

# Repeated Cocaine Administration Decreases 5-HT<sub>2A</sub> Receptor-Mediated Serotonergic Enhancement of Synaptic Activity in Rat Medial Prefrontal Cortex

Chiung-Chun Huang<sup>\*1</sup>, Ying-Ching Liang<sup>2</sup>, Cheng-Che Lee<sup>2</sup>, Mei-Ying Wu<sup>1</sup> and Kuei-Sen Hsu<sup>\*,1,2,3</sup>

<sup>1</sup>Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; <sup>2</sup>Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan; <sup>3</sup>Center for Gene Regulation and Signal Transduction Research, National Cheng Kung University, Tainan, Taiwan

Neural adaptations in the medial prefrontal cortex (mPFC) are thought to be crucial in the development and maintenance of addictive behaviors. The mPFC receives a dense serotonergic (5-hydroxytryptamine, 5-HT) innervation from raphe nuclei and 5-HT exerts complex actions on mPFC pyramidal neurons. The present study, using a rat model of behavioral sensitization to cocaine, was designed to determine whether repeated cocaine exposure *in vivo* is capable of altering 5-HT-induced regulation of glutamatergic transmission in the mPFC. In layer V pyramidal neurons of the mPFC, application of 5-HT, through activation of 5-HT<sub>2A</sub> receptors, induced a massive enhancement of spontaneous excitatory postsynaptic currents (sEPSCs). Repeated cocaine administration for 5 days resulted in an attenuation in the ability of 5-HT to enhance sEPSCs. This effect was prevented when cocaine was co-administered with the selective 5-HT<sub>2A</sub> receptor antagonist ketanserin and was mimicked by repeated 5-HT<sub>2A</sub> receptor agonist (–)4-iodo-2,5-dimethoxyphenylisopropylamine administration. Repeated cocaine administration is not associated with any changes in the levels of 5-HT<sub>2A</sub> receptors or regulator of GTP-binding protein signaling 4. These results suggest that cocaine-induced inhibition of 5-HT<sub>2A</sub> receptor-mediated enhancement of glutamatergic transmission in the mPFC may be caused, at least in part, by the impairment of coupling of 5-HT<sub>2A</sub> receptors with GTP-binding proteins during cocaine withdrawal. These alterations in 5-HT<sub>2A</sub> receptor responsiveness in the mPFC may be relevant to the development of behavioral sensitization and withdrawal effects following repeated cocaine administration. *Neuropsychopharmacology* (2009) **34**, 1979–1992; doi:10.1038/npp.2009.10; published online 11 February 2009

**Keywords:** cocaine; serotonin; 5-HT<sub>2A</sub> receptor; glutamate; medial prefrontal cortex; addiction

## INTRODUCTION

Drug-induced long-lasting neural adaptations in the brain reward circuitry are widely proposed to contribute to several core features of addictive behaviors. There is now considerable evidence that neurotransmitter receptors, receptor-mediated signaling, intrinsic neuronal excitability, neuronal morphology, synaptic strength, and gene expression can be substantially altered in response to single or repeated exposure to addictive drugs (Nestler, 2002; Thomas and Malenka, 2003; Dong *et al*, 2005; Hyman

*et al*, 2006). Although the mesolimbic dopamine system, consisting of the ventral tegmental area and nucleus accumbens, is considered to be the major brain substrates for the neural adaptations that may underlie drug addiction (Hyman *et al*, 2006; Kauer and Malenka, 2007), several recent studies have shown that the neuronal excitability and synaptic functions of the medial prefrontal cortex (mPFC) were also changed during the development of addictive behaviors (Wolf, 1998; Steketee, 2003; Nasif *et al*, 2005; Peterson *et al*, 2006; Huang *et al*, 2007a, b), suggesting that mPFC is critically involved in drug addiction.

The mPFC receives a moderate to dense 5-hydroxytryptamine (5-HT) innervation originating from raphe nuclei of the midbrain in both primates and rodents (Azmitia and Segal, 1978). In addition, the raphe nuclei also receive reciprocal glutamatergic projections from the mPFC (Sesack *et al*, 1989). 5-HT has been reported to produce complex actions on pyramidal neurons of the mPFC presumably by acting at different receptor subtypes. For example, electrophysiological study has shown that 5-HT hyperpolarizes and depolarizes layer V pyramidal neurons of the mPFC by

\*Correspondence: Professor K-S Hsu, Department of Pharmacology, College of Medicine, National Cheng Kung University, No. 1, University Road, Tainan 701, Taiwan, Tel: +886 6 2353535 ext. 5498, Fax: +886 6 2749296, E-mail: richard@mail.ncku.edu.tw or Dr C-C Huang, Department of Pharmacology, College of Medicine, National Cheng Kung University, No. 1, University Road, Tainan 701, Taiwan, Tel: +886 6 2353535 ext. 5478, Fax: +886 6 2749296, E-mail: Chun19682003@yahoo.com.tw  
Received 23 September 2008; revised 5 January 2009; accepted 12 January 2009

acting on 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors, respectively (Araneda and Andrade, 1991). In addition, previous studies have shown that activation of 5-HT<sub>2A</sub> receptors in layer V pyramidal neurons of the mPFC can induce a rapid and robust increase in spontaneous glutamatergic synaptic activity (Aghajanian and Marek, 1997; Zhou and Hablitz, 1999; Lambe and Aghajanian, 2001; Marek *et al*, 2001; Béique *et al*, 2004, 2007), although multiple mechanistic interpretations have been proposed to account for such effect.

In addition to the well-recognized dopaminergic mechanisms in the development of cocaine addiction, an increasing number of evidence indicates that cocaine-induced alterations in 5-HT system in the brain may also contribute to behavioral sequelae of addiction. For example, behavioral studies have shown that withdrawal from intermittent cocaine administration enhances the 5-HT<sub>2A</sub> receptor-associated head-shake response (Baumann and Rothman, 1996) and locomotor activity (Filip *et al*, 2001). Furthermore, administration of 5-HT<sub>2A</sub> receptor antagonists has been reported to attenuate relapse to cocaine and cocaine-induced reinstatement of cocaine-seeking behavior (Fletcher *et al*, 2002; Burmeister *et al*, 2004). It is therefore reasonable to speculate that dysregulation of 5-HT<sub>2A</sub> receptor function would have a significant role in the development of cocaine addiction. To test this hypothesis, we examine the effect of repeated cocaine administration and withdrawal on the 5-HT<sub>2A</sub> receptor-mediated facilitation of glutamatergic transmission in mPFC layer V pyramidal neurons. Here, we provide the first evidence that withdrawal from repeated cocaine administration induces a severe impairment of 5-HT<sub>2A</sub> receptor responsiveness to regulate mPFC glutamatergic transmission.

## MATERIALS AND METHODS

### Animals and Treatment

Male Sprague-Dawley rats (21–23 days old) were housed in groups of four in a humidity- and temperature-controlled (22°C) vivarium on a 12 h light–dark cycle (light on at 0600 hours) with access to food and water *ad libitum*. After a 3-day acclimation period, animals were assigned randomly to two groups that received an intraperitoneal injection of saline (1 ml/kg) or cocaine HCl (15 mg/kg) once per day for 5 consecutive days followed by 1–14 day(s) of withdrawal before the experiments. During this time, they were handled and weighed daily. All comparisons between saline- and cocaine-treated groups were performed by experimenters blind to group assignment. All procedures were conducted in adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University. All efforts were made to minimize the number of animals used and their suffering.

### Locomotor Activity

Following each intraperitoneal injection of cocaine (15 mg/kg), (–)-4-iodo-2,5-dimethoxyphenylisopropylamine (DOI; 1 mg/kg), or saline, rats were immediately placed in the activity chamber and horizontal locomotor activity was

monitored with a video tracking system (EthoVision; Noldus, the Netherlands) for 20 min. Distance traveled was analyzed for estimates of locomotor response. After 2 days of saline injections to habituate the chamber, rats were divided into two groups that received five daily injections of cocaine, DOI, or saline (during 1000–1200 hours). To assess the persistence of the effect of repeated cocaine or DOI administration, following 3 days without injections, all groups received cocaine (15 mg/kg) injections and locomotor activities were assessed.

### Slice Preparation and Electrophysiology

Slice preparation and whole-cell patch-clamp recordings were conducted as described previously (Huang and Hsu, 2006). Briefly, rats were deeply anesthetized with halothane and decapitated with guillotine, and coronal slices (250  $\mu$ m thick) containing the prelimbic area of the mPFC (2.0–3.5 mm from the bregma) were prepared using a vibrating microtome (Leica VT1000S or VT1200S; Leica, Nussloch, Germany). The slices were placed in a holding chamber of aCSF oxygenated with 95% O<sub>2</sub>–5% CO<sub>2</sub> and kept at room temperature for at least 1 h before recording. The composition of the aCSF solution was (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, and 11 glucose at pH 7.3–7.4 and equilibrated with 95% O<sub>2</sub>–5% CO<sub>2</sub>. For whole-cell patch-clamp recording, one slice was transferred to a submerged recording chamber and fixed at the glass bottom of the chamber with a nylon grid on a platinum wire frame. The chamber consisted of a circular well of low volume (1–2 ml) and was perfused constantly at 32.0  $\pm$  0.5°C with a rate of 2–3 ml/min. Visualized whole-cell patch-clamp recording of spontaneous excitatory postsynaptic currents (sEPSCs) and miniature EPSCs (mEPSCs) was conducted using standard methods as described previously (Huang and Hsu *et al*, 2006). The layer V pyramidal neurons were identified using differential interference contrast image on a fixed-stage upright microscope (Olympus BX50WI; Olympus, Tokyo, Japan). The recording pipettes were pulled from borosilicate capillary tubing and heat-polished. The electrode resistance was typically 3–6 M $\Omega$ . The composition of intracellular solution was (mM): 115 potassium gluconate, 20 KCl, 10 HEPES, 2 MgCl<sub>2</sub>, 4 Na<sub>2</sub>ATP, 0.3 Na<sub>3</sub>GTP, 10 sodium phosphocreatine and sucrose to bring osmolarity to 290–295 mOsm and pH to 7.3. After a high resistance seal (>2 G $\Omega$  before breaking into whole-cell mode) was obtained, suction was applied lightly through the pipette to break through the membrane. Neurons were held at –70 mV and spontaneous activity was recorded. Recordings were made using a patch-clamp amplifier (Axopatch 200B; Axon Instruments, Union City, CA). Electrical signals were low-pass filtered at 1 kHz and digitized at 10 kHz using a 12-bit analog-to-digital converter (Digidata 1320; Axon Instruments). An Intel Pentium-based computer with pCLAMP software (version 8.0; Axon Instruments) was used for online acquisition of the data. sEPSCs were recorded in the presence of bicuculline methiodide (10  $\mu$ M) and analyzed offline using a commercially available software (Mini Analysis 4.3; Synaptosoft, Leonia, NJ). In the experiments involving recordings of mEPSCs, slices were bathed in the presence of bicuculline methiodide (10  $\mu$ M) and tetrodotoxin (1  $\mu$ M). The software

detects events based on amplitudes exceeding a threshold set just above the baseline noise of the recording ( $-3$  pA). All detected events were re-examined and accepted or rejected based on subjective visual examination. The program then measured amplitudes and intervals between successive detected events. Background current noise was estimated from the baseline with no clear event and was subtracted from signals for analysis. The sEPSCs frequencies were calculated by dividing the total number of detected events by the total time sampled. Periods of 2 min were analyzed for 5-HT or DOI treatment. Events were ranked by amplitude and interevent interval for preparation of cumulative probability distribution. Experiments that access resistance changed by more than 20% were discarded.

