Small Changes in Ambient Temperature Cause Large Changes in 3,4-Methylenedioxymethamphetamine (MDMA)-Induced Serotonin Neurotoxicity and Core Body Temperature in the Rat

Jessica E. Malberg and Lewis S. Seiden
University of Chicago, Department of Pharmacological and Physiological Sciences, Chicago, Illinois 60637

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) is a drug of abuse and has been shown to be neurotoxic to 5-HT terminals in many species. MDMA-engendered neurotoxicity has been shown to be affected by both ambient temperature and core body temperature. We now report that small (2°C) changes in ambient temperature produce changes in core temperature in MDMA-treated rats, but the same changes in ambient temperature do not affect core temperature of saline-treated animals. Furthermore, increases in core temperature of MDMA-treated animals increase neurotoxicity. Rats were given MDMA (20 or 40 mg/kg) or saline and placed in an ambient temperature of 20, 22, 24, 26, 28, or 30°C using a novel temperature measurement apparatus that controls ambient temperature ±0.5°C. Two weeks after MDMA treatment, the rats were killed, and regional 5-HT and 5-hydroxyindole acetic acid levels were analyzed as a measure of neurotoxicity. Rats treated with MDMA at 20 and 22°C showed a hypothermic core temperature response. Treatment with MDMA at 28 and 30°C produced a hyperthermic response. At ambient temperatures of 20–24°C, neurotoxicity was not observed in the frontal cortex, somatosensory cortex, hippocampus, or striatum. At ambient temperatures of 26–30°C, neurotoxicity was seen and correlated with core temperature in all regions examined. These data indicate that ambient temperature has a significant affect on MDMA neurotoxicity, core temperature, and thermoregulation in rats. This finding has implications on both the temperature dependence of the mechanism of MDMA neurotoxicity and human use because fatal hyperthermia is associated with MDMA use in humans.

Key words: MDMA; amphetamine; core body temperature; ambient temperature; neurotoxicity; 5-HT; thermoregulation

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Correspondence should be addressed to Dr. Lewis S. Seiden, University of Chicago, 947 East 58th Street, Department of Pharmacological and Physiological Sciences, Abbott 109, Chicago, IL 60637.

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AMB TEMP ±0.5°C and (2) measures CORE TEMP once per minute using a noninvasive technique.

The purpose of this study was to investigate the interaction of AMB TEMP, CORE TEMP, and MDMA-induced 5-HT neurotoxicity. Twenty or 40 mg/kg MDMA was administered to rats in controlled AMB TEMPs of 20, 22, 24, 26, 28, and 30°C; 2 weeks later, regional brain 5-HT and 5-HIAA levels were analyzed to assess neurotoxicity. A preliminary presentation of this work has been made in abstract form (Malberg and Seiden, 1996).

MATERIALS AND METHODS

Animals

One hundred forty-six male Holtzman (Madison, WI) rats were used, each weighing 250–300 g at time of injection. Rats were group-housed (four to five per cage) in plastic cages with a room temperature of 22–24°C, except on the drug injection days, when they were housed individually in the temperature measurement apparatus at different AMB TEMPs (see Temperature measurement apparatus and Experimental procedure below). Throughout the experiment, rats had access to food (LabDiet 5008; Charm City Lab, Inc., Madison, WI) and water ad libitum and were maintained on a 12 hr light/dark cycle. Housing and experimental treatment of the rats were in accordance with National Institutes of Health guidelines.

Drugs

(±)-3,4-Methylenedioxymethamphetamine HCl was obtained from the National Institute on Drug Abuse. Ketamine and xylazine were obtained from Abbott Labs (Chicago, IL). All chemicals used were of analytical grade. Drug dosages are expressed as the weight of the salt, and drugs were dissolved in 0.9% NaCl.

Temperature measurement apparatus

A novel temperature measurement chamber was developed. This consists of an arrangement of components (computer, computer interface cards, AMB TEMP and CORE TEMP temperature sensors, housing chamber, and Visual Basic software) that forms an integrated system to perform the following functions: (1) measure and maintain a constant AMB TEMP (any temperature from 5 to 45°C, ±0.5°C) using a feedback system that monitors AMB TEMP on a minute-to-minute basis; and (2) measure CORE TEMP once per minute in freely moving animals.

