AD_____

Award Number: DAMD17-99-1-9479

TITLE: The Nigrostriatal Dopamine System and Methamphetamine: Roles for Excitoxicity and Environmental, Metabolic and Oxidative Stress

PRINCIPAL INVESTIGATOR: Bryan K. Yamamoto, Ph.D.

CONTRACTING ORGANIZATION: Case Western Reserve University
Cleveland, Ohio 44106

REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
The Nigrostriatal Dopamine System and Methamphetamine: Roles for Excitoxicity and Environmental, Metabolic and Oxidative Stress

5. FUNDING NUMBERS
DAMD17-99-1-9479

6. AUTHOR(S)
Bryan K. Yamamoto, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University
Cleveland, Ohio 44106

E-Mail: bkyam@bu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

Degeneration of the nigrostriatal dopamine system is linked to the pathophysiology of Parkinson's disease. Similarly, the psychostimulant drug, methamphetamine also produces relatively selective damage to nigrostriatal dopamine neurons and is a widespread problem and drug of abuse throughout the U.S. However, the neurochemical underpinnings that mediate methamphetamine toxicity and Parkinson's disease are unknown.

Several variables common to methamphetamine toxicity and Parkinson's disease, each of which may be important but alone are insufficient, may account for the neurodegeneration of the nigrostriatal dopamine path. It is hypothesized that the convergence of excitotoxicity, free radicals and a depleted bioenergetic state produces damage to dopamine neurons. Moreover, environmental stressors, which also increase free radicals and excitatory amino acids predispose dopamine neurons to damage. Consequently, environmental stress may be synergistic with oxidative and metabolic insults as well as glutamate to culminate in dopamine cell death. ***The major objective is to examine the interaction between environmental stress and methamphetamine and the convergent action of excitotoxicity and bioenergetic and oxidative stress to produce damage to nigrostriatal dopamine neurons.*** A multidisciplinary approach will be used as well as pharmacological strategies that we posit to be neuroprotective against methamphetamine, excitotoxicity, and bioenergetic and oxidative stress will be examined.

14. SUBJECT TERMS	15. NUMBER OF PAGES
neurotoxin	58
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

Degeneration of the nigrostriatal dopamine system is linked to the pathophysiology of Parkinson's disease. Similarly, the psychostimulant drug, methamphetamine, also produces relatively selective damage to nigrostriatal dopamine neurons and is rapidly becoming a widespread problem and drug of abuse throughout the U.S. However, the neurochemical underpinnings that mediate methamphetamine toxicity and Parkinson's disease have escaped definition.

We propose that several variables common to methamphetamine toxicity and Parkinson's disease, each of which may be important but alone are insufficient, account for the neurodegeneration of the nigrostriatal dopamine path. It is hypothesized that the convergence of excitotoxicity, free radicals and a depleted bioenergetic state produces damage to dopamine neurons. Moreover, environmental stressors, which also increase free radicals, excitatory amino acids, and alter energy metabolism, predispose dopamine neurons to damage. Consequently, environmental stress may be synergistic with oxidative and metabolic insults as well as glutamate to culminate in dopamine cell death. *The major objective is to examine in rats the interaction between environmental stress and methamphetamine and the convergent action of excitotoxicity, bioenergetic stress, and oxidative stress to produce damage to nigrostriatal dopamine neurons.* A multidisciplinary approach of *in vivo* and *in vitro* biochemical and histochemical methods will be used. In addition, pharmacological strategies that we posit to be neuroprotective against methamphetamine, excitotoxicity, and bioenergetic and oxidative stress will be examined.

ANNUAL PROGRESS REPORT

In September, 2001, I moved to the Department of Pharmacology at the Boston University School of Medicine. Despite my continued requests over the past year to have the funds transferred as quickly as possible and due to a variety of administrative issues, funds for the proposed research were temporarily unavailable from July 2001 until recently. On June 15, 2002, these funds were officially transferred to Boston University from Case Western Reserve University. Consequently, progress towards the objectives described in the Statement of Work is very limited. However, despite the lack of funding during the last 11 months, we still were able to make modest progress toward the stated objectives.

OBJECTIVE 1:

To develop a chronic unpredictable stress model to study the interaction between stress and methamphetamine to dopamine neurons.

Results:

The current study tested the hypotheses that 10 days of unpredictable stress 1) augments the acute increase in extracellular striatal dopamine concentrations in response to injections of 7.5 or 10 mg/kg METH and 2) produces greater depletions of striatal dopamine 7 days following the injection regimen. Male rats exposed to unpredictable stress had an increased mortality rate (33%) compared to non-stressed controls (16.7%) following 4 injections of 10 mg/kg METH. Stressed rats also had increased hyperthermic responses and dopamine efflux in the striatum during the METH injections when compared to non-stressed control rats. Furthermore, one week following either a 7.5 mg/kg or 10 mg/kg METH injection regimen, stressed rats showed greater depletions in striatal dopamine tissue content than non-stressed controls injected with METH. The enhanced acute and longer-term effects of METH in stressed rats was not due to a greater concentrations of METH in the striatum, as extracellular levels of METH during the injection regimen did not differ between the 2 groups.

A copy of the preprint that has been submitted to the Journal of Neuroscience is attached.

Discussion
Overall, several mechanisms may contribute to the potentiated decreases in dopamine tissue content and the acute increases in hyperthermia or extracellular dopamine in the striatum. The precise effects of repeated, unpredictable stress on the brain are unknown but alterations in 5-HT receptors, the dopaminergic system, or excitatory amino acid transmission may account for the enhanced hyperthermia, mortality, extracellular dopamine concentrations, or depletions of dopamine content in the striatum. Due to the broad overlap of stress and drug use, the increased vulnerability of the brain by exposure to unpredictable stressors may be important for understanding the potential detrimental effects of drugs of abuse as well as the etiology of Parkinson's disease.

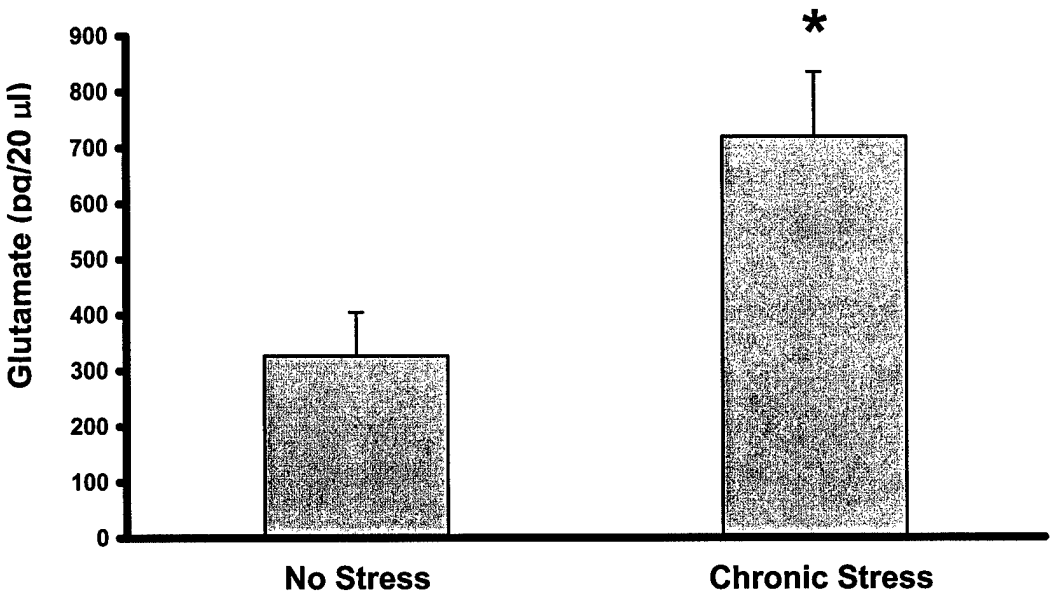
Objective 2:
To examine the effects of chronic restraint stress on basal concentrations of glutamate in the hippocampus.

Evidence is accumulating that stress is associated with the onset of depression, a dysregulation of the hypothalamic-pituitary-adrenal axis, and possible neurodegeneration. With regard to the latter, McEwen and colleagues have described a model of stress-induced morphological reorganization in the hippocampus that appears to be mediated by excitatory amino acids and adrenal steroids (McEwen, 1997). Unpublished observations indicate that chronic stress increases mRNA expression of the glial glutamate transporter (GLT-1) in the CA3 region of the hippocampus (Reagan and McEwen) and further support the role of excitatory amino acids in mediating the neurodegeneration observed in this area following chronic stress. Consistent with these findings of stress-induced increases in excitatory amino acid transmission in the hippocampus, we have shown that acute restraint stress increases hippocampal glutamate release

measured in vivo; an effect that is reversed by adrenalectomy (Lowy et al., 1993).

Results:

Figure 1 shows that chronic restraint stress for 21 days using a commercially available plastic rat restrainer increases the basal concentrations of glutamate in the hippocampus (*$p<0.05$).



Discussion:

These findings illustrate that chronic stress can increase the extracellular concentrations of glutamate and perhaps account for the hippocampal remodeling and apical dendritic atrophy that has been observed by others (McEwen, 1997). Future studies will focus on the mechanisms mediating this increase in extracellular glutamate and will focus on glutamate release processes as well as glutamate uptake systems.

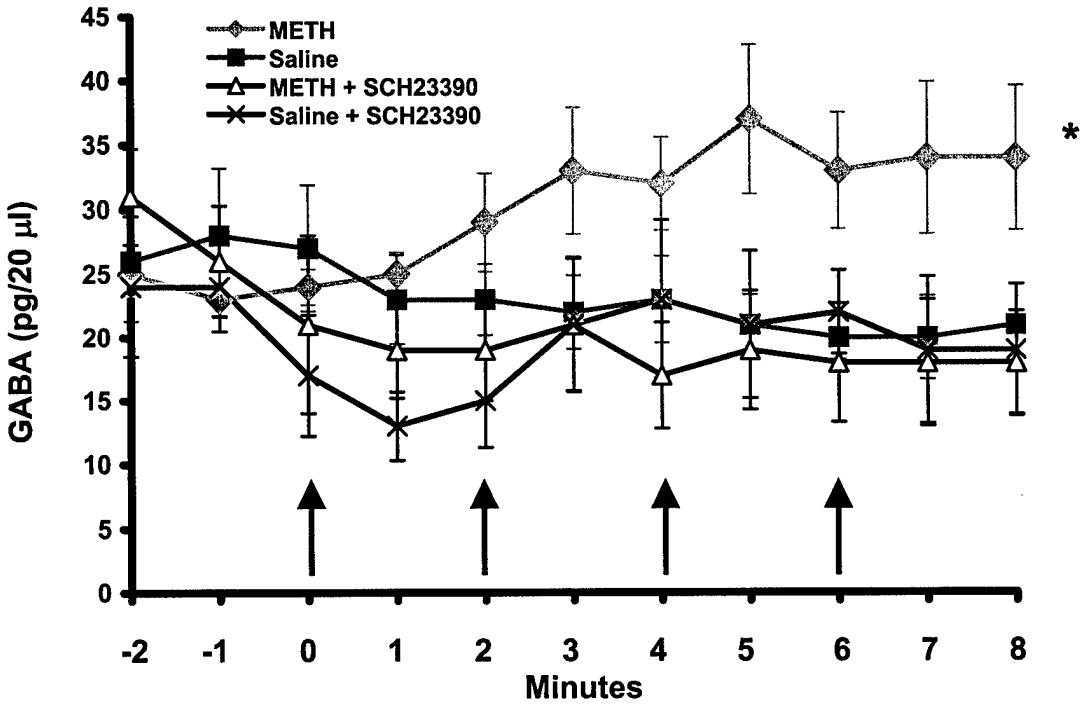
Objective 3:

To examine the effect of methamphetamine on GABA release in the substantia nigra (SN) and the local regulation of GABA by DA as an index of the outflow activity of the basal ganglia.

High extracellular concentrations of DA and the excitatory amino acid glutamate (GLU) have been implicated in mediating METH toxicity (Nash and Yamamoto, 1992). Systemic administration of METH increases both DA and GLU release. However, while local perfusions of METH directly into the striatum do produce an increase in DA release they do not produce an increase in GLU and do not produce long term depletions of striatal DA tissue content (Burrows et al., 2000). This suggests that increases in both DA and GLU are necessary to produce neurotoxicity.

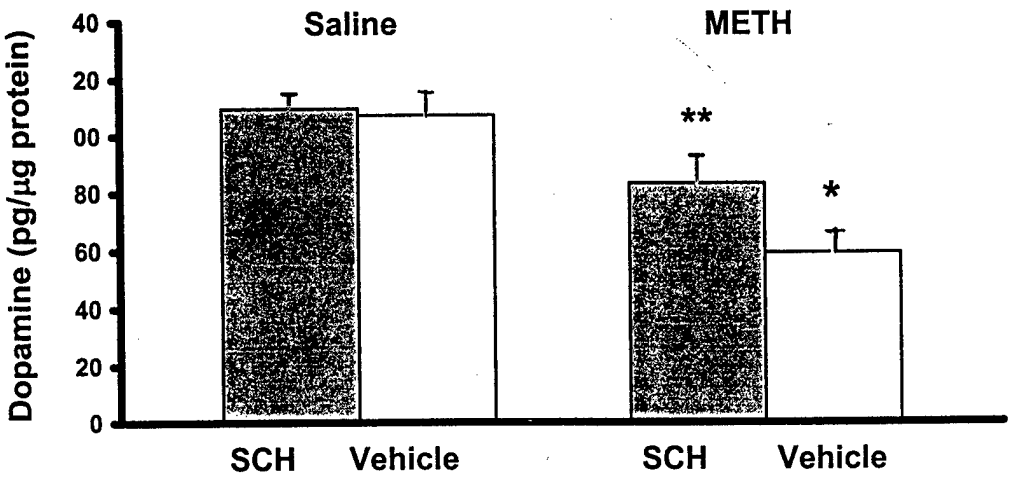
Although GLU appears to be significant in mediating METH toxicity, it is still unclear how METH increases GLU. Our hypothesis is that METH will increase extracellular GLU via the striatal outflow pathways, specifically the striatonigral efferents. We predicted that stimulation of the D1 receptors in the SN will increase GABA release in the SN.

Results:

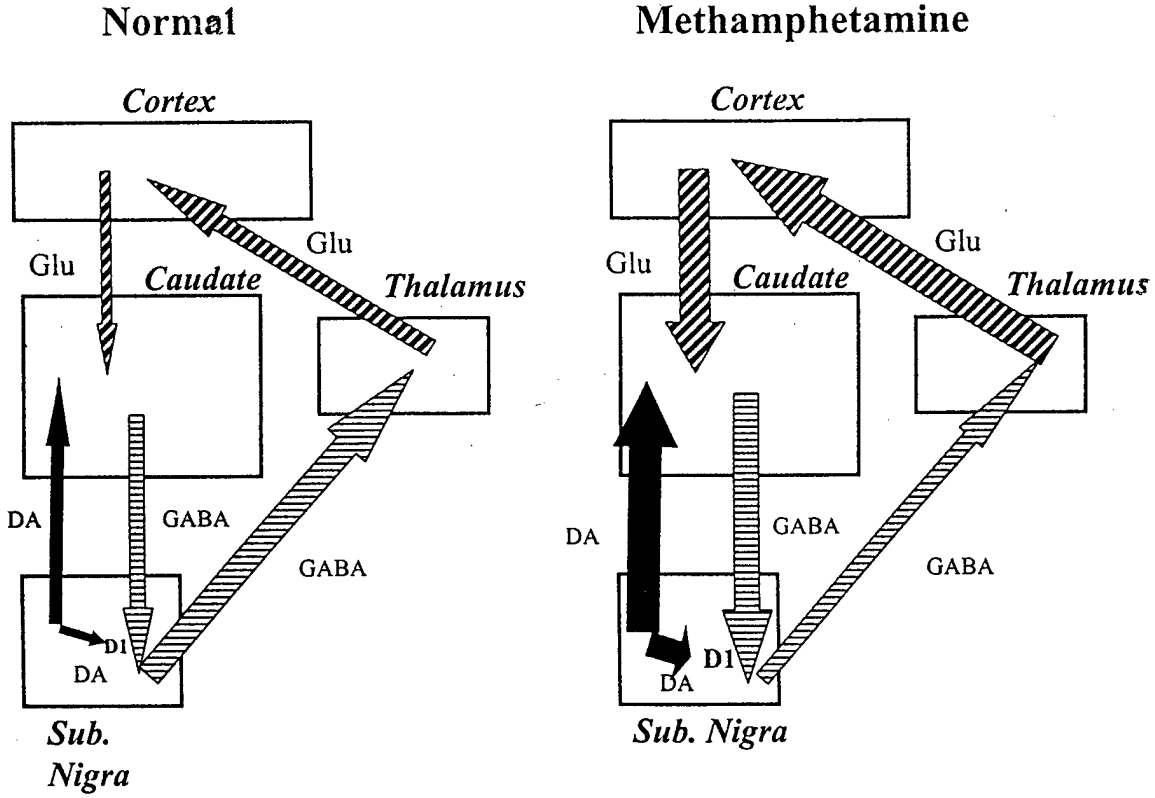


Systemic administration of METH alone produced an increase of extracellular GABA release in the substantia nigra (*p<0.05). The local perfusion of D1 antagonist, SCH23390 (SCH), alone into the SN decreased the basal concentrations of GABA and attenuated the METH induced increase of extracellular GABA.

