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ORIGINAL INVESTIGATION

The effects of methamphetamine on core body temperature in the rat—Part 2: an escalating regimen

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Abstract
Rationale Methamphetamine (METH) induces hyperthermia, which is diminished with chronic treatment in a dose-dependent manner. Our objective was to determine whether the temperature responses produced by a chronic, escalating-dose METH regimen and a chronic, 5.0 mg/kg dose regimen.
Methods Rats received pretreatment injections of saline, 5.0 mg/kg METH, 10.0 mg/kg METH (second comparison group), or an escalating-METH regimen (2–9 mg/kg) for 12 days. On day 13, all four groups were challenged with 10.0 mg/kg METH. Temperature measurements were made telemetrically at 24°C ambient temperature.
Results Escalating pretreatment produced hyperthermia; with successive exposures, the hyperthermic peak shifted to the right. The 5.0-mg/kg-pretreatment group initially showed no change in temperature at 60 min post-treatment but developed hypothermia at 60 min with chronic treatment; at 3 h post-treatment, significant hyperthermia was present and did not diminish with chronic treatment. After the 10.0-mg/kg-METH challenge, the saline-pretreatment group was hyperthermic, and the 10.0-mg/kg-pretreatment group was hypothermic; the 5.0 mg/kg and escalating pretreatment groups were intermediate and were not different from each other. At 3 h post-challenge, no group differences were apparent. Dopamine (DA) and serotonin (5-HT) were not depleted when measured 2 weeks after treatment ended.
Conclusions (1) Escalating and 5.0-mg/kg regimens produced different temperature profiles during the 12-day pretreatment period but a similar diminished response to the 10.0-mg/kg-METH challenge on day 13. (2) The diminished temperature responses with chronic treatment occurred in the absence of long-term DA and 5-HT depletions.

Keywords Methamphetamine · Drug abuse · Rat · Temperature · Tolerance

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Humans who abuse the amphetamines often begin by taking small doses; however, with repeated use, tolerance occurs, causing users to consume larger amounts. This develops into a secondary high-dose pattern of multiple dosing throughout the day. Users typically self-administer the drug chronically throughout the day (consuming up to 1 g until later in the day, then sleeping at night) or in a pattern of multiple dosing over several days (consuming up to 2–4 g over an approximate 3-day interval of periodic administration) (Cho and Melega 2002). Studies with laboratory animals indicate that tolerance develops to methamphetamine (METH)-induced hyperthermia. Because human drug users escalate their doses, we were interested in evaluating tolerance to hyperthermia in relation to an escalating-dose METH regimen in the laboratory rat.

In animal studies of temperature and neurotoxicity, many researchers choose to mimic human-dosing patterns of established users. Thus, researchers have used multiple high-dose patterns administering a total of four doses at 2-hour intervals to study drug effects (Albers and Sonsalla 1995; Bowyer et al. 1992; Gygi et al. 1996; Johnson-Davis et al. 2002, 2003; Riddle et al. 2002; Sabol et al. 2000, 2001; Schmidt et al. 1985).

In addition to mimicking the patterns and dosing intervals of the established user, research has also focused

on simulating the initial escalating patterns of use, which reflect the early phase of drug abuse. Accordingly, patterns of escalation have been used, which range from once-daily treatments (Robinson et al. 1988) to multiple-dosing regimens (Gygi et al. 1996; Johnson-Davis et al. 2003; Segal and Kuczenski 1997). Regimens of once-daily treatments may yield greater clarity in data (Cho et al. 2001; Miller and O'Callaghan 2003). Because temperature changes occur up to 6 h post-injection, changes occurring during a rapid multiple-injection paradigm would be difficult to interpret. We examined the core temperature effects of an escalating-METH pretreatment regimen, which incremented once per day across 12 days.

Previously, we showed that chronic treatment with 10 mg/kg/day METH but not 1.0 mg/kg/day resulted in diminished hyperthermia over a 12-day period at 24°C ambient temperature (Myles et al. 2008). The effect with 5.0 mg/kg was intermediate. Furthermore, when using an intermittent, escalating pattern of METH pretreatment, the development of tolerance to hyperthermia was evident (Johnson-Davis et al. 2003). The purpose of this study was to determine whether (1) an escalating-dose regimen (increasing from 2.0 to 9.0 mg/kg METH) and (2) a constant daily dose regimen of 5.0 mg/kg would result in similar changes in core temperature after a 12-day period of chronic exposure. Both regimens had equivalent total doses. We also wanted to determine if the altered temperature response with chronic treatment was independent of the neurotoxic effects of METH.

Materials and methods

Subjects

Twenty-four male Sprague–Dawley rats, 300–325 g upon arrival (Harlan, Indianapolis, IN, USA), were used. The rats were individually housed in hanging stainless steel wire cages, maintained on a 12-h light/dark cycle (lights on 7:00 AM). They had free access to food and water except during the 7-h test session. All procedures were carried out according to the Guide for the Animal Care and Use of Laboratory Animals (NRC 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi.

Surgery

For the measurement of core temperature, radio transmitters (Mini-mitter, model #VM-FH disc) were implanted into the abdomen of all rats. Animals were anesthetized with ketamine (80 mg/kg), xylazine (10 mg/kg), and atropine (5 mg/kg). Supplemental anesthesia was with ketamine as needed. Ketoprofen (5 mg/kg) was given as post-surgical analgesia.

