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ORIGINAL INVESTIGATION

The effects of methamphetamine on core body temperature in the rat—PART 1: chronic treatment and ambient temperature

Benita J. Myles · Lee Ann Jarrett · Susan L. Broom · H. Anton Speaker · Karen E. Sabol

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Abstract
Rationale Stimulants such as methamphetamine (METH) alter core temperature in a manner that is dependent on ambient temperature and that shows tolerance after chronic use. Our objectives were to (1) determine whether tolerance to METH-induced hyperthermia was a consequence of neurotoxicity to dopamine or serotonin and (2) determine the relationship between ambient temperature and chronic treatment on the METH-induced temperature response.
Materials and methods Rats were treated with 1.0, 5.0, or 10.0 mg/kg METH at 24°C (experiment 1) or treated with 5.0 mg/kg METH at 20°C, 24°C, or 28°C (experiment 2). Treatment occurred for 12 days, and temperature measurements were made once per minute telemetrically during 7-h sessions in computer-regulated environments.
Results Peak increases in core temperature occurred at 60 min post-treatment for the 1.0 and 10.0 mg/kg doses, and at 180 min for the 5.0 mg/kg dose. Tolerance-like effects were seen with chronic 5.0 (mixed results) and 10.0 mg/kg METH in the absence of dopamine or serotonin depletions measured 2 weeks after the completion of treatment. After 5.0 mg/kg METH, variations in ambient temperature resulted in an early flexible change in core temperature (phase 1) (hyperthermia at 28° and hypothermia at 20°) and a later inflexible hyperthermia (phase 2).
Conclusions The results suggest that (1) the peak effect of different doses of METH occurs at different times (24°), (2) the diminished temperature response with chronic METH treatment was not associated with long-term dopamine and serotonin depletions, and (3) a two-phase temperature response to METH may reflect two independent mechanisms.

Keywords Drug abuse · Methamphetamine · Temperature · Rat · Tolerance

L. A. Jarrett · S. L. Broom · H. A. Speaker · K. E. Sabol (✉)
Department of Psychology, University of Mississippi, University, MS 38677, USA
e-mail: ksabol@olemiss.edu

B. J. Myles · K. E. Sabol
Department of Pharmacology, University of Mississippi, University, MS 38677, USA

Methamphetamine (METH) and amphetamine (AMPH) are drugs of abuse. Humans who use the AMPHs sometimes develop patterns of chronic use and tolerance (Brecht et al. 2000; Comer et al. 2001; Perez-Reyes et al. 1991; Reiber et al. 2000; Simon et al. 2002b). After long-term use cognitive, neurophysiological, and neuroanatomical deficits have been reported (Chang et al. 2007; Chang et al. 2002; Paulus et al. 2002; Salo et al. 2002; Simon et al. 2002a; Thompson et al. 2004; Volkow et al. 2001). Hyperthermia also occurs, which can be lethal (Ishigami et al. 2003) and enhance neurophysiological deficits (Bowyer et al. 1994). In order to understand the acute and long-term effects of METH, an understanding of its effects on core temperature is important. In this report, we studied the development of tolerance to METH-induced hyperthermia and the influence of ambient temperature on this process.

After chronic AMPH, tolerance to hyperthermia develops in a dose-dependent manner. Thornhill et al. (1977) found no tolerance to 1.0 and 5.0 mg/kg AMPH, once per day for 10 days, whereas Lewander (1971) reported tolerance after 16.0 mg/kg, twice/day for 12 days.

High-dose METH sometimes leads to tolerance in other paradigms. In vivo dialysis experiments showed decreased extracellular accumulation of dopamine (DA) upon AMPH (Cass et al. 1998) or METH challenge (Sabol et al. 2001) in animals pre-exposed to high-dose METH. Ando et al.

(1985) and Finnegan et al. (1982) treated monkeys chronically with METH and found tolerance to the effects of METH on operant responding. In each of these studies, post-mortem analysis indicated DA and/or serotonin (5-HT) depletions. It is therefore possible that the doses used by Lewander (1971) were high enough to be neurotoxic. The purpose of our first experiment was to determine whether tolerance to METH-induced hyperthermia can occur in the absence of neurotoxicity as measured by long-term DA and 5-HT depletions.

For METH and its analogs, ambient temperature affects drug-induced changes in body temperature. AMPH (7.5 or 15 mg/kg) caused hyperthermia at 20–37°C but hypothermia at 4–15°C (Yehuda and Wurtman 1972). METH (5.0 mg/kg×4) caused hyperthermia at 23°C and hypothermia at 4°C (Bowyer et al. 1992), whereas 3,4-methylenedioxy-*N*-methamphetamine (MDMA) induced hypothermia at 20–22°C and hyperthermia at 28–30°C (Malberg and Seiden 1998). The purpose of experiment 2 was to manipulate ambient temperature to help clarify our understanding of METH-induced hyperthermia and subsequent tolerance.

Rodent temperature measurements are often made using rectal probes (Johnson-Davis et al. 2004; Lewander 1971; Riddle et al. 2002; Thornhill et al. 1977). In this report, we recorded temperature using telemetric measurements of radio frequency signals emitted by abdominal probes. This method allowed frequent measurements without temperature changes that may be related to stress (handling the rats) (Gordon 1990, 1993).

Experiment 1

As stated above, the tolerance to AMPH-induced hyperthermia reported by Lewander (1971) may have been secondary to AMPH-induced neurotoxicity. DA and 5-HT, targets for METH-induced neurotoxicity, have a role in temperature regulation (Gudelsky et al. 1985; Hansen and Whishaw 1973; Lee et al. 1985; Salmi and Ahlenius 1998). We selected a dose range that was not likely to cause long-term depletions of DA or 5-HT (index of neurotoxicity) yet would maintain a potential for the development of tolerance to hyperthermia. Fukumura et al. (1998) reported that a single dose of 10 mg/kg did not deplete DA or 5-HT, but single administrations of 20, 30, or 40 mg/kg did cause depletions. Wagner et al. (1980) reported that daily injections of 12.5 mg/kg did not deplete striatal DA after 40 days of treatment; however, daily injections of 25 mg/kg for 20 days did cause depletions. Based on these reports, we selected the following treatments: daily doses of saline and 1.0, 5.0, or 10.0 mg/kg METH for 12 days at an ambient temperature of 24°C. In order to evaluate possible neurotoxic effects of the chronic regimens, post-mortem tissue concentrations of DA and 5-HT were measured 2 weeks after the completion of treatment. We chose a post-treatment survival interval of 2 weeks for several reasons. First, this interval has been associated with long-term depletions of DA and 5-HT after high-dose regimens (Sabol et al. 2000; Wagner et al. 1980). It is also the interval used in the Wagner et al. (1980) report discussed above, which showed no depletions after daily 12.5 mg/kg injections. Second, we wanted to avoid short-term reversible depletions. For example, using MDMA (10.0 mg/kg), Schmidt (1987) reported temporary 5-HT depletions at 6-h post-treatment, which recovered at 24 h; a second depletion occurred between 24 h and 7 days.

