Neuropsychopharmacology (2004) 29, 1675–1687

www.neuropsychopharmacology.org

Dissociable Roles for the Dorsal and Median Raphé in the Facilitatory Effect of 5-HT1A Receptor Stimulation upon Cocaine-Induced Locomotion and Sensitization

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A distinct role for serotonin transmission from the dorsal and median raphé nuclei (DRN and MRN, respectively) was identified in regulating the behavioral and neurochemical effects of acute and repeated cocaine administration. Serotonin 1A (5-hydroxytryptophan (5-HT)1A) receptors were stimulated by intraraphé microinjection of 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT; 5 or 10 μg) and behavior, as well as extracellular neurotransmitter content in the nucleus accumbens was measured. Pretreatment of the DRN with DPAT caused a sensitization-like potentiation of acute cocaine-induced motor activity and an elevation in extracellular dopamine and glutamate. In contrast, DPAT microinjection into the MRN did not alter acute cocaine-induced motor activity or extracellular levels of dopamine or glutamate. Acutely, DPAT microinjection into either raphé nucleus reduced the basal and acute cocaine-stimulated levels of extracellular serotonin. Pretreatment with DPAT before systemic cocaine administration was continued for 5 days, and 3 weeks after the last injection, all rats were administered a cocaine challenge injection. The sensitized behavioral and neurochemical response produced by repeated cocaine in control subjects was unaffected by the intra-DRN administration of DPAT. However, in animals administered DPAT into the MRN, both the sensitized motor response and the increase in glutamate were augmented, while the sensitized serotonin response was blocked, without altering dopamine sensitization. These data show a differential role for 5-HT1A receptors in the DRN and MRN in the acute and sensitized effects of cocaine. While the DRN is involved in the acute effects of cocaine, neuroadaptations in the MRN may regulate the long-term consequences of repeated cocaine exposure.
Neuropsychopharmacology (2004) **29,** 1675–1687, advance online publication, 5 May 2004; doi:10.1038/sj.npp.1300473

Keywords: 5-HT1A receptors; sensitization; dopamine; glutamate; serotonin; raphé nuclei

INTRODUCTION

The expression of behavioral sensitization to repeated cocaine administration is most frequently associated with neuroadaptations in mesocorticolimbic dopamine and corticofugal glutamate transmission (Di Chiara, 1995; Wolf, 1998; Vanderschuren and Kalivas, 2000; Everitt and Wolf, 2002; Tzschentke and Schmidt, 2003). This includes an enhanced ability of cocaine to elevate extracellular levels of dopamine and glutamate in the nucleus accumbens (Heidbreder *et al*, 1996; Pierce *et al*, 1996), a brain region critical for the expression of drug-induced motor activation (Koob and Le Moal, 2001). In addition, repeated cocaine administration enhances the capacity of a subsequent cocaine injection to elevate extracellular serotonin in the accumbens (eg Parsons and Justice, 1993; Carey and Damianopoulos, 1994; Dworkin *et al*, 1995; Parsons *et al*, 1995, 1996). These effects on extracellular serotonin are consistent with the pharmacological blockade of serotonin reuptake by cocaine (Koe, 1976) and the potential involvement of impulse-modulating somatodendritic serotonin 1A (5-hydroxytryptophan (5-HT)1A) receptors (eg Pitts and Marwah, 1986, 1987; Cunningham and Lakoski, 1988, 1990). Accordingly, behavioral, biochemical, and electrophysiological evidence indicates alterations in 5-HT1A autoreceptor function following withdrawal from repeated cocaine (Cunningham *et al*, 1992; Johnson *et al*, 1993; King *et al*, 1993; Baumann *et al*, 1995; Baumann and Rothman, 1995; Cunningham and Lakoski, 1988, 1990). Also, cocaine-induced locomotor sensitization is potentiated by the systemic administration of the 5-HT1A agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT) and inhibited by the systemic administration of 5-HT1A antagonist WAY 100635 (De La Garza and Cunningham, 2000; Muller *et al*, 2003).

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E-mail: szumlink@musc.edu
Received 28 August 2003; revised 22 March 2004; accepted 23 March 2004
Online publication: 29 March 2004 at http://www.acnp.org/citations/Npp03290403394/default.pdf

While 5-HT1A receptors have been implicated in the behavioral consequences of repeated cocaine administration, the experiments to date have not identified specific anatomical site(s) of action. Somatodendritic 5HT1A autoreceptors are found in both the dorsal (DRN) and median (MRN) raphé nuclei, which send topographically distinct, as well as overlapping, serotonin projections to glutamatergic nuclei that innervate the accumbens (eg Azmitia and Segal, 1978; Steinbusch *et al*, 1978; Herve *et al*, 1987; Vertes, 1991; Vertes *et al*, 1999). Whereas DRN and MRN neurons dually innervate some aspects of the frontal cortex (eg cingulate, infralimbic, and prelimbic cortices), the hippocampus, the mediodorsal nucleus of the thalamus, and the basolateral and central nuclei of the amygdala (Vertes, 1991; Vertes *et al*, 1999), the DRN innervates strongly the majority of thalamic and amygdalar nuclei, as well as other cortical regions (incl., parietal, occipital, frontal orbital, and piriform cortices) (Vertes, 1991; Vertes *et al*, 1999). Whereas the DRN innervates more selectively the substantia nigra pars compacta, the primary dopaminergic projection to the dorsal striatum (Fallon and Moore, 1978), both the DRN and the MRN send dense projections to the ventral tegmental area, the primary dopaminergic afferent to the accumbens (Azmitia and Segal, 1978; Herve *et al*, 1987; Vertes, 1991; Vertes *et al*, 1999). The nucleus accumbens itself receives a very dense and diffuse innervation from the DRN, but receives only sparse innervation from the MRN (Takada and Hattori, 1987; Mamounas and Molliver, 1988; Vertes, 1991; Vertes *et al*, 1999). Given some divergence in the innervation patterns of DRN and MRN neurons, the possibility exists that cocaine-induced activation of serotonin transmission in the two raphé nuclei and in their respective terminal fields might differentially contribute to the behavioral and neurochemical consequences of acute and repeated cocaine administration. In order to examine this possibility, the 5-HT1A autoreceptor agonist DPAT was microinjected into the MRN or DRN in order to inactivate these nuclei prior to acute and repeated cocaine administration. The capacity of intra-raphé DPAT to alter the effect of acute systemic cocaine administration was determined by measuring locomotor activity and the extracellular content of serotonin, dopamine, and glutamate in the nucleus accumbens using *in vivo* microdialysis. Similarly, the ability of DPAT to alter the development of sensitized locomotor and neurochemical responses to a cocaine injection was examined 3 weeks after discontinuing the daily DPAT and cocaine injections.

MATERIALS AND METHODS

Animals

Experimentally naïve male Sprague–Dawley rats (Harlan, Indianapolis, IN; weighing 250–275 g at the start of the experiment) were housed individually in polyethylene cages ($35 \times 30 \times 16\,cm^3$) in an AAALAC-approved animal facility. The colony rooms were temperature controlled (25°C) and under a 12 h day–12 h night cycle (lights off: 1900). 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 1200. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina 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 was a generous gift from the National Institute on Drug Abuse and DPAT and 5-HT were purchased from Sigma-RBI Chemical Co. (Natick, MA). Cocaine was dissolved in 0.9% sterile saline (SAL) and saline served for the systemic control injections (volume = 1.0 ml/kg). DPAT was dissolved in sterile water for injection and water served for the control intracranial injections (VEH; volume = 0.25 μl). 5-HT was dissolved in microdialysis buffer (pH = 7.4) for infusion via the microdialysis probe.