Drugs

(+)-Methamphetamine hydrochloride (SIGMA, St. Louis, MO, USA) was dissolved in saline vehicle (0.09% NaCl solution). A constant injection volume of 1 ml/kg was used. METH doses were calculated as the METH HCl salt. Each rat (n=6/treatment) received intraperitoneal (i.p.) injections of vehicle, 5.0 mg/kg, 10.0 mg/kg, or an escalating regimen of METH (2.0, 2.5, 3.0, and 3.5 mg/kg on days 1, 2, 3, and 4, respectively; 4.0, 4.5, 5.0, and 5.5 mg/kg on days 5, 6, 7, and 8, respectively; 6.0, 7.0, 8.0, and 9.0 mg/kg on days 9, 10, 11, and 12, respectively) for 12 consecutive days, once/day, followed by a challenge dose of 10 mg/kg METH on day 13.

Procedure

After 1-week quarantine, surgery was performed. Two weeks after surgery, the rats began treatments. Each rat received once-daily saline injections for 4 days before each regimen (HAB day-block 1–4). This was followed by one of the following four regimens: daily injections of saline, 5.0 mg/kg, 10.0 mg/kg, or an escalating regimen of METH for 12 pretreatment days. On day 13, rats from all pretreatment groups were challenged with 10.0 mg/kg METH. This resulted in a total of 17 test-days/rat. Note that the saline-pretreatment group received 16 daily injections of saline, followed by 10.0 mg/kg METH on the challenge day.

Body weight was measured at the start of each test day. The rats were housed in computer-controlled environmental chambers maintained at 24°C for 7 h/day during the 17-day test period. Treatment occurred 2 h after the rats were placed into the chambers. Telemetric temperature signals were automatically recorded once per minute.

Two procedures described above were different from those used in our companion paper (Myles et al. 2008): the addition of the HAB1–4 period allowed us to investigate whether repeated saline or METH resulted in carry-over effects at the beginning of successive sessions; recording baseline temperature for 2 h instead of 1 h each day, allowed core temperature to level off and stabilize at the time of daily METH or saline treatment.

In prior research (Myles et al. 2008), we noticed the presence of salivation after treatment with METH. Because salivation is a mechanism for heat loss, we were interested in systematically studying its role in METH's effects on core temperature in this report. Animals were given a score ranging from 0 to 3 depending on the degree of salivation observed at 15-min intervals during the temperature

measurement periods: 0 = no salivation; 1 = saliva noted on floor of test chamber, with a small amount on the body; 2 = wet forelimbs and hind limbs; 3 = wet ventral body surface. The rater was not blind to treatment. The highest value recorded over each 4-day block was used for data analysis.

Two weeks after the completion of testing, the animals were euthanized by decapitation, having their brains rapidly dissected on ice for high-performance liquid chromatography (HPLC) neurochemical analysis.

Neurochemical analysis

Dopamine (DA) and 5-HT concentrations in the striatum, hippocampus, and hypothalamus were analyzed post-mortem using HPLC. Internal standard (DHBA) and 0.01 N $HClO_4$ were added to individual brain regions. Samples were homogenized with an ultrasonic tissue disrupter and centrifuged twice at 14,000 rpm for 20 min. The supernatant was drawn off and stored on ice for HPLC analysis. Instrumentation included ESA HPLC pump (model #580); Rheodyne injection valve (model #7010); Adsorbosphere Catecholamine 100×4.6 mm column (C_{18}, 3-μm particle size); ESA Coulochem II coulometric detector (model #5200), with applied potentials of −100 (E1) and +330 mV (E2); and EZ-Chrome, Chromatography Data System (version 6.7). Mobile phase consisted of 20.7 g/2 L $Na_2H_2PO_4$, 0.5513 g/2 L OSA, and 500 μL of 100 mM EDTA/2 L. The volatile component of the mobile phase was 7.5% acetonitrile. The solution was titrated to a pH of 3.1 using phosphoric acid.

Data analysis

Temperature

Data for 4 days were combined for analysis; this resulted in four, 4-day blocks, namely one habituation saline day-block (HAB day-block 1–4), followed by three METH (M1–4, M5–8, M9–12), or saline (S1–4, S5–8, S9–12) day-blocks. For each pretreatment regimen, a two-way within-subjects analysis of variance (ANOVA) was used to analyze the effect of day-block and session time (−105 and −60 and 0 min pre-injection, 30, 60, 120, 180, 240, and 300 min post-injection).

To evaluate changes in baseline performance for the saline-pretreatment group, we followed up significant main effects of session time (collapsed across day-blocks) with the modified Bonferroni post hoc test ($p=(df)(.05)$/number of comparisons (Keppel 1991); $p<.05$) comparing time 0 (immediately before injection) with −105, −60, +30, and +60 min.

To evaluate carry-over effects in the METH pretreatment groups, we followed up significant main effects of day-block or significant interactions with one-way ANOVAs at specific time points before injection (times −105, −60, and 0 min). Significant one-way ANOVAs at each time point were followed by three post hoc pair-wise comparisons: HAB day-block 1–4 was compared to METH day-blocks 1–4, 5–8, and 9–12 (modified Bonferroni correction, $p<0.05$). By comparing each day-block to HAB1–4, we demonstrated whether previous drug exposure influenced core temperature at the start of each session.

