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Prior Exposure to Chronic Stress and MDMA Potentiates Mesoaccumbens Dopamine Release Mediated by the 5-HT_{1B} Receptor

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(+) 3,4,-Methylenedioxymethamphetamine (MDMA) is an abused drug that acutely releases serotonin (5-HT) and dopamine (DA) but produces long-term damage to 5-HT terminals. MDMA-induced DA release has been shown to be dampened by 5-HT. Although stress also activates the mesolimbic DA pathway, it is unknown if chronic stress after exposure to neurotoxic doses of MDMA will augment MDMA-induced DA release in the nucleus accumbens shell (NAcc(sh)). Rats were pretreated with MDMA (10 mg/kg × 4, intraperitoneal (i.p.)). After 7 days, rats were subjected to 10 days of chronic unpredictable stress. DA release in the NAcc(sh) and 5-HT in the ventral tegmental area (VTA) were measured after a challenge injection of MDMA (5 mg/kg, i.p.). The combination of pretreatment with MDMA + stress decreased basal concentrations of 5-HT in the VTA and DA in the NAcc(sh) and enhanced MDMA-stimulated DA release in the NAcc(sh). Pretreatment with MDMA or stress alone blunted MDMA-induced 5-HT release in the VTA. The augmentation of MDMA-induced DA release in rats pretreated with MDMA + chronic stress was attenuated by perfusion of the 5-HT_{1B} antagonist, GR127935 into the VTA before the MDMA challenge injection. These results suggest that prior exposure to both MDMA and stress can produce a long-term augmentation in mesolimbic DA transmission and enhanced drug abuse vulnerability that is mediated, in part, by the 5-HT_{1B} receptor in the VTA.
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INTRODUCTION

(+) 3, 4-Methylenedioxymethamphetamine (MDMA) is a psychostimulant that is abused worldwide. The stimulant and rewarding properties of abused psychostimulants such as MDMA, arise in part, from the release of dopamine from neurons that originate in the ventral tegmental area (VTA) and project to the nucleus accumbens shell (NAcc(sh)) (Hoebel, 1985; Wise and Bozarth, 1985; Koob, 1992). MDMA binds to and reverses the dopamine or serotonin transporters, to produce impulse-independent/carrier-mediated efflux of dopamine or 5-HT, respectively (Johnson *et al*, 1991). However, MDMA-induced dopamine release is not entirely carrier mediated and involves an impulse-dependent component that is mediated by 5-HT (Koch and Galloway, 1997; Gudelsky and Nash, 1996). Thus, MDMA-induced dopamine release is both carrier mediated via the reversal of the dopamine transporter and impulse dependent through the stimulation of dopamine neurons by 5-HT.

Serotonin neurons from the dorsal raphe nucleus project to the mesolimbic DA cell bodies of the VTA and substantia nigra (Gervais and Rouillard, 2000). Multiple 5-HT receptors are believed to modulate basal (Gervais and Rouillard, 2000; Cameron and Williams, 1994; Doherty and Pickel, 2000; Di Giovanni *et al*, 2000) and MDMA-induced DA release (Yamamoto *et al*, 1995; Bankson and Yamamoto, 2004). In particular, activation of 5-HT_{1B} receptors in the VTA produces DA efflux in the nACC (Yan *et al*, 2005; O'Dell and Parsons, 2004) through inhibition of GABA release in the VTA and the consequent disinhibition of DA neurons (Yan and Yan, 2001a; Cameron and Williams, 1995; Cameron and Williams, 1994). In addition, behavioral studies have shown that mice lacking the 5-HT_{1B} receptor or the administration of the 5-HT_{1B} antagonist GR127935 to rats blocks MDMA-induced hyperactivity (McCreary *et al*, 1999; Scearce-Levie *et al*, 1999).

Moderate to high doses of MDMA produce selective damage to 5-HT neurons as evidenced by the reduction of 5-HT transporter binding sites (Battaglia *et al*, 1987; Schmidt, 1987), 5-HT transporter immunoreactivity,

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(O'Hearn *et al*, 1988), and reductions of 5-HT content (Schmidt, 1987; O'Hearn *et al*, 1988) as well as tryptophan hydroxylase activity (Schmidt and Taylor, 1988). It follows that these deficits in mesolimbic 5-HT neurotransmission could alter the interaction of 5-HT with dopamine to affect the stimulant and rewarding effects of a subsequent MDMA administration. In fact, rats exposed to a neurotoxic regimen of MDMA exhibit enhanced sensitivity to MDMA-induced reinstatement of amphetamine self-administration (Morley *et al*, 2004) and attenuate serotonergic reactivity to a challenge dose of MDMA (Shankaran and Gudelsky, 1999) or fenfluramine (Series *et al*, 1994). Therefore, damage to 5-HT neurons might enhance vulnerability to the addictive properties of MDMA and other drugs of abuse by altering mesolimbic dopamine function.