We hypothesized that hyperthermia would develop to all doses of chronic METH; neither tolerance to METH-induced hyperthermia nor neurotransmitter depletions would occur at 1.0 and 5.0 mg/kg. Tolerance would develop at 10.0 mg/kg in the absence of DA and 5-HT depletions.

Materials and methods: experiments 1 and 2

Subjects

Sixty male Sprague–Dawley rats (Harlan, Indianapolis, Indiana, USA) weighing 300–325 g upon arrival were used (24 rats in experiment 1, 36 rats in experiment 2). Rats were individually housed in hanging stainless steel cages, maintained on a 12-h light/dark cycle (lights on at 7:00 AM), and had free access to food and water except during the 7-h test sessions (see below). 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 body temperature, radio transmitters (Mini-mitter, model #VM-FH disc) were implanted into the abdomen of all rats. Animals were anesthetized with sodium pentobarbital (50 mg/kg). Supplemental anesthesia was with ketamine at 10.0-mg doses as needed. Ketoprofen (5.0 mg/kg) was given for postsurgical analgesia in experiment 2.

Drugs

(+)-Methamphetamine hydrochloride (Sigma, St. Louis, MO, USA) was dissolved in physiological saline. A constant injection volume of 1 ml/kg was used. Each rat

(n=6/treatment) received intraperitoneal (i.p.) injections of saline or dose of METH once per day for 12 consecutive days (doses expressed as the salt).

Procedure

Seven days after arrival, surgery was performed. Two weeks after surgery, the rats received treatments for 12 consecutive days. In experiment 1, saline, 1.0, 5.0, or 10.0 mg/kg METH was administered at 24°C ambient temperature. In experiment 2, rats received saline or 5.0 mg/kg METH at 20°C, 24°C, or 28°C ambient temperature once per day. Treatments were administered in computer-controlled environmental chambers, where the rats were monitored 7 h per day. Treatment sessions occurred during the light cycle and started between 7:00 AM and 9:00 AM. Daily injections occurred 1 h after the rats were placed into the chambers. Temperature signals emitted by the abdominal temperature transmitters were automatically recorded once per minute. This procedure eliminated the need for handling the rats during temperature measurements. Two weeks after the completion of testing, the animals were euthanized by decapitation and their brains rapidly dissected on ice for high-performance liquid chromatography (HPLC) neurochemical analysis.

Neurochemical analysis

DA and 5-HT were analyzed post-mortem using HPLC. After the addition of an internal standard (3,4-dihydroxybenzylamine hydrobromide) and 0.01 N HClO4, brain samples were homogenized and centrifuged (20 min, 14,000 rpm, two times). HPLC instrumentation included ESA model 580 pump, Rheodyne model 7010 injection valve, Adsorbosphere Catecholamine 100×4.6 mm column (C_{18}, 3 μm particle size), ESA Coulochem II model 5200 coulometric detector, with applied potentials of −100 mV (E1), and +330 mV (E2), EZ-Chrome, Chromatography Data System version 6.7. Mobile phase for experiment 1 consisted of 14.2 g/l monochloroacetic acid, 5.25 g/l NaOH, 0.15 g/l octyl sodium sulfate, 0.745 g/l ethylenediaminetetraacetic acid (EDTA), 10% MeOH, pH=3.0. Mobile phase for experiment 2 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, 7.5% acetonitrile, pH=3.1, using phosphoric acid.

Data analysis: experiment 1

For temperature analysis, data for 4 days were combined; this resulted in three 4-day blocks: day-blocks 1–4, 5–8, and 9–12. To determine whether treatment with METH resulted in an initial hyperthermia, a two-way analysis of variance (ANOVA) was used for day-block 1–4. A within-subjects factor was session time (0, 30, 60, 120, 180, 240, 300, and 360 min post-treatment), and a between-subjects factor was dose of METH (saline, 1.0, 5.0, or 10.0 mg/kg). Significant effects were followed by one-way ANOVAs at specific time points. Where appropriate, t tests (modified Bonferroni correction: $p=(df)(0.05)$/number of comparisons; Keppel 1991) were applied to determine which METH treatments were different from saline: $p<0.05$.

To determine the effect of chronic treatment within each dose, a three-way ANOVA was used. Within subjects factors were day block (1–4, 5–8, and 9–12) and session time (0, 30, 60, 120, 180, 240, 300, and 360 min post-treatment). A between-subjects factor was treatment (saline, 1.0, 5.0, or 10.0 mg/kg METH). Follow-up two-way ANOVAs (treatment×day block) were conducted to explore significant interactions. One-way ANOVAs and modified Bonferroni t tests were performed at specific session times: $p<0.033$.

For the neurochemical data, one-way ANOVAs (saline, 1.0, 5.0, and 10.0 mg/kg METH) were used to analyze DA and 5-HT tissue concentrations in each brain region. Brain regions analyzed were nucleus accumbens, hippocampus, striatum, hypothalamus, substantia nigra/ventral tegmental area, and somatosensory cortex (Sabol et al. 2000).

Data analysis: experiment 2

For temperature analysis, data for 4 days were combined; this resulted in three 4-day blocks: day-blocks 1–4, 5–8, and 9–12. To determine whether initial treatment with 5.0 mg/kg METH at different ambient temperatures resulted in differences in the core temperature profile, a three-way ANOVA was used to analyze the effect of treatment (between subjects factor, METH vs. saline), ambient temperature (between subjects factor, 20°, 24°, and 28°C), and session time (within subjects factor, time points 0, 30, 60, 180, and 360 min post-injection) for day-block 1–4. A significant interaction was followed by two-way ANOVAs at specific time points (treatment×ambient temperature). Each significant two-way ANOVA was followed by six post hoc pairwise comparisons (the three METH ambient temperatures compared to each other; saline compared to METH at each ambient temperature), where significance was determined using a modified Bonferroni correction of $p<0.0167$.

