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Biphasic alterations in Serotonin-1B (5-HT_{1B}) receptor function during abstinence from extended cocaine self-administration

Laura E. O'Dell,† Ann M. Manzardo,§ Ilham Polis,* David G. Stouffer* and Loren H. Parsons*

**Molecular and Integrative Neurosciences Department, The Scripps Research Institute, La Jolla, California, USA*

†Department of Psychology, The University of Texas at El Paso, El Paso, Texas, USA

§Department of Psychiatry and Behavioral Sciences, The University of Kansas Medical Center, Kansas City

Abstract

Alterations in 5-HT_{1B} receptor function during cocaine abstinence were evaluated in rats given either limited- or extended access (LA and EA, respectively) to cocaine self-administration. The locomotor response to the 5-$HT_{1B/1A}$ agonist RU24969 was significantly reduced in cocaine-experienced animals relative to cocaine-naïve controls following 6 h of abstinence but became sensitized over the subsequent 14 days of abstinence. Both the early phase subsensitivity and later phase supersensivity to RU 24969-induced activity were greater in EA versus LA animals. Intra-nucleus accumbens administration of the 5-HT_{1B} agonist CP 93, 129 produced significantly greater increases in dialysate dopamine levels in EA versus control animals following 14 days of abstinence. However, there was no difference between EA and cocaine-naïve control animals in the augmentation of cocaine-induced increases in nucleus accumbens DA produced by intra-VTA CP 93, 129. Collectively these findings demonstrate that 5-HT_{1B} receptor function is persistently altered by cocaine self-administration.

Keywords: cocaine, dopamine, microdialysis, self-administration, serotonin$_{1B}$, withdrawal.

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Humans frequently use cocaine in repetitive episodes or 'binges' lasting from 12 h to several days during which the drug is continuously re-administered. In contrast to the effects produced by intermittent 'recreational' cocaine use, binge patterns of cocaine use result in increased impulsivity, sexuality and aggression while abstinence from binge cocaine use is associated with severe withdrawal symptoms and high rates of relapse (for a review see Gawin and Ellinwood 1988; Coccaro *et al*. 1989; Fishbein *et al*. 1989; Moeller *et al*. 1994). These effects may be produced by adaptations in molecular and/or cellular processes that develop during long-term drug abuse which are believed to oppose the pharmacological effects of the drug, and to persist during abstinence after cocaine has cleared from the brain (Solomon and Corbit 1974; Koob *et al*. 1998). When left un-opposed in this manner, these adaptations are thought to suppress the neurochemical systems that mediate the acute neurophysiological and behavioral effects of cocaine, thereby producing symptoms of withdrawal. However, the nature of these adaptive changes as well as the neurochemical systems that are affected by this process are not yet fully understood.

Substantial evidence suggests that the positive reinforcing effects of cocaine are dependent on the ability of this drug to enhance extracellular dopamine (DA) concentrations in the mesocorticolimbic system (for a review see Wise 1984; Koob and Bloom 1988; Kuhar *et al*. 1991). Consistent with the neuroadaptation theory, both clinical and preclinical evidence suggest that reductions in DA neurotransmission contribute to symptoms of cocaine withdrawal (for a review see Dackis and Gold 1985; Weiss *et al*. 1995). Preclinical studies indicate that in addition to DA deficits, extracellular serotonin (5-HT) levels are also severely decreased during abstinence from extended cocaine exposure (Egan *et al*. 1994; Parsons *et al*. 1995). Because there is serotonergic regulation of the behavioral effects of psychostimulants in

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Address correspondence and reprint requests to Loren H. Parsons, Molecular and Integrative Neurosciences Department, SP-2120, The Scripps Research Institute, La Jolla, CA, 92037, USA.
E-mail: lparsons@scripps.edu.

Abbreviations used: DA, dopamine; EA, extended access; LA, limited access; NAC, nucleus accumbens; VTA, ventral tegmental area.

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humans (Walsh *et al*. 1994; Aronson *et al*. 1995; Satel *et al*. 1995; Buydens-Branchey *et al*. 1997) as well as in rats and monkeys (Carroll *et al*. 1990a,b; Loh and Roberts 1990; Spealman 1993; Callahan and Cunningham 1995, 1997; Parsons *et al*. 1996; 1998), it may be hypothesized that decrements in 5-HT neurotransmission also contribute to cocaine withdrawal symptoms. However, in contrast to dopaminergic mechanisms, relatively little is known about the specific serotonergic mechanisms which contribute to the behavioral and physiological effects of cocaine, and thus potentially to the symptoms of cocaine withdrawal.

Recent findings suggest that serotonin$_{1B}$ (5-HT$_{1B}$) receptors, in particular, are involved in the generation of the behavioral and neurophysiological effects produced by cocaine. 5-HT$_{1B}$ receptors are the rodent homologue of the human 5-HT$_{1D\beta}$ receptor (for a review see Hartig *et al*. 1992) and are the most abundant 5-HT receptor subtype in the ventral midbrain (for a review see Hoyer *et al*. 1994). Activation of midbrain 5-HT$_{1B}$ receptors results in disinhibition of DA neurons in the substantia nigra and VTA (Johnson *et al*. 1992; Cameron and Williams 1994, 1995) and facilitation of DA release in the dorsal and ventral striatum (Guan and McBride 1989; Higgins *et al*. 1991; Yan *et al*. 2004). 5-HT$_{1B}$ agonists have been shown to enhance the locomotor activating (Przegalinski *et al*. 2001), place conditioning (Cervo *et al*. 2002), discriminative stimulus (Callahan and Cunningham 1995, 1997; Filip *et al*. 2001) reinforcing (Parsons *et al*. 1998) and neurochemical (Parsons *et al*. 1999; O'Dell and Parsons 2004) effects produced by cocaine. Moreover, there is evidence for a specific involvement of 5-HT$_{1B}$ receptors in the cocaine-induced expression of the immediate early gene *c-fos* in several brain regions (Lucas *et al*. 1997). Together, these findings provide strong evidence for an involvement of 5-HT$_{1B}$ receptors in the behavioral and cellular responses elicited by cocaine. Based on the neuroadaptation theory, it may therefore be hypothesized that alterations in 5-HT$_{1B}$ receptor function contribute to behavioral and physiological abnormalities associated with abstinence from long-term cocaine use. Supporting this hypothesis, 5-HT$_{1B}$ receptor dysfunction in rodents has been shown to produce many of the symptoms which are present in humans during chronic cocaine use and withdrawal such as aggression, depression and anxiety (Pellow *et al*. 1985; Chopin and Briley 1987; Benjamin *et al*. 1990; Edwards *et al*. 1991; Saudou *et al*. 1994; Buhot and Naili 1995; Ramboz *et al*. 1996).

The present experiments were designed to test the hypothesis that 5-HT$_{1B}$ receptor function is altered after long-term exposure to cocaine. In an effort to model both high-dose cocaine 'binging' and more intermittent 'recreational' cocaine use in humans, two groups of cocaine self-administering rats were examined. One group was trained to self-administer cocaine in daily 3 h sessions and was given additional access to cocaine for a 12 h session (extended access group, EA), while the other group was given only 3 h cocaine sessions throughout the self-administration period (limited access group, LA). Alterations in 5-HT$_{1B}$ receptor function during cocaine abstinence in these cocaine-exposed groups (relative to cocaine-naïve controls) were investigated by evaluating (1) the locomotor response to the 5-HT$_{1B/1A}$ agonist RU 24969 over the course of 14 days of cocaine abstinence; (2) alterations in the effect of intra-NAC CP 93, 129 (a selective 5-HT$_{1B}$ agonist; administered via retrodialysis) on dialysate DA levels after 14 days of cocaine abstinence; (3) alterations in the effect of intra-VTA CP 93, 129 administration on cocaine-induced increases in NAC DA levels and decreases in VTA GABA levels after 14 days of cocaine abstinence.

