The effects of repeated MDMA (3,4-methylenedioxymethamphetamine) administration on neurotransmitter efflux and sensory-evoked discharge in the ventral posterior medial thalamus

Starr M.A, Page M.E., Waterhouse B.D

Drexel University College of Medicine

Laboratory of Barry D. Waterhouse, Ph.D (B.D.W., M.E.P., M.A.S.)
Effects of MDMA on sensory processing in VPM thalamus

Barry D. Waterhouse
Drexel University College of Medicine
2900 Queen Lane; Department of Neurobiology and Anatomy
Philadelphia, PA 19129
1(215)991-8411 (work)
1(215)843-9082 (fax)
waterhouse@drexelmed.edu

7 Figures
39 pages
Abstract: 250 words
Introduction: 496 words
Discussion: 1460 words
References: 30

Recommended section assignment: Neuropharmacology

**List of Abbreviations**
- aCSF: artificial cerebrospinal fluid
- CNS: central nervous system
- EDTA: ethylene diamine tetraacetic acid
- MDMA: 3,4-methylenedioxyamphetamine
- MDA: methylenedioxyamphetamine
- NET: norepinephrine transporter
- PSTH: post stimulus time histogram
- PTSD: Post-Traumatic Stress Disorder
- SED: sensory-evoked discharge
- SERT: serotonin transporter
- SFR: spontaneous firing rate
- VPM: ventral posterior medial
- WED: whisker-evoked discharge

**List of Chemical Compounds**
- Butyric Anhydride: (CH₃CH₂CH₂CO)₂O
- Chloral Hydrate: C₂H₃Cl₃O₂
- 4-dimethylaminopyridine: C₇H₁₀N₂
- Halothane: C₂HBrClF₃
- Hydrochloric Acid: HCL
- Isopropanol: C₃H₈O
- MDMA: C₁₁H₁₅NO₂
- Methanol: CH₃OH
- Methylene Chloride: CH₂Cl₂
- Methylene Chloride: CH₂Cl₂
- Norepinephrine: C₈H₁₁NO₃
- Petroleum Ether: C₆H₆
- Propylamphetamine: C₁₂H₁₉N
- Serotonin: C₁₀H₁₂N₂O
- Sodium Chloride: NaCl
- Sodium Hydroxide: NaOH
- Sodium Pentobarbital: C₁₁H₁₈N₂O₃
- Toluene: C₇H₈ (C₆H₅CH₃)
Abstract

MDMA is known to enhance tactile sensory perception, an effect that contributes to its popularity as a recreational drug. The neurophysiological basis for MDMA’s effects on somatosensation are unknown. However, MDMA interactions with the serotonin transporter (SERT) and subsequent enhancement of serotonin neurotransmission are well known. The rat trigeminal somatosensory system receives serotonergic afferents from the dorsal raphe nucleus. Since these fibers express SERT, they should be vulnerable to MDMA-induced effects. We found that administration of a challenge injection of MDMA (3 mg/kg i.p.) following repeated MDMA treatment (3 mg/kg per day; 4 days) elicits both 5-HT and NE efflux in the VPM thalamus of Long Evans Hooded rats, the main relay along the lemniscal portion of the rodent trigeminal somatosensory pathway. We evaluated the potential for repeated MDMA administration to modulate whisker-evoked discharge of individual neurons in this region. After surgically implanting stainless steel 8-wire multi-channel electrode bundles, we recorded spike train activity of single cells while activating the whisker pathway using a piezoelectric mechanical stimulator. We found that repeated MDMA administration increased spontaneous firing rate, but reduced both magnitude and duration of whisker-evoked discharge in individual VPM thalamic neurons. The timecourse of drug action on neuronal firing patterns was generally consistent with fluctuations in neurotransmitter efflux as shown from our microdialysis studies. Based on these results, we propose that single use and repeated administration of MDMA may “distort”, rather than enhance, tactile experiences in humans, in part, by disrupting normal spike firing patterns through somatosensory thalamic relay circuits.
Introduction

In human subjects the popular recreational drug ‘ecstasy’ (MDMA) produces powerful, yet poorly understood effects on somatosensation (reviewed in Starr et al, 2008). We reported previously that acute (single dose, naïve subject) systemic administration of MDMA suppresses the transmission of sensory signals through the rat somatosensory thalamus (Starr et al, 2008). Since human users of MDMA rarely consume the drug only once, the present study employed the same rodent model to examine the effects of repeated, MDMA administration on serotonin (5-HT) and norepinephrine (NE) efflux and somatosensory-evoked discharge in the ventral posterior medial nucleus (VPM) of rat thalamus.

In the previous report (Starr et al., 2008) we found that acute MDMA administration led to a significant, rapid dose-dependent increase of 5-HT levels in the VPM thalamus of waking male Long-Evans Hooded rats. However, acute administration caused increased NE efflux in this region only at the highest dose tested (10 mg/kg). In conjunction with these results we found that, injection of 3 mg/kg MDMA leads to significant increases in plasma levels of MDMA and its major metabolite MDA. In addition, we determined that acute (3 mg/kg) MDMA administration led to decreased responsiveness of individual neurons to whisker stimulation.

The methods used in the present study are identical to those described in the previous report (Starr et al., 2008) with the exception of the repeated drug regimen. Briefly, we used the rat trigeminal somatosensory pathway as a model to investigate the effects of repeated MDMA administration on 5-HT and NE efflux, and sensory-evoked discharge (SED). This system transmits tactile information from the rat’s facial whiskers
to the contralateral somatosensory cortex (SSC). Sensory information from individual whiskers is first relayed to the brainstem and then projected to the contralateral VPM thalamus. Whisker-related information is then relayed to the SSC. All regions along this pathway receive serotonergic and noradrenergic projections (Simpson et al., 1997; Kirifides et al., 2001), and thus are vulnerable to the pharmacological effects of MDMA.

