

Blockade of nucleus accumbens 5-HT_{2A} and 5-HT_{2C} receptors prevents the expression of cocaine-induced behavioral and neurochemical sensitization in rats

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Abstract

Rationale The serotonin 5-HT_{2A} and 5-HT_{2C} receptors regulate the capacity of acute cocaine to augment behavior and monoamine levels within the nucleus accumbens (NAC), a brain region involved in cocaine's addictive and psychotogenic properties.

Objectives In the present study, we tested the hypothesis that NAC 5-HT_{2A} and 5-HT_{2C} receptor activation is involved in the expression of cocaine-induced neuroplasticity following protracted withdrawal from a sensitizing repeated cocaine regimen (days 1 and 7, 15 mg/kg; days 2–6, 30 mg/kg, i.p.).

Methods The effects of intra-NAC infusions of the 5-HT_{2A} antagonist *R*-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine methanol (MDL 100907; 0, 50, 100, 500 nM) or the 5-HT_{2C} antagonist [6-chloro-5-methyl-1-(6-(2-methylpyridin-3-yloxy)pyridine-3-yl carbamoyl)] inodoline dihydrochloride (SB 242084; 0, 50, 100, 500 nM) were first assessed upon the expression of locomotor activity elicited by a 15-mg/kg cocaine challenge injection administered at 3-week withdrawal. A follow-up in vivo microdialysis exper-

iment then compared the effects of the local perfusion of 0, 50, or 100 nM of each antagonist upon cocaine-induced dopamine and glutamate sensitization in the NAC.

Results Although neither MDL 100907 nor SB 242084 altered acute cocaine-induced locomotion, SB 242084 reduced acute cocaine-elevated NAC dopamine and glutamate levels. Intra-NAC perfusion with either compound blocked the expression of cocaine-induced locomotor and glutamate sensitization, but only MDL 100907 pretreatment prevented the expression of cocaine-induced dopamine sensitization.

Conclusions These data provide the first evidence that NAC 5-HT_{2A} and 5-HT_{2C} receptors are critical for the expression of cocaine-induced neuroplasticity following protracted withdrawal, which has relevance for their therapeutic utility in the treatment of addiction.

Keywords 5-HT_{2A} receptor · 5-HT_{2C} receptor · Cocaine · Sensitization · Glutamate · Dopamine · Nucleus accumbens · Addiction · Psychosis

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Behavioral sensitization refers to the progressive increase in the psychomotor-activating effects of a drug with repeated treatment and is a putative mechanism underlying both the addictive and psychotogenic properties of cocaine (e.g., Post and Weiss 1988; Kalivas et al. 1998; Robinson and Berridge 2000). Moreover, the study of behavioral sensitization is a technically facile animal model with which to investigate the short- and long-term molecular and cellular consequences of repeated cocaine exposure. Enduring increases in the capacity of cocaine to elevate extracellular levels of monoamines and glutamate within the nucleus accumbens (NAC) are considered critical for cocaine's behavioral-sensitizing property (e.g., Robinson and Berridge

2000; Vanderschuren and Kalivas 2000). The expression of cocaine-induced behavioral and NAC neurochemical sensitization is modulated, in a time-dependent manner, by ascending serotonin projections from the median raphe nucleus (Szumlinski et al. 2004). However, the precise receptor mechanism(s) through which serotonin influences the expression of cocaine-induced sensitization is not well understood. Currently, at least 16 different serotonin receptor subtypes are identified (e.g., Bockaert et al. 2006), of which the 5-HT_{2A} and 5-HT_{2C} receptors have received considerable experimental attention vis-à-vis their interactions with drugs of abuse (e.g., Di Giovanni et al. 2006; Berg et al. 2008). Indeed, studies employing selective ligands for the 5-HT_{2A} and 5-HT_{2C} receptors implicate these receptors as potential sites through which cocaine-induced increases in serotonin might impinge upon cocaine-induced changes in behavior and NAC neurotransmission of relevance to addiction.

In general, systemic pretreatment with 5-HT_{2A} agonists enhances, while antagonist pretreatment attenuates, cocaine addiction-related behaviors (O'Neill et al. 1999; Filip and Cunningham 2002, 2003; McMahon and Cunningham 2001; Fletcher et al. 2002; Filip et al. 2004), and the capacity of 5-HT_{2A} receptor stimulation to facilitate NAC dopamine release has been implicated in this regard (Schmidt et al. 1992). In contrast, 5-HT_{2C} receptor stimulation inhibits stimulated dopamine release within striatal regions (e.g., Di Matteo et al. 1999; Gobert et al. 2000; Navailles et al. 2004) and attenuates various measures of cocaine-induced psychomotor activation, reward, and reinforcement (Callahan and Cunningham 1995; Grottick et al. 2000; Filip and Cunningham 2003; Filip et al. 2004; Fletcher et al. 2004, 2008; Neisewander and Acosta 2007). Conversely, systemic pretreatment with selective 5-HT_{2C} receptor antagonists augments the NAC dopamine-elevating effects of cocaine (Navailles et al. 2004) and increases acute cocaine-induced locomotor activity, cocaine self-administration, as well as cocaine-primed reinstatement of cocaine-seeking behavior (Fletcher et al. 2002, 2006; Filip et al. 2004), but does not significantly affect the development or expression of cocaine-induced locomotor sensitization (Filip et al. 2004).

5-HT_{2A} and 5-HT_{2C} receptors exhibit moderate to high expression within the ventral tegmental area (VTA) and its major forebrain terminal regions, including the NAC (Compan et al. 1998a, b; Eberle-Wang et al. 1997; Clemett et al. 2000; Bubar and Cunningham 2006). While the effects of intra-VTA infusions of 5-HT_{2A} and 5-HT_{2C} ligands upon cocaine-induced changes in behavior and NAC dopamine levels more or less parallel those observed upon systemic pretreatment (McMahon et al. 2001; Fletcher et al. 2004; Navailles et al. 2008), data obtained from studies aimed at the NAC are either inconclusive or opposite those observed upon systemic ligand pretreatment (McMahon et al. 2001;

Filip and Cunningham 2002). Such discrepancies might relate to biphasic effects of receptor ligands upon cocaine's capacity to elevate NAC dopamine levels (Navailles et al. 2008), but this possibility has not been thoroughly investigated. To gain a deeper understanding of how NAC 5-HT_{2A} and 5-HT_{2C} receptors modulate the enduring behavioral and neurochemical effects of repeated cocaine, the present study established the dose–effect functions for the selective 5-HT_{2A} antagonist MDL 100907 (Kehne et al. 1996) and the selective 5-HT_{2C} antagonist SB 242084 (Kennett et al. 1997) upon the long-term expression of cocaine-induced locomotor sensitization, as well as the sensitization of cocaine-induced increases in NAC dopamine and glutamate.

Materials and methods

Subjects Experimentally naïve male Sprague-Dawley rats (Charles River, Hollister, CA; weighing 225–250 g at the start of the experiment) were housed in pairs in polyethylene cages (35×30×16 cm) in a temperature-controlled (25°C) colony room, under a 12-h day–12-h night cycle (lights off 1900 hours). Food and water were available ad libitum. Rats were allowed to acclimatize to the colony room for 4–5 days following arrival. All treatment sessions occurred during the light phase of the day–night cycle, beginning at 0900 hours. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of California at Santa Barbara and were consistent with the guidelines of the NIH Guide for Care and Use of Laboratory Animals (NIH Publication no. 80-23, revised 1996).

Drugs Cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% physiological saline, and saline served for control injections (volume=1.0 ml/kg). The 5-HT_{2A} antagonist MDL 100907 [*R*-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine methanol] and the 5-HT_{2C} antagonist SB 242084 ([6-chloro-5-methyl-1-(6-(2-methylpyridin-3-yloxy)pyridine-3-yl carbamoyl] inodoline dihydrochloride) (Solvay Pharmaceuticals Research, Weesp, The Netherlands) were dissolved in 45% (w/v) cyclodextrin (Sigma-Aldrich) and were diluted to the final working concentrations of 50, 100, and 500 nM using saline (for behavioral experiments) or aCSF (146 nM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂, pH=7.4; for microdialysis experiments) and a 45% cyclodextrin solution (in saline or aCSF) served for control intra-NAC injections (vol=0.25 μ l/site) and microdialysis perfusions, respectively.