Materials and methods

Animals and surgery

Male Wistar rats (Charles River, Hollister, CA, USA; $n = 82$) weighing 275–300 g upon delivery were housed in groups of 2–3 in a humidity and temperature-controlled (22°C) vivarium on a 12 h light/dark cycle (lights off 10AM). All animals were surgically prepared with chronic indwelling jugular catheters under anesthesia (1.0–1.5% isofluorane/oxygen mixture) as previously described (Parsons *et al*. 1998). Animals used in the microdialysis experiments were also stereotaxically implanted with 21-gauge stainless steel microdialysis guide cannulae (Plastics One, Roanoke, VA, USA) using coordinates based on the atlas by Paxinos and Watson (1998). Animals in Experiment 2 received a single guide cannula lowered to the dorsal surface of the NAC (from bregma AP, + 1.7 mm; ML ± 1.2 mm; from dura V, − 6.1 mm). Animals in Experiment 3 were implanted with two guide cannulae: one lowered to the dorsal surface of the NAC as described above and the other lowered to the dorsal surface of the ipsilateral VTA (from Bregma AP, − 4.8 mm; ML, ± 0.8 mm; V, − 7.4 mm). During recovery, rats received 0.2 mL of the antibiotic ticarcillin (100 mg/mL, i.v) and catheters were flushed daily with sterile heparinized saline (30 USP units/mL) for the duration of the experiments. A minimum of 7 post operative days for recovery were allowed prior to any training or testing, and all procedures were conducted in strict adherence to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Drugs and reagents

Cocaine HCl was obtained from the National Institute on Drug Abuse (Washington D.C., USA). RU 24969 was generously provided by Roussel UCLAF (Romainville, France). CP 93, 129 was purchased from Tocris Cookson (Ellisville, MO, USA). Sterile saline (0.9% solution) was the vehicle for all systemically administered drugs used in this study, and all doses refer to the weights of the respective salts. All reagents used in the preparation of artificial cerebral spinal fluid (aCSF), high performance liquid chromatography (HPLC) mobile phases, capillary electrophoresis buffers and neurotransmitter standards were obtained from Sigma-Aldrich (St Louis, MO, USA).

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Intravenous cocaine self-administration

Prior to catheterization surgery all rats were food restricted (20 g/rat/day) and allowed to lever press for 0.45 mg food pellets (Bio-Serve, Frenchtown, NJ, USA). Once stable responding for food on a fixed ratio 5 (FR-5) schedule of reinforcement was achieved, the rats were given *ad libitum* food for the remainder of the experiment. The animals were then surgically prepared with chronic indwelling jugular catheters under anesthesia (1.0–1.5% isofluorane/oxygen mixture) as previously described (Parsons *et al*. 1998). The animals were subsequently trained to self-administer cocaine in daily 3 h sessions where lever pressing was reinforced by 0.25 mg/infusion cocaine on an FR-5 schedule of reinforcement (see Parsons *et al*. 1998 for additional details). Stable patterns of cocaine intake were achieved in all animals within 7 training sessions and all animals were given a total of 14 daily 3 h cocaine sessions prior to the start of testing. Subsequently the animals were divided into one of two groups: (1) the Limited Access (LA) group, that received an additional 3 h cocaine self-administration session; and (2) the Extended Access (EA) group that received an additional 12 h cocaine self-administration session. Assignment to these groups was made based on cocaine intake during self-administration training to ensure similar cocaine exposure prior to the final self-administration session. For each experiment a third group of catheterized but cocaine-naïve animals was also prepared as a control comparison group. All operant sessions were conducted during the animals' dark cycle.

Locomotor activity testing

Motor activity was monitored in wire-grid cages (25 × 20 × 36 cm) equipped with 2 horizontal infrared photocell beams located across the long axis of the cages 2 cm above the floor and spaced 16 cm apart from one another. All activity tests were conducted in the animals' dark cycle and in the presence of a 70-dB white noise. The room containing the motor test cages was dimly illuminated by red light to permit observation of the animals from a darkened corridor adjacent to the testing room through a sound-attenuating window. Observational measures of stereotypy were made at 20-minute intervals following RU 24969 administration using the rating scale of Creese and Iversen (1973). Locomotor data were collected by a computer in a separate room. Prior to the first drug test, all animals were habituated to the locomotor activity cages for 3 h per day for two days. These habituation sessions took place on the last 2 days of the 14-day self-administration training period, and commenced 5 h after the self-administration session for that day.

In vivo microdialysis

At least 12 h prior to dialysis testing, microdialysis probes were lowered into each brain region (active membrane length was 2 mm for NAC and 1 mm for VTA, as described in O'Dell and Parsons 2004) and perfused overnight with aCSF at a rate of 0.2 μL/min. The following day, the perfusate flowrate was increased to 0.6 μL/min for NAC probes and 1.1 μL/min for VTA probes and allowed to equilibrate for at least 1 h prior to the experiment. Dialysate samples were then collected at 10-min intervals throughout each phase of the experiment and were immediately frozen and stored for subsequent analysis. Dialysate neurotransmitter levels were not adjusted by *in vitro* recovery values. After completion of the experiments the rats were sacrificed and dialysis probe placement was confirmed histologically. Only those subjects with accurate placement of the dialysis membrane within the region of interest were included in the final data analyses.

Analytical Procedures

Analysis of dialysate DA by HPLC with electrochemical detection

Dialysate DA concentrations were quantified from 5 μL volumes of dialysate injected onto a microbore high performance liquid chromatography (HPLC) system equipped with a 1 × 100 mm column (3 μm BetaBasic packing material, C-18 stationary phase, Keystone, Bellefonte, PA, USA) and eluted using a mobile phase composed of a 50-mM NaH_2PO_4 (monohydrate) buffer (pH 3.92) with 17% (v/v) acetonitrile, 0.27 mM Na_2-EDTA, 0.4% (v/v) triethylamine and 3.27 mM decane sulfonic acid delivered at 30 μL/min by an Isco model 500D syringe pump (Lincoln, NE, USA). DA was detected with an amperometric detector (model MP 1304, Princeton Applied Research) using dual glassy carbon working electrodes (BAS, Lafayette, IN, USA), set at 700 and − 10 mV. DA concentrations were determined using an external calibration curve. The limit of quantitation was approximately 0.2 nM.

Analysis of dialysate amino acid content by capillary electrophoresis with laser-induced fluorescence (CE-LIF)

Dialysate amino acid content was determined using capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection. Derivatization of the amino acids was achieved by mixing 6 μL of microdialysate with 9 μL of 40 mM borate buffer (pH 10.5) containing 3.8 mM KCN and 1 μL of 5 mM naphthalene-2,3-dicarboxaldehyde in MeOH. This mixture was allowed to react at room temperature in the dark for 30 min prior to placing the samples in the refrigerated (10°C) sample tray of the CE instrument (Agilent Technologies, Wilmington, DE, USA). The derivatized dialysate was subsequently loaded onto a 90-cm fused silica capillary (30 μm inner diameter; sample loading by 50 mbar pressure for 10 s) and the amino acids were separated using + 30 kV and a background electrolyte solution consisting of 200 mM borate buffer (pH 9.2) containing 36 mM SDS and 3 mM hydroxypropyl-b-cyclodextrin. The amino acids were detected using a laser-induced fluorescence detector (Zetalif, Picometrics, Ramon Ville, France) equipped with a 442-nm HeCd laser (30 mW, Melles Griot, Carlsbad, CA, USA). External calibration standards were run in duplicate and were interspersed throughout the sample run. The limits of quantitation were approximately 1 nM for each amino acid.