Similar to many addictive drugs, stress also affects mesolimbic dopamine neurotransmission by eliciting immediate increases in dopamine and 5-HT release (Kalivas and Duffy, 1995; Abercrombie *et al*, 1989; Broom and Yamamoto, 2005; Bland *et al*, 2003). Numerous studies have demonstrated that stressful life events are associated with increased drug addiction in humans (Rhoads, 1983; Kosten *et al*, 1986; Wilsnack *et al*, 1997; Najavits *et al*, 1998) and enhanced self-administration of various drugs of abuse by animals (Piazza and Le, 1998). Thus, chronic stress may enhance mesolimbic functions that mediate reward and drug-seeking behavior. However, at present, no studies have assessed if chronic stress alters the mesolimbic dopamine response to MDMA.

Nothing is known about the combined effects of neurotoxic doses of MDMA and chronic stress exposure on MDMA-induced increases in mesolimbic dopamine and 5-HT or the influence of 5-HT receptors in mediating the impulse-dependent component of MDMA-induced dopamine release. Although several animal models of chronic stress exposure have been used, the chronic unpredictable stress (CUS) paradigm has added validity because the type and time of stress exposures are varied (Katz *et al*, 1981; Willner *et al*, 1992; Ortiz *et al*, 1996) to mimic the exposure to unexpected stressful life events. Moreover, the paradigm is not confounded with learning, adaptation, and tolerance of the hypothalamo-adrenal axis (Herman *et al*, 1995) typically observed with repeated restraint stress (Bielajew *et al*, 2002; Lopez *et al*, 1998). CUS also produces neurochemical changes in the mesolimbic dopamine system, increases place preference for cocaine at low doses, and enhances cocaine-induced locomotor activation (Haile *et al*, 2001), whereas repeated restraint stress does not affect these measures (Ortiz *et al*, 1996; Haile *et al*, 2001).

The present study used *in vivo*, dual-probe microdialysis to assess changes in mesolimbic responses to MDMA in rats with prior exposure to a neurotoxic regimen of MDMA and/or CUS. Based on the known ability of stress to enhance drug-seeking behavior, it is predicted that CUS, in combination with a serotonergic lesion produced by prior administration of neurotoxic doses of MDMA, will enhance the effect of a challenge dose of MDMA on NAcc(sh) shell dopamine release. Furthermore, it is posited that the augmentation of mesolimbic dopaminergic responsiveness to a challenge injection of MDMA is mediated by 5-HT_{1B} receptors in the VTA.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) weighing 175–220 g at the beginning of the experimental procedures were housed in pairs in a temperature controlled environment (21–23°C) for 5 days before the experiments. Food and water were available ad libitum. Lighting was maintained under a 12 h light–dark cycle (lights on 0700–1900 hours). All procedures were performed during the light cycle and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Boston University IACUC.

Drugs

MDMA was obtained from the National Institutes of Drug Abuse (NIDA, Research Triangle Park, NC, USA). Doses refer to the weight of the salt. MDMA was administered intraperitoneally (i.p.) in a volume of 1 ml per kg of body weight. GR127935 (N-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-carboxamide was purchased from Tocris Cookson (Ellisville, MO)) and used as a selective antagonist for the 5-HT_{1b} receptor since there are no 5-HT_{1d} receptors in the rat mesolimbic system. GR127935 was administered by reverse dialysis in modified Dulbecco's buffered saline (137 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1.2 mM $CaCl_2$ and 0.5 mM d-glucose, pH 7.4; Sigma Chemical Co.; St Louis, MO).

Pretreatment of MDMA

After acclimation to the colony room, rats were administered i.p. injections of 0.9% NaCl or MDMA (10 mg/kg) once every 2 h for a total of four injections. Temperatures were recorded every 2 h, on the off hour of injections, to ensure rats reached hyperthermia (at least 40°C) after MDMA. Temperatures were measured via a rectal probe digital thermometer (Thermalert TH-8; Physitemp Instruments Inc., Clifton, NJ). Rats were monitored over night before they were returned to the colony.

CUS Paradigm

Seven days after MDMA administration, stressed rats were exposed to stressors that varied by day and time for 10 days (Matuszewich and Yamamoto, 2003). Rats were subjected to the following procedure—Day 1 1000 hours 50 min cold room (40°C), 1300 hours 60 min cage rotation; Day 2 1400 hours 4 min swim stress, 1800 hours lights on overnight (12 h); Day 3 0900 hours 3 h lights off, 1300 hours 60 min restraint stress; Day 4 1100 hours 20 min cage rotation, 1800 hours lights on overnight (12 h); Day 5 1500 hours 20 min cold room (40°C), 1800 hours isolation housing overnight (12 h); Day 6 1300 hours 60 min restraint stress, 1800 hours food and water deprivation overnight (12 h); Day 7 1000 hours 2 h lights off, 1400 hours 3 min swim stress; Day 8 1100 hours 50 min cold room, 1800 hours lights on overnight (12 h); Day 9 1000 hours 30 min cage rotation, 1300 hours 30 min cold room (40°C); Day 10 0900 hours

intracranial surgery, 1800 hours food and water deprivation overnight (12 h).