Apparatus Locomotor activity was monitored in a non-colony room containing four black Plexiglas activity chambers (22×43×33 cm), above which were mounted

two digital video cameras interfaced to a PC-type computer, and the total distance traveled by the animal (in meter) was tabulated with ANYMaze software (Stoelting Company, Wood Dale, IL). In vivo microdialysis was conducted in a different non-colony room containing eight Rubbermaid™ microdialysis chambers (45×90×70 cm), each equipped with a liquid swivel suspended from a balance arm (CMA Microdialysis, Chelmsford, MA). aCSF or antagonist was delivered through PE50 tubing that was connected to an infusion pump and then routed through a 3-port liquid switch (CMA Microdialysis) to the swivel. The HPLC system consisted of a Coularray detector, a Model 542 autosampler, and two Model 582 solvent delivery systems (ESA Inc., Bedford, MA), which enabled the sequential detection of monoamines and amino acids from each dialysate sample (detection limit for each cell=0.01 ng/sample; e.g., Szumlinski et al. 2008). For dopamine (27 µl/sample onto column), the mobile phase consisted of 0.075 µM NaH₂PO₄, 25 µM EDTA, 0.0017 µM 1-octanesulfonic acid, 10% acetonitrile (v/v), pH 3.0, and monoamines were separated using a MD-150×3.2 column (15 cm; ESA Inc.) and an ESA 5014B analytical cell with two electrodes (E1, -150 mV; E2, +220 mV) was used. For glutamate (20 µl/sample onto column), the mobile phase consisted of 100 mM NaH₂PO₄, 22% methanol (v/v), 3.5% acetonitrile (v/v) pH=6.75, and amino acids were separated using a CAPCELL PAK C18 MG column (5 cm; Shiseido Company Ltd., Tokyo Japan). An ESA 5011A analytical cell with two electrodes (E1, +150 mV; E2, +550 mV) was used for the electrochemical detection of glutamate, following precolumn derivatization with *o*-phthalaldehyde (2.7 mg/ml) using the autosampler. Neurotransmitter content in each sample was analyzed by peak height and was compared with external standard curves (one for dopamine and one for glutamate) for quantification using ESA Coularray for Windows software.

Surgery The ventromedial portion of the NAC (the shell) expresses a greater degree of serotonin innervation (Brown and Molliver 2000) and expresses higher levels of both 5-HT_{2A} and 5-HT_{2C} receptors compared to the more lateral (core) subregion (e.g., Compan et al. 1998a, b; Clemett et al. 2000). Thus, under isoflurane anesthesia (4% for induction; 1.5–2.5% for maintenance), microinjector guide cannulae (26 gauge, 20 mm; Plastics One, Roanoke VA) were implanted bilaterally 2 mm over the NAC shell for animals slated for the behavioral experiments, and microdialysis guide cannulae (20 gauge, 20 mm, Plastics One) were implanted bilaterally 3 mm over the NAC shell for the neurochemical experiments using the following coordinates: AP, +1.1 mm; ML, ±2.5 mm; DV for behavior, -5.7 mm; DV for microdialysis, -4.7 mm; 6° angle from vertical (e.g., Szumlinski et al. 2003, 2004). The coordinates were based on the rat brain atlas of Paxinos and Watson (2000). The AP and

ML coordinates are relative to Bregma, while the DV coordinates are relative to the surface of the skull. The guide cannulae were fixed to the skull with four stainless steel skull screws (Small Parts, Roanoke, VA) and dental acrylic. Appropriately sized dummy cannulae were inserted into the guide cannulae to prevent externalization. Animals were monitored for changes in health for at least 5 days prior to beginning drug treatment.

Behavioral sensitization procedures Following recovery from surgery, animals were habituated to the activity monitors for 60 min. Animals then received seven, once daily, injections of either saline or cocaine (days 1 and 7, 15 mg/kg; days 2–6, 30 mg/kg), using a repeated treatment regimen demonstrated previously to elicit robust long-term sensitization in rats (e.g., Szumlinski et al. 2003, 2004). On injections 1 and 7, the locomotor activity of the animals was monitored for 2 h, while on injections 2–6, animals were returned to their home cages following injection. A comparison of the total distance traveled from injections 1 to 7 of repeated treatment served to index the extent of locomotor habituation and locomotor sensitization exhibited respectively by repeated saline- and cocaine-treated animals (data not shown). A 2-h test for behavioral sensitization was conducted 3 weeks later (Szumlinski et al. 2003, 2004), in which the effects of an intra-NAC infusion of 0, 50, 100, or 500 nM MDL 100907 or SB 242084 were assessed upon the capacity of a 15-mg/kg cocaine challenge injection to elicit locomotor activity. Assignment of rats to their intra-NAC pretreatments was such that each repeated saline and repeated cocaine group exhibited equivalent locomotor behavior prior to the test for sensitization (i.e., based on the change in locomotor activity from injections 1 to 7 of repeated treatment). The intra-NAC infusion procedures were similar to those employed in a previous intra-cranial infusion study (Szumlinski et al. 2004) and involved careful removal of the dummy cannulae, bilateral insertion of injector cannulae (33 gauge, 22 mm), and the infusion of 0.25 µl/side of the appropriate test dose at a rate of 0.25 µl/min. Injectors remained in place for an additional 60 s to allow for diffusion of the drug away from the injector tip, at which time the injectors were removed and animals injected i.p. with 15 mg/kg cocaine. Cocaine-induced locomotion was then monitored in 10-min bins for 2 h. Sensitization on test day was defined as a significant increase in the locomotor activity of animals with repeated cocaine experience, relative to their saline-pretreated counterparts receiving cocaine for the first time.

Neurochemical sensitization procedures Rats were treated repeatedly with either saline or cocaine as described for the behavioral study above with the exception that all injections occurred in the colony room, and locomotor activity was

not assessed. As in the locomotor study, 3 weeks were allowed prior to in vivo microdialysis procedures. Animals were randomly assigned to three groups receiving 0, 50, or 100 nM MDL 100907 or three groups receiving 0, 50, or 100 nM SB 242084. The effects of the 500 nM dose of either antagonist were not assessed as the behavioral data indicated that a maximal effect upon behavior was produced by the 100 nM dose of both compounds (see “Results” section below). Similar to earlier microdialysis studies (e.g., Szumlinski et al. 2003, 2004), microdialysis probes (24 gauge; 23 mm in length incl. 1.0–1.5 mm active membrane) were inserted unilaterally into the NAC and perfused overnight (at least 12 h prior to sample collection) at a rate of 0.2 μ l/min with aCSF (see Drugs). Probe insertion was counterbalanced across all treatment groups to reduce asymmetry confounds (Glick and Carlson 1989). The following morning (~0900 hours), the flow rate was increased to 2.0 μ l/min for a minimum of 2 h prior to sample collection. Testing began with a 1-h baseline collection period in which dialysate was sampled every 20 min. The assigned concentration of MDL 100907 or SB 242084 was then perfused via the microdialysis probe for 20 min, after which time all animals were injected i.p. with 15 mg/kg cocaine. Perfusion of the assigned concentration of antagonist then continued for 3 h post-cocaine injection, and samples were collected in 20-min fractions. As conducted previously (e.g., Szumlinski et al. 2003, 2004, 2008), oxidation of dopamine in the dialysate was minimized by the addition of 10 μ l of preservative prior to sample collection (4.76 mM citric acid, 150 mM NaH₂PO₄, 50 μ M EDTA, 3 mM sodium dodecyl sulfate, 10% methanol (v/v), 15% acetonitrile (v/v), pH 5.6). The microdialysis procedures were repeated 3–4 days later via unilateral probe insertion into the opposite side of the cranium. On the second session, animals received vehicle or a different antagonist test dose than that infused during the first microdialysis session. As there were three doses per antagonist (0, 50, and 100 nM), the assignment of the second dose was done in a pseudo-counterbalanced fashion such that half of the animals administered the 0 nM dose on session 1 received the 50 nM dose on session 2, while the remaining half received the 100 nM dose, etc. In this way, each animal was tested with two out of three possible antagonist concentrations over the two microdialysis sessions.