The temperature chambers are modified refrigerators (0.20 cubic meters) that have a 1-inch-thick Plexiglas window in the door to allow observation of the rats and to keep the light/dark cycle in synchrony with rat housing light sources. The rat is unrestrained and allowed to move freely within a cage (20.3 cm wide, 15.2 cm high, and 16.5 cm deep) inside the chamber. The cage is large enough to allow the rat to circle, rear, and show exploratory locomotion. The cage has a hardware cloth (1.3 cm mesh) floor, top, and back wall to ensure air flow within the cage and has a Plexiglas lid and side walls. Each refrigerator has been modified so that in addition to a compressor to cool the chamber, there is also a strip heater and a fan to ensure that the AMB TEMP is even throughout the chamber. A thermistor to record AMB TEMP is mounted in the chamber 10.2 cm from the rat cage. To measure CORE TEMP, a temperature-sensitive transmitter (Minimiter Co., Sunriver, OR) that emits an AM radio signal proportional to the CORE TEMP of the rat is implanted in the peritoneal cavity of the rat. Four AM radios (Radio Shack) surrounding the outside of the rat cage receive signals from the transmitter.

The heating and cooling elements, thermistor, and CORE TEMP radio receivers are interfaced to a standard microcomputer (33 MHz, 386 DX), which has an analog-to-digital interface allowing the environmental and CORE TEMPs to be recorded. Both CORE TEMP and AMB TEMP readings are recorded once per second and then averaged to produce one reading per minute.

The computer also has digital output lines that turn the compressor or heater off and on inside the chamber. To maintain AMB TEMP, the heating or cooling is turned on or off as necessary to maintain the desired AMB TEMP ±0.5°C. This monitoring of AMB TEMP is done every minute. In addition to monitoring AMB TEMP, the software can also be programmed to prevent the rat from reaching lethal hyperthermia. If the rat CORE TEMP increases over a certain temperature point (set by the experimenter), the AMB TEMP decreases until the rat CORE TEMP drops below that point. The entire system is run by a novel Visual Basic computer program. Eight temperature chambers are used and run independently of each other, allowing up to eight different experiments to be run at the same time.

The use of minimitters to measure rat CORE TEMP has been demonstrated as valid and reliable (Dilsaver et al., 1992). The minimitters have a resolution of ±0.01°C (Clement et al., 1989).

Surgery

To implant the CORE TEMP transmitters, rats were anesthetized with ketamine (0.6 mg/ml; 1 ml/kg) and xylazine (100 mg/ml; 0.33 ml/kg) and were given supplemental 1 ml injections of ketamine as needed. A midline cut was made in the peritoneum, and a sterilized transmitter was inserted into the peritoneal cavity, as described by Farfel and Seiden (1995a). Rats were allowed a minimum of 3 d to recover from the surgeries before drug injections.

Experimental procedure

Parametric experiment: effect of different AMB TEMPs on CORE TEMP and neurotoxicity. At 9:00 A.M., rats were placed in the temperature measurement chamber at an AMB TEMP of 24°C (the usual AMB TEMP in the laboratory) for a baseline CORE TEMP measurement and to prevent any hyperthermia induced by exploratory locomotion from influencing any change with the effects of the drug. After 1 hr, the AMB TEMP was set at one of the following: 20, 22, 24, 26, 28, or 30°C. Once the chamber reached the desired AMB TEMP, rats were given a subcutaneous injection of MDMA (20 or 40 mg/kg) or saline (SAL; 1 ml/kg). The rats remained at that AMB TEMP for 24 hr after the injection. After 24 hr, the rats were returned to their group-housing conditions. Two weeks after the injection, the rats were killed by decapitation, and the following regions were dissected, as described by Sabol et al. (1996): frontal cortex, somatosensory cortex, hippocampus, and striatum. Tissue sections were stored in liquid nitrogen until ready for neurochemical analysis.