Surgical Procedures

Both unstressed and stressed rats underwent intracranial surgery performed on the last day of CUS (Day 10). All rats were anesthetized with a combination of ketamine (80 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.) before being placed into a Kopf stereotaxic frame. The skull was exposed and microdialysis probes were slowly lowered into the VTA (4.8 mm posterior, $\pm$0.9 mm medial to bregma and 9.0 mm ventral to the brain surface) and ipsilateral nucleus accumbens shell (NAcc(sh)) (1.7 mm anterior, $\pm$0.9 mm medial to bregma and 7.6 mm ventral to the brain surface). The probes were constructed as described by Yamamoto and Pehek (1990). Probes had an active/exposed membrane length of 2.0 mm for those targeted at NAcc(sh) and 1.5 mm for probes targeted at the VTA. The probes and a male metal connector used to attach the rat to a steel spring cable and liquid swivel were secured to the skull with three stainless steel screws and cranioplast cement (Plastics One Inc., Roanoke, VA).

In Vivo Microdialysis

The day after probe insertion, modified Dulbecco's phosphate buffered saline medium was pumped through the microdialysis probes with a Harvard Model 22 syringe infusion pump (Hollison, MA, USA) set at a flow rate of 1.5 µl/min. A 2-h perfusion period was performed before collecting baseline samples. Dialysate samples from the NAcc(sh) and VTA were then collected every 30 min. Four baseline samples were collected before all rats were injected with an MDMA challenge (5 mg/kg, i.p.). Samples from the VTA and NAcc(sh) were simultaneously collected for 2.5 h. In some experiments, GR127935 (1 µM) was reverse dialyzed through the dialysis probe in the VTA for two baseline measures and throughout the period following the systemic injection of an MDMA challenge (5 mg/kg).

HPLC Analysis of 5-HT and Dopamine

Microdialysis samples were assayed for 5-HT and dopamine by high-performance liquid chromatography with electrochemical detection. Separation was achieved with a C18 column (100 $\times$ 2.0 mm, 3 µm particle size; Phenomenex, Torrance, CA, USA). The mobile phase for detection of 5-HT and dopamine and metabolites (pH 4.2) consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sodium sulfate, and 3% methanol. Detection was accomplished with a DECADE or Intro^{R} electrochemical detector (Antec Leyden; GBC Scientific Equipment Inc., Hubbardston, MA) and peaks were analyzed with EZChrom^{R} Scientific Software Inc. (Pleasanton, CA).

Tissue Content of 5-HT and Dopamine in NAcc

Twenty-two days after rats were pretreated with MDMA (ie 1 week after dialysis experiments), rats were killed by rapid decapitation and brains were removed and quick frozen on dry ice. Brains were sectioned in 40 µm increments using a cryostat microtome (−20°C), and probe placements were recorded for both NAcc and VTA. Once the probe tract in the NAcc was identified, NAcc tissue at 1.7 anterior to bregma was removed from the frozen coronal section using a 16 ga punch. Samples were stored at −80°C until assayed. Tissues were sonicated in cold 0.25 N $HClO_4$ and centrifuged at 12 000*g* for 20 min. The supernatant was analyzed for 5-HT and dopamine using high-performance liquid chromatography with electrochemical detection as described above. Protein was determined by the Bradford Method using Bradford protein dye (Bio-Rad, Hercules, CA). Concentrations are expressed as picograms (pg) per microgram of protein.

Statistical Analyses

Two-way ANOVAs and two-way ANOVAs with repeated measures were used to compare rats pretreated with MDMA or saline, stress or no stress, and over time including the time during drug administrations. Tukey's test was used to conduct *post hoc* analyses for any significant treatments at specific time points. Statistical significance was set at $p<0.05$ for all conditions and experiments. The overall effects of MDMA and/or stress on basal extracellular DA and 5-HT are represented by the average of the four baseline samples taken before MDMA challenge. The overall effects of MDMA and/or stress on MDMA-induced changes in extracellular DA and 5-HT over the 2.5 h following MDMA challenge are represented by the average cumulative change from baseline. The group differences at specific time points are represented by the timecourse plots of percent average basal concentration of DA or 5-HT.

DA and 5-HT concentrations for the microdialysis studies represent concentrations of neurotransmitter recovered in dialysis fluid and are not actual/absolute extracellular concentrations. Thus, treatments were balanced across all groups and across time to control for any change in microdialysis conditions which might occur over time.

RESULTS

MDMA and Stress Pretreatment on Basal Extracellular 5-HT Concentrations in VTA and Dopamine Concentrations in NAcc(sh)

Pretreatment with MDMA followed by stress significantly decreased basal extracellular 5-HT in the VTA (Tukey's *post hoc*, $p<0.05$) compared to stress alone (Figure 1). Pretreatment with MDMA produced an overall significant decrease in basal extracellular dopamine concentrations in the NAcc(sh) ($F_{1,21}=6.78$, $p<0.05$) compared to saline groups (Figure 2). In contrast, there was no effect of stress on basal extracellular dopamine concentrations in either the saline or MDMA pretreated groups.

