direct and indirect DA receptor agonists as well as ethanol (Wang et al., 1997). These mice, along with normal mice treated with repeated intermittent psychostimulants (Figure 1A), offer an excellent set of genetic and pharmacological models to explore the general mechanisms underlying core features of behavioral plasticity elicited by psychostimulants.

Here we undertook a phenotype-driven, microarray-based genomic approach to search for the common mechanisms underlying this form of behavioral plasticity using *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ as well as mice chronically treated with cocaine. We demonstrate a concurrent diminution of PSD-95 and enhancement of nucleus accumbens LTP in both the genetically and pharmacologically "sensitized" mice. Reciprocally, functional ablation of PSD-95 in mice enhances LTP, augments the stimulating effects of acute cocaine, and prevents any further increase in locomotor response to cocaine following repeated administration. Our data identify a molecular and cellular mechanism common to several forms of behavioral supersensitivity and illustrate a novel paradigm by which drugs of abuse can usurp the reward circuits through altering the PSD-95-mediated glutamatergic synaptic plasticity.

Results

Microarray Screen for Commonly Affected Striatal Genes

To obtain striatal gene expression profiles for each strain of mice in a uniform genetic background, we back-

crossed each strain of mutants for at least 10 generations to derive *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ on a pure C57BL/6J background. This strategy would minimize the influence of genetic background on gene expression (Sandberg et al., 2000). To derive an expression profile that emphasizes the lasting effect of chronic cocaine administration, we treated C57BL/6J wild-type mice with cocaine (20 mg/kg/day, i.p.) for 5 consecutive days and allowed a 14-day withdrawal period from the drug before microarray analyses. This standard protocol (Wang et al., 1997; Xu et al., 2000; Gainetdinov et al., 2003) produces robust behavioral sensitization that lasts for weeks. Striatal mRNA was harvested from the five congenic mouse strains, biotinylated cRNA prepared, and Affymetrix mouse GeneChips representing ~36,000 gene clusters (MG_U74v2 Set) used to probe the labeled cRNA. Three independent RNA samples, each pooled from five mice, were prepared for each condition.

We used a pair-wise comparison algorithm to identify candidate genes that exhibit altered expression in the striatum of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice relative to saline-treated wild-type controls. This strategy recognizes genes that show highly consistent changes at the probe level and at least 20% change in overall expression levels (see Supplemental Experimental Procedures at http://www.neuron.org/cgi/content/full/41/4/625/DC1). We identified 116, 273, 149, and 27 transcripts in *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice, respectively (Figure 1C). We clustered the annotated genes into groups by biological function (Figure 1C and Supplemental Tables S1–S4). Expectedly, the majority of affected genes encode proteins involved in mechanisms of signaling, transport, transcription, ion channels and receptors, neural transmission, and cytoskeleton (Figure 1C). These genes account for 61.3%, 65.9%, 69.0%, and 73.3% of the total numbers of annotated genes affected in *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice, respectively.

We focused our analyses on overlapping genes. Overall, 44.8%, 25.6%, and 40.3% of genes that exhibit altered expression in *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ mice are also affected in at least one other mutant strain, respectively (Figure 1D). Twenty-six transcripts are altered in all three knockouts, six of which are also similarly altered in chronically cocaine treated mice, accounting for 22% of the 27 genes in this group (Figures 1D and 1E, Table 1). Three of the six genes encode, respectively, adenylate cyclase 1 (upregulated), a key component in the cAMP signaling pathway; Pin/Dlc-2 (protein inhibitor of neuronal nitric oxide synthase/dynein light chain-2, downregulated), a cytoskeletal protein that may participate in scaffolding in inhibitory synapses (Fuhrmann et al., 2002); and PSD-95 (downregulated), a postsynaptic scaffolding protein at excitatory glutamatergic synapses. The potential contribution of cAMP-dependent pathways and NO signaling to reward, addiction, and behavioral sensitization has been documented previously (summarized in Table 1), immediately validating our approach and results. More remarkably, cAMP and nitric oxide (NO) signaling and PSD-95 are all implicated in experience-dependent plasticity, such as learning and memory (Table 1). The remaining three genes encode, respectively, a postsynaptic calcium-sensing membrane protein (calsyntenin 1, upregulated), a neurotrophic and myelinotrophic glycoprotein (prosaposin, downregulated), as well as a protein of unknown function (RIKEN 1200007D18, downregulated).

We pursued PSD-95 for further investigation for the following reasons. First, PSD-95 plays an indispensable role in learning (Migaud et al., 1998), a process proposed to share similarities with the molecular mechanisms underlying reward and addiction (Berke and Hyman, 2000; Nestler, 2001; Schultz, 2002). Second, PSD-95 is an abundant postsynaptic density protein (Cho et al., 1992) at glutamatergic synapses, regulating the maturation (El-Husseini et al., 2000) and strength (Migaud et al., 1998; Beique and Andrade, 2003; Stein et al., 2003) of the excitatory synapses. The cortical glutamatergic system has been implicated in behavioral sensitization (Wolf, 1998; Thomas et al., 2001; Vanderschuren and Kalivas, 2000). Third, as a scaffolding protein of the membrane-associated MAGUK protein family, PSD-95 contains discrete protein-protein interaction domains, including three PDZ domains, an SH3 domain, and a guanylate kinase (GK) homology domain (Garner and Kindler, 1996) that participate in interactions with NMDA receptors, ion channels, and signal transduction molecules, thus organizing an intricate protein network into the postsynaptic density of excitatory synapses (Sheng and Kim, 2002). We reasoned that dysregulation of PSD-95 may alter the signaling profiles of many pathways in the synapse, ultimately leading to altered plasticity in neural circuits critical for reward.

Reduction of PSD-95 Transcripts and Proteins: Generality and Specificity

We validated the microarray results with two traditional methods. In situ hybridization using a PSD-95-specific probe reveals significant downregulation of PSD-95 in both the nucleus accumbens and the caudate putamen of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and chronic cocaine-treated mice (20 mg/kg/day, 5 days, i.p., 14-day withdrawal; Figures 2A and 2B). Using the more quantitative and accurate real-time RT-PCR assay, we found that the amount of PSD-95 mRNA was reduced to 65.7% $\pm$ 4.7%, 47.6% $\pm$ 2.7%, 52.0% $\pm$ 7.0%, and 58.3% $\pm$ 6.2% in the striatum of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and chronic cocaine-treated mice, respectively (Figure 2C).

We then examined PSD-95 protein using Western blot analysis. As shown in Figures 2D and 2F, total striatal PSD-95 proteins were diminished to 44.6%, 67.3%, and 55.7% in *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ mice, respectively, in comparison to wild-type littermates. Consistently, repeated cocaine administration induced a similar reduction (51.3% $\pm$ 8.3%, $p < 0.05$) in striatal PSD-95 protein levels (Figures 2E and 2F). The decrease of PSD-95 was not confined to either the caudate putamen or the nucleus accumbens of the striatum, as PSD-95 protein levels were diminished in both the dorsal and ventral structures (Figure 2D). Despite functional differences, both structures receive extensive, converging dopamine innervation from midbrain dopaminergic projections and glutamatergic input from the cortex.