Heating experiment: effect of hyperthermia on neurotoxicity. In a separate experiment to investigate the effect of hyperthermia on 5-HT, 5-HIAA, dopamine (DA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE) levels, saline-treated rats were placed in the temperature chamber at 24°C for 1 hr to establish a baseline CORE TEMP. The AMB TEMP was then either increased to 40°C or remained at 24°C. The rats remained at that AMB TEMP (24 or 40°C) for 8 hr. For rats exposed to 40°C AMB TEMP, we prevented lethality by keeping the rat CORE TEMP at ≤41°C. This maximum CORE TEMP was chosen because it has been reported that a CORE TEMP over 41.3°C in amphetamine-treated rats produces lethal hyperthermia and heatstroke (Bowyer et al., 1994). To keep rat CORE TEMP at ≤41°C, the AMB TEMP was kept at 40°C unless the rat CORE TEMP exceeded 41°C. At that point, the AMB TEMP was automatically decreased until the rat CORE TEMP was <41°C. When the rat was at ≤41°C CORE TEMP, the AMB TEMP was again increased to 40°C. Two weeks after this treatment, rats were killed by decapitation, and the frontal cortex and somatosensory cortex were dissected as described above.

Neurochemical assays

5-HT, 5-HIAA, DA, HVA, DOPAC, and NE in the various rat brain regions were assayed by HPLC with electrochemical detection (HPLC-EC) according to the method of Sabol et al. (1996) and Kotake (1985).

Core temperature analysis

The CORE TEMP data were quantified by using an area under the curve analysis. For each rat, a CORE TEMP versus time graph was generated, and the area between each CORE TEMP versus time curve and y = 0 was calculated using a trapezoidal area under the curve analysis. In this way, the CORE TEMP response of each rat could be quantified and assigned a number value so that the CORE TEMP data could be analyzed using inferential statistics. This area under the curve value was denoted as the “total CORE TEMP” response for each rat. The CORE TEMP responses for all rats in each experimental group (n = 8) were summed together so that statistical analysis could be performed. This area under the curve value more accurately reflects the CORE TEMP changes over time for all the groups as opposed to using the mean CORE TEMP for each group (Clement et al., 1989; Dilsaver et al., 1990).

Statistics

For neurotoxicity data, differences were determined by ANOVA followed by a Tukey post hoc test (Instat for Macintosh computers, Graph-
turned to a CORE TEMP of 37.5

the rats to thermoregulate is impaired when given 20 or 40 mg/kg

together, these data indicate that small (2°C) changes in AMB TEMPs can be readily seen for each dose (Fig. 2

statistical analysis of the CORE TEMP results include only the

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Table 1. Maximum and minimum CORE TEMP and area-under-the-curve (AUC) values for all treatment groups 10 hr after injection

<table>
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<tr>
<th></th>
<th>20°C AMB TEMP</th>
<th>22°C AMB TEMP</th>
<th>24°C AMB TEMP</th>
<th>26°C AMB TEMP</th>
<th>28°C AMB TEMP</th>
<th>30°C AMB TEMP</th>
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<tr>
<td>SAL</td>
<td>Min: 36.55</td>
<td>Min: 37.03</td>
<td>Min: 36.81</td>
<td>Min: 37.05</td>
<td>Min: 36.97</td>
<td>Min: 37.04</td>
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<td>Max: 38.01</td>
<td>Max: 37.54</td>
<td>Max: 37.57</td>
<td>Max: 37.80</td>
<td>Max: 37.73</td>
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<tr>
<td>AUC: 4925 ± 40</td>
<td>AUC: 4940 ± 55</td>
<td>AUC: 4915 ± 90</td>
<td>AUC: 4927 ± 65</td>
<td>AUC: 4937 ± 58</td>
<td>AUC: 4938 ± 40</td>
<td></td>
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<tr>
<td>20 mg/kg MDMA</td>
<td>Min: 35.22</td>
<td>Min: 35.61</td>
<td>Min: 36.55</td>
<td>Min: 36.6</td>
<td>Min: 36.79</td>
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<td>Max: 36.73</td>
<td>Max: 37.32</td>
<td>Max: 37.36</td>
<td>Max: 37.76</td>
<td>Max: 37.82</td>
<td>Max: 39.07</td>
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<tr>
<td>AUC: 4734 ± 110*</td>
<td>AUC: 4756 ± 98*</td>
<td>AUC: 4871 ± 58</td>
<td>AUC: 4932 ± 87</td>
<td>AUC: 4996 ± 46</td>
<td>AUC: 5192 ± 109*</td>
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<tr>
<td>40 mg/kg MDMA</td>
<td>Min: 35.45</td>
<td>Min: 36.54</td>
<td>Min: 36.78</td>
<td>Min: 37.25</td>
<td>Min: 37.39</td>
<td>Min: 37.3</td>
</tr>
<tr>
<td>Max: 37.52</td>
<td>Max: 37.43</td>
<td>Max: 37.86</td>
<td>Max: 38.13</td>
<td>Max: 38.56</td>
<td>Max: 38.99</td>
<td></td>
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<tr>
<td>AUC: 4716 ± 64*</td>
<td>AUC: 4831 ± 11</td>
<td>AUC: 4948 ± 44</td>
<td>AUC: 4977 ± 78</td>
<td>AUC: 5102 ± 75*</td>
<td>AUC: 5181 ± 65*</td>
<td></td>
</tr>
</tbody>
</table>