MDMA and Stress Pretreatment on Extracellular 5-HT Concentrations in VTA after an MDMA Challenge

An MDMA challenge (5 mg/kg, i.p.) produced an overall increase in extracellular 5-HT concentrations in the VTA when analyzed across all MDMA groups and compared to

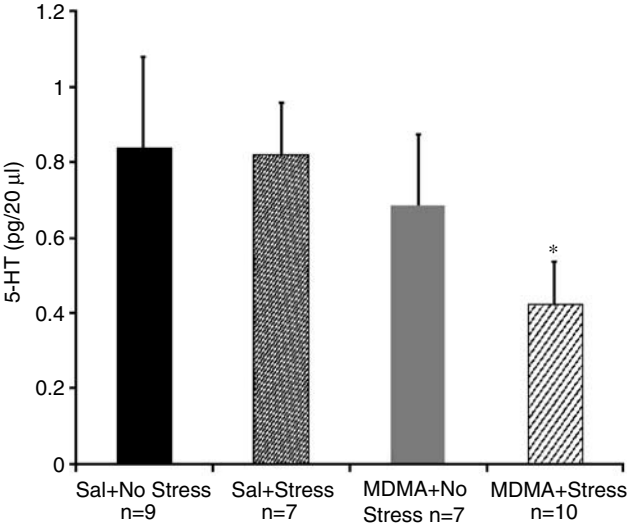

Figure 1 Basal extracellular concentrations of 5-HT in the VTA after pretreatment with saline, MDMA and/or chronic stress. Saline (Sal) or MDMA, 10 mg/kg, every 2 h × 4 (i.p.) was injected 7 days before the start of CUS. Chronic stress was administered for 10 days before basal extracellular 5-HT was collected via microdialysis of the VTA. Bars represent an average of four baseline samples per rat after 17 days following pretreatment with drug and/or stress (7 days after saline or MDMA + 10 days of CUS/No CUS). *n* = number of rats. Values are mean pg/20 µl of dialysate. **p* < 0.05 compared to Sal + stress treatment.

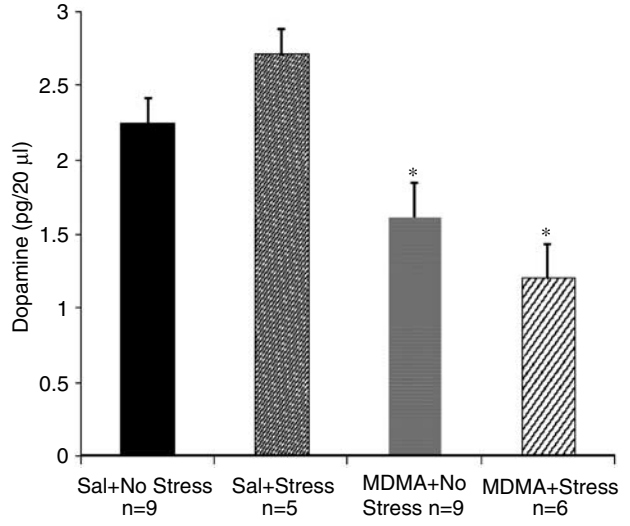

Figure 2 Basal extracellular concentrations of dopamine in Nacc(sh) after pretreatment with saline, MDMA and/or chronic stress. Saline (Sal) or MDMA, 10 mg/kg, every 2 h × 4 (i.p.) was injected 7 days before the start of CUS. Chronic stress was administered for 10 days before basal extracellular DA was collected in the NAcc(sh) via microdialysis. Bars represent an average of four baseline samples per rat after 17 days following pretreatment with drug and/or stress (7 days after saline or MDMA + 10 days of CUS/No CUS). *n* = number of rats. Values are mean pg/20 µl of dialysate. **p* < 0.05 compared to saline treatments.

baseline concentrations ($F_{2,52} = 17.29$, $p < 0.05$) (Figure 3a). Pretreatment with MDMA + stress produced a maximal increase of 921% after a challenge injection of MDMA and the overall increase after an MDMA challenge was significantly greater than the MDMA + no stress and saline + stress groups (Tukey's *post hoc*, $p < 0.05$).

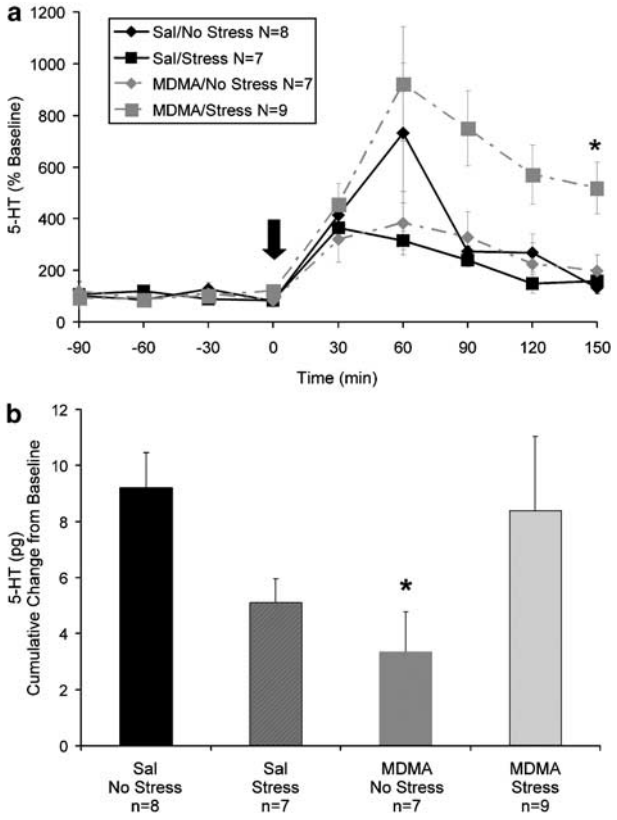

Figure 3 Extracellular 5-HT in the VTA before and after a challenge injection of MDMA. Dialysis samples were collected from the VTA of rats pretreated with saline (Sal) or MDMA (10 mg/kg, every 2 h s × 4, i.p.) and then 7 days later exposed to 10 days of CUS (stress). Experiments were performed after 17 days following pretreatment with drug and/or stress (7 days after saline or MDMA + 10 days of CUS/No CUS) (a) A challenge dose of MDMA (5 mg/kg, i.p.) was injected (as indicated by the black arrow) after a 2 h baseline. **p* < 0.05 compared to MDMA + no stress and saline + no stress pretreatment. (b) Mean cumulative picogram increase from the average of four baseline samples for 2.5 h after a challenge injection of MDMA. **p* < 0.05 compared to saline + no stress treatment.