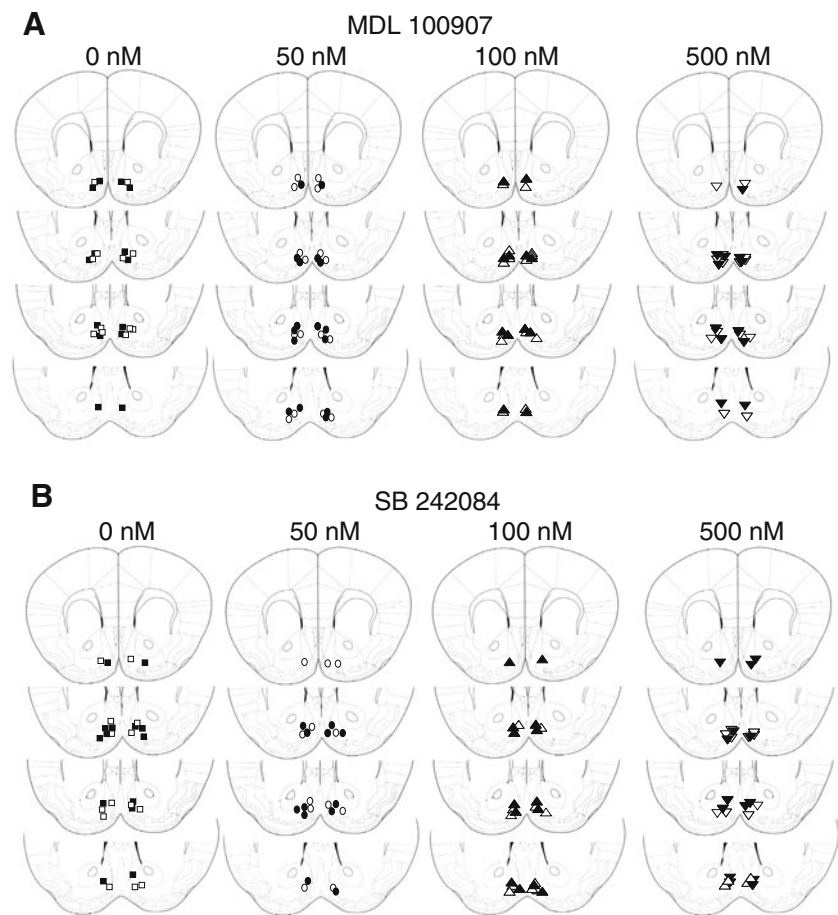
Histology Following the tests for sensitization, rats were euthanized and their brains removed and placed in a 10% formalin solution. After fixation, the brains were sectioned along the coronal plane on a vibratome at the level of the NAC (100 μ m; AP +2.2 to 1.0 mm, relative to Bregma), according to the atlas of Paxinos and Watson (2000). Sections were stained with cresyl violet for histological examination under a light microscope. Only rats whose

injector cannulae (Fig. 1) or microdialysis probes (Fig. 2) were located within the boundaries of the ventromedial NAC (i.e., the shell) were included in the statistical analysis of the data. The final samples sizes employed in the statistical analysis of the data are indicated in their corresponding figures (see “Results” section).

Statistical analysis For the locomotor studies, the 2-h time-course of the distance traveled throughout the entire activity chamber (distance in meter) on the test for sensitization served as the principal variable of interest. For each antagonist, the time-courses of locomotion were analyzed by a pretreatment (0, 50, 100, or 500 nM) \times repeated treatment (saline vs. cocaine) \times time analysis of variance (ANOVA), with repeated measures on the time factor (12, 10-min bins). To facilitate group comparisons, the total distance traveled during the 2-h test session was also analyzed using a pretreatment \times repeated treatment ANOVA. To confirm the presence of sensitization, a priori comparisons were conducted on the time-course data using repeated treatment \times time ANOVAs and on the total locomotor data using a univariate ANOVA between repeated saline- and cocaine-treated animals, separately for each pretreatment group.

For the in vivo microdialysis experiments, the average basal concentration of each neurotransmitter was determined based upon the three samples collected during baseline sampling and analyzed using a pretreatment (0, 50, or 100 nM) \times repeated treatment (saline vs. cocaine) \times hemisphere (left vs. right) ANOVA. As expected, there was no main effect of, or interaction with, the hemisphere factor for either basal dopamine (for MDL 100907, $p > 0.09$; for SB 242084, $p > 0.13$) or glutamate (for MDL 100907, $p > 0.25$; for SB 242084, $p > 0.53$). Thus, to simplify analyses of the time-course data of cocaine’s effects upon dopamine and glutamate, hemisphere was not included in any further statistical analyses of the neurochemical findings. The time-course of cocaine-induced changes in dopamine and glutamate was analyzed using a pretreatment \times repeated treatment \times time ANOVA, with repeated measures on the time factor (12, 20-min samples; 3 baseline+9 post-systemic injection). To facilitate visualization of the effects of intra-NAC pretreatment upon the magnitude of neurochemical sensitization, the data post-cocaine injection were expressed as the percent change from the average of the three baseline samples (Szumlinski et al. 2004) and the time-course of the neurochemical changes subjected to a pretreatment \times repeated treatment \times time ANOVA. The area under the curve (AUC or cumulative percent change from baseline) for the time-course data was also calculated and then subjected to a pretreatment \times repeated treatment ANOVA. Again, to confirm the presence of neurochemical sensitization, a priori comparisons were also conducted on

Fig. 1 Summary of the microinjector tip placements within the nucleus accumbens of rats pretreated with MDL 100907 (a) or SB 242084 (b). The placements of saline-treated animals are indicated by *open symbols*, while those of cocaine-treated animals are indicated by *closed symbols*



the time-course and AUC data between repeated saline- and cocaine-treated animals using, respectively, a repeated treatment \times time ANOVA and an univariate ANOVA, separately for each pretreatment group.

For both the behavioral and neurochemical data, significant interactions were deconstructed for main effects, followed by post hoc analyses using *t* tests. $\alpha=0.05$ for all analyses.

Results

The effects of intra-NAC 5-HT_{2A} blockade with MDL 100907 upon the expression of cocaine-induced behavioral sensitization Statistical analysis of the data indicated that pretreatment with MDL 100907 did not influence the capacity of repeated cocaine to elicit locomotor sensitization when animals were challenged with 15 mg/kg cocaine at 3 weeks withdrawal (repeated treatment \times time: $F(11,484)=1.88$, $p=0.04$; repeated treatment \times pretreatment \times time: $p>0.90$). As illustrated in Fig. 3, intra-NAC MDL 100907 reduced cocaine-induced locomotion, but this effect appeared to be independent of the repeated

cocaine experience of the animal (pretreatment effect: $F(3,44)=4.73$, $p=0.006$; repeated treatment \times pretreatment interactions, $p>0.05$). However, planned comparisons conducted between repeated saline and repeated cocaine groups independently for each MDL 100907 pretreatment indicated saline–cocaine differences for rats pretreated with vehicle (repeated treatment effect: $F(1,11)=4.98$, $p=0.05$) and 50 nM MDL 100907 (repeated treatment \times time: $F(11,154)=1.89$, $p=0.04$), but not for rats pretreated with 100 or 500 nM MDL 100907 (compare open vs. closed symbols in Fig. 3). A two-way repeated treatment \times pretreatment ANOVA conducted on the total distance traveled by the animals during the 2-h session yielded similar statistical results as the time-course analysis (Fig. 3c; pretreatment effect: $F(3,54)=5.61$, $p=0.002$; repeated treatment effect: $F(1,54)=8.82$, $p=0.005$; interaction: $p>0.10$), but planned comparisons indicated significant cocaine–saline differences only for rats pretreated with vehicle ($F(1,12)=5.95$, $p=0.03$; for all other MDL 100907 doses, $p>0.05$). These data indicate that blocking 5-HT_{2A} receptors within the NAC reduces cocaine-induced locomotion and, importantly, completely prevents the expression of cocaine-induced locomotor sensitization.

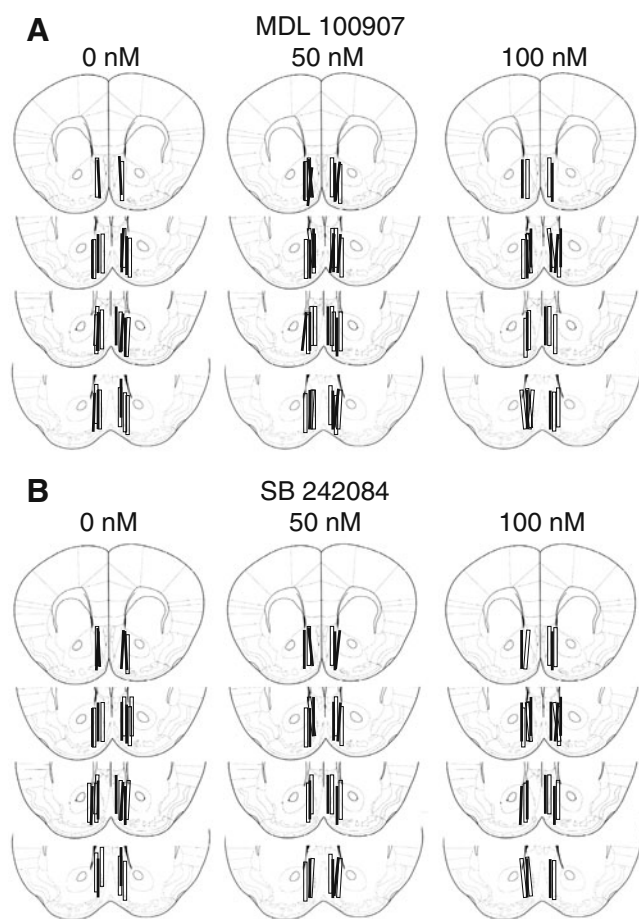


Fig. 2 Summary of the microdialysis active membrane placements within the nucleus accumbens of rats pretreated with MDL 100907 (a) or SB 242084 (b). The placements of saline-treated animals are indicated by open symbols, while those of cocaine-treated animals are indicated by closed symbols

The effects of intra-NAC 5-HT_{2A} blockade with MDL 100907 upon the expression of cocaine-induced neurochemical sensitization An analysis of baseline NAC extracellular dopamine levels prior to intra-NAC MDL 100907 infusion and the administration of the cocaine challenge injection yielded no differences between the six groups (Table 1).