A significant effect of treatment on total core temperature changes [area under the curve (AUC)] is seen at AMB TEMPs of 20 ($F = 6.80; p < 0.0001$), 22 ($F = 12.15; p < 0.0010$), 28 ($F = 5.36; p < 0.0145$), and 30°C ($F = 9.85; p < 0.001$). At 20°C, both the 20 and 40 mg/kg groups had significant decreases in CORE TEMP. At 22°C, only the 20 mg/kg group had a significant decrease in CORE TEMP. Treatment at 24 and 26°C did not produce an overall effect of CORE TEMP change, although the 40 mg/kg rats at 26°C seemed to have a hyperthermic peak temperature. At 28°C, only the 40 mg/kg group had a significantly higher CORE TEMP response than the SAL group, and at 30°C, both the 20 and 40 mg/kg groups had significantly higher CORE TEMP response.

* $p < 0.01$ Total CORE TEMP change, as measured by the AUC compared with SAL group at that AMB TEMP.

Pad). For the CORE TEMP analysis, two-way ANOVA for repeated measures was used to compare treatment groups (SuperAnova). Single-time point comparisons between groups were made using a Tukey post hoc test. Statistical significance was considered to be $p < 0.05$ unless indicated otherwise.

RESULTS

Parametric experiment: effects of MDMA and different AMB TEMPs on rat CORE TEMP

Each rat was given SAL or MDMA (20 or 40 mg/kg) at one of six different AMB TEMPs of 20, 22, 24, 26, 28, or 30°C. For all rats, a 1 hr baseline CORE TEMP was recorded. In that time, the average CORE TEMP was 37.8 ± 0.05°C. After the baseline hour the AMB TEMP was adjusted, and animals were injected with MDMA or SAL in the different AMB TEMPs.

There were no effects on CORE TEMP in any of the SAL-treated rats at any of the six different AMB TEMPs. The average CORE TEMP for all SAL-treated rats ($n = 48$) was 37.3 ± 0.07°C for the entire 24 hr spent in the temperature measurement apparatus. This indicates that AMB TEMPs of 20–30°C do not affect the CORE TEMP of SAL-treated animals. In contrast, MDMA-treated animals had a markedly different CORE TEMP profile in different AMB TEMPs. MDMA administration in AMB TEMPs of 20 and 22°C produced a hyperthermia compared with SAL controls. MDMA administration in AMB TEMPs of 28–30°C produced a hyperthermia compared with SAL controls (Table 1; Fig. 1A–F). Both SAL- and MDMA-treated rats returned to a CORE TEMP of 37.5 ± 0.01°C by 11 hr (660 min) after the MDMA was injected. For this reason, all figures and statistical analysis of the CORE TEMP results include only the first 11 hr of the experiment.

The effects of incrementally higher AMB TEMPs on CORE TEMPs can be readily seen for each dose (Fig. 2A,B). Taken together, these data indicate that small (2°C) changes in AMB TEMP affect the CORE TEMP of the rat and that the ability of the rats to thermoregulate is impaired when given 20 or 40 mg/kg MDMA.