To determine the effect of chronic treatment, three-way ANOVAs were used for each ambient temperature tested, comparing drug treatment (between subjects factor, METH vs. saline), day-block (within subjects factor, day-blocks 1–4, 5–8, and 9–12), and session time (within subjects factor, time points 0, 30, 60, 180, and 360 min post-injection). Significant interactions were followed by two-way ANOVAs (treatment×day-block) at specific session times

(0, 30, 60, 180, and 360 min). Significant two-way interactions were followed by six pairwise comparisons, comparing day-blocks 1–4 to 5–8, 1–4 to 9–12, and 5–8 to 9–12 for both METH and saline treatments. This resulted in a modified Bonferroni correction of $p<0.0167$. Significant main effects of day-block but non-significant interactions were followed by pairwise comparisons of day-blocks at specific time points (day-block 1–4 vs. 5–8, 1–4 vs. 9–12, and 5–8 vs. 9–12) after collapsing across treatments, modified Bonferroni correction $p<0.033$.

For the neurochemical data, two-way ANOVAs were used to analyze the effect of drug treatment (METH or saline) and ambient temperature (20°C, 24°C, or 28°C) for each brain region. Regions analyzed were the striatum, hippocampus, and hypothalamus.

Results: experiment 1

Rats were chronically treated with saline or METH (1.0, 5.0, or 10.0 mg/kg), once per day, for 12 days. We evaluated the initial change in core temperature (day-block 1–4, each dose compared to saline), as well as subsequent changes that developed as a result of chronic treatment (day-block 1–4 compared to day-blocks 5–8 and 9–12 within each dose).

Temperature: initial METH treatment (day-block 1–4)

A significant main effect of treatment [$F(3,20)=4.1$, $p=0.02$], main effect of session time [$F(7,140)=20.6$, $p=0.0000$], and interaction [$F(21,140)=14$, $p=0.0000$] were identified. Follow-up one-way ANOVAs uncovered significant effects of treatment at 60 min [$F(3,20)=7.8$, $p=0.0012$], 120 min [$F(3,20)=3.36$, $p=0.039$], 180 min [$F(3,20)=9.7$, $p=0.0004$], 240 min [$F(3,20)=13$, $p=0.0001$], 300 min [$F(3,20)=8.2$, $p=0.0010$], and 360 min post-treatment [$F(3,20)=4.78$, $p=0.011$]. Pairwise comparisons indicated that 1.0 mg/kg METH significantly increased core temperature above saline at 60 and 120 min post-treatment, 5.0 mg/kg METH increased temperature at 180 and 240 (but not 60) min post-treatment, and 10.0 mg/kg METH increased temperature at 60, 120, 180, 240, 300, and 360 min post-treatment. See Fig. 1.

Temperature: chronic METH treatment

For the three-way ANOVA, significant main effects of day-block [$F(2,40)=13$, $p=0.0000$] and session time [$F(7,140)=14$, $p=0.0000$] were found. Significant interactions were also found: treatment×day-block [$F(6,140)=2.9$, $p=0.018$], treatment×session time [$F(21,140)=16$, $p=0.0000$], day-block×session time [$F(14,280)=2.1$, $p=0.011$] and treatment×day-block×session time [$F(42,280)=1.7$, $p=0.008$]. Follow-up two-way ANOVAs were conducted at 0, 30, 60, 120, 180, 240, 300, and 360 min post-treatment. At 0 min, a significant main effect of day-block [$F(2,40)=3.7$, $p=0.035$] was found. At 30 min, significant main effects of treatment [$F(3,20)=3.30$, $p=0.042$] and day-block [$F(2,40)=15$, $p=0.0000$] and a significant treatment×day-block interaction [$F(6,40)=2.8$, $p=0.023$] were identified. At 60 min, main effects of treatment [$F(3,20)=6.7$, $p=0.003$] and day block [$F(2,40)=9.5$, $p=0.0004$], and the interaction [$F(6,40)=4$, $p=0.003$] were significant. At 120 min, the main effect of treatment was borderline [$F(3,20)=3$, $p=0.053$], the main effect of day-block was significant [$F(2,40)=4$, $p=0.027$], and the interaction was borderline [$F(6,40)=2.3$, $p=0.057$]. At 180 min, significant main effects of treatment [$F(3,20)=6$, $p=0.004$] and day-block [$F(2,40)=6.7$, $p=0.003$] were found; the interaction was borderline [$F(6,40)=2.3$, $p=0.054$]. At 240 min, significant main effects of treatment [$F(3,20)=8$, $p=0.0008$] and day-block [$F(2,40)=3.8$, $p=0.032$] were identified. At 300 min [$F(3,20)=6.8$, $p=0.002$] and 360 min [$F(3,20)=4.6$, $p=0.013$], significant main effects of treatment were found.

The significant two-way interactions between treatment and day-block at 30 and 60 min post-treatment were further analyzed. Chronic saline decreased temperature at 30 min: day-blocks 5–8 and 9–12 were lower than day-block 1–4. Temperature did not change over day-blocks with chronic 1.0 mg/kg METH. Temperature did not change over day-blocks with chronic 5.0 mg/kg METH; however, a borderline chronic effect was seen at 60 min ($p=0.053$). Chronic 10.0 mg/kg METH decreased temperature: day-blocks 5–8 and 9–12 were lower than day-block 1–4 at 30 and 60 min post-treatment. See Fig. 2.