### Preparation of Detergent-Resistant Membrane

Detergent-resistant, caveolin-enriched membrane fractions were isolated using the Caveolae/Rafts isolation kit (catalogue no. CS0750; Sigma-Aldrich, St Louis, MO) according to the manufacturer's instruction. The mPFC slices were homogenized in ice-cold lysis buffer solution (catalogue no. L7667) containing a cocktail of protease inhibitors (catalogue no. P8340) to avoid protein degradation, and ground with a pellet pestle (Kontes glassware, Vineland, NJ). Samples were sonicated and spun down at 15 000 g for 15 min at 4°C. The crude membrane pellet was extracted with TNE buffer (50 mM Tris-HCl, 150 mM NaCl, and 4 mM EDTA, pH 7.4) containing 1% Triton X-100 and incubated on ice for 30 min. Samples were mixed with cold Optiprep density gradient medium (catalogue no. D1556), transferred into centrifuge tubes, and overlaid with 2 ml each of 35, 30, 25, 20, and 0% Optiprep in TNE buffer. The gradients were spun at 50 000 r.p.m. in a Ti 70.1 rotor (Beckman Instruments, Fullerton, CA) for 3.5 h at 4°C. After centrifugation, nine fractions (1 ml) were collected from top to the bottom of centrifuge tubes. Equal volumes of the fractions were separated by SDS-PAGE followed by immunoblot analysis with appropriate antibodies. Specific antibody that recognized cholesterol-binding protein caveolin-1 (1:1000; Sigma-Aldrich) was used as the detergent-resistant membrane (DRM) marker to determine the cholesterol-enriched membrane subdomains. Transferrin receptor (1:500; Zymed Laboratories, Carlsbad, CA) was used as the non-NRM marker.

### Western Blotting

For each experimental group, homogenates from at least three slices were pooled. The prelimbic cortices were lysed in ice-cold Tris-HCl buffer solution (TBS; pH 7.4) containing a cocktail of protein phosphatase and proteinase inhibitors (50 mM Tris-HCl, 100 mM NaCl, 15 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 5 mM EGTA, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1  $\mu$ M microcystin-LR, 1  $\mu$ M okadaic acid, 0.5% Triton X-100, 2 mM benzamidin, 60  $\mu$ g/ml aprotinin, and 60  $\mu$ g/ml leupeptin) to avoid dephosphorylation and degradation of proteins, and ground with a pellet pestle (Kontes glassware). Samples were sonicated and spun down at 15 000 g for 10 min at 4°C. The supernatant was

then assayed for total protein concentration using Bio-Rad Bradford Protein Assay Kit (Hercules, CA). Each sample was separated in 8% SDS-PAGE. Following the transfer on nitrocellulose membranes, blots were blocked in TBS containing 3% bovine serum albumin and 0.01% Tween 20 for 1 h and then blotted for 2 h at room temperature with antibodies that recognize 5-HT<sub>2A</sub> receptor (1:1000; BD Biosciences, Franklin Lakes, NJ) or regulator of GTP-binding protein signaling 4 (RGS4, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA). It was then probed with HRP-conjugated secondary antibody for 1 h and developed using the ECL immunoblotting detection system. The immunoblots were subsequently stripped and reprobed with  $\beta$ -actin antibody (1:20 000; Sigma-Aldrich). Expression of 5-HT<sub>2A</sub> receptor and RGS4 was evaluated relative to that for  $\beta$ -actin. Immunoblots were analyzed by densitometry using Bio-profile BioLight PC software. Only film exposures that were in the linear range of the ECL reaction were used for quantification analysis.

### Drug Treatment

Cocaine HCl (15 mg/kg), DOI (1 mg/kg), (*R*)-(+) -chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390, 0.5 mg/kg), raclopride (0.5 mg/kg), 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenyl)sulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione (RS102221, 2 mg/kg), ketanserin (1 mg/kg), and prazosin (1 mg/kg) were dissolved in 0.9% NaCl and administered intraperitoneally. Drug doses were selected on the basis of published studies (Essman *et al*, 1994; Filip *et al*, 2001; Wellman *et al*, 2002; Dong *et al*, 2004; Conductier *et al*, 2005; Berg *et al*, 2006; Huang *et al*, 2007a). All drugs used in *in vitro* experiments were applied by manually switching the superfusate. Drugs were diluted from stock solutions just before application. DOI and 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine (NAN-190) were dissolved in DMSO stock solutions and stored at  $-20^{\circ}\text{C}$  until the day of experiment. Other drugs used in this study were dissolved in distilled water. The concentration of DMSO in the perfusion medium was 0.1%, which alone had no effect on basal synaptic transmission (Huang and Hsu, 2006). SCH23390, RS102221, (2*R*)-1-[(3-hydroxy-phenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine (SB 269970), raclopride, ketanserin, prazosin, and tetrodotoxin were purchased from Tocris Cookson (Bristol, UK); cocaine HCl, DOI, and bicuculline methiodide were obtained from Sigma (St Louis, MO).

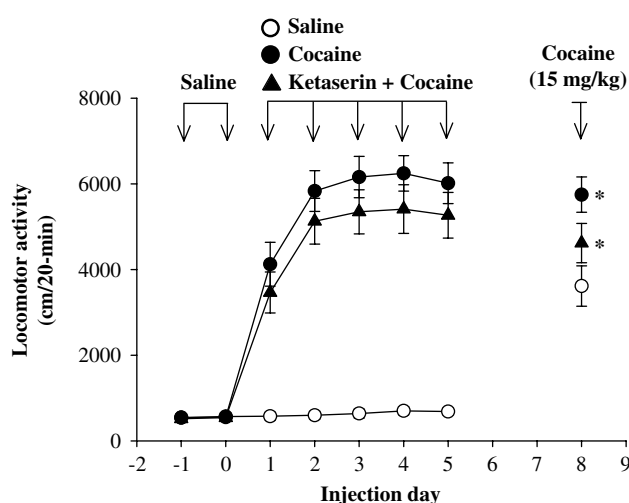
### Statistical Analysis

The data for each experiment were normalized relative to baseline, and are presented as mean  $\pm$  SEM. The significance of the difference between the mean was calculated by unpaired Student's *t*-test. Probability values (*p*) of less than 0.05 were considered to represent significant differences. Number of animals used is indicated by *n*. Statistical comparisons of the sEPSCs and mEPSCs were made using the Kolmogorov-Smirnov (K-S) test. Distributions were considered different using a conservative critical probability level of *p* < 0.01.

## RESULTS

Repeated Cocaine Administration Impairs 5-HT<sub>2A</sub> Receptor-Mediated Serotonergic Enhancement of Glutamatergic Synaptic Activity

To study the effect of repeated cocaine administration on 5-HT system in the mPFC, we examined 5-HT-induced enhancement of sEPSCs in mPFC layer V pyramidal neurons in slices from rats that received daily administration of saline (1 mg/ml) or cocaine (15 mg/kg) intraperitoneally for 5 consecutive days followed by a 3-day withdrawal period. This cocaine regimen is widely used in the literature to induce behavioral sensitization (Beurrier and Malenka, 2002; Dong *et al*, 2005; Nasif *et al*, 2005; Huang *et al*, 2007a). The effectiveness of the repeated cocaine treatment on rats was shown by their heightened locomotor activity in response to cocaine challenge injection (Figure 1). Consistent with previous reports (Aghajanian and Marek, 1997; Zhou and Hablitz, 1999; Béique *et al*, 2007), bath application of 5-HT (30  $\mu$ M) for 3 min elicited a rapid and robust increase in both the amplitude (from  $6.74 \pm 0.32$  to  $9.28 \pm 0.38$  pA,  $n = 12$ ) and frequency (from  $4.78 \pm 0.48$  to  $15.78 \pm 1.38$  Hz,  $n = 12$ ) of sEPSCs of layer V pyramidal neurons in slices from rats treated with saline for 5 days (Figure 2). This enhancing effect persisted through the application of 5-HT and was almost completely reversed after drug washout. Interestingly, the ability of 5-HT to increase the frequency and amplitude of sEPSCs was markedly attenuated in slices from age-matched rats treated with cocaine for 5 days (Figure 3a–d). To assess whether changes in 5-HT sensitivity observed in short-term cocaine withdrawal persist after a long-term withdrawal, the effects