We found that administration of a challenge injection (3 mg/kg i.p.) of MDMA following repeated MDMA treatment elicits both 5-HT and NE efflux in the VPM thalamus. Repeated systemic MDMA administration led to decreases in magnitude and timing of individual sensory neuron responses to whisker stimulation. Importantly, following repeated MDMA administration the effects of the drug on spontaneous firing rate are blunted compared to acute, single dose treatment. The net effect of suppressed evoked discharge and increased spontaneous firing is a pronounced reduction in the signal to noise ratio for sensory signals. Therefore, it appears that the net effect of both single and repeated MDMA administration is to reduce neuronal responsiveness to sensory afferents. Overall, there is evidence of changes in response to MDMA or baseline neuronal activity that suggest persistent drug effects in repetitively treated animals. Although psychophysical studies are needed, we speculate that the blunted electrophysiological effects observed following repeated MDMA treatment could potentially help explain tolerance to MDMA’s positive effects as reported by many human MDMA users (Parrott, 2005).

Methods
**Animals.** Male, Long-Evans Hooded rats (Charles River Laboratories, Wilmington, Mass.) weighing 250-300g at the start of the experiment were housed individually with *ad libitum* access to food and water. The animal facility was maintained at 21 degrees Celsius with a 12:12 hour light: dark cycle with the light period beginning at 07.00h. All procedures were conducted in accordance with the guidelines published in the *NIH Guide for Care and Use of Laboratory Animals.* All protocols were approved by the Drexel University College of Medicine Institutional Animal Care and Use Committee.

**Dialysis Probe Construction and Calibration.** Vertical concentric microdialysis probes were utilized (Abercrombie et al. 1988). Briefly, a piece of fused silica (Polymicro Technologies, Phoenix, Ariz.) was inserted through PE10 tubing (Clay Adams, Parsippany, N.J.) and a semi permeable membrane of hollow cuprammonium rayon fibers with a 224-μm o.d. and 35,000-MW cutoff (C series; Terumo Corp., Somerset, N.J.) was fixed over the fused silica into the PE10 tubing with epoxy. The open end of the dialysis fiber was sealed with a 0.5-mm epoxy plug and a region was coated with epoxy, leaving an active area of 1mm for exchange across the membrane. The *in vitro* recovery rate was determined by placing the probe in a beaker filled with artificial cerebrospinal fluid (aCSF) containing a known concentration of NE or 5-HT standard. The concentration of NE or 5-HT in the perfusate was compared with the amount in the bath. Probes that did not correspond to the typical range of recovery (11-21%) were identified and eliminated. Because the diffusion properties of neurochemicals in brain tissue are likely different from *in vitro* conditions, dialysate values reported were not corrected for the recovery of the probe.

**Surgeries.**
Microdialysis Studies. Animals were allowed to acclimate to the animal facility for at least one week prior to surgery. The day before an experiment, rats were anesthetized with halothane (0.75% in oxygen), positioned in a stereotaxic apparatus with the skull flat, and allowed to breathe spontaneously. Under anesthesia, microdialysis probes were surgically implanted into the VPM (AP-3.5, ML-2.9, DV-5.5) thalamus. The rats were then placed in cylindrical Plexiglas containers lined with bedding material, connected to a liquid swivel by a spring tether, and allowed to recover overnight.

Electrophysiology Studies. Adult male (150-300gm) Long Evans hooded rats were anesthetized with sodium pentobarbital (17.5 mg/kg, i.p.) and choral hydrate (400 mg/kg, i.p.). The animal’s body temperature was monitored using a rectal probe and was maintained at 37 degrees Celsius by a heating pad. Anesthesia was maintained throughout the surgery such that animals were not responsive to foot pinch, the blinking reflex was absent, and the breathing was slow and regular. Supplemental doses of sodium pentobarbital and choral hydrate were alternately administered as needed. Rats were positioned in a stereotaxic apparatus with the skull flat. An incision was made down the scalp, and the skull and dura mater overlying the VPM thalamus was removed to expose the brain tissue. Lidocaine gel was used to locally anesthetize incised skin. A small hole was drilled in the skull, centered 3.5 mm posterior and 2.9 mm lateral to bregma. Four stainless steel screws were fixed to the skull to serve as electrical grounds and anchors for cementing the electrodes. Microelectrode bundles (8 microwires per bundle) were implanted in the VPM thalamus. The microelectrode bundles were placed initially using stereotaxic coordinates (A3.5, L2.9, DV5.5); however the final position of each bundle was determined using electrophysiological verification that the site contained
cells that met established criteria for VPM thalamic neurons. Microelectrode bundles were permanently attached to the head using a connector and dental cement. The wound was sutured and the animal was administered topical antibiotics in order to prevent infection. All animals were allowed to recover for one week before the beginning of unit recording sessions.

**Repeated Drug Administration and Microdialysis Experiments.** We utilized a repeated drug administration protocol similar to that described by Kalivas et al. (1998). Animals were injected with MDMA (3mg/kg) i.p. once per day for 4 days. Importantly, i.p. injection of 3 mg/kg MDMA results in peak plasma levels of MDMA (322.5 ± 19.738) that are similar to those seen in humans (223 ± 48 ng/ml human) following an oral dose of 100mg (Pizarro et al., 2002). On day 5, microdialysis probes were surgically implanted into the VPM thalamus (as described above). Baseline sample collection began the following morning (day 6). Rats received a challenge injection of MDMA (3mg/kg, i.p.) or vehicle following 2 hours of baseline sampling. Diasylate samples were collected every 20 minutes for 7 hours and frozen at -80 degrees Celsius for subsequent HPLC analysis.