The reduction in PSD-95 levels in the striatum could reflect an overall loss of glutamatergic synapses in this brain region. To evaluate this possibility, we analyzed the protein levels of several established presynaptic and postsynaptic markers (Figure 3). The amounts of synapsin (a synaptic vesicle protein), syntaxin (a presynaptic

Table 1. Genes Commonly Affected in the Striatum of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and Chronic Cocaine-Treated Mice Identified by Microarrays

GenBank ID	Description	Map Location	Changes	Cellular Function	Behavioral Associations
AW123151	Adenylate cyclase 1	11 A2	up	signal transduction, synaptic plasticity	learning and memory,[a] addiction,[b] pain processing[c]
D50621	PSD-95	11 B4	down	synaptic plasticity, scaffolding	learning,[d] pain processing[e]
AW047674	Pin/Dlc-2	11 C	down	NOS activity/NO signaling, microtubule-based process	learning and memory,[f,g] addiction,[f,h] responses to psychostimulants,[f,i] behavioral sensitization[f,i]
AW048171	Calsyntenin 1	4	up	Ca^{2+}-dependent process	unknown
AF037437	Prosaposin	10	down	neurotrophic factor, sphingolipids degradation	Gaucher's disease[j]
AA815795	RIKEN 1200007D18	17 A3.3	down	unknown	unknown

Map location: chromosome and position mapped with NetAffx (http://www.Affymetrix.com).
[a] Livingston et al., 1984; Wong et al., 1999
[b] Moore et al., 1998; Berke and Hyman, 2000; Nestler, 2001
[c] Wei et al., 2002
[d] Migaud et al., 1998
[e] Garry et al., 2003
[f] Based on modulation of NOS activity and NO signaling.
[g] Prast and Philippu, 2001
[h] Collins and Kantak, 2001; Gholami et al., 2002
[i] Wolf, 1998
[j] O'Brien and Kishimoto, 1991

plasma membrane protein), and NF-H (neurofilament heavy chain subunit, an axonal marker) did not differ between wild-type controls and any of the "genetically" or pharmacologically sensitized mice (Figure 3A), suggesting that the presynaptic structures are largely intact in the striatum of these mice. Similarly, postsynaptic proteins, represented by three PSD-95 homologs in the MAGUK family that includes chapsyn-110/PSD-93, SAP97, and SAP102 (Garner and Kindler, 1996) did not show discernable differences. Another postsynaptic protein, MAP-2 (microtubule-associated protein-2, a dendritic marker) did not differ in the genetically modified mice, but exhibited a nearly 2-fold increase in mice chronically treated with cocaine. This cocaine-induced increase in a dendritic structural protein is consistent with the increased spine densities and sizes following repeated cocaine administration (Robinson and Kolb, 1999). Consequently, the spines in the drug-treated group may be inevitably less enriched in PSD-95, which is expected to favor the formation of silent synapses (Stein et al., 2003). Together, our data suggest a selective decrease of a specific scaffolding protein, rather than a global loss of synapses, in the striatum of several behaviorally sensitized mouse strains.

Effects of Chronic Cocaine on PSD-95: Spatial and Temporal Characteristics

In addition to the striatum, the cortex and hippocampus are also key components of the reward circuits and are involved in drug-dependent behavioral plasticity (Berke and Hyman, 2000; Wise, 2002). We asked whether cocaine elicits PSD-95 dysregulation in these regions. PSD-95 transcripts were selectively decreased in the striatum of chronic cocaine-treated mice, including the nucleus accumbens and the caudate putamen, but were statistically unchanged in the cortex and hippocampus (Figure 2A and data not shown). Similarly, PSD-95 protein levels were also significantly decreased specifically in the striatum, but not in the cortex and hippocampus, of cocaine-treated animals (Figure 4A).

We next determined the number of cocaine injections needed to establish the sustained decline in PSD-95. Mice were given increasing number of cocaine injections and were analyzed for PSD-95 levels 14 days after the last injection. One or two injections of cocaine at 20 mg/kg/day did not induce noticeable changes in PSD-95 proteins (Figure 4B). In contrast, mice receiving three or more injections exhibited substantial decreases in PSD-95 protein levels compared to saline-treated controls. Interestingly, ten consecutive cocaine injections did not suppress PSD-95 protein levels more than fewer treatments (Figure 4B). Considering that mice receiving three or more cocaine injections developed sustained enhancement of responsiveness to cocaine (data not shown), these data suggest that striatal PSD-95 alterations might be correlated with the behavioral plasticity elicited by chronic, but not acute, cocaine administration.

One key characteristic of behavioral plasticity associated with repeated psychostimulant administration is its extremely long-lived nature. While considerable efforts have been made to identify the molecular mechanisms that contribute to the persistent neural and behavioral plasticity, specific changes identified so far are not sufficiently long lasting to account for the nearly permanent behavioral modification (Nestler, 2001). We thus explored the candidacy of PSD-95 downregulation as a mechanism underlying this phenomenon. Striatal PSD-95 levels were measured at different time of withdrawal following the standard 5 day sensitization paradigm in wild-type mice. The PSD-95 protein levels were significantly lower the next day (53% $\pm$ 6%, $p < 0.01$) following the last injection and continued to be significantly lower than saline-treated controls at 10–14 days and 28–35 days after the last cocaine dose (Figure 4C). Remark-

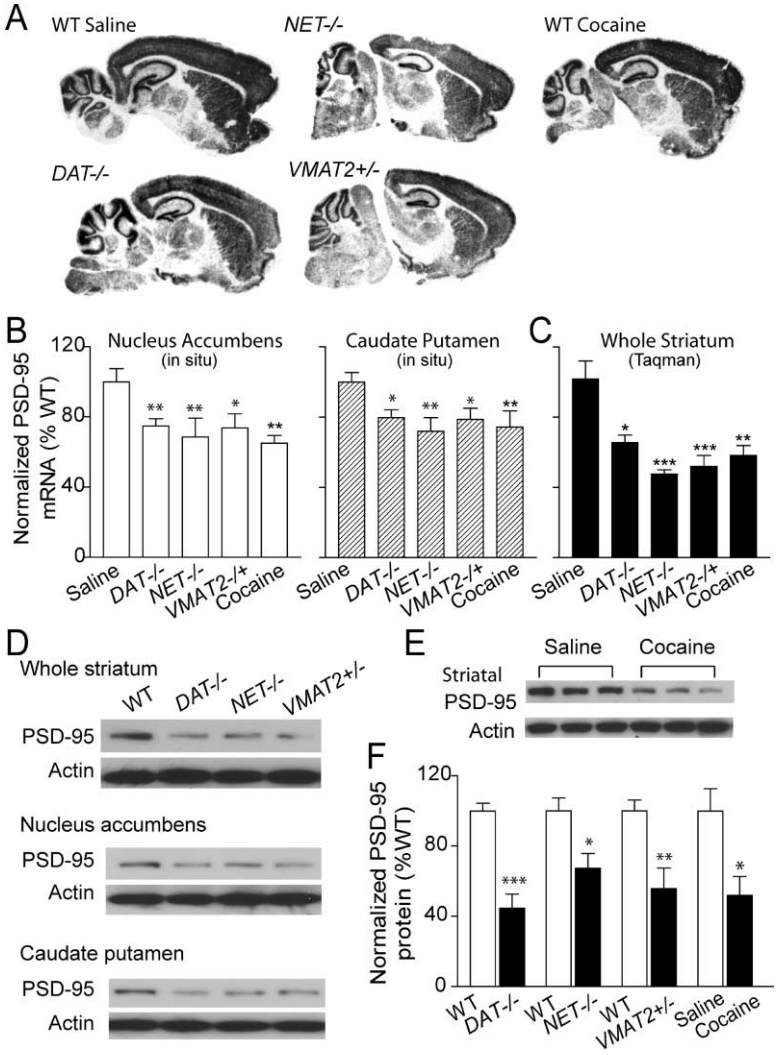

Figure 2. Assessment of mRNA and Protein Levels of PSD-95 in the Striatum of *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and Chronic Cocaine-Treated Mice

(A) In situ hybridization analysis of PSD-95 mRNA. Bright-field images of representative whole-brain sagittal sections are shown in which mRNA signals are in black.