To determine the effects of initial METH exposure (day-block 1–4) and chronic METH exposure (day-blocks 1–4, 5–8, and 9–12), we followed up significant main effects of day-block or interactions with one-way ANOVAs at specific time points post-injection. Significant one-way ANOVAs were followed by four post hoc tests. HAB day-block 1–4 was compared to METH day-block 1–4 to determine initial drug-induced changes in temperature; METH day-block 1–4, METH day-block 5–8, and METH day-block 9–12 were compared to each other to determine the effect of chronic exposure (modified Bonferroni correction, $p<0.0375$). These tests were carried out at different session times post-treatment depending on the dose of METH.

To assess the effect of the challenge injection on the 13th METH treatment day, two-way ANOVAs were conducted. One of the two-way ANOVAs analyzed the saline group: day-block (saline day-block 9–12 vs. day 13)×session time (−105, −60, 0, 30, 60, 120, 180, 240, and 300 min post-injection). This was followed by a one-way within-subjects ANOVA comparing saline day-block 9–12 to METH challenge day 13 at each time point (modified Bonferroni correction of $p<0.044$). This analysis allowed us to demonstrate the hyperthermic challenge effect in animals without a history of METH exposure. The other two-way ANOVA analyzed the METH challenge day (pretreatment× session time) to determine the effects of different pretreatment regimens upon challenge. This was followed by a one-way between-subjects ANOVA at each time point. Before injection, saline was compared to each METH pretreatment group (modified Bonferroni correction of 0.05); after injection, all four groups were compared to each other (modified Bonferroni correction of 0.025).

Salivation

Salivation data were analyzed using the non-parametric test of Kruskal–Wallis for each day-block, followed by the Mann–Whitney U (modified Bonferroni, $p<.05$).

Body weight

A two-way ANOVA (pretreatment×day-block) was used to analyze the body weights of treated animals. Follow-up

one-way ANOVAs were conducted, and pair-wise comparisons were made between the different pretreatment groups at each of the three pretreatment day-blocks (modified Bonferroni correction, $p<0.05$).

Neurochemistry

Using one-way ANOVAs for each brain region (striatum, hippocampus, and hypothalamus), we analyzed the post-mortem tissue concentrations of DA and 5-HT for the following four groups: saline-pretreatment/METH challenge, escalating-METH-pretreatment/METH challenge, 5.0-mg/kg-METH pretreatment/METH challenge, and 10.0-mg/kg-METH pretreatment/METH challenge.

Results

Temperature

METH pretreatment

Rats received chronic treatment with one of the following four regimens: saline, 5.0 mg/kg METH, escalating-METH doses, or 10.0 mg/kg METH. For each pretreatment regimen, we analyzed the effects of chronic exposure on core temperature using one-way ANOVAs (day-block× session time).

For saline pretreatment, the main effect of session time was significant [$F(8,32)=87.8$, $p=0.0000$]. The main effect of day-block and the session time×day-block interaction were not significant. For 5.0-mg/kg-METH pretreatment, the main effect of session time [$F(8,32)=19.3$, $p=0.000$] and the interaction between session time and day-block [$F(24,96)=11.8$, $p=0.0000$] were significant, but the main effect of day-block was borderline [$F(3,12)=3.2$, $p=0.061$]. For escalating pretreatment, there were significant main effects of day-block [$F(3,15)=19.8$, $p=0.0000$] and session time [$F(8,40)=9.0$, $p=0.000$]; the interaction was also significant [$F(24,120)=19.2$, $p=0.0000$]. For 10.0-mg/kg-METH pretreatment, there were significant main effects of day-block [$F(3,15)=6.8$, $p=0.004$] and session time [$F(8,40)=17.9$, $p=0.0000$]; the interaction was also significant [$F(24,120)=10.2$, $p=0.0000$].

Analysis of temperature before and after injection for saline pretreatment

We explored the significant main effect of session time in the saline-pretreatment group by comparing baseline time 0 to −105, −60 (pre-injection), +30, and +60 min (post-injection), collapsed across day-blocks. Only the 0 vs. +60 min post-injection comparison failed to reach significance. At −105 and −60 min (pre-injection), core temperature decreased as the session progressed. Temperature increased at 30 min post-treatment, reflecting the effects of stress (handling) or arousal from sleep on core temperature. Note that this effect reversed itself at 60 min post-injection, at which point there was no difference from baseline time 0 (data not shown).