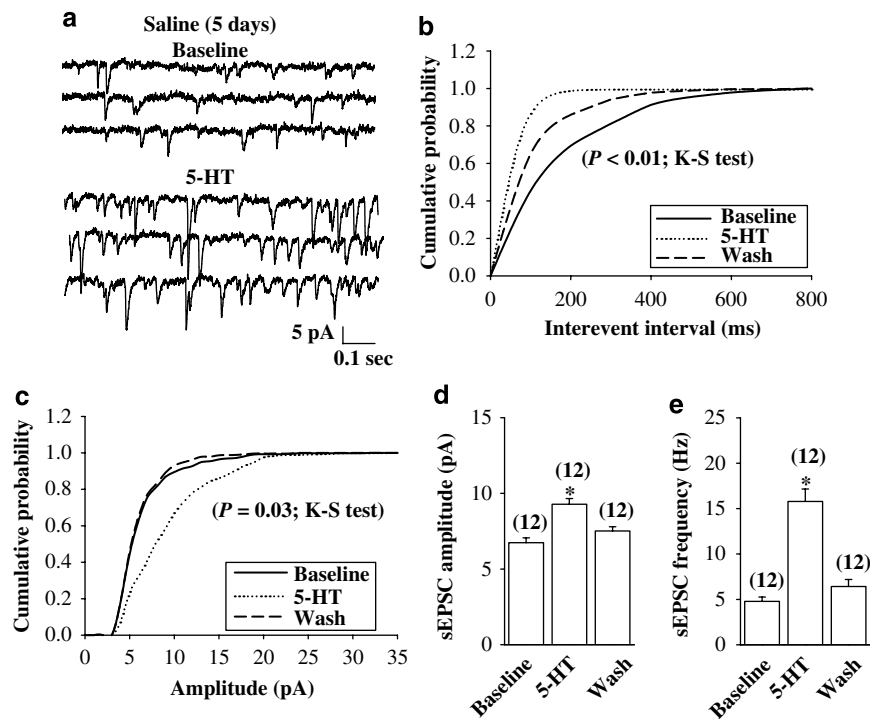
**Figure 1** Behavioral sensitization induced by repeated cocaine administration. Following each intraperitoneal injection of cocaine or saline, rats were immediately placed in the activity chamber and horizontal locomotor activity was monitored for 20 min. After 2 days of saline injections to habituate the chamber, rats were divided into three groups that received saline, vehicle (1 ml/kg), or ketanserin (1 mg/kg) 15 min before each of the five daily cocaine injections. To examine the persistence of the effects of repeated cocaine exposure, three groups of rats were kept for additional 3 days without injection and the locomotor activity was measured at day 8 with a single injection of cocaine (15 mg/kg) ( $n = 5$  for each group). Note that cocaine-induced locomotor sensitization was partially prevented by ketanserin pretreatment. Asterisk (\*) shows significant difference from saline control at  $p < 0.05$ .

of repeated cocaine treatment on the ability of 5-HT to increase sEPSC activity were evaluated following 1–14 days of withdrawal. We found that the slices from rats withdrawn from repeated cocaine treatment for 1, 3, 7, or 14 days exhibited comparable impairment of the enhancing effects of 5-HT on the amplitude and frequency of sEPSCs (Figure 3f and g). In contrast, no change in the extent of 5-HT-induced enhancement of sEPSCs was observed in slices from rats that were given a single injection of saline or cocaine (Supplementary Figure S1). These results suggest that repeated but not single cocaine administration impairs the ability of 5-HT to enhance the spontaneous glutamatergic synaptic activity in mPFC layer V pyramidal neurons and that this effect persists after long-term withdrawal.

On the basis of gene sequence and pharmacological profile, 5-HT receptors could be divided into seven families (designated 5-HT<sub>1</sub> to 5-HT<sub>7</sub>) with at least 14 subtypes (Roth, 1994). To identify the cellular mechanisms underlying the decreased ability of 5-HT to increase sEPSCs in mPFC layer V pyramidal neurons after cocaine withdrawal, we first pharmacologically examined the subtype of 5-HT receptors responsible for the synaptic potentiation. As shown in Figure 4, none of the 5-HT<sub>1</sub> receptor antagonist NAN-190 (10  $\mu$ M), 5-HT<sub>2A</sub> receptor antagonist R-96544 (1  $\mu$ M), 5-HT<sub>2A/2C</sub> receptor antagonist ketanserin (10  $\mu$ M), 5-HT<sub>2C</sub> receptor antagonist RS102221 (5  $\mu$ M), and 5-HT<sub>7</sub> receptor antagonist SB 269970 (1  $\mu$ M), given alone, had a significant effect on sEPSCs in slices from naive rats. Pretreatment of the slices with R-96544 or ketanserin, but not NAN-190, RS102221, or SB 269970, completely suppressed the 5-HT-induced increase of the frequency (Figure 4a) or amplitude of sEPSCs (Figure 4b). Drug concentrations were selected on the basis of published studies (Inoue *et al*, 1999; Campbell and Walker, 2001; Dawson *et al*, 2002; Chen *et al*, 2003; Béique *et al*, 2004). Attempts were also conducted to see whether the direct activation of 5-HT<sub>2A</sub> receptors by application of the selective 5-HT<sub>2A</sub> receptor agonist DOI could mimic the effect of 5-HT to facilitate sEPSCs. As shown in Figure 4c, application of DOI (10  $\mu$ M) for 3 min also produced a massive increase in the frequency and amplitude of EPSCs of layer V pyramidal neurons in slices from rats treated with saline for 5 days similar to that induced by 5-HT. Pretreatment of the slices with ketanserin (10  $\mu$ M) completely prevented the effects of DOI ( $n = 4$ ; data not shown). These results suggest that 5-HT-induced enhancement of sEPSCs in mPFC layer V pyramidal neurons is predominantly mediated by the activation of 5-HT<sub>2A</sub> receptors.

In addition, the enhancing effect of DOI on sEPSCs was also markedly impaired by repeated cocaine treatment and withdrawal. As illustrated in Figure 4c and d, application of DOI (10  $\mu$ M) increased the frequency of sEPSCs only from  $5.64 \pm 0.32$  to  $8.69 \pm 0.59$  Hz ( $n = 6$ ) in slices from rats treated with cocaine for 5 days followed by a 3-day withdrawal, which was significantly less than the effect of DOI in slices from saline-treated rats (from  $5.12 \pm 0.56$  to  $14.38 \pm 1.43$  Hz,  $n = 6$ ).

If cocaine withdrawal attenuates the modulation of 5-HT on sEPSCs by reducing the activation of 5-HT<sub>2A</sub> receptors, then increasing the activation of 5-HT<sub>2A</sub> receptors in slices from cocaine-treated rats can be expected to mimic the effect of 5-HT on sEPSCs in saline-treated rats. To test this



**Figure 2** 5-Hydroxytryptamine (5-HT) enhances spontaneous excitatory postsynaptic currents (sEPSCs) in medial prefrontal cortex (mPFC) layer V pyramidal neurons. (a) A representative neuron showing that application of 5-HT (30  $\mu$ M) for 3 min dramatically increases both the frequency and amplitude of sEPSCs. (b) Cumulative interevent interval distribution illustrating a significant decrease in the interevent interval (ie increased frequency;  $p < 0.01$ , Kolmogorov–Smirnov test) during 5-HT application. (c) Cumulative distribution of sEPSC amplitude before and after 5-HT application. (d, e) The bar graph showing the summary data of the effect of 5-HT on the average amplitude and frequency of sEPSCs. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from baseline at  $p < 0.05$ . The data shown in (a–c) were taken from the same neuron of a rat treated with saline for 5 days followed by a 3-day withdrawal. Holding potential,  $-70$  mV.

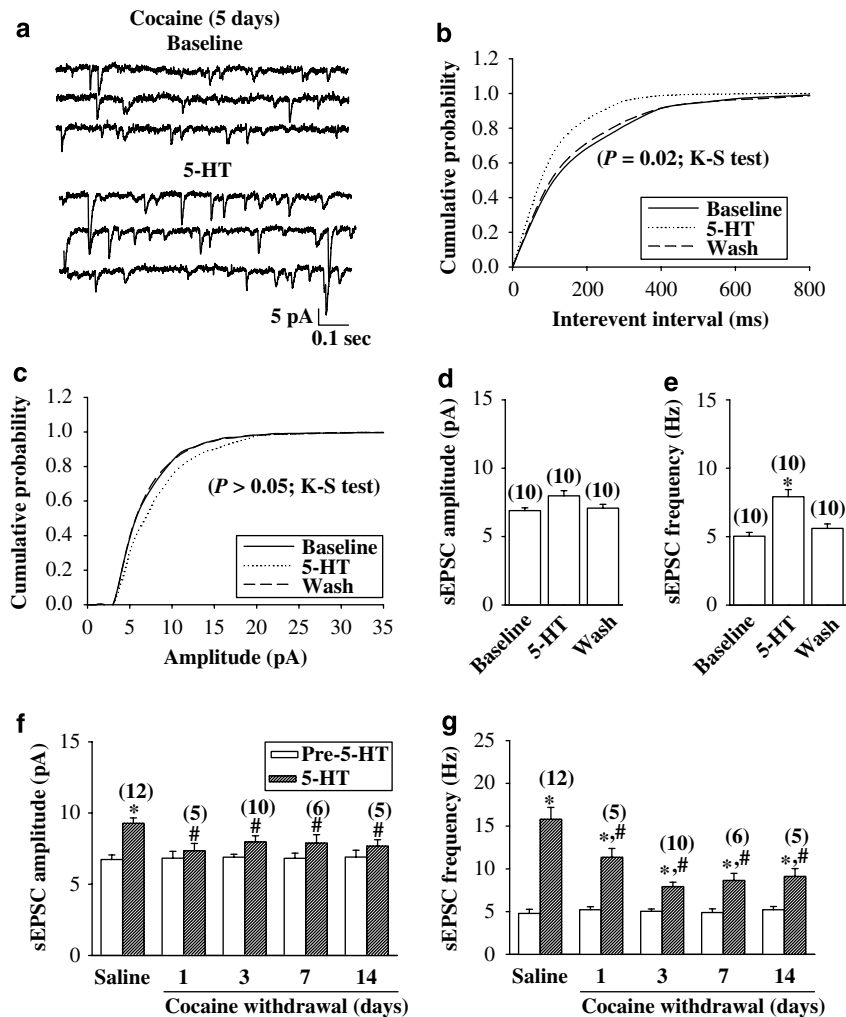
prediction, the effect of a higher concentration of 5-HT on sEPSCs was examined. Accordingly, we demonstrate that application of nearly saturation concentration of 5-HT (100  $\mu$ M) (Zhou and Hablitz, 1999) for 3 min increased in both the amplitude (from  $6.13 \pm 0.36$  to  $8.76 \pm 0.52$  pA,  $n = 6$ ) and frequency (from  $4.75 \pm 0.46$  to  $12.35 \pm 1.92$  Hz,  $n = 6$ ) of sEPSCs in slices from rats treated with cocaine for 5 days followed by a 3-day withdrawal (Figure 5a and b), which was not significantly different from the effect of 5-HT (30  $\mu$ M) on sEPSCs in slices from saline-treated rats (Figure 2). In slices from saline-treated rats, 5-HT (100  $\mu$ M) enhanced the amplitude of sEPSCs from  $6.54 \pm 0.43$  to  $11.32 \pm 0.54$  pA ( $n = 4$ ) and the frequency from  $5.12 \pm 0.57$  to  $29.12 \pm 2.32$  Hz ( $n = 4$ ), respectively. Dose–response curves in slices from saline- and cocaine-treated rats showed that the maximum effects of 5-HT on the amplitude and frequency of sEPSCs were markedly attenuated by repeated cocaine exposure (Figure 5c and d). Together, these results are consistent with the idea that that repeated cocaine exposure may reduce 5-HT<sub>2A</sub> receptor signaling at the mPFC excitatory synapses.