(B) Quantification of mRNA signals of in situ hybridization reveals significant reduction of PSD-95 mRNA in the nucleus accumbens and caudate putamen in transgenic mutants (n = 4–5) and chronic cocaine-treated mice (n = 3) relative to saline-treated wild-type controls (n = 7).

(C) Real-time RT-PCR (Taqman) analysis of PSD-95 mRNA. Significant reduction of PSD-95 mRNAs was found in the whole striatum of transgenic mutants and chronic cocaine-treated mice relative to saline-treated controls (n = 5–10 mice for each group).

(D) Western blot analysis of extracts prepared from the whole striatum, caudate putamen, and nucleus accumbens shows lower amounts of PSD-95 proteins in transgenic mutants (n = 8–12) compared to wild-type controls (n = 10–14).

(E) Western blots show decreased striatal PSD-95 levels in cocaine-treated animals (n = 7) in comparison to saline controls (n = 8).

(F) Densitometric quantification of Western blots.

Except when specified otherwise, C57BL/6J males were treated with cocaine (20 mg/kg, i.p.) or saline for 5 consecutive days and brain mRNA and protein were analyzed 14 days after discontinuing cocaine treatments. *p < 0.05, **p < 0.01, ***p < 0.001; two-tail Student's t tests against wild-type controls.

ably, the PSD-95 levels remained at 66.7% ± 2.7% ($p < 0.05$) of the control levels even 2 months after the cessation of cocaine administration, without returning to normal levels (Figure 4C). Thus, modulation of PSD-95 at excitatory synapses could represent a long-lasting molecular adaptation that may contribute to the altered responsiveness to psychostimulants.

Altered Cortico-Accumbal Glutamatergic Synaptic Plasticity Associated with PSD-95 Reduction

We investigated whether the decreases in striatal PSD-95 levels in the "genetically" and pharmacologically sensitized mice translate to modification of synaptic plasticity. The excitatory cortico-accumbal glutamatergic pathway has been implicated in the expression of drug-induced sensitization (White et al., 1995; Wolf, 1998; Vanderschuren and Kalivas, 2000; Thomas et al., 2001) in particular and aberrant rewarding processes (Robbins and Everitt, 2002; Schultz, 2002) in general. We examined two parameters of synaptic plasticity of this pathway in adult mice: paired-pulse facilitation (PPF) and long-term potentiation (LTP). PPF is a sensitive measure of changes in residual Ca^{2+} dynamics and release probability, a form of short-term presynaptic plasticity. LTP reflects a persistent enhancement in synaptic strength in which both presynaptic and postsynaptic mechanisms might be involved (Bekkers and Stevens, 1990; Malenka and Nicoll, 1999).

The paired-pulse ratio did not differ at interpulse intervals of 20, 50, and 100 ms between *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ mice and wild-type controls (Figure 5A), nor between cocaine- and saline-treated wild-type mice (Figure 5B), suggesting no gross changes in presynaptic function in both the genetically and pharmacologically sensitized mice. In contrast, LTP, induced by a minimal tetanus protocol (1 s, 100 Hz), was substantially enhanced in slices prepared from *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ mice relative to their wild-type littermates (Figures 5C and 5D). Furthermore, LTP was also larger in slices from the cocaine group compared to the saline controls (Figures 5E and 5F). It is interesting to note, however, that while the posttetanic potentiation at both the early (immediately following tetanus) and the late (30 min posttetanus) time course are enhanced in the three mutants, only the late LTP is significantly enhanced by cocaine exposure (Figure 5F), suggesting

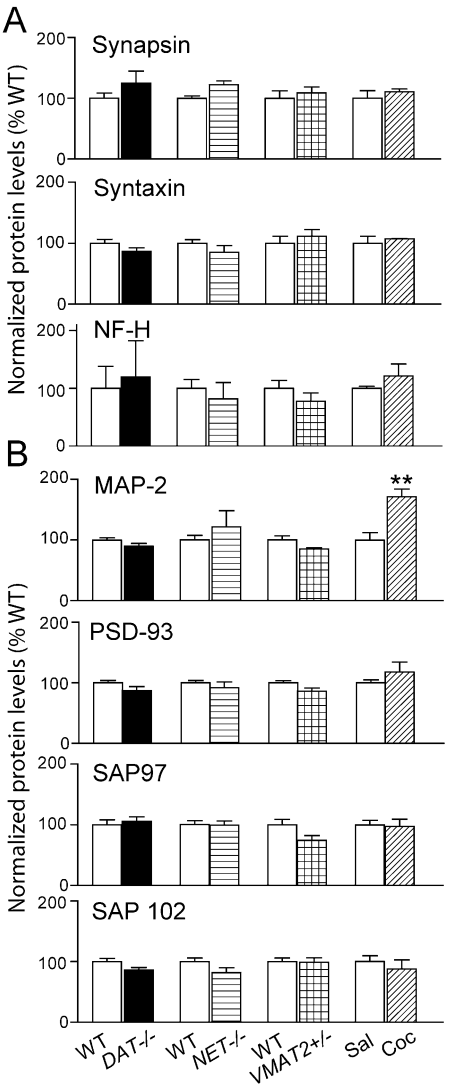

Figure 3. Western Blot Analysis of Presynaptic and Postsynaptic Protein Markers

(A) Western blot analysis of selected presynaptic marker proteins shows no differences among transgenic mutants, chronic cocaine-treated mice, and wild-type controls.

(B) Western blot analysis of selected postsynaptic proteins shows no differences among transgenic mutants, chronic cocaine-treated mice, and wild-type controls. One exception is MAP-2, which is increased by nearly 2-fold following repeated cocaine administration.

that the genetic models may not completely recapitulate the synaptic alterations caused by repeated cocaine administration.

To further explore whether the enhanced LTP is due to a reduction in PSD-95 levels, we studied the PPF and LTP in this cortical pathway in a genetically engineered mouse line (*PSD-95-GK*) in which the GK domain of the PSD-95 gene is deleted (Figure 6). Mice carrying the truncated form of PSD-95 gene (Figure 6A) do not produce any detectable PSD-95 proteins in the striatum, as revealed by two independent antibodies recognizing different protein sequences of PSD-95 (Figures 6B and 6C). The levels of other MAGUK proteins in the NMDA receptor complex (PSD-93 and SAP102) (Figure 6C) as well as NMDA receptor subunits (data not shown) were unaltered in the mutant mice. When compared to their wild-type littermates, *PSD-95-GK* mice showed no difference in PPF (Figure 6D) but demonstrated striking enhancements in tetanus-induced cortico-accumbal LTP throughout the posttetanic recording session (Figure 6E). Importantly, the complete deletion of PSD-95 in the mutant mice did not result in more LTP enhancements than those observed for *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice at 30 min posttetanus (Figure 6F; means ± SEMs are 133.0% ± 5.0%, 222.0% ± 28.5%, 188.6% ± 13.7%, 201.0% ± 37.1%, 155.8% ± 11.8%, and 175.4% ± 18.5% for control, *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, cocaine-treated, and *PSD-95-GK* mice, respectively). Thus, much like removing functional PSD-95 enhances LTP in the hippocampus (Migaud et al., 1998), decreasing the level of PSD-95 in the striatum may also account for the enhanced cortico-accumbal LTP.