To examine whether the inhibition of GABA release in the substantia nigra pars reticulata can eventually decrease the long-term depletion of dopamine produced by methamphetamine, dopamine content in the striatum was measured 7 days after the dialysis experiment in METH or saline pretreated rats. SCH (METH-SCH group) significantly attenuated (**P<0.05 from METH-Vehicle group) the METH-induced dopamine depletions (*p<0.05; METH pretreated-vehicle infused group) in the striatum.



Discussion:
Basal and stimulated GABA release in the SN is modulated by D1 receptors. Furthermore, neurotoxicity to DA terminals in the STR after METH is partially mediated by activation of D1 receptors and GABA release in the SN.
It can be postulated from the circuitry model illustrated below that an increase of extracellular GABA in the SN will result in the activation of the thalamocortical projections and a subsequent increase in extracellular GLU release in the striatum.



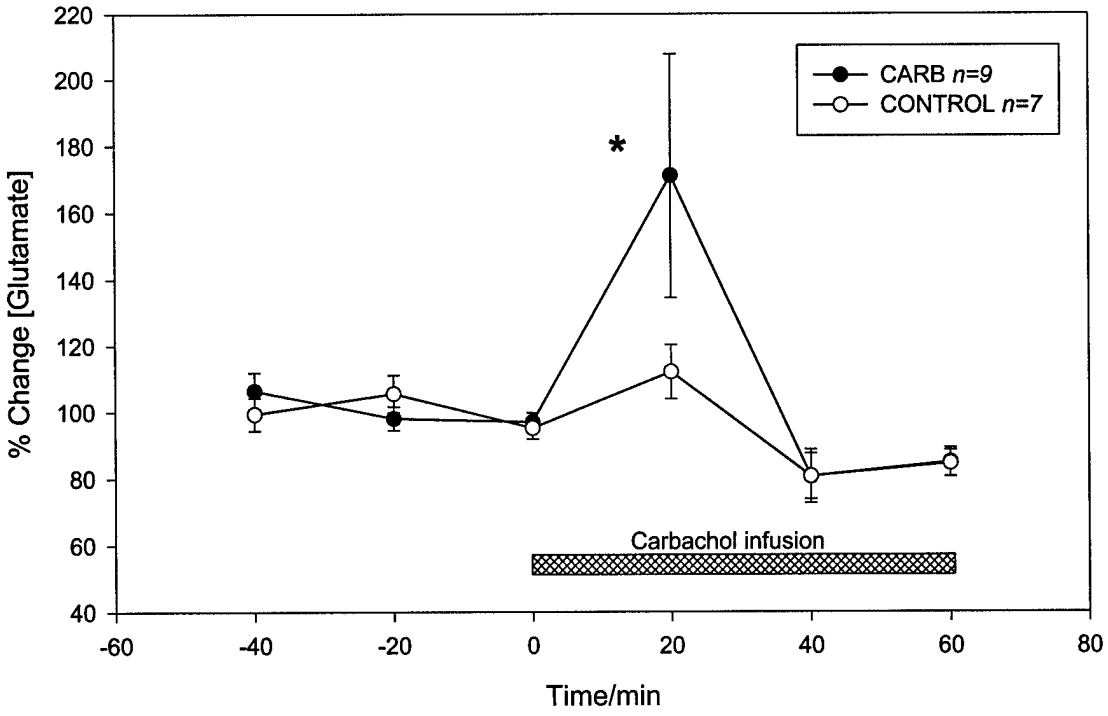
It remains to be determined if disinhibition of the thalamocortical path via decreased inhibitory input to the thalamus from the SN results in an increase in striatal glutamate release.

Objective 4:
To characterize the input from the subthalamic nucleus to the substantia nigra.

Results:

Using dual probe microdialysis, we examined the effect of the reverse dialysis of carbachol into the subthalamic nucleus while simultaneously measuring glutamate release in the substantia nigra. As illustrated below, carbachol infusion significantly increased (*$p<0.05$) glutamate release in the substantia nigra.

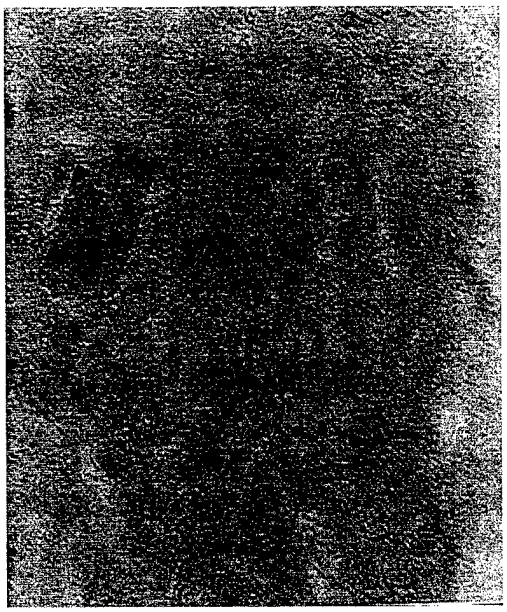
Effect of carbachol (1mM) administration in the subthalamic nucleus on extracellular glutamate concentration in the substantia nigra



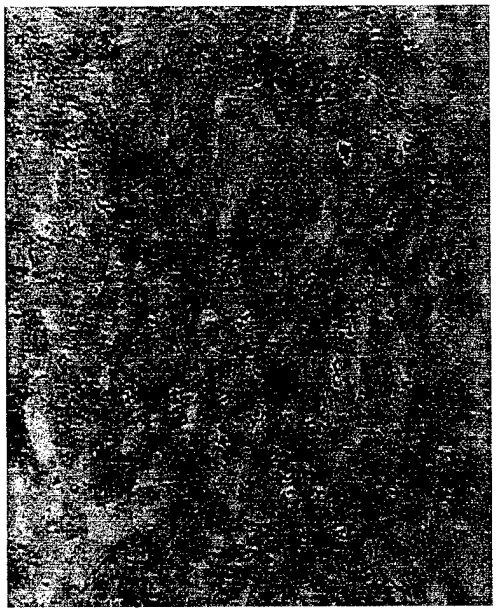
Discussion
These data indicate that stimulation of muscarinic receptors in the subthalamic nucleus results in an increase in subthalamic activity to increase glutamate release in the substantia nigra. Future studies will investigate whether carbachol infusions are additive/synergistic with methamphetamine to augment glutamate release in subtstantia nigra and produce subsequent excitotoxicity to dopamine neurons. Moreover, we plan to examine the regulation of the subthalamonigral pathway by metabotropic and dopaminergic receptors in the substantia nigra.

Objective 5:
To examine the effect of methamphetamine on oxidative damage in the striatum as measured by protein nitration.
As described in the Progress Report last year, methamphetamine increased the formation of nitrotyrosine in the striatum, as measured by HPLC analysis of acid hydrolyzed protein. To verify this finding with another method, we employed the immunohistochemical detection of an anti-nitrotyrosine antibody 24 hrs after the administration of methamphetamine.

Results:
As can be observed in the figure below, there was increased immunohistochemical staining observed in the striatum of methamphetamine treated rats.



Saline



Methamphetamine

Discussion:
The results indicate that methamphetamine produces evidence of oxidative stress as indicated by the nitration of protein in the form of nitrotyrosine.

KEY RESEARCH ACCOMPLISHMENTS

- Chronic unpredictable stress enhances the neurotoxicity of methamphetamine to dopamine terminals
- Chronic restraint stress elevates basal extracellular glutamate concentrations in the hippocampus
- Methamphetamine increases GABA release in the substantia nigra via the D1 receptor. The increase in GABA release in the substantia nigra plays a critical role in the long-term loss of dopaminergic innervation to the striatum following methamphetamine.
- Methamphetamine produces protein oxidation in the striatum.
- An experimental design and methodology was established to study *the in vivo* regulation of the glutamatergic innervation of the substantia nigra from the subthalamic nucleus

REPORTABLE OUTCOMES

Published paper

Matuszewich, L., Filon, M.E., Finn, D.A., and Yamamoto, B.K. Altered forebrain neurotransmitter responses to immobilization stress following 5-HT depletions with MDMA. Neuroscience 110: 41-48, 2002

Submitted Paper

Matuszewich, L. and Yamamoto, B.K. Chronic Stress Augments the Acute and Long-term effects of methamphetamine. Submitted to J. Neuroscience.

Abstracts

Matuszewich, L. and Yamamoto, BK, Chronic unpredictable stress produces persistent DOI-induced hyperthermia in rats. Society for Neuroscience, 2001

Gallaugher, L, Stamm, M and Yamamoto, BK Dopamine-GABA interactions in the substantia nigra: Effects of methamphetamine. Society for Neuroscience, 2001

CONCLUSIONS

The results illustrated in this progress report support the conclusion that chronic environmental stress can potentiate the neurotoxic effects of methamphetamine on dopamine terminals. The mechanisms underlying the enhanced vulnerability produced by stress on methamphetamine-induced damage to dopamine neurons may include excitotoxicity mediated by an enhanced release of glutamate in the striatum or substantia nigra. Our studies described in this progress report have begun to characterize how the release of dopamine within the substantia nigra can alter the outflow of the basal ganglia through the activation of D1 receptors that in turn, could lead to an increase in corticostriatal glutamate release via a multisynaptic process to culminate in excitotoxic damage to dopamine terminals. Moreover, we have established a methodology to stimulate glutamate efferents from the subthalamic nucleus that innervate the substantia nigra while simultaneously measuring glutamate release. As a result, we are now positioned to determine (1) if methamphetamine also increases glutamate release from subthalamic efferents in the substantia nigra and (2) if chronic stress exacerbates this increase and enhances the vulnerability of dopamine soma in the substantia nigra to excitotoxicity and oxidative damage and (3) the regulatory mechanisms of the glutamatergic efferents of the subthalamic nucleus to the substantia nigra. Overall, these studies continue to address the hypothesis that the synergistic interaction between chronic stress and methamphetamine damages dopamine neurons at both the terminal (striatum) and cell body (substantia nigra).

REFERENCES

Burrows, K., Nixdorf, W., and Yamamoto, B.K. Central administration of methamphetamine synergizes with metabolic inhibition to deplete striatal monoamines. Journal of Pharm. and Exptl. Therapeutics, 292: 853-860, 2000

McEwen, B.S. Possible mechanisms for atrophy of the human hippocampus. Mol Psychiatry 3: 255-62, 1997.

Nash J.F. and Yamamoto, B.K. Methamphetamine neurotoxicity and striatal glutamate release: Comparison to 3,4-methylenedioxymethamphetamine. Brain Research 581: 237-243, 1992.

LIST OF APPENDICES

Matuszewich, L., Filon, M.E., Finn, D.A., and Yamamoto, B.K. Altered forebrain neurotransmitter responses to immobilization stress following 5-HT depletions with MDMA. Neuroscience 110: 41-48, 2002.

Matuszewich, L. and Yamamoto, B.K. Chronic Stress Augments the Acute and Long-term effects of methamphetamine. Submitted to J. Neuroscience.

Pergamon

PII: S0306-4522(01)00539-5

www.neuroscience-ibro.com

Neuroscience Vol. 110, No. 1, pp. 41–48, 2002

0306-4522/02 $22.00+0.00

ALTERED FOREBRAIN NEUROTRANSMITTER RESPONSES TO IMMOBILIZATION STRESS FOLLOWING 3,4-METHYLENEDIOXYMETHAMPHETAMINE

L. MATUSZEWICH,[a]* M. E. FILON,[b] D. A. FINN[c] and B. K. YAMAMOTO[a,1]

[a]Program in Basic and Clinical Neuroscience, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106, USA

[b]Department of Anatomy, Case Western Reserve University, Cleveland, OH 44106, USA

[c]Veteran Affairs Medical Center and Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97201, USA

Abstract—(±)3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an increasingly popular drug of abuse that acts as a neurotoxin to forebrain serotonin neurons. The neurochemical effects of the serotonin depletion following high doses of MDMA were investigated in response to acute immobilization stress. Male rats were treated with a neurotoxic dosing regimen of MDMA (10 mg/kg, i.p. every 2 h for four injections) or equivalent doses of saline. Seven days after treatment, *in vivo* microdialysis was used to assess extracellular dopamine and serotonin in the dorsal hippocampus and prefrontal cortex during 1 h of immobilization stress. In saline treated control rats, serotonin in the hippocampus and serotonin and dopamine in the prefrontal cortex were increased during immobilization stress. Rats pretreated with MDMA, however, showed blunted neurotransmitter responses in the hippocampus and the prefrontal cortex. In the drug pretreated rats, basal serotonin levels in the hippocampus, but not the prefrontal cortex, were lower compared to saline pretreated controls. Stress-induced increases in plasma corticosterone and body temperature were not affected by the pretreatment condition.

From these studies we suggest that depletion of serotonin stores in terminal regions with the neurotoxin MDMA compromises the ability of the serotonergic neurons to activate central systems that respond to stressful stimuli. This altered responsiveness may have implications for long-term functional consequences of MDMA abuse as well as the interactions between the serotonergic system and stress.

Key words: MDMA, immobilization stress, prefrontal cortex, hippocampus, serotonin, dopamine.

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is a commonly abused 'club drug' with long-term neurochemical effects on the CNS. Administration of high doses of MDMA results in depletions of serotonin (5-hydroxytryptamine or 5-HT) in the frontal cortex, hippocampus, and striatum of rodents and non-human primates (Callahan et al., 2001; Gibb et al., 1990; Schmidt, 1987; Stone et al., 1986). Neuronal damage to the serotonergic system is evidenced by decreased tryptophan hydroxylase activity, decreased number of 5-HT reuptake sites and immunocytochemical damage to 5-HT axons following high doses of MDMA (Battaglia et al., 1987; Commins et al., 1987; O'Hearn et al., 1988; Schmidt and Taylor, 1987; Stone et al., 1987). The degeneration of 5-HT axons has been reported to be limited to fine-diameter axons from the dorsal raphe nucleus that project to forebrain regions, such as the hippocampus and frontal cortex (Mamounas et al., 1991; O'Hearn et al., 1988; Wilson et al., 1989).

Although the neurochemical alterations following neurotoxic treatment with MDMA have been well characterized, the functional consequences of MDMA at neurotoxic doses are relatively unknown. Several studies have reported that 7 days after treatment with a neurotoxic regimen of MDMA, extracellular 5-HT responses to an acute injection of MDMA or D-fenfluramine are suppressed, as are behavioral and hyperthermic responses (Series et al., 1994; Shankaran and Gudelsky, 1999). Prior exposure to a neurotoxic regimen of MDMA also decreased the sensitivity of rhesus monkeys to the subsequent effects of an acute injection of dexfenfluramine on a behavioral task, or of rats to MDMA in a

[1] Present address: Boston University Medical School, Department of Pharmacology, Boston, MA 02118, USA.

*Correspondence to: L. Matuszewich. Present address: Department of Psychology, Northern Illinois University, DeKalb, IL 60115, USA. Tel.: +1-815-753-7072; fax: +1-815-753-8088.

E-mail address: imatusze@niu.edu (L. Matuszewich).