### Repeated Cocaine Exposure Decreases Serotonergic Enhancement of sEPSCs by the Activation of 5-HT<sub>2A</sub> Receptors

A critical question is how the repeated cocaine administration could reduce the 5-HT<sub>2A</sub> receptor responsiveness in layer V pyramidal neurons of the mPFC. Considering that

cocaine blocks the dopamine reuptake transporter and, thus, acutely increases local dopamine concentrations in brain areas receiving dopaminergic inputs (Hyman, 1996), it is therefore possible that activation of dopaminergic receptors in the critical brain areas is required for the aforementioned cocaine-induced decrease of 5-HT<sub>2A</sub> receptor function. Because dopaminergic receptors could be divided into two families, D<sub>1</sub>- and D<sub>2</sub>-like receptors (Civelli *et al*, 1993), we administered specific D<sub>1</sub>- or D<sub>2</sub>-like receptor antagonists before cocaine injection. As illustrated in Figure 6a and b, neither the D<sub>1</sub> receptor antagonist SCH23390 (0.5 mg/kg) nor D<sub>2</sub> receptor antagonist raclopride (0.5 mg/kg) had a significant effect on 5-HT-induced facilitation when co-administered with saline or cocaine for 5 days, excluding a role of the dopaminergic receptor activation in the development of cocaine-induced decrease of 5-HT<sub>2A</sub> receptor responsiveness at the mPFC excitatory synapses.

It is known that cocaine also alters 5-HT neurotransmission through blockade of 5-HT transporters and elevation of extracellular levels of 5-HT in reward-related brain regions (Bradberry *et al*, 1993; Teneud *et al*, 1996). Additional evidence indicated that 5-HT<sub>2</sub> receptors may be involved in the reinforcing properties of cocaine (Meert *et al*, 1991). To determine the role of 5-HT<sub>2</sub> receptor activation in the development of cocaine-induced decrease of 5-HT<sub>2A</sub> receptor responsiveness, we administered specific 5-HT<sub>2A/2C</sub> antagonist ketanserin (1 mg/kg) or 5-HT<sub>2C</sub> receptor antagonist RS102221 (2 mg/kg) 30 min before cocaine injection. As shown in Figure 6a and b, neither ketanserin

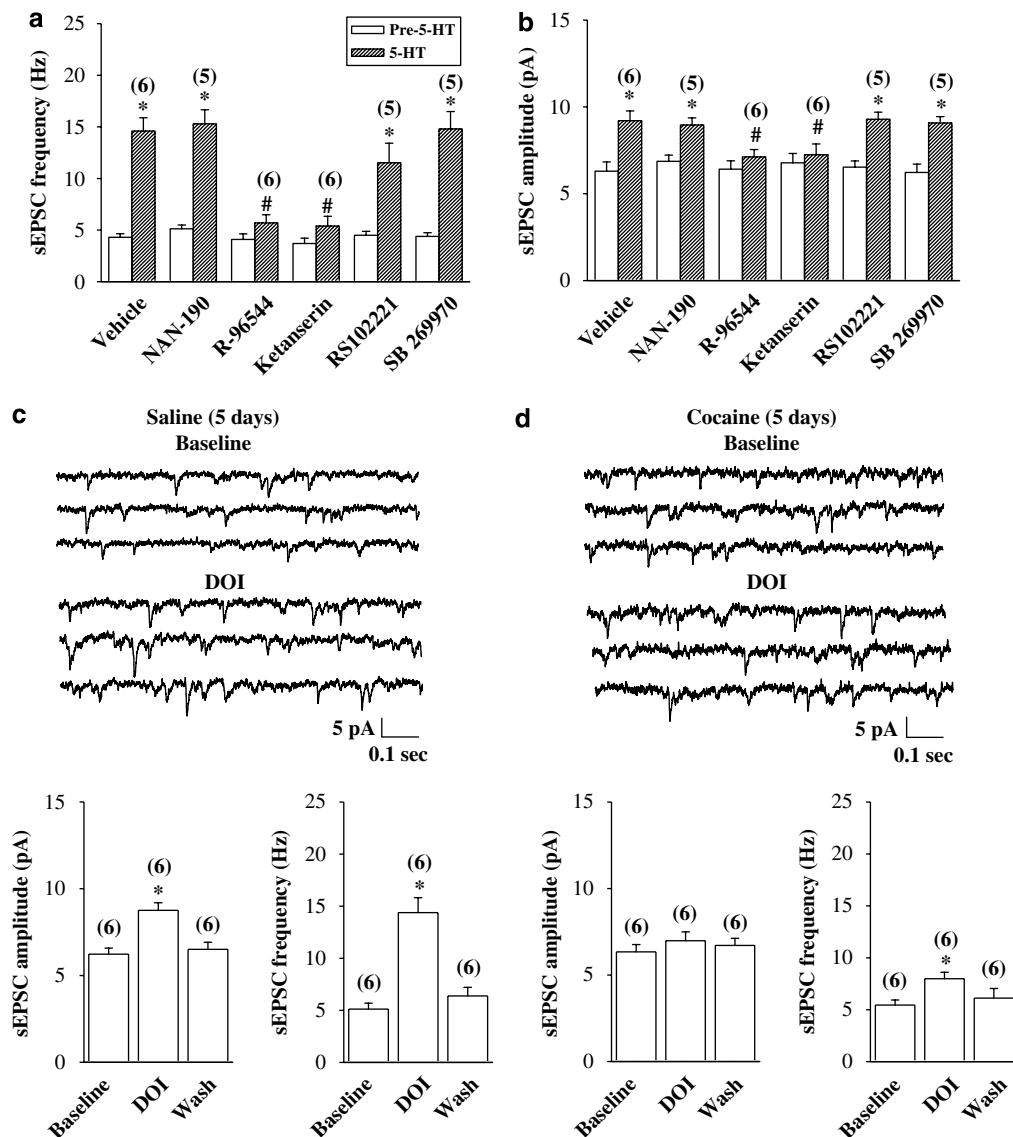


**Figure 3** Repeated cocaine administration decreases 5-hydroxytryptamine (5-HT)-induced enhancement of spontaneous excitatory postsynaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC). (a) An example experiment showing the effect of 5-HT (30  $\mu$ M) on sEPSCs recorded from an mPFC layer V pyramidal neuron of a rat treated with cocaine for 5 days followed by a 3-day withdrawal. (b) Cumulative probability plots depicting the effect of 5-HT on the distribution of sEPSC interevent interval for the experiment illustrated in (a). (c) Cumulative distribution of sEPSC amplitude recorded before and after application of 5-HT for the same experiment. (d, e) The bar graph showing the summary data of the effect of 5-HT on the average amplitude and frequency of sEPSCs. (f, g) The bar graph showing the summary data of the effect of 5-HT on the average amplitude and frequency of sEPSCs from rats that were injected daily with saline or cocaine for 5 days and then examined at 1, 3, 7, or 14 days after withdrawal. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from pre-5-HT baseline at  $p < 0.05$ . #Significant difference from saline-treated group at  $p < 0.05$ . Holding potential,  $-70$  mV.

nor RS102221 treatment alone had a significant effect on 5-HT-induced enhancement of sEPSCs. When co-administered with cocaine, ketanserin, but not RS102221, the 5-HT-induced enhancement of sEPSCs was not impaired in slices from 5-day cocaine-treated rats. In addition, cocaine-induced locomotor sensitization was partially prevented by ketanserin pretreatment (Figure 1). It has been shown that ketanserin also blocks the activation of  $\alpha_1$ -adrenergic receptors (Nishimura *et al*, 1987). To exclude a role for the  $\alpha_1$ -adrenergic receptor activation in the development of cocaine-induced decrease of 5-HT-induced enhancement of sEPSCs, we administered specific  $\alpha_1$ -adrenergic receptor antagonist prazosin (1 mg/kg) 30 min before cocaine injection. However, in contrast to ketanserin, prazosin did not significantly affect the decrease of 5-HT-induced enhancement of sEPSCs in slice from 5-day cocaine-treated rats (Figure 6a and b). Together, these findings indicate that the

activation of 5-HT<sub>2A</sub> receptor is responsible for the inhibitory effect of repeated cocaine administration on 5-HT-induced enhancement of glutamatergic synaptic activity in the mPFC.

If cocaine-induced impairment of 5-HT<sub>2A</sub> receptor responsiveness is caused by an elevated level of 5-HT, repeated 5-HT agonist treatment would likely mimic the effect of cocaine to decrease the 5-HT-induced enhancement of sEPSCs. To test this prediction, we injected rats with DOI (1 mg/kg per day) for 5 days and then examined slices 1–7 days after the termination of the last injection. As reported previously (Baumann and Rothman, 1996), intraperitoneal injection of DOI produced significant head-shake and skin-jerk behaviors. Our results revealed that repeated DOI administration also effectively decreased 5-HT-induced enhancement of sEPSCs, and this reduction persisted for at least 3 days after withdrawal from the 5-day