Role of PSD-95 in Responsiveness to Psychostimulants and Drug-Dependent Behavioral Plasticity

We extended our molecular and electrophysiological studies to behavioral and pharmacological analysis of *PSD-95-GK* mice to investigate whether and how PSD-95 regulates the acute and chronic responsiveness to cocaine. In open field locomotor activity assays, unchallenged *PSD-95-GK* were hypoactive, displaying significantly lower horizontal (Figure 7A) and vertical (data not shown) activities and stereotypy (Figure 7B) than wild-type littermates. However, acute administration of cocaine elicited a significantly higher behavioral response in *PSD-95-GK* mice than the wild-type littermates at all doses tested (Figures 7C and 7D). The mutant mice exhibited a significantly higher locomotor activation, as measured by horizontal activity in response to 5 and 10 mg/kg cocaine than did wild-type littermates (Figure 7C). Cocaine at 20 mg/kg induced a horizontal activity that was similar to that of the wild-type response. However, this dose elicited significantly increased stereotypy, characterized by intensive sniffing, rearing, grooming, licking, and paw chewing in *PSD-95-GK* mice in comparison to wild-type littermates (Figure 7D). Stereotypy has been shown to compete with and antagonize locomotor activities at higher concentrations of psychostimulants and is an indication of higher degree of drug sensitivity. These data show that PSD-95 null mice are supersensitive to cocaine, indicating that selective reduction of PSD-95 levels at glutamatergic synapses is sufficient to confer the enhanced stimulating action of cocaine, mediated predominantly by monoamine systems.

To explore the role of the glutamatergic PSD-95 in behavioral sensitization, we examined the locomotor responses before and after a standard sensitization regimen that consists of five consecutive daily cocaine administration (Figure 8A). As shown in Figure 8, the chronic cocaine-induced behavioral plasticity is absent in *PSD-95-GK* mutants. Compared to the drug challenge prior to the treatment regimen, wild-type animals exhibited enhanced locomotor responses to cocaine on day 7, indicative of the development of sensitization. In contrast, PSD-95 mutants displayed nearly identical loco-

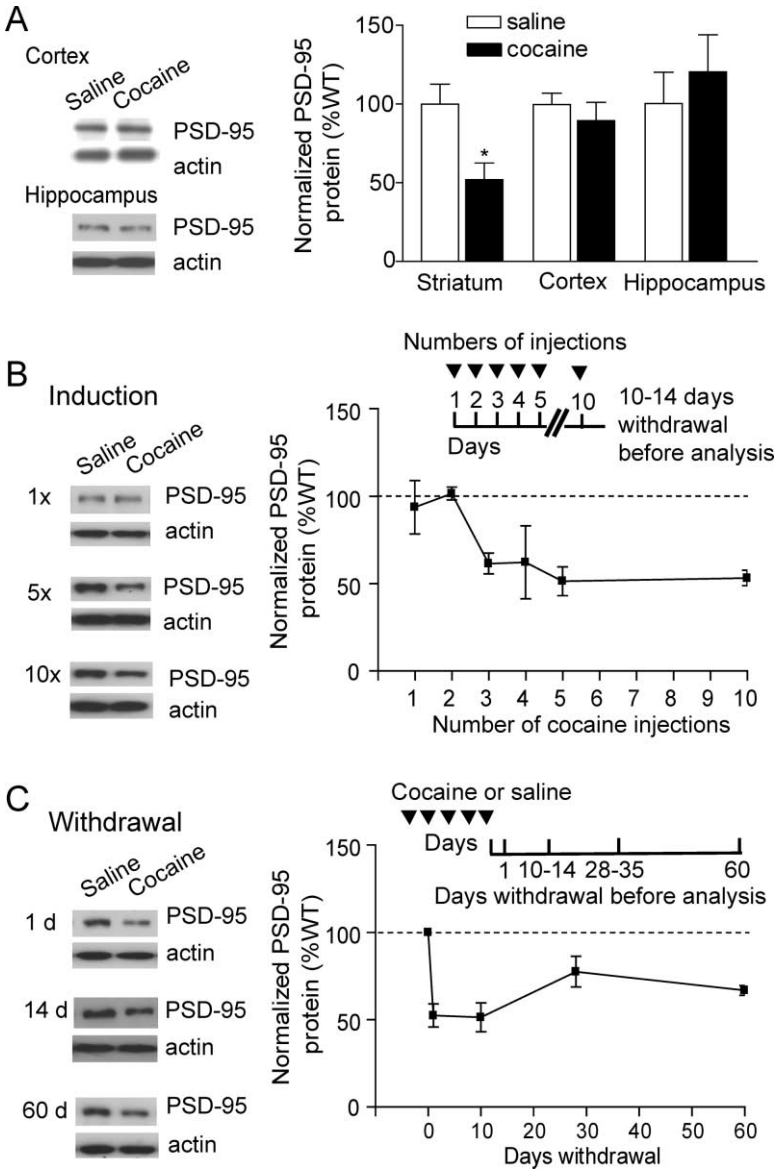

Figure 4. Spatial and Temporal Characteristics of Striatal PSD-95 Following Repeated Cocaine Administration

(A) PSD-95 protein levels were reduced in the striatum, but not in the cerebral cortex and hippocampus following 5-day cocaine administration and 14-day withdrawal (n = 5–9 mice for each group).

(B) Effects of increasing amount of cocaine exposures on PSD-95 levels in the striatum. Mice that received consecutive daily cocaine injections over 3, 4, 5, or 10 days demonstrated significantly lower levels of PSD-95 proteins in their striatum 10–14 days following the last injection when compared to saline controls. In contrast, PSD-95 levels were unaltered in the striatum of mice that received one or two cocaine doses.

(C) Long-lasting reduction in PSD-95 levels following cessation of chronic cocaine exposure. Mice were given daily cocaine for 5 days and allowed for various periods of withdrawal. The decrease of PSD-95 levels was evident 1 day, 10–14 days (pooled), 28–35 days (pooled), and persisted for at least 2 months following the cessation of the last cocaine dose (n = 4–8 saline- or cocaine-treated mice for each data point).

All results shown were from analyses of Western blots. Insets in (B) and (C) are treatment and analysis protocols.

motor responses to the same cocaine challenges before and after the sensitization protocol (Figures 8B and 8C). Thus, while supersensitive to the acute stimulating effect of cocaine, *PSD-95-GK* mice showed no evidence for further sensitization under this sensitization routine. The requirement of PSD-95 for the full expression of behavioral sensitization suggests that intact PSD-95-mediated mechanisms at glutamatergic synapses are an integral component of drug-dependent, monoamine-mediated behavioral plasticity.