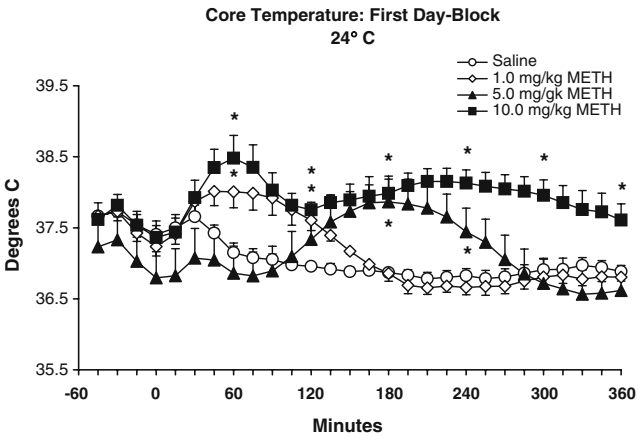

Fig. 1 Initial METH effects (day-block 1–4) on core temperature (mean ± SEM) after single daily injections of saline, 1.0 mg/kg, 5.0 mg/kg, or 10.0 mg/kg of METH. *Significantly different from saline during the same post-injection time period

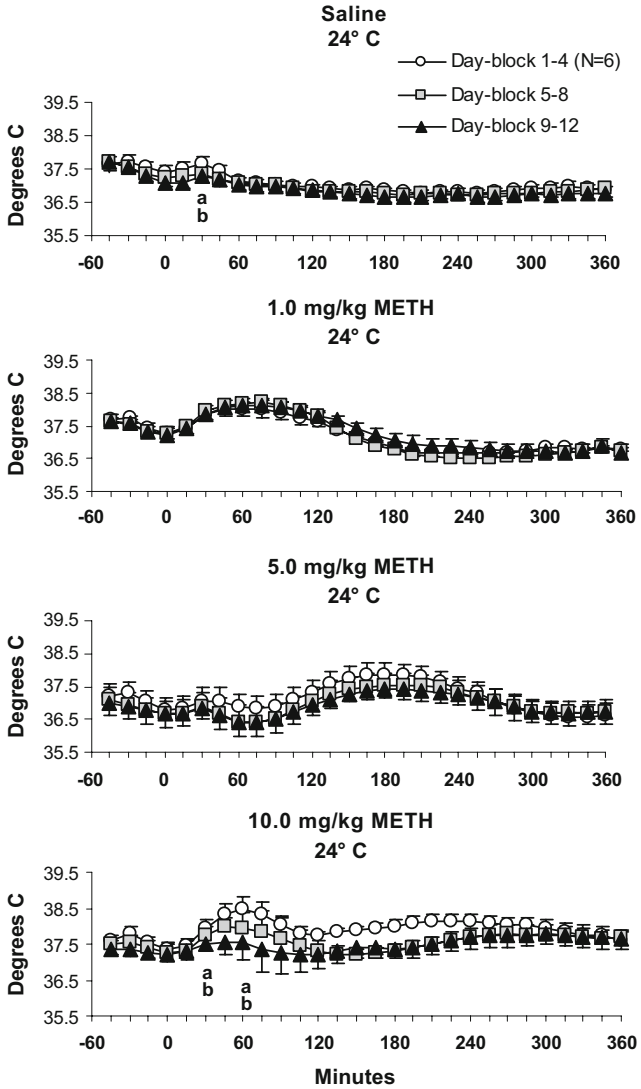

Fig. 2 Chronic METH effects (day-blocks 1–4, 5–8, and 9–12) on core temperature (mean ± SEM): saline, 1.0 mg/kg METH, 5.0 mg/kg METH, or 10.0 mg/kg METH. *a* Day-block 1–4 was significantly different from 5–8, same dose comparison; *b* day-block 1–4 was significantly different from 9–12, same dose comparison

A descriptive example of the 12 individual days across the treatment period for 10.0 mg/kg METH is depicted in Fig. 3. The greatest changes in temperature early in the session occurred between days 1 and 2 and again between days 10, 11, and 12. Day 4 appears higher than day 3, perhaps reflecting a nonrepresentative rise between days 3–4. Days 10, 11, and 12 appear different from each other in a natural progression, suggesting that the individual day analysis may be more sensitive to changes later in the 12-day sequence. In the later part of the session, the first 4 days remained higher than the remaining 8 days, but the distinction between the last two day-blocks has become less clear.

Neurochemical analyses

There were no significant changes in DA or 5-HT 2 weeks after completion of chronic treatment with saline, 1.0, 5.0, or 10.0 mg/kg METH (see Table 1).

Experiment 2

The findings of experiment 1 showed that 1.0, 5.0, and 10.0 mg/kg METH (12 daily injections) produced hyperthermia, and 10.0 mg/kg METH produced tolerance to hyperthermia at an ambient temperature of 24°C. The maximum increase in temperature after 5.0 mg/kg was at 3 h post-treatment; the maximum response after 1.0 and 10.0 mg/kg METH was at 60 min. In addition, borderline effects of chronic 5.0 mg/kg METH (p=0.053) suggested an enhancement of hypothermia rather than a tolerance to hyperthermia at 60 min (Fig. 2). Ambient temperature alters the direction of temperature change induced by METH (Bowyer et al. 1992). In experiment 2, we focused on the 5.0 mg/kg dose, and manipulated ambient temperature to help clarify the timing of the core temperature response and the effects of chronic METH on temperature. We hypothesized that ambient temperature would affect the core temperature response to initial (day-block 1–4) and chronic METH treatment (day-block 1–4 vs. 5–8 and 9–12).

Results: experiment 2

Rats were treated with saline or 5.0 mg/kg METH at three different ambient temperatures (20°C, 24°C, or 28°C), once per day for 12 days. We evaluated initial changes (day-block 1–4) and chronic changes (day-blocks 1–4 vs. 5–8, and 9–12) in core temperature.

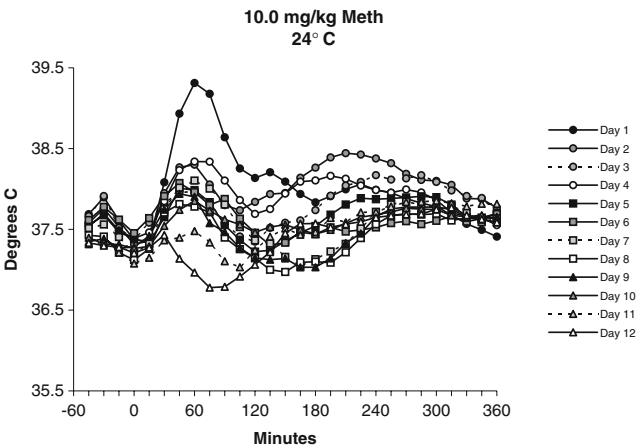

Fig. 3 Chronic METH effects (10.0 mg/kg) on core temperature (mean): 12 individual days