Tests for carry-over effects from preceding treatment days

As stated above, for the saline-pretreatment group, the main effect of day-block and the day-block×session time interaction were not significant. For 5.0-mg/kg-METH pretreatment, day-blocks were significantly different at −105 [$F(3,12)=26.2$, $p=0.0000$] and −60 min [$F(3,12)=8.3$, $p=0.003$]. At −105 min, HAB day-block 1–4 was greater than M1–4, M5–8, and M9–12. At −60 min, HAB day-block 1–4 was greater than M5–8 and M9–12 (Fig. 1). For escalating pretreatment, day-blocks were significantly different at −105 [$F(3,15)=33.3$, $p=0.0000$] and −60 min [$F(3,15)=3.5$, $p=0.041$]. At −105 min, HAB day-block 1–4 was greater than M1–4, M5–8, and M9–12. At −60 min, HAB day-block 1–4 was greater than M1–4 and M9–12 (Fig. 2). For 10.0-mg/kg-METH pretreatment, day-blocks were significantly different at −105 [$F(3,15)=30.0$, $p=0.0000$] and −60 min [$F(3,15)=9.7$, $p=0.0008$]. At −105 min, HAB day-block 1–4 was greater than M1–4, M5–8, and M9–12. At −60 min, HAB day-block 1–4 was greater than M1–4, M5–8, and M9–12 (Fig. 3). These data demonstrate that for all pretreatments except saline, carry-over effects were seen at all 3 day-blocks: M1–4, M5–8,

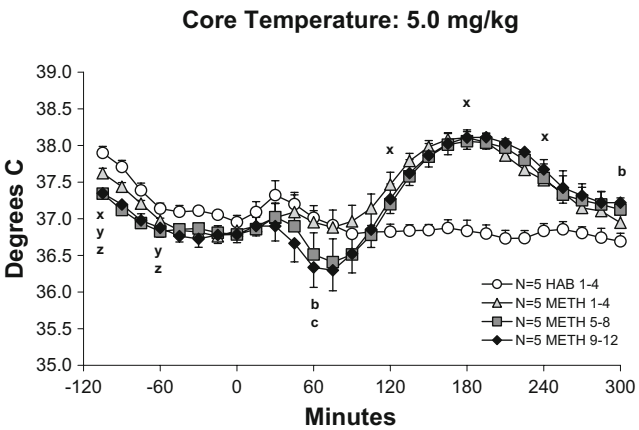

Fig. 1 Core temperature (mean±SEM). Rats received 4 days of saline injection (HAB 1–4) followed by 12 days of 5.0 mg/kg METH (METH 1–4, 5–8, and 9–12). *x* HAB 1–4 was significantly different from METH 1–4, *y* HAB 1–4 was significantly different from METH 5–8, *z* HAB1–4 was significantly different from METH 9–12, *a* METH 1–4 was significantly different from METH 5–8, *b* METH 1–4 was significantly different from METH 9–12, *c* METH 5–8 was significantly different from METH 9–12. *HAB 1–4* Habituation day-block 1–4, *METH 1–4* METH day-block 1–4, *METH 5–8* METH day-block 5–8, *METH 9–12* METH day-block 9–12

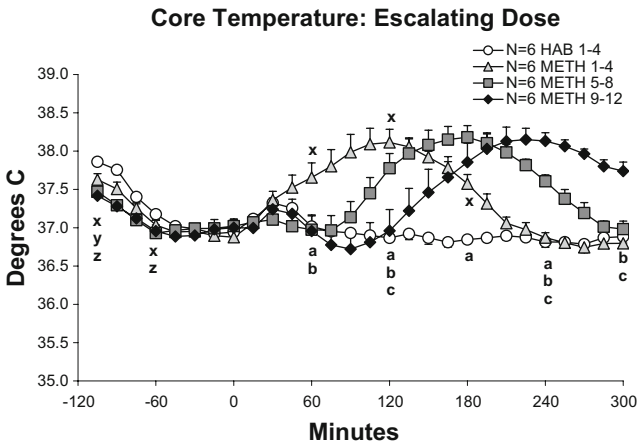

Fig. 2 Core temperature (mean±SEM). Rats received 4 days of saline injection followed by 12 days of an escalating-METH regimen. *x* HAB 1–4 was significantly different from METH 1–4, *y* HAB 1–4 was significantly different from METH 5–8, *z* HAB1–4 was significantly different from METH 9–12, *a* METH 1–4 was significantly different from METH 5–8, *b* METH 1–4 was significantly different from METH 9–12, *c* METH 5–8 was significantly different from METH 9–12. *HAB 1–4* Habituation day-block 1–4, *METH 1–4* METH day-block 1–4, *METH 5–8* METH day-block 5–8, *METH 9–12* METH day-block 9–12

and M9–12. The significance of this finding is that some aspect of chronic drug exposure resulted in a diminished core temperature at the start of the next test session, before new drug exposure.

Tests for initial hyperthermia (day-block 1–4) and effects due to chronic treatment

As stated above, for the saline-pretreatment group, the main effect of day-block and the day-block×session time interaction were not significant.

For the 5.0-mg/kg-pretreatment regimen, follow-up one-way ANOVAs showed significant effects of day-block at 60 min [$F(3,12)=5.6$, $p=0.0125$], 120 min [$F(3,12)=4.3$, $p=.0276$], 180 min [$F(3,12)=45.0$, $p=0.0000$], 240 min [$F(3,12)=11.5$, $p=0.0008$], and 300 [$F(3,12)=6.8$, $p=0.0063$]. Pairwise comparisons showed that 5.0-mg/kg-METH pretreatment resulted in hyperthermia during the initial day-block at 120, 180, and 240 min post-treatment (M1–4 was greater than HAB day-block 1–4). Chronic effects were seen at 60 min where M9–12 was lower than M1–4 and M5–8 and at 300 min where M9–12 was greater than M1–4 (Fig. 1).