NAC perfusion with MDL 100907 affected the dopamine response to cocaine in a manner dependent upon the prior cocaine experience of the animal (Fig. 4a, b) (raw data: repeated treatment×pretreatment×time: $F(22,407)=2.24$, $p=0.001$; normalized data: $F(22,407)=2.55$, $p<0.0001$). Deconstruction of the significant three-way interaction along the repeated treatment factor indicated a reduction in the NAC dopamine response to cocaine by MDL 100907 in cocaine-treated animals (raw data: repeated treatment×time: $F(22,220)=2.89$, $p<0.0001$; normalized data: $F(22,220)=3.83$, $p=0.006$), but not in saline-treated controls. In vehicle-perfused animals, the 15 mg/kg cocaine challenge produced an approximately 500% increase in NAC extracellular

dopamine levels in cocaine-treated rats, compared to the approximately 200% increase in dopamine observed in saline-treated controls (Fig. 4b), and planned comparisons between these two vehicle groups indicated dopamine sensitization (raw data: repeated treatment×time: $F(11,143)=2.40$, $p=0.009$; normalized data: $F(11,143)=2.59$, $p=0.005$). In contrast, cocaine–saline differences in the NAC dopamine response to cocaine were not apparent for animals perfused with either dose of MDL 100907 (Fig. 4b; repeated treatment×time interactions, $p>0.5$). The differential effects of MDL 100907 infusion upon the dopamine response to cocaine between repeated saline- and cocaine-treated rats were even more apparent when the AUC for the data presented in Fig. 4b was considered (repeated treatment×pretreatment: $F(2,42)=9.0$, $p<0.001$). As depicted in Fig. 4c, the cumulative cocaine-elicited rise in dopamine was greater in vehicle-treated cocaine animals, relative not only to their saline controls, but also in comparison to their cocaine counterparts pretreated with either 50 or 100 nM MDL 100907 (pretreatment effect: $F(2,22)=13.32$, $p<0.0001$). In contrast, the cumulative cocaine-elicited rise in dopamine did not vary across the pretreatment groups in repeated saline-treated animals (pretreatment effect, $p>0.05$). These data indicate that intra-NAC 5-HT_{2A} blockade prevents the expression of cocaine-induced dopamine sensitization, without influencing the capacity of acute cocaine to elevate NAC dopamine levels.

Withdrawal from repeated cocaine treatment reduced basal NAC extracellular glutamate levels, but the magnitude of this cocaine effect did not vary across the three pretreatment groups (Table 1; repeated treatment effect: $F(1,47)=12.32$, $p=0.001$). The effects of intra-NAC MDL 100907 upon the glutamate response to the cocaine challenge injection paralleled those observed for dopamine, despite the cocaine-induced reduction in NAC extracellular levels of glutamate (Fig. 4d, e; raw data: pretreatment×repeated treatment×time: $F(22,462)=1.82$, $p=0.01$; normalized data: $F(22,462)=2.67$, $p<0.0001$). Deconstruction of the significant three-way interaction along the repeated treatment factor indicated no effect of MDL 100907 upon the marginal rise in NAC glutamate produced by an acute injection of 15 mg/kg cocaine. In contrast, intra-NAC MDL 100907 completely abolished the approximately 500% increase in extracellular glutamate produced by the 15 mg/kg cocaine challenge in cocaine-treated animals (raw data: pretreatment×time: $F(22,253)=5.09$, $p<0.0001$; normalized data: $F(22,253)=4.91$, $p<0.0001$). Planned comparisons indicated cocaine–saline differences in vehicle-perfused animals (raw data: repeated treatment×time: $F(11,165)=2.28$, $p=0.02$; normalized data: $F(11,165)=3.32$, $p<0.0001$), but not in animals perfused with either dose of MDL 100907 (Fig. 4d, e). Again, the selective effect of intra-NAC MDL 100907 pretreatment upon the

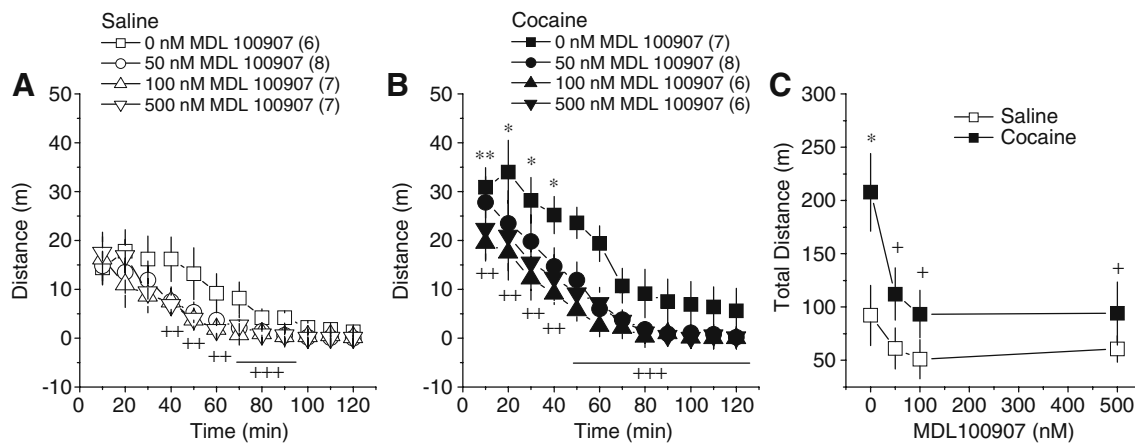


Fig. 3 Effects of intra-NAC 5-HT_{2A} blockade with MDL 100907 upon the acute and sensitized locomotor response to cocaine. **a** Summary of the effects of 0, 50, 100, and 500 nM of the 5-HT_{2A} antagonist MDL 100907 administered into the NAC upon the time-course of locomotor hyperactivity elicited by an acute injection of 15 mg/kg cocaine, administered 3 weeks following repeated saline treatment. **b** Summary of the effects of intra-NAC MDL 100907 upon the time-course of locomotion elicited by a 15-mg/kg cocaine challenge injection, administered 3 weeks following repeated cocaine treatment. * $p < 0.05$ for 0 nM cocaine vs. respective saline; ** $p < 0.05$

for both 0 and 50 nM cocaine vs. respective saline groups (sensitization); + $p < 0.05$ for 500 nM vs. respective 0 nM pretreatment; ++ $p < 0.05$ for both 100 and 500 nM vs. respective 0 nM pretreatment; +++ $p < 0.05$ for 50, 100, and 500 nM vs. respective 0 nM pretreatment. **c** Effect of intra-NAC MDL 100907 upon the total distance traveled during the 2-h test session by repeated saline- and cocaine-treated rats. * $p < 0.05$ cocaine vs. saline (sensitization); + $p < 0.05$ vs. respective 0 nM pretreatment (antagonist effect). The data represent the mean \pm SEM of the number of animals indicated in parentheses

cocaine-sensitized rise in glutamate was very apparent from an AUC analysis of the data presented in Fig. 4e (repeated treatment \times pretreatment: $F(2,47) = 3.12$, $p = 0.04$). As depicted in Fig. 4f, a cocaine-elicited rise in glutamate was only apparent in vehicle-pretreated cocaine controls, and this rise was abolished by intra-NAC pretreatment with either 50 or 100 nM MDL 100907 (pretreatment effect: $F(2,25) = 7.64$, $p = 0.003$). In contrast, the cumulative change in glutamate levels exhibited by repeated saline-treated animals did not vary with intra-NAC MDL 100907 pretreatment (pretreatment effect, $p > 0.05$). Thus, intra-NAC 5HT_{2A} antagonism also completely blocks the

expression of cocaine-induced glutamate sensitization within the NAC.