Table 1 Experiment 1: post-mortem 5-HT and DA tissue concentrations

	NA/OT	HIPPO	STR	HYPOTHAL	SN/VTA	SSCTX
Serotonin (mean ± SEM, ng/mg tissue)						
Saline	0.895 (±0.110)	0.375 (±0.031)	0.389 (±0.008)	0.690 (±0.024)	0.720 (±0.057)	0.351 (±0.017)
1.0 METH	0.790 (±0.071)	0.328 (±0.009)	0.367 (±0.017)	0.672 (±0.029)	0.720 (±0.052)	0.346 (±0.024)
5.0 METH	0.805 (±0.118)	0.357 (±0.022)	0.381 (±0.024)	0.706 (±0.060)	0.770 (±0.067)	0.327 (±0.019)
10.0 METH	0.789 (±0.050)	0.367 (±0.015)	0.382 (±0.015)	0.649 (±0.026)	0.683 (±0.046)	0.354 (±0.018)
Dopamine (mean ± SEM, ng/mg tissue)						
Saline	5.38 (±0.85)		11.71 (±0.32)	0.394 (±0.022)	0.316 (±0.039)	
1.0 METH	4.63 (±0.38)		11.27 (±0.56)	0.355 (±0.027)	0.326 (±0.049)	
5.0 METH	5.15 (±0.91)		11.69 (±0.35)	0.385 (±0.026)	0.420 (±0.067)	
10.0 METH	4.65 (±0.42)		10.67 (±0.45)	0.434 (±0.016)	0.311 (±0.062)	

No differences between METH and saline treatment were found (ambient temperature 24°C).
NA/OT nucleus accumbens/olfactory tubercle, *HIPPO* hippocampus, *STR* striatum, *HYPOTHAL* hypothalamus, *SN/VTA* substantia nigra/ventral tegmental area, *SSCTX* somatosensory cortex

Core temperature: initial METH treatment (day-block 1–4)

A three-way ANOVA resulted in significant main effects of treatment [$F(1,30)=15$, $p=0.0006$] and session time [$F(4,120)=57$, $p=0.0000$]. Significant interactions were also identified: treatment×ambient temperature [$F(2,30)=5.5$, $p=0.010$], treatment×session time [$F(4,120)=75$, $p=0.0000$], ambient temperature×session time [$F(8,120)=5.5$, $p=0.0000$], and treatment×ambient temperature×session time [$F(8,120)=7.3$, $p=0.0000$]. Follow-up two-way ANOVAs showed a significant ambient temperature×treatment interaction at 30 min [$F(2,30)=4.9$, $p=0.015$], a significant main effect of ambient temperature [$F(2,30)=6.1$, $p=0.006$] and a significant ambient temperature×treatment interaction at 60 min [$F(2,30)=17$, $p=0.0000$], a significant main effect of treatment [$F(1,30)=174$, $p=0.0000$] and a significant ambient temperature×treatment interaction at 180 min [$F(2,30)=4.5$, $p=0.019$].

Pairwise comparisons showed that at 30 min, 5.0 mg/kg METH at 20°C significantly decreased temperature relative to 5.0 mg/kg METH at 28°C. At 60 min, 5.0 mg/kg METH at 20°C significantly decreased temperature relative to 5.0 mg/kg METH at 24°C and 28°C; 5.0 mg/kg METH decreased core temperature relative to saline at 20° and increased temperature relative to saline at 28°C. At 180 min, 5.0 mg/kg METH at all three ambient temperatures increased temperature above saline, but the three METH groups were not different from each other (Fig. 4).

Core temperature: chronic METH treatment

Ambient temperature of 20°C. The main effects of day-block [$F(2,20)=9.6$, $p=0.0012$] and session time [$F(4,40)=43.9$, $p=0.0000$] were significant. There were also significant interactions between treatment and session time [$F(4,40)=84.9$, $p=0.0000$], day-block and session time [$F(8,80)=4.0$, $p=0.0005$], and treatment, day-block, and session time [$F(8,80)=3.3$, $p=0.0026$]. Follow-up two-way ANOVAs at 0 min showed a significant main effect of day-block [$F(2,20)=10.5$, $p=0.0008$]. This was followed by analysis of marginal means (collapsed across treatment), where day-blocks 5–8 and 9–12 were significantly lower than day-block 1–4. At 30 min, there were significant main effects of drug treatment [$F(1,10)=11.2$, $p=0.0074$] and day-block [$F(2,20)=9.7$, $p=0.0012$]. Day-blocks 5–8 and 9–12 (marginal means) were significantly lower than day-block 1–4. At 60 min post-injection, there were significant main effects of drug treatment [$F(1,10)=61.4$, $p=0.0000$] and day-block [$F(2,20)=6.0$, $p=0.0091$]. Day-block 5–8 (marginal means) was significantly lower than day-block 1–4. At 180 min post-injection, the main effect of drug treatment [$F(1,10)=38.1$, $p=0.0001$] and the treatment×

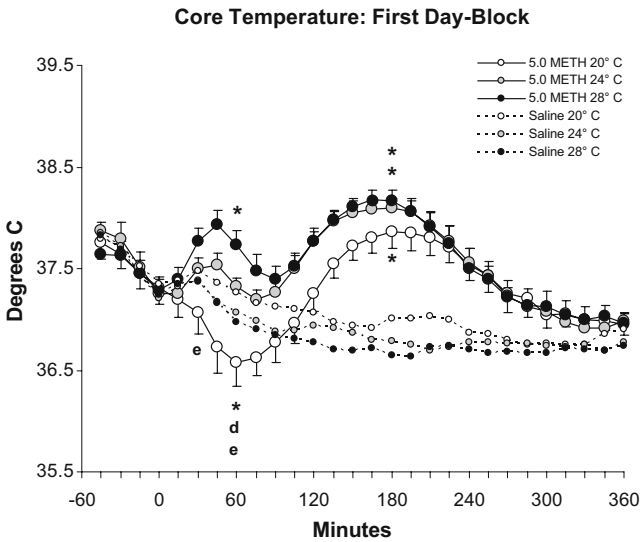

Fig. 4 Initial effects of ambient temperature (day-block 1–4) on core temperature after single daily injections of saline (mean) or 5.0 mg/kg METH (mean ± SEM). *METH was significantly different from saline, same ambient temperature; *d* METH20°C was significantly different from METH24°C; *e* METH20°C was significantly different from METH28°C

day-block interaction [$F(2,20)=5.1$, $p=0.0158$] were significant. Pairwise comparisons between day-blocks were not significant. There were no significant findings at 360 min post-injection (Fig. 5).