Experimental design

Experiment 1: motor response to RU 24969 during cocaine abstinence

Following the 14-day cocaine self-administration training period and habituation to the locomotor testing apparatus, the animals were divided into the naive, LA, and EA groups ($n = 8$ per group). The locomotor response to RU 24969 was examined in all animals on 5 separate test sessions over the course of 15 days. Each locomotor test began with a 1-h period during which drug-free baseline motor activity was monitored. All animals were then injected with 1 mg/kg RU

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24969, and motor activity was subsequently monitored for an additional 2 h. Animals were returned to their home cages in the vivarium between locomotor tests.

The 5 locomotor tests are referred to with regard to their proximity to the final cocaine self-administration session that delineates the LA and EA groups. The first locomotor test [Day (− 1)] commenced 5 h after the last 3 h training session on the day prior to the final LA or EA cocaine session. The animals in the EA group were allowed to self-administer cocaine for 12 h beginning 4 h after this first locomotor test, and animals in the LA group were allowed to self-administer cocaine for 3 h beginning 13 h after this first locomotor test. Five hours after this final self-administration session all animals were given the second locomotor test with RU 24969 (Day 0). The subsequent 3 locomotor tests occurred on Day 3, 7 and 14 after the last cocaine self-administration session.

Experiment 2: CP 93,129-induced increases in NAC DA efflux during cocaine abstinence

An initial test was conducted in cocaine-naïve subjects to determine the dose-dependent effects of the 5-HT_{1B} agonist CP 93, 129 on NAC dialysate DA ($n = 5$). Following collection of 6 baseline samples, the dialysate perfusate was switched to an aCSF solution containing 30 μM CP 93, 129 for 30 min, followed by 60 min washout with drug-free aCSF, a second 30 min perfusion with an aCSF containing 100 μM CP 93, 129 and an additional 40 min washout with drug-free aCSF. A second experiment was conducted in cocaine-naïve subjects ($n = 5$) to evaluate the ability of the selective 5-HT_{1B} antagonist GR 55562 (Lamothe *et al*. 1997) to block the effect produced by perfusate CP 93, 129. Following collection of 6 baseline samples, the dialysate perfusate was switched to an aCSF solution containing 100 μM CP 93, 129 for 30 min, followed by 60 min of washout with drug-free aCSF, a second 30 min perfusion with an aCSF containing both 100 μM CP 93, 129 and 300 μM GR 55562, and an additional 40 min washout with drug-free aCSF. The doses of 5-HT_{1B} compounds were chosen based on the reported affinity of these compounds for 5-HT_{1B} receptors (Macor *et al*. 1990; Lamothe *et al*. 1997) and on previous reports that have observed significant 5-HT_{1B} receptor-mediated neurochemical effects of these compounds when delivered locally by reverse dialysis (Yan and Yan 2001a; O'Dell and Parsons 2004). It should be noted that the drug concentration in the tissue surrounding the dialysis probe is substantially lower than the drug concentration in the perfusion medium due to limited drug diffusion across the probe membrane (estimated at 5–10% efficiency; data not shown) and dilution as the drug diffuses into the extracellular space.

In order to compare the effect of intra-NAC CP 93, 129 on dialysate DA during cocaine abstinence groups of cocaine-naïve ($n = 8$), LA ($n = 11$) and EA ($n = 8$) animals were prepared as described earlier. Microdialysis probes were implanted into the NAC on the evening of the thirteenth day of cocaine abstinence, and the effects of intra-NAC CP 93, 129 administration on dialysate DA levels were determined on the morning of the fourteenth day of abstinence. Following a 30-min baseline period, animals received 20-min perfusions of increasing concentrations of CP 93, 129 (10, 30, and 100 μM) each separated by 30 min washout periods with drug-free aCSF. Dialysates were collected at 10 min intervals, immediately frozen on dry ice and stored at −70C until subsequent HPLC analysis was performed.

Experiment 3: effect of intra-VTA CP 93, 129 administration on peripheral cocaine-induced alterations in NAC DA efflux and VTA amino acid efflux during cocaine abstinence

In order to compare the effect of intra-VTA CP 93, 129 on cocaine-induced increases in NAC dialysate DA during cocaine abstinence, groups of cocaine-naïve ($n = 11$) and EA ($n = 10$) animals were prepared as described earlier. Microdialysis probes were implanted through previously implanted guide cannulae into the NAC and ipsilateral VTA on the evening of the thirteenth day of cocaine abstinence. The following morning 4 baseline dialysate samples were collected from both probes, and subsequently the perfusate flowing through the VTA was switched to either 100 μM CP 93, 129 in aCSF ($n = 5$ for the naïve and EA groups, respectively) or to drug-free fresh aCSF ($n = 6$ for the naïve group, $n = 5$ for the EA group). Twenty min later all animals received an intraperitoneal injection of 10 mg/kg cocaine, followed by an additional 90 min of dialysate collection. The perfusate concentration of CP 93, 129 was chosen based on previous results obtained by our laboratory (O'Dell and Parsons 2004) and others (Yan and Yan 2001a). To provide functional verification of correct probe placement (Rahman and McBride 2002) the $GABA_A$ antagonist bicuculline (100 μM) was perfused through the VTA probe upon completion of the 5-HT_{1B} – cocaine manipulations. Only rats that exhibited at least a 2-fold bicuculline-induced increase in NAC DA levels were included in our analyses.

Statistical analyses

Cocaine intake was compared using ANOVA with experiment and group (i.e. non-binge or binge) as a between subjects factors. Comparisons of locomotor activity were made using mixed-factorial ANOVA with treatment group as the between subjects factor and test day as the repeated measure. Subsequently, the effect of repeated RU 24969 administration on locomotor activity in the naive group was analyzed by one-way ANOVA with test day as the independent variable. The mean locomotor response to RU 24969 of the naive group over test days was then used as a comparison for examining changes in the motor response of the two

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cocaine-experienced groups to RU 24969 at different points of cocaine abstinence using one-way ANOVA with the test day as the repeated measure. In experiments 2 and 3 baseline dialysate neurotransmitter levels between experimental groups were compared by ANOVA, and following confirmation of no group differences in dialysate concentration the data were transformed to the percent change from baseline. For comparison of drug treatment condition the area under the curve (AUC) was calculated for each animal by subtracting 100 from the percent of baseline value for each data point, and subsequently summing all data points collected during the experimental manipulation. In experiment 2 the AUC was calculated by summing all data obtained during intra-NAC perfusion with CP 93, 129 and AUC values for each CP 93, 129 manipulation were compared by ANOVA. In experiment 3 the AUC was calculated for each neurotransmitter by summing all data points from time 0–90 min (e.g. all data gathered following peripheral cocaine administration). These data were then evaluated by two-way ANOVA with cocaine group (naïve versus EA) and CP 93, 129 condition as factors. Where appropriate post hoc analyses were made using Fisher's protected least significant difference test.

Results

Cocaine Intake

A summary of self-administered cocaine intake for all animals in this report is shown in Table 1. As revealed by ANOVA there was no significant difference between experiments in the average daily cocaine intake during the 14-day self-administration training period for either the LA ($F_{1,17} = 0.25$; n.s.) or EA ($F_{2,23} = 0.918$; n.s.) groups of animals (note that there are no LA animals included in the third experiment, see below). Similarly, there was no significant difference between experiments in cocaine intake during the final self-administration session ($F_{1,17} = 2.171$; for the LA groups; $F_{2,23} = 2.02$; n.s.. for the EA groups) or as assessed by total cocaine intake over the entire history of self-administration ($F_{1,17} = 0.06$ for the LA groups; $F_{2,23} = 1.187$ for the EA groups). Thus animals in both the LA and EA groups received comparable amounts of cocaine exposure in each of the 3 experiments described below. Further evaluation of cocaine intake in each individual experiment demonstrated that there was no significant difference in cocaine intake between any of the experimental groups during the 14d training period, though animals in the EA groups self-administered significantly more cocaine than those in the LA groups during the final self-administration session. Despite this group difference in intake during the final session there was no significant difference in total cocaine intake between LA and EA groups when entire cocaine histories were compared. Thus, in terms of cocaine consumption the LA and EA groups were differentiated only by the duration of the final self-administration session.