When the overall effect of MDMA and/or stress during the 2.5 h following MDMA challenge is analyzed as a cumulative change from baseline (Figure 3b), there was a significant interaction effect between the pretreatment with MDMA and the pretreatment with stress ($F_{1,27} = 5.147$, $p < 0.05$) (Figure 3b). Stress had a differential effect on the total increase in 5-HT after a MDMA challenge such that stress tended to attenuate the increase in 5-HT in the saline pretreated group but restored the increase in the MDMA + stress pretreated group (Figure 3b).

MDMA and Stress Pretreatment on Extracellular Dopamine Concentrations in NAcc(sh) after an MDMA Challenge

An MDMA challenge (5 mg/kg) produced an increase in extracellular dopamine concentrations in the NAcc(sh) over baseline concentrations ($F_{2,40} = 35.31$, $p < 0.05$) (Figure 4). Pretreatment with MDMA + stress produced a maximal increase in dopamine of 1010% after the MDMA challenge that was significantly greater than the MDMA-induced increase in extracellular dopamine concentrations observed

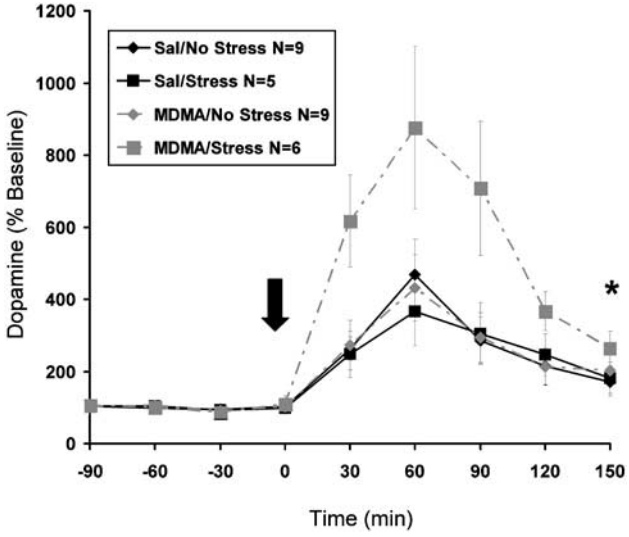

Figure 4 Extracellular dopamine in the NAcc(sh) before and after a challenge injection of MDMA. Dialysis samples were collected from the NAcc(sh) of rats pretreated with saline (Sal) or MDMA (10 mg/kg, every 2 h s × 4, i.p.) and then 7 days later exposed to 10 days of CUS (stress). Experiments were performed after 17 days following pretreatment with drug and/or stress (7 days after saline or MDMA + 10 days of CUS/No CUS). A challenge dose of MDMA (5 mg/kg, i.p.) was injected after a 2 h baseline (as indicated by the black arrow). *$p < 0.05$ compared to all groups.

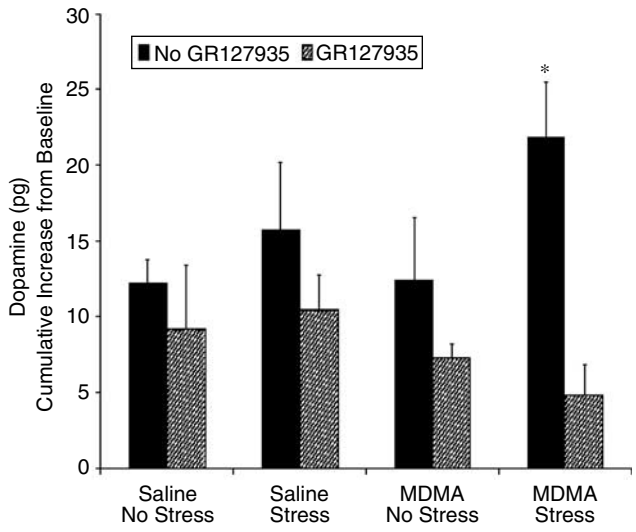

Figure 5 Effect of GR127935 perfusion in VTA on MDMA-induced dopamine release after pretreatment with MDMA + chronic stress. Dialysis samples were collected from the NAcc(sh) of rats pretreated with saline or MDMA (10 mg/kg, every 2 h × 4, i.p.) and then 7 days later treated with 10 days of CUS (stress) or no stress. Experiments were performed after 17 days following pretreatment with drug and/or stress (7 days after saline or MDMA + 10 days of stress/no stress). GR127935 (1 μM) was reverse dialyzed into the VTA for 1 h before the challenge dose of MDMA (5 mg/kg, i.p.) and continued for 2.5 h. Values are mean cumulative picogram increase from the average of four baseline samples for 2.5 h after a challenge injection of MDMA. In No GR127935 groups, $n = 9$ for Sal + no stress, $n = 5$ for Sal + stress, $n = 9$ for MDMA + no stress, $n = 6$ for MDMA + stress; In GR127935 groups, $n = 5$ for Sal + no stress, $n = 7$ for Sal + stress, $n = 5$ for MDMA + no stress, $n = 4$ for MDMA + stress. *$p < 0.05$ compared to MDMA + stress with GR127935 treatment.

for any of the other pretreatment groups (Tukey's *post hoc*, $p < 0.05$).