Effects of different AMB TEMPs on MDMA-induced 5-HT neurotoxicity

The effect of different AMB TEMPs on MDMA-induced neurotoxicity is shown in Figures 3 and 4. In this experiment, decreases in 5-HT and 5-HIAA levels were measured as indicators of neurotoxicity; we have found this to be a reliable marker of MDMA neurotoxicity (Lew et al., 1996; Sabol et al., 1996).

There were no significant depletions of 5-HT at AMB TEMPs of 20, 22, or 24°C in any of the brain regions examined. As AMB TEMP increased above 24°C, significant depletions were seen, with higher AMB TEMPs inducing greater depletions. At 26°C, there were significant depletions of 5-HT compared with control in the frontal cortex (87% of control) ($p < 0.05$ for all post hoc tests), hippocampus (80%), and striatum (72%) in the 40 mg/kg group and in the somatosensory cortex in both the 20 and 40 mg/kg groups (90 and 82%, respectively) (see Figures 3 and 4 for $F$ values).

At 28°C, there were significant 5-HT depletions compared with control in the frontal cortex (75% of control) in the 40 mg/kg group. All other regions had depletions in both the 20 and 40 mg/kg group: somatosensory cortex, 80 and 66%, respectively; hippocampus, 75 and 65%, respectively; and striatum, 71 and 61%, respectively.

At 30°C, both the 20 kg and 40 mg/kg MDMA-treated groups had decreases in 5-HT levels compared with control in all regions examined: somatosensory cortex, 70 and 65%, respectively; hippocampus, 58 and 42%, respectively; frontal cortex, 63 and 70%, respectively; and striatum, 74 and 66%, respectively.

The 5-HIAA level data were very similar to the 5-HT levels data; treatment at 20 and 22°C AMB TEMP produced no changes in 5-HIAA levels, and higher AMB TEMPs produced decreases in 5-HIAA levels. The one difference was that at 24°C, 5-HIAA levels were affected, whereas 5-HT levels were not affected until the AMB TEMP reached 26°C. At 24°C AMB TEMP, there was a significant decrease in 5-HIAA levels compared with control in the 40 mg/kg group in the frontal cortex (72.7% of control), somatosensory cortex (77%), and hippocampus (79%).

At 26°C AMB TEMP, the 40 mg/kg group showed significant depletions compared with 5-HIAA depletions to control in the frontal cortex (70% of control), hippocampus (79%), and striatum (64%).

Treatment at 28°C AMB TEMP produced decreases in 5-HIAA levels compared with control in the 40 mg/kg group in all regions: somatosensory cortex, 69% of control; hippocampus, 72%; frontal cortex, 70%; and striatum, 64%.­
At 30°C AMB TEMP, both the 20 and 40 mg/kg MDMA-treated groups had significant decreases in 5-HIAA levels in all regions: somatosensory cortex, 70 and 73%, respectively; hippocampus, 58 and 56%, respectively; frontal cortex, 63 and 60%, respectively; and striatum, 54 and 70%, respectively.

These results clearly indicate that at 20–24°C, there is protection against MDMA-induced decreases in 5-HT levels, and at 20–22°C there is protection against 5-HIAA decreases. At 26–28°C there is increased neurotoxicity, and by 28–30°C all treatment groups are significantly affected. These results point to a large effect of AMB TEMP in determination of MDMA-induced neurotoxicity.

Correlation between AMB TEMP and neurotoxicity
Regression analysis was used to determine a correlation between the area under the curve and neurotoxicity (decrease in 5-HT and 5-HIAA levels) in all MDMA-treated animals. All regions had significant negative correlations (Table 2) for both 5-HT and 5-HIAA levels. These data indicate that as the CORE TEMP of animals treated with MDMA increases above a normal CORE TEMP, 5-HT and 5-HIAA levels decrease.

Lethality
In the parametric experiment, lethality was not prevented as it was in the heating experiment so that the percentage of lethality for each AMB TEMP-dose combination could be determined. In the 40 mg/kg 30°C AMB TEMP group, we obtained 20% lethality. This is less lethality than Gordon et al. (1991) obtained at the same AMB TEMP. This may be attributable to the fact that in our temperature measurement chamber, there was a constant movement of air because of the fan, so that any lethality attributable to additional humidity was attenuated in our temperature chamber.