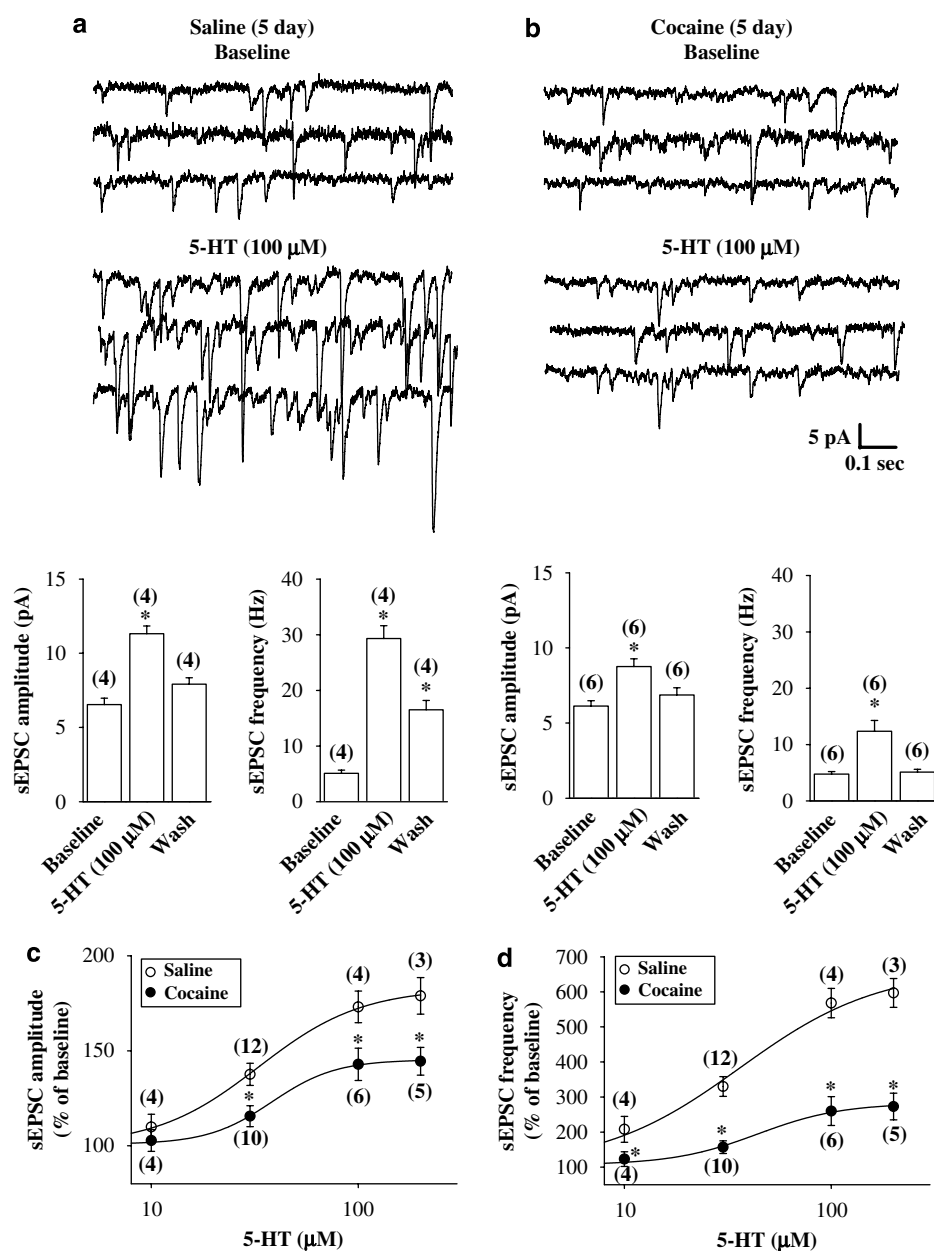
**Figure 4** Activation of 5-HT<sub>2A</sub> receptors mediates the 5-hydroxytryptamine (5-HT)-induced enhancement of spontaneous excitatory postsynaptic currents (sEPSCs) in medial prefrontal cortex (mPFC) layer V pyramidal neurons. (a, b) The bar graph summarizing the effects of 5-HT (30  $\mu$ M) on the average amplitude and frequency of sEPSCs in the presence or absence of various 5-HT receptor antagonists. (c) Top, an example experiment showing the effect of (–)-4-iodo-2,5-dimethoxyphenylisopropylamine (DOI; 10  $\mu$ M) on sEPSCs recorded from an mPFC layer V pyramidal neuron of a saline-treated rat. Bottom, the bar graph summarizing the effect of DOI on the average amplitude and frequency of sEPSCs. (d) Top, an example experiment showing the effect of DOI on sEPSCs recorded from an mPFC layer V pyramidal neuron of a rat 3 days after withdrawal from 5 days of cocaine treatment. Bottom, the bar graph summarizing the effect of DOI on the average amplitude and frequency of sEPSCs. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from pre-DOI baseline at  $p < 0.05$ . Holding potential,  $-70$  mV.

DOI injection (Figure 7). Moreover, although repeated DOI administration produced subtle effect on basal locomotor activity, it caused slight but significant suppression of cocaine-induced locomotor sensitivity at the withdrawal time (Supplementary Figure S2).

#### Repeated Cocaine Exposure does not Alter the Levels of 5-HT<sub>2A</sub> Receptors and RGS4

We then proceeded to investigate the cellular adaptations triggered by repeated cocaine treatment to alter the responsiveness of 5-HT<sub>2A</sub> receptors in the mPFC. One possible mechanism that might account for the cocaine-induced impairment of 5-HT<sub>2A</sub> receptor responsiveness is

the reduced expression of 5-HT<sub>2A</sub> receptors. To explore this possibility, we examined the protein levels of 5-HT<sub>2A</sub> receptors in mPFC slices. As shown in Figure 8a, we found no significant difference between cocaine- and saline-treated rats on the levels of 5-HT<sub>2A</sub> receptors after 3-day withdrawal. We further explored whether repeated cocaine treatment may alter the membrane distribution of 5-HT<sub>2A</sub> receptors using density gradient centrifugation. Immunoblot analysis of gradient fractions showed that most of 5-HT<sub>2A</sub> receptors were concentrated at the bottom fractions along with the DRM marker cavelolin-1. The distribution pattern of 5-HT<sub>2A</sub> receptors was not significantly changed by repeated cocaine administration and withdrawal when compared with saline-treated control rats (Figure 8b). These



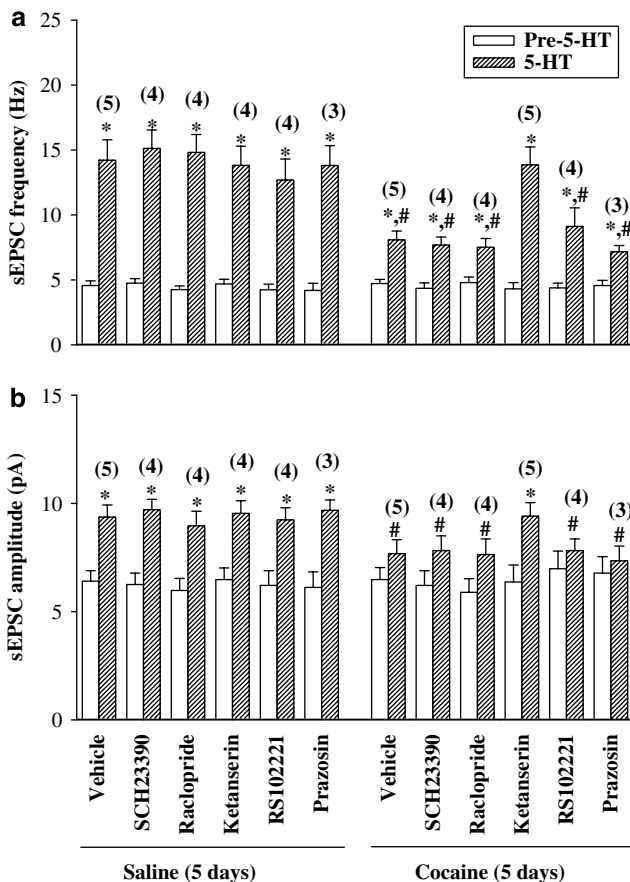
**Figure 5** The maximum effect of 5-hydroxytryptamine (5-HT) on spontaneous excitatory postsynaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC) is decreased by repeated cocaine administration. (a) Top, an example experiment showing the effect of 5-HT (100  $\mu$ M) on sEPSCs recorded from an mPFC layer V pyramidal neuron of a rat treated with saline for 5 days followed by a 3-day withdrawal. Bottom, the bar graph summarizing the effect of 5-HT on the average amplitude and frequency of sEPSCs. (b) Top, an example experiment showing the effect of 5-HT on sEPSCs recorded from an mPFC layer V pyramidal neuron of a rat treated with cocaine (15 mg/kg) for 5 days followed by a 3-day withdrawal. Bottom, the bar graph summarizing the effect of 5-HT on the average amplitude and frequency of sEPSCs. (c, d) The dose-response relationship of the effect of 5-HT on the average amplitude and frequency of sEPSCs in slices from rats treated with saline or cocaine for 5 days followed by a 3-day withdrawal. The total number of neurons examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from pre-5-HT baseline at  $p < 0.05$ . Holding potential,  $-70$  mV.

results suggest that the impaired responsiveness of 5-HT<sub>2A</sub> receptors in the mPFC during withdrawal from chronic cocaine administration is not associated with changes in the expression or membrane distribution of 5-HT<sub>2A</sub> receptors.

RGS proteins are known to function as GTPase accelerating proteins for G $\alpha$  subunits, attenuating GTP-binding protein-coupled receptor signaling cascades (De Vries *et al*, 2000). RGS proteins consist of four structurally and functionally distinct subfamilies (Ross and Wilkie, 2000).

RGS4, a member of R4 subfamily, is enriched in the PFC (Erdely *et al*, 2004) and has been shown to regulate 5-HT signaling negatively in PFC pyramidal neurons in the rat phencyclidine model of schizophrenia (Gu *et al*, 2007). To explore the possible involvement of RGS4 in the impairment of the 5-HT<sub>2A</sub> receptor responsiveness after repeated cocaine administration, we examined the change of RGS4 expression in rats withdrawn from repeated cocaine administration for 3 days. As illustrated in Figure 8c, no





**Figure 6** Repeated cocaine administration decreases 5-HT<sub>2A</sub> receptor-mediated serotonergic enhancement of spontaneous excitatory postsynaptic currents (sEPSCs) by the activation of 5-HT<sub>2A</sub> receptors. (a, b) Summary of experiments showing the effects of 5-hydroxytryptamine (5-HT; 30  $\mu$ M) on the average amplitude and frequency of sEPSCs in medial prefrontal cortex (mPFC) layer V pyramidal neurons of rats receiving vehicle, SCH23390 (0.5 mg/kg), raclopride (0.5 mg/kg), ketanserin (1 mg/kg), RS102221 (2 mg/kg), or prazosin (1 mg/kg) 30 min before saline or cocaine injection for 5 days followed by a 3-day withdrawal. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from pre-5-HT baseline at  $p < 0.05$ . #Significant difference from saline-treated group at  $p < 0.05$ . Holding potential,  $-70$  mV.