Apparatus

Motor activity was monitored in a noncolony room containing 24 Plexiglas activity chambers ($22 \times 43 \times 33\,cm^3$) (Omnitech Electronics, Columbus, OH). A series of 16 photobeams (eight on each horizontal axis) tabulated horizontal movements and estimated the total distance traveled by the animal (in cm). These chambers were interfaced to a Digiscan monitor (Omnitech Electronics, Columbus, OH) and IBM PC computers provided automated recording of the motor behavior.

Surgery

Using ketamine (100 mg/kg) and xylazine (3 mg/kg) anesthesia, microinjector guide cannluae (26 gauge, 20 mm; Plastics One, Roanoke, VA) were implanted unilaterally 2 mm over the DRN or the MRN using the following coordinates, according to the atlas of Paxinos and Watson (1986) (DRN, AP: −7.8 mm; ML: +1.4 mm; DV: −6.4 mm; MRN, AP: −8.2 mm, ML: +1.8 mm; DV: −8.0 mm, both at 12° angle from vertical). For the microdialysis studies, rats were also implanted bilaterally with microdialysis guide cannulae (20 gauge, 20 mm, Plastics One) 3 mm over the nucleus accumbens using the following coordinates (AP: +1.1 mm; ML: ±2.5 mm; DV: −4.7 mm; 6° angle from vertical) (Paxinos and Watson, 1986). All AP and ML coordinates are relative to Bregma and all 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. Animals were monitored for changes in health for at least 5 days prior to beginning the experiments.

IntraRaphé Pretreatment and Locomotor Sensitization Procedures

Locomotor studies commenced with a day of habituation, in which rats were placed into the activity chambers for 60 min. Animals were removed from the activity monitors and lightly restrained for insertion of an injector cannula (33 gauge, 22 mm in length). VEH (0.25 μl) was then infused at a rate of 0.3 μl/min and the injector remained in place for

an additional 60 s to allow for diffusion of the drug away from the injector tip. During intraraphé injection, animals were allowed to locomote freely in an empty housing cage. Rats were then lightly restrained for removal of the injector and returned to their home cages for 15 min. Rats were then injected with saline intraperitoneally (i.p.) and behavior was recorded for an additional 2 h. On Injections 1 and 5 of the repeated treatment phase of the experiment, animals were treated in an identical manner as described for the habituation day with the exception that animals were randomly assigned to receive an intraraphé injection of DPAT (5 or 10 μg/0.25 μl) or VEH, followed 15 min later by an i.p. injection of cocaine (15 mg/kg, i.p.) or saline. Thus, six groups of rats were tested for each raphé nucleus in the behavioral studies: VEH–saline, 5 μg DPAT–saline, 10 μg DPAT–saline VEH-cocaine, 5 μg DPAT–cocaine and 10 μg DPAT–cocaine ($n = 6$–11/group). On Injections 2–4, rats were transported to the behavioral room where they were injected intraraphé with their respective treatment, followed 15 min later by a systemic injection of cocaine (30 mg/kg, i.p.) or saline as appropriate, and then were returned immediately to the colony room. Following Injection 5, drug treatment was withdrawn for 3 weeks and a test for sensitization was conducted in the activity chambers, in which all groups received a challenge injection of cocaine (15 mg/kg, i.p.), without intraraphé pretreatment, using procedures identical to those described above.

In Vivo Microdialysis Procedures

Microdialysis probes (24 gauge; 23 mm in length incl. 1.5–2.0 mm active membrane) were constructed as described by Robinson and Whishaw (1988) with some modifications. For the study examining the effects of intraraphé DPAT pretreatment upon cocaine-induced changes in nucleus accumbens neurotransmission, microdialysis sessions were conducted on Injection 1 of repeated cocaine or saline treatment and on the test day for sensitization, following 3 weeks withdrawal from Injection 5 of repeated treatment. For all microdialysis sessions, microdialysis probes were inserted unilaterally into the nucleus accumbens and perfused overnight (at least 12 h prior to sample collection) at a rate of 0.5 μl/min with a microdialysis buffer (5 mM glucose, 2.5 mM KCl, 140 mM NaCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.15% phosphate-buffered saline, pH 7.4). Probe insertion was counterbalanced across all treatment groups to reduce asymmetry confounds (Glick and Carlson, 1989). The following morning (~0900), the flow rate was increased to 2.0 μl/min for 2 h prior to sample collection. Dialysate was then collected in 20-min fractions into 10 μl of preservative (4.76 mM citric acid, 150 mM NaH_2PO_4, 50 μM EDTA, 3 mM sodium dodecyl sulfate, 10% methanol (v/v), 15% acetonitrile (v/v), pH 5.6) for 2 h.

For the DPAT studies, rats were injected intraraphé with either 10 μg DPAT or VEH as described above. The 10 μg dose of DPAT dose was selected for the microdialysis study as this dose produced the largest effect upon behavior (see Figure 2a and d). Consistent with the procedures employed in the locomotor studies, 15 min following intraraphé injection, rats were injected i.p. with either saline or cocaine (15 mg/kg) and dialysate was collected in 20-min fractions for an additional 3 h. Following the end of the first microdialysis session, rats were lightly restrained for probe removal and returned to the colony room. For the next 4 days, rats were transported in their home cages to the microdialysis room (~1300) where they received the appropriate intraraphé pretreatment and systemic drug treatment (saline *vs* 30 mg/kg cocaine, i.p.). Animals remained in the microdialysis room for 3 h, at which time they were returned to the colony room. This procedure was followed to allow for the formation of cocaine–context associations, which might promote the expression of neurochemical sensitization on test day (Duvauchelle *et al*, 2000). Following 3 weeks of withdrawal, the second microdialysis session was conducted using procedures identical to those employed on Injection 1 with the following exceptions: (1) the microdialysis probe was inserted on the side of the head opposite to that used on Injection 1; (2) no VEH or DPAT pretreatment was administered; and (3) all rats received a challenge injection of cocaine (15 mg/kg, i.p.).

For the no net-flux study, rats were treated repeatedly with either saline or cocaine as described for the behavioral studies above, with the exception that all injections were administered in the colony room. The microdialysis session was conducted following 3 weeks withdrawal from the 5th cocaine or saline injection. Increasing concentrations of serotonin (0, 0.05, 0.25, 0.55, 0.75, 1.15 nM) was delivered via the microdialysis probe for 1 h at each concentration.