Discussion

Striatal Transcript Profiles Associated with Monoamine Dysfunction

Genome-wide expression profiling provides a powerful unbiased approach to identify putative molecular and cellular changes that may underlie phenotypic properties of a cell or an organism (Mirnics et al., 2000; McGill et al., 2002; Whitfield et al., 2003). This approach is even more amenable to the comparison of multiple samples with identical or different phenotypes. In such studies with mouse CNS tissue, it would seem that starting with a homogeneous genetic background and multiple replicates would be essential to assure quality and reliability of the microarray data. In this study, all mice used for microarray analysis were C57BL/6J congenic mice and all experiments were repeated three times using independent samples. In addition, indirect validation of the observations presented here came from the fact that numerous genes previously implicated in psychostimulant-induced striatal neuroadaptations are contained in our data set (see Supplemental Tables S1–S4 at http://www.neuron.org/cgi/content/full/41/4/625/DC1). Examples include, but are not limited to, transcripts for cholecystokinin (Beinfeld et al., 2002), prodynorphin (Daunais and McGinty, 1994), brain-derived neurotrophic factor (Grimm et al., 2003), *per1* and *per2* (Abarca

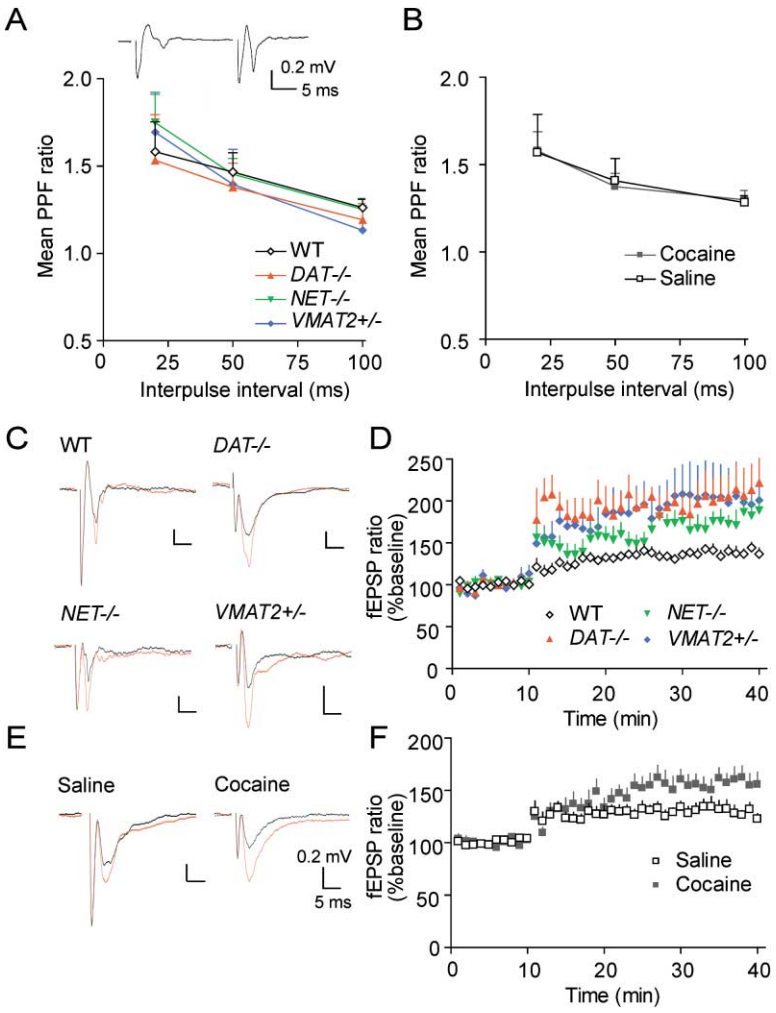

Figure 5. Altered Synaptic Plasticity in the Nucleus Accumbens in *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and Chronic Cocaine-Treated Mice

(A and B) Paired-pulse facilitation is normal. PPF was measured with pairs of presynaptic cortical stimulation pulses at intervals of 20, 50, and 100 ms. A representative recording from a wild-type slice is shown as inset. n = 6–16 slices from 4–7 mice for each data point.

(C and D) Enhanced LTP in *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ mice. LTP was induced by a high-frequency tetanus (100 Hz, 1 s) delivered at the 10 min mark. Representative traces during baseline recordings (black) and 30 min after the tetanus (red) are shown for each strain of mice (C) and summary graph is shown in (D). Data are from five *DAT*$^{-/-}$ slices (n = 5 mice), six *NET*$^{-/-}$ slices (n = 4 mice), seven *VMAT2*$^{+/-}$ slices (n = 6 mice), and ten wild-type slices (n = 7 mice), all on C57BL/6J background.

(E and F) Enhanced LTP in chronic cocaine-treated mice. Sample recordings are shown in (E) and summary graph for 7 saline slices and 9 cocaine slices are shown in (F). Adult C57BL/6J wild-type mice were treated with either cocaine (n = 5) (20 mg/kg, i.p.) or saline (n = 5) for 5 consecutive days and were used for recordings 2–3 days following the last injection.

et al., 2002), synaptotagmin IV (Yuferov et al., 2003), and Fos-like antigen 2 (Hope et al., 1994), as well as components of the MAPK, PKA, and CaMKII pathways. Further internal validation of the data set is the observation that different probe sets against the same gene clusters often produced the similar transcriptional changes (Supplemental Tables S1–S4). Finally, McClung and Nestler (2003) recently reported microarray analyses of the ΔFosB- and CREB-regulated genes using two inducible mouse models of cocaine reward. Interestingly, there is a striking overlap between the genes induced by ΔFosB and CREB overexpression and the genes altered by monoamine dysregulation in our study.

Our data reveals interesting features about the transcriptional profiles related to dysregulation of monoamine homeostasis and dynamics. First, among many biological processes defined by the Gene Ontology Consortium (http://www.geneontology.org), we found that alterations of monoamine homeostasis and signaling primarily affect a relatively restricted subset of processes, namely intracellular or transmembrane signal transduction, transport, transcriptional regulation, ion channels, receptors and transporters, neurotransmitters and synaptic transmission, as well as cytoskeletal rearrangement (Figure 1C and Supplemental Tables S1–S4 at http://www.neuron.org/cgi/content/full/41/4/625/DC1). Second, there are substantial overlaps of affected genes between different lines of mutants (Figure 1D), supporting our hypothesis that common molecular adaptations may occur in these mutants despite distinct lesions in their monoamine systems. Third, in several instances, different members of the same gene family are affected in different mutants, suggesting an overlap at the functional level. For example, the gene encoding the voltage-dependent calcium channel α 2/delta subunit 2 is upregulated in *DAT*$^{-/-}$, whereas the α 1A subunit of P/Q type is upregulated in both *NET*$^{-/-}$ and *VMAT2*$^{+/-}$ mice. Furthermore, more than one member of the same gene families is often altered in a mutant.

Five of the six genes commonly affected in the three mutants as well as cocaine-treated mice all encode synaptic or neuronal proteins. The subcellular localization, molecular function, and interacting partners of these proteins predict a high likelihood that they may play a role in neural development and/or plasticity. Adenylate cyclases are best known for their potential contributions to learning and memory (Livingstone et al., 1984; Wong et al., 1999), ethanol intoxication (Moore et al., 1998), pain processing (Wei et al., 2002), and addiction. Upregulation of the cAMP pathway is arguably the best-estab-

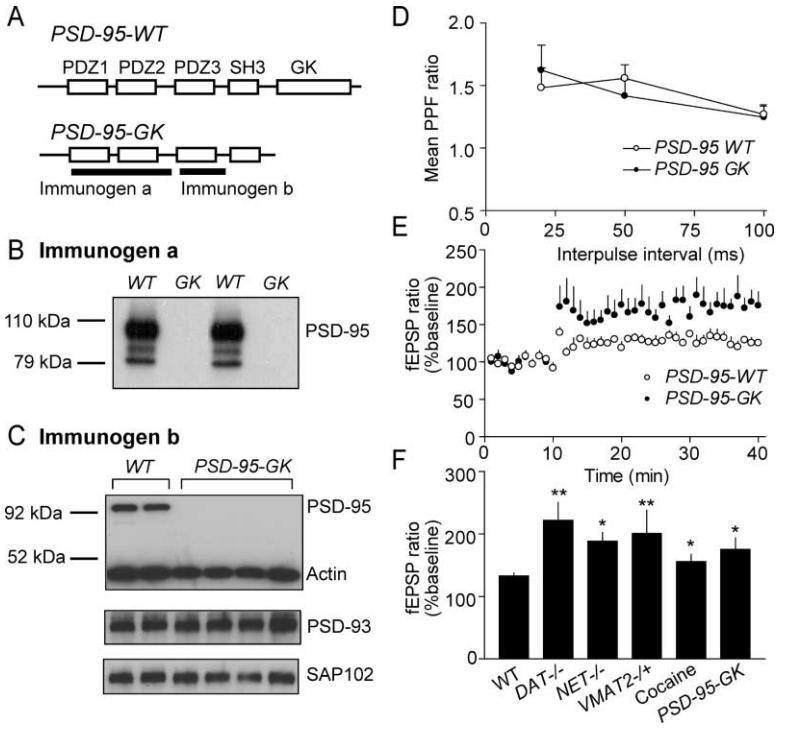

Figure 6. Assessment of Synaptic Plasticity in the Nucleus Accumbens of PSD-95 Null Mice

(A) The *PSD-95-GK* mice. A schematic diagram illustrating the targeted deletion of the GK domain of the PSD-95 gene in the mutant mice.