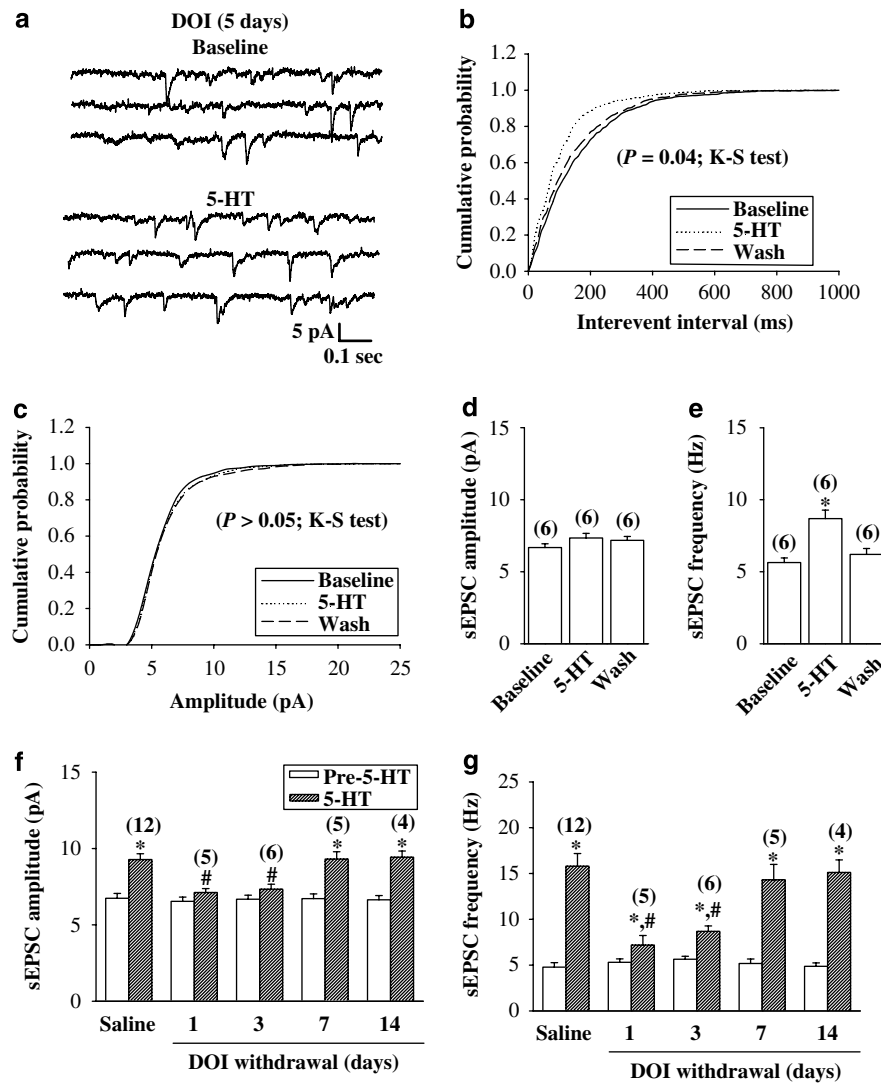
detectable changes in the levels of RGS4 were observed in the mPFC from rats treated with cocaine as compared with rats treated with saline.

## DISCUSSION

In this study, we provide the first evidence that withdrawal from repeated cocaine treatment *in vivo* decreases 5-HT<sub>2A</sub> receptor-mediated serotonergic enhancement of glutamatergic synaptic activity in layer V pyramidal neurons of the mPFC *in vitro*. The cellular mechanism mediating this change may result from an increased extracellular concentration of 5-HT on the days of cocaine treatment, which leads to a sustained increase in the activation of 5-HT<sub>2A</sub> receptors and thereby decreases the coupling of 5-HT<sub>2A</sub> receptors with GTP-binding proteins during the cocaine withdrawal period. We further demonstrate that the levels

of 5-HT<sub>2A</sub> receptors and RGS4 are not changed during withdrawal from repeated cocaine administration.

Cocaine binds with high affinity to the monoamine transporters and blocks the uptake of extracellular dopamine, serotonin, and norepinephrine (Hyman, 1996). Although adaptive changes in mesolimbic and mesocortical dopaminergic systems are thought to contribute to the development and expression of cocaine-induced behavioral sensitization and addiction, recent findings suggest that alterations in serotonin and norepinephrine systems may also be involved (Drouin *et al*, 2002; Fletcher *et al*, 2002; Burmeister *et al*, 2004). By extending these observations, we show here that withdrawal from repeated cocaine administration reduces 5-HT<sub>2A</sub> receptor-mediated serotonergic facilitation of sEPSCs in the mPFC and this effect is likely mediated by the enhanced 5-HT<sub>2A</sub> receptor activation in response to repeated cocaine treatment. Two lines of pharmacological evidence support this conclusion. First, the cocaine-induced decrease of 5-HT-induced enhancement of sEPSCs was prevented after pharmacological blockade of 5-HT<sub>2A</sub>, but not of D<sub>1</sub>-like, D<sub>2</sub>-like, or  $\alpha_1$ -adrenergic receptors during daily cocaine injection (Figure 4). Second, repeated DOI administration mimicked the effect of cocaine to decrease the 5-HT-induced enhancement of sEPSCs, and this reduction persisted for at least 3 days after withdrawal from the 5-day DOI injection (Figure 7). Given that ketanserin also exhibits a high affinity to  $\alpha_1$ -adrenergic receptors ( $K_i = 8.3$  nM for  $\alpha_1$ -adrenergic receptors) (Nishimura *et al*, 1987), it might be thought that the antagonizing effect of ketanserin on cocaine's effect seen in the present study is in part through its action on  $\alpha_1$ -adrenergic receptors. However, this possibility appears unlikely because the selective  $\alpha_1$ -adrenergic receptor antagonist prazosin did not significantly affect the cocaine-induced decrease of 5-HT-induced enhancement of sEPSCs. Thus, a persistent decrease observed in 5-HT<sub>2A</sub> receptor responsiveness in cocaine-withdrawn mPFC neurons may be attributed to a cocaine-induced increase in extracellular 5-HT concentration in the mPFC to activate specifically 5-HT<sub>2A</sub> receptor-dependent signaling cascade during chronic cocaine treatment period. This observation is consistent with a recent biochemical study showing that rats receiving daily intraperitoneal injections of 5-HT<sub>2A</sub> receptor agonist DOI (1 mg/kg) for 7 days caused a significant reduction in the serotonergic responsiveness in the PFC (Shi *et al*, 2007a). It is noteworthy that a single exposure to cocaine produced no change in the ability of 5-HT<sub>2A</sub> receptors to mediate serotonergic enhancement of sEPSCs, indicating marked drug administration paradigm specificity of the cocaine-induced adaptations of the serotonergic systems. Although we found that repeated DOI administration mimics the effects of cocaine to attenuate 5-HT<sub>2A</sub> receptor-mediated enhancement of sEPSCs, the time courses of their action are not completely consistent. Repeated cocaine administration suppressed 5-HT<sub>2A</sub> receptor function for at least 2 weeks whereas DOI was only effective for 3 days after withdrawal. The reason for this discrepancy is not clear but could be attributable to, at least in part, the different levels of 5-HT<sub>2A</sub> receptor signaling activation by repeated cocaine and DOI administration, resulting in stimulating different cellular processes that may vary in their mode of action. However, we could

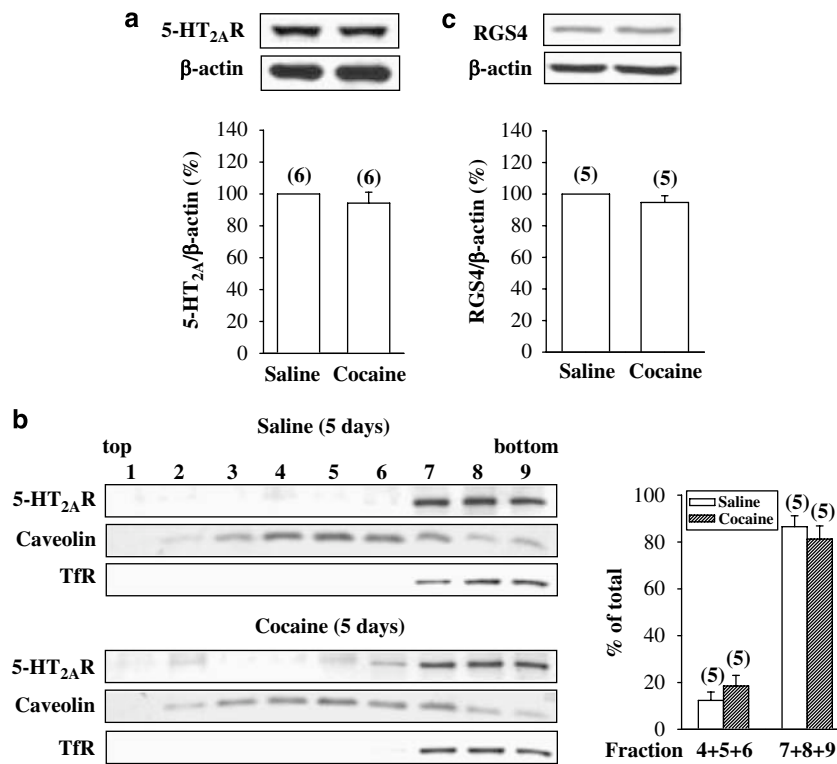


**Figure 7** Repeated (–)-4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) administration decreases 5-hydroxytryptamine (5-HT)-induced enhancement of spontaneous excitatory postsynaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC). (a) An example experiment showing the effect of 5-HT (30  $\mu$ M) on sEPSCs recorded from an mPFC layer V pyramidal neuron of a rat treated DOI (1 mg/kg per day) for 5 days followed by a 3-day withdrawal. (b) Cumulative probability plots depicting the effect of 5-HT on the distribution of sEPSC interevent interval for the experiment illustrated in (a). (c) Cumulative distribution of sEPSC amplitude recorded before and after application of 5-HT for the same experiment. (d, e) The bar graph showing the summary data of the effect of 5-HT on the average amplitude and frequency of sEPSCs. (f, g) The bar graph showing the summary data of the effect of 5-HT on the average amplitude and frequency of sEPSCs from rats that were injected daily with saline or DOI for 5 days and then examined at 1, 3, 7, or 14 days after withdrawal. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM. Asterisk (\*) shows significant difference from pre-5-HT baseline at  $p < 0.05$ . #Significant difference from saline-treated group at  $p < 0.05$ . Holding potential,  $-70$  mV.

not exclude the possibility that other mechanisms of action, in addition to the control of 5-HT<sub>2A</sub> receptor signaling, may contribute to cocaine-induced prolonged reduction of 5-HT<sub>2A</sub> receptor responsiveness in the mPFC. Further work is needed to assess this possibility. Interestingly, repeated DOI administration did not elicit cross-sensitization with cocaine but resulted in a reduction of locomotor sensitivity to an acute dose of cocaine (Supplementary Figure S2), supporting the concept that sole stimulation of 5-HT<sub>2A</sub> receptors is not sufficient to underlie behavioral sensitization to cocaine. Because antagonism of 5-HT<sub>2A</sub> receptors can significantly attenuate the locomotor stimulant effect of cocaine (Figure 1; McMahon and Cunningham, 2001), the reduction of cocaine-induced locomotor

sensitivity following repeated DOI administration may be related to the impairment of 5-HT<sub>2A</sub> receptor responsiveness.