Abbreviations: 5-HT, 5-hydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; ANOVA, analysis of variance; EDTA, ethylenediaminetetra-acetate; HPLC-EC, high-performance liquid chromatography with electrochemical detection; MDMA, (±)3,4-methylenedioxymethamphetamine; VTA, ventral tegmental area.

drug discrimination task (Frederick et al., 1995; Schechter, 1991). However, in a behavioral paradigm without the use of a drug challenge, MDMA pretreatment in rats had no effect on a place navigational learning-set task, or maze performance (Ricaurte et al., 1993; Robinson et al., 1993; Seiden et al., 1993; Slikker et al., 1989). Thus, the effects of MDMA on the functioning of the CNS may only be apparent during an activated or stimulated state, such as following a drug injection or an environmental stressor.

The integrity of the central serotonergic system appears to be important for some types of neurochemical responses to the presentation of an acute stressor. In rats, lesions of hypothalamic 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) attenuate the increase in plasma corticosterone concentrations following photic stimuli (Feldman, 1985; Feldman et al., 1984). In contrast, dopamine metabolism in prefrontal cortex and nucleus accumbens following 30 min of restraint stress was enhanced in rats that received injections of 5,7-DHT into the dorsal raphe (Morrow and Roth, 1996). Therefore, a sufficient serotonergic tone may be important in maintaining the appropriate neurochemical responses to an acute stressor.

Exposure to an environmental stressor increases 5-HT and dopamine levels in several brain regions, including areas innervated by 5-HT and depleted by MDMA (for review see Chaouloff, 1993). The application of acute stressors has been correlated with increased 5-HT release in the prefrontal cortex, hippocampus, striatum, hypothalamus, amygdala and periaqueductal gray of the rat as measured by *in vivo* microdialysis or voltammetry (Adell et al., 1997; Amat et al., 1998; Boutelle et al., 1990; Joseph and Kennett, 1983, 1986; Kawahara et al., 1993; Kirby et al., 1997; Shimizu et al., 1992; Takahashi et al., 1998; Yoshioka et al., 1995). In a similar manner, extracellular dopamine and dopamine turnover increase in the prefrontal cortex, striatum and nucleus accumbens in response to tail shock/pressure, restraint, foot shock or handling stress (Abercrombie et al., 1989; Cenci et al., 1992; Finlay et al., 1995; Finlay and Zigmond, 1997; Gresch et al., 1994; Imperato et al., 1991; Kawahara et al., 1999; Keefe et al., 1993; Nakahara and Nakamura, 1999; Sorg and Kalivas, 1993; Taber and Fibiger, 1997; Thierry et al., 1976). The hippocampus and prefrontal cortex may play important roles in the interaction between neurotransmitter and neuroendocrine responses to stress due to the large number of corticosterone receptor sites in each, their projections to hypothalamic structures, and their dense monoamine innervation (Chaouloff, 1993; Diorio et al., 1993; Herman and Cullinan, 1997; McEwen et al., 1986).

It remains to be determined whether 5-HT terminal damage produced by exposure to high doses of MDMA alters the stress-induced release of monoamines in brain regions targeted by MDMA. The objective of the present study was to measure extracellular concentrations of 5-HT in the hippocampus, as well as 5-HT and dopamine in the prefrontal cortex, during immobilization stress of rats that were administered MDMA 7 days earlier.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (175–250 g) were purchased from Zivic Miller Labs (Allison Park, PA, USA). Rats were housed individually, with food and water available *ad libitum*, on a 12-h light–dark cycle in a temperature controlled room. All procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the local institutional animal care committee.

Drugs

All rats were given i.p. injections with 10 mg/kg MDMA hydrochloride salt (National Institutes of Drug Abuse, Bethesda MD, USA) or an equivalent volume of saline (0.9% NaCl), every 2 h for a total of four injections. Drug injections were given in a volume of 1 ml/kg. The above injection procedure has been shown previously to decrease 5-HT neurotransmitter content in the striatum (Shankaran and Gudelsky, 1999).

Surgical procedures

Three days after the i.p. injections, all rats were anesthetized with a combination of xylazine (6 mg/kg) and ketamine (70 mg/kg) and placed into a Kopf stereotaxic frame. The skull was exposed and two 21-gauge stainless steel guide cannulae (Small Parts, Miami Lakes, FL, USA) were positioned above the cortex (prefrontal cortex: 3.2 mm anterior and 0.5 mm medial to bregma) and dorsal hippocampus (3.2 mm posterior and 2.0 mm medial to bregma). The cannulae and a metal female connector were secured to the skull with three stainless steel screws and cranioplastic cement. Obturators fashioned from 31-gauge stainless steel wire, ending flush with the guide cannulae, were inserted into the cannulae after surgery.

Experiment 1: Measurement of monoamines with in vivo microdialysis during restraint stress

Three days after surgery, the obturators were removed from the guide cannulae and replaced with microdialysis probes. The probes were constructed as previously described (Lowy et al., 1993) from a 27-gauge thin wall stainless steel tube, fitted with a dialysis membrane (13 000 Da cut off, 210 μm outer diameter; Spectrum Laboratories, Rancho Domingues, CA, USA) at one end, and a 3 cm piece of polyethylene 20 tubing (Fisher Scientific, Pittsburg, PA, USA) at the other end, to serve as the inlet for the perfusion medium. The dialysis membrane was 4 mm or 2 mm × 210 μm diameter for the prefrontal cortex or dorsal hippocampus, respectively. A 4 cm length of capillary tubing (125 μm outer diameter, 50 μm inner diameter; Polymicro Technologies, Phoenix, AZ, USA) served as the outlet from the dialysis membrane. The vertical placement of the microdialysis probe was determined during construction of the probe by gluing a ring of polyethylene 20 tubing, which acts as a mechanical 'stop', at a measured distance along the length of the probe. The positioning permitted the exposed portion of the dialysis membrane to extend beyond the guide cannulae and into the prefrontal cortex (ventral from dura −1.0 to −5.0) or dorsal hippocampus (ventral from dura −2.0 to −4.0). The rats were placed in microdialysis cages and attached to a swivel (Instech Laboratories, Plymouth Meeting, PA, USA), with food and water available *ad libitum*.

Eighteen hours after probe insertion, Dulbecco's phosphate-buffered saline medium (138 mM NaCl, 2.1 mM KCl, 0.5 mM

$MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM $NaHPO_4$, 1.2 mM $CaCl_2$, and 5 mM D-glucose, pH 7.4) was pumped through the microdialysis probes with a Harvard Model 22 syringe infusion pump (Hollison, MA, USA). The Dulbecco's medium was perfused at a rate of 1.5 µl/min. A 3-h perfusion period was allowed prior to sample collection. Twenty-minute samples were then collected during the following conditions: three baseline samples, three samples during immobilization stress, and three samples following the immobilization stress. The immobilization stress procedure was initiated between 11:30 h and 13:00 h. For the immobilization stress, rats were placed with their ventral surface on a Plexiglas board and secured with a 2-inch Velcro strap across their midregion and a 1-inch Velcro strap behind their head. Tape was used to secure their paws. In five saline and five MDMA pretreated rats, rectal temperatures were monitored every 10 min during the 1-h immobilization stress with a Thermalert TH-8 monitor (Physitemp Instruments, Clinton, NJ, USA). After the 1-h immobilization, the tape and Velcro strips were removed and the rats returned to their microdialysis cages for the remaining three samples.

Experiment 2: Plasma corticosterone concentrations prior to and during restraint stress

Separate groups of rats given injections of either MDMA or saline were killed and trunk blood assayed for corticosterone concentrations. These rats did not have microdialysis cannulae implanted. One week after injections, saline ($n = 12$) or MDMA treated ($n = 12$) rats were killed by rapid decapitation either without immobilization stress ($n = 12$) or 10 min into the immobilization stress procedure ($n = 12$). Trunk blood was collected into a 4-ml vial with 0.1 ml heparin sodium sulfate (1000 U/ml, Pharmacia&Upjohn, Kalamazoo, MI, USA) and centrifuged for 15 min ($4000 \times g$).

Histology

After the microdialysis study or the blood collection, brains were quickly removed from the skull and frozen on dry ice. For the microdialysis experiment, probe placements were verified from frozen coronal sections and only rats with probes located in the prefrontal cortex or dorsal hippocampus were used for statistical analysis.

Neurochemical analysis of monoamines (high-performance liquid chromatography)

Microdialysis samples were assayed for dopamine and/or 5-HT by high-performance liquid chromatography with electrochemical detection (HPLC-EC). Samples from the prefrontal cortex were assayed for dopamine and 5-HT, and samples from the hippocampus were assayed for 5-HT only. Samples (20 µl) were loaded via a Rheodyne injector (Cotati, CA, USA) onto a 3-µm C18 column (100×2.0 mm, Phenomenex, Torrance, CA, USA). The mobile phase (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. Compounds were detected with an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), with a 6 mm glassy carbon working electrode maintained at a potential of +0.7 V relative to an Ag/AgCl reference electrode. Data were collected using a Hewlett Packard Integrator.

Analysis of 5-HT and dopamine tissue concentrations of the frontal cortex and 5-HT tissue concentrations of the dorsal hippocampus were performed on an HPLC-EC, as described above for the monoamines. The tissues were sonicated in 0.1 M perchloric acid, centrifuged at $13000 \times g$ for 6 min. The supernatant was assayed for 5-HT and dopamine. The pellet was resuspended in 1 N NaOH and protein content determined by a Bradford Protein Assay.

Radioimmunoassay of corticosterone

Corticosterone was measured in plasma (5 µl) samples that were diluted with 100 µl sterile water and stored at 4°C until assayed. The radioimmunoassay was adapted from a previously reported procedure (Keith et al., 1978) and employed [^{125}I]corticosterone from ICN Pharmaceuticals (Costa Mesa, CA, USA) and antisera from Ventrex (Portland, ME, USA). Counts per minute were normalized and fit to a least-squares regression equation produced by log–logit transformation of the standards. Mass of samples was calculated by interpolation of the standards. The detectable range of the assay was from 0.1 to 400 µg corticosterone per 100 ml plasma. Intra- and inter-assay coefficients of variation were less than 10%. The specificity of the assay is very high, with only 4% cross-reactivity to deoxycorticosterone, 1% cross-reactivity to 5β-pregnanedione, and less than 0.6% cross-reactivity to other endogenous steroids.

Statistical analysis

Thirty rats were used for the microdialysis experiment. Of the 15 rats treated with MDMA and the 15 rats treated with saline prior to surgery, six rats were excluded from data analysis due to misplaced cannulae or microdialysis difficulties. Further data were excluded from analysis if the average basal monoamine concentrations were below a 3:1 signal-to-noise limit of detection. Twenty-four rats were used to determine the concentration of corticosterone in plasma.

Two-way, repeated-measures analyses of variance (ANOVAs) were computed to compare rats pretreated with MDMA to those pretreated with saline, across all samples (baseline samples, stress samples and post-stress samples) for each neurotransmitter. Individual one-way repeated-measures ANOVAs were also computed separately when appropriate for the saline and MDMA pretreated groups to assess possible differences due to immobilization stress. Post-hoc Newman–Keuls pairwise tests were used to analyze any significant treatments. Independent *t*-tests were used to compare the pretreatment groups (MDMA vs. saline) on the following measures: 5-HT and dopamine tissue content in the frontal cortex or the dorsal hippocampus; the average of the baseline samples of each neurotransmitter; and peak rectal temperatures. A two-way ANOVA was used to compare the corticosterone plasma concentrations prior to and during immobilization stress, in saline and MDMA pretreated rats. Statistical significance was fixed at $P < 0.05$ for all tests.

RESULTS

Neurochemical content of monoamines

Serotonin tissue content of rats treated with MDMA

Table 1. Serotonin and dopamine tissue levels (µg/g protein) in the dorsal hippocampus and frontal cortex 7 days following treatment with MDMA (10 mg/kg i.p. × 4 injections, every 2 h) or an equivalent volume of saline

Brain region and neurotransmitter measured	Saline treated	MDMA treated
Hippocampus 5-HT	2.38 ± 0.15	1.08 ± 0.13*
Frontal cortex 5-HT	2.44 ± 0.30	1.27 ± 0.22*
Frontal cortex dopamine	0.82 ± 0.18	0.72 ± 0.07

All data are presented as the mean ± S.E.M. *$P < 0.05$ vs. saline injected control by an independent Student's *t*-test.

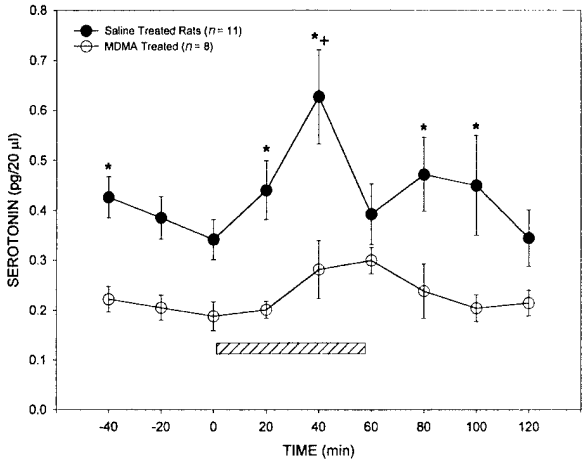


Fig. 1. Effects of 1-h immobilization stress on extracellular levels of 5-HT in the dorsal hippocampus of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Basal concentrations of 5-HT were higher in saline compared to MDMA pretreated rats ($P < 0.05$). Concentrations of 5-HT were elevated in saline pretreated rats during the second period of immobilization stress (time = 40 min) compared to the final baseline (time = 0 min) ($^{*}P < 0.05$ versus respective MDMA pretreated group; $^{+}P < 0.05$ versus time = 0 of saline treated group).

was significantly decreased by 55% in the dorsal hippocampus ($t(28) = 6.28$, $P < 0.01$) and 48% in the frontal cortex ($t(28) = 3.09$, $P < 0.01$) compared to saline treated rats (Table 1). There was no effect of MDMA pretreatment on the concentration of dopamine in the frontal cortex ($t(26) = 0.674$).

Extracellular serotonin response to stress

In the dorsal hippocampus, there was a significant difference between the treatment groups in 5-HT concentrations throughout the experiment as indicated by the main effect of treatment ($F(1,109) = 14.63$, $P < 0.001$; Fig. 1) and through post-hoc comparisons at −40, 20, 40 80 and 100 min ($P < 0.05$). This difference was highlighted by a significant increase in extracellular 5-HT after 40 min of the immobilization stress compared to baseline (time = 0 min) in saline treated rats ($F(8,109) = 2.46$, $P < 0.05$), but not of MDMA treated rats.

In the prefrontal cortex, there was a significant interaction between drug pretreatment (saline or MDMA) across time for extracellular 5-HT concentrations ($F(8,147) = 2.52$, $P < 0.05$, Fig. 2). Post-hoc comparisons indicate that there was a significant increase in 5-HT in the saline, but not the MDMA pretreated group, during the first 20 min of immobilization stress compared to baseline.

Extracellular dopamine response to stress

There was an overall increase in extracellular dopamine concentrations in the prefrontal cortex ($F(8,105) = 2.33$, $P < 0.05$). The saline pretreated group showed a significant increase in dopamine during the first 20 min of immobilization compared to baseline (one-way ANOVA, $F(8,59) = 3.06$, $P < 0.01$; Fig. 3). In contrast, extracellular dopamine concentrations in the MDMA treated group were unaffected by stress and did not change across time ($F(8,46) = 0.66$).

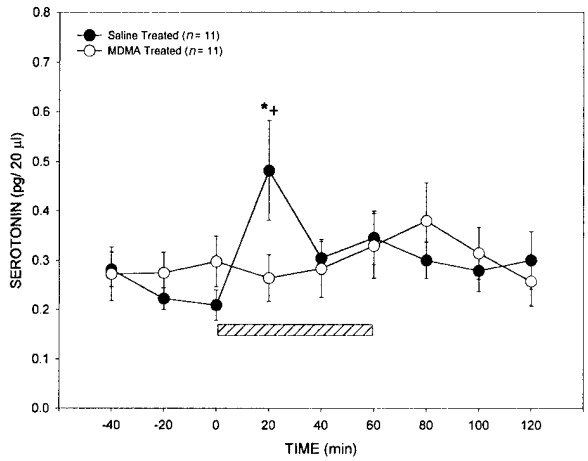


Fig. 2. Effects of 1-h immobilization stress on extracellular levels of serotonin in the prefrontal cortex of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Saline pretreated rats showed an increase in 5-HT concentrations during the first period of immobilization stress (time = 20 min). No change in 5-HT was observed in MDMA pretreated rats ($^{*}P < 0.05$ versus respective MDMA pretreated group; $^{+}P < 0.05$ versus time = 0 of saline treated group).