The effects of intra-NAC 5-HT_{2C} blockade with SB 242084 upon the expression of cocaine-induced locomotor sensitization As illustrated in Fig. 5, intra-NAC SB 242084 pretreatment reduced cocaine-induced locomotion selectively in rats with repeated cocaine experience (pretreatment \times repeated treatment \times time: $F(33,484) = 3.25$, $p < 0.0001$). Deconstruction of the three-way interaction along the repeated treatment factor confirmed an SB 242084 effect in cocaine-treated rats (pretreatment \times time: F

Table 1 Summary of the extracellular dopamine and glutamate levels exhibited by the different repeated saline- and cocaine-treated groups prior to NAC perfusion with MDL 100907 and SB 242084

	Dopamine (pg/sample)	Glutamate (ng/sample)
MDL 100907		
Saline 0 nM	2.9 \pm 0.9 (6)	4.0 \pm 1.1 (7)
Saline 50 nM	3.9 \pm 0.8 (8)	3.3 \pm 1.1 (7)
Saline 100 nM	3.2 \pm 0.7 (6)	3.4 \pm 1.1 (8)
Cocaine 0 nM	3.9 \pm 0.8 (9)	2.0 \pm 0.2 (10)
Cocaine 50 nM	5.0 \pm 1.2 (6)	1.3 \pm 0.2 (7)
Cocaine 100 nM	4.2 \pm 0.5 (8)	1.1 \pm 0.2 (9)
SB 242084		
Saline 0 nM	3.8 \pm 1.1 (7)	3.1 \pm 0.6 (7)
Saline 50 nM	3.7 \pm 0.6 (7)	3.9 \pm 0.3 (11)
Saline 100 nM	2.6 \pm 0.3 (7)	3.8 \pm 0.7 (8)
Cocaine 0 nM	3.9 \pm 0.8 (9)	2.0 \pm 0.1 (10)
Cocaine 50 nM	5.2 \pm 0.8 (7)	2.9 \pm 0.3 (9)
Cocaine 100 nM	4.0 \pm 0.5 (7)	2.2 \pm 0.2 (6)

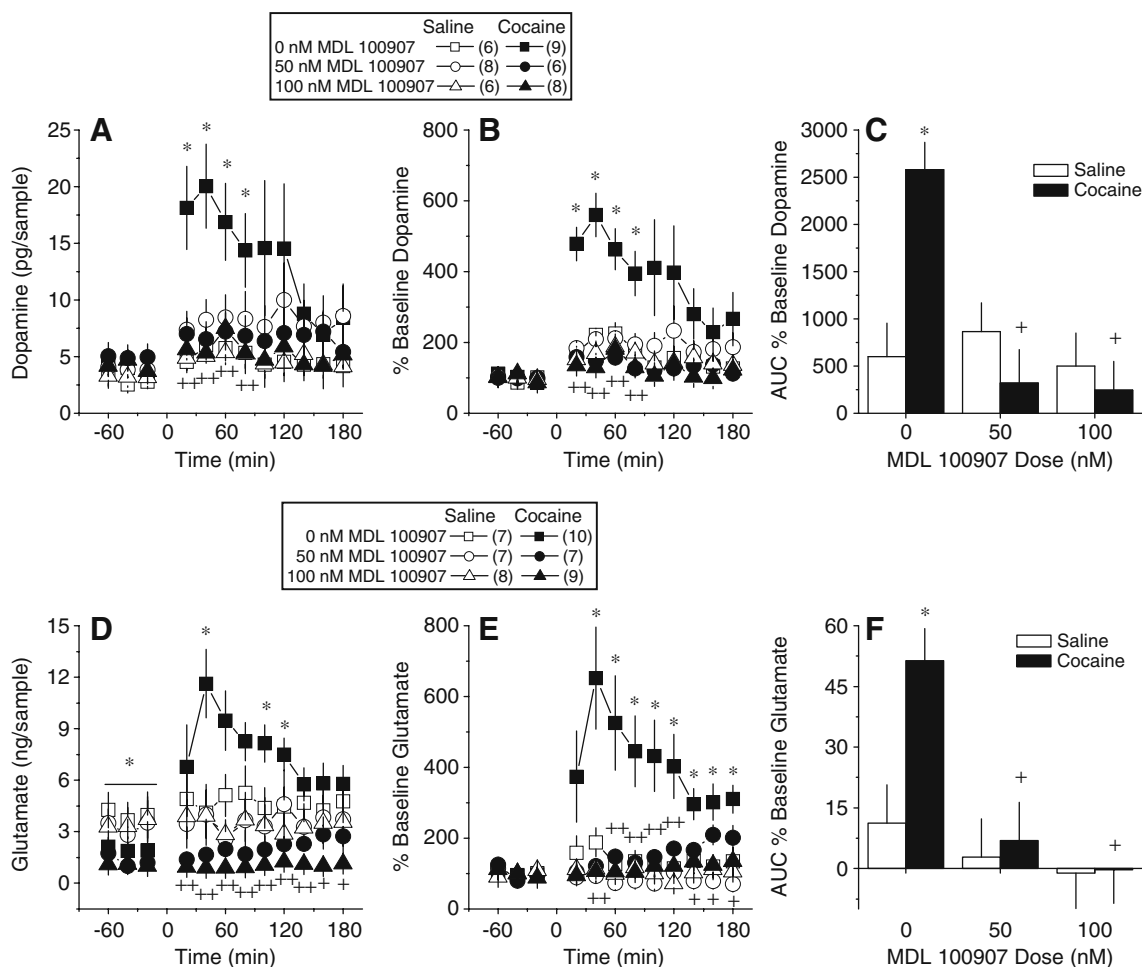


Fig. 4 Effects of intra-NAC 5-HT_{2A} blockade with MDL 100907 upon cocaine-induced neurochemical sensitization. **a** Summary of the effects of perfusing the NAC, via the microdialysis probe, with 0, 50, or 100 nM of the 5-HT_{2A} antagonist MDL 100907 upon the time-course of changes in extracellular dopamine elicited by a 15-mg/kg cocaine challenge injection, administered 3 weeks following repeated saline/cocaine treatment. **b** Data from **a** expressed as a percent change from the average baseline values. **c** Area under the curve (AUC) for the data from **b**. **d** Summary of the effects of perfusing the NAC with MDL 100907 upon the time-course of changes in extracellular glutamate elicited by the 15 mg/kg cocaine challenge. **e** Data from **b**

expressed as a percent change from the average baseline glutamate values. **f** AUC for the data from **e**. For all panels, saline-treated animals are represented by *open symbols*, and cocaine-treated animals are represented by *closed symbols*. The data represent the mean±SEM of the number of animals indicated in *parentheses*. For **a**, **b**, **d**, **e**, * $p < 0.05$ for 0 nM cocaine vs. respective saline (cocaine effect/sensitization); + $p < 0.05$ for 100 nM vs. 0 nM pretreatment; ++ $p < 0.05$ for both 50 and 100 nM vs. respective 0 nM pretreatment. For **c** and **f**, * $p < 0.05$ for saline vs. cocaine (cocaine effect/sensitization); + $p < 0.05$ for 50 or 100 nM vs. 0 nM pretreatment

(33,253)=5.21, $p < 0.0001$), but not in saline animals, and deconstruction along the pretreatment factor indicated the presence of sensitization in vehicle-perfused controls (Fig. 5a; repeated treatment×time, $p > 0.05$), in rats perfused with 50 nM SB 242084 (Fig. 5b; repeated treatment×time: $F(11,110)=14.66$, $p < 0.0001$), and in rats perfused with 100 nM SB 242084 (Fig. 5c; repeated treatment×time: $F(11,121)=3.65$, $p < 0.001$), although this latter interaction reflected saline–cocaine differences only during the first 10-min time-bin (Fig. 5a vs. b, triangles; post hoc tests). No sensitization was apparent in cocaine-treated rats perfused with 500 nM SB 242084. Similar statistical results were obtained upon analysis of the total distance traveled by the

animals (Fig. 5c) (pretreatment×repeated treatment: $F(3,51)=6.56$, $p = 0.001$), with planned comparisons revealing cocaine–saline differences for rats pretreated with vehicle ($F(1,13)=8.93$, $p = 0.01$) and 50 nM SB 242084 ($F(1,11)=28.93$, $p < 0.0001$), but not the two higher SB 242084 doses. Thus, blocking 5-HT_{2C} receptors within the NAC also attenuates the expression of cocaine-induced locomotor sensitization following protracted withdrawal.