Effect of hyperthermia on SAL-treated rats
Saline-treated rats in an AMB TEMP of 24°C had an average CORE TEMP of 37.4 ± 0.02°C, and saline-treated rats treated at an AMB TEMP of 40°C had an average CORE TEMP of 39 ± 0.08°C, with a peak CORE TEMP of 40.89°C. There was a significant difference (p < 0.0001) in CORE TEMP response between the rats treated at 24 and 40°C, indicating that an AMB TEMP of 40°C produces hyperthermia in SAL-treated rats. Twenty-four hours after the rats were placed in the temperature measurement apparatus, all rats had average CORE TEMPs of 37.5 ± 0.03°C.

There was no change in any neurotransmitter level (5-HT, 5-HIAA, DA, DOPAC, HVA, and NE) between the rats treated at 24 and 40°C AMB TEMP in all of the brain regions examined. In the interest of space, only the frontal cortex and somatosensory cortex data are shown (Fig. 5A,B). This indicates that the significant CORE TEMP hyperthermia induced by a 40°C AMB TEMP causes no changes in neurotransmitter levels. This is in
agreement with Wilkinson et al. (1991), who looked at pyrogen-induced fever on 5-HT levels and found no significant effect. This control experiment demonstrates that the changes in neurotransmitter levels seen in our MDMA experiment are not attributable to the CORE TEMP hyperthermia alone but are attributable to the CORE TEMP hyperthermia interacting with the MDMA. The hyperthermia itself does not cause depletions in neurotransmitter levels.

**DISCUSSION**

The main finding of this study is that a small (2°C) change in AMB TEMP produces marked changes in both CORE TEMP and MDMA-induced 5-HT neurotoxicity. Although previous studies (Gordon et al., 1991; Bowyer et al., 1994; Miller and O’Callaghan, 1994) have demonstrated that large changes in AMB TEMP affect CORE TEMP and neurotoxicity, the present study is the first to investigate the effect of small controlled changes in AMB TEMP. We demonstrate that MDMA disrupts thermoregulatory ability, and this in turn makes the CORE TEMP dependent on the AMB TEMP. The resulting changes in CORE TEMP affect MDMA-induced neurotoxicity. This study underscores the importance of a constant and controlled AMB TEMP in any experimental paradigm investigating MDMA neurotoxicity.

It can be seen that above 24°C AMB TEMP, changes in AMB TEMP increase the neurotoxicity. We hypothesize that there is a critical AMB TEMP, which we term “break point,” below which protection is seen. Increases in AMB TEMP from this point increase MDMA-induced neurotoxicity. The break point for a single dose of 20 or 40 mg/kg MDMA seems to be 24°C, although most likely this break point is different for other MDMA doses and amphetamine analogs and may differ in different-sized animals and species.

It is of interest that at 24°C AMB TEMP, 5-HIAA levels are decreased, whereas 5-HT levels are not affected until 26°C AMB TEMP. It may be that the decrease in 5-HIAA levels near the break point is indicative of partial damage to cellular metabolism, although further research is needed to determine its extent and characterization.

MDMA and other amphetamines have been shown to disrupt thermoregulation in both rats and humans (Gordon et al., 1991;
Dafters, 1995; McCann et al., 1996). The MDMA-induced loss of thermoregulation in rats has been shown to occur not only in neurotoxic doses (Gordon et al., 1991; Gordon and Fogelson, 1994) but also in non-neurotoxic doses (Dafters, 1994; Ainsworth et al., 1997). This has received much attention because of the number of MDMA-related fatalities that have involved hyperthermia and heatstroke (Henry, 1992; Dar and McBrien, 1996). Many, but not all, of these overdoses arise from MDMA use at rave parties (Green et al., 1995; McCann et al., 1996). The use of MDMA or other amphetamines combined with high AMB TEMP seen at rave parties and hyperthermia induced by dancing (Sternbach, 1991) may all contribute to the hyperthermia-associated problems with MDMA use.