High-Pressure Liquid Chromatography (HPLC) Analysis of Glutamate, Serotonin, and Dopamine

Glutamate in the dialysis sample was measured using an HPLC system with fluorescent detection. The mobile phase consisted of 13% acetylnitrile (v/v), 100 mM NaH_2PO_4, 0.1 mM EDTA, pH 6.0. A reversed-phase column (10 cm, 3 μm ODS; Bioanalytical Systems, West Lafayette, IN) was used to separate the amino acids, and precolumn derivatization of amino acids with *o*-phthalaldehyde was performed using an ESA Model 540 autosampler. Glutamate was detected by a fluorescence spectrophotometer (LINEAR FLOUR LC 305, from ESA Inc.) using an excitation wavelength of 336 nm and an emission wavelength of 420 nm. Glutamate content in each sample was analyzed by peak height and was compared with an external standard curve for quantification. Dopamine and serotonin in the dialysate sample were measured using an HPLC system with electrochemical detection. The mobile phase consisted of 4.76 mM citric acid, 150 mM NaH_2PO_4, 50 μM EDTA, 3 mM sodium dodecyl sulfate, 10% methanol (v/v), 15% acetylnitrile (v/v) at a pH = 5.6. Dopamine and 5-HT were separated using a reversed-phase column (10 cm, C18; Bioanalytical Systems, West LaFayette, IN) and oxidized/reduced using coulometric detection (Coulochem II, ESA Inc., Bedford, MA). Three electrodes were used: a preinjection port guard cell (+0.1 V) to oxidize the mobile phase; an oxidation analytical electrode (E1, −0.075 V); and a reduction analytical electrode (E2, +0.3 V). The area under curve of the dopamine and serotonin peaks were measured with ESA 501 Chromatography Data System, and the dopamine and serotonin values were compared with an external standard curve for quantification.

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 nucleus accumbens (100 μm; AP: +2.2 to 1.0 mm, relative to Bregma) and at the level of the raphé nuclei (50 μm; AP: −7.0 to −9.0 mm, relative to Bregma), according to the atlas of Paxinos and Watson (1986). Sections were stained with cresyl violet for histological examination under a light microscope. Only rats whose injector cannulae were located within the boundaries of the DRN or the MRN (incl. the peri-MRN region; Vertes *et al*, 1999) and only rats in which the microdialysis probes were located within the boundaries of the nucleus accumbens were included in the statistical analysis.

Statistical Analysis

For the locomotor studies, the 2-h time course of the distance traveled throughout the entire activity chamber (total distance in cm) served as the principal variable of interest. Owing to the design of the study, the data from Injection 1 and the test for sensitization were analyzed independently. For both test sessions, the time courses of locomotion were analyzed by a Brain region (DRN *vs* MRN) × Pretreatment (0, 5, or 10 μg DPAT) × Repeated treatment (saline *vs* cocaine) × Time analysis of variance (ANOVA), with repeated measures on the Time factor (12, 10-min bins). As significant Brain region × Pretreatment × Repeated treatment interactions were observed in the statistical analyses of behavior ($p<0.05$), the behavioral data for each brain region were analyzed and presented separately for clarity.

Statistical analysis of the data from the DPAT locomotor studies revealed no significant differences between DRN and MRN infusions of VEH for either VEH–cocaine or VEH–saline rats (Group effect: $p>0.05$). In order to reduce the number of rats employed in the microdialysis studies, the DRN- and MRN-cannulated VEH–saline and VEH–cocaine rats were combined to form one VEH–saline control group and one VEH–cocaine control group to conserve animal numbers. Thus, six groups of animals were included in the statistical analyses of the neurochemical data: VEH–saline, VEH–cocaine, MRN–DPAT–saline, MRN–DPAT–cocaine, DRN–DPAT–saline, and DRN–DPAT–cocaine. The average basal concentration of each neurotransmitter was determined based on the three samples collected in the second hour of baseline sampling. The average basal concentration of each neurotransmitter was analyzed using a Group × Injection number ANOVA. Owing to attrition that occurred during testing and technical difficulties with the HPLC systems, the sample sizes for all groups were not equal on Injection 1 and on the test for sensitization. Thus, in order to include the data from all animals in the statistical analyses, the Injection number factor was treated as a between-subjects factor for the analysis of basal neurotransmitter levels. The neurochemical data were expressed as the percent change from the average basal concentration and analyzed using a Group × Time ANOVA, with repeated measures on the Time factor (12, 20-min samples; three baseline + nine postsystemic injection). For both the DPAT locomotor and neurochemical studies, planned comparisons between repeated saline and repeated cocaine groups were conducted for each pretreatment independently to confirm the presence of sensitization on test day.

For the serotonin no net-flux study, the net flux of serotonin diffusing into or out of the probe was calculated by subtracting the concentration of serotonin added to the buffer from the concentration of serotonin in the sample. The plot of the serotonin flux at each concentration of serotonin added to the buffer yields an estimate of basal serotonin levels ($y=0$ is the point at which there is no net flux of serotonin into, or out of, the probe) and elimination (slope is the rate of clearance of serotonin from the probe) (eg Parsons *et al*, 1996). Linear regression analyses were performed and the individual slopes of the linear regressions and their corresponding *y*-intercepts were determined and the means analyzed by two-tailed Student's *t*-tests between the two repeated treatment groups (repeated saline *vs* repeated cocaine).

RESULTS

Regionally Selective Effects of IntraRaphé DPAT Pretreatment Upon Cocaine-Induced Locomotion and Locomotor Sensitization

Consistent with the results of earlier studies employing intraraphé serotonin depletion or WAY 100635 administration (Jacobs *et al*, 1974; Geyer *et al*, 1976; Herges and Taylor, 1999a, b), microinjection of DPAT into either the DRN (Figure 1a) or MRN (Figure 1b) produced no effect on the acute motor response associated with a saline injection. Similarly, daily intraraphé DPAT plus systemic saline injections did not alter the acute motor response to a cocaine injection given 3 weeks after the last daily injection (Figure 1c and d).

In contrast to systemic saline-treated animals, intraraphé DPAT enhanced the acute and sensitized locomotor effects of systemic cocaine in a regionally selective manner. Intra-DRN microinjection of DPAT augmented dose dependently the locomotor response to acute cocaine (Figure 2a), whereas intra-MRN DPAT pretreatment was without any effect (Figure 2b). Conversely, daily pretreatment with the highest dose of DPAT into the MRN augmented the sensitized locomotor response to a cocaine injection administered 3 weeks after the last daily injection (Figure 2d), while DPAT in the DRN did not alter the sensitized behavioral response, compared to their respective VEH-treated cocaine controls (Figure 2c).

Differential Effects of DPAT into the DRN *vs* MRN on Accumbens Glutamate and Dopamine

The mean concentrations of basal extracellular levels of dopamine were equivalent between all treatment groups (Table 1). Consistent with previous reports (Pierce *et al*, 1996; McFarland *et al*, 2003), all groups pretreated with daily systemic cocaine demonstrated a reduction in basal extracellular glutamate, regardless of the intraraphé pretreatment (Figure 3c; Table 1).