MDMA and Stress Pretreatment on Extracellular Dopamine Concentrations in NAcc(sh) after an MDMA Challenge: Effect of 5-HT$_{1B}$ Antagonism in VTA

A challenge injection of MDMA to rats pretreated with MDMA + stress produced a cumulative increase during the 2.5 h following MDMA in extracellular dopamine in the NAcc(sh) to 22 ± 3.7 pg over baseline during the 2.5 h period after the MDMA challenge (Figure 5). GR127935 perfusion in the VTA of rats pretreated with MDMA + stress resulted in a mean increase above baseline to 4.9 ± 2.0 pg/2.5 h in response to an MDMA challenge that was significantly less than the MDMA-induced increase in dopamine in the NAcc(sh) of rats pretreated with MDMA + stress (Tukey's *post hoc*, $p < 0.05$) (Figure 5).

5-HT and Dopamine Tissue Content in the NAcc after MDMA and Stress Pretreatment

Pretreatment with MDMA (10 mg/kg, every 2 h × 4, i.p.) produced a significant decrease in 5-HT tissue content in the NAcc when analyzed across both MDMA groups and compared to both saline groups ($F_{1,66} = 6.46$, $p < 0.05$) (Figure 6). Pretreatment with MDMA and/or stress did not produce a change in dopamine tissue content in the NAcc (Figure 7).

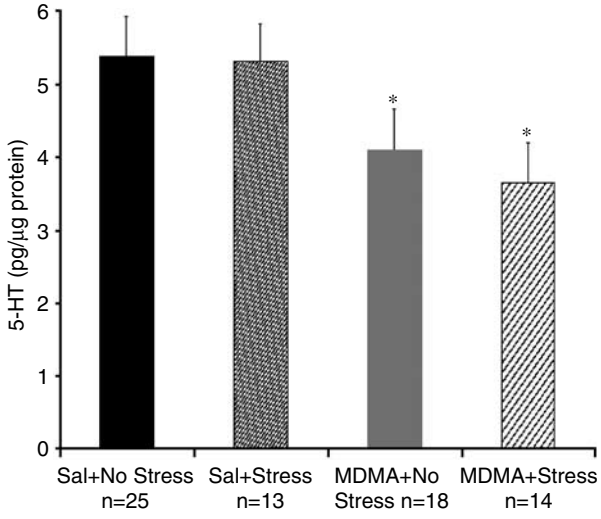

Figure 6 5-HT content in the NAcc after pretreatment with saline or MDMA and chronic stress (stress) or no stress. Rats were killed 3 weeks after pretreatment with saline (Sal) or MDMA (10 mg/kg, every 2 h × 4, i.p.) followed by stress/no stress. *$p < 0.05$ compared to saline groups.

DISCUSSION

These results are the first to demonstrate that CUS after previous exposure to neurotoxic doses of MDMA alters basal and MDMA-stimulated mesolimbic dopamine and 5-HT release, and suggest that stress can alter the neuronal mechanisms associated with MDMA reward and abuse. Neurotoxic MDMA followed by stress produced a

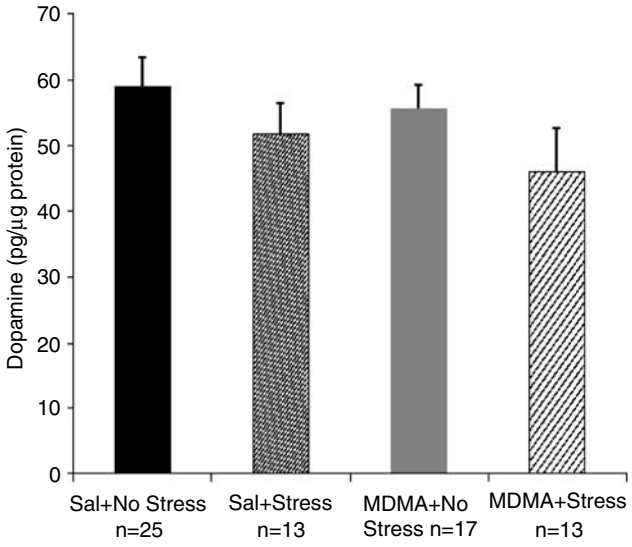

Figure 7 Dopamine content in the NAcc after pretreatment with saline or MDMA and chronic stress (stress) or no stress. Rats were killed 3 weeks after pretreatment with saline (Sal) or MDMA (10 mg/kg, every 2 h × 4, i.p.) followed by stress/no stress.

significant reduction of baseline extracellular 5-HT in the VTA and dopamine in NAcc shell (NAcc(sh)) (Figures 1 and 2). In contrast, MDMA-induced dopamine efflux in the NAcc(sh) was enhanced by the combined pretreatments with neurotoxic MDMA and chronic stress (Figure 4). Overall, these data show that pre-exposure to MDMA and stress is not simply additive but synergize to augment mesolimbic dopamine neurotransmission in a manner that is both quantitatively and qualitatively different from either the effects of MDMA or stress alone. Furthermore, stress exposure after a neurotoxic regimen of MDMA did not affect the neurotoxicty of MDMA as indicated by the unaltered long-term depletion of 5-HT tissue content (Figure 6). Thus, the effects of stress exposure after MDMA are not the result of added damage to 5-HT that is produced by MDMA.