**HPLC**

**5-HT Detection and Quantification.** Samples of 15-μl (each sample was divided in half in order to analyze both 5-HT and NE) were injected directly onto the HPLC column using an autosampler (model 542 ESA, Chelmsford, MA). The HPLC consisted of an ESA solvent delivery system, and an ESA column (model MD-150; 150 X 2mm). The
mobile phase consisted of 32 mM NaH2PO4, 0.67 μM ethylene diamine tetraacetic acid (EDTA), 0.43 mM octyl sulfate, and 19% methanol adjusted to a pH of 5.6. The detection system consisted of an ESA Coulochem II electrochemical detector with two electrodes in series – the guard cell set at +150 mV, and the compounds of interest quantified at the analytical cell (model 5041, ESA, Chelmsford, MA) which was set at +500 mV. Peak heights were measured and compared with the peak height of a 10⁻⁸-M standard calibrated daily. The detection limit, defined as the sample amount producing a peak height that was twice the height of background noise, was approximately 0.5pg 5-HT.

**NE Detection and Quantification.** Samples of 15-μl were injected directly onto the HPLC column using an autosampler (model 542 ESA, Chelmsford, MA.). The HPLC consisted of an ESA solvent delivery system, and an ESA column (model MD-150; 150 X 2mm). The mobile phase consisted of 60mM sodium phosphate buffer (pH 4.2) with 100μM EDTA, 1.5mM sodium octyl-sulfate, and 3.5% (v/v) methanol. The detection system consisted of an ESA Coulochem II electrochemical detector with two electrodes in series – the applied potential of the guard cell set at −150mV and the compounds of interest quantified at the analytical cell (model 5041, ESA, Chelmsford, MA) which was set at +220 mV. Peak heights were measured and compared with the peak height of a 10⁻⁸-M standard calibrated daily. The detection limit, defined as the sample amount producing a peak height that was twice the height of background noise, was approximately 0.5pg NE.
Extracellular Unit Recordings. After one week recovery animals were anesthetized with isoflurane, positioned in a stereotaxic apparatus, and allowed to breathe spontaneously. Previously implanted microelectrode bundles were used to record the extracellular activity from ensembles of single neurons in the VPM thalamus. Output from the electrodes was amplified and displayed on an oscilloscope and sent to a window discriminator for spike isolation. Neuronal activity was recorded from the eight microelectrodes simultaneously using the multi-channel acquisition processor (MAP) hardware and real-time acquisition system programs for unit timing in neuroscience (RASPUTIN) from Plexon Inc (Dallas, TX, USA). Data acquisition parameters (amplification, filtering, and threshold of detection) were set independently for each channel. Multiple methods for real-time spike sorting online were available, such as time-voltage window discrimination and template based discrimination. Alternatively, we stored all waveforms that crossed the detection threshold and sorted them later using offline sorter. Output from the window discriminator was sent to a digital computer in order to build real-time post stimulus time histograms (PSTHs) and raster records of neuronal activity.

Single-unit recordings in our data were verified off-line by analyzing waveforms, as well as interspike interval histograms. A recording was identified as a single-unit if it met all three of the following criteria: (i) the presence of reproducible waveforms; (ii) interspike interval histograms showing that <5% of the interspike intervals shorter than 1.2ms; and (iii) the amplitude of the waveform being analyzed being at least three times the average background noise levels.

We generated peri-stimulus time histograms (PSTHs) in NeuroEXplorer (Plexon, Dallas, TX, USA), using the whisker pad stimulation as the reference event and a bin
width of 1ms. Two hundred, 2 second trials (20 minutes total) were used to build PSTHs.

Each recording session consisted of one twenty minute control period, one twenty minute period immediately following a saline (0.09%, i.p.) injection, and eleven twenty minute periods following a challenge injection of MDMA (3 mg/kg, i.p.). All animals received repeated MDMA treatment (3mg/kg, i.p. once per day x 4 days). The duration of the chronic recordings was determined from the results of our microdialysis studies. We chose recording periods based on the timecourse of MDMA-induced increases in extracellular 5-HT and NE.

The response to the whisker deflection (see below) usually consisted of a short latency excitation (E1), followed by a long latency excitation (E2). Numerical data from PSTHs were sent to MatLab (The Mathworks, Inc., Natick, MA) for the determination of the amplitude of the whisker evoked response and the spontaneous firing rate (SFR) during 400ms preceding whisker deflection. The amplitude of E1 (PeakE1) was defined as the maximum amplitude during the first 25ms following whisker stimulation minus the SFR. The onset, offset, latency, and duration of whisker-evoked responses were determined using a Gaussian 95% confidence interval. The onset (OnsetE1) and offset (OffsetE1) of the whisker-evoked response was defined as the time where the firing rate increased and decreased above and below the 95% confidence interval limit, respectively. The duration of E1 (DurE1) was determined by subtracting the OnsetE1 from OffsetE1. The latency of E1 (LatE1) was defined as the time in milliseconds (beginning 2ms after whisker stimulation to account for any potential artifact from the whisker stimulator) that it took to reach to the peak amplitude of the E1 response. The onset of E1 occurred
between 4 and 9ms following whisker stimulation and the latency of E1 was between 4 and 20ms.

Thalamic field potentials were collected throughout the duration of each recording in order to determine the effects of MDMA administration on the animal’s level of arousal. Power Spectrum Analysis was performed for each 20 minute interval using a maximum value of 100 Hz and 128 frequency values.