For the escalating-dose-pretreatment regimen, follow-up one-way ANOVAs showed significant effects of day-block at 60 min [$F(3,15)=8.5$, $p=0.0016$], 120 min [$F(3,15)=25.8$, $p=0.0000$], 180 min [$F(3,15)=19.0$, $p=0.0000$], 240 min [$F(3,15)=58.3$, $p=0.0000$], and 300 min [$F(3,15)=32.7$, $p=0.0000$]. Pairwise comparisons indicated that escalating-METH pretreatment resulted in hyperthermia during the initial day-block at 60, 120, and 180 min post-treatment (M1–4 was greater than HAB day-block 1–4). There were also significant effects of chronic drug exposure at several time points. At 60 min, M5–8 and M9–12 were less than M1–4. At 120 min, M5–8 and M9–12 were less than M1–4; M9–12 was less than M5–8. At 180 min, M1–4 was less than M5–8. At 240 min, M1–4 was less than M5–8 and M9–12; M5–8 was less than M9–12. At 300 min, M1–4 and M5–8 were less than M9–12. As can be seen in Fig. 2, the escalating regimen resulted in a right-shift of the peak temperature response over day-blocks.

For the 10.0-mg/kg-pretreatment regimen, follow-up one-way ANOVAs showed significant effects of day-block at 30 min [$F(3,15)=4.3$, $p=0.0215$], 60 min [$F(3,15)=5.2$, $p=0.0115$], 90 min [$F(3,15)=7.5$, $p=0.0027$], 120 min [$F(3,15)=4.6$, $p=0.0173$], 180 min [$F(3,15)=6.8$, $p=0.0041$], 240 min [$F(3,15)=44.0$, $p=0.0000$], and 300 min [$F(3,15)=49.2$, $p=0.0000$]. Pairwise comparisons indicated that 10.0-mg/kg-METH pretreatment resulted in hyperthermia during the initial day-block at 120, 180, 240, and 300 min (M1–4 was greater than HAB day-block 1–4). There were also significant effects of chronic drug exposure at the following time points: 30 min, M9–12 was less than M1–4; 60 min, M5–8 and M9–12 were less than M1–4; 90 min, M5–8 and M9–12 were less than M1–4; 120 min, M5–8 and M9–12 were less than M1–4; 180 min, M9–12 was less than M1–4 (Fig. 3).

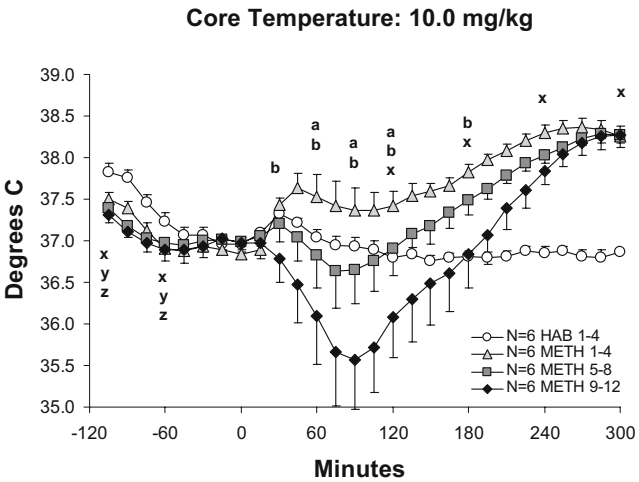

Fig. 3 Core temperature (mean±SEM). Rats received 4 days of saline injection followed by 12 days of 10.0 mg/kg METH. *x* HAB 1–4 was significantly different from METH 1–4, *y* HAB 1–4 was significantly different from METH 5–8, *z* HAB1–4 was significantly different from METH 9–12, *a* METH 1–4 was significantly different from METH 5–8, *b* METH 1–4 was significantly different from METH 9–12, *c* METH 5–8 was significantly different from METH 9–12. *HAB 1–4* Habituation day-block 1–4, *METH 1–4* METH day-block 1–4, *METH 5–8* METH day-block 5–8, *METH 9–12* METH day-block 9–12

The 10.0-mg/kg challenge on day 13

To determine the effects of 10.0 mg/kg METH in rats without a prior history of METH, a two-way ANOVA was performed on the data from the saline-pretreatment group: day-block (day-block 9–12 vs. day 13) and session time. There were significant effects of day-block [$F(1,4)=103$, $p=0.0005$] and session time [$F(8,32)=9.0$, $p=0.0000$], and there was a significant interaction [$F(8,32)=11.7$, $p=0.0000$]. Post hoc analysis showed no significant effects of day-block at −105 min, −60 min, and injection time 0 min. However, at 30, 60, 120, 180, 240, and 300 min, significant drug-induced hyperthermia was evident after the 10.0-mg/kg challenge (Fig. 4; note that saline day-block 9–12 is not included in this figure).