The MDMA-induced hyperthermia has been theorized to be linked to the “serotonin syndrome” (Ames and Wirshing, 1993; Friedman, 1993). This syndrome consists of hyperthermia and other symptoms (Sternbach, 1991) and is thought to result from excess 5-HT at the 5-HT1A receptor (Ames and Wirshing, 1993; Sporer, 1995). Given that MDMA induces 5-HT release, it has been theorized that there is a link between MDMA-induced
hyperthermia and serotonin syndrome (Kaskey, 1992; Ames and Wirshing, 1993; Friedman, 1993). This has been supported by studies in the rat in which 5-HT syndrome behaviors such as forepaw treading, head weaving, and a low body posture were induced by MDMA administration (Spanos and Yamamoto, 1989; Colado et al., 1993). These behaviors increased in intensity and duration of response with increasing doses of MDMA (Spanos and Yamamoto, 1989).

Our study indicates that MDMA-induced hyperthermia leads to neurotoxicity. We hypothesize that once MDMA enters the neuron via the 5-HT transporter (Rudnick and Wall, 1992), the hyperthermia would then affect and possibly increase the rate of reactions leading to MDMA-induced neurotoxicity. In support of this, we (Malberg et al., 1996) have shown that if MDMA is prevented from entering the 5-HT neuron by fluoxetine pretreatment, the rats still display hyperthermia, although no neurotoxicity is seen. Under these circumstances, the hyperthermia may be from MDMA stimulating 5-HT2A/C receptors. DOI and M-CPP, selective agonists to 5-HT2A and 5-HT2C receptors, respectively, have been shown to produce a hyperthermia (Mazzola-Pomietto et al., 1997), and MDMA has been shown to act as an agonist to 5-HT2A and 5-HT2C receptors (Nash et al., 1994). This 5-HT2A/C-mediated hyperthermia could contribute to the neurotoxicity observed.

In contrast to hyperthermia increasing reactions leading to neurotoxicity, hypothermia may also protect by decreasing the rate of reactions leading to MDMA-induced neurotoxicity. A cold AMB TEMP has been shown to protect against neurotoxicity (Bowyer et al., 1993; Ali et al., 1994; Che et al., 1995), and many drugs that protect against neurotoxicity do so by lowering the rats’ CORE TEMP (Bowyer et al., 1994; Miller and O’Callaghan, 1994; Farfel and Seiden, 1995a,b; Malberg et al., 1996). Hypothermia has also been shown to be neuroprotective in other models of brain injury (Ginsberg et al., 1992). We hypothesize that hypothermia may slow down some of the reactions leading to METH-induced neurotoxicity. It is not currently known how the hypothermia is produced. In a cold AMB TEMP, the hypothermia may come from an MDMA-induced deficit in the thermoregulatory system that results in an inability to produce normal heat-conservation or heat-production responses, which would make an animal hypothermic. Further experiments are necessary to determine how and where MDMA would affect thermoregulatory responses.

There are several hypothesized mechanisms of neurotoxicity. For many of the reactions leading to neurotoxicity that have been experimentally investigated, hypothermia and hyperthermia may affect the rate of reactions. MDMA has been shown to bind to the 5-HT transporter (Rudnick and Wall, 1992). It is not known whether the CORE TEMP hypothermia or hyperthermia would affect in vivo binding of MDMA to the transporter. It has been shown, however, that plasma levels of MDMA are not affected by either a CORE TEMP hypothermia or hyperthermia (Colado et al., 1995) so that MDMA pharmacokinetics are not affected by changes in CORE TEMP.

Temperature has an affect on amphetamine-induced 5-HT and DA release, which may be important to neurotoxicity. Clauing et al. (1996) have shown using in vivo microdialysis that amphetamine-induced striatal DA levels correlate with maximum brain temperature, indicating that hyperthermia increases

<table>
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<th>Region</th>
<th>$R^2$</th>
<th>$p$</th>
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<tr>
<td>Frontal cortex</td>
<td>0.47</td>
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<tr>
<td>5-HT</td>
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<td></td>
</tr>
<tr>
<td>5-HIAA</td>
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<td>Hippocampus</td>
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</tr>
<tr>
<td>5-HIAA</td>
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<td>Striatum</td>
<td>0.39</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>5-HT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA</td>
<td>0.33</td>
<td>&lt;0.013</td>
</tr>
</tbody>
</table>

Correlation of total CORE TEMP response (area under the curve value) and 5-HT or 5-HIAA levels for all regions. Group means were used for calculation of $R^2$ values.