Experiment 1: motor response to RU 24969 during cocaine abstinence

There was no significant effect of prior cocaine exposure on baseline motor activity over the period of analyses performed in this study ($F_{2,21} = 0.534$; n.s.). In contrast, there was a significant effect of prior cocaine exposure (main effect of groups: $F_{2.21} = 3.503$; $p < 0.05$) and cocaine abstinence (main effect of test day: $F_{4.84} = 33.798$; $p < 0.0001$) on the locomotor response to RU 24969. There was also a significant interaction between cocaine group and test day ($F_{8,84} = 9.727$, $p < 0.0001$) indicating that the degree of alteration in the response to RU 24969 during abstinence was dependent on the amount of prior cocaine exposure. Post-hoc analyses demonstrated that the motor response to RU 24969 was significantly reduced in cocaine-experienced animals relative to naïve controls after 14 daily 3 h cocaine self-administration training sessions [Day (− 1)], and there was no significant difference between the LA and EA groups at this time point. Subsequent exposure to 12 h of cocaine self-administration led to an even greater deficit in the motor response to RU 24969 by the EA group while no further change was observed in the LA group that received only 3 h access to cocaine as on all prior days. During the first 14 days of cocaine abstinence the motor response of the cocaine-exposed groups to RU 24969 returned not only to

Table 1 Summary of self-administered cocaine intake for each exposure group across all three experiments

Experiment	Exposure Group	Daily Training (mg/kg)	Final Session (mg/kg)	Total Intake (mg/kg)
1	LA	21.4 ± 2.6	21.7 ± 1.8	450.2 ± 52.5
	EA	22.9 ± 0.8	88.8 ± 5.6	547.6 ± 17.4
2	LA	21.8 ± 1.2	25.8 ± 2.1	462.5 ± 25.2
	EA	19.6 ± 1.5	75.6 ± 4.6	467.6 ± 32.7
3	EA (VTA VEH)	22.4 ± 4.4	78.2 ± 8.4	525.8 ± 93.9
	EA (VTA CP 93, 129)	20.0 ± 2.2	80.4 ± 5.6	479.2 ± 47.4

LA = Limited Access Group. EA = Extended Access Group. Daily Training values are the average daily cocaine intake during the 14 × 3 h training sessions that occurred prior to separation into LA and EA groups.

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levels observed in cocaine-naïve subjects, but steadily increased to significantly higher levels over time. The onset and overall magnitude of this enhanced response was significantly greater in animals given extended access to cocaine self-administration relative to those given only limited access. Observational measures of behavior provided no evidence of stereotypy following RU 24969 administration in any group on any test day (data not shown). The behavioral response to RU 24969 consisted predominantly of forward locomotion around the perimeter of the locomotor test cage with occasional changes in direction similar to previous descriptions of RU 24969-induced behavior (Rempel *et al.* 1993). The reduced number of cage crossings in cocaine-experienced animals 6 h after their final exposure to cocaine was associated with an overall reduction in the behavior described above, without a concomitant increase in stereotyped sniffing, rearing, gnawing or licking. Accordingly, the reduction in the motor response to RU 24969 which occurred in the early stages of cocaine abstinence reflects a blunting of the motor activating effects of this compound, rather than a narrowing of the behavioral repertoire to restricted repetitive motions (i.e. an increase in stereotypy).

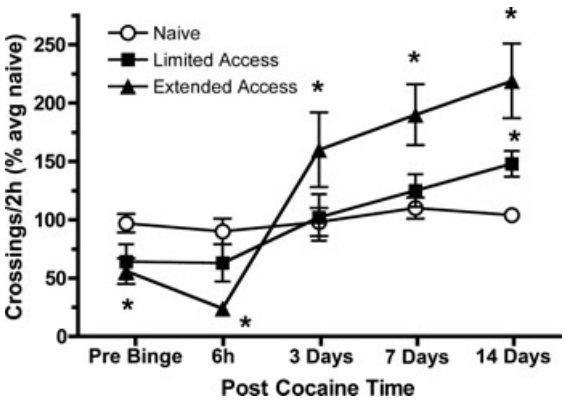

Fig. 1 The locomotor response to RU 24969 (1 mg/kg, s.c) during different times of abstinence from cocaine self-administration. Test days are referred to relative to their proximity in time to the 12 h cocaine self-administration session given to the EA group (see text for details). Data represent the total number of cage crossings that occurred in a 2 h period following RU 24969 administration, and are expressed as a percentage (mean ± SEM) of the average motor response of cocaine-naïve control rats. The experimental groups ($n = 8$/group) were cocaine-naïve controls (open circles), animals given 15 × 3 h self-administration sessions (LA; filled squares), and animals given 14 × 3 h and one 12 h self-administration sessions (EA; filled triangles). *Denotes a significantly different ($p < 0.05$) motor response to RU 24969 relative to that observed in cocaine-naïve control animals as determined by Fisher's protected least significant difference post hoc test.

Experiment 2: CP 93,129-induced increases in NAC DA efflux during cocaine abstinence

To provide a neurochemical test of alterations in 5-HT_{1B} receptor function in the NAC during cocaine abstinence the effects of local administration (by reverse dialysis) of the 5-HT_{1B} agonist CP 93, 129 on dialysate DA levels were investigated. An initial test was conducted in cocaine-naïve subjects to determine the dose-dependent effects of CP 93, 129 on dialysate DA. Baseline dialysate DA concentrations were 2.1 ± 0.3 nM in these animals ($n = 5$), and these levels were significantly ($p < 0.0001$) and dose-dependently enhanced to 225 ± 16 and 505 ± 34% of baseline by perfusates containing 30 and 100 μM CP 93,129, respectively (Fig. 1a). Dialysate DA concentrations returned to predrug baseline levels during the 60 minute 'washout' period between CP 93, 129 pulses. In a separate group of cocaine-naïve animals ($n = 5$; baseline DA 2.3 ± 0.6 nM) the DA-enhancing effect of perfusate CP 93, 129 (100 μM) was significantly ($p < 0.0001$) attenuated by coperfusion with the 5-HT_{1B} receptor antagonist GR 55562 (300 μM; Fig. 1b), suggesting that the effect of perfusate CP 92, 129 on NAC dialysate DA levels is mediated through activation of 5-HT_{1B} receptors.

Subsequent to these tests the effects of perfusate CP 93, 129 on NAC dialysate DA levels in cocaine-naïve ($n = 8$), LA ($n = 11$) and EA ($n = 8$) rats was examined 14 days after the final cocaine self-administration session. There was no statistical difference ($F_{2,29} = 0.716$; n.s.) in the baseline dialysate DA concentrations between groups (naïve = 2.2 ± 0.5 nM; LA = 2.2 ± 0.5 nM; EA = 1.6 ± 0.4 nM). A 2-way ANOVA with cocaine group as a between subjects factor and CP 93, 129 concentration as the within subjects factor revealed a significant effect of CP 93, 129 concentration ($F_{2,48} = 125.6$, $p < 0.0001$), a significant effect of cocaine group ($F_{2,24} = 3.510$, $p < 0.05$) and a significant interaction between cocaine group and CP 93, 129 concentration ($F_{4,48} = 3.473$, $p < 0.05$). Post-hoc comparisons indicated that the effect of CP 93, 129 was significantly greater in the EA group than the cocaine-naïve group for the 30 and 100 μM CP concentrations, and in the EA group relative to the LA group at the 100 μM CP concentration.