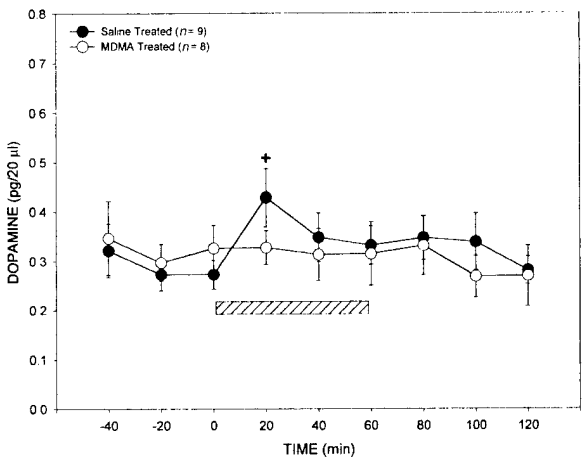


Fig. 3. Effects of 1-h immobilization stress on extracellular levels of dopamine in the prefrontal cortex of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Saline pretreated rats showed an increase in dopamine concentrations during the first period of immobilization stress (time = 20 min). No change in dopamine was observed in MDMA pretreated rats ($^{+}P < 0.05$ versus time = 0 of saline pretreated group).

Basal serotonin and dopamine levels following MDMA or saline pretreatment

Extracellular concentrations of 5-HT in the dorsal hippocampus were significantly lower in rats treated with MDMA compared to saline treated rats during baseline (0.21 ± 0.02 pg/20 µl vs. 0.38 ± 0.02 pg/20 µl; $t(47) = 4.91$, $P < 0.001$). There were no differences in basal concentrations of dopamine or 5-HT in the prefrontal cortex between MDMA and saline pretreated rats.

Corticosterone and hyperthermic responses to stress

No significant differences in plasma corticosterone concentrations were observed between the saline and MDMA treated rats, either prior to or 10 min into the immobilization stress procedure ($F(1,20) = 0.80$). Immobilization stress increased plasma corticosterone levels in both groups ($F(1,20) = 23.41$, $P < 0.001$, Table 2). There was no difference in peak rectal temperatures during the immobilization procedure between the two groups: saline treated 38.50 ± 0.16, MDMA treated 38.3 ± 0.22°C ($t(9) = 0.04$).

DISCUSSION

MDMA pretreatment attenuates stress-induced neurotransmitter release

The primary, novel finding of the current study is that MDMA pretreatment can inhibit acute 5-HT and dopamine responses to a behavioral challenge, i.e. immobilization stress. The attenuated 5-HT responsiveness is consistent with previous studies using less subtle pharmacological challenges that activate striatal and cortical 5-HT systems (Series et al., 1994; Shankaran and Gudelsky, 1999). A similar attenuation of 5-HT release following a pharmacological challenge has also been reported following other neurochemical lesions of 5-HT neurons, e.g. 5,7-DHT, *p*-chloramphetamine, or fenfluramine (Baumann et al., 1998; Kirby et al., 1995; Romero et al., 1998; Sabol et al., 1992; Series et al., 1994).

To our knowledge, this is the first report that depleting 5-HT with a serotonergic neurotoxin leads to an attenuation of extracellular dopamine concentrations in response to an environmental challenge. Other reports have suggested 5-HT depletions may augment the responsiveness of dopamine neurons to dopaminergic agents. In MDMA- or 5,7-DHT-induced 5-HT lesioned rats, a challenge injection of cocaine enhanced extracellular levels of dopamine in the nucleus accumbens, increased dopamine metabolism in the prefrontal cortex, and increased conditioned place preference (Horan et al., 1997, 2000; Morrow and Roth, 1996). Restraint stress also increased dopamine metabolism to a greater extent in rats with 5,7-DHT lesions compared to sham controls (Morrow and Roth, 1996). The assessment of dopaminergic activity with ex vivo tissue concentrations rather than *in vivo* microdialysis may explain the discrepancies between Morrow and Roth (1996) and the present findings. Of more interest is the possibility that the toxicity of MDMA to the serotonergic system produces a unique response profile with regard to its longer-term impact on dopaminergic activity.

The blunted stress-induced dopamine response observed in our study may be due to the attenuated stress-induced release of 5-HT in the prefrontal cortex. Increasing 5-HT levels through blockade of 5-HT uptake with fluoxetine or the perfusion of 5-HT itself through a microdialysis probe in the prefrontal cortex increased dopamine levels (Iyer and Bradberry, 1996; Matsumoto et al., 1999). Alternatively, the removal of the normal 5-HT mediated inhibition of GABAergic tone on dopamine cell bodies of the ventral tegmental area (VTA) (Johnson et al., 1992; Kalivas, 1993) may explain the inhibition of stress-induced cortical dopamine release after neurotoxic doses of MDMA. The VTA contains 5-HT fibers, which in part, originate from the dorsal raphe nucleus, a region targeted by MDMA (Herve et al., 1987; Imai et al., 1986; Vertes, 1991). If 5-HT release in the VTA is attenuated following MDMA, GABAergic tone would be enhanced and terminal dopamine release inhibited.

MDMA pretreatment affects basal serotonin levels in the dorsal hippocampus

In addition to the effects on stimulated 5-HT release, basal 5-HT concentrations were lower in rats pretreated with MDMA in the dorsal hippocampus, but not in the prefrontal cortex. The magnitude of 5-HT depletion produced by MDMA may contribute to these regional differences in basal 5-HT concentrations 1 week after MDMA. Decreased basal 5-hydroxyindoleacetic acid levels but not 5-HT concentrations were reported in rats treated with high doses of MDMA in the ventral hippocampus, prefrontal cortex or striatum (Gartside et al., 1996; Series et al., 1994; Shankaran and Gudelsky, 1999). It is possible that a 40–50% decrease in tissue 5-HT as observed previously in the striatum (Shankaran and Gudelsky, 1999) or frontal cortex (Table 2) is not

Table 2. Corticosterone concentrations (µg/dl) in trunk blood plasma collected 7 days following treatment with MDMA (10 mg/kg ip. ×4 injections, every 2 h) or an equivalent volume of saline

Time of corticosterone measurement	Saline treated	MDMA treated
Prior to restraint stress	3.0 ± 2.2	4.5 ± 4.4
During exposure to restraint stress	20.3 ± 4.1*	20.6 ± 3.2*

All data are presented as the mean ± S.E.M. *$P < 0.05$ vs. prior to restraint stress by a two-way ANOVA.

sufficient to produce changes in basal extracellular concentrations of 5-HT.

A similar relationship between the severity of tissue depletion and the extracellular basal concentrations has been observed following other 5-HT neurotoxic regimens and other monoamine neurotoxins, such as 6-hydroxydopamine. As reported for hippocampal 5-HT in the present study, depletions of tissue norepinephrine content greater than 50% seem to be required for alterations in basal hippocampal norepinephrine (Abercrombie and Zigmond, 1989). In the striatum, Hall et al. (1999) found decreased basal extracellular 5-HT levels following 65% or greater depletions of striatal 5-HT tissue content following 5,7-DHT lesions. However, another study reported no changes in extracellular levels of 5-HT after depletions of 76–93% in striatal 5-HT (Kirby et al., 1995). This later study measured 5-HT levels in anesthetized rats 3 h following probe implantation, rather than in freely moving, awake animals 18 h after probe insertion. Therefore, a longer period for stabilization of extracellular 5-HT concentrations, such as used in this study, may be necessary to measure subtle differences in basal extracellular concentrations of neurotransmitters (Hall et al., 1999).

MDMA pretreatment does not affect stress-induced endocrine or autonomic responses

Central serotonergic systems can modulate stress-stimulated activity of the hypothalamic–pituitary–adrenal axis (for review see Chaouloff, 1993). MDMA, possibly through its ability to increase extracellular 5-HT levels, acutely increases plasma corticosterone (Aguirre et al., 1997; McNamara et al., 1995; Nash et al., 1988). However, these effects of MDMA on corticosterone are not persistent. There were no differences in basal or stress-stimulated corticosterone responses 7 days following MDMA pretreatment (Table 2; Aguirre et al., 1997). This lack of a long-term effect of MDMA on corticosterone responsiveness may be due to the regional selectivity of MDMA-induced 5-HT depletions. MDMA more severely damages 5-HT axons in cortical and limbic terminal regions than in hypothalamic regions (for review see Sprague et al., 1998; Battaglia et al., 1987; O'Hearn et al., 1988; Stone et al., 1986; however see Callahan et al., 2001). Previous studies have reported variable effects of 5-HT lesions on corticosterone levels depending on the neurotoxin used to produce the lesion and the type of stimulus (Baumann et al., 1998; Chung et al., 1999; Poland, 1990). These differences in corticosterone responses may also be due to the varying degrees of 5-HT depletions in hypothalamic nuclei observed in the studies (Baumann et al., 1998; Chung et al., 1999).

Acute stressors have been shown to produce hyperthermic responses (Kluger et al., 1987; Long et al., 1990; Morimoto et al., 1991; Terlouw et al., 1996). In this experiment, the stress-induced rise in core body temperature occurred in both saline and MDMA pretreated groups. These findings corroborate previous reports that basal, acute stress- or drug-stimulated thermic responses remained intact following 5-HT depletions (McNamara et al., 1995; Chung et al., 1999). Thus, while 5-HT depletions with MDMA may alter neurotransmitter responses to an acute stressor, the autonomic responses appear to remain intact.

OVERALL CONCLUSIONS

Pretreatment with MDMA resulted in 5-HT tissue depletions in the hippocampus and the frontal cortex. MDMA pretreatment blunted the dopamine and 5-HT increases during acute immobilization stress observed in non-depleted or saline pretreated rats. While previous studies have shown that the release of 5-HT in response to a pharmacological agent may be impaired by MDMA pretreatment, the present study indicates that the release of neurotransmitters induced by an environmental change (i.e. stress) also is disrupted. The importance of the various neurotransmitter responses that have been correlated with the onset of an acute stressor is unknown. The increases in neurotransmitter release may be specific to a particular stressor, or may be a more general response to any relevant arousing stimuli. Regardless of the specific behavioral significance, MDMA treated rats exhibited abnormal physiological responses that may have significant implications for long-term functional consequences of MDMA abuse as well as the interactions between the serotonergic system and stress.

Acknowledgements—This work was supported by the National Institutes of Health DA07606, DA07427, DA05837 and AA10760, the Department of Defense DAMD 17-99-1-9479, and the Department of Veterans Affairs.

REFERENCES

Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on *in vivo* dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52, 1655–1658.

Abercrombie, E.D., Zigmond, M.J., 1989. Partial injury to central noradrenergic neurons: reduction of tissue norepinephrine content is greater than reduction of extracellular norepinephrine measured by microdialysis. J. Neurosci. 9, 4062–4067.

Adell, A., Casanovas, J.M., Artigas, F., 1997. Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. Neuropharmacology 36, 735–741.

Aguirre, N., Frechilla, D., García-Osta, A., Lasheras, B., Río, J.D., 1997. Differential regulation by methylenedioxymethamphetamine of 5-hydroxytryptamine1A receptor density and mRNA expression in rat hippocampus, frontal cortex, and brainstem: the role of corticosteroids. J. Neurochem. 68, 1099–1105.

Amat, J., Matus-Amat, P., Watkins, L.R., Maier, S.F., 1998. Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. Brain Res. 797, 12–22.

Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J., De Souza, E.B., 1987. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin termins in rat brain: quantification of neurodegeneration by measurement of [^{3}H]-paroxetine-labeled serotonin uptake sites. J. Pharmacol. Exp. Ther. 242, 911–916.

Baumann, M.H., Ayestas, M.A., Rothman, R.B., 1998. Functional consequences of central serotonin depletion produced by repeated fenfluramine administration in rats. J. Neurosci. 18, 9069–9077.

Boutelle, M.G., Zetterstrom, T., Pei, Q., Svensson, L., Fillenz, M., 1990. *In vivo* neurochemical effects of tail pinch. J. Neurosci. Methods 34, 151–157.

Callahan, B.T., Cord, B.J., Ricaurte, G.A., 2001. Long-term impairment of anterograde axonal transport along fiber projections originating in the rostral raphe nuclei after treatment with fenfluramine or methylenedioxymethamphetamine. Synapse 40, 113–121.

Cenci, M.A., Kalen, P., Mandel, R.J., Bjorklund, A., 1992. Regional differences in the regulation of dopamine and noradrenaline release in medial prefrontal cortex, nucleus accumbens and cautdate-putamen: a microdialysis study in the rat. Brain Res. 581, 217–228.

Chaouloff, F., 1993. Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res. Rev. 18, 1–32.

Chung, K.K., Martinez, M., Herbert, J., 1999. Central serotonin depletion modulates the behavioural, endocrine and physiological responses to repeated social stress and subsequent c-fos expression in the brains of male rats. Neuroscience 92, 613–625.

Commins, D.L., Yosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R., Seiden, L.S., 1987. Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. J. Pharmacol. Exp. Ther. 241, 338–345.

Diorio, D., Viau, V., Meaney, M.J., 1993. The role of the medial prefrontal cortex (cingulate cortex) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J. Neurosci. 13, 3839–3947.

Feldman, S., 1985. Neural pathways mediating adrenocortical responses. Fed. Proc. 44, 169–175.

Feldman, S., Melamed, E., Conforti, N., Weidenfeld, J., 1984. Effect of central serotonin depletion on adrenocortical responses to neural stimuli. Exp. Neurol. 85, 661–666.

Finlay, J.M., Zigmond, M.J., 1997. The effects of stress on central dopaminergic neurons: possible clinical implications. Neurochem. Res. 22, 1387–1394.

Finlay, J.M., Zigmond, M.J., Abercrombie, E.D., 1995. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. Neuroscience 64, 619–628.

Frederick, D.L., Ali, S.F., Gillam, M.P., Gossett, J., Slikker, W.Jr., Paule, M.G., 1995. Acute effects of dexfenfluramine (d-FEN) and methylenedioxymethamphetamine (MDMA) before and after short-course, high dose treatment. Ann. NY Acad. Sci. 844, 183–190.

Gartside, S.E., McQuade, R., Sharp, T., 1996. Effects of repeated administration of 3,4-methylenedioxymethamphetamine on 5-hydroxytryptamine neuronal activity and release in the rat brain *in vivo*. J. Pharmacol. Exp. Ther. 279, 277–283.

Gibb, J.W., Johnson, M., Stone, D., Hanson, G.R., 1990. MDMA: historical perspectives. Ann. NY Acad. Sci. 600, 601–611.

Gresch, P.J., Sved, A.F., Zigmond, M.J., Finlay, J.M., 1994. Stress-induced sensitization of dopamine and norepinephrine efflux in the medial prefrontal cortex of the rat. J. Neurochem. 63, 575–583.

Hall, F.S., DeVries, A.C., Fong, G.W., Huang, S., Pert, A., 1999. Effects of 5,7-dihydroxytryptamine depletion of tissue serotonin levels on extracellular serotonin in the striatum assessed with *in vivo* microdialysis: relationship to behavior. Synapse 33, 16–25.

Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of hypothalamic-pituitary-adrenocortical axis. Trends Neurosci. 20, 78–84.

Herve, D., Pickel, V.M., Joh, T.H., Beaudet, A., 1987. Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res. 435, 71–83.

Horan, B., Gardner, E., Ashby, C.R., 2000. Enhancement of conditioned place preference response to cocaine in rats following subchronic administration of 3,4-methylenedioxymethamphetamine (MDMA). Synapse 35, 160–162.

Horan, B., Morgan, A.E., Dewey, S.L., Jr., Ashby, C.R., 1997. Repeated administration of 3,4-methylenedioxymethamphetamine augments cocaine's action on dopamine in the nucleus accumbens: a microdialysis study. Eur. J. Pharmacol. 331, R1–R3.

Imai, H., Steindler, D.A., Kitai, S.T., 1986. The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. J. Comp. Neurol. 243, 363–380.

Imperato, A., Puglisi-Allegra, S., Casolini, P., Angelucci, L., 1991. Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. Brain Res. 538, 111–117.