A second two-way ANOVA was conducted to compare the effects of the four different pretreatment conditions (saline, 5.0 mg/kg, escalating, 10.0 mg/kg METH) on the temperature response after a 10.0-mg/kg-METH-challenge injection. There were significant effects of pretreatment [$F(3,18)=7.9$, $p=0.0014$] and session time [$F(8,144)=29.5$, $p=0.0000$], and there was a significant interaction [$F(24,144)=4.6$, $p=0.0000$]. Follow-up one-way ANOVAs indicated significant carry-over effects at −105 min [$F(3,18)=6.0$, $p=0.0049$]; significant post-injection effects were found at 30 min [$F(3,18)=4.5$, $p=0.0159$], 60 min [$F(3,18)=19.2$, $p=0.0000$], and 120 min [$F(3,18)=4.5$, $p=0.0161$]. Before injection, at −105 min (carry-over effects), the saline-pretreatment group was greater than all METH pretreatment groups. At 30 min post-injection (challenge effects), the 10.0-mg/kg-METH-pretreatment group was less than the saline-pretreatment group, with the saline group exhibiting hyperthermia and the 10.0 mg/kg group exhibiting hypothermia. At 60 min, the saline group was significantly greater than the 5.0 mg/kg, the escalating regimen, and the 10.0-mg/kg-METH-pretreatment groups; the 5.0 mg/kg and the escalating-METH groups were greater than the 10.0-mg/kg-METH group; however, there was no difference between the 5.0 mg/kg and escalating-METH groups. At 120 min, the saline group was greater than the escalating regimen and 10.0 mg/kg groups (Fig. 4).

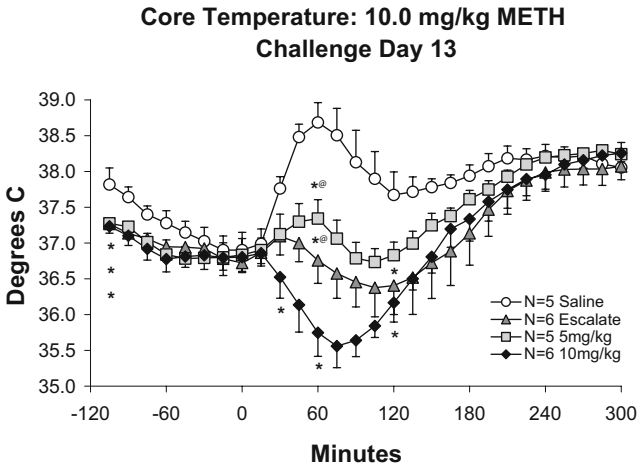

Fig. 4 Core temperature (mean±SEM). All rats received a 10.0-mg/kg-METH challenge on day 13 after 12 days of pretreatment with saline, 5.0 mg/kg METH, an escalating-dose-METH regimen, or 10.0 mg/kg METH. * Significantly different from the chronic saline-pretreatment group; @ significantly different from the chronic 10.0-mg/kg-METH-pretreatment group

Table 1 Observed salivation during drug administration

Pretreatment	Degree of salivation	Day of earliest appearance	Number of rats
Saline	2	Day 13 challenge	1 of 5
5.0 mg/kg	1–3	8th METH day	1 of 5
Escalating	1–3	9th and 11th METH day	2 of 5
10.0 mg/kg	1–3	1st, 2nd, 3rd, and 8th METH day	5 of 5

0 No salivation, animal does not appear wet, *1* salivation onto the floor, small amount on the body, *2* wet forelimbs and hind limbs, *3* ventral body surface appears wet

Observations: salivation

Five of six rats in each group were observed for salivation. There were significant main effects of treatment at day-block 1–4 ($p=.0018$), day-block 5–8 ($p=.0095$), day-block 9–12 ($p=.0149$), but not on challenge day 13. Post hoc analysis showed that salivation was increased in the 10.0-mg/kg-pretreatment group compared to the saline-pretreatment group at all 3 day-blocks (Table 1).

Weight measurements

A two-way ANOVA (pretreatment×day-block) was performed. There was a significant effect of pretreatment [$F(3,18)=3.4$, $p=0.038810$], and the interaction was significant [$F(6,36)=13.2$, $p=0.0000$]. Post hoc analysis indicated that for day-block 5–8, saline was only different from 10.0-mg/kg-METH pretreatment. At day-block 9–12, saline was different from the three METH pretreatment groups (Table 2).

Table 2 Average daily weight (grams, mean±SEM) during the 12-day pretreatment period

Pretreatment	Day-block 1–4	Day-block 5–8	Day-block 9–12
Saline	377±8.2	383±8.4	389±9.6
5.0 mg/kg	370±2.4	367±2.4	367±1.8[a]
Escalating	375±5.5	372±4.4	370±3.9[a]
10.0 mg/kg	355±8.6	348±9.3[a]	350±9.7[a]

[a] Different from saline, same day-block

Table 3 Post-mortem DA and 5-HT tissue concentrations (nanogram per milligram tissue, mean±SEM) in the hypothalamus, hippocampus, and striatum

		Saline pretreatment	5.0-mg/kg pretreatment	Escalating pretreatment	10.0-mg/kg pretreatment
Hypothalamus	DA	0.311±0.02	0.321±0.01	0.315±0.01	0.345±0.01
	5-HT	0.523±0.04	0.544±0.03	0.506±0.03	0.522±0.01
Hippocampus	DA	–	–	–	–
	5-HT	0.311±0.01	0.302±0.02	0.301±0.02	0.295±0.01
Striatum	DA	11.964±0.42	10.962±0.46	11.966±0.27	11.703±0.12
	5-HT	0.302±0.02	0.303±0.01	0.296±0.01	0.300±0.01

There were no significant differences between pretreatment groups.

Neurochemistry

There were no significant depletions of DA or 5-HT in striatum, hippocampus, or hypothalamus for any of the METH pretreatment groups (Table 3).