Experiment 3: Effect of intra-VTA CP 93, 129 administration on peripheral cocaine-induced alterations in NAC DA efflux and VTA amino acid efflux during cocaine abstinence

Previous work by our group has demonstrated that the effect of peripherally administered cocaine on NAC DA levels is dose-dependently enhanced by intra-VTA CP 93, 129 administration and that this effect is mediated through an activation of VTA 5-HT_{1B} receptors (O'Dell and Parsons 2004). The present experiment utilized a similar protocol to evaluate alterations in the influence of VTA 5-HT_{1B} receptors on cocaine-induced increases in NAC DA after a period of 14 days' abstinence from extended cocaine self-administration. There was no significant difference in baseline NAC

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dialysate DA concentrations between the cocaine naïve (1.5 ± 0.4 nM; $n = 11$) and EA groups (0.97 ± 0.2 nM; $n = 10$) ($F_{1,19} = 3.899$, n.s.). Moreover, there were no significant differences in baseline dialysate DA between the vehicle and CP 93, 129 subgroups of the naïve ($F_{1,9} = 1.771$, n.s.) or EA ($F_{1,8} = 0.009$, n.s.) groups. A 2-way ANOVA with cocaine group and CP 93, 129 condition as between subjects factors revealed a significant effect of intra-VTA CP 93, 129 on cocaine-induced increases in NAC DA ($F_{1,17} = 13.067$, $p < 0.005$), but no significant effect of cocaine group ($F_{1,17} = 2.88$, n.s.) and no significant interaction between CP 93, 129 condition and cocaine group ($F_{1,17} = 0.083$, n.s.). As shown in the top panels of Fig. 4, cocaine administration increased NAC DA levels in cocaine-naïve animals, and this effect was enhanced by intra-VTA CP 93, 129 administration as confirmed by one-way ANOVA ($F_{1,9} = 6.385$, $p < 0.05$). Cocaine administration also enhanced NAC DA levels in EA animals and this effect was also potentiated by intra-VTA CP 93, 129 administration ($F_{1,8} = 6.685$, $p < 0.05$). There was no significant difference in cocaine-induced increases in NAC DA between cocaine naïve and EA animals under either vehicle ($F_{1,9} = 0.910$, n.s.) or CP 93, 129 ($F_{1,9} = 3.897$, n.s.) conditions.

VTA dialysate samples were also analyzed for GABA and glutamate content since previous work has suggested that VTA 5-HT_{1B} receptors influence mesolimbic DA activity through a modulation of amino acid neurotransmission (Johnson *et al*. 1992; Cameron and Williams 1994; Yan and Yan 2001b; O'Dell and Parsons 2004). There was no significant difference in baseline dialysate VTA GABA levels between the cocaine naïve (35.6 ± 3.1 nM; $n = 11$) and EA groups (35.4 ± 5.4 nM; $n = 10$) ($F_{1,19} = 0.002$, n.s.). Moreover, there were no significant differences in baseline dialysate GABA between the vehicle and CP 93, 129 subgroups of the naïve ($F_{1,9} = 0.272$, n.s.) and EA ($F_{1,8} = 0.089$, n.s.) groups. A 2-way ANOVA with cocaine group and CP 93, 129 condition as between subjects factors revealed no significant effect of CP 93, 129 condition ($F_{1,17} = 0.624$, n.s.) but a significant effect of cocaine history (e.g. naïve versus EA) on cocaine-induced decreases in VTA GABA ($F_{1,17} = 16.582$, $p < 0.0001$) and a significant interaction between CP 93, 129 condition and cocaine group ($F_{1,17} = 5.702$, $p < 0.05$). As shown in Fig. 4, cocaine administration decreased VTA GABA levels in cocaine-naïve animals, and this effect was enhanced by intra-VTA CP 93, 129 administration as confirmed by one-way ANOVA on data from these animals ($F_{1,9} = 9.346$, $p < 0.05$). In contrast, cocaine administration tended to enhance VTA GABA levels in EA animals though this effect was unaltered by intra-VTA CP 93, 129 administration ($F_{1,8} = 2.542$, n.s.). There was a significant difference in cocaine-induced alterations in VTA GABA between cocaine naïve and EA animals when CP 93, 129 was present in the VTA perfusate ($F_{1,9} = 20.756$, $p < 0.005$), but no significant difference in cocaine-induced alterations in VTA GABA between cocaine naïve and EA animals under the vehicle condition ($F_{1,9} = 1.192$, n.s.).

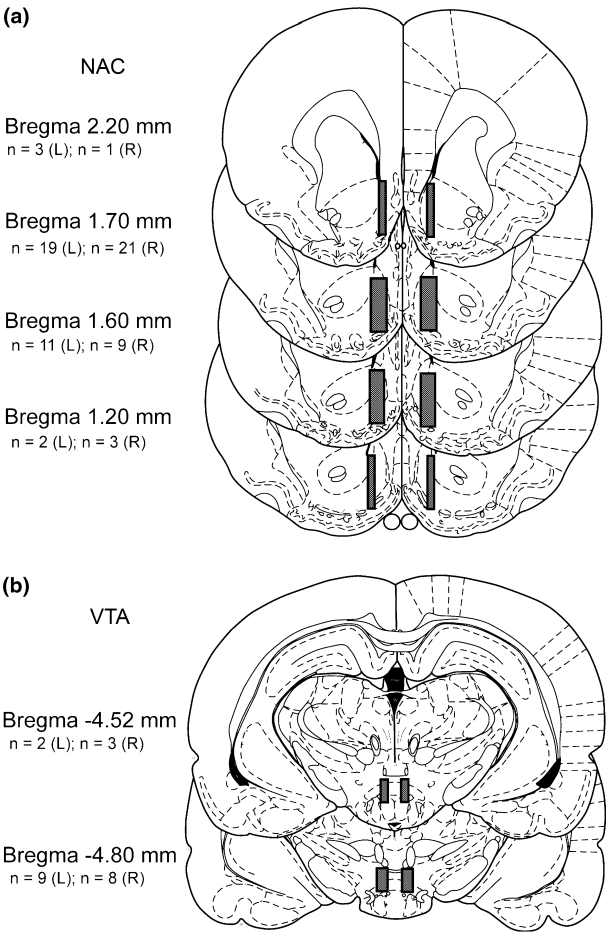

Fig. 2 Schematic diagram of microdialysis probe placements for experiments 2 and 3. Panel (a) shows placement of the 2 mm dialysis probes within the NAC ($n = 58$ probes). The majority of these probes were located between 1.6 and 1.7 mm anterior to Bregma, with the active membrane area placed within the shaded boxes depicted in the diagram. Panel (b) shows placement of the 1 mm dialysis probes within the VTA for experiment 3 ($n = 21$ probes). All VTA probes were located between 4.5 and 4.8 mm posterior to Bregma with the active membrane area placed within the shaded boxes. For each panel, the number of probes located in the left and right hemispheres is listed for each coronal slice. Diagrams are from Paxinos and Watson (1998).

Baseline levels of glutamate in VTA dialysates did not differ between the cocaine naïve (285 ± 27 nM; $n = 11$) and EA groups (304 ± 55 nM; $n = 10$) ($F_{1,19} = 0.102$, n.s.). Moreover, there were no significant differences in baseline dialysate glutamate between the vehicle and CP 93, 129 subgroups of the naïve ($F_{1,9} = 0.008$, n.s.) and EA ($F_{1,8} = 1.13$, n.s.) groups. A 2-way ANOVA with cocaine group and CP 93, 129 condition as between subjects factors revealed no significant effect of CP 93, 129 condition ($F_{1,17} = 0.189$,

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n.s.) but a significant effect of cocaine history (e.g. naïve versus EA) on cocaine-induced alterations in VTA glutamate ($F_{1,17} = 5.722$, $p < 0.05$) and no significant interaction between CP 93, 129 condition and cocaine group ($F_{1,17} = 1.503$, n.s.). As shown in the bottom panels of Fig. 4, cocaine administration produced no alteration in VTA glutamate levels in cocaine-naïve animals, and there was no effect of intra-VTA CP 93, 129 administration as confirmed by one-way ANOVA on data from these animals ($F_{1,9} = 2.235$, n.s.). In contrast, cocaine administration enhanced VTA glutamate levels in EA animals though this effect was unaltered by intra-VTA CP 93, 129 administration ($F_{1,8} = 0.797$, n.s.). There was a significant difference in cocaine-induced alterations in VTA glutamate between cocaine naïve and EA animals when CP 93, 129 was present in the VTA perfusate ($F_{1,9} = 7.263$, $p < 0.05$), but no significant difference in cocaine-induced decreases in VTA glutamate between cocaine naïve and EA animals under the vehicle condition ($F_{1,9} = 0.710$, n.s.).