Iyer, R.N., Bradberry, C.W., 1996. Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. J. Pharmacol. Exp. Ther. 277, 40–47.

Johnson, S.W., Mercuri, N.B., North, R.A., 1992. 5-Hydroxytryptamine1B receptors block the GABA synaptic potential in rat dopamine neurons. J. Neurosci. 12, 2000–2006.

Joseph, M.H., Kennett, G.A., 1983. Stress-induced release of 5-HT in the hippocampus and its dependence on increased tryptophan availability: an *in vivo* electrochemical study. Brain Res. 270, 251–257.

Joseph, M.H., Kennett, G.A., 1986. Serotonin release in rat hippocampus examined by *in vivo* voltammetry: serotonergic function and tryptophan availability. Ann. NY Acad. Sci. 473, 256–266.

Kalivas, P.W., 1993. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res. Rev. 18, 75–113.

Kawahara, Y., Kawahara, H., Westerink, B.H.C., 1999. Comparison of effects of hypotension and handling stress on the release of noradrenaline and dopamine in the locus coeruleus and medial prefrontal cortex of the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 42–49.

Kawahara, H., Yoshida, M., Yokoo, H., Nishi, M., Tanaka, M., 1993. Psychological stress increases serotonin release in the rat amygdala and prefrontal cortex assessed by *in vivo* microdialysis. Neurosci. Lett. 162, 81–84.

Keefe, K.A., Sved, A.F., Zigmond, M.J., Abercrombie, E.D., 1993. Stress-induced dopamine release in the neostriatum: evaluation of the role of action potentials in nigrostriatal dopamine neurons or local initiation by endogenous excitatory amino acids. J. Neurochem. 61, 1943–1952.

Keith, L.D., Winslow, J.R., Reynolds, R.W., 1978. A general procedure for estimation of corticosteroid response in individual rats. Steroids 31, 523–531.

Kirby, L.G., Chou-Green, J.M., Davis, K., Lucki, I., 1997. The effects of different stressors on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Brain Res. 760, 218–230.

Kirby, L.G., Kreiss, D.S., Singh, A., Lucki, I., 1995. Effect of destruction of serotonin neurons on basal and fenfluramine-induced serotonin release in striatum. Synapse 20, 99–105.

Kluger, M.J., O'Reilly, B., Shope, T.R., Vander, A.J., 1987. Further evidence that stress hyperthermia is a fever. Physiol. Behav. 39, 763–766.

Long, N.C., Vander, A.J., Kluger, M.J., 1990. Stress-induced rise of body temperature in rats is the same in warm and cool environments. Physiol. Behav. 47, 773–775.

Lowy, M.T., Gault, L., Yamamoto, B.K., 1993. Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. J. Neurochem. 61, 1957–1960.

Mamounas, L.A., Mullen, C.A., O'Hearn, E., Molliver, M.E., 1991. Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J. Comp. Neurol. 314, 558–586.

Matsumoto, M., Togashi, H., Mori, K., Ueno, K.-I., Miyamoto, A., Yoskioka, M., 1999. Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. Eur. J. Pharmacol. 383, 39–48.

McEwen, B.S., DeKloet, E.R., Rostene, W., 1986. Adrenal steroid receptors and actions in the nervous system. Physiol. Rev. 66, 1121–1188.

McNamara, M.G., Kelly, J.P., Leonard, B.E., 1995. Some behavioural and neurochemical aspects of subacute (+)3,4-methylenedioxymethamphetamine administration in rats. Pharmacol. Biochem. Behav. 52, 479–484.

Morimoto, A., Watanabe, T., Morimoto, K., Nakamoria, T., Murakami, N., 1991. Possible involvement of prostaglandins in psychological stress-induced responses in rats. J. Physiol. 443, 421–429.

Morrow, B.A., Roth, R.H., 1996. Serotonergic lesions alter cocaine-induced locomotor behavior and stress-activation of the mesocorticolimbic dopamine system. Synapse 23, 174–181.

Nakahara, D., Nakamura, M., 1999. Differential effect of immobilization stress on *in vivo* synthesis rate of monoamines in medial prefrontal cortex and nucleus accumbens of conscious rats. Synapse 32, 238–242.

Nash, J.F., Meltzer, H.Y., Gudelsky, G.A., 1988. Elevation of serum prolactin and corticosterone concentrations after administration of 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 245, 873–879.

O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Molliver, M.E., 1988. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) ablation cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J. Neurosci. 8, 2788–2803.

Poland, R.E., 1990. Diminished corticotropin and enhanced prolactin responses to 8-hydroxy-2(di-n-propylamino)tetralin in methylenedioxymethamphetamine pretreated rats. Neuropharmacology 29, 1099–1101.

Ricaurte, G.A., Markowska, A.L., Wenk, G.L., Hatzidimitriou, G., Wlos, J., Olton, D.S., 1993. 3,4-Methylenedioxy-methamphetamine, serotonin and memory. J. Parmacol. Exp. Ther. 266, 1097–1105.

Robinson, T.E., Castaneda, E., Whishaw, I.Q., 1993. Effects of cortical serotonin depletion induced by 3,4-methylenedioxymethamphetamine (MDMA) on behavior, before and after additional cholinergic blockade. Neuropsychopharmacology 8, 77–85.

Romero, L., Jernej, B., Bei, N., Cicin-Sain, L., Cortes, R., Artigas, F., 1998. Basal and stimulated extracellular serotonin concentration in the brain of rats with altered serotonin uptake. Synapse 28, 313–321.

Sabol, K.E., Richards, J.B., Seiden, L.S., 1992. Fenfluramine-induced increases in extracellular hippocampal serotonin are progressively attenuated *in vivo* during a four-day fenfluramine regimen in rats. Brain Res. 571, 64–72.

Schechter, M.D., 1991. Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. Pharmacol. Biochem. Behav. 38, 539–544.

Schmidt, C.J., 1987. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 240, 1–7.

Schmidt, C.J., Taylor, V.L., 1987. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem. Pharmacol. 36, 4095–5102.

Seiden, L.S., Woolverton, W.L., Lorens, S., Williams, J.E.F., Corwin, R.L., Hata, N., Olimski, M., 1993. Behavioral consequences of partial monoamine depletion in the CNS after methamphetamine-like drugs: the conflict between pharmacology and toxicology. NIDA Res. Monogr. 136, 34–52.

Series, H.G., Cowen, P.J., Sharp, T., 1994. *p*-Chloroamphetamine (PCA), 3,4-methylenedioxymethamphetamine (MDMA) and D-fenfluramine pretreatment attenuates D-fenfluramine-evoked release of 5-HT *in vivo*. Psychopharmacology 16, 508–514.

Shankaran, M., Gudelsky, G.A., 1999. A neurotoxic regimen of MDMA suppresses behavioral, thermal and neurochemical responses to subsequent MDMA administration. Psychopharmacology 147, 66–72.

Shimizu, N., Take, S., Hori, T., Oomura, Y., 1992. *In vivo* measurement of hypothalamic serotonin release by intracerebral microdialysis: significant enhancement by immobilization stress in rats. Brain Res. Bull. 28, 727–734.

Slikker, W., Jr., Holson, R.R., Ali, S.F., Kolta, M.G., Paule, M.G., Scallet, A.C., McMillan, D.E., Bailey, J.R., Hong, J.S., Scalzo, F.M., 1989. Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology 10, 529–542.

Sorg, B.A., Kalivas, P.W., 1993. Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53, 695–703.

Sprague, J.E., Everman, S.L., Nichols, D.E., 1998. An integrated hypothesis for serotonin axonal loss induced by 3,4-methylenedioxymethamphetamine. Neurotoxicology 19, 427–442.

Stone, D.M., Merchant, K.M., Hanson, G.R., Gibb, J.W., 1987. Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26, 1677–1683.

Stone, D.M., Stahl, D.C., Hanson, G.R., Gibb, J.W., 1986. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur. J. Pharmacol. 128, 41–48.

Taber, M.T., Fibiger, H.C., 1997. Activation of the mesocortical dopamine system by feeding: lack of a selective response to stress. Neuroscience 77, 295–298.

Takahashi, H., Takada, Y., Nagai, N., Urano, T., Takada, A., 1998. Extracellular serotonin in the striatum increased after immobilization stress only in the nighttime. Behav. Brain Res. 91, 185–191.

Terlouw, E.M.C., Kent, S., Cremona, S., Dantzer, R., 1996. Effect of intracerebroventricular administration of vasopressin on stress-induced hyperthermia in rats. Physiol. Behav. 60, 417–424.

Thierry, A.M., Tassin, J.P., Blank, G., Glowinski, J., 1976. Selective activation of mesocortical DA system by stress. Nature 263, 242–244.

Vertes, R.P., 1991. A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J. Comp. Neurol. 313, 643–668.

Wilson, M.A., Ricaurte, G.A., Molliver, M.E., 1989. Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine. Neuroscience 28, 121–137.

Yoshioka, M., Matsumoto, M., Togashi, H., Saito, H., 1995. Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. Pharmacol. Biochem. Behav. 51, 515–519.

(*Accepted* 8 *October* 2001)

Behavioral Neuroscience
Dr. Francis J. White

Chronic Stress Augments the Acute and Long-Term Effects of Methamphetamine

Leslie Matuszewich[1] and Bryan K. Yamamoto[2]

Department of Psychiatry, Case Western Reserve University,

11100 Euclid Avenue, Cleveland OH 44106

[1] Current Address: Department of Psychology, Northern Illinois University, DeKalb, IL 60115

[2] Current Address: Boston University, School of Medicine, Department of Pharmacology, Boston, MA 02118

Abbreviated Title: Effects of Chronic Stress on METH

Number of Text pages: 32

Number of Words in Abstract: 248

Introduction: 448

Discussion: 1456

No. of Figures: 4

No. of Tables: 0

Corresponding Author: Dr. Leslie Matuszewich, Department of Psychology, Northern Illinois University, DeKalb, IL 60115. Phone: 815-753-7072. Fax: 815-753-8088. Email: **lmatusze@niu.edu**.

Acknowledgements: This work was supported by National Institutes of Health grants DA07606 and DA05937-02 and by Department of Defense grant 17-99-1-9479.

Key Words: Chronic Stress, Methamphetamine, Psychostimulants, Neurotoxicity, Dopamine, Striatum

Abstract:

There is growing evidence that stress may alter the effects of abused drugs on the central nervous system. Animal studies have shown that exposure to repeated stress alters the acute behavioral or neurochemical responses to drugs of abuse. The psychostimulant methamphetamine (METH) at high doses can have acute and sustained neurochemical effects on forebrain dopamine concentrations. The current study tested the hypotheses that 10 days of unpredictable stress 1) augments the acute increase in extracellular striatal dopamine concentrations in response to injections of 7.5 or 10 mg/kg METH and 2) produces greater depletions of striatal dopamine 7 days following the injection regimen. Male rats exposed to unpredictable stress had an increased mortality rate (33%) compared to non-stressed controls (16.7%) following 4 injections of 10 mg/kg METH. Stressed rats also had increased hyperthermic responses and dopamine efflux in the striatum during the METH injections when compared to non-stressed control rats. Furthermore, one week following either a 7.5 mg/kg or 10 mg/kg METH injection regimen, stressed rats showed greater depletions in striatal dopamine tissue content than non-stressed controls injected with METH. The enhanced acute and longer-term effects of METH in stressed rats was not due to a greater concentrations of METH in the striatum, as extracellular levels of METH during the injection regimen did not differ between the 2 groups. These findings suggest that chronic stress increases the responsiveness of the brain to the acute pharmacological effects of METH and enhances the vulnerability of the brain to the neurotoxic effects of psychostimulants.

There is increasing evidence that repeated exposure to environmental stressors can alter behavioral and neurochemical responses to drugs of abuse. Prior exposure to stress enhances locomotor activity following a systemic injection of amphetamine, morphine or cocaine (Campbell and Fibiger, 1971; Antelman et al., 1980; Herman et al., 1984; Robinson and Becker, 1986; Kalivas and Stewart, 1991; Deroche et al., 1993; Robinson and Berridge, 1993; Stewart and Badiani, 1993; Deroche et al., 1995). Likewise, drug-induced dopamine release in several forebrain regions is augmented by pre-exposure to stress (Kalivas and Duffy, 1989; Sorg and Kalivas, 1991; Hamamura and Fibiger, 1993; Rouge-Pont et al., 1995). Exposure to stress also facilitates the propensity of rats to self-administer drugs of abuse (for review see Piazza and LeMoal, 1998) and several studies have reported recently that prior exposure to METH may augment subsequent stress-induced responses in rats and human METH users (Tsuchiya et al., 1996; Yui et al., 1999; Wallace et al., 2001; Yui et al., 2001).

Although chronic stress has been shown to potentiate the damage of some neurotoxins (Stein-Behrens et al., 1994; Mizoguchi et al., 2000), it is unknown if exposure to chronic stress enhances the vulnerability of the dopamine system to the neurotoxic effects of drugs of abuse, in particular, methamphetamine (METH). The psychostimulant METH can act as a neurotoxin to monoamine neurons when administered at high doses or repeatedly in rodents and non-human primates (Ricaurte and McCann, 1992; Gibb et al., 1993) as evidenced by decreases in the number of tyrosine hydroxylase immunoreactive fibers (Hotchkiss and Gibb, 1980), in the density of dopamine terminals and uptake sites (Bittner et al., 1981; Pu et al., 1994), and in the amount of dopamine in striatal tissue (Ricaurte et al., 1980; Stephans and Yamamoto, 1994). The alterations in these biochemical markers have been reported to endure for months and are most pronounced in the striatum (Seiden

et al., 1975/76; Ricaurte et al., 1980; Bittner et al., 1981; Villemagne et al., 1998). Whether chronic stress can modulate either the long-term damaging effects of METH to striatal dopamine content or the acute effects of METH on extracellular dopamine release has not been assessed.

Therefore, the present study investigated the effects of chronic unpredictable stress on the immediate and longer-term neurochemical changes in the striatum associated with high doses of METH. Chronic unpredictable stress, during which the type and timing of different mild stressors are varied, was chosen based on evidence that unpredictability is important in determining the responsiveness to psychostimulant drugs (MacLennan and Maier, 1983; Halle et al., 2001). We hypothesize that exposure to 10 days of unpredictable stress will enhance the vulnerability of striatal neurons to METH neurotoxicity as evidenced by greater dopamine depletions.

MATERIALS AND METHODS

Animals and stress exposure. Male Sprague-Dawley rats (175 – 250 g) were purchased from Zivic Miller Laboratories (Allison Park, PA). Rats were pair housed until intracranial surgery with food and water available ad lib, on a 12/12 h light/dark cycle in a temperature controlled room. All procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the local institutional animal care committee.

Stressed rats were exposed to stressors that varied by day and time for 10 days (Willner, 1984; Stein-Behrens et al., 1994; Fitzgerald et al., 1996; Halle et al., 2001). The following schedule was followed for rats used in the microdialysis experiments: Day 1 11:00 a.m. 50 min cold room (4°C), and 1:00 p.m. 60 min cage rotation; Day 2 1:00 p.m. 4 min swim stress, and 6:00 p.m. lights on overnight; Day 3 12:00 p.m. 3 h

lights off, and 3:00 p.m. 60 min restraint stress; Day 4 6: 00 p.m. 50 min cage rotation, and food and water deprivation overnight; Day 5 3:00 p.m. 15 min cold room isolation, and 4:00 p.m. isolation housing overnight; Day 6 11:00 a.m. 3 min swim stress, and 3:00 p.m. 60 min restraint stress; Day 7 intracranial surgery; Day 8 10:00 a.m. 20 min cage rotation, and 3:00 p.m. 2 h lights off; Day 9 10:00 a.m. 3 min swim stress, and 6:00 p.m. food and water deprivation over night; Day 10 12:00 p.m. 3 h lights off, and 6:00 p.m. lights on overnight. Unstressed and stressed rats were weighed daily and both groups underwent intracranial surgery on Day 7, as listed above. For the hyperthermia and ex vivo tissue experiments that did not require intracranial surgery, the same stress procedure was followed until Day 6, after which the following schedule was used: Day 6 11:00 a.m. 3 min swim stress, and 3:00 p.m. 2 h lights off; Day 7 1:00 p.m. 30 min cage rotation, and 6:00 p.m. 1 h lights on; Day 8 10:00 a.m. 20 min cage rotation, and 3:00 p.m. 60 min restraint stress; Day 9 10:00 a.m. 3 min swim stress, and 6:00 p.m. food and water deprivation; Day 10 6:00 p.m. isolation housing and lights on overnight.