The effects of intra-NAC 5-HT_{2C} blockade with SB 242084 upon the expression of cocaine-induced neurochemical sensitization A comparison of the basal dopamine levels between the different groups failed to reveal group differences

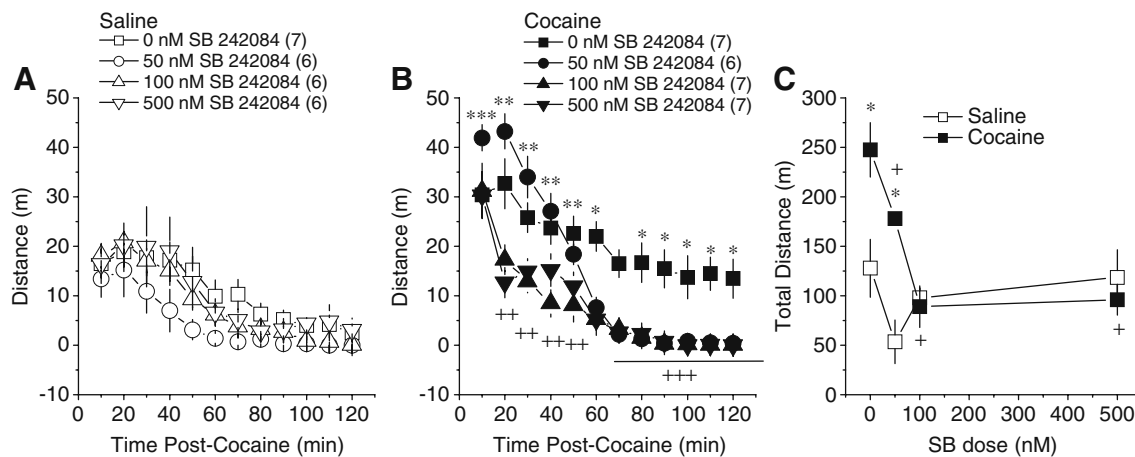


Fig. 5 Effects of intra-NAC 5-HT_{2C} blockade with SB 242084 upon the acute and sensitized locomotor response to cocaine. **a** Summary of the effects of 0, 50, 100 and, 500 nM of the 5-HT_{2C} antagonist SB 242084 administered into the NAC upon the time-course of locomotor hyperactivity elicited by an acute injection of 15 mg/kg cocaine, administered 3 weeks following repeated saline treatment. **b** Summary of the effects of intra-NAC SB 242084 upon the time-course of locomotion elicited by a 15 mg/kg cocaine challenge injection, administered 3 weeks following repeated cocaine treatment. * $p < 0.05$ for 0 nM-Cocaine vs. respective Saline; ** $p < 0.05$ for both 0 &

50 nM-Cocaine vs. respective Saline groups (sensitization); + $p < 0.05$ for 500 nM vs. respective 0 nM pretreatment; ++ $p < 0.05$ for both 100 and 500 nM vs. respective 0 nM pretreatment; +++ $p < 0.05$ for 50, 100 and 500 nM vs. respective 0 nM pretreatment. **c** Effect of intra-NAC MDL 100907 upon the total distance traveled during the 2-h test session by repeated saline- and cocaine-treated rats. * $p < 0.05$ Cocaine vs. Saline (sensitization); + $p < 0.05$ vs. 0 nM (pretreatment effect). The data represent the mean \pm SEM of the number of animals indicated in parentheses

(Table 1). The effects of an intra-NAC infusion of SB 242840 upon the dopamine response to cocaine depended upon the prior cocaine history of the animal (Fig. 6a, b; raw data: pretreatment \times repeated treatment \times time: $F(22,418) = 2.70$, $p < 0.0001$; normalized data: $F(22,418) = 2.24$, $p = 0.001$). Deconstruction of the three-way interaction along the repeated treatment factor revealed SB 242084 effects in cocaine-treated rats (Fig. 6b; raw data: $F(22,220) = 3.92$, $p < 0.0001$; normalized data: $F(22,220) = 2.58$, $p < 0.0001$), but not in saline animals (Fig. 6a). Planned comparisons indicated dopamine sensitization in vehicle-perfused controls (raw data: repeated treatment \times time: $F(11,154) = 3.59$, $p < 0.0001$; normalized data: $F(11,154) = 2.35$, $p = 0.01$) and in rats perfused with 50 nM SB 242840 (raw data: $F(11,132) = 2.42$, $p = 0.009$; normalized data: $F(11,132) = 1.98$, $p = 0.04$). In rats perfused with intra-NAC with 100 nM SB 242840, a significant repeated treatment \times time interaction was observed when the raw time-course data were examined ($F(11,132) = 4.16$, $p < 0.0001$). However, post hoc analyses failed to indicate cocaine–saline differences at any time-point prior to, or following, cocaine injection (Fig. 5a), and an analysis of the normalized data made it apparent that the interaction at 100 nM SB 242084 reflected a blunted cocaine-induced rise in dopamine in cocaine-treated vs. saline-treated rats (Fig. 5b; $F(11,132) = 6.83$, $p < 0.0001$; post hoc tests). As was found for the MDL 100907 study (see Fig. 4c), the differential effects of SB 242084 infusion upon the dopamine response to cocaine between repeated saline- and cocaine-treated rats were more apparent when the AUC for the data presented in Fig. 6b was considered (repeated treatment \times

pretreatment: $F(2,43) = 8.78$, $p = 0.001$). As depicted in Fig. 6c, the cumulative cocaine-elicited rise in dopamine has greater in vehicle-treated cocaine animals, relative not only to their saline controls, but also in comparison to their cocaine counterparts pretreated with either 50 or 100 nM MDL 100907 (pretreatment effect: $F(2,22) = 10.07$, $p = 0.001$). In contrast, the cumulative cocaine-elicited rise in dopamine did not vary across the pretreatment groups in repeated saline-treated animals (pretreatment effect, $p > 0.05$). These data indicate that intra-NAC 5-HT_{2C} blockade with SB242084 prevents the expression of cocaine-induced dopamine sensitization in the NAC, without significantly influencing the capacity of acute cocaine to elevate NAC dopamine levels.

As observed in the MDL 100907 study (Table 1), withdrawal from repeated cocaine treatment reduced basal extracellular levels of NAC glutamate also in the SB 242084 study, but the magnitude of this cocaine effect did not vary across the three pretreatment groups (Table 1; repeated treatment effect: $F(1,50) = 3.84$, $p = 0.03$). As illustrated in Fig. 6d, e, intra-NAC SB 242084 perfusion differentially affected the glutamate response to cocaine in repeated saline and cocaine animals (pretreatment \times treatment \times time: $F(22,495) = 2.02$, $p = 0.004$; normalized data: $F(22,495) = 3.25$, $p < 0.0001$). Deconstruction of the three-way interaction along the repeated treatment factor supported an SB242084 effect in rats treated repeatedly with cocaine (raw data: pretreatment \times time: $F(22,242) = 5.22$, $p < 0.0001$; normalized data: $F(22,242) = 5.13$, $p < 0.0001$), but not in saline-treated rats (interactions, $p > 0.10$). Planned

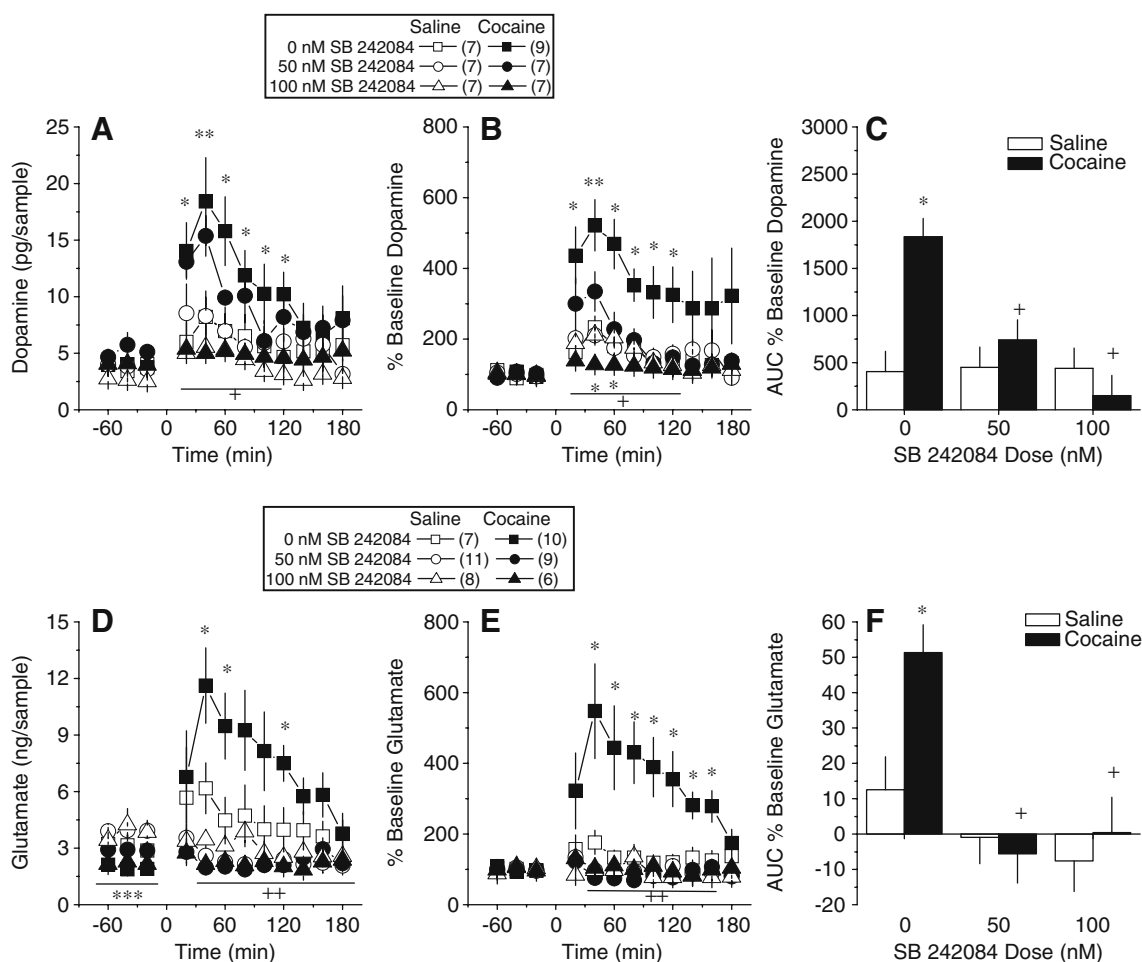


Fig. 6 Effects of intra-NAC 5-HT_{2C} blockade with SB 242084 upon cocaine-induced neurochemical sensitization. **a** Summary of the effects of perfusing the NAC, via the microdialysis probe, with 0, 50, or 100 nM of the 5-HT_{2C} antagonist SB 242084 upon the time-course of changes in extracellular dopamine elicited by a 15-mg/kg cocaine challenge injection, administered 3 weeks following repeated saline/cocaine treatment. **b** Data from **a** expressed as a percent change from the average baseline values. **c** AUC for the cumulative data in **b**. **d** Summary of the effects of perfusing the NAC with SB 242084 upon the time-course of changes in extracellular glutamate elicited by the 15 mg/kg cocaine challenge. **e** Data from **d** expressed as a percent