(B and C) Absence of striatal PSD-95 proteins in *PSD-95-GK* mice. Western blot analyses of striatal extracts using two antibodies against different regions of PSD-95 show that *PSD-95-GK* mutant mice do not produce any detectable PSD-95 proteins. Immunogen a recognizes the amino acid residues 77–299 at the N terminus; immunogen b recognizes the amino acid residues 353–504 within the PDZ3 domain. PSD-93 and SAP102 are not affected in the mutant mice.

(D) Paired-pulse facilitation is not affected in *PSD-95-GK* mice. n = 9–12 slices from 4–5 mice for each data point.

(E) Cortical-accumbal LTP is enhanced in *PSD-95-GK* mice. Mean ± SEM are presented for five *PSD-95-GK* slices (n = 4 mice) and four slices from the wild-type littermates (n = 4 mice).

(F) Summary of LTP data. LTP was measured at 30 min posttetanus and presented as mean ± SEM. Wild-type data used in this graph were combinations of C57BL/6J WT (Figure 5D) and PSD-95-WT mice (E). *$p < 0.05$, **$p < 0.01$; two-tail Student's t tests against wild-type.

lished adaptation to drugs of abuse such as morphine (Berke and Hyman, 2000; Nestler, 2001). The common increase of adenylate cyclase 1 expression in *DAT*$^{-/-}$, *NET*$^{-/-}$, *VMAT2*$^{+/-}$, and cocaine-treated mice is in line with this theme. Pin/Dlc-2, a close homolog of the original protein inhibitor of neuronal nitric oxide synthase (Pin or Dlc-1; Jaffrey and Snyder, 1996) may play important roles in drug responses and plasticity. Pharmacological inhibition of neuronal NO synthase has been shown to attenuate sensitization to cocaine and several drugs of abuse (reviewed in Wolf, 1998), as well as cocaine self-administration (Collins and Kantak, 2002) and morphine-induced place preference (Gholami et al., 2002). In our microarray analysis, striatal Pin/Dlc-2 is

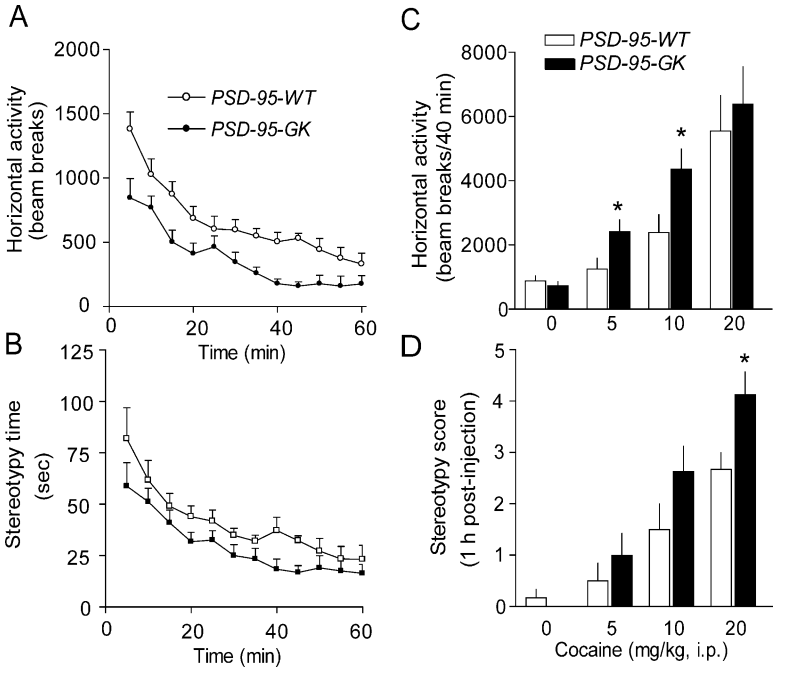

Figure 7. Enhanced Acute Responses to Cocaine in *PSD-95-GK* Mutants

(A and B) Locomotor activity in the open field of wild-type (n = 6) and *PSD-95-GK* (n = 8) mice. While habituating to a similar degree, both the horizontal activity (A) and stereotypy time (B) were lower in *PSD-95-GK* compared to wild-type mice.

(C) Locomotor responses to different concentrations of cocaine stimulation. Although less active before drug challenges, *PSD-95-GK* mice (n = 7–8) displayed significantly higher horizontal locomotor activities in response to 5 mg/kg and 10 mg/kg cocaine than did wild-type littermates (n = 6). Cocaine at 20 mg/kg elicited only slightly higher locomotor responses in *PSD-95-GK* (n = 10) mice when compared to wild-type mice (n = 9).

(D) Increased stereotypy in *PSD-95-GK* mice (n = 8), measured 60 min postinjection, in response to different concentrations of cocaine, compared to wild-type littermates (n = 6).

*$p < 0.05$; two-tail Student's t tests against wild-type littermates.

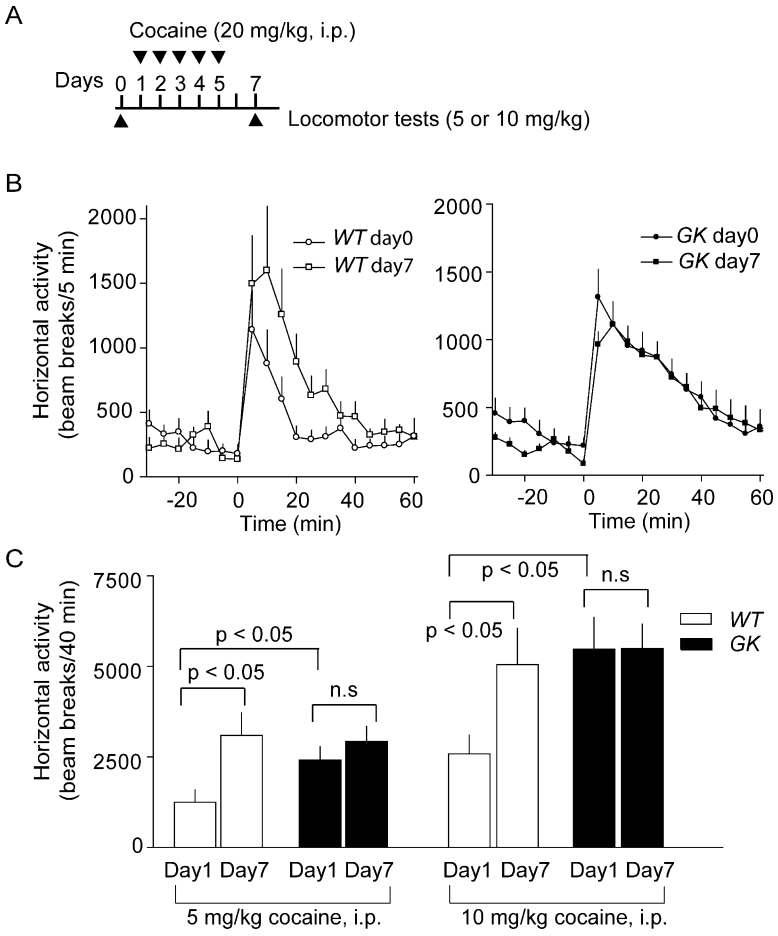

Figure 8. Absence of Cocaine-Induced Behavioral Plasticity in *PSD-95-GK* Mutants