Neurotoxic MDMA, either alone or followed by stress, decreased the basal release of dopamine in the nACC shell (Figure 2). In contrast, pretreatment with stress did not affect basal extracellular dopamine concentrations regardless of drug pretreatment. The reduced basal extracellular dopamine concentrations in the MDMA + stress group may be secondary to MDMA-induced damage to 5-HT neurons, depletion of tissue 5-HT content, and a consequent reduction in the activation of 5-HT_{1B} receptors in the VTA (Yan *et al*, 2004; Yan and Yan, 2001a). This possibility is consistent with the lower basal extracellular 5-HT observed after neurotoxic doses of MDMA followed by stress (Figure 1). However, it remains unclear how neurotoxic MDMA alone reduced basal dopamine but had no effect on basal 5-HT. Therefore, a neurotoxic regimen of MDMA lowers basal extracellular dopamine concentrations in a manner independent of changes in basal 5-HT concentrations. In fact, repeated doses of the non-neurotoxic stimulant cocaine (Zhang *et al*, 2003) or low, nontoxic doses of methamphetamine (Broom and Yamamoto, 2005) also lower basal dopamine release in the NAcc(sh). This effect may be an adaptive response to psychostimulant drugs resulting from an upregulation of accumbal dopamine transporter expression (Broom and Yamamoto, 2005) and suggests that long-term changes in stimulant-induced decreases in basal extracellular dopamine are due to enhanced reuptake of dopamine from the extracellular space.

In contrast to the reduced basal extracellular concentrations of dopamine which appear to be linked to neurotoxic MDMA treatment, a reduction in basal 5-HT in the VTA occurs only after the pretreatment with MDMA and stress. Other studies have reported decreased basal 5-HT release in hippocampus after MDMA (Matuszewich *et al*, 2002) or chronic stress (Mangiavacchi *et al*, 2001). The present studies demonstrating decreased basal 5-HT release only after both MDMA and stress indicates a synergistic diminution of extracellular 5-HT in the VTA. It can be speculated that one contributory factor may be a stress-induced reduction in the vesicular monoamine transporter-2 (VMAT-2) (Zucker *et al*, 2005) resulting in decreased filling of 5-HT vesicles (Pothos *et al*, 2000; Fon *et al*, 1997). This reduction of VMAT-2 and 5-HT quantal size may decrease basal release to a greater degree in neurons previously compromised by MDMA, particularly since neurotoxic amphetamines have also been shown to reduce VMAT-2 (Hansen *et al*, 2002; Eyerman and Yamamoto, 2005; Frey *et al*, 1997). Regardless, further studies are necessary to examine the impact of stress and MDMA on VMAT-2 as one of several mechanisms that are likely contributors to the observed decrease in basal 5-HT. It is also important to note that the present results of dialysate concentrations of monoamines, unlike those collected with no-net-flux microdialysis, does not measure actual concentrations of neurotransmitters in the extracellular space of the brain.

Neurotoxic MDMA alone produced a significant reduction in MDMA-induced release of 5-HT (Figure 3). This finding is consistent with similar studies in the striatum (Shankaran and Gudelsky, 1999) and suggests that MDMA-induced damage to 5-HT terminals is sufficient to limit transporter-mediated release. Surprisingly, when analyzed as a cumulative increase from baseline (Figure 3b), there was a more pronounced increase in MDMA-stimulated release of 5-HT in rats pretreated with MDMA and stress compared to rats pretreated with MDMA alone (MDMA + no stress). Further studies are needed to determine if stress actually ameliorates MDMA-induced deficits in stimulated 5-HT release. However, when the lower basal extracellular 5-HT concentrations of the neurotoxic MDMA + stress group are considered, the relative increase from basal 5-HT concentrations is the greatest of all the conditions examined. Thus, the combination of neurotoxic MDMA followed by stress produced a relative greater increase in MDMA-induced 5-HT than either MDMA or stress alone. Neurotoxic doses of MDMA followed by chronic stress also appeared to delay the return of VTA 5-HT levels to baseline, suggesting that MDMA followed by stress enhances both the relative 5-HT response to an MDMA challenge as well as the duration of the response. Moreover, the large relative change from baseline VTA 5-HT observed in the MDMA + stress group corresponds to a greatly enhanced dopamine release in the NAcc(sh) (Figure 4) and suggests that mesolimbic dopamine release may be more responsive to relative rather than absolute changes in 5-HT.