Discussion

The present observations provide evidence that cocaine self-administration induces transient alterations in 5-HT_{1B} receptor function that persist for at least two weeks into cocaine abstinence. The locomotor response to the 5-$HT_{1B/1A}$ agonist RU 24969 was altered in a biphasic manner during abstinence from cocaine self-administration, with an initial subsensitivity followed by a supersensitive response that developed over the course of the first seven days of abstinence and intensified during the subsequent week of abstinence. Each of these effects was more pronounced in animals given extended access to cocaine self-administration as compared with those given only limited access to cocaine. Following 2 weeks of cocaine abstinence local administration of the selective 5-HT_{1B} agonist CP 93, 129 into the NAC by retrodialysis produced a significantly greater increase in dialysate DA levels in animals previously exposed to cocaine self-administration, and again this effect was greatest in those animals given extended access to cocaine. Finally, consistent with recent data from our group local CP 93, 129 administration into the VTA enhanced the ability of peripherally administered cocaine to increase NAC DA levels and to decrease VTA GABA levels. However, although the effect of peripheral cocaine on VTA GABA and GLU levels was altered in animals given extended access to cocaine relative to cocaine-naïve controls, there was no significant difference in the potentiating effect of intra-VTA CP 93, 129 on cocaine-induced increases in NAC DA between cocaine-experienced and cocaine-naïve animals. Collectively these findings suggest that cocaine self-administration persistently alters 5-HT_{1B} receptor function.

Several lines of evidence indicate that the motor stimulation induced by RU 24969 is mediated specifically by 5-HT_{1B} receptors, despite the activity of this agonist at both 5-HT_{1B} and 5-HT_{1A} receptors (Hoyer *et al.* 1994). For example, RU 24969 does not increase motor activity in mutant mice that lack 5-HT_{1B} receptors but have functional 5-HT_{1A} receptors (Ramboz *et al.* 1996). In addition, the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 blocks the motor activating effect of RU 24969 in rats and mice (O'Neill *et al.* 1996; Chaouloff *et al.* 1999), but the selective 5-HT1A receptor antagonist WAY 100135 does not (Cheetham and Heal 1993; O'Neill *et al.* 1996). Together with the observations that 5-HT_2 and 5-HT_3 receptor antagonists do not alter RU 24969-induced motor stimulation (Cheetham and Heal 1993), these findings suggest that alterations in the motor response to RU 24969 during cocaine abstinence likely reflect specific changes in 5-HT_{1B} receptor function.

It is important to note that there was no significant change in the locomotor response to RU 24969 across test days in cocaine-naive animals (Fig. 3). Although tolerance to the locomotor activating effects of RU 24969 has been reported (Oberlander *et al.* 1987; Frances and Monier 1991; Callaway and Geyer 1992), the low RU 24969 dose and relatively infrequent agonist administration employed in the present study did not produce RU 24969 tolerance. There was also no indication of sensitization to repeated RU 24969 treatment in cocaine-naive animals. Thus, the changes in the response of the cocaine-experienced groups to RU 24969 were likely specifically related to the effects of cocaine exposure, rather than repeated RU 24969 administration.

The biphasic response to RU 24969 observed during abstinence from cocaine may have resulted from cocaine-induced changes in interstitial 5-HT concentrations, since adaptations in 5-HT_{1B} receptor function are known to occur following prolonged elevations or decrements in extracellular 5-HT levels. For example, chronic 5-HT_{1B} receptor stimulation decreases the number of 5-HT_{1B} binding sites in several brain structures (Pranzatelli and Razi 1994) and produces tolerance to the behavioral effects produced by 5-HT_{1B} receptor agonists (De Souza *et al.* 1986; Oberlander *et al.* 1987; Frances and Monier 1991; Callaway and Geyer 1992). Because cocaine self-administration produces sustained elevations in extracellular 5-HT concentrations sufficient to stimulate 5-HT_{1B} receptors (Macor *et al.* 1990; Parsons *et al.* 1995), the blunted behavioral response to RU 24969 observed immediately following cocaine self-administration may reflect a down-regulation of 5-HT_{1B} receptor number or a decrease in the efficacy of their G-protein coupling. Conversely, depletion of extracellular 5-HT levels has been shown to increase 5-HT_{1B} receptor density (Weissmann *et al.* 1986; Offord *et al.* 1988; Crino *et al.* 1990; Frankfurt *et al.* 1993; Manrique *et al.* 1993, 1994) and produce supersensitive locomotor and neuroendocrine responses to 5-HT_{1B} receptor agonist administration (Van de Kar *et al.* 1989; Oberlander *et al.* 1986, 1987; but see Nisbet and Marsden 1984; Cheetham and Heal 1993).

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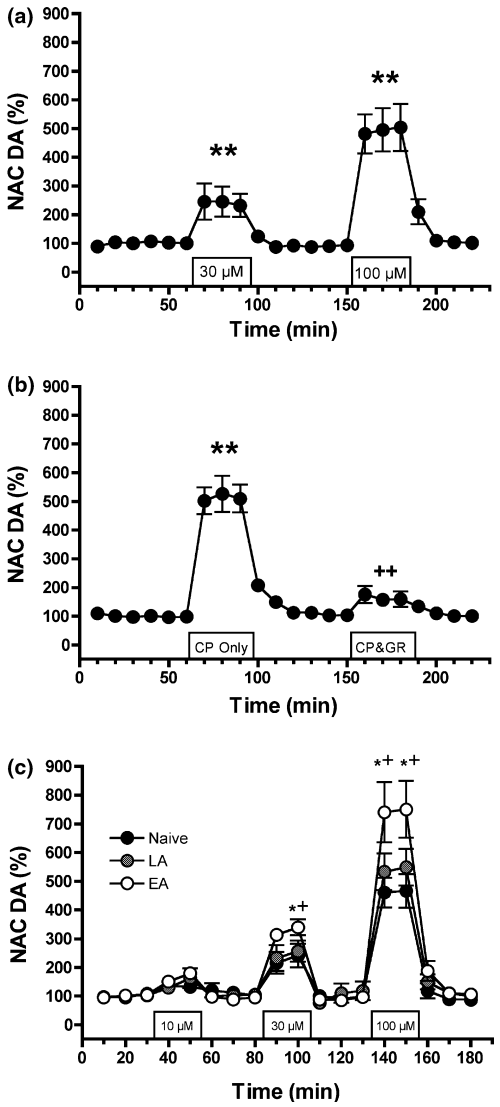

Fig. 3 Alterations in CP 93,129-induced increases in NAC DA efflux during cocaine abstinence. Initial tests in cocaine-naïve animals demonstrated that local administration of the selective 5-HT_{1B} agonist CP 93, 129 by retrodialysis dose-dependently (30 and 100 μM) increases NAC dialysate DA levels (panel a; $n = 5$) and that this effect of CP 93, 129 (100 μM) is blocked by coadministration of the 5-HT_{1B} receptor antagonist GR 55562 (300 μM; panel b; $n = 5$). Alterations in the effect of locally administered CP 93, 129 following 14 days of abstinence from cocaine self-administration are shown in panel (c). CP 93, 129 induced significantly greater increases in dialysate DA in animals given previous exposure to extended cocaine self-administration (open circles; $n = 8$) relative to the effect observed in cocaine-naïve control animals (filled circles; $n = 8$). There was no significant alteration in the effect of CP 93, 129 on dialysate DA levels in LA animals (grey circles; $n = 11$) as compared with cocaine-naïve controls. *Denotes $p < 0.05$ relative to cocaine-naïve controls, + denotes $p < 0.05$ relative to the LA group.