(A) Sensitization regimen. Mice were first tested for their locomotor sensitivity to 10 mg/kg or 5 mg/kg cocaine on day 0. Mice were then injected daily with cocaine (20 mg/kg, i.p.) for 5 days. Mice were challenged on day 7 with 10 mg/kg or 5 mg/kg cocaine for the development of behavioral sensitization.

(B) Locomotor response of *PSD-95-GK* (n = 10) and wild-type (n = 7) littermates to cocaine administration (10 mg/kg, i.p., injected at 0 min) before and after the sensitization protocol. Wild-type mice displayed substantial potentiation in their response to the same test dose of cocaine following a sensitization regimen, while the PSD-95 mutants did not.

(C) Accumulated horizontal activity after cocaine administration (5 or 10 mg/kg, i.p.) on days 0 or 7 over a 40 min period (between 10 and 50 min postinjection), during which mice are fully activated (n = 7–11 mice in each group). Significance assessed by two-tail Student's t tests.

downregulated in every mutant and following cocaine exposure, which in principle may promote the expression of sensitization. Lastly, although the exact contributing molecular mechanisms have not previously been identified, changes in LTP and LTD in brain reward circuits have been reported, and changes in the levels of PSD-95 are known to lead to bidirectional modulation of synaptic plasticity (see below). Interestingly, adenylate cyclase 1, Pin/Dlc-2, and PSD-95 are all located on chromosome 11 (equivalent to human chromosome 17). The possible vulnerability of this chromosome to addictive drugs will require genetic linkage and gene association studies.

Under our filtering criteria, we found a surprisingly low number of genes that are consistently up- or downregulated 14 days after the 5 day cocaine administration regimen. Among 36,000 genes/EST clusters profiled, only 27 genes (or 0.08%) showed consistent alteration in the striatum of cocaine-treated mice. Moreover, no gene showed more than 2-fold changes relative to the saline controls. Strikingly, however, 11 of the 27 genes (40.7%) were also similarly affected in at least one of the mutants, and 6 genes (22.2%) were affected in all the mutants. These data suggest that while a 14-day withdrawal period has washed away many of the transient transcriptional adaptations caused by chronic cocaine, a significant portion of the remaining genes might be involved in core features of sensitization. The subtle transcriptional alterations in chronic cocaine-treated mice suggest that the robust behavioral plasticity in these animals may primarily depend on posttranscriptional mechanisms and modifications of related neural circuits.

Glutamatergic Plasticity Associated with Behavioral Sensitization

Generally, monoamines act as modulators of fast synaptic transmission such as glutamatergic neurotransmission. Our findings illustrate a profound modification of glutamatergic synaptic plasticity, highlighted by enhanced cortico-accumbal LTP, as a consequence of genetic or pharmacological perturbations of monoamine systems. Methamphetamine sensitization of rats converts corticostriatal LTD into LTP (Nishioku et al., 1999). Chronic cocaine administration elicits depressed AMPA receptor-mediated synaptic currents accompanied by a reduced long-term depression (LTD) in the nucleus accumbens (Thomas et al., 2001). Consistently, after chronic psychostimulant treatments, nucleus accumbens neurons become significantly less responsive to glutamate (White et al., 1995). Taken together, drugs of abuse may alter brain reward circuits by impairing the bidirectional plasticity of excitatory synapses.

A PSD-95-mediated dual-role scheme can explain the multifaceted glutamatergic synaptic modifications following chronic drug administration. First, a host of

receptors, ion channels, and signal transduction molecules interacting with PSD-95 at the postsynaptic density might be involved in synaptic plasticity (Husi et al., 2000; Sheng and Kim, 2002). Genetic dissection of the NMDA receptor-PSD-95 signaling complex shows that pathways controlling the bidirectional modification of glutamatergic synaptic strength are regulated by PSD-95 and its binding partners (Migaud et al., 1998; Komiyama et al., 2002). A targeted disruption of PSD-95 favors induction of hippocampal LTP (Migaud et al., 1998). Second, PSD-95 may control the synaptic AMPA receptor number, and thus basal transmission, independent of total available AMPA receptors (Schnell et al., 2002). The basal state and recent history of a synapse may influence its potential for undergoing subsequent plastic changes (Montgomery and Madison, 2002). Consistently, expression of exogenous PSD-95 increases synaptic AMPA receptors and occludes LTP and enhances LTD in both the cortex (Beique and Andrade, 2003) and hippocampus (Stein et al., 2003). If these two complementary mechanisms hold in the basal ganglia as suggested by the enhanced cortico-accumbal LTP in the *PSD-95-GK* mice, diminished PSD-95 levels could contribute to the observed decrease of basal glutamatergic transmission and reduced LTD, as well as enhanced LTP at cortico-accumbal synapses following repeated cocaine administration. Further investigation of the NMDA and AMPA currents in the genetically modified mice using whole-cell patch-clamping will help dissect the contribution of basal synaptic transmission and signaling complexes/pathways to the enhanced LTP observed in these mice.

Role of PSD-95 and Glutamatergic Transmission in Dopamine Signaling and Drug-Induced Behavioral Plasticity

Changes of both the dopamine and glutamate systems are implicated in drug-induced plasticity. While the contribution of dopaminergic transmission to behavioral sensitization has been recognized, how plastic changes of the glutamatergic systems contribute to this process remains elusive. The removal of PSD-95 in mice leads to two major behavioral manifestations. First, it augments the acute locomotor stimulating effect of cocaine, and second, it prevents the development of further sensitization following chronic cocaine administration. These data suggest that the changes in PSD-95 levels elicited by repeated drug exposure must serve as a contributing mechanism that promotes behavioral sensitization. This contrasts with the compensatory mechanism in which some drug-induced neuroadaptations counteract the behavioral manifestation. For example, chronic cocaine administration induces Cdk5 (Bibb et al., 2001), which appears to serve a homeostatic response to dampen sensitivity to subsequent stimulation.

Two potential mechanisms could underlie the PSD-95 regulation of psychostimulant responsiveness. First, PSD-95 may directly or indirectly interact with subtypes of dopamine receptors and affects dopamine signaling. For example, PSD-95 modulates the internalization of NMDA receptors (Roche et al., 2001), which in turn may interact with dopamine receptors (Lee et al., 2002) and influence the desensitization, trafficking, and signaling of dopamine receptors (Fiorentini et al., 2003). Second, glutamatergic and dopaminergic axons form a "synaptic triad" at postsynaptic dendritic spines in the striatum. This close spatial association may be required for the converging and coordinating actions of the two afferent pathways. The altered postsynaptic architecture due to the lack of PSD-95 may change the interplay of the two systems, engaging the glutamate synapses to a state more responsive to DA modulation. Aberrant DA-glutamate interaction is implicated in a number of dopamine-related disorders, including schizophrenia (Mohn et al., 1999; Carlsson et al., 2001). The potential role of PSD-95 in these brain disorders will require further investigation.