**Vibrissae Stimulation.** Somatosensory afferent pathways were activated by mechanical displacement of single whiskers on the side of the muzzle contralateral to recording sites in the VPM thalamus. Initially, a cell’s receptive field was characterized by deflecting individual whiskers with a hand-held probe while listening to spike discharge on an audio monitor. Following initial characterization of a cell’s receptive field, a multi-angular piezoelectric stimulator was used to produce uniform displacement of individual whiskers. Activation of the piezoelectric stimulator triggered computer collection of spike train data. This device has the ability to reliably generate a range of small whisker deflections (1-1000 micron) in any direction (360 degrees) or velocity (1-1440 deg/sec) of motion as a linear function of input voltage. In order to determine whether MDMA administration differentially effects responsiveness of VPM thalamic neurons to whisker stimulation, we stimulated the primary whisker using two randomly presented stimulation intensities that we termed ‘medium’ and ‘high’. Medium and high intensity whisker stimulation caused whisker deflection of ~200 and 500 microns, respectively. Deflection of the whisker with the medium intensity stimulus produced a less robust response in VPM thalamic neurons compared to high intensity whisker stimulation. In all cases,
single whisker deflection was achieved without perturbation of surrounding whiskers. For detailed information on this device, see Armstrong-James and Fox (1987).

**Histology.** After each experiment, the probe location was marked by infusion of 2% pontamine sky blue dye through the microdialysis probe. Following euthanasia the brains were removed, sectioned, and stained with neutral red for histological verification of probe or electrode placement within the VPM thalamus (See Fig. 1A).

**Data Analysis**

**Microdialysis Experiments.** The baseline value against which MDMA administration was compared was derived from the average of three samples immediately prior to drug injection. Extracellular neurotransmitter levels are expressed as the mean + S.E.M. The overall effect of MDMA on 5-HT and NE efflux, and locomotor activity were analyzed using two-way analysis of variance (ANOVA) with repeated measures. The absolute amount of neurotransmitter measured in dialsylates (pg/15μl) was used as the dependent variable for assessment of within group effects, and the absolute change was measured using a 1-way ANOVA with a Dunnett’s post hoc test.

**Electrophysiology Experiments.** Computer generated PSTHs and cumulative raster records were used to characterize stimulus-evoked responses and to quantitate evoked activity as both spikes/stimulus (excitations only), and percentage of baseline spontaneous firing rate (excitations and inhibitions). Rasters and histograms were
generated preceding and following i.p. administration of MDMA. Equal numbers of stimuli were used to compute each histogram.

Changes in evoked and spontaneous activity were calculated for each cell by comparing discharges in identical portions of histograms computed during saline and MDMA post-injection periods. Spontaneous and evoked discharge rates were calculated as described above. These rates were computed from control and post-MDMA injection histograms, compared, and the difference expressed as percentage increase or decrease from control response. Spikes per stimulus comparisons were made between histograms using a similar method. Changes in stimulus bound activity between saline and MDMA post-injection periods were assessed for statistical significance using one-way ANOVA. Such analytical procedures have been used previously in our laboratory to define 5-HT and NE actions on rodent somatosensory and visual cortical neurons.

Results

Effects of repeated MDMA administration on 5-HT efflux in VPM thalamus. A challenge injection of MDMA following repeated systemic MDMA administration (3 mg/kg once per day x 4 days) led to a rapid prolonged increase in 5-HT efflux in the VPM thalamus (F(6,15) = 13.53; p = 0.0001; one-way repeated measures ANOVA (See Fig. 1B). MDMA (3 mg/kg) increased the concentration of extracellular 5-HT in diasylates from 1.337 ± 0.145 pg/15μl to 4.145 ± 0.649 pg/15μl. These effects were evident within 20 minutes of drug administration. 5-HT levels peaked 40 minutes following MDMA administration. Levels returned to baseline within 3 hours and 20 minutes (Dunnett’s; p < .05). Thus, a challenge injection of MDMA following repeated
treatment leads to prolonged increases in 5-HT efflux compared to single treatment animals (See Fig. 2A) and - Starr et al., 2008). Extracellular levels of 5-HT did not change significantly after saline injection (1.341 ± 0.250 pg/15μl to 1.416 ± 0.228 pg/15μl) (See Fig. 1B).

**Effects of repeated MDMA administration on NE efflux in VPM thalamus.** A challenge injection of MDMA following repeated systemic MDMA administration led to a rapid prolonged increase in NE efflux in the VPM thalamus (F(5,15) = 19.31; p = 0.0001; one-way repeated measures ANOVA (See Fig. 1C). MDMA (3 mg/kg) increased the concentration of extracellular NE in dialysates from 1.338 ± 0.171 pg/15μl to 4.715 ± 1.205 pg/15μl. These effects were evident within 20 minutes of drug administration and peaked 1 hour and 20 minutes following MDMA administration. NE levels returned to baseline within 3 hours and 20 minutes (Dunnett’s; p < .05). Extracellular levels of NE did not change significantly after saline injection (1.326 ± 0.291 pg/15μl to 1.459 ± 0.518 pg/15μl). (See Fig. 1C). Thus, administration of a challenge injection following repeated drug treatment elicits both 5-HT and NE efflux in the VPM thalamus. This was in contrast to the selective increase in 5-HT efflux that was observed following a single injection of the same dose (3 mg/kg, i.p.) in naïve animals (See Fig. 2A and 2B – Starr et al, 2008). In addition, a challenge injection of MDMA following repeated low-dose treatment produces similar effects on NE efflux as a single acute high-dose (10 mg/kg, i.p.) injection of MDMA (See Fig. 2B and Starr et al., 2008).
Effects of repeated MDMA administration on VPM thalamic unit responsiveness (Peak E1) to whisker deflection. A challenge injection of MDMA (3 mg/kg) following repeated MDMA treatment (3mg/kg x 4 days) led to a reduction in the magnitude of short latency responses (peak E1) to medium and high intensity whisker stimulation (F(66,12) = 4.783; p<.0001; one-way ANOVA); (F(66,12) = 3.844; p<.0001; one-way ANOVA), respectively (See Fig. 3A and 3B). Unlike naïve animals treated with a single dose of the drug, MDMA’s suppressant effects on whisker responsiveness to high intensity whisker stimulation (n=7 animals; 45 cells; 67 responses) were blunted following repeated drug treatment (See Fig. 5A). Peak E1 was defined as the maximum firing rate that occurs from 2 – 25 milliseconds following stimulus onset minus the spontaneous firing rate.