There is accumulating evidence that activation of 5-HT<sub>2A</sub> receptors in the mPFC can result in an enhancement of spontaneous glutamatergic synaptic activity (Aghajanian and Marek, 1997; Zhou and Hablitz, 1999; Lambe and Aghajanian, 2001; Marek *et al*, 2001; Béique *et al*, 2004, 2007), but the cellular processes underlying this enhancement have remained inconclusive. A leading hypothesis is that activation of postsynaptic 5-HT<sub>2A</sub> receptors would elicit the release of an excitatory retrograde messenger, which leads to increased glutamate release from thalamocortical terminals (Zhou and Hablitz, 1999; Lambe and Aghajanian,



**Figure 8** The effect of repeated cocaine treatment on the levels of 5-HT<sub>2A</sub> receptors and RGS4 in the medial prefrontal cortex (mPFC). (a) Top, representative immunoblot showing the levels of 5-HT<sub>2A</sub> receptors in the mPFC slices from rats 3 days after withdrawal from 5 days of saline or cocaine treatment. Bottom, group data showing the normalization of 5-HT<sub>2A</sub> receptors to the  $\beta$ -actin were determined in each group of six separate experiments. (b) Left, the representative immunoblot showing the localization of 5-HT<sub>2A</sub> receptors in the detergent-resistant membrane (DRM) fractions. Nine fractions were collected from the top and immunoblotted for the indicated proteins. Caveolin-1 was used as DRM marker, whereas transferrin receptor (TfR) was used as the non-DRM marker. Right, quantitative analysis of the 5-HT<sub>2A</sub> receptor distribution was performed by densitometry and calculated in the percentage of the total protein amount in all fractions. (c) Top, representative immunoblot showing the levels of RGS4 in the mPFC slices from rats 3 days after withdrawal from 5 days of saline or cocaine treatment. Bottom, group data showing the normalization of RGS4 to the  $\beta$ -actin were determined in each group of five separate experiments. The total number of animals examined is shown in parentheses. Data are presented as mean  $\pm$  SEM.

2001; Marek *et al*, 2001). A recent study examined this hypothesis in more detail and offered a new interpretation by using molecular intervention strategies (Béïque *et al*, 2007). The results of this study support the idea that the increase in sEPSCs in response to 5-HT<sub>2A</sub> receptor activation results from the depolarization and excitation of a subpopulation of pyramidal neurons in the deep layers of the mPFC to increase in glutamatergic recurrent network activity and not through activation of the thalamocortical afferents by a retrograde messenger. If activation of postsynaptic 5-HT<sub>2A</sub> receptors elicits an increase in sEPSCs by inducing the release of a retrograde messenger, a manipulation of 5-HT<sub>2A</sub> G-protein-coupled receptor signaling cascade in the recorded neurons should equally alter the ability of 5-HT<sub>2A</sub> receptors to induce an inward current and the release of a retrograde messenger. As reported previously (Béïque *et al*, 2007), we found that intracellular infusion of GTP $\gamma$ S into mPFC layer V pyramidal neurons resulted in an augmented 5-HT-induced inward current but failed to affect the ability of 5-HT to elicit an increase in EPSCs (Huang and Hsu, unpublished observations). As such, the 5-HT<sub>2A</sub> receptor-mediated enhancement of sEPSCs in mPFC layer V pyramidal neurons is unlikely mediated by inducing the release of a retrograde messenger.

An intriguing question is as to what mechanism might give rise to cocaine-induced increase in extracellular 5-HT concentration during repeated cocaine administration to regulate 5-HT<sub>2A</sub> receptor function and hence to impair persistently the 5-HT-mediated facilitation of sEPSCs during cocaine withdrawal. This is likely achieved through the desensitization of 5-HT<sub>2A</sub> receptors on overexposure to 5-HT. Multiple mechanisms have been proposed for the desensitization of GTP-binding protein-coupled receptors, including decreasing the coupling of receptors with GTP-binding proteins, increasing receptor internalization from the cell surface, or reducing the total receptor number (Gainetdinov *et al*, 2004). Although the molecular mechanisms underlying the desensitization of 5-HT<sub>2A</sub> receptors have not yet been clearly established, our results indicate that 5-HT<sub>2A</sub> receptor desensitization induced by chronic cocaine administration appears to be due to the reduced ability of 5-HT<sub>2A</sub> receptors to couple to GTP-binding proteins, but does not involve receptor downregulation. Our results showed that the levels of 5-HT<sub>2A</sub> receptors and RGS4 in the mPFC were not changed by repeated cocaine administration and withdrawal (Figure 8). It is noteworthy that the decreased coupling of 5-HT<sub>2A</sub> receptors with GTP-binding proteins may occur as a result of the phosphorylation of 5-HT<sub>2A</sub> receptors, phosphorylation of coupling

GTP-binding proteins, or a reduction of GTP-binding protein levels (Shi *et al*, 2007a,b). Additional studies are needed to determine which mechanism may account for the impairment of 5-HT<sub>2A</sub> receptor signaling in the mPFC following repeated cocaine administration.

What could be the functional significance of cocaine-induced decrease of 5-HT<sub>2A</sub> receptor-mediated serotonergic enhancement of sEPSCs in the mPFC? The mPFC is known to have an important function in the development of cocaine-induced behavioral sensitization and withdrawal effects (Tzschentke, 2001). As 5-HT<sub>2A</sub> receptors in the mPFC are important for the regulation of moods, this withdrawal-induced deficit of 5-HT<sub>2A</sub> receptor function may be clinically relevant with respect to behavioral depression observed after termination of chronic cocaine administration (Barr and Markou, 2005). This study also provides novel evidence in supporting the view that cocaine withdrawal is associated with desensitization of 5-HT<sub>2A</sub> receptor signaling, which may provide a mechanism to fine-tune the effects of 5-HT on glutamatergic synaptic plasticity in the mPFC. Analogous results were also observed in studies examining the synaptic effects following chronic 5-HT<sub>2A</sub> receptor agonist administration. However, our findings seemingly conflict with previous reports showing that withdrawal from chronic cocaine treatment enhanced 5-HT<sub>2A</sub> receptor-mediated ACTH, prolactin, and corticosterone release in the hypothalamic paraventricular nucleus (Levy *et al*, 1992; Baumann and Rothman, 1996, 1998; Carrasco *et al*, 2003) and dopamine release in the nucleus accumbens (Yan *et al*, 2000), suggesting that chronic cocaine administration can produce a withdrawal-induced supersensitivity of 5-HT<sub>2A</sub> receptor function. The reason for this discrepancy is unclear but could be attributable to variation among the assay systems used in different brain regions, resulting in stimulating different cellular processes that may vary in their mode of action. We could not exclude the possibility that differences in the basal or cocaine-induced increase in extracellular 5-HT levels among brain regions may also involve in the occurrence of this apparent discrepancy.

In conclusion, our data indicate that withdrawal from repeated cocaine treatment *in vivo* may decrease 5-HT<sub>2A</sub> receptor-mediated serotonergic enhancement of glutamatergic synaptic activity in layer V pyramidal neurons of the mPFC. Our data also indicate that cocaine-induced increase in extracellular 5-HT levels may cause profound activation of 5-HT<sub>2A</sub> receptor, and thereby desensitize 5-HT<sub>2A</sub> receptors. Over the past few years, there has been evidence that enhanced membrane excitability of mPFC pyramidal neurons and excitatory output from the mPFC may contribute to the development of behavioral sensitization to cocaine (Pierce *et al*, 1996; Kalivas and Duffy, 1998; Dong *et al*, 2005; Nasif *et al*, 2005). The impairment of 5-HT<sub>2A</sub> receptor-mediated enhancement of mPFC glutamatergic synaptic transmission after cocaine withdrawal may result in reduced excitability of the intrinsic mPFC circuitry and thus prevent the development of cocaine-induced behavioral sensitization. These findings provide a major advance in establishing correlation and possible link between cocaine-induced neural adaptive changes of 5-HT<sub>2A</sub> receptor function in the mPFC and the development of behavioral sensitization and withdrawal effects after chronic cocaine exposure.

## ACKNOWLEDGEMENTS

This work was supported by research grants (NSC97-2321-B-006-002-MY2; principal investigator, CCH and NSC97-2752-B-006-002-PAE; principal investigator, KSH) from the National Science Council, Taiwan. We thank Dr TP Su for critical comments on an earlier version of this paper.

## DISCLOSURE/CONFLICT OF INTEREST

The authors declare that there are no actual or potential conflicts of interest. The authors affirm that there are no financial, personal, or other relationships with other people or organizations that have inappropriately influenced or biased the work.