The failure to develop further sensitization to cocaine by PSD-95 null mice following repeated cocaine administration highlights the indispensable role of PSD-95-mediated glutamatergic mechanisms in drug-dependent behavioral plasticity. The loss of behavioral plasticity might be expected, given the predisposed cocaine supersensitivity in PSD-95 mutants. Lack of PSD-95 promotes LTP and may also maximally facilitate the dopamine modulation of cortical glutamatergic input, effectively uncoupling the glutamate system from additional dopamine system modulation. In this regard, the behavioral consequence of chronic drug exposure may be equivalent to reducing glutamatergic PSD-95, which occludes sensitization. Regardless of the mechanism, our work illustrates a molecular and cellular paradigm by which drugs of abuse can usurp the reward circuits through altering the cortical glutamatergic system, leading to profound behavioral alterations in drug action.

Molecular and Cellular Mechanisms Underlying Drug-Dependent Plasticity, Learning, and Memory

Persistent modification of synaptic strength, such as LTP and LTD, has been considered a cellular mechanism underlying learning and memory (Chen and Tonegawa, 1997; Kandel, 1997). Interestingly, half of the commonly affected genes identified in our sensitization-based forward genetic screen are also implicated in synaptic plasticity and learning and memory. Adenylate cyclase I regulates synaptic efficacy (Zhong and Wu, 1991) and learning and memory in *Drosophila* (Livingstone et al., 1984) and hippocampus-dependent LTP and memory in mice (Wong et al., 1999). Nitric oxide and NOS activity regulate LTP and LTD in several brain regions and appear essential in various memory tasks (reviewed in Prast and Philippu, 2001). PSD-95 mutation leads to altered hippocampal LTP and impaired spatial learning (Migaud et al., 1998), as well as altered striatal LTP and diminished drug-induced behavioral plasticity (this study). Together, our work provides direct support for the notion that the molecular and cellular mechanisms underling the long-lasting behavioral plasticity associated with psychostimulant action may be similar to those implicated in learning and memory (Berke and Hyman, 2000; Nestler, 2001; Schultz, 2002), and more importantly, identifies specific molecules to support this contention. It is likely that in both cases, impairment of the bidirectional synaptic plasticity at related synapses might deteriorate the performance of related brain reward circuits, leading to abnormal experience-dependent neural plasticity, including that induced by addictive drugs.

Experimental Procedures

Mice

All experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of animals and with an approved animal protocol from the Duke University Animal Care and Use Committee. Generation of *DAT*$^{-/-}$, *NET*$^{-/-}$, and *VMAT2*$^{+/-}$ was described previously (Giros et al., 1996; Wang et al., 1997; Xu et al., 2000). All three lines of genetically modified mice were backcrossed at least ten generations on the C57BL/6J background. Age-matched (3–5 month) C57BL/6J mice were used as controls to these three mutant strains. Detailed description of the generation of *PSD-95-GK* mutant mice will be reported elsewhere (M.I.A. and S.G.N.G.). PSD-95 wt and GK littermates were used for the experiments presented in Figures 6–8. Mice were housed at standard lab conditions (12 hr light/dark cycle) with food and water provided ad libitum.

Microarray Experiments

MIAME (minimal information about a microarray experiment)-compliant format regarding experimental design, sample and probe preparation, hybridization procedures and parameters, data analysis, and array design as defined in the guidelines established by MGED (http://www.mged.org) is detailed in Supplemental Data at http://www.neuron.org/cgi/content/full/41/4/625/DC1.

Real Time RT-PCR

The procedure for real-time RT-PCR has been described previously (Kim et al., 2002). One or two striatum per mouse were homogenized in RNA lyses buffer (PE Biosystems). Tissue homogenates were further processed to remove tissue debris followed by RNA isolations in an RNA purification tray, using the ABI Prism 6700 automated nucleic acid workstation (PE Biosystems). The detailed information of the PCR primers and fluorogenic probes for PSD-95, amplification parameters, and measurement and quantification is described in Supplemental Data at http://www.neuron.org/cgi/content/full/41/4/625/DC1.

In Situ Hybridization

Procedures for [^{35}S]UTP-labeled antisense cRNA probe preparation and in situ hybridization are detailed in Supplemental Data at http://www.neuron.org/cgi/content/full/41/4/625/DC1.

Biochemistry and Western Blots

Methods for protein extraction, Western blotting, antibodies, detection, and quantification are described in Supplemental Data at http://www.neuron.org/cgi/content/full/41/4/625/DC1.

Animal Treatments and Behavioral Assessments

Locomotion was evaluated in an automated Omnitech Digiscan apparatus (AccuScan Instruments) under illuminated conditions (Gainetdinov et al., 1999b). Mice were placed in an activity chamber and their horizontal and vertical activities were recorded at 5 min intervals. In each experiment, mice were habituated to the chamber environment and their activities monitored for 30 or 60 min. Each of the mice then received a dose of cocaine or saline and their locomotor activities were measured for 120 min. Stereotypy was detected using two measurements: computer-generated stereotypy time (Gainetdinov et al., 1999b) recorded by Omnitech monitors and ethological stereotypy score (McNamara et al., 2002) recorded visually. Stereotypy time refers to the total time that stereotypic behaviors (repetitive beam breaks of a given beam or beams with intervals less than 1 s) were observed (Gainetdinov et al., 1999b). Alternatively, behavioral responses in each mouse after cocaine or saline injections were recorded for 30 s with a camcorder every 15 min. Each animal was then evaluated by an independent investigator based on the recorded movie and received a stereotypy score using the conventional 0–6 point stereotypy scale as described (McNamara et al., 2002). In all experiments involving cocaine sensitization, mice were treated with cocaine (20 mg/kg) for 5 consecutive days (except specified otherwise) in home cages and their responses to the challenging dose of cocaine were analyzed 48 hr after the last injection. All injections were performed i.p. Age-matched mice received saline and served as controls.

Brain Slices and Electrophysiology

Following deep isofluorene anesthesia, adult mice (3–5 month) were quickly decapitated and the brain removed to ice-cold ACSF consisting of (in mM): 126 NaCl, 18 $NaHCO_3$, 1.6 KCl, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 11 glucose, saturated with 95% O_2/5% CO_2. Sagittal slices (200–400 μm) containing the nucleus accumbens were prepared using a vibrotome and stored in ACSF at room temperature. Following recovery for at least 1 hr, slices were transferred to a recording chamber filled with constantly perfused (1.5–2 ml/min) ACSF saturated with 95% O_2/5% CO_2. Picrotoxin (100 μM) was included to block $GABA_A$ receptors in all experiments.

Extracellular field recordings were made at the nucleus accumbens with a glass micropipette filled with 1 M NaCl ($\sim$5 MΩ). Signals were amplified with an Axoclamp 2B at the BRIDGE mode. Stimuli were delivered at 0.05 Hz with a bipolar stimulating electrode placed at the prefrontal cortex (PFC) near the PFC-accumbens border 0.5–3 mm dorsal to the recording electrode. At the beginning of each experiment, the stimulus intensity was adjusted to evoke a half-maximal field response. fEPSP amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak negativity, and the amplitude from the peak negativity to the peak late positivity. LTP was induced by tetanus that consisted of 100 pulses at 100 Hz at an intensity that induced maximum population spikes (approximately 1.5-fold of the test stimulus). Data were filtered at 3 kHz and digitized at 10 kHz using pCLAMP.

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