change from the average baseline glutamate values. **f** AUC for the cumulative data in **e**. For all panels, saline-treated animals are represented by *open symbols*, and cocaine-treated animals are represented by *closed symbols*. The data represent the mean±SEM of the number of animals indicated in parentheses. For panels **a**, **b**, **d**, **e**, * $p < 0.05$ for 0 nM-Cocaine vs. respective Saline (cocaine effect/sensitization); + $p < 0.05$ for 100 nM vs. respective 0 nM pretreatment; ++ $p < 0.05$ for both 50 and 100 nM vs. respective 0 nM pretreatment. For **c** and **f**: * $p < 0.05$ for saline vs. cocaine (cocaine effect/sensitization); + $p < 0.05$ for 50 or 100 nM vs. 0 nM pretreatment

comparisons revealed cocaine–saline differences between animals perfused with vehicle (0 mM: raw data: treatment×time: $F(11,165)=2.04$, $p=0.03$; normalized data: $F(11,165)=3.44$, $p<0.0001$), but not in the groups infused with either 50 nM (Fig. 6d, e; interactions, $p>0.40$) or 100 mM SB242084 (interactions, $p>0.15$). Interestingly, an analysis of the effects of intra-NAC SB 242084 upon the cumulative rise in NAC glutamate produced by the cocaine challenge (from Fig. 6e) revealed a significant repeated treatment×pretreatment interaction ($F(2,45)=3.62$, $p=0.04$), suggesting a differential effect of SB 242084 upon cocaine-induced glutamate release. However, as depicted in Fig. 6f, intra-NAC SB 242084 not only abolished the cocaine-sensitized rise in glutamate in repeated cocaine-treated animals (pre-

treatment effect: $F(2,24)=8.69$, $p=0.002$), but the 100 nM dose also significantly reduced cumulative glutamate levels in repeated saline-treated controls (pretreatment effect: $F(2,25)=4.00$, $p=0.03$). Thus, intra-NAC blockade of 5HT_{2C} receptors by SB 242084 completely blocks the expression of glutamate sensitization, as well as reduces basal extracellular levels of glutamate in the NAC.

Discussion

Both 5-HT_{2A} and 5-HT_{2C} receptors are expressed in moderate to high abundance within both the cell body and

terminal regions of the mesolimbic dopamine system, including the NAC (Compan et al. 1998a, b; Eberle-Wang et al. 1997; Clemett et al. 2000; Bubar and Cunningham 2006). Repeated cocaine administration sensitizes the capacity of this drug to elevate extracellular levels of serotonin within the NAC (Parsons and Justice 1993; Szumlinski et al. 2004), and ascending serotonin projections to the NAC from both the median and dorsal raphe modulate the long-term expression of cocaine-induced behavioral and neurochemical sensitization (Szumlinski et al. 2004). However, studies designed to address the precise role for NAC 5-HT_{2A} and 5-HT_{2C} receptors have yielded conflicting results with respect to the psychomotor-activating properties of cocaine, and little study has focused on their role in the manifestation of cocaine-induced behavioral sensitization following protracted periods of withdrawal from repeated cocaine treatment. Thus, the present study employed intra-NAC infusion of selective 5-HT_{2A} and 5-HT_{2C} antagonists to examine the functional relevance of these receptors for the acute and sensitized locomotor and neurochemical responses to cocaine.

NAC 5-HT_{2A} receptors and cocaine-induced neuroplasticity

In contrast to the attenuating effect of systemic 5-HT_{2A} antagonist pretreatment upon acute cocaine-induced locomotion (O'Neill et al. 1999; Filip and Cunningham 2002; McMahan and Cunningham 2001; Filip et al. 2004) and the expression of cocaine-induced locomotor sensitization in rats withdrawn for 5 days from repeated cocaine treatment (Filip et al. 2004), intra-NAC 5-HT_{2A} blockade was reported to produce negligible effects upon acute cocaine-induced locomotion (McMahan et al. 2001). The results of the present locomotor study, using a fivefold dose-range of the selective 5-HT_{2A} antagonist MDL 100907, are more consistent with those of Filip et al. (2004) in that intra-NAC MDL 100907 reduced cocaine-induced locomotion, regardless of the prior cocaine history of the animal (Fig. 3). Even at the lowest dose tested (50 nM), intra-NAC MDL 100907 reduced the expression of cocaine-induced locomotion by approximately 50% in both repeated treatment groups (Fig. 3c), and all MDL 100907 doses reduced the level of cocaine-induced locomotion exhibited by repeated cocaine-treated rats down to that of their repeated saline counterparts (Fig. 3c). While the MDL 100907 attenuation of cocaine-induced locomotion might reflect some disruption in motor capacity, this possibility is mitigated by existing evidence that neither systemic nor intra-cranial MDL 100907 pretreatment affects spontaneous/saline-induced locomotor activity (e.g., McMahan and Cunningham 2001; McMahan et al. 2001; Filip et al. 2004). The present data point to the activation of NAC shell 5-HT_{2A} receptors by cocaine-elicited increases in 5-HT as a neural substrate

mediating both the acute and the persistent effects of cocaine upon psychomotor activity.

5-HT_{2A} receptors facilitate neuronal excitability within the cell body and terminal regions of the mesolimbic dopamine system; the local perfusion of the NAC with the non-selective 5-HT₂ agonist DOI elevates extracellular dopamine levels (Bowers et al. 2000; Yan et al. 2000), while systemic pretreatment with both selective and non-selective 5-HT_{2A} antagonists (but not mixed 5-HT_{2B/2C}) reduce the NAC dopamine response elicited by dorsal raphe stimulation (De Deurwaerdère and Spampinato 1999). Of relevance to this study, systemic pretreatment with the non-selective 5-HT_{2A/2C} antagonist ketanserin is reported to block the capacity of acute cocaine to elevate NAC levels of both dopamine and serotonin (Broderick et al. 2004), and systemic pretreatment with the 5-HT_{2A} preferring antagonist SR 46349B reduces both amphetamine- and morphine-induced dopamine release in the NAC (Auclair et al. 2004a, b; Porras et al. 2002). Moreover, repeated cocaine-treated rats exhibit an increased capacity of DOI to elevate NAC dopamine levels (Yan et al. 2000), indicating a sensitization of this effect by repeated cocaine experience. The results of the present study extend these earlier dopamine findings by demonstrating that the intra-NAC perfusion of the selective 5-HT_{2A} antagonist MDL 100907 reduces the capacity of cocaine to elevate NAC extracellular dopamine levels but, interestingly, only in animals with repeated cocaine experience (Fig. 4a–c).