## REFERENCES

- Aghajanian GK, Marek GJ (1997). Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **36**: 589–599.
- Araneda R, Andrade R (1991). 5-Hydroxytryptamine<sub>2</sub> and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* **40**: 399–412.
- Azmitia EC, Segal M (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* **179**: 641–667.
- Barr AM, Markou A (2005). Psychostimulant withdrawal as an inducing condition in animal models of depression. *Neurosci Biobehav Rev* **29**: 675–706.
- Baumann MH, Rothman RB (1996). Chronic cocaine exposure potentiates prolactin and head shake responses to 5-HT<sub>2</sub> receptor stimulation in rats. *Neuropharmacology* **35**: 295–301.
- Baumann MH, Rothman RB (1998). Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. *Biol Psychiatry* **44**: 578–591.
- Béïque JC, Chapin-Penick EM, Mladenovic L, Andrade R (2004). Serotonergic facilitation of synaptic activity in the developing rat prefrontal cortex. *J Physiol* **556**: 739–754.
- Béïque JC, Imad M, Mladenovic L, Gingrich JA, Andrade R (2007). Mechanism of the 5-hydroxytryptamine<sub>2A</sub> receptor-mediated facilitation of synaptic activity in prefrontal cortex. *Proc Natl Acad Sci USA* **104**: 9870–9875.
- Berg KA, Navailles S, Sanchez TA, Silva YM, Wood MD, Spampinato U *et al* (2006). Differential effects of 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]carbamoyl]-6-trifluoromethylindone (SB 243213) on 5-hydroxytryptamine<sub>2C</sub> receptor-mediated responses. *J Pharmacol Exp Ther* **319**: 260–268.
- Beurrier C, Malenka RC (2002). Enhanced inhibition of synaptic transmission by dopamine in the nucleus accumbens during behavioral sensitization to cocaine. *J Neurosci* **22**: 5817–5822.
- Bradberry CW, Nobiletti JB, Elsworth JD, Murphy B, Jatlow P, Roth RH (1993). Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. *J Neurochem* **60**: 1429–1435.
- Burmeister JJ, Lungren EM, Kirschner KF, Neisewander JL (2004). Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology* **29**: 660–668.
- Campbell BM, Walker PD (2001). MK-801 prevents dopamine D1 but not serotonin 2A stimulation of striatal preprotachykinin mRNA expression. *Neuroreport* **12**: 953–955.
- Carrasco GA, Zhang Y, Damjanoska KJ, D'Souza DN, Garcia F, Battaglia G *et al*. (2003). A region-specific increase in Galphaq

- and Galphal1 proteins in brains of rats during cocaine withdrawal. *J Pharmacol Exp Ther* 307: 1012–1019.
- Chen A, Hough CJ, Li H (2003). Serotonin type II receptor activation facilitates synaptic plasticity via *N*-methyl-D-aspartate-mediated mechanism in the rat basolateral amygdala. *Neuroscience* 119: 53–63.
- Civelli O, Bunzow JR, Grandy DK (1993). Molecular diversity of the dopamine receptors. *Annu Rev Pharmacol Toxicol* 33: 281–307.
- Conductier G, Crosson C, Hen R, Bockaert J, Compan V (2005). 3,4-*N*-Methylenedioxymethamphetamine-induced hypophagia is maintained in 5-HT<sub>1B</sub> receptor knockout mice, but suppressed by the 5-HT<sub>2C</sub> receptor antagonist RS102221. *Neuropsychopharmacology* 30: 1056–1063.
- Dawson LA, Galandak J, Djali S (2002). Attenuation of ischemic efflux of endogenous amino acids by the novel 5-HT<sub>1A</sub>/5-HT<sub>2</sub> receptor ligand adatsersin. *Neurochem Int* 40: 203–209.
- De Vries L, Zheng B, Fischer T, Elenko E, Farquhar MG (2000). The regulator of G protein signaling family. *Annu Rev Pharmacol Toxicol* 40: 235–2371.
- Dong Y, Nasif FJ, Tsui JJ, Ju WY, Cooper DC, Hu XT et al (2005). Cocaine-induced plasticity of intrinsic membrane properties in prefrontal cortex pyramidal neurons: adaptations in potassium currents. *J Neurosci* 25: 936–940.
- Dong Y, Saal D, Thomas M, Faust R, Bonci A, Robinson T et al (2004). Cocaine-induced potentiation of synaptic strength in dopamine neurons: behavioral correlates in GluRA(–/–) mice. *Proc Natl Acad Sci USA* 101: 14282–14287.
- Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S et al (2002).  $\alpha_{1b}$ -Adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J Neurosci* 22: 2873–2884.
- Erdely HA, Lahti RA, Lopez MB, Myers CS, Roberts RC, Tamminga CA et al (2004). Regional expression of RGS4 mRNA in human brain. *Eur J Neurosci* 19: 3125–3128.
- Essman WD, Singh A, Lucki I (1994). Serotonergic properties of cocaine: effects on a 5-HT<sub>2</sub> receptor-mediated behavior and on extracellular concentrations of serotonin and dopamine. *Pharmacol Biochem Behav* 49: 107–113.
- Filip M, Nowak E, Papla I (2001). On the role of serotonin2A/2C receptors in the sensitization to cocaine. *J Physiol Pharmacol* 52: 471–481.
- Fletcher PJ, Grottick AJ, Higgins GA (2002). Differential effects of the 5-HT<sub>2A</sub> receptor antagonist M100907 and the 5-HT<sub>2C</sub> receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology* 27: 576–586.
- Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG (2004). Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* 27: 107–144.
- Gu Z, Jiang Q, Yan Z (2007). RGS4 modulates serotonin signaling in prefrontal cortex and links to serotonin dysfunction in a rat model of schizophrenia. *Mol Pharmacol* 71: 1030–1039.
- Huang CC, Hsu KS (2006). Presynaptic mechanism underlying cAMP-induced synaptic potentiation in medial prefrontal cortex pyramidal neurons. *Mol Pharmacol* 69: 846–856.
- Huang CC, Lin HJ, Hsu KS (2007a). Repeated cocaine administration promotes long-term potentiation induction in rat medial prefrontal cortex. *Cereb Cortex* 17: 1877–1888.
- Huang CC, Yang PC, Lin HJ, Hsu KS (2007b). Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. *J Neurosci* 27: 2958–2968.
- Hyman SE (1996). Addiction to cocaine and amphetamine. *Neuron* 16: 901–904.
- Hyman SE, Malenka RC, Nestler EJ (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* 29: 565–598.
- Inoue T, Itoh S, Kobayashi M, Kang Y, Matsuo R, Wakisaka S et al (1999). Serotonergic modulation of the hyperpolarizing spike afterpotential in rat jaw-closing motoneurons by PKA and PKC. *J Neurophysiol* 82: 626–637.
- Kalivas PW, Duffy P (1998). Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. *J Neurochem* 70: 1497–1502.
- Kauer JA, Malenka RC (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci* 8: 844–858.
- Lambe EK, Aghajanian GK (2001). The role of Kv1.2-containing potassium channels in serotonin-induced glutamate release from thalamocortical terminals in rat frontal cortex. *J Neurosci* 21: 9955–9963.
- Levy AD, Li Q, Alvarez Sanz MC, Rittenhouse PA, Brownfield MS, Van de Kar LD (1992). Repeated cocaine modifies the neuroendocrine responses to the 5-HT<sub>1C</sub>/5-HT<sub>2</sub> receptor agonist DOI. *Eur J Pharmacol* 221: 121–127.
- Marek GJ, Wright RA, Gewirtz JC, Schoepp DD (2001). A major role for thalamocortical afferents in serotonergic hallucinogen receptor function in the rat neocortex. *Neuroscience* 105: 379–392.
- McMahon LR, Cunningham KA (2001). Antagonism of 5-hydroxytryptamine<sub>2A</sub> receptors attenuates the behavioral effects of cocaine in rats. *J Pharmacol Exp Ther* 297: 357–363.
- Meert TF, Awouters F, Niemegeers CJ, Schellekens KH, Janssen PA (1991). Ritanserin reduces abuse of alcohol, cocaine, and fentanyl in rats. *Pharmacopsychiatry* 24: 159–163.
- Nasif FJ, Hu XT, White FJ (2005). Repeated cocaine administration increases voltage-sensitive calcium currents in response to membrane depolarization in medial prefrontal cortex pyramidal neurons. *J Neurosci* 25: 3674–3679.
- Nestler EJ (2002). From neurobiology to treatment: progress against addiction. *Nat Neurosci* 5(Suppl): 1076–1079.
- Nishimura J, Kanaide H, Shogakiuchi Y, Nakamura M (1987). Ketanserin blocks  $\alpha_1$ -adrenoceptors of porcine vascular smooth muscle cells. *Eur J Pharmacol* 133: 235–238.
- Peterson JD, Wolf ME, White FJ (2006). Repeated amphetamine administration decreases D1 dopamine receptor-mediated inhibition of voltage-gated sodium currents in the prefrontal cortex. *J Neurosci* 26: 3164–3168.
- Pierce RC, Bell K, Duffy P, Kalivas PW (1996). Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci* 16: 1550–1560.
- Ross EM, Wilkie TM (2000). GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem* 69: 795–827.
- Roth BL (1994). Multiple serotonin receptors: clinical and experimental aspects. *Ann Clin Psychiatry* 6: 67–78.
- Sesack SR, Deutch AY, Roth RH, Bunney BS (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. *J Comp Neurol* 290: 213–242.
- Shi J, Damjanoska KJ, Singh RK, Carrasco GA, Garcia F, Grippo AJ et al (2007a). Agonist induced-phosphorylation of Galphal1 protein reduces coupling to 5-HT<sub>2A</sub> receptors. *J Pharmacol Exp Ther* 323: 248–256.
- Shi J, Zemaitaitis B, Muma NA (2007b). Phosphorylation of G<sub>z11</sub> protein contributes to agonist-induced desensitization of 5-HT<sub>2A</sub> receptor signaling. *Mol Pharmacol* 71: 303–313.
- Steketee JD (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev* 41: 203–228.
- Teneud LM, Baptista T, Murzi E, Hoebel BG, Hernandez L (1996). Systemic and local cocaine increase extracellular serotonin in the nucleus accumbens. *Pharmacol Biochem Behav* 53: 747–752.

- Thomas MJ, Malenka RC (2003). Synaptic plasticity in the mesolimbic dopamine system. *Philos Trans R Soc Lond B Biol Sci* **358**: 815–819.
- Tzschentke TM (2001). Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog Neurobiol* **63**: 241–320.
- Wellman P, Ho D, Cepeda-Benito A, Bellinger L, Nation J (2002). Cocaine-induced hypophagia and hyperlocomotion in rats are attenuated by prazosin. *Eur J Pharmacol* **455**: 117–126.
- Wolf ME (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog Neurobiol* **54**: 679–720.
- Yan Q, Reith ME, Yan S (2000). Enhanced accumbal dopamine release following 5-HT<sub>2A</sub> receptor stimulation in rats pretreated with intermittent cocaine. *Brain Res* **863**: 254–258.
- Zhou FM, Hablitz JJ (1999). Activation of serotonin receptors modulates synaptic transmission in rat cerebral cortex. *J Neurophysiol* **82**: 2989–2999.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)