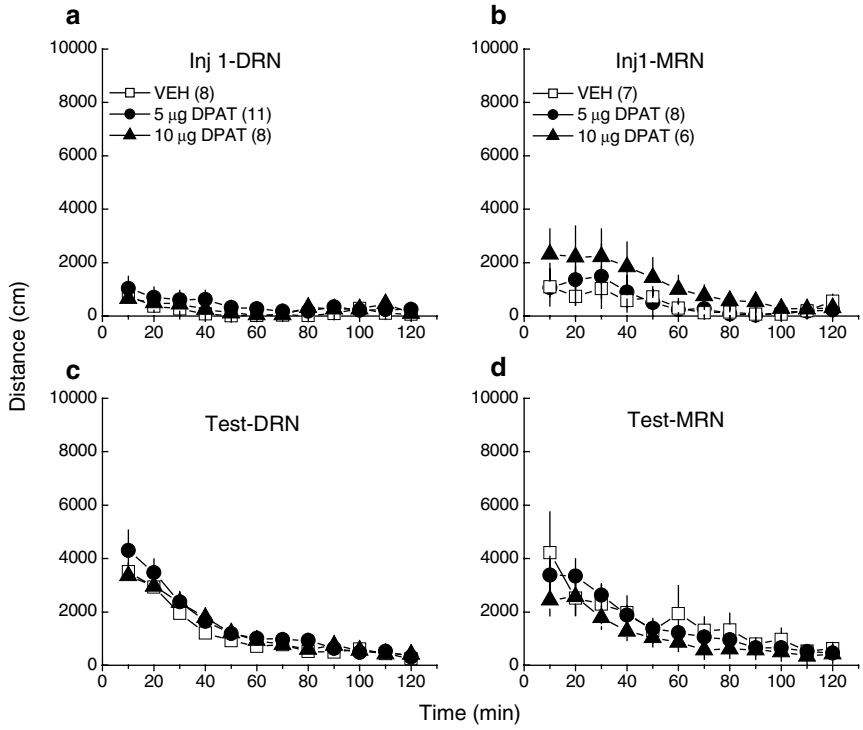

Figure 1 Intraraphé DPAT pretreatment does not alter locomotion in repeated saline-treated rats. On Injection 1 (Inj 1) of repeated saline treatment, DPAT injected into either the DRN (a) or the MRN (b) produced no observable effects upon saline-induced locomotion. Likewise, DPAT injected into the DRN (c) or into the MRN (d) produced no observable effects upon cocaine-induced locomotion on a test for sensitization conducted 3 weeks following repeated DPAT plus saline treatment (Test). Data represent the mean ($\pm$SEM) of the number of animals indicated within parentheses in the legends.

Figure 3a and b show that acute microinjection of DPAT into neither the DRN nor the MRN altered the levels of extracellular glutamate in animals that were injected systemically with saline. However, pretreatment of the DRN with DPAT in combination with an acute systemic cocaine injection produced a marked and enduring increase in extracellular glutamate, while acute cocaine in combination with MRN microinjection of DPAT or intraraphé VEH did not change glutamate levels (Figure 3a and b). As the peak effect of intra-DRN DPAT upon cocaine-induced changes in nucleus accumbens glutamate was late relative to its effect upon cocaine-induced changes in behavior (Figure 2a), the elevation in accumbens glutamate appears to be unrelated to the behavioral potentiating effect of intra-DRN DPAT treatment. After 3 weeks withdrawal, cocaine did not elicit an increase in extracellular glutamate in any of the repeated saline groups (Figure 3c and d). In contrast, relative to baseline, all repeated cocaine groups demonstrated a cocaine-induced increase in glutamate regardless of the intraraphé pretreatment (Figure 3d). The cocaine-induced increase in accumbens glutamate was of a relatively short duration (0–40 min postinjection) in the intra-DRN DPAT group and the intraraphé VEH controls; in contrast, the increase was more enduring (0–120 min) and of higher magnitude in the intra-MRN DPAT group (Figure 3d).

Acute cocaine administration elevated extracellular dopamine levels in all intraraphé pretreatment groups during the first 40 min postinjection (Figure 4a). However, similar to extracellular glutamate (Figure 3b), the cocaine-induced increase in dopamine was greater in the intra-DRN pretreatment group than the other two pretreatment groups. At 3 weeks after the last daily injection, the cocaine injection elevated dopamine levels in all treatment groups during the first 60 min postinjection, compared to their respective baseline dopamine concentrations (Figure 4b). Akin to previous studies (Vanderschuren and Kalivas, 2000, for a review), all groups of animals treated repeatedly with daily cocaine showed a sensitized increase in extracellular dopamine during the first 40–60 min postinjection, compared to daily saline groups (Figure 4b). However, there was no observable effect of DPAT pretreatment into either the DRN or the MRN on the cocaine-induced increase in dopamine for either repeated treatment group.

Extracellular Serotonin Levels and IntraRaphé DPAT

The basal levels of extracellular serotonin were statistically equivalent between all acute and repeated drug treatment groups (Table 1). Since this appeared contradictory to previous studies that showed a reduction in basal extracellular serotonin in the accumbens at 1–7 days after discontinuing daily cocaine treatment (Dworkin *et al*, 1995; Parsons *et al*, 1995, 1996), no net-flux microdialysis was conducted to more accurately quantify extracellular serotonin content. Rats were administered daily cocaine or saline for 5 days and no net-flux microdialysis was conducted in the nucleus accumbens 3 weeks later. Consistent with an earlier report (Parsons *et al*, 1996), the slopes of the two lines were parallel (repeated saline: 0.49 ± 0.04; repeated cocaine: 0.46 ± 0.04; $p = 0.50$), indicating similar probe recovery of serotonin (Figure 5a). In addition, there was no significant difference in basal extracellular serotonin concentrations between the two repeated treatment groups (repeated saline: 0.27 ± 0.02 nM;

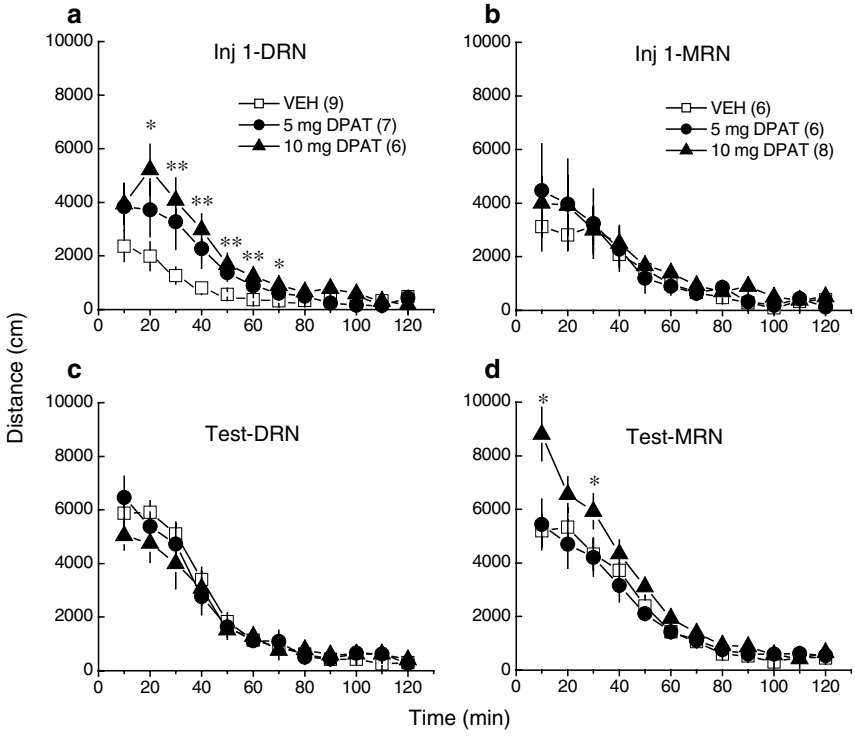

Figure 2 Regional selectivity of the effects of intraraphé DPAT pretreatment upon cocaine-induced locomotion and locomotor sensitization. On Injection 1 of repeated cocaine treatment, DPAT administered into the DRN increased dose dependently cocaine-induced locomotion (a; Treatment × Time: $F(11,473) = 25.85$, $p < 0.0001$; Pretreatment × Treatment × Time: $F(22,473) = 2.50$, $p < 0.0001$), whereas DPAT administered into the MRN was without effect MRN (b; Treatment × Time: $F(11,385) = 12.45$, $p < 0.0001$; Pretreatment × Treatment × Time: $F(22,385) = 0.86$, $p > 0.05$). In contrast to Injection 1, DPAT administered into the DRN was without effect upon cocaine-sensitized locomotion (c; Treatment × Time: $F(11,473) = 16.57$, $p < 0.0001$; Pretreatment × Treatment × Time: $F(22,473) = 0.63$, $p > 0.05$), whereas DPAT administered into the MRN augmented cocaine-sensitized locomotion (d; Treatment × Time: $F(11,385) = 26.23$, $p < 0.0001$; Pretreatment × Treatment × Time: $F(22,385) = 19.43$, $p < 0.0001$) on a test for sensitization conducted 3 weeks following the end of repeated DPAT plus cocaine treatment (Test). Data represent the mean (±SEM) of the number of animals indicated within parentheses in the legends. *$p < 0.05$ for 10 μg DPAT *vs* VEH; **$p < 0.05$ for both 5 and 10 μg DPAT *vs* VEH (LSD *post hoc* tests).