Ambient temperature of 24°C The main effects of treatment [$F(1,10)=8.1$, $p=0.0172$], day-block [$F(2,20)=13.8$, $p=0.0002$] and session time [$F(4,40)=19.5$, $p=0.0000$] were significant. There were also significant interactions between treatment and session time [$F(4,40)=35.1$, $p=0.0000$], day-block and session time [$F(8,80)=5.3$, $p=0.0000$], and between treatment, day-block, and session time [$F(8,80)=4.6$, $p=0.0001$]. Follow-up two-way ANOVAs at 0 min showed a significant main effect of day-block [$F(2,20)=5.5$, $p=0.013$]; day-blocks 5–8 and 9–12 (marginal means) were significantly decreased relative to day-block 1–4. At 30 min post-injection, the main effect of day-block [$F(2,20)=17.9$, $p=0.0000$] and the treatment×day-block interaction [$F(2,20)=3.9$, $p=0.0359$] were significant. At 60 min post-injection, the main effect of day-block [$F(2,20)=17.8$, $p=0.0000$] and the treatment×day-block interaction [$F(2,20)=12.7$, $p=0.0003$] were significant. At both the 30- and 60-min time points, pairwise comparisons showed that day-blocks 5–8 and 9–12 were significantly lower than day-block 1–4, only in the METH treatment group. At 180 min [$F(1,10)=147$, $p=0.0000$] and 360 min [$F(1,10)=7.9$, $p=0.0185$], there were significant main effects of treatment (Fig. 6).

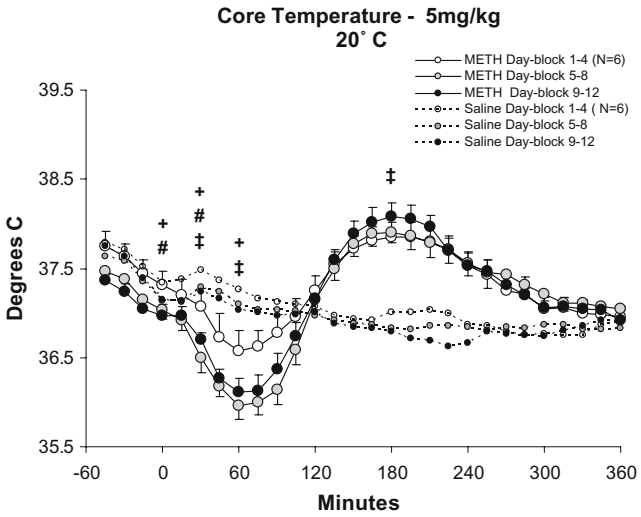

Fig. 5 Effects of 20°C ambient temperature and chronic treatment (day-blocks 1–4, 5–8, and 9–12) on core temperature during a 12-day period of single daily injections with saline (mean) or 5.0 mg/kg METH (mean ± SEM). +, Main effect of day-block, 1–4 was significantly different from 5–8, collapsed across treatment; #, main effect of day-block, 1–4 was significantly different from 9–12, collapsed across treatment; ‡, main effect of treatment, 5.0 mg/kg METH was significantly different from saline, collapsed across day-blocks

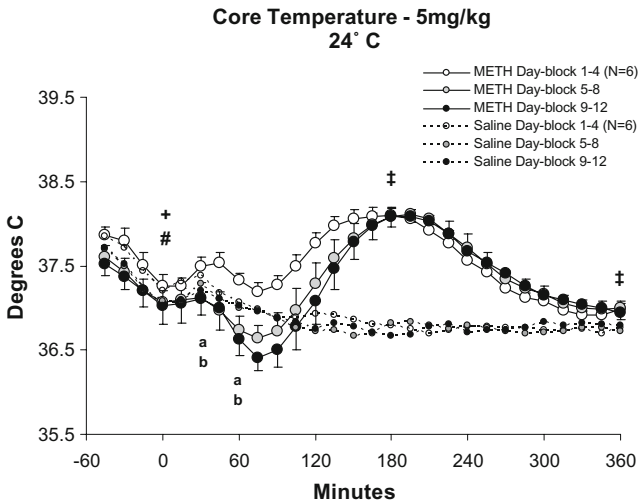

Fig. 6 Effects of 24°C ambient temperature and chronic treatment (day-blocks 1–4, 5–8, and 9–12) on core temperature during a 12-day period of single daily injections with saline (mean) or 5.0 mg/kg METH (mean ± SEM). *a*, METH day-block 1–4 was significantly different from METH 5–8; *b*, METH day-block 1–4 was significantly different from METH 9–12; +, main effect of day-block, 1–4 was significantly different from 5–8, collapsed across treatment; #, main effect of day-block, 1–4 was significantly different from 9–12, collapsed across treatment; ‡, main effect of treatment, 5.0 mg/kg METH was significantly different from saline, collapsed across day-blocks

Ambient temperature of 28°C The main effects of drug treatment [$F(1,10)=14.6$, $p=0.0033$], day-block [$F(2,20)=10.9$, $p=0.0006$], and session time [$F(4,40)=19.5$, $p=0.0000$] were significant. Significant interactions were also present between treatment and session time [$F(4,40)=35.12$, $p=0.000$], and day-block and session time [$F(8,800)=9.7$, $p=0.0000$]. Follow-up two-way ANOVAs at 0 min [$F(2,20)=8.1$, $p=0.0026$] and 30 min [$F(2,20)=19$, $p=0.0000$] showed significant main effects of day-block. Day-blocks 5–8 and 9–12 (marginal means, collapsed across treatment) were significantly decreased relative to day-block 1–4. At 60 min post-injection, there were significant main effects of treatment [$F(1,10)=8.6$, $p=0.015$] and day-block [$F(2,20)=15.8$, $p=0.0001$]; the interaction was also significant [$F(2,20)=6.5$, $p=0.0065$]. Pairwise comparisons showed that day-blocks 5–8 and 9–12 were lower than day-block 1–4, only in the METH treatment group. At 180 min, there was a significant main effect of treatment [$F(1,10)=112$, $p=0.0000$]. There were no significant findings at 360 min post-injection (Fig. 7).

Neurochemical analyses

There were no DA or 5-HT depletions 2 weeks after the completion of chronic treatment with 5.0 mg/kg METH compared to saline at 20°C, 24°C, or 28°C (Table 2).