Effects of repeated MDMA administration on the spontaneous firing rate (SFR) of neurons in the VPM thalamus. A challenge injection of MDMA following repeated MDMA treatment increased the spontaneous firing rate of individual neurons during medium and high intensity whisker stimulation, followed by a return to control levels (F(37,12) = 2.088; p<.01; one-way ANOVA); (F(44,12) = 2.43; p<.004; one-way ANOVA), respectively (See Fig. 3C). In addition, a challenge injection led to a second significant increase in the spontaneous firing rate 180-220 minutes post-drug (See Fig. 3C). Interestingly, the effects on spontaneous firing were blunted compared to single MDMA treatment conditions (See Fig. 5B). SFR was defined as the average firing rate 400 milliseconds prior to stimulation onset. Scatter plots were generated in order to illustrate the change in Peak E1 and SFR for each unit. Percent change in Peak E1 and SFR were calculated using the following formulas; % change Peak E1 = control Peak E1
– Peak E1 (during peak drug effect) * 100; and % change SFR = control SFR – drug SFR (during peak drug effect) *100, respectively. Following a challenge injection of MDMA the majority of points plotted from this analysis lie above a 45 degree equivalence line indicating cases where MDMA suppressed evoked discharge and increased the spontaneous firing rate of the recorded neuron (See Fig. 4A and 4B).

The effect of repeated MDMA administration on the onset latency of the whisker evoked response. A challenge injection of MDMA following repeated MDMA treatment led to an increase in the onset latency during medium and high intensity whisker stimulation (F(66,12) = 8.345; p<.0001; one-way ANOVA); (F(66,12) = 9.561; p<.0001; one-way ANOVA), respectively (See Fig. 3E). However, the onset latency to medium and high intensity whisker stimulation was shorter during the control period following repeated drug treatment compared to that observed in the single treatment, drug naïve animal (control period on day 1) (t(66) = 2.343; p=.0227); (t(66) = 2.392; p = .0195), respectively (See Fig. 5C). *Onset Latency* was defined as the time in milliseconds from stimulation onset to when the firing rate first crosses the 95% Gaussian confidence interval minus 2 milliseconds to control for stimulation-related artifact.

The effects of repeated MDMA administration on the offset latency of the whisker-evoked response. A challenge injection of MDMA following repeated drug treatment led to a significant decrease in the offset latency after repeated drug treatment, but this effect was observed only during the last 60 minutes of the recording session (Dunnett’s; p<.05) (See Fig. 3F). Overall MDMA’s effects on offset latency appear to be greater in
the single treatment, drug naive animals described in Starr et. al, (2008) (See Fig 5D). Offset Latency was defined as the time in milliseconds from stimulation onset to when the firing rate first falls below the 95% Gaussian confidence interval (after the peak response) minus 2 milliseconds to control for stimulation-related artifact.

The effects of repeated MDMA administration on the duration of the whisker-evoked response. Similar to the single treatment, drug naive animals described in the previous study (Starr et al., 2008), a challenge injection of MDMA following repeated drug treatment also reduced the duration of the whisker-evoked response during medium and high intensity whisker stimulation (F(66,12) = 5.439; p<.0001; one-way ANOVA); (F(66,12) = 10.44; p<.0001; one-way ANOVA), respectively (See Fig. 3D). In order for a decrease in duration of the whisker-evoked response to occur, either the onset latency alone must increase, the offset latency alone must decrease, or the onset latency must increase combined with a decrease in the offset latency. It appears that single MDMA administration reduces the duration of the whisker-evoked response by causing a decrease in the offset latency, while repeated MDMA treatment increases the onset latency of the response (See Fig. 5E). Duration was defined as the Offset latency minus the Onset latency in milliseconds.

The effects of repeated MDMA administration on thalamic field potential activity. Power spectral density (PSD) analysis was performed in order to determine the effects of repeated MDMA administration on thalamic field potential activity. Similar to the effects of single MDMA administration in drug naïve animals, a challenge injection of
MDMA following repeated MDMA administration also led to decreases in the mean PSD values (See Fig 6). In other words, MDMA administration causes a reduction in high frequency field potential activity compared to control periods. This suggests that administration of MDMA to an anesthetized animal causes the animal to transition to a “lighter” level of anesthesia and, as a result, more excitable sensory pathways and increased spontaneous discharge as reported by Chapin et al, (1981). Instead we observed suppression of sensory evoked discharge in the VPM thalamus following MDMA administration. Time had no effect on the mean PSD values in the thalamus (See Fig 7A from Starr et al., 2008).