Table 1 Comparison of Basal Extracellular Levels of Serotonin (5-HT), Glutamate, and Dopamine in the NAC on Injection 1 of Repeated Treatment (Inj 1) and on the Test for Sensitization, Conducted following 3 Weeks Withdrawal from Repeated Treatment (Test)

		5-HT (fmol)		Glutamate (pmol)		Dopamine (fmol)	
		Inj 1	Test	Inj 1	Test	Inj 1	Test
	VEH–SAL	7.7±0.6(14)	6.2±0.6(7)	129.2±12.5(11)	118.2±15.9(5)	25.1±0.9(14)	19.5±2.6 (7)
	VEH–COC	6.3±0.4(17)	7.3±0.9(7)	127.3±9.3(12)	68.1±5.9*(8)	23.0±1.6(17)	38.1±14.9(8)
DRN	DPAT–SAL	8.0±0.7(7)	8.7±0.9(8)	128.2±13.7(13)	115.9±6.2(6)	22.8±1.8(7)	22.0±1.9(8)
	DPAT–COC	9.5±1.2(8)	7.3±0.8(9)	128.4±12.8(10)	48.7±6.1*(5)	26.6±2.7(8)	21.1±1.8(7)
MRN	DPAT–SAL	7.0±0.9(6)	7.5±1.2(7)	138.8±10.7(6)	127.0±9.4(5)	22.5±2.4(11)	23.8±2.6(7)
	DPAT–COC	7.8±0.5(10)	8.9±1.3(12)	128.9±13.7(5)	65.7±13.3*(7)	25.2±1.3(11)	22.0±2.4(12)

Samples sizes are indicated within parentheses. Numbers represent the mean±SEM of the three dialysate samples prior to intracranial infusion.
*$p < 0.05$ *vs* Injection 1.

repeated cocaine: 0.24±0.04 nM; $p = 0.48$). One difference that may account for distinct findings between this experiment and previous studies (Dworkin *et al*, 1995; Parsons *et al*, 1995, 1996) may relate to differences in the duration of cocaine withdrawal (3 weeks *vs* 1 week, respectively).

Consistent with an autoregulatory role for raphé 5-HT1A receptors (Sprouse and Aghajanian, 1986; De Montigny *et al*, 1993), acute microinjection of DPAT into either the DRN or the MRN decreased the extracellular levels of serotonin in the nucleus accumbens of animals treated with systemic saline (Figure 5b). Furthermore, the increase in serotonin levels elicited by acute systemic cocaine administration was significantly reduced by pretreatment of either raphé nucleus with DPAT (Figure 5b). Intraraphé microinjections and systemic saline or cocaine were continued for 5 days

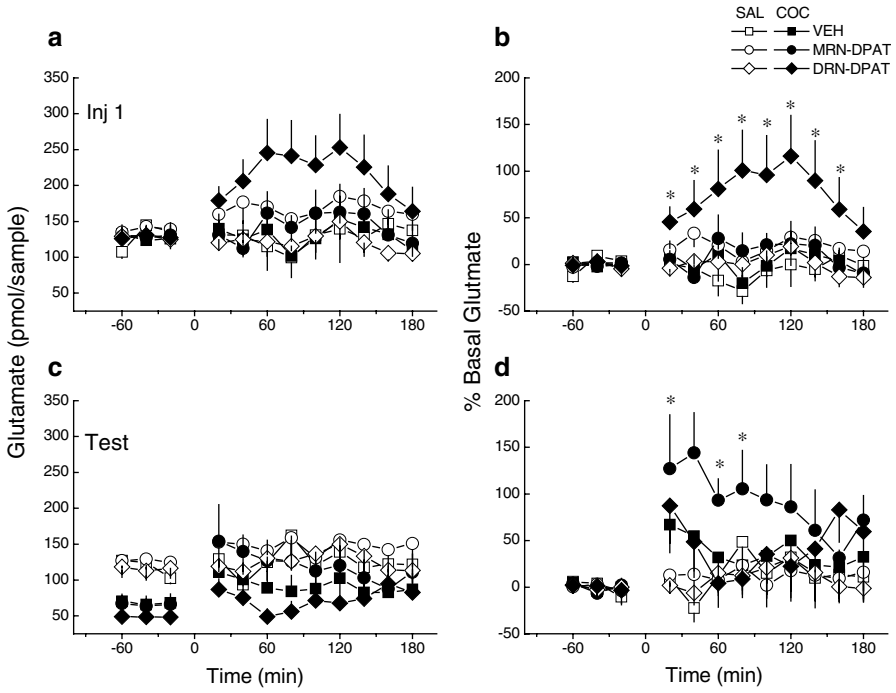

Figure 3 Differential effects of intra-DRN and intra-MRN DPAT upon extracellular levels of glutamate in the nucleus accumbens. On Injection 1 of repeated treatment (a, b), DPAT administered into the DRN, but not into the MRN, caused a cocaine-induced increase in extracellular glutamate in the nucleus accumbens, without altering glutamate levels in saline-treated groups (for glutamate content (a): Group effect: $F(5,51) = 2.3$, $p = 0.06$; Group × Time: $F(55,561) = 1.31$, $p = 0.07$; for the percent change from baseline (b): Group effect: $F(5,51) = 3.87$, $p = 0.005$; Group × Time: $F(55,561) = 1.39$, $p = 0.04$). In contrast, on the test for sensitization conducted 3 weeks following the end of repeated drug treatment (c, d), DPAT administered into the MRN, but not into the DRN, enhanced the cocaine-sensitized increase in accumbens glutamate, without altering the glutamate response to acute cocaine (for glutamate content (c): Group effect: $F(5,30) = 2.23$, $p = 0.07$; Group × Time: $F(55,330) = 0.69$, $p > 0.05$; for the percent change from baseline (d): Group effect: $F(5,30) = 3.21$, $p = 0.05$; Group × Time: $F(55,330) = 1.084$, $p > 0.05$). Data represent the mean (±SEM). Sample sizes are presented in Table 1. *$p < 0.05$ *vs* respective VEH group (LSD *post hoc* tests).