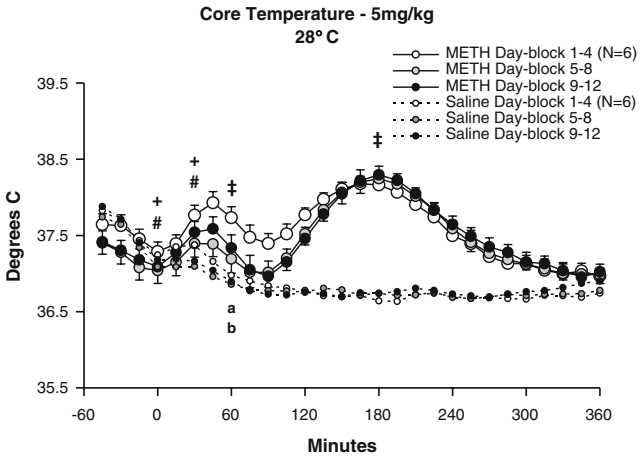

Fig. 7 Effects of 28°C ambient temperature and chronic treatment (day-blocks 1–4, 5–8, and 9–12) on core temperature during a 12-day period of single daily injections with saline (mean) or 5.0 mg/kg METH (mean ± SEM). *a*, METH day-block 1–4 was significantly different from METH 5–8; *b*, METH day-block 1–4 was significantly different from METH 9–12; +, main effect of day-block, 1–4 was significantly different from 5–8, collapsed across treatment; #, main effect of day-block, 1–4 was significantly different from 9–12, collapsed across treatment; ‡, main effect of treatment, 5.0 mg/kg METH was significantly different from saline, collapsed across day-blocks

Discussion

METH caused hyperthermia at 60 min post-injection for the 1.0 and 10.0 mg/kg doses. For 5.0 mg/kg METH, hyperthermia was delayed until 3 h post-injection. Tolerance developed to the 10.0 but not the 1.0 mg/kg dose; the effects of chronic 5.0 mg/kg METH were mixed. In experiment 1, 5.0 mg/kg METH failed to decrease temperature over the 12-day period (borderline, $p=0.053$), whereas in experiment 2, core temperature did diminish over day blocks (24°C and 28°C, 60 min post-treatment). Similar to the work of Thornhill et al. (1977) and Lewander (1971), these findings indicate that tolerance to METH-induced hyperthermia is dose dependent.

At 30 min post-treatment, experiment 1, saline-treated rats responded to chronic treatment. This decrease in core temperature may represent an adaptation to handling or arousal caused by the injection. The chronic saline effect was no longer present at 60 min post-injection, when the effects of chronic 10.0 mg/kg METH peaked (Fig. 2). The saline response, therefore, demonstrates that factors in addition to chronic METH exposure contributed to the decrease in core temperature over the 12-day treatment period. Note that in our companion paper (Myles and Sabol 2008) with a longer baseline each day and the addition of a habituation procedure, this effect of chronic saline treatment was not present.

The effects of ambient temperature were most apparent at 60 min post-treatment (5.0 mg/kg METH, Fig. 4). At 20°C (day-block 1–4) METH induced hypothermia, whereas at 28°C, hyperthermia was seen. At 24°C, 5.0 mg/kg METH failed to differ significantly from saline. Consistent with prior research, these findings demonstrate that the direction of core-temperature change was dependent on ambient temperature (Bowyer et al. 1992; Malberg and Seiden 1998; Yehuda and Wurtman 1972). At 24°C and 28°C, we found a diminishing temperature response with chronic METH treatment (see Figs. 6 and 7). For the 20°C condition, we failed to uncover a significant treatment by day-block interaction. Further work, therefore, will be needed to clarify the enhanced hypothermia for day-blocks 5–8 and 9–12, 30–60 min post-treatment at an ambient temperature of 20°C (see Fig. 5).

Mechanisms of tolerance to METH-induced hyperthermia

METH releases DA and 5-HT (Sabol et al. 2001; Schmidt et al. 1991). Central nervous system DA and 5-HT may have roles in both hypothermia and hyperthermia (Crawshaw 1972; Faunt and Crocker 1987; Gudelsky et al. 1986; Hansen and Whishaw 1973; Mechan et al. 2002; Salmi and Ahlenius 1998; Salmi et al. 1993). METH and its analogs also induce short-term depletions of DA and 5-HT. For

Table 2 Experiment 2, post-mortem 5-HT and DA tissue concentrations

	Serotonin (mean ± SEM, ng/mg tissue)			Dopamine (mean ± SEM, ng/mg tissue)	
	HIPPO	STR	HYPOTHAL	STR	HYPOTHAL
Saline, 20°C	0.349 (±0.03)	0.353 (±0.02)	0.593 (±0.03)	11.616 (±0.32)	0.289 (±0.01)
Saline, 24°C	0.347 (±0.03)	0.373 (±0.01)	0.602 (±0.03)	11.571 (±0.39)	0.295 (±0.02)
Saline, 28°C	0.347 (±0.03)	0.371 (±0.02)	0.574 (±0.03)	11.973 (±0.28)	0.276 (±0.01)
5.0 METH, 20°C	0.355 (±0.02)	0.342 (±0.01)	0.640 (±0.03)	11.090 (±0.64)	0.272 (±0.02)
5.0 METH, 24°C	0.356 (±0.03)	0.352 (±0.01)	0.635 (±0.04)	11.446 (±0.59)	0.317 (±0.03)
5.0 METH, 28°C	0.365 (±0.03)	0.368 (±0.01)	0.624 (±0.03)	12.121 (±0.44)	0.305 (±0.02)

There were no significant differences between treatment groups.
HIPPO hippocampus, *STR* striatum, *HYPOTHAL* hypothalamus

example, using the METH analog MDMA, Schmidt (1987) reported that a transient 5-HT depletion (6 h) was followed by recovery at 24 h, and partial depletion again at 7 days. Similarly, Bowyer et al. (1992) reported DA depletions after a METH regimen that showed partial recovery by 14 days. Bowyer et al. (1992) indicated that depletions observed at 3 days post-treatment may have, in part, been due to decreases in DA synthesis.

When administered in high doses, METH also causes neurotoxic changes to DA and 5-HT neurons, including long-term DA and 5-HT depletions (Axt and Molliver 1991; Bowyer et al. 1994; Ricaurte et al. 1980; Sabol et al. 2001; Wagner et al. 1980). Since the tolerance to AMPH-induced hyperthermia reported by Lewander (1971) may have been from a neurotoxic regimen (16 mg/kg twice per day for 12 days), we investigated whether tolerance to METH-induced hyperthermia was dependent on neurotoxicity to DA and 5-HT axon terminals. We measured post-mortem DA and 5-HT tissue concentrations 2 weeks after the end of treatment and found no depletions.