Figure 5. 5-HT, 5-HIAA, DA, DOPAC, HVA, and NE levels from SAL-treated rats exposed to an AMB TEMP of 24 or 40°C for 8 hr. Frontal cortex (A) and somatosensory cortex levels (B) were obtained 2 weeks after exposure. All tissue weights are expressed in nanograms per milligram of wet tissue weight. $n = 8$ for all experimental groups. All values are mean ± SEM.
ampetamine-induced DA release. Conversely, Bowyer et al. (1992) have reported that a cold AMB TEMP, which lowers CORE TEMP, prevents METH-induced DA and 5-HT release. Changes in CORE TEMP, then, may affect the step of amphetamine-induced neurotransmitter release.

Free radical formation is hypothesized to be a major reaction leading to neurotoxicity (Halliwell, 1992; Cadet et al., 1994; Gudelsky, 1996). Therefore, the effect of CORE TEMP on the formation of free radicals may be a major point in preventing or increasing neurotoxicity. It has been shown that hypothermia prevents free radical formation; maintaining the brain at a hypothermic temperature prevents ischemia-induced 2,3- and 2,5-dihydroxybenzoic acid formation. Conversely, free radical formation is increased in rats with hyperthermic brain temperature (Globus et al., 1995; Kil et al., 1996). These results show that formation of hydroxyl radicals in the brain is a temperature-dependent process. In addition, CORE TEMP hypothermia could decrease and hyperthermia could increase free radical formation such as 6-OHDA, 5,7-DHT, and NO (Halliwell, 1992). At least some, if not all, of these free radicals are theorized to participate in amphetamine-induced neurotoxicity (Seiden and Vosmer, 1984; Commings et al., 1987a; Dawson et al., 1993; Cadet et al., 1994).

Activation of excitatory amino acids and Ca$^{2+}$ accumulation have been implicated in amphetamine-induced neurotoxicity (Sonsalla et al., 1989), and hypothermia may also prevent the accumulation of calcium ions. Hypothermia reduces glutamate and glycine release (Illievich et al., 1994) and Ca$^{2+}$ accumulation and release (Corbett et al., 1990; Mitani et al., 1991), and this may contribute to hypothemic protection against neurotoxicity.

Kramer et al. (1995, 1997) have reported that MDMA induces translocation of the calcium- and phospholipid-dependent enzyme PKC and theorize that this prolonged kinase activation may contribute to the neurotoxicity. Interestingly, it has been reported that in hypothermic rats, ischemia-induced translocation of PKC was completely abolished (Cardell et al., 1991). This again points to hypothermia decreasing or preventing another neurotoxic reaction.

Another theory of neurotoxicity has been advanced by Carlsson (1993), who posits a feed-forward loop that is excited by amphetamines and causes continued release of neurotransmitter. This requires energy, and eventually the cell goes into ATP depletion and dies. Hypothermia may slow down this reaction; it has been shown that reduction of brain temperature significantly delays decreases in ATP throughout the hippocampus (Busto et al., 1987; Zeevak and Nicklas, 1993). Therefore, hypothemic prevention of the ATP depletion may be another mechanism of protection against neurotoxicity.

In summary, this experiment used a novel temperature measurement apparatus to measure and control AMB TEMP and to measure CORE TEMP. We have shown that small (2°C) changes in AMB TEMP have a large affect on CORE TEMP neurotoxicity in MDMA-treated animals. At low AMB TEMPs, hypothermia and protection against neurotoxicity is seen, and at high AMB TEMPs we report a hypothermia that correlates with increased neurotoxicity. This is in line with clinical evidence indicating reduced thermoregulation and lethality from MDMA use and 5-HT syndrome, and we offer a hypothesis that the hypothermia and hyperthermia may be acting on one or more of the reactions leading to neurotoxicity.

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