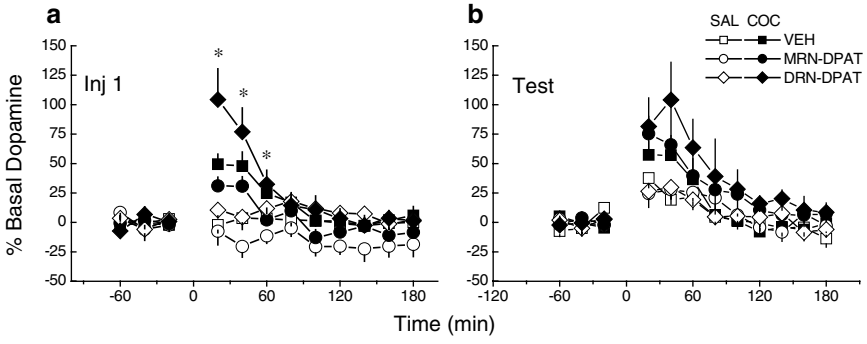

Figure 4 Differential effects of intra-DRN and intra-MRN DPAT upon extracellular levels of dopamine in the nucleus accumbens. On Injection 1 of repeated treatment (a), DPAT administered into the DRN, but not into the MRN, facilitated the cocaine-induced increase in extracellular dopamine in the nucleus accumbens, without altering glutamate levels in saline-treated groups (Group effect: $F(5,57) = 6.36$, $p < 0.001$; Group × Time: $F(55,627) = 4.27$, $p < 0.0001$). On the test for sensitization conducted 3 weeks following the end of repeated drug treatment (b), DPAT administered into either raphé nuclei failed to alter significantly either the acute or the sensitized increase in accumbens dopamine (Group effect: $F(5,43) = 4.27$, $p = 0.003$; Group × Time: $F(55,473) = 1.65$, $p = 0.003$). Data points represent the mean percent change from baseline ±SEM. Sample sizes are presented in Table 1. *$p < 0.05$ *vs* respective VEH (LSD *post hoc* tests).

and 3 weeks later, all animals were administered cocaine. Animals pretreated with intraraphé VEH or intra-DRN DPAT plus repeated cocaine demonstrated a greater increase in extracellular serotonin during the first 60 min postinjection than did animals treated with repeated saline (Figure 5c). In contrast, the sensitized increase in serotonin was almost completely absent in animals pretreated with daily DPAT microinjection into the MRN.

Histology

Figure 6 illustrates microinjection cannulae placement in the raphé nuclei and the location of the 2 mm active membrane of the microdialysis probes within the nucleus accumbens. The location of microinjections into the DRN and MRN was equivalent between the behavioral (Figure 6a) and microdialysis (Figure 6b) experiments. Similarly, the

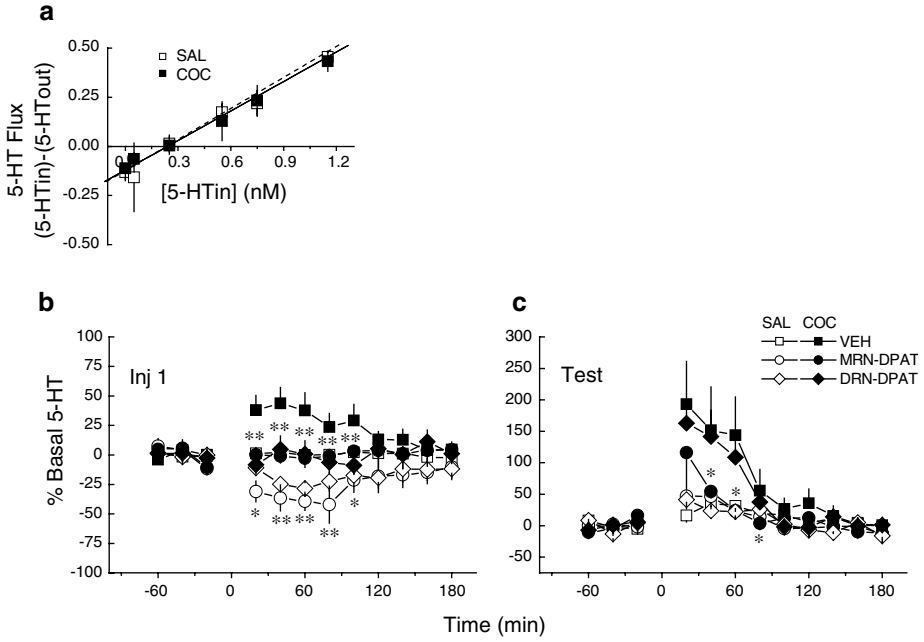

Figure 5 Intra-MRN DPAT blocks the development of serotonin sensitization. (a) When examined at 3 weeks withdrawal, repeated cocaine treatment produced no observable effects upon either the basal extracellular concentration of serotonin ($y = 0$; $p = 0.48$) or the probe recovery (slope; $p = 0.5$) when compared to repeated saline treatment. On Injection 1 of repeated treatment (b), DPAT administered into either raphé nucleus reduced extracellular levels of serotonin in control animals and inhibited the rise in serotonin elicited by acute cocaine (Group effect: $F(5,100) = 8.52$, $p < 0.0001$; Group × Time: $F(55,1309) = 3.49$, $p = 0.006$). On the test for sensitization conducted 3 weeks following the end of repeated drug treatment (c), DPAT administered into the MRN selectively blocked the development of serotonin sensitization in repeated cocaine rats, whereas DPAT was without effect in any other treatment group (time course: Group effect: $F(5,93) = 3.98$, $p = 0.003$; Group × Time: $F(55,1023) = 2.99$, $p < 0.0001$). Data points represent the mean percent change from baseline ± SEM. Sample sizes are presented in Table 1. *$p < 0.05$ MRN-DPAT *vs* respective VEH; **$p < 0.05$ both MRN-DPAT and DRN-DPAT *vs* respective VEH (LSD *post hoc* tests).

active membranes of the microdialysis probes were located in both the core and the shell compartments of the nucleus accumbens in both the DPAT (Figure 6c) and the no net-flux (Figure 6d) experiments.

DISCUSSION

Alterations in forebrain serotonin transmission are implicated in both the acute and the enduring psychomotor-activating effects of cocaine (for reviews, Walsh and Cunningham, 1997; Carroll *et al*, 1999; Weiss *et al*, 2001; Lima *et al*, 2003). Thus, the present study determined the relative contribution of the serotonergic projections emanating from the DRN and the MRN to the acute and long-term behavioral and neurochemical consequences of cocaine administration. Inactivation of the DRN and the MRN via microinjection of the 5-HT1A autoreceptor agonist DPAT differentially affected the capacity of acute and repeated cocaine to alter motor behavior and extracellular levels of serotonin, glutamate, and dopamine. Interestingly, DPAT microinjected into the DRN, but not into the MRN, produced a sensitization-like response to acute cocaine administration. Intra-DRN microinjection of DPAT potentiated the acute locomotor stimulant effect of cocaine and produced an augmentation in the cocaine-induced rise in extracellular dopamine and glutamate that has been reported to accompany locomotor sensitization (Vanderschuren and Kalivas, 2000, for a review). While 5-HT1A receptor stimulation in the DRN influenced markedly the effect of acute cocaine, intra-DRN microinjection of DPAT did not alter the development of behavioral sensitization to repeated cocaine. However, daily treatment with DPAT into the MRN in combination with daily systemic cocaine injections potentiated the sensitized locomotor response to a subsequent cocaine injection on a test conducted following 3 weeks withdrawal, in the absence of any further intra-MRN pretreatment. Moreover, the DPAT-induced potentiation of the cocaine-sensitized motor response in MRN pretreated animals was paralleled by an augmented cocaine-induced increase in extracellular glutamate and a blockade of cocaine-induced sensitization of serotonin.