Another factor which may account for the enhanced relative release of 5-HT after the combination of MDMA and stress may be a decreased 5-HT_{1A} autoreceptor-mediated inhibition of raphe neuron firing rate (Sprouse and Aghajanian, 1987; Sotelo *et al*, 1990). Neurotoxic doses of MDMA (Aguirre *et al*, 1995) or chronic stress (Laaris *et al*, 1997; Lanfumey *et al*, 1999) decrease 5-HT_{1A} receptor activity. Under normal conditions, MDMA stimulated 5-HT release would act at 5-HT_{1A} autoreceptors to inhibit cell firing rates. However, pretreatment with neurotoxic doses of MDMA and exposure to chronic stress may reduce or eliminate 5-HT_{1A} receptor-mediated autoinhibition of 5-HT neurons. The resultant continued firing of 5-HT neurons would enhance the calcium-dependent component of MDMA-induced 5-HT release (Azmitia *et al*, 1990; Crespi *et al*, 1997), and together with MDMA-induced reductions in SERT (Battaglia *et al*, 1987), could be sufficient to reverse the deficits in MDMA-induced 5-HT release observed after neurotoxic MDMA or stress alone.

MDMA + stress pretreatment produced a dramatic increase in MDMA-induced dopamine release in the NAcc(sh). The lack of effect observed with either treatment alone clearly demonstrates a synergism between the combined effects of MDMA and stress pretreatments to enhance MDMA-induced dopamine (Figure 4). It is interesting to note that this effect was blocked by the perfusion of the 5-HT_{1B} receptor antagonist GR 127935 (de Vries *et al*, 1997) into the VTA (Figure 5). This finding is consistent with the conclusion that 5-HT_{1B} receptor activation is required for the MDMA + stress enhancement of accumbal dopamine release and is supported by studies showing that 5-HT_{1B} activation in the VTA can disinhibit dopamine neurons (Yan *et al*, 2005; Yan and Yan, 2001a) via a reduction of local GABA release (Yan and Yan, 2001b; Yan *et al*, 2004).

5-HT_{2C} receptors are thought to oppose 5-HT_{1B} receptors and inhibit mesolimbic DA (De Deurwaerdere *et al*, 2004; Di Giovanni *et al*, 2000; Di Matteo *et al*, 1999). We have shown that blockade of 5-HT_{2C} receptors in the VTA inhibits local GABA release while simultaneously enhancing MDMA-induced dopamine release in NAcc(sh) (Bankson and Yamamoto, 2004). Therefore, the present data demonstrating enhanced 5-HT_{1B}-dependent mesolimbic dopamine efflux after a challenge dose of MDMA are consistent with a relative increase of 5-HT_{1B} over 5-HT_{2C} receptor function. It remains to be seen if the enhanced 5-HT_{1B} response to an MDMA challenge is the result of 5-HT_{1B} receptor sensitization due to the lower basal extracellular concentrations of 5-HT noted in this group. Regardless, increased 5-HT_{1B} or decreased 5-HT_{2C} activation in the VTA should result in an enhanced accumbal dopamine response via decreased GABA release in the VTA (Bankson and Yamamoto, 2004; Yan and Yan, 2001b). The role of 5-HT_{2C} or other receptors is unknown, but prevention of the enhanced MDMA-induced dopamine release by GR 127935 demonstrates that 5-HT_{1B} receptor activation is necessary for the synergistic increase in accumbal dopamine release produced by the pretreatment of MDMA and chronic stress.

A common feature of MDMA (Cadoni *et al*, 2005; Bankson and Yamamoto, 2004) and other drugs of abuse is their ability to elevate extracellular dopamine in the shell region of the nACC. This elevation of NAcc(sh) dopamine is widely accepted as the basis for drug-induced reward (Wise and Bozarth, 1984; Wise, 1984; Hoebel, 1985; Koob and Weiss, 1992; Carlezon and Wise, 1996; Everitt *et al*, 1999; Di Chiara and Imperato, 1988). Conversely, disruption of dopamine transmission attenuates the rewarding effects of MDMA in rats trained to self-administer MDMA (Daniela *et al*, 2004). Therefore, the enhanced MDMA-induced dopamine efflux observed after previous exposure to MDMA + chronic stress could reflect an increase in the rewarding and addictive properties of MDMA. The lower basal extracellular dopamine observed after MDMA and stress may also facilitate and sustain drug-seeking behavior. Reduced basal extracellular dopamine concentrations in the NAcc triggers drug-seeking behavior in animals trained to self-administer stimulants and is thought to represent drug craving (Gratton and Wise, 1994; Wise, 1994; Wise *et al*, 1995; Gerrits *et al*, 2002). Thus, the combination of neurotoxic MDMA followed by chronic stress may exacerbate craving and drug-seeking behavior as well as enhance the rewarding and addictive properties of MDMA or other addictive drugs.

In conclusion, prior exposure to a neurotoxic regimen of MDMA followed by CUS may enhance the rewarding and addictive properties of MDMA or other drugs through changes in mesolimbic monoamine transmission, which are quantitatively and qualitatively different from the effects of either treatment alone. The enhanced dopamine release observed in the NAcc(sh) after pre-exposure to MDMA and chronic stress is dependent upon 5-HT_{1B} activation in the VTA and implicates this receptor as a new critical mediator of chronic stress-induced changes in the neurophysiological and behavioral effects of MDMA.

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