Drug injections and Temperature Measurements. On day 11, intraperitoneal (i.p.) injections of 7.5 mg/kg or 10 mg/kg d-METH hydrochloride salt (National Institute of Drug Abuse, Bethesda MD), or an equivalent volume of saline (0.9% NaCl), were given every two hours for a total of 4 injections. METH was dissolved in saline and given in a volume of 1 ml/kg. The high METH dosing regimen (1o mg/kg x 4 injections) was selected due to its reliability for causing long-term dopamine depletions in the striatum (Stephans et al., 1998). The lower dosing regimen (7.5 mg/kg x 4 injections) was selected due to its lower mortality rate. Rectal temperature was measured 30 and 60 min following each i.p. injection with a Thermalert TH-8 monitor (Physitemp Instruments, Inc., Clinton NJ).

Microdialysis Procedures. Rats used in the microdialysis experiments were anesthetized with a combination of xylazine (6 mg/kg) and ketamine (70 mg/kg) and placed into a Kopf stereotaxic frame. The skull was exposed and a 21-gauge stainless steel guide cannula (11 mm in length, Small Parts, Inc., Miami Lakes FL) was positioned above the striatum (2.0 mm anterior and 3.2 mm medial to bregma). The cannula and a metal female connector were secured to the skull with 3 stainless steel screws and cranioplastic cement. An obturator fashioned from 31-gauge stainless steel wire, ending flush with the guide cannula, was inserted into the cannula after surgery.

Four days following surgery, the obturator was removed from the guide cannula and replaced with a microdialysis probe. The microdialysis probes were constructed as previously described (Lowy et al., 1993) from a 27-gauge thin wall stainless steel tube, fitted with a dialysis membrane (13,000 dalton cut off, 210 μm o.d.; Spectrum Laboratories, Inc., Rancho Domingues CA) at one end, and a 3 cm piece of polyethylene (PE) 20 tubing (Fisher Scientific, Inc., Pittsburgh PA) at the other end, to serve as the inlet for the perfusion medium. The dialysis membrane was 4 mm x 210 μm diameter. A 4 cm length of capillary tubing (125 μm o.d., 50 μm i.d.; Polymicro Technologies, Phoenix AZ) served as the outlet from the dialysis membrane. The vertical placement of the microdialysis probe was determined during construction of the probe by gluing a ring of PE 20 tubing, which acts as a mechanical "stop", at a measured distance along the length of the probe. The positioning permitted the exposed portion of the dialysis membrane to extend beyond the guide cannula and into the striatum (ventral from dura –1.0 to –5.0). The rats were placed in microdialysis cages and attached via a spring-covered tether to a swivel (Instech Laboratories, Inc., Plymouth Meeting PA). Dulbecco's phosphate-buffered saline

medium (NaCl 138 mM, 2.1 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM $NaHPO_4$, 1.2 mM $CaCl_2$, and 5 mM d-glucose, pH 7.4) was pumped immediately through the microdialysis probe with a Harvard Model 22 syringe infusion pump (Hollison MA) at a rate of 2.0 µl/min. After a 3 h equilibration period, the following 1 hour samples were collected: 2 baseline samples and 8 samples during systemic METH injections.

High Performance Liquid Chromatograph. Microdialysis samples from the striatum were assayed for dopamine by high performance liquid chromatography with electrochemical detection (HPLC-EC). For catecholamine detection, samples (22 µl) were loaded via a Rheodyne injector (Cotati CA) onto a 3 µm C18 column (100 x 2 mm, Phenomenex, Torrance CA). The mobile phase (pH 4.2) consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM ethylenediaminetetraacetic acid (EDTA), 0.215 mM octyl sodium sulfate and 3 % methanol. Compounds were detected with an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette IN), with a 6 mm glassy carbon working electrode maintained at a potential of +0.7 V relative to an Ag/AgCl reference electrode. Data were collected using a Hewlett Packard Integrator.

To assess METH concentrations in dialysate, 40 µl of sample was loaded via a Rheodyne injector onto a 5µ C18 column (150 x 2 mm, Phenomenex, Torrance CA). A Hewlett Packard 1050 pump delivered 0.455 ml/min of mobile phase (50 mM NaH_2PO_4, 6% acetonitrile, pH = 4.6) to the column. Striatal concentrations of METH were detected with a Waters 486 Tunable Absorbance Detector (Milford, MA), with the wavelength set at 245nm.

Analysis of dopamine tissue concentrations in striatal tissue was performed on an HPLC-EC, as described above. The tissues were sonicated in 0.1 M perchloric acid, centrifuged at 13,000 x g for 6 min and the supernatant assayed for dopamine. The

pellet was re-suspended in 1 N NaOH and protein content determined by a Bradford Protein Assay.

Histology One week after systemic METH or saline injections, rats were killed and the brains quickly removed from the skull and frozen on dry ice. Probe placements were verified from frozen coronal sections and only rats with probes located in the striatum were used for statistical analysis. Striatal tissue was dissected from a 400 μm slice. The tissue was frozen at -80°C for later analysis of neurotransmitter content. *Statistical analysis.* Independent t-tests were used to compare body weight differences (weight on Day 11-weight on Day 1) and baseline concentrations of dopamine from chronically stressed rats and non-stressed controls. Two-way, Repeated Measures Analysis of Variances (ANOVA) were computed to compare dialysate dopamine or METH concentrations or rectal temperatures over time. Dopamine concentration in dialysate was compared in 10 samples (2 predrug samples and 8 samples during systemic administration of METH). METH concentration in dialysate was compared in 9 samples (1 predrug sample and 8 samples during systemic administration of METH) because there was no detectable level of METH in the striatum prior to drug injection. Rectal temperature was compared at 9 time points (30 min before the first drug injection and 30 and 60 min after each systemic administration of METH). Dopamine tissue concentrations between chronically stressed rats and non-stressed controls were compared in a 2 Way ANOVA. Post hoc Tukey's pairwise tests were used to analyze any significant treatments. Statistical significance was fixed at $p < 0.05$ for all tests.

RESULTS

Effects of chronic stress on body weight

Across all experiments, rats exposed to the 10 day stress protocol showed lower total body weight gain compared to non-stressed controls ($t(1) = 10.655$). Non-stressed controls gained 79.77 $\pm$ 2.28 g over 11 days, while the stressed rats gained 46.69 $\pm$ 2.12 g.

Effects of chronic stress on METH-induced mortality and hyperthermia

Of the rats exposed to chronic stress, 8 rats that received 4 METH injections of 10 mg/kg died (33.3%) compared to 4 of the non-stressed controls (16.7%). Two chronically stressed rats died following 7.5 mg/kg METH (20%), while one non-stressed control rat died (8.3%). None of the rats injected with saline from either group died.

Both stressed and non-stressed rats showed overall increases in rectal temperature after METH injections (7.5 mg/kg: $F(8,128)=8.81$, $p < .05$; 10 mg/kg: $F(8,56)=13.79$, $p < .05$). However, chronically stressed rats showed higher rectal temperatures during either 7.5 or 10 mg/kg METH injection regimens compared to non-stressed controls (7.5 mg/kg: $F(8,128)=2.51$, $p < .05$, Figure 1a; 10 mg/kg: $F(8,56)=4.56$, $p < .05$, Figure 1b). The rectal temperature of stressed rats peaked at 39.2°C compared to 38.5°C for non-stressed rats during the 4 injections of 7.5 mg/kg METH. Likewise, during injections of 10 mg/kg METH, peak rectal temperature of stressed rats reached 40.6°C while control non-stressed rats reached 39.6°C.

Effects of chronic stress on dopamine in the striatum

Extracellular levels of dopamine increased after injections of 7.5 or 10 mg/kg METH (7.5 mg/kg: $F(9,126)=49.9$, $p < .05$, Figure 2a; 10 mg/kg: $F(9,90)=35.02$, $p<.05$, Figure 2b). Peak dopamine content in dialysis samples was dose-dependent with dopamine

concentrations peaking at 66 pg/20 µl following 7.5 mg/kg x 4 METH, and 110 pg/20 µl following 10 mg/kg x 4 METH. Chronically stressed rats injected with 10 mg/kg METH showed greater dopamine increases compared to non-stressed controls ($F(1,9)=2.78$, $p<.05$; Figure 2b). There was no difference in basal concentrations of dopamine in striatum between stressed and non-stressed rats ($t(29)=0.5$, n.s.).

Prior exposure to stress potentiated METH-induced dopamine decreases in striatal tissue collected 7 days after 7.5 or 10 mg/kg METH. Dopamine concentrations in the striatum did not differ between stressed or non-stressed rats following saline injections ($t(28)=0.23$; stressed: 145.5 ± 14.9 ng/mg protein; non-stressed: 141.1 ± 11.8 ng/mg protein). Therefore, dopamine content is presented graphically as a percentage of the dopamine content in saline-injected striata. Four injections of 7.5 mg/kg METH decreased dopamine content of striatal tissue in stressed rats compared to non-stressed rats injected with METH ($F(1,35)=6.094$, $p<.05$, Figure 3a). Both non-stressed and stressed rats had lower striatal dopamine levels following 4 injections of 10 mg/kg METH relative to saline injected rats ($F(1,31)=44.54$, $p<.05$). Dopamine concentrations in the striatum of stressed rats were significantly decreased compared to non-stressed rats treated with 4 injections of 10 mg/kg METH ($F(1,31)=4.195$, $p<.05$, Figure 3b).

Effects of chronic stress on METH concentrations in the striatum

The concentration of METH in the striatum increased following the 4 injections of 10 mg/kg METH in both stressed and non-stressed rats ($F(8,104)=15.65$, $p<.01$; Figure 4), peaking after the 3rd METH injection. However, there were no significant differences in the extracellular concentrations of METH during the injection regimen between stressed and non-stressed rats ($F(1,13)=0.11$, n.s.).

Discussion

Chronic unpredictable stress augmented the acute and long-term effects of repeated METH injections on striatal dopamine. Systemic injections of METH elevated extracellular levels of dopamine in the striatum to a greater extent in chronically stressed rats compared to non-stressed controls. One week after administration of METH, decreases in striatal dopamine content was potentiated in rats exposed to chronic stress compared to non-stressed control rats. Previous studies in our and other laboratories have shown acute increases in extracellular dopamine during METH administration and subsequent long-term depletions of striatal dopamine (Seiden et al., 1975/76; Ricaurte et al., 1980; Stephans and Yamamoto, 1994). However, to our knowledge, this is the first report that prior exposure to chronic unpredictable stress augments the acute release of dopamine during high doses of METH, as well as the depletion of striatal dopamine content 1 week after drug treatment.

Potentiation of Acute Extracellular Dopamine Levels

The potentiation of METH-induced extracellular dopamine levels in rats exposed to stress parallels other findings following a challenge injection of amphetamine or cocaine (for a review see Kalivas and Stewart, 1991). Repeated exposure to stress may contribute to the enhanced dopamine release by increasing tyrosine hydroxylase and/or the releasable stores of dopamine, inhibiting dopamine catabolism, decreasing dopamine uptake, or increasing impulse generation in dopaminergic neurons. Ortiz and colleagues (1996) reported an increase of tyrosine hydroxlyase in the ventral tegmental area, but not the substantia nigra, following the same 10-day stress procedure as used for the current study. Although increases in dopamine synthesis may explain elevated extracellular dopamine concentrations in the mesolimbic terminal regions, such as the nucleus accumbens, other mechanisms may be

operative in the nigrostriatal system (Beitner-Johnson et al., 1991; Beitner-Johnson and Nestler, 1991; Sorg and Kalivas, 1991; Beitner-Johnson et al., 1992).

Due to the ability of METH to release dopamine through reverse transport (Fischer and Cho, 1979), stress-induced alterations in the dopamine transporter (DAT) may account for the augmented release of dopamine during METH injections. However, acute social stress in mice housed in isolation reduced DAT binding in the striatum (Isovich et al., 2001), as did exposure of male tree shrews to chronic subordinate stress (Isovich et al., 2001). These studies collectively suggest that the observed increase in striatal extracellular dopamine in chronically stressed rats is not due to increases in DAT. Interestingly, elevated body temperature also can influence the function of DAT by increasing the intracellular accumulation of METH (Metzger et al., 2000; Xie et al., 2000). Therefore, the augmented hyperthermic responses in the stressed rats (Figure 1) may enhance the function of DAT and subsequently contribute to observed increases in extracellular dopamine during METH treatment, irrespective of the number of transporters.

Chronic stress may enhance impulse-dependent dopamine release rather than altering reuptake by redistributing the releasable pool of dopamine in the terminal regions. Alterations of various neurotransmitters, such as glutamate, γ-aminobutyric acid (GABA) or serotonin, in the substantia nigra could affect impulse-dependent release of striatal dopamine in a manner similar to that observed after another amphetamine derivative, 3,4-methyelendioxymethamphetamine (Yamamoto et al., 1995). Chronic stress has been shown to increase glutamate receptor subunits in the ventral tegmental area, but not the substantia nigra (Fitzgerald et al., 1996), however alterations of other neurotransmitter systems have not been investigated. On the other hand, chronic stress may affect the distribution or amount of releasable

dopamine stores in terminal regions (Connor and Kuzcenski, 1986; Robinson and Becker, 1986). Rats sensitized with low doses of METH showed an enhanced dopamine response to potassium stimulation (80 mM) in the prefrontal cortex, but not in the striatum (Stephans and Yamamoto, 1995). The balance between these different mechanisms may account for regional effects of either drug- or stress-induced increases in extracellular dopamine release.

The acute increases or delayed depletions of dopamine observed in the rats exposed to chronic stress do not appear to be due to increased bioavailability of METH in the striatum. METH concentrations in the striatum of stressed and non-stressed rats were similar throughout the METH injection regimen (Figure 4). While METH concentrations measured with in vivo microdialysis suggest that the extracellular concentrations are similar between stressed and non-stressed rats, this technique does not assess the concentrations of METH in dopamine terminals. The concentration of METH in the terminals and subsequent alterations of vesicular pH gradients may be more critical to the longer-term dopamine depletions than extracellular METH concentrations (Sulzer and Rayport, 1990).

Potentiaton of METH-induced dopamine depletions.

Several factors proposed to mediate dopamine depletions following high doses of METH also may be responsible for the observed potentiated depletions of dopamine in chronically stressed rats. A long-term depletion of dopamine content in the striatum after METH is correlated with elevated body temperatures during METH administration (Itoh et al., 1986; Bowyer et al., 1994). Pharmacological agents that lower body temperature attenuate METH-induced dopamine depletions in the striatum, as do lower ambient temperatures (Sonsalla et al., 1991; Bowyer et al., 1992; Bowyer et al., 1994). In the present study, stressed rats showed greater

hyperthermia during METH administration (Figure 1) and this increase in rectal temperature may contribute to the enhanced dopamine damage observed 1 week after METH injections (Figure 3).

The increased hyperthermic response also may account for the elevated mortality of stressed rats observed during and following the 10 mg/kg METH injections. Thirty-three percent of the rats exposed to unpredictable stress died compared to 16.7% of the non-stressed rats injected with 10 mg/kg METH, even though cooling was used for both groups to decrease mortality. These results are consistent with the finding that hyperthermia mediates the lethal effects of amphetamines in rodents (Askew, 1962). Central 5-HT receptors contribute to temperature regulation in rats (Salmi and Ahlenius, 1998) and are increased following exposure to acute or chronic stressors (Papp et al., 1994; Ossowska et al., 2001). Following the 10-day unpredictable stress paradigm, stressed rats showed a greater increase in rectal temperature after a systemic injection of a 5-HT_2 receptor agonist compared to non-stressed rats (Matuszewich and Yamamoto, 2001). Although stimulation of 5-HT_2 receptors may mediate acute hyperthermia associated with METH, the relationship between 5-HT_2 receptor activation and longer-term neurotransmitter content changes after METH remains to be established. Pretreatment with the 5-HT_2 receptor antagonist ritanserin failed to prevent METH-induced decreases in tyrosine and tryptophan hydroxylase activities in the neostriatum, 1-20 h after the last METH injection (Johnson et al., 1988; Johnson et al., 1994).