Serotonin Projections from the DRN Regulate the Acute Effects of Cocaine

Similar to previous reports with systemic DPAT administration (De La Garza and Cunningham, 2000; Muller *et al*, 2003), stimulating 5-HT1A receptors locally in the DRN, but not in the MRN, potentiated the acute motor response to cocaine. An acute injection of cocaine increases extracellular dopamine without altering extracellular glutamate levels (Smith *et al*, 1995; Reid and Berger, 1996; Pierce *et al*, 1996; McFarland *et al*, 2003) and behavioral sensitization to repeated cocaine administration is associated with an augmentation in the capacity of cocaine to increase extracellular dopamine and glutamate (Heidbreder *et al*, 1996; Pierce *et al*, 1996; Reid and Berger, 1996). Similar to the neurochemical changes reported for cocaine-sensitized rats, the sensitization-like increase in acute cocaine-induced

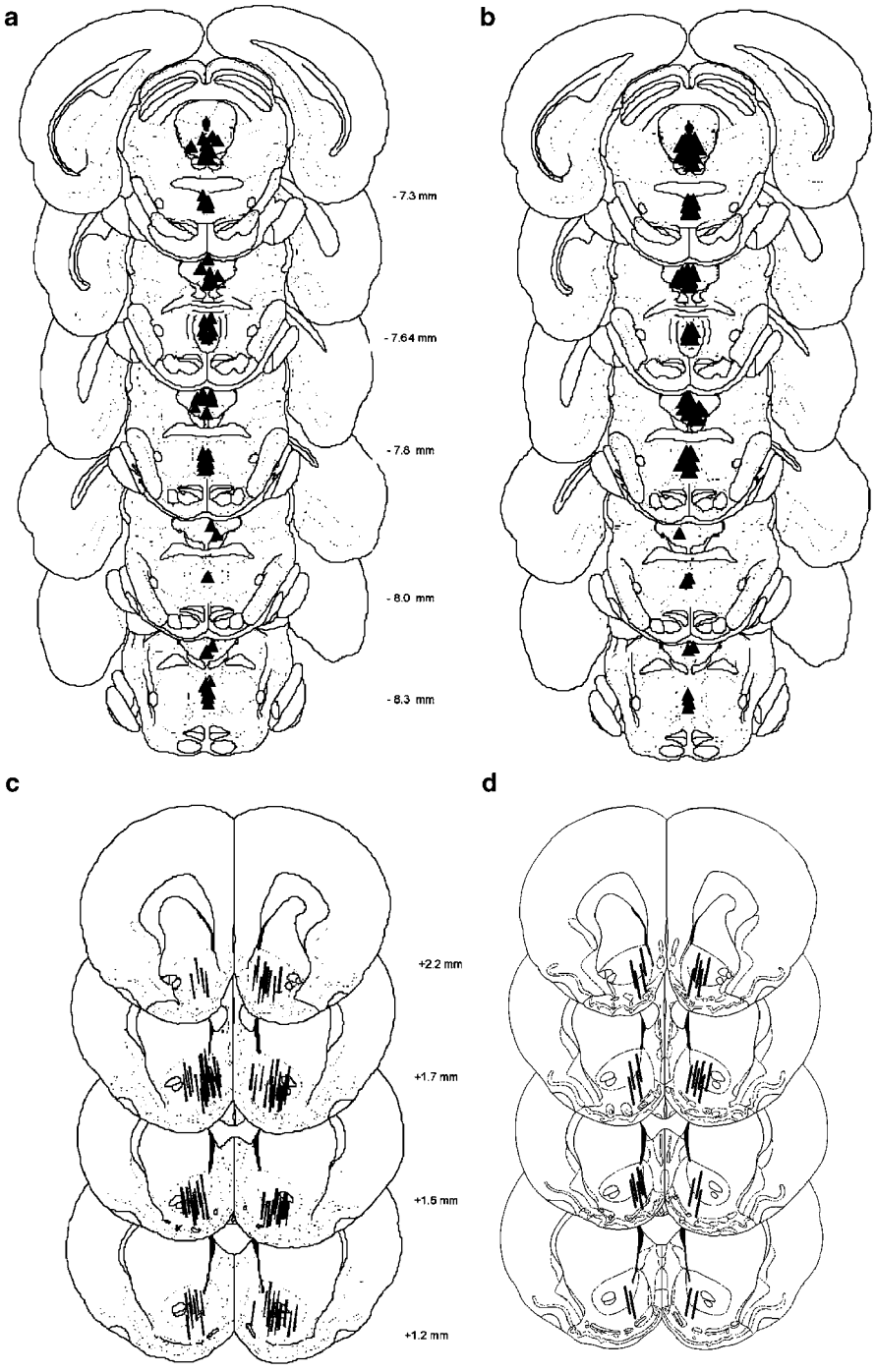

Figure 6 Localization of injector cannulae tips within the DRN and MRN of animals employed in the DPAT locomotor studies (a) and the *in vivo* microdialysis studies (b). Localization the active membrane (2–2.5 mm) of the microdialysis probes within the nucleus accumbens of animals employed in the DPAT microdialysis experiment (c) and in the no net-flux microdialysis experiment (d).

motor activity produced by DPAT administration into the DRN was associated with an augmentation in the cocaine-induced increase in extracellular dopamine and an increase in extracellular glutamate in the nucleus accumbens. In cocaine-sensitized rats, the expression of behavioral sensitization appears to depend not only on increased dopamine release but also on on the emergence of cocaine-induced glutamate release, since the sensitized response is prevented by intraaccumbens administration of glutamate antagonists or lesions of the prefrontal cortex (Vanderschuren and Kalivas, 2000; Tzschentke and Schmidt, 2003, for reviews). The cocaine-sensitized increase in extracellular glutamate observed following repeated cocaine administration consists of both an early Na^+-independent and a later Na^+-dependent component (Pierce *et al*, 1996). Intra-DRN DPAT facilitated only the latent component of the glutamate response to cocaine, suggesting that 5-HT from the DRN facilitates selectively the effect of cocaine upon Na^+-dependent glutamate release. The similarities in the effects of cocaine on accumbens neurotransmission between

animals injected with repeated cocaine and animals administered acute cocaine plus DPAT into the DRN argues that augmented dopamine and perhaps glutamate transmission produced by the inhibition of DRN serotonin transmission may contribute to the augmentation in acute cocaine-induced motor behavior produced by intra-DRN DPAT pretreatment.