Discussion

The effect of a chronic, escalating-METH regimen on core temperature was investigated in rats. METH treatment began after all groups received 4 days of saline exposure (HAB day-block 1–4) to habituate the animals to the testing/injection procedures. Both initial (METH or saline day-blocks 1–4) and chronic (METH or saline day-blocks 5–8 and 9–12) pretreatment regimens were then evaluated. A final challenge dose of 10.0 mg/kg METH was administered to all pretreatment groups on the 13th treatment day, for a total of 17 days of temperature testing. Consistent with prior research, initial administration resulted in hyperthermia (Lewander 1971; Myles et al. 2008; Thornhill et al. 1977), chronic administration resulted in a subsequent diminished core temperature response (Lewander 1971; Myles et al. 2008), and the maximum temperature increase after the 5.0 mg/kg dose (24°C) occurred at 3 h post-treatment (Myles et al. 2008).

Escalating-dose regimen

The primary focus of this report was to determine (a) whether an escalating-METH regimen would result in a diminishing temperature response over a chronic treatment period and (b) how the degree of temperature change would relate to that found with a chronic, non-escalating regimen with a total dose that was equivalent to the escalating regimen. The escalating regimen involved one injection per day, increasing the dose each day; the total-dose comparison group involved the same dose (5.0 mg/kg) each day. The individual patterns demonstrated by these two groups differed across the 12-day pretreatment period. With successive days, the 5.0-mg/kg-pretreatment group demonstrated a diminishing core temperature at 1 h post-injection and a consistent hyperthermia at 3 h (Fig. 1). The escalating regimen resulted in a right-shift in the core temperature profile over the 12-day pretreatment period (Fig. 2). We recently showed that single injections (0.5 to 5.0 mg/kg METH) resulted in longer peak delays with increasing dose (Phelps et al. 2005). This finding suggested that, in addition to chronic treatment, the shift seen here with the escalating regimen was because of the increasing dose.

We also demonstrated that even though the 5.0 mg/kg and the escalating regimen groups (equivalent total-dose groups) showed different temperature profiles during the 12-day regimen, they demonstrated similar patterns when challenged with 10.0 mg/kg METH (treatment day 13, Fig. 4). This is particularly evident at the 60 min post-treatment time point, when the chronic 5.0 mg/kg and escalating pretreatment groups were different from the saline and 10.0-mg/kg-pretreatment groups, but not from each other. These findings suggest that a gradually increasing regimen and a constant-amount daily regimen have similar long-term effects on core temperature, when the total dose and duration of exposure (once a day over a 12-day period) are held constant. Whether this conclusion can be generalized to higher doses will need further testing.

Two-phase temporal response to METH administration

Also important in this study was the time-dependent response of core temperature for the different pretreatment groups. Chronic METH (5.0 and 10.0 mg/kg) resulted in a diminishing core temperature response at 1 h post-treatment. By 2 and 4 h post-treatment (5.0 and 10.0 mg/kg METH, respectively), METH day-block 1–4 showed hyperthermia, yet none of the METH day-blocks differed from each other (Figs. 1 and 3). This finding indicates that chronic treatment did not diminish the temperature response 2–4 h into the session. In addition, on the 10.0-mg/kg challenge day, at 1 h post-METH, the saline-pretreatment group demonstrated hyperthermia, the 10-mg/kg-pretreatment group showed hypothermia, and the 5.0-mg/kg and escalating regimens showed an intermediate core temperature response. How-

ever, by 3 h post-injection, no group differences existed (Fig. 4). This pattern of results supports the suggestion of our prior work (Myles et al. 2008) that METH induces a two-phase temperature response: a malleable early phase and a less flexible later phase.

Long-term neurochemical, neuroanatomical, and cognitive changes have been identified in humans with a history of METH use (Chang et al. 2007; Chang et al. 2005; Chang et al. 2002; Simon et al. 2002; Thompson et al. 2004; Volkow et al. 2001; Wilson et al. 1996). Neurochemical changes are altered by differences in core temperature (Bowyer et al. 1994). Intermittent pretreatment with METH results in tolerance to neurotoxicity induced by subsequent high-dose treatment; this tolerance to neurotoxic consequences, in part, may be due to tolerance to hyperthermia (Danaceau et al. 2007; Riddle et al. 2002). After pretreatment with an escalating intermittent regimen, Johnson-Davis et al. (2003) reported a significant tolerance to METH-induced hyperthermia at 90 min post-treatment but not later during a high-dose regimen; the METH pre-exposure also protected against damage induced by the high-dose regimen. Based on the results of Johnson-Davis et al. (2003) and our findings that the phase 1 temperature response may be more flexible than the phase 2 response, we suggest that phase 1 temperature responses might be responsible for enhanced neurotoxicity induced by METH, as well as the protection afforded by cooling procedures; early intervention may wield greater influence on the long-term consequences of METH.

Mechanisms of METH-induced chronic temperature changes

Reductions in core temperature, after chronic treatment with stimulants, are often discussed as tolerance to an acute hyperthermic response**.** In our companion report (Myles et al. 2008), we found that the change in temperature after chronic METH treatment was sometimes an induction of hypothermia relative to a previously neutral baseline instead of a diminished hyperthermia. This finding was supported in the present report as well. At 60 min post-treatment, the animals receiving chronic 5.0 mg/kg METH failed to show an initial increased core temperature (day-block 1–4) but demonstrated a decreased temperature response with repeated treatment (Fig. 1). See Myles et al. (2008) for further discussion.