Accordingly, the supersensitive response to RU 24969 observed after several days of cocaine abstinence may reflect an up-regulation of 5-HT_{1B} receptors induced by prolonged deficits in extracellular 5-HT which occur following extended exposure to cocaine (Egan *et al.* 1994; Parsons *et al.* 1995). Based on this hypothesis, the greater alterations in the response to RU 24969 following a 12-h cocaine session would be expected given that 5-HT levels are robustly elevated for the *duration* of this length of session, and that postcocaine deficits in extracellular 5-HT are more severe following a single 12 h session than after daily limited-access self-administration (Parsons *et al.* 1995). Together, these findings suggest that the biphasic change in the locomotor response to RU 24969 during abstinence from cocaine reflects a biphasic alteration in the function of 5-HT_{1B} receptors induced by prolonged alterations in extracellular 5-HT concentrations.

The present findings are consistent with the observation of Przegalinski and colleagues of increased 5-HT_{1B} ligand binding in the NAC, VTA, substantia nigra and subiculum of cocaine-treated animals relative to cocaine-naïve subjects (Przegalinski *et al.* 2003). Moreover, these authors found a positive relationship between the relative increase in 5-HT_{1B} binding and the duration of cocaine exposure and observed that increased 5-HT_{1B} receptor binding developed during a period of cocaine abstinence. This is consistent with the hypothesis that deficits in interstitial 5-HT during cocaine abstinence result in an up-regulation of 5-HT_{1B} expression/function. This hypothesis is also supported by reports of increased 5-HT_{1B} receptor binding in rat brain following regimens of either ethanol (Nevo *et al.* 1995) or (+)3,4-methylenedioxy-methamphetamine (Sexton *et al.* 1999) administration that produce sustained deficits in interstitial 5-HT.

Because 5-HT_{1B} receptors in the NAC and VTA have been found to be up-regulated during cocaine abstinence (Przegalinski *et al.* 2003) and evidence that 5-HT_{1B} receptors in each of these regions can modulate the behavioral and neurochemical effects produced by cocaine (Przegalinski *et al.* 2002a, 2002b, 2004; Filip *et al.* 2003; O'Dell and Parsons 2004) we evaluated alterations in the neurochemical response to local administration of the selective 5-HT_{1B} agonist CP 93, 129 into the NAC and VTA by retrodialysis following 14 days of abstinence from cocaine self-administration.

Perfusate CP 93, 129 dose-dependently increased DA levels in NAC dialysates, consistent with previous reports by others in the rat NAC (Yan and Yan 2001a), rat DStr (Benloucif *et al.* 1993; Galloway *et al.* 1993; Bentue-Ferrer *et al.* 1998), rat prefrontal cortex (Iyer and Bradberry 1996) and mouse DStr (De Groote *et al.* 2003). The stimulatory effect of perfusate CP 93, 129 on NAC dialysate DA levels was significantly enhanced in cocaine-experienced animals given 14 days of cocaine abstinence relative to cocaine-naïve control animals and this effect was greater in animals that had been given extended access to cocaine relative to those given only limited access, consistent with the heightened locomotor

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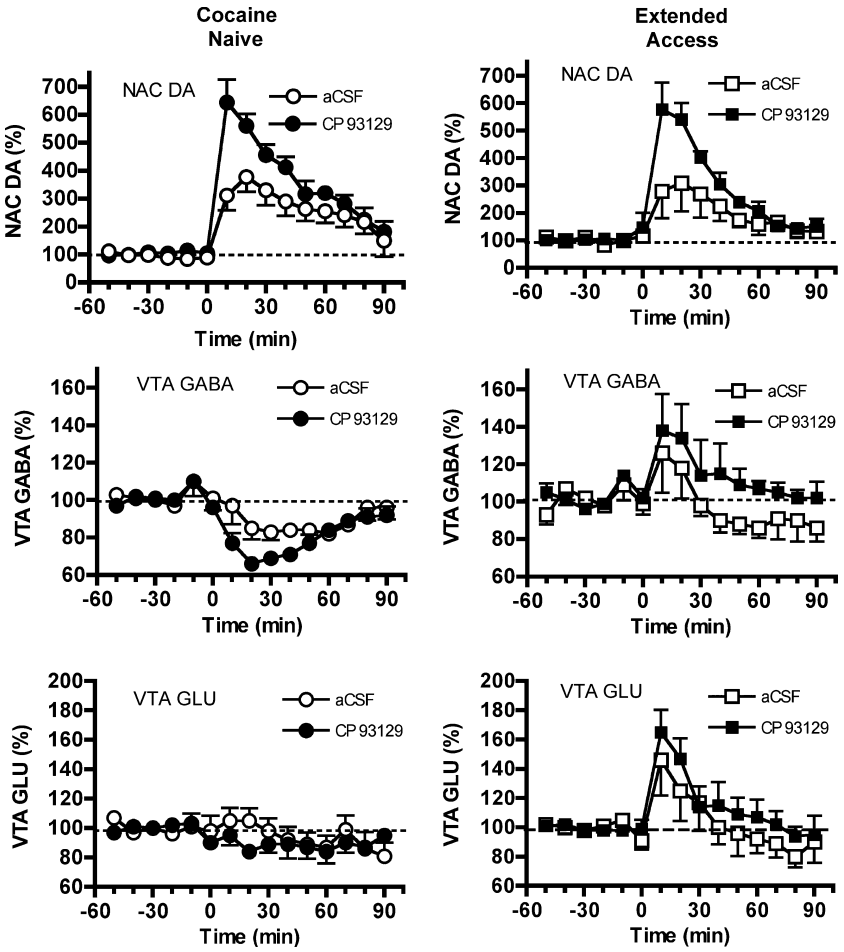

Fig. 4 Alterations in the effects of intra-VTA CP 93, 129 administration on cocaine-induced increases in NAC DA efflux observed following 14 days of cocaine abstinence. Consistent with previous observations (O'Dell and Parsons 2004) intra-VTA CP 93, 129 administration by retrodialysis (100 μM) significantly enhanced the effect of peripherally administered cocaine (10 mg/kg, i.p., administered at time zero) on NAC DA levels and VTA GABA levels in cocaine-naïve control rats (left column; CP 93, 129 group $n = 5$; aCSF control group $n = 6$). There was no significant alteration in the effect of intra-VTA CP 93, 129 on cocaine-induced increases in NAC DA following 14 days of abstinence from extended cocaine self-administration (top right panel) relative to that observed in cocaine-naïve controls (top left panel). There were significant alterations in the effects of cocaine on VTA GABA (middle panels) and VTA glutamate (bottom panels) efflux after 14 days of cocaine abstinence, and the ability of intra-VTA CP 93, 129 to potentiate cocaine-induced decrements in VTA GABA was absent in animals previously given EA to cocaine self-administration. See text for discussion.