Alternatively, chronic stress may enhance METH-induced dopamine depletions in the striatum by increasing the acute release of dopamine. Extracellular dopamine concentrations were greater following METH injections in rats exposed to

unpredictable stress, than in non-stressed controls (Figure 2). Blocking dopamine transmission through inhibition of synthesis, blockade of transporter-mediated uptake or co-administration of dopamine antagonists attenuates METH-induced dopamine depletions (Buening and Gibb, 1974; Schmidt et al., 1985; Sonsalla et al., 1986; Marek et al., 1990; Pu et al., 1994). The acute increase in extracellular dopamine may contribute to longer-term dopamine depletions through the generation of free radical species and quinones (Cubells et al., 1994; Hirata et al., 1995; Huang et al., 1997; Yamamoto and Zhu, 1998; Fumagalli et al., 1999).

Exposure to chronic stress or high levels of glucocorticoids potentiate neuronal damage due to other neurotoxins (Johnson et al., 1989; Hortnagl et al., 1993; Stein-Behrens et al., 1994; Huang et al., 1997), hypoxia-ischemia (Sapolsky and Pulsinelli, 1985; Koide et al., 1986) or seizure (Sapolsky, 1985; Stein and Sapolsky, 1988). An increase in glucocorticoids through direct application, or indirectly via stress (Halle et al., 2001), may stimulate excitatory amino acids that lead to high levels of free cytosolic calcium, which can damage cytoskeletal proteins (for review see (Sapolsky, 1999; Thome et al., 2001). METH also increases extracellular concentrations of glutamate in the striatum (Nash and Yamamoto, 1992; Stephans and Yamamoto, 1994) and glutamate antagonists attenuate METH-induced decreases in dopamine terminals (Sonsalla et al., 1989; Sonsalla et al., 1991; Bowyer et al., 1994). The increases in glutamate following METH injections may increase calcium levels that in turn contribute to the damage of dopamine terminals.

Overall, several mechanisms may contribute to the potentiated decreases in dopamine tissue content and the acute increases in hyperthermia or extracellular dopamine in the striatum. The precise effects of repeated, unpredictable stress on the brain are unknown but alterations in 5-HT receptors (Ossowska et al., 2001), the

dopaminergic system (Ortiz et al., 1996; Ossowska et al., 2001), or excitatory amino acid transmission (Fitzgerald et al., 1996) may account for the enhanced hyperthermia, mortality, extracellular dopamine concentrations, or depletions of dopamine content in the striatum. Due to the broad overlap of stress and drug use (Piazza and LeMoal, 1998; Yui et al., 1999; Yui et al., 2001), the increased vulnerability of the brain by exposure to unpredictable stressors may be important for understanding the potential detrimental effects of drugs of abuse.

Figure Legends.

Figure 1. Rectal temperature increased during injections of (a) 7.5 mg/kg or (b) 10 mg/kg METH (i.p., every 2 h for a total of 4 injections) compared to temperatures prior to the 1st injection (time = 0). Rats exposed to chronic unpredictable stress showed greater hyperthermic responses compared to non-stressed controls (+, $p<.05$). Values are expressed as means $\pm$ SEM. Arrow indicates each injection of METH.

Figure 2. In vivo extracellular dopamine concentrations in the striatum prior to and during injections of (a) 7.5 mg/kg or (b) 10 mg/kg METH (i.p., every 2 h for a total of 4 injections). METH increased dopamine levels for stressed and non-stressed rats relative to baseline concentrations. Rats exposed to chronic unpredictable stress showed greater increases of extracellular dopamine compared to non-stressed controls (+, $p<.05$). Values are expressed as means $\pm$ SEM. Arrow indicates each injection of METH.

Figure 3. Ex vivo dopamine concentrations in the striatum 7 days following injections of (a) 7.5 mg/kg or (b) 10 mg/kg METH (i.p., every 2 h for a total of 4 injections). Injections of 10 mg/kg METH depleted dopamine levels in the striatum for stressed and non-stressed rats relative to rats treated with saline (*, $p<.05$). Rats exposed to chronic unpredictable stress showed greater decrease of striatal dopamine content compared to non-stressed controls following 7.5 or 10 mg/kg METH (+, $p<.05$). Values are expressed as means $\pm$ SEM.

Figure 4. In vivo extracellular METH concentrations in the striatum prior to and during injections of 10 mg/kg METH (i.p., every 2 h for a total of 4 injections). Striatal levels of METH increased for stressed and non-stressed rats relative to baseline concentrations (time = 0)(*, $p<.05$). There was no difference in METH concentrations

xposed to chronic unpredictable stress and non-stressed controls.

ressed as means ± SEM. Arrow indicates each injection of METH.

References

Antelman SM, Eichler AJ, Black CA, Kocan D (1980) interchangeability of stress and amphetamine in sensitization. Sci 207:329-331.

Askew BM (1962) Hyperpyrexia as a contributing factor in the toxicity of amphetamine in aggregated mice. Br J Pharmacol 19:245-257.

Beitner-Johnson D, Nestler EJ (1991) Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J Neurochem 57:344-347.

Beitner-Johnson D, Guitart X, Nestler EJ (1991) Dopaminergic brain reward regions of Lewis and Fischer rats display different levels of tyrosine hydroxylase and other morphine- and cocaine-regulated phosphoproteins. Br Res 561:147-150.

Beitner-Johnson D, Guitart X, Nestler EJ (1992) Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. J Neurosci 12:2165-2176.

Bittner SE, Wagner GC, Aigner TG, Seiden LS (1981) Effects of high-dose treatment methamphetamine on caudate dopamine and anorexia in rats. Pharmacol Biochem Behav 14:481-486.

Bowyer JF, Tank AW, Newport GD, Slikker JW, Ali SF, Holson RR (1992) The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels and dopamine release in rat striatum. J Pharmacol Exper Ther 260:817-824.

Bowyer JF, Davies DL, Schmued L, Broening HW, Newport GD, Slikker JW, Holson RR (1994) Further studies of the role of hyperthermia in methamphetamine neurotoxicity. J Pharmacol Exper Ther 268:1571-1580.

Buening MK, Gibb JW (1974) Influence of methamphetamine and neuroleptic drugs on tyrosine hydroxylase activity. European Journal of Pharmacology 26:30-34.

Campbell BA, Fibiger HC (1971) Potentiation of amphetamine induced arousal by starvation. Nature 233:424-425.

Connor CE, Kuzcenski R (1986) Evidence that amphetamine and Na+ gradient reversal increase striatal synaptosomal dopamine synthesis through carrier-mediated efflux of dopamine. Biochem Pharmacol 35:3123-3130.

Cubells JF, Rayport S, Rajendran G, Sulzer D (1994) Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. J Neurosci 14:2260-2271.

Deroche V, Piazza PV, Casolini P, LeMoal M, Simon H (1993) Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. Br Res 611:352-356.

Deroche V, Marinelli M, Maccari S, LeMoal M, Simon H, Piazza PV (1995) Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. J Neurosci 15:7181-7188.

Fischer JF, Cho AK (1979) Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. J Pharmacol Exper Ther 208:203-209.

Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ (1996) Drugs of abuse and stress increase the expression of GluR1 and NDMAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. J Neurosci 16:274-282.

Fumagalli F, Gainetdinov RR, Wang YM, Valenzano KJ, Miller GW, Caron MG (1999) Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice. J Neurosci 19:2424-2431.

Gibb JW, Johnson M, Stone DS, Hanson GR (1993) Mechanisms mediating biogenic amine deficits induced by amphetamine and its congeners. NIDA Res Mono 136:226-236.

Halle CN, GrandPre T, Kosten TA (2001) Chronic unpredictable stress, but not chronic predictable stress, enhances the sensitivity to the behavioral effects of cocaine in rats. Psychopharmacol 154:213-220.

Hamamura T, Fibiger HC (1993) Enhanced stress-induced dopamine release in the prefrontal cortex of amphetamine-sensitized rats. Eur J Pharmacol 237:65-71.

Herman J-P, Stinus L, LeMoal M (1984) Repeated stress increases locomotor response to amphetamine. Psychopharmacol 84:431-435.

Hirata H, Ladenheim B, Rothman RB, Epstein C, Cadet JL (1995) Methamphetamine-induced serotonin neurotoxicity in mediated by superoxide radicals. Br Res 677:345-347.

Hortnagl H, Berger ML, Havelec L, Hornykiewicz (1993) Role of glucocorticoids in the cholinergic degeneration in rat hippocampus induced by ethylcholine aziridinium (AF64A). J Neurosci 13:2939-2945.

Hotchkiss AJ, Gibb JW (1980) Long-term effects of multiple doses of methamphetamine on tryptophan hydroxlyase and tyrosine hydroxylase activity in rat brain. J Pharmacol Exper Ther 214:257-262.

Huang NK, Wan FJ, Tseng CJ, Tung CS (1997) Amphetamine induces hydroxyl radical formation in the striatum of rats. Life Sci 61:2219-2229.

Isovich E, Englemann M, Landgraf R, Fuchs E (2001) Social isolation after a single defeat reduces striatal dopamine transporter binding in rats. Eur J Neurosci 13:1254-1256.

Itoh Y, Oishi R, Nishibori M, Saeki K (1986) Comparison of effects of phencyclidine and methamphetamine on body temperature in mice: a possible role for histamine neurons in thermoregulation. Naun Schmiedebergs Arch Pharmacol 332:293-296.

Johnson M, Hanson GR, Gibb JW (1988) Effects of dopaminergic and serotonergic receptor blockade on neurochemical changes induced by acute administration of methamphetamine and 3,4-methylenedioxymethamphetamine. Neuropharmacol 27:1089-1096.

Johnson M, Stone DS, Bush LG, Hanson GR, Gibb JW (1989) Glucocorticoids and 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity. Eur J Pharmacol 161:181-188.

Johnson M, Sonsalla PK, Letter AA, Hanson GR, Gibb JW (1994) Role of the 5-HT2 receptor in the methamphetamine-induced neurochemical alterations. J Pharmacol Exper Ther 270:97-103.

Kalivas PW, Duffy P (1989) Similar effects of daily cocaine and stress on mesocortical dopamine neurotransmission in the rat. Biological Psychiatry 25:913-928.

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Br Res Rev 16:223-244.

Koide T, Wieloch TW, Siesjo BK (1986) Chronic dexamethasone pretreatment aggravates ischemic neuronal necrosis. J Cerebr Blood Flow Metabol 6:395-404.

Lowy MT, Gault L, Yamamoto BK (1993) Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. J Neurochem 61:1957-1960.

MacLennan AJ, Maier SF (1983) Coping and the stress-induced potentiation of stimulant stereotypy in the rat. Science 219:1091-1093.

Marek GJ, Vosmer G, Seiden LS (1990) Dopamine uptake inhibitors block long-term neurotoxic effects of methamphetamine upon dopaminergic neurons. Br Res 513:274-279.

Matuszewich L, Yamamoto BK (2001) Chronic unpredictable stress produces persistent DOI-induced hyperthermia in rats. In: Society for Neuroscience. SanDiego, CA.

Metzger RR, Haughey HM, Wilkins DG, Gibb JW, Hanson GR, Fleckenstein AE (2000) Methamphetamine-induced rapid decrease in dopamine transporter function: role of dopamine and hyperthermia. J Pharmacol Exper Ther 295:1077-1085.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui D-H, Tabira T (2000) Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci 20:1568-1574.

Nash JF, Yamamoto BK (1992) Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. Br Res 581:237-243.

Ortiz J, Fitzgerald LW, Lane S, Terwilliger R, Nestler EJ (1996) Biochemical adaptations in the mesolimbic dopamine system in response to repeated stress. Neuropsychopharmacol 14:443-452.

Ossowska G, Nowak G, Kata R, Klenk-Majewska B, Danilszuk Z, Zebrowska-Lupina I (2001) Brain monoamine receptors in chronic unpredictable stress model in rats. J Neural Trans 108:311-319.

Papp M, Klimek V, Willner P (1994) Effects of imipramine on serotonergic and beta-adrenergic receptor binding in a realistic animal model of depression. Psychopharmacol 114:309-314.

Piazza PV, LeMoal M (1998) The role of stress in drug self-administration. Trends in Pharmacological Sciences 19:67-74.

Pu C, Fisher JE, Cappon GD, Vorhees CV (1994) The effects of amfonelic acid, a dopamine uptake inhibitor, on methamphetamine-induced dopaminergic terminal degeneration and astrocytic response in rat striatum. Br Res 649:217-224.

Ricaurte GA, McCann UD (1992) Neurotoxic amphetamine analogues: effects in monkeys and implications for humans. Ann NY Acad Sci 648:371-382.

Ricaurte GA, Schuster CR, Seiden LS (1980) Long-term effects of repeated methamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. Br Res 193:153-163.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Br Res Rev 11:157-198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive sensitization theory of addiction. Br Res Rev 18:247-291.

Rouge-Pont F, Marinelli M, LeMoal M, Simon H, Piazza PV (1995) Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. J Neurosci 15:7189-7195.

Salmi P, Ahlenius S (1998) Evidence of functional interactions between 5-HT_{1A} and 5-HT_{2A} receptors in rat thermoregulatory mechanisms. Pharmacol Toxicol 82:122-127.

Sapolsky R, Pulsinelli WA (1985) Glucocorticoids potentiate ischemia injury to neurons: therapeutic implications. Science 229:1397-1340.

Sapolsky RM (1985) A mechanism for glucocorticoid toxicity in the hippocampus: increased neuronal vulnerability to metabolic insults. J Neurosci 5:1228-1232.

Sapolsky RM (1999) Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. Exper Gerontol 34:721-732.

Schmidt CJ, Sonsalla PK, Hanson GR, Gibb JW (1985) Role of dopamine in the neurotoxic effects of methamphetamine. J Pharmacol Exper Ther 233:539-544.

Seiden LS, Fishman MW, Schuster CR (1975/76) Long-term methamphetamine induced changes in brain catecholamines in tolerant rhesus monkeys. Drug and Alcohol Dependence 1:215-219.

Sonsalla PK, Gibb JW, Hanson GR (1986) Roles of D1 and D2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems. J Pharmacol Exper Ther 238:932-937.

Sonsalla PK, Nicklas WJ, Heikkila RE (1989) Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. Science 243:398-400.

Sonsalla PK, Riordan DE, Heikkila RE (1991) Competitive and noncompetitive antagonists at N-methyl-D-aspartate receptors protect against

methamphetamine-induced dopaminergic damage in mice. J Pharmacol Exper Ther 256:506-512.

Sorg BA, Kalivas PW (1991) Behavioral and neurochemical cross-sensitization between foot shock stress and cocaine. Br Res 559:29-36.

Stein B, Sapolsky R (1988) Chemical adrenalectomy reduces hippocampal damage induced by kainic acid. Br Res 473:175-180.

Stein-Behrens B, Mattson MP, Yeh CM, Sapolsky R (1994) Stress exacerbates neuron loss and cytoskeletal pathology in the hippocampus. J Neurosci 14:5373-5380.

Stephans SE, Yamamoto BK (1994) Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. Synapse 17:203-209.

Stephans SE, Yamamoto BK (1995) Effect of repeated methamphetamine administrations on dopamine and glutamate efflux in rate prefrontal cortex. Br Res 700:99-106.

Stephans SE, Whittingham TS, Douglas AJ, Lust WD, Yamamoto BK (1998) Substrates of energy metabolism attenuate methamphetamine-induced neurotoxicity in striatum. J Neurochem 71:613-621.

Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4:289-312.

Sulzer D, Rayport S (1990) Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. Neuron 5:797-808.

Thome J, Pesold B, Baader M, Hu M, Gerwitz JC, Duman RS, Henn FA (2001) Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus. Biological Psychiatry 50:809-812.

Tsuchiya K, Inoue T, Koyama T (1996) Effect of repeated methamphetamine pretreatment on freezing behavior induced by conditioned fear stress. Pharmacol Biochem Behav 54:687-691.

Villemagne C, Yuan J, Wong DF, Dannals RF, Hatzidimitriou G, Matthews WB, H.T. R, Musachio J, McCann UD, Ricaurte GA (1998) Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: evidence from [^{11}C]WIN-35,428 positron emission topography studies and direct in vitro determinations. J Neurosci 18:419-427.

Wallace TL, Gudelsky GA, Vorhees CV (2001) Neurotoxic regimen of methamphetamine produces evidence of behavioral sensitization in the rat. Synapse 39:1-7.

Willner P (1984) The validity of animal models of depression. Psychopharmacol 83:1-16.