Salivation When testing at the 10.0 mg/kg dose, two different temperature patterns emerged at 60 min post-treatment. The initial treatment period (METH day-block 1–4, Fig. 3) failed to show an increased temperature until 120 min post-treatment. This finding represents a divergence from our previous work (Myles et al. 2008), in which treatment (day-block 1–4) resulted in a significant increase at 60 min, and from this report where the saline-pretreatment group showed a significant increase 60 min after the 10.0-mg/kg-METH challenge. The reason for this discrepancy is not clear but may be related to factors not controlled for across the experiments. For example, environmental variables such as humidity or subject factors such as salivation may have played a role. In the rat, spreading saliva on the fur contributes to heat loss during thermal stress, and continuous exposure to heat can cause hypertrophy of the salivary glands (Gordon 1990, 1993). In our companion report, we noted the presence of salivation but did not systematically measure it. We did so in this report. Rats receiving chronic 10.0 mg/kg (but not chronic 5.0 mg/kg or the escalating regimen) salivated significantly more than saline-treated rats during all 3 day-blocks of the METH pretreatment period. In this study, salivation may have attenuated METH-induced hyperthermia for the 10.0 mg/kg pretreatment group (day-block 1–4) and contributed to subsequent temperature decreases throughout the METH treatment period. The extent to which salivation plays a role in cooling rats chronically treated with METH will require further study.

Anorexia It has been shown that calorie-restricted rats show lower body temperatures than rats fed ad libitum (Duffy et al. 1989; Duffy et al. 1997). Rats in our chronic regimen groups experienced significant weight loss during the pretreatment period (Table 2). During the 5–8 day-block, only the 10.0-mg/kg group differed significantly from saline, but at the 9–12 day-block, all METH pretreatment groups were different from saline. Anorexia, therefore, may be a contributing factor to the development of hypothermia during the phase 1 response.

Neurochemistry METH induces transient DA depletions, which, in part, may be because of diminished synthesis (Bowyer et al. 1992). METH-induced 5-HT and DA depletions are also associated with neurotoxicity (Bowyer et al. 1994; O'Callaghan and Miller 1994; Sabol et al. 2001; Wagner et al. 1980)**.** In this report, DA and 5-HT post-mortem tissue concentrations were not depleted when measured 2 weeks after completion of treatment (Table 3). We have not addressed the role of transient decreases in monoamine tissue levels post-METH; however, our findings that depletions were not present 2 weeks after the completion of treatment suggest that the effects of chronic METH treatment on core temperature are not because of the onset of a longer-term degeneration of DA or 5-HT axon terminals. See Myles et al. (2008).

Context Environmental context has been identified as an important determinant for tolerance to certain drug effects

(Poulos and Cappell 1991). In particular, Hinson et al. (1991) found that tolerance developed to amphetamine-induced hyperthermia in one environment, but hyperthermia was reinstated when injected with amphetamine in an environment previously associated with saline injections. One aspect of our data suggests that environmental context may have been a factor in the temperature reductions we observed with chronic exposure. In our prior report (Myles et al. 2008), we found that both saline and METH decreased baseline temperature during a chronic treatment regimen (baseline is defined as temperature measurements before daily injections). In the present report, we modified our design (4 days of saline alone for all groups (HAB day-block 1–4) and longer baseline periods within each daily session) and found that chronic treatment with METH, but not saline, decreased baseline temperature. This pattern is demonstrated in Figs. 1–3, where the habituation days (HAB day-block 1–4) showed significantly higher core temperature compared to the METH treatment day-blocks (2 h before treatment). The same finding is also evident in Fig. 4 on challenge day 13: the saline-pretreatment animals had a significantly higher core body temperature at 2 h before injection compared to all other pretreatment groups. Context (temperature test chambers) may have triggered this preparatory response. Alternatively, this decreased core temperature during baseline periods may have been triggered by internal responses to repeated METH treatment rather than the environment.

In sum, while the absence of long-lasting DA/5-HT depletions suggests an absence of neurotoxicity, other factors including short-term monoamine depletions, salivation, anorexia, and context may have contributed to the altered temperature response after chronic METH.

Summary

Initial exposure to METH (day-block 1–4) increased core temperature at 24°C ambience. The timing of this increase was dose dependent. Subsequent decreases in temperature were evident with chronic exposure. A constant daily regimen (5.0 mg/kg/day) and an escalating regimen of the same total dosage produced different temporal patterns of core temperature response; however, these regimens demonstrated a similar decrease in temperature (relative to the saline-pretreatment group) when given a challenge dose of METH.

The differential response to chronic METH at 1 vs. 3 h post-treatment suggests two separate mechanisms of thermoregulation. The attenuated temperature response after chronic METH treatment is unrelated to neurotoxicity as indexed by central DA or 5-HT tissue concentrations measured 2 weeks after the end of treatment. Other potential contributors to the chronic temperature response include short-term monoamine depletion, salivation, and anorexia. Chronic exposure to METH also produced a decrease in baseline temperature, possibly related to Pavlovian conditioning.

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