response to RU 24969 in the EA versus LA groups. Previous work has shown the effect of CP 93, 129 on dialysate DA to be sensitive to Na^+ channel inhibition (Yan and Yan 2001a; De Groote *et al*. 2003), indicating that this agonist enhances impulse-dependent DA release. Moreover, several lines of evidence indicate that the CP 93,129-induced increase in DA efflux is mediated through 5-HT_{1B} receptors. CP 93, 129 is highly selective for 5-HT_{1B} receptors over other 5-HT receptor subtypes (Macor *et al*. 1990; Koe *et al*. 1992; Chopin *et al*. 1994) and has no appreciable interaction with DA, noradrenaline or opiate receptors (Macor *et al*. 1990). The effect of this agonist on dialysate DA levels is blocked by coperfusion with either the 5-$HT_{1B/1D}$ receptor antagonist GR 127 935 (Iyer and Bradberry 1996) or the selective and neutral 5-HT_{1B} antagonist GR 55562 (present results). In addition, the ability of perfusate CP 93, 129 to increase NAC DA levels is blocked by coperfusion with the 5-$HT_{1B/1A}$ receptor antagonist cyanopindolol but not by the highly selective 5-HT_{1A} receptor antagonist WAY 100 635 (Yan and Yan 2001a). Collectively these findings indicate that locally administered CP 93, 129 increases DA release by activating 5-HT_{1B} receptors. The mechanism through which NAC 5-HT_{1B} receptors increase local DA release has not been characterized, though it is likely that the 5-HT_{1B} receptors that mediate this effect of CP 93, 129 are not on DA terminals since 5-HT_{1B} receptor stimulation reduces the functional activity of the host cell (Bouhelal *et al*. 1988). Moreover, in contrast to the effects of this agonist *in vivo,* CP 93, 129 dose-dependently reduces DA release in a rat striatal synaptosomal preparation (Sarhan *et al*. 1999). A more likely mechanism for the effect of CP 93, 129 on DA release is via a 5-HT_{1B} receptor-induced reduction in GABAergic neurotransmission. Presynaptic 5-HT_{1B} receptors also inhibit excitatory glutamatergic neurotransmission in the NAC (Muramatsu *et al*. 1998; Morikawa *et al*. 2000) and it is possible this mechanism plays a role in the effect of CP 93, 129 on local DA release (Karasawa *et al*. 2006). Regardless of mechanism, however, the present data suggest that 5-HT_{1B} receptor expression and/or function is increased in the NAC following a 14-day period of cocaine abstinence.

Consistent with our previous observations (O'Dell and Parsons 2004), retrodialysis of CP 93, 129 into the VTA significantly increased the effect of peripherally administered cocaine on NAC DA and VTA GABA levels in cocaine-naïve rats. Specifically, intra-VTA CP 93, 129 administration led to significantly greater cocaine-induced increases in NAC

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DA and significantly greater cocaine-induced decreases in VTA GABA in cocaine naïve rats. However, in contrast to the hypothesized up-regulation of 5-HT_{1B} influence in the VTA following cocaine exposure there was no significant alteration in the ability of intra-VTA CP 93, 129 to potentiate cocaine-induced increases in NAC DA after 14 days of abstinence from extended cocaine self-administration. Interestingly, the effects of CP 93, 129 on cocaine-induced alterations in VTA GABA levels were absent in the cocaine-experienced rats. This observation suggests that in contrast to the effects of this agonist observed in cocaine-naïve rats (O'Dell and Parsons 2004; Yan *et al*. 2004) the effect of intra-VTA CP 93, 129 on cocaine-induced increases in NAC DA in cocaine-experienced rats does not involve a GABAergic link. However, as discussed below the profile of cocaine-induced alterations in VTA GABA and glutamate levels differ substantially between cocaine-naïve and cocaine-experienced animals.

In control animals (e.g. drug-free perfusate in the VTA dialysis probe) cocaine challenge induced a significant increase in VTA glutamate levels in cocaine-experienced, but not cocaine-naïve rats. This is consistent with previous observations by others (Pierce *et al*. 1996; Kalivas and Duffy 1998; Bell *et al*. 2000; but see Kalivas and Duffy 1995). Similarly, there was a substantial difference in the effects of a cocaine challenge injection on VTA GABA levels between cocaine-naïve and cocaine-experienced animals. In naïve animals acute cocaine administration significantly reduced VTA GABA levels (see also Parsons *et al*. 1999; O'Dell and Parsons 2004) while in cocaine experienced animals cocaine transiently increased VTA GABA levels. The mechanism(s) underlying the differential effects of cocaine challenge on VTA amino acid levels in naïve versus cocaine experienced animals are not known. However, recent evidence demonstrates that $GABA_B$ receptor function is decreased in the NAC (Xi *et al*. 2003) and dorsolateral septum (Shoji *et al*. 1997, 1998) following long-term cocaine exposure. $GABA_B$ receptors function as both presynaptic auto- and heteroreceptors (Potashner 1979; Anderson and Mitchell 1985) and provide inhibitory control over the release of both GABA and glutamate (Misgeld *et al*. 1995; Wu *et al*. 1999). Thus, although it is not known whether $GABA_B$ receptor function in the VTA is altered by extended cocaine exposure, it is possible that a down-regulation of $GABA_B$ auto- and heteroreceptors underlies the cocaine-induced increase in both GABA and glutamate release presently observed in the VTA of animals previously given extended access to cocaine self-administration. This dysregulation of amino acid neurotransmission following extended cocaine exposure may mask the observation of 5-HT_{1B} receptor-mediated neurochemical effects by *in vivo* microdialysis measures.

In summary, the present observations provide evidence that 5-HT_{1B} receptor function is altered during abstinence from cocaine self-administration in rats, and that these alterations in 5-HT_{1B} function are more severe following extended exposure to cocaine intake, consistent with recent findings by others (Przegalinski *et al*. 2003). 5-HT_{1B} receptor activation has been shown to potentiate the locomotor activating (Przegalinski *et al*. 2001), place conditioning (Cervo *et al*. 2002), discriminative stimulus (Callahan and Cunningham 1995, 1997; Filip *et al*. 2001), reinforcing (Parsons *et al*. 1998) and neurochemical (Parsons *et al*. 1999; O'Dell and Parsons 2004) effects of cocaine. Moreover, 5-HT_{1B} receptors in the NAC have been implicated in the locomotor activating (Neumaier *et al*. 2002; Przegalinski *et al*. 2002a), interoreceptive (Filip *et al*. 2002) and conditioning (Neumaier *et al*. 2002) effects of cocaine, and recent evidence points to NAC 5-HT_{1B} receptors in the regulation of cocaine self-administration behavior (Parsons *et al.* unpublished observation). Thus it is possible that increased NAC 5-HT_{1B} receptor function during cocaine abstinence contributes to sensitization of the behavioral and reinforcing effects of cocaine that are present during abstinence from extended cocaine self-administration (Zapata *et al*. 2003; Morgan and Roberts 2004; Morgan *et al*. 2005a, 2005b). 5-HT_{1B} receptors have been implicated in modulating the conditioning (Cervo *et al*. 2002; Neumaier *et al*. 2002) and discriminative stimulus (Callahan and Cunningham 1995, 1997; Filip *et al*. 2001) properties of cocaine. However, recent evidence from animal models does not support a role for 5-HT_{1B} receptors in either cocaine priming or conditioned cue-induced reinstatement of cocaine-seeking behavior (Acosta *et al*. 2005). Nonetheless, the present observation of increased 5-HT_{1B} receptor function during cocaine abstinence may have relevance to the symptoms of aggression, anxiety and depression associated with chronic cocaine use and withdrawal (Fishbein *et al*. 1989; Miller *et al*. 1993; Gillin *et al*. 1994; Moeller *et al*. 1994) in light of the proposed involvement of 5-$HT_{1B/1D}$ receptors in the generation of these states (Pellow *et al*. 1985; Chopin and Briley 1987; Benjamin *et al*. 1990; Edwards *et al*. 1991; Saudou *et al*. 1994; Buhot and Naili 1995; Ramboz *et al*. 1996). Accordingly, the present findings suggest that 5-HT_{1B} receptors may be a viable target for the pharmacotherapeutic treatment of cocaine addiction.

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