While no published report exists concerning 5-HT_{2A} receptor regulation of NAC extracellular glutamate, considerable electrophysiological and neurochemical data indicate that in various frontal cortical regions, 5-HT_{2A} receptor activation stimulates glutamate release and/or increases glutamate neuron excitability via both pre- and postsynaptic mechanisms (Aghajanian and Marek 1997; Hasuo et al. 2002; Marek and Aghajanian 1994, 1999; Marek et al. 2001, 2006; Martin-Ruiz et al. 2001; Scruggs et al. 2000, 2003; Wang 2005; Wang et al. 2006). Consistent with the possibility that 5-HT_{2A} receptors exert a facilitatory role also over NAC extracellular levels of glutamate, intra-NAC infusion of MDL 100907 completely blocked the expression of cocaine-induced glutamate sensitization, without influencing NAC glutamate levels in animals treated acutely with this stimulant (Fig. 4d–f). These data provide the first evidence that 5-HT_{2A} receptors, localized specifically to the NAC shell, are critical for the expression of cocaine-induced dopamine and glutamate sensitization and pose an important role for a cocaine-sensitized increase in 5-HT_{2A} function within the NAC shell (Yan et al. 2000) in mediating these neurochemical adaptations. Lesions of the cell body or terminals of thalamocortical glutamatergic projections are reported to upregulate 5-HT_{2A} receptor expression within the prefrontal

cortex (Marek et al. 2001), indicating that a reduction in glutamate tone, at least within prefrontal cortex, is sufficient to sensitize 5-HT_{2A} receptors. Protracted withdrawal from repeated cocaine is well characterized to reduce glutamate tone within the NAC (see Vanderschuren and Kalivas 2000 for review; Table 1). This raises the intriguing possibility that a cocaine-induced reduction in basal NAC glutamate may trigger an increase in 5-HT_{2A} receptor expression/function that then promotes or facilitates subsequent glutamate and dopamine release within this region. Given the putative roles for cocaine-induced NAC dopamine and glutamate sensitization in mediating both the addictive and the behavioral-sensitizing properties of this drug (for reviews Vanderschuren and Kalivas 2000; Everitt and Wolf 2002; Kalivas 2007), such a mechanism may have relevance for both the anti-psychotic and anti-addictive properties of 5-HT_{2A} antagonists (for reviews, de Angelis 2002; Seeman 2002; Bubar and Cunningham 2006).

NAC 5-HT_{2C} receptors and cocaine-induced neuroplasticity

The data concerning the effects of 5-HT_{2C} antagonists upon cocaine-induced locomotion are highly discrepant; systemic pretreatment with 5-HT_{2C} antagonists augments acute cocaine-induced locomotion (Filip et al. 2004; Fletcher et al. 2006) but reduces (albeit moderately) the expression of cocaine-induced sensitization in animals withdrawn for 5 days (Filip et al. 2004). In contrast, intra-NAC pretreatment with the 5-HT_{2C} antagonist RS 102221 into the shell subregion can attenuate acute cocaine-induced locomotor hyperactivity (McMahon et al. 2001); however, the present study failed to detect an effect of intra-NAC 5-HT_{2C} blockade upon acute cocaine-induced locomotion using a fivefold dose-range of a different, but also selective, antagonist SB 242084 (Fig. 5). A comparison of the microinjector placements within the NAC revealed no obvious discrepancies in target sites between the two studies (Fig. 1 vs. McMahon et al. 2001) nor are the differential results easily explained by differences in the relative affinities of RS 102221 vs. SB 242084 for the 5-HT_{2C} receptor (both nM, with an approximately 30-fold lower affinity for other 5-HT receptor subtypes; Bonhaus et al. 1997; Kennett et al. 1997). In the present study, acute cocaine animals were microinjected intra-NAC with SB 242084 only once, and this occurred at 3-week withdrawal from repeated saline treatment. In contrast, acute cocaine animals in the earlier study by McMahon and colleagues (2001) were first habituated to the microinjection handling procedures prior to the commencement of testing, and rats were tested repeatedly either under the influence of saline or cocaine, every 3 days, for a total of eight tests. Thus, differences in the extent to which rats were habituated to the microinjection procedures, possible receptor adaptations secondary to repeated intra-

NAC antagonist administration and/or possible development of cocaine-induced locomotor sensitization with once weekly repeated testing, might all have contributed to the discrepancies in results. In support of the latter possibility, intra-NAC SB 242084 blocked cocaine-induced locomotion in repeated cocaine-treated animals when tested at 3-week withdrawal (Fig. 5). Such data are more or less consistent with the moderate attenuation of shorter-term cocaine-induced sensitization observed upon systemic pretreatment with 5-HT_{2C} antagonists (Filip et al. 2004) and point to an important role for 5-HT_{2C} receptors, located within the NAC shell, in mediating this form of cocaine-induced behavioral plasticity.

In contrast to 5-HT_{2A} ligands, systemic pretreatment with 5-HT_{2C} compounds does not appear to affect indices of basal or stimulated glutamate release (at least within cortical regions) (Hasuo et al. 2002; Marek and Aghajanian 1994, 1999). However, systemic pretreatment with either non-selective 5-HT_{2C/2B} agonists or selective 5-HT_{2C} agonists decreases indices of basal and evoked dopamine neuronal activity (Di Giovanni et al. 2000; Di Matteo et al. 1999; Di Matteo et al. 2000a, b), while systemic pretreatment with either non-selective 5-HT_{2C/2B} antagonists or certain selective 5-HT_{2C} antagonists dose-dependently increases indices of basal and electrically evoked dopamine neuronal activity (Di Giovanni et al. 1999; Di Matteo et al. 1998; 1999). Of more direct relevance to addiction, pretreatment with 5-HT_{2C} antagonists fails to alter amphetamine-induced dopamine release (Porrás et al. 2002), but both 5-HT_{2C} antagonists and 5-HT_{2C} gene deletion potentiate the dopamine responses to acute cocaine and morphine (Navailles et al. 2004; Porrás et al. 2002; Rocha et al. 2002). Conversely, 5-HT_{2C} receptor activation reduces nicotine-induced activation of VTA neurons (Pierucci et al. 2004) and prevents the expression of nicotine-induced dopamine sensitization in the NAC (Di Matteo et al. 2004). Thus, 5-HT_{2C} receptors appear to be important regulators of impulse-dependent dopamine release within the NAC (Porrás et al. 2002). Interestingly, recent study of the effects of perfusing the NAC with the selective 5-HT_{2C} agonist Ro 60-0175 and the selective 5-HT_{2C} antagonist SB 242084 upon cocaine-induced changes in NAC dopamine revealed effects that were biphasic with respect to dose; 100 nM of the agonist increased, while 100 nM of the antagonist decreased. The cocaine-induced rise in NAC dopamine and polar opposite effects were observed when a 1.0- μ M concentration of each drug was perfused (Navailles et al. 2008). While we failed to detect significant effects of intra-NAC perfusion with SB 242084 upon extracellular levels of dopamine, we did observe a reduction in extracellular glutamate at the 100 nM SB 242084 dose in rats treated acutely with cocaine (Fig. 6f), as well as an attenuating effect of both doses of SB 242084 upon the sensitized dopamine and glutamate responses exhibited by

cocaine-sensitized animals (Fig. 6). In fact, pretreatment with either SB 242084 dose completely prevented the expression of both dopamine and glutamate sensitization when assessed at 3-week withdrawal. While similarities in the effects of NAC 5-HT_{2A} and 5-HT_{2C} blockade (see Fig. 4 vs. Fig. 6) might suggest a non-selective effect of antagonist infusion, the likelihood that the observed attenuation of the cocaine-sensitized rise in NAC dopamine by intra-NAC SB 242084 infusion is due to blockade of the 5-HT_{2A} receptor is low, given that this drug exhibits a 160-fold higher affinity for the 5-HT_{2C} vs. 5-HT_{2A} receptor (Kennett et al. 1997) and higher doses of SB 242084 elevate, not reduce, the cocaine-induced rise in NAC dopamine (Navailles et al. 2008). The control of NAC dopamine activity by 5-HT_{2C} receptors was considered to be indirect and involve activation of GABAergic interneurons within the VTA (Di Matteo et al. 2000a, b; Navailles et al. 2004). As 5-HT_{2C} receptors are localized primarily to GABAergic interneurons within the two major sources of glutamate to the NAC—the prefrontal cortex (Liu et al. 2000; Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999) and the basolateral amygdala (Stein et al. 2000)—one might speculate on an indirect, GABAergic, mechanism within these limbic sites to account also for an effect of 5-HT_{2C} ligands upon NAC glutamate transmission. However, the present data and the recent report by Navailles et al. (2008) clearly indicate that 5-HT_{2C} receptors within the NAC properly facilitate NAC dopamine/glutamate release. While the precise mechanisms through which NAC 5-HT_{2C} receptors regulate cocaine-induced neurotransmitter release within the NAC require considerable investigation (see Berg et al. 2008 for discussion), the parallels in the effects of NAC 5-HT_{2C} blockade upon the expression of cocaine-induced behavioral and neurochemical sensitization implicate drug-induced alterations in NAC 5-HT_{2C} function as a potential neural substrate mediating the addictive and psychotogenic properties of this drug.

Conclusions

Intra-NAC infusions of the selective 5-HT_{2A} antagonist MDL 100907 and the selective 5-HT_{2C} silent antagonist SB 242084 both reduced the expression of cocaine-induced behavioral and neurochemical sensitization observed in rats following protracted withdrawal from repeated cocaine exposure. As the sensitization of dopamine and glutamate within the NAC is considered a critical cocaine-induced neurochemical adaptation underlying the development of addiction and related neuropsychiatric conditions (including psychosis, depression, and possibly anxiety), the ability of 5-HT_{2A} and 5-HT_{2C} antagonists to reverse the neurochemical consequences of repeated cocaine might contribute to their putative anti-addictive or anti-psychotic effects.

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