Xie T, McCann UD, Kim S, Yuan J, Ricaurte GA (2000) Effect of temperature on dopamine transporter function and intracellular accumulation of methamphetamine: implications for methamphetamine-induced dopaminergic neurotoxicity. J Neurosci 20:7838-7845.

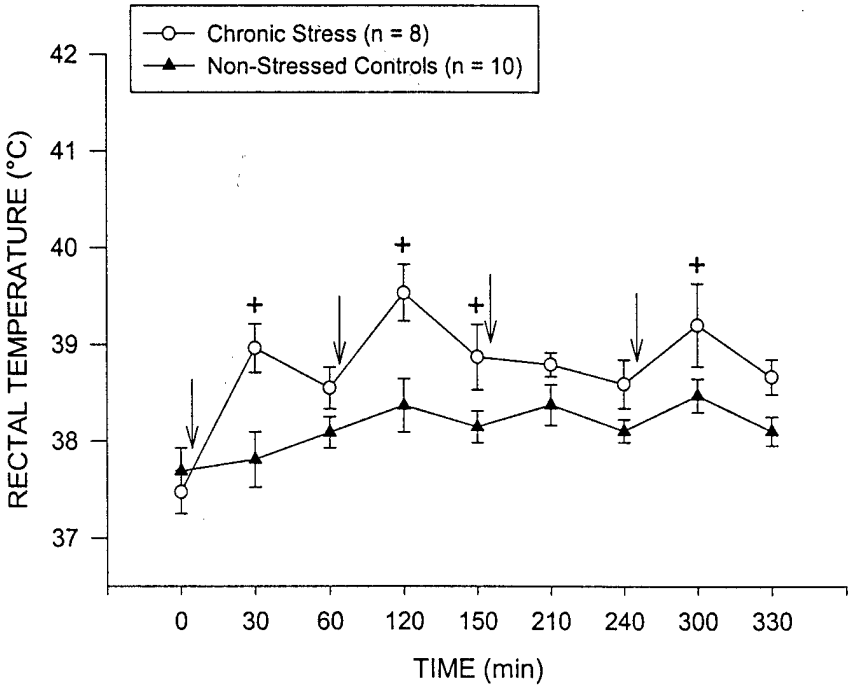
Yamamoto BK, Zhu W (1998) The effects of methamphetamine on the production of free radicals and oxidative stress. J Pharmacol Exper Ther 287:107-114.

Yamamoto BK, Nash JF, Gudelsky GA (1995) Modulation of methyelendioxymethamphetamine-induced striatal dopamine release by the interaction between serotonin and γ-aminobutyric acid in the substantia nigra. J Pharmacol Exper Ther 273:1063-1070.

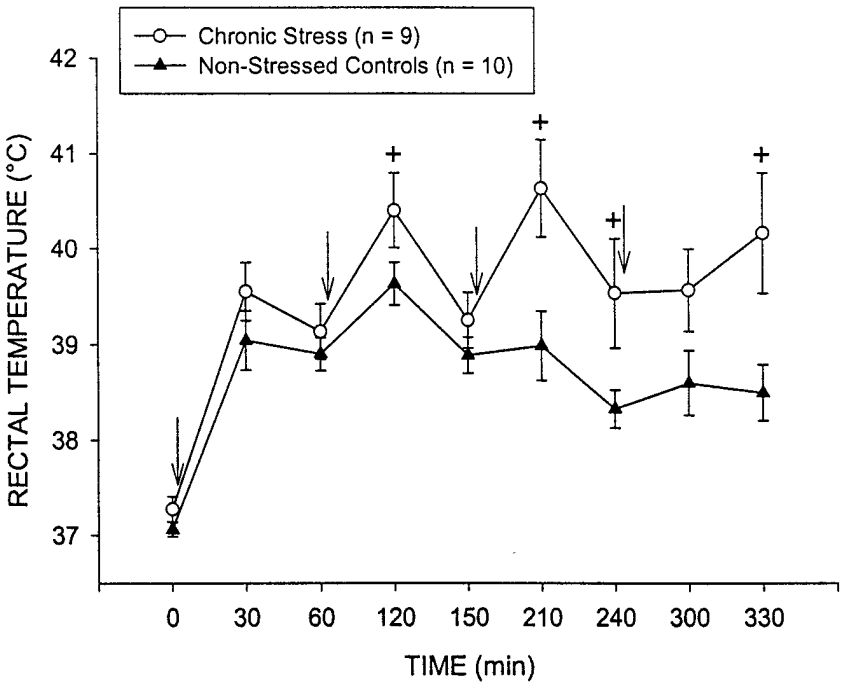
Yui K, Goto K, Ikemoto S, Ishiguro T, Kamada Y (1999) Increased sensitivity to stress and episode recurrence in spontaneous recurrence of methamphetamine psychosis. Psychopharmacol 145:267-272.

Yui K, Goto K, Ikemoto S, Ishiguro T, Kamata Y (2001) Increased sensitivity to stress in spontaneous recurrence of methamphetamine psychosis: noradrenergic hyperactivity with contribution from dopaminergic hyperactivity. J Clin Psychopharmacol 20:165-174.

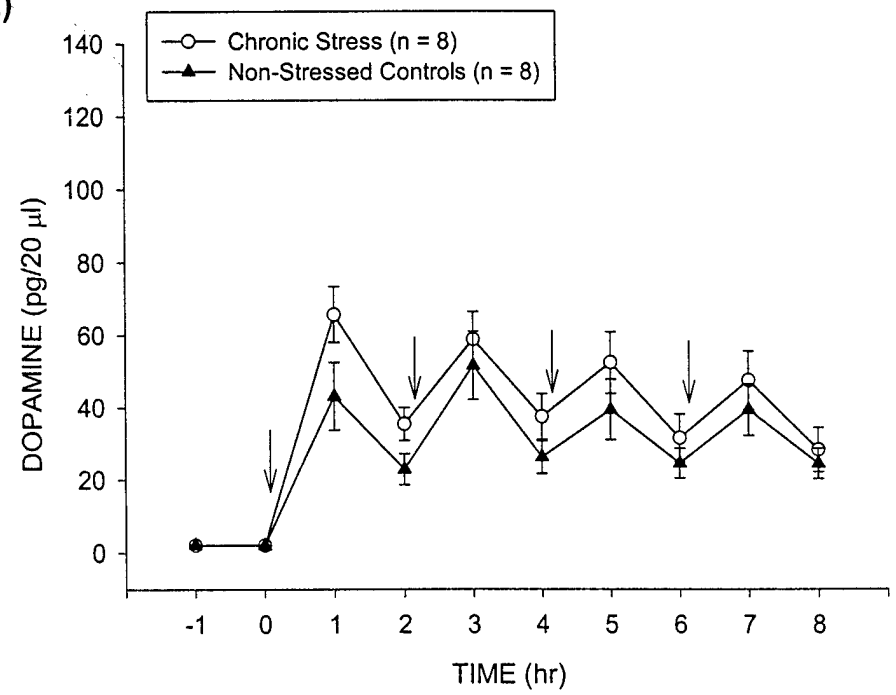
a)


Chronic Stress (n = 8)
Non-Stressed Controls (n = 10)
RECTAL TEMPERATURE (°C)
42
41
40
39
38
37
0
30
60
120
150
210
240
300
330
TIME (min)

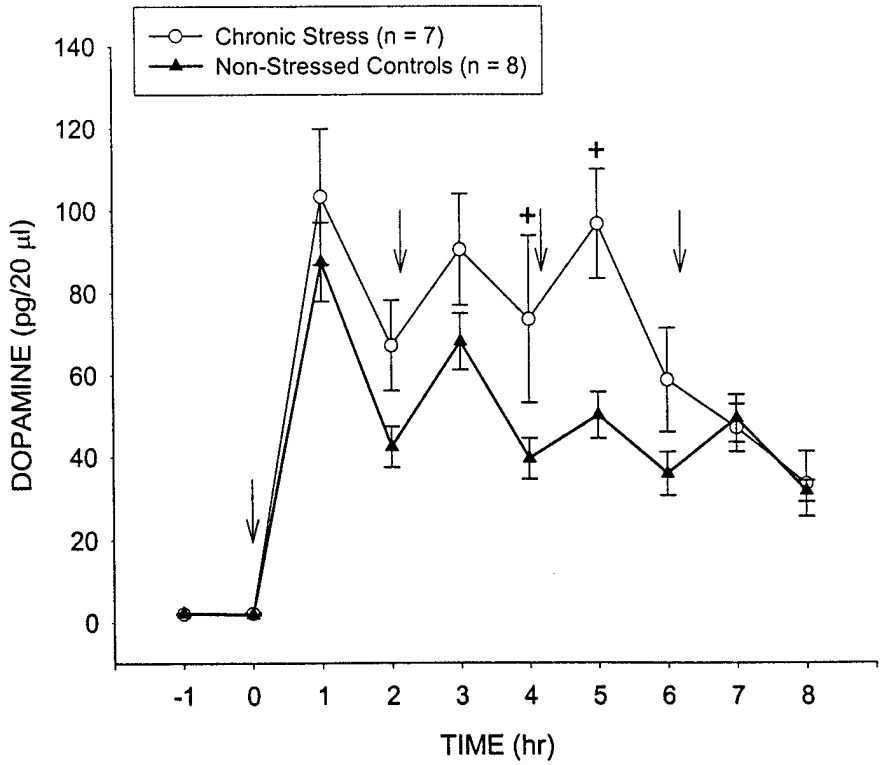
b)


Chronic Stress (n = 9)
Non-Stressed Controls (n = 10)
RECTAL TEMPERATURE (°C)
42
41
40
39
38
37
0
30
60
120
150
210
240
300
330
TIME (min)

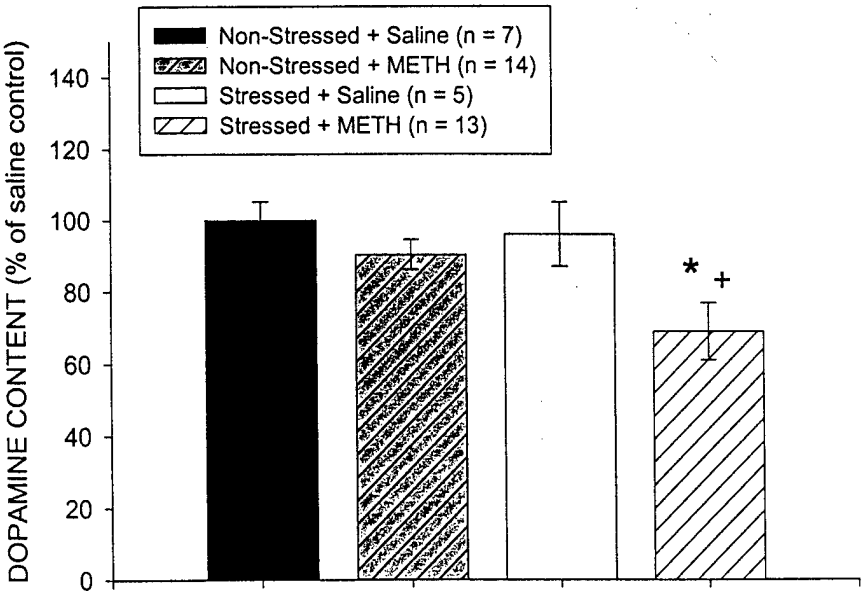
a)


Chronic Stress (n = 8)
Non-Stressed Controls (n = 8)
DOPAMINE (pg/20 µl)
140
120
100
80
60
40
20
0
-1
0
1
2
3
4
5
6
7
8
TIME (hr)

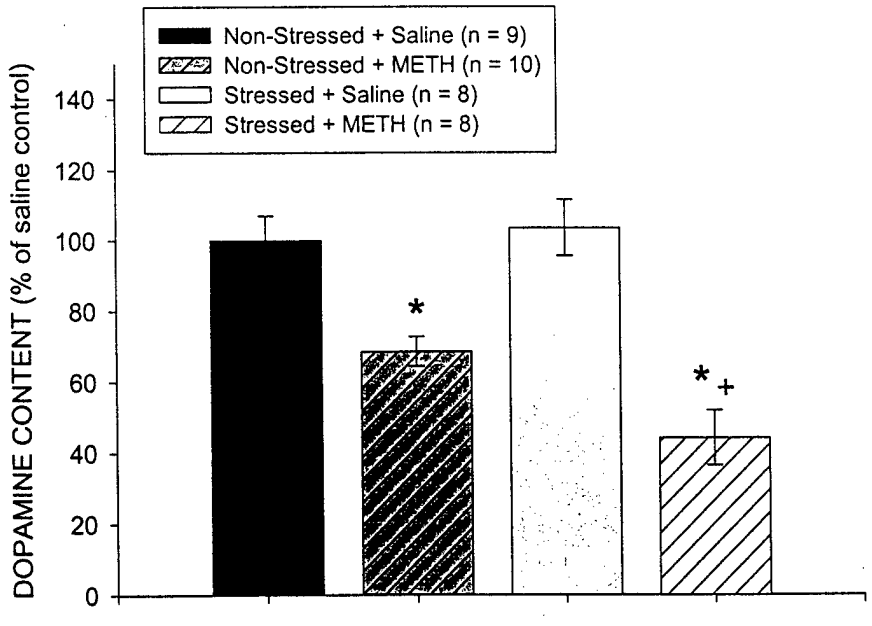
b)

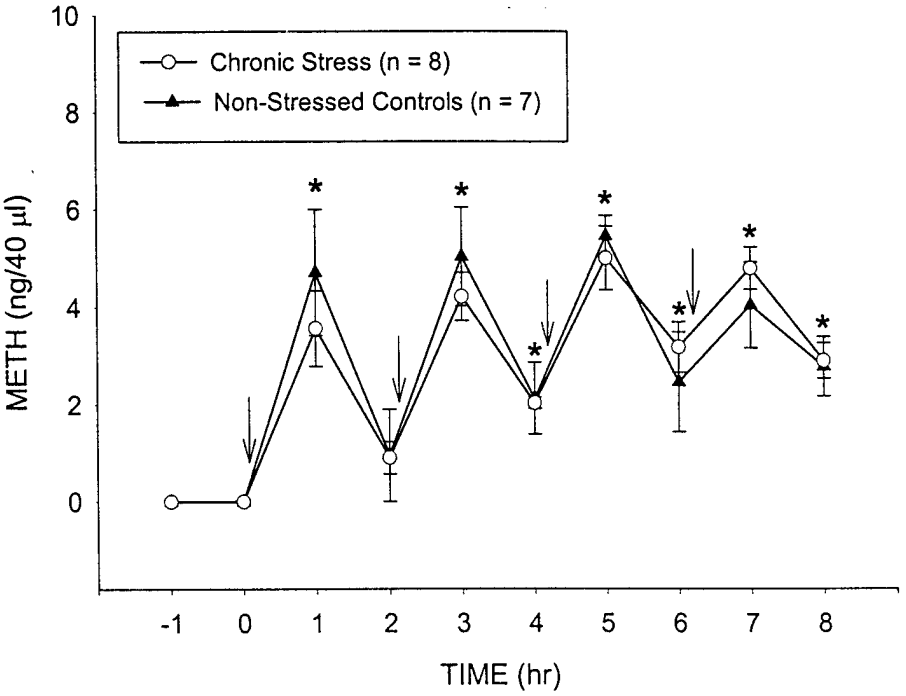

Chronic Stress (n = 7)
Non-Stressed Controls (n = 8)
DOPAMINE (pg/20 µl)
140
120
100
80
60
40
20
0
+
+
-1
0
1
2
3
4
5
6
7
8
TIME (hr)

a)


Non-Stressed + Saline (n = 7)
Non-Stressed + METH (n = 14)
Stressed + Saline (n = 5)
Stressed + METH (n = 13)
DOPAMINE CONTENT (% of saline control)
0
20
40
60
80
100
120
140
* +

b)


Non-Stressed + Saline (n = 9)
Non-Stressed + METH (n = 10)
Stressed + Saline (n = 8)
Stressed + METH (n = 8)
DOPAMINE CONTENT (% of saline control)
0
20
40
60
80
100
120
140
*
* +


Chronic Stress (n = 8)
Non-Stressed Controls (n = 7)
METH (ng/40 µl)
10
8
6
4
2
0
-1
0
1
2
3
4
5
6
7
8
TIME (hr)