Microinjection of DPAT into the raphé stimulates 5-HT1A autoreceptors (Sprouse and Aghajanian, 1986; De Montigny *et al*, 1993), an effect that was apparent in the reduction in accumbens extracellular serotonin by intraraphé DPAT administration in control animals. The DRN sends serotonin projections throughout the forebrain, including the nucleus accumbens and prefrontal cortex, in addition to the dopamine cells in the ventral tegmental area (eg Herve *et al*, 1987; Vertes *et al*, 1999). The dense innervation of both the cell body and terminal regions of the mesoaccumbens dopamine system by the DRN is consistent with the capacity of intra-DRN administration of DPAT to potentiate the effect of acute cocaine on accumbens dopamine transmission. Previous reports on the electrophysiological and neurochemical effects of reducing serotonin tone in the ventral tegmental area and accumbens, respectively, indicate mixed effects depending in part on the serotonin receptor subtype examined and the basal activity of the dopamine cells (Imperato and Angelucci, 1989; Chen and Reith, 1994; Di Mascio and Esposito, 1997; Lejeune and Millan, 1998; Parsons *et al*, 1999). A contributing factor to the enhanced dopamine release may be the rise in extracellular glutamate produced by the combination of intra-DRN DPAT and acute cocaine since, in some studies, glutamate receptor stimulation enhances dopamine release (Meltzer *et al*, 1997; Whitton, 1997 for reviews). While the source of elevated glutamate was not identified in the present study, serotonergic projections from the DRN innervate densely the cortical and allocortical regions supplying glutamate to the accumbens, including the prefrontal cortex, hippocampus, thalamus, and amygdala (eg Vertes, 1991). Indeed, *in vivo* microdialysis and electrophysiological studies indicate that activation of several different subtypes of 5-HT heteroreceptors in these glutamatergic nuclei can inhibit excitatory neurotransmission (eg Dijk *et al*, 1995; Cheng *et al*, 1998; Dawson *et al*, 2001; Golembiowska and Dziubina, 2002; Hajos *et al*, 2003), a finding consistent with the observed facilitation of cocaine-induced glutamate release upon DRN inactivation.

Serotonin Projections from the MRN Regulate the Development of Behavioral Sensitization

In contrast with a selective influence of the DRN on the acute motor and neurochemical responses to cocaine, stimulating 5-HT1A receptors in the MRN, but not in the DRN, promoted the development of behavioral sensitization to repeated cocaine administration. This behavioral facilitation was associated with an augmented cocaine-induced increase in glutamate. Despite the dense innervation of the ventral tegmental area by the MRN (Vertes *et al*, 1999), the facilitation of cocaine-induced behavioral sensitization produced by DPAT into the MRN occurred in the absence of any DPAT effect upon the cocaine-sensitized increase in accumbens dopamine. This observation is consistent with a more critical role for mesoaccumbens dopamine transmission in the expression, rather than in the development, of cocaine-induced behavioral sensitization (eg, Mattingly *et al*, 1994; White *et al*, 1998; Szumlinski *et al*, 2003). Similar to the sensitization-like increase in the motor response to the first cocaine injection that was produced by intra-DRN pretreatment with DPAT, these MRN data pose enhanced glutamate transmission in the accumbens as a contributing factor mediating the potentiated behavioral sensitization produced by daily intra-MRN administration of DPAT with cocaine. Supporting mediation by glutamatergic inputs to the accumbens, there is overlapping serotonin innervation from the DRN and MRN to cortical and allocortical regions supplying glutamate to the accumbens, notably the prefrontal cortex, mediodorsal thalamus, basolateral and central amygdala, and hippocampus, whereas the MRN sends a very sparse projection to the accumbens itself (Mamounas and Molliver, 1988; Vertes *et al*, 1999). Only in the case of the MRN did DPAT microinjection during repeated cocaine administration prevent the development of serotonin sensitization in the nucleus accumbens indicating that long-term cellular changes in MRN serotonin neurons that project to allocortical or cortical glutamatergic neurons innervating the accumbens are inhibitory upon the development of glutamate sensitization produced by repeated cocaine administration. The sensitization of prefrontal corticoaccumbens glutamate transmission is associated with increased sensitivity to cocaine reward and psychomotor activation (eg Pierce *et al*, 1996; McFarland *et al*, 2003). Thus, the present data are consistent with the reported effectiveness of selective serotonin reuptake inhibitors to attenuate cocaine self-administration and/or cocaine seeking in animal models of relapse (Baker *et al*, 2001; Tran-Nguyen *et al*, 2001; Czoty *et al*, 2002; Glatz *et al*, 2002) and pose a critical and selective role for MRN serotonin regulation of prefrontal cortex glutamate transmission in the enduring behavioral consequences of repeated cocaine administration.

Reduced Extracellular Serotonin in the Accumbens by Stimulating 5-HT1A Autoreceptors is without Consequence on Cocaine-Induced Behaviors

Consistent with the fact that stimulating 5-HT1A receptors in the DRN inhibits serotonin cell firing (Sprouse and Aghajanian, 1986; De Montigny *et al*, 1993), extracellular levels of serotonin in the accumbens were reduced by the intra-DRN administration of DPAT. Similarly, stimulating DRN 5-HT1A receptors reduced the rise in serotonin produced by acute cocaine administration. These functional data are consistent with the dense serotonergic innervation by the DRN of both the core and shell regions of the accumbens (eg Vertes, 1991). Surprisingly, as the MRN sends very sparse serotonergic projections to the accumbens (Vertes *et al*, 1999), DPAT microinjection into the MRN also reduced extracellular serotonin in the accumbens. Given the weak innervation of the accumbens by MRN serotonin neurons, the DPAT-induced decrease in accumbens serotonin most likely resulted via a multisynaptic mechanism. One possibility is the projection from the MRN to the DRN (Vertes *et al*, 1999), which could regulate serotonin projections directly to the accumbens. Another

multisynaptic mechanism could involve MRN projections to the cortex to affect corticofugal glutamatergic projections regulating presynaptic sertonin release from axon terminals originating in the DRN (Celada *et al*, 2001). Regardless of the mechanisms underlying the reduction in accumbens serotonin levels produced by the administration of DPAT into the MRN, the changes in serotonin did not parallel the acute behavioral effects of cocaine since DPAT administered into the MRN and the DRN produced equivalent reductions in accumbens serotonin, but only DPAT administration into the DRN augmented acute cocaine-induced locomotion.

The previously reported capacity of repeated cocaine to augment cocaine-induced elevation in extracellular serotonin in the accumbens (Parsons and Justice, 1993) was attenuated if 5-HT1A receptors in the MRN, but not DRN, were stimulated prior to the daily cocaine injections. One possible mechanism for this blunting is that repeated DPAT may have augmented the elevation in 5-HT1A autoreceptor function produced by repeated cocaine administration. In support of this, electrophysiological recordings (eg Cunningham *et al*, 1992) as well as autoradiographic (Cunningham *et al*, 1992; Perret *et al*, 1998), behavioral (King *et al*, 1993), and biochemical studies (Levy *et al*, 1994; Parsons *et al*, 1995, 1996; Baumann and Rothman, 1998) indicate that repeated cocaine administration sensitizes 5-HT1A autoreceptor function, at least following short periods of cocaine withdrawal.

Conclusions

Stimulating 5-HT1A somatodendritic receptors differentially affected the behavioral and neurochemical consequences of acute and repeated cocaine administration depending on whether the receptors were in the MRN or the DRN. While stimulating 5-HT1A receptors in the DRN augmented the capacity of acute cocaine to produce locomotor activity and elevate extracellular dopamine and glutamate in the accumbens, 5-HT1A receptor stimulation in the MRN promoted the development of behavioral sensitization and the capacity of cocaine to increase accumbens glutamate. These data are consistent with a modulatory role for ascending serotonin projections on cocaine-induced neuroplasticity, and pose that serotonergic modulation of glutamatergic afferents to the accumbens as a shared substrate modulating both the acute and sensitized motor stimulant effect of cocaine.

ACKNOWLEDGEMENTS

This work was supported by grants from NIDA to PWK (DA-03906; DA-14185). KKS is supported by a postdoctoral fellowship from the CIHR.

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