Discussion

Summary of Findings. First, we found that administration of a challenge injection (3 mg/kg i.p.) of MDMA following repeated MDMA treatment elicits both 5-HT and NE efflux in the VPM thalamus. Importantly, extracellular 5-HT levels remain elevated longer in the repeated treatment rats compared to the single treatment animals as described in the previous report (Starr et al., 2008). Furthermore, the observed increases in NE efflux following challenge injection in repeated treatment animals were similar in time course and magnitude to a single high dose injection of MDMA (10 mg/kg) in naïve animals. Since we conducted the electrophysiology recordings in the anesthetized rat, we performed a series of control microdialysis experiments using an anesthetized preparation in order to ensure that MDMA-induced efflux of 5-HT and NE in the VPM thalamus is comparable to that observed in the waking rat. Importantly, both 5-HT and NE efflux were increased by a challenge dose of MDMA in the VPM thalamus of a repetitively-treated, anesthetized animal (see Supplemental Figure 1).
Second, we demonstrated that a challenge injection of MDMA following repeated systemic MDMA administration leads to decreases in magnitude and timing of individual sensory neuron responses to whisker stimulation. However, the increase in responsiveness to whisker stimulation 160-180 minutes post-drug administration that was observed in the acutely treated animals was absent following a challenge injection of MDMA in animals receiving repeated drug treatment. Interestingly, MDMA’s suppressant effects on whisker responsiveness to high intensity whisker stimulation are blunted following repeated drug treatment. Following repeated MDMA administration the effects of the drug on spontaneous firing rate are also blunted compared to acute, single dose treatment. The picture that emerges is one in which repetitive drug treatment sensitizes neurochemical responses and desensitizes electrophysiological responses in the brain to subsequent drug administration. Nevertheless, as with acute drug administration in naïve animals, the net effect of suppressed evoked discharge and increased spontaneous firing following challenge injections in repetitively treated animals is a pronounced reduction in the signal to noise ratio for sensory signals within the VPM thalamus.

MDMA Pharmacokinetics Following Repeated MDMA Administration In the present study we did not measure plasma levels of MDMA following challenge doses of the drug in repetitively treated animals. However, other studies have reported non-linear pharmacokinetics of MDMA (Baumann et al, 2009; de la Torre et al, 2000; Mueller et al, 2008) and suppressed liver function following repeated MDMA administration (Betia et al, 2000; Cerretani et al, 2011; Moon et al, 2008). In combination after several days of repeated MDMA administration these effects might result in elevated circulating MDMA
levels following challenge drug injection and subsequent enhancement of neurochemical and electrophysiological effects.

**Impact of Repeated MDMA on VPM Neuronal Responses to Sensory Input.** The initial decrease in response latency and decrease in offset latency that was observed in the acutely treated animals was absent following a challenge injection of MDMA after repeated drug treatment. However, similar to acute MDMA administration, repeated drug treatment also decreases the duration of the whisker-evoked response. In single and repeated treatment animals, MDMA administration decreases the duration of the whisker-evoked response through a reduction in the offset latency and increase in the onset latency, respectively. Therefore, it appears that the net effect of both single and repeated MDMA administration is to reduce neuronal responsiveness to sensory-driven afferent inputs. Furthermore, there is evidence of changes in response to MDMA or baseline neuronal activity that suggest persistent drug effects in repeated treatment animals.

The observation that many of the electrophysiological effects observed with acute drug administration are blunted after repetitive MDMA treatment could help explain tolerance to MDMA’s positive effects as reported by human MDMA users (Parrott, 2005). In humans tolerance to MDMA’s positive effects occurs within the first few drug taking sessions (Greer and Tolbert, 1986); so it is possible that the blunted neurophysiological effects of MDMA following our repeated dosing regimen represents a similar form of tolerance in the rat.
Importantly, our microdialysis studies demonstrated that MDMA selectively increases 5-HT efflux when administered acutely at low doses (3 mg/kg, i.p) and increases both 5-HT and NE efflux in the VPM thalamus when a challenge injection of MDMA (3 mg/kg, i.p. once per day x 4 days) is administered after repeated drug treatment. Therefore, the current study was the first to examine indirectly, via repeated systemic MDMA administration, the electrophysiological effects of simultaneous 5-HT and NE efflux in the VPM thalamus. Since it has been shown previously that 5-HT increases spontaneous firing rate and suppresses sensory-evoked discharge, and since NE has been shown to produce the opposite effects (Waterhouse et al., 1986; 1990; Waterhouse and Woodward, 1980), it is possible that the efflux of both transmitters following challenge doses in repetitively treated animals act to counterbalance each other’s effects on neuronal responsiveness. Such mitigating actions could explain the blunting of electrophysiological effects in repetitively treated rats and some aspects of tolerance to drug effects reported by human recreational users. In general, this effect is consistent with evidence of tolerance to various MDMA effects following repeated drug administration in humans (Parrott, 2005), non-human primates (Fantegrossi, 2008; Frederick et al, 1995) and rats (Baumann et al, 2008; Callaway and Geyer, 1992; Marston et al, 1999; Reveron et al, 2010).

To our knowledge this is the first demonstration of sensitization of noradrenergic neurotransmission following repeated MDMA administration. There is however a previous report (Kalivas et al, 1998) of behavioral (increased motor activity) and neurochemical (dopamine transmission in the nucleus accumbens) sensitization to repeated MDMA administration. These findings are generally consistent with other
reports of changes in catecholamine transmitter responses following repeated exposure to psychostimulant drugs (Cadoni et al., 2000; Pierce and Kalivas, 1998). In our study we observed increased monoamine neurotransmission and blunted electrophysiological responses to MDMA challenge in animals receiving repeated administrations of MDMA. One might expect parallel increases in these measures of brain activity however, the complex interplay and net outcome of drug-induced increases in NE and 5HT neurotransmission on neuronal discharge in intact animals is difficult to predict. As described above increased release of NE following challenge MDMA administration in repetitively treated animals could offset 5HT-mediated effects on spontaneous and evoked discharge, thereby providing the appearance of blunted electrophysiological responses.