From the above discussion, it can be seen that our METH regimens may have depleted DA and 5-HT in the short-term, and regulatory changes in these systems may have contributed to the development of tolerance to METH-induced hyperthermia; at the same time, the absence of DA and 5-HT depletion at 14 days post-treatment suggests that the tolerance was not due to the onset of a long-term, neurotoxic (degenerative) process.

Pharmacokinetic explanations for the development of tolerance to METH have also been studied. After chronic treatment, increases in METH concentrations were found in the brain (in vivo dialysis) and plasma (Kitaichi et al. 2003). Gygi et al. (1996) reported decreases in brain tissue but increases in plasma METH concentrations. Cook et al. (1992) reported no change in plasma METH concentration after a low dose but increases after a high dose following a chronic regimen. Finally, Riddle et al. (2002) and Danaceau et al. (2007) reported no change in brain METH concentrations. Further work will be necessary to clarify this issue as the evidence described above both supports or fails to support a pharmacokinetic explanation for the development of tolerance to METH.

Ambient temperature

Chronic effects: tolerance or enhanced hypothermia? At 1 h post-treatment, during day-block 1–4, 5.0 mg/kg METH did not cause hyperthermia at 24°C ambient temperature; yet, with chronic treatment core temperature decreased. This chronic effect was seen in experiment 2 (but not experiment 1) and in our companion report (Myles and Sabol 2008) and raises the possibility that the effect of chronic treatment at 24°C is better described as an induction of hypothermia rather than tolerance to hyperthermia. At 28°C, 5.0 mg/kg METH caused hyperthermia, followed by a decrease in temperature with chronic treatment (1 h post-treatment). Therefore, at 28°C, either tolerance to hyperthermia or induction of hypothermia may equally explain the outcome. The significance of the findings at 24°C is that tolerance to hyperthermia may not adequately explain the change in core temperature after chronic METH treatment. Alternatively, hyperthermic mechanisms may be active even when not apparent. While 5.0 mg/kg METH (day-block 1–4) did not change temperature 60 min post-injection at 24°C, the same dose resulted in hypothermia at 20°C and hyperthermia at 28°C. These findings suggested that a hyperthermic component may have been masked by a hypothermic response at 24°C. DA and 5-HT have both been implicated in hyperthermic and hypothermic mechanisms (Crawshaw 1972; Faunt and Crocker 1987; Gudelsky et al. 1986; Hansen and Whishaw 1973; Mechan et al. 2002; Salmi and Ahlenius 1998; Salmi et al. 1993). In addition, Rusyniak et al. (2007) recently prevented hypothermia induced by a low MDMA dose with 5-HT1A antagonist pretreatment. Rusyniak et al. (2007) hypothesized that at higher doses MDMA-induced hyperthermia may overcome the 5-HT1A-mediated hypothermia. The initial lack of response to METH at 24°C in our report (60 min post-treatment) may be due to competing neurotransmitter systems (or competing receptor subtypes), canceling each other's effects. Conceivably, tolerance may develop to the system(s) mediating hyperthermia, or sensitization may develop to the system(s) mediating hypothermia.

Two-phase temporal response to METH administration In experiment 1 we found that 5.0 mg/kg METH (24°C) resulted in a late hyperthermic peak at 3 h post-treatment. A primary focus of experiment 2 was to determine whether there was a relationship between ambient temperature and the timing of the temperature response seen with 5.0 mg/kg. At 60 min, we saw the emergence of a hyperthermic peak in a warm environment (28°C) and a hypothermic peak in a cool environment (20°C). As stated above, at 24°C the hypothermic effects may have counteracted hyperthermia, or perhaps neither hypothermia nor hyperthermia was activated under these dose and ambient conditions. In either event, the temperature response appeared neutral at 24°C. The 3-h peak, however, was present at all ambient temperatures and was always hyperthermic. From these findings we concluded that at this dose and ambient temperature range, the core temperature response had two distinct phases: a malleable phase at 1 h post-treatment that could be hyper- or hypothermic depending on ambient temperature (phase 1) and an inflexible phase at 3 h that was always hyperthermic (phase 2). It is noteworthy that, at 20°C ambient temperature,

this reflects opposite changes in core temperature at 1 vs. 3 h. See Fig. 4.

In addition to ambient temperature, chronic treatment itself may selectively affect the phase 1 response. As seen in Figs. 6 and 7, chronic METH resulted in a decreased temperature response at 60 min post-treatment but not at 180 min. This point may be contradicted by our companion paper (Myles and Sabol 2008), Fig. 3, which showed that tolerance after chronic 10.0 mg/kg METH extended up to 3 h post-injection. However, the *duration* of phase 1 may also be flexible, or factors not yet identified may contribute to tolerance in phase 2.

If METH-induced temperature responses can be divided into these two phases, one would expect that separate mechanisms are responsible for the first and second phases. One possibility is that the second peak reflects a compensatory response to the first; however, since the second is routinely hyperthermic and the first ranges between hypo- and hyperthermic, this explanation is unlikely. As monoamines (see above), gluccocorticoids (Makisumi et al. 1998), and thyroid hormone (Sprague et al. 2003; Sprague et al. 2007) are all involved in temperature regulation, investigation of their contributions to the two phases will be of interest.

Summary

We used telemetry to measure the effects of repeated METH injections on core temperature at different doses and different ambient temperatures. Studying the effects of different doses of METH on temperature demonstrated a temporal shift between 1.0 and 5.0 mg/kg. Studying 5.0 mg/kg METH at different ambient temperatures demonstrated that an early peak was present at 28°C (hyperthermia) and at 20°C (hypothermia), and absent at 24°C. While the dependence of core temperature on ambient temperature has been well documented in the stimulant literature, the relatively stable presence of a late hyperthermic peak is less well characterized. In other words, a flexible response to 5.0 mg/kg METH was restricted to an early time point (60 min), whereas a later peak (180 min) remained hyperthermic regardless of ambient temperature and did not diminish with chronic treatment**.** This finding suggests that independent mechanisms mediate early and late phases of the METH-induced temperature response. Finally, the decreased phase 1 temperature response after chronic treatment was not due to the onset of a degenerative process indexed by an absence of long-term (14 days) DA or 5-HT depletions.

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