**MDMA and Sensory Perception in Human Subjects.** At first glance, it appears that there is a paradox between the results presented here and human reports of MDMA’s effects on tactile sensations. Why do humans report feelings of “enhanced” tactile sensations, when the results here and previously (Starr et al., 2008) show that MDMA administration ultimately results in suppressed responsiveness of VPM thalamocortical neurons to tactile stimulation? While there is considerable risk in extrapolating from single unit recording studies in animal models to drug influences on human perception, there are several points worth mentioning in the context of the current study.

First, as mentioned previously, it is unknown what effects MDMA has on other components of the primary somatosensory pathway, e.g. somatosensory cortical, posterior medial (POm)/nucleus reticularis (nRT) thalamic, and trigeminal ganglion neurons. Despite MDMA’s potent effects on VPM thalamocortical neurons, the
perceived effects of the drug on tactile awareness may emerge from combinatorial effects on multiple brain regions. This is likely considering that MDMA affects neurotransmitter systems (i.e. serotonergic and noradrenergic) that send widespread projections to multiple levels of the neuraxis, including all levels of the trigeminal somatosensory pathway.

Second, it must be taken into account that there could be a discrepancy between human subjective reports and what is actually occurring in sensory circuits following MDMA consumption. Although humans report an “enhanced” sense of tactile awareness following MDMA consumption, the reality may be a “distortion” of tactile sensations deriving from reduced neuronal responsiveness to sensory-driven synaptic input. This distortion may be perceived as enhancement by virtue of the fact that it represents an altered sensory experience but not necessarily an augmented signal. By analogy, humans sometimes report that they can drive a vehicle better when they are intoxicated (Flemen, 1997); but in fact many studies have demonstrated that the opposite is true (Ogden and Moskowitz, 2004; Ferrara et al., 1994).

Third, while we demonstrated MDMA-induced suppression of sensory-evoked VPM thalamic responses, we also showed that the basal discharge of these cells increased. This means that there is a generalized increase in thalamocortical transmission following MDMA administration, albeit non-specific with respect to the stimulus. This increase in the amount of “noise” reaching the cortex could, in part, be responsible for the distorted tactile sensations reported by human recreational users.

**Conclusion.** In summary, both single dose and repetitive administration of MDMA alters levels of monoamine transmitters across broad regions of the brain and specifically modifies transmission of somatosensory signals through the thalamus. A question
remaining for future study is why such actions are not prominently expressed in other sensory modalities.
Author Contributions

Participated in research design: Starr, Page, Waterhouse

Conducted experiments: Starr

Contributed new reagents or analytic tools: N/A

Performed data analysis: Starr

Wrote or contributed to the writing of the manuscript: Starr, Page, Waterhouse


Exp Neurol 67: 11-34.
Footnotes

This work was supported by the National Institute of Health National Institute of Drug Abuse [Grants 1 F31 DA018469-01A, R21 DA023711]
Figure legends

Figure 1. A. Coronal section of the rat brain illustrating electrode placement in the VPM thalamus. Low power photomicrograph demonstrating the tip of the microelectrode bundle in the VPM thalamus. Section was stained with neutral red. B. The effects of repeated systemic administration of MDMA on extracellular 5-HT levels in the rat VPM thalamus. Serotonin levels were measured using in vivo microdialysis with HPLC. Significant prolonged fluctuating increases in extracellular 5-HT were elicited by a challenge injection of MDMA following repeated MDMA administration (n=6 animals). Repeated administration of saline (n=4 animals), did not elicit any significant changes in extracellular 5-HT. C. The effects of repeated systemic administration of MDMA on extracellular NE levels in the rat VPM thalamus. Norepinephrine levels in the rat VPM thalamus were measured using in vivo microdialysis with HPLC. Significant increases in extracellular NE were elicited by a challenge injection of MDMA following repeated MDMA administration (n=6 animals). Repeated administration of saline (n=4 animals), did not elicit any significant changes in extracellular NE. All data are presented as the mean ± S.E.M.; asterisks (*) indicate significant changes (Dunnett’s, p>0.05).

Figure 2. A. Comparison of the effects of single (acute) versus repeated MDMA administration on 5-HT efflux in the VPM thalamus. Both single (n=6 animals) and repeated (n=6 animals) MDMA administration led to significant increases in 5-HT efflux. However, prolonged fluctuating increases in extracellular 5-HT were elicited by a challenge injection of MDMA in repetitively treated animals compared to those receiving
a single administration of the drug. The dashed and solid vertical lines indicate the time at which 5-HT levels returned to baseline following single and repeated MDMA administration, respectively. B. The effects of single versus repeated MDMA administration on NE efflux in the VPM thalamus. Single low-dose (3mg/kg; n=6 animals) MDMA administration had no significant effects on NE efflux, whereas single high-dose (10 mg/kg; n=6 animals) and repeated low-dose (3 mg/kg once per day x 4 days; n=6 animals) MDMA administration produced significant prolonged increases in NE efflux. All data are presented as the mean ± S.E.M. (Single administration data taken from Starr et al 2008, JPET 327:20-31 where it was reported as acute administration, hence the labeling remains the same in this figure. Repeated administration data are from Fig. 1)

Figure 3. A. Representative example of the effects of a challenge injection of MDMA (3mg/kg) following repeated MDMA administration (3mg/kg once per day x 4 days) on VPM thalamic neuron responsiveness to mechanical whisker stimulation. Peri-event histograms (above) and raster records (below) illustrate the response of a single VPM neuron to whisker deflection, before (control) and after challenge injections of saline and MDMA. Time zero represents stimulus onset. Each vertical dash in the raster record represents the occurrence of a single action potential. Peri-event histograms represent cell activity during 20 minute intervals. The black diamond in the raster record corresponds to the time of MDMA injection. Spikes were collected for 3 hours and 20 minutes post-drug injection. Note that MDMA-induced increases in spontaneous discharge are blunted following a challenge injection in animals receiving repeated drug
administrations. B. *The effects of repeated MDMA administration on VPM thalamic unit responsiveness (Peak E1) to mechanical whisker deflection.* Repeated MDMA (3mg/kg x 4 days) administration led to decreased responsiveness to medium and high intensity whisker stimulation. C. *The effects of repeated MDMA administration on the spontaneous firing rate (SFR) of neurons in the VPM thalamus.* Repeated MDMA administration led to an increased level of spontaneous firing during medium and high intensity whisker stimulation, followed by a return to control levels. D. *The effects of repeated MDMA administration on the duration of the response.* MDMA administration decreased the duration of the whisker-evoked response during medium and high intensity whisker stimulation. E. *The effects of repeated MDMA administration on the onset latency of the response.* Repeated MDMA administration led to an increase in the onset latency during medium and high intensity whisker stimulation. F. *The effects of repeated MDMA administration on the offset latency of the response.* There was a significant decrease in the offset latency after repeated drug treatment, but this effect was observed only during the last 60 minutes of the recording session. In B. through F. asterisks (*) and open circles (O) indicate significant changes from control (Dunnet’s,  p>0.05) for high vs medium intensity whisker stimulation, respectively. The vertical dashed lined indicates the time of challenge MDMA injection.
MDMA administration on responsiveness to high intensity whisker stimulation. The results shown here are taken from 40-60 minutes post-MDMA administration, where the drug’s strongest effects on whisker responsiveness were observed. In both cases the majority of cells lie above the 45% line, which indicates that MDMA suppresses evoked discharge and increases the spontaneous firing rate of VPM neurons.

Figure 5. A. The effects of single versus repeated MDMA administration on VPM thalamic unit responsiveness (Peak E1) to mechanical whisker deflection. A single (3mg/kg) MDMA administration decreased responsiveness to medium and high (not shown) intensity whisker stimulation 10-120 minutes post-drug and increased responsiveness at 160-180 minutes post-drug administration. A challenge injection of MDMA (3 mg/kg) following repeated drug administration (3mg/kg x 4 days) led to a reduction in the response magnitude (peak E1) to medium and high (not shown) intensity whisker stimulation, respectively. Unlike the single drug treatment condition, MDMA’s effects on whisker responsiveness to high intensity whisker stimulation were blunted following repeated MDMA administration. B. The effects of single versus repeated MDMA administration on the spontaneous firing rate (SFR) of neurons in the VPM thalamus. Acute MDMA administration led to an increased level of spontaneous firing during medium and high (not shown) intensity whisker stimulation, followed by a return to control levels. Repeated MDMA administration increased the spontaneous firing rate of individual neurons during medium and high (not shown) intensity whisker stimulation, followed by a return to control levels, respectively. However, following repeated MDMA administration the effects on spontaneous firing were blunted compared to acute,
single dose treatment. 

C. The effects of single versus repeated MDMA administration on the onset latency of the response. Single administration of MDMA had no significant effect on the onset latency of the whisker-evoked response. A challenge injection of MDMA following repeated MDMA administration led to an increase in the onset latency during medium and high (not shown) intensity whisker stimulation, respectively. However, the onset latency to medium and high (not shown) intensity whisker stimulation was shorter during the control period following repeated MDMA administration compared to that observed in the drug naïve animal.

D. The effects of single versus repeated MDMA administration on the offset latency of the response. MDMA administration led to an initial decrease in the offset latency during medium and high (not shown) intensity whisker stimulation. A challenge injection of MDMA following repeated MDMA administration led to a significant decrease in the offset latency after repeated MDMA administration, but this effect was observed only during the last 60 minutes of the recording session. MDMA’s effects on offset latency appear to be greater in the single treatment animals.

E. The effects of single versus repeated MDMA administration on the duration of the response. Single administration of MDMA decreased the duration of the whisker-evoked response during medium and high (not shown) intensity whisker stimulation. Repeated MDMA administration also decreased the duration of the whisker-evoked response during medium and high (not shown) intensity whisker stimulation. The dashed vertical lines indicate the time of MDMA injection. The closed and open circles indicate significant changes from control following single versus repeated MDMA administration, respectively (Dunnett’s, p>0.05). (Single administration data taken from Starr et al 2008, JPET 327:20-31)
Figure 6. Representative example showing the effects of a challenge injection of MDMA following repeated MDMA administration on thalamic field potential activity. Power spectral density (PSD) analysis was performed at twenty minute intervals before and after a challenge injection of MDMA. The halothane level remained constant throughout the recording session. A challenge injection of MDMA led to a reduction in low frequency activity (n=6 animals).
Figure 1

A. Image of brain region labeled VPM Thalamus.

B. Graph showing extracellular 5-HT levels with repeated 3mg/kg MDMA and repeated saline.

C. Graph showing extracellular NE levels with repeated 3mg/kg MDMA and repeated saline.
Figure 2

A. Extracellular 5-HT (pg/15 μl)

- Single 3mg/kg
- Repeated 3mg/kg

B. Extracellular NE (pg/15 μl)

- Repeated 3mg/kg
- Single 10mg/kg
- Single 3mg/kg
Figure 3
Figure 4
Figure 5

(A) Whisker Response Magnitude

(B) Spontaneous Firing Rate

(C) Onset Latency

(D) Offset Latency

(E) Duration

- Repeated
- Single

Interval (minutes)