EFFECTS OF (±)3,4-METHYLENEDIOXYMETHAMPHETAMINE, (±)3,4-METHYLENEDIOXYAMPHETAMINE AND METHAMPHETAMINE ON TEMPERATURE AND ACTIVITY IN RHESUS MACAQUES

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Abstract—Severe and malignant hyperthermia is a frequently reported factor in emergency department (ED) visits and fatalities in which use of amphetamine drugs, such as (±)3,4-methylenedioxymethamphetamine (MDMA), (±)3,4-methylenedioxyamphetamine (MDA) and (+)-methamphetamine (METH), is confirmed. Individuals who use "ecstasy" are also often exposed, intentionally or otherwise, to several of these structurally-related compounds alone or in combination. In animal studies the degree of (subcritical) hyperthermia is often related to the severity of amphetamine-induced neurotoxicity, suggesting health risks to the human user even when emergency medical services are not invoked. A clear distinction of thermoregulatory risks posed by different amphetamines is therefore critical to understand factors that may produce medical emergency related to hyperthermia. The objective of this study was therefore to determine the relative thermoregulatory disruption produced by recreational doses of MDMA, MDA and METH in nonhuman primates. Body temperature and spontaneous home cage activity were monitored continuously in six male rhesus monkeys via radiotelemetric devices. The subjects were challenged intramuscularly with 0.56–2.4 mg/kg MDMA, 0.56–2.4 mg/kg MDA and 0.1–1.0 mg/kg METH. All three amphetamines significantly elevated temperature; however the time course of effects differed. The acute effect of METH lasted hours longer than MDA or MDMA and a disruption of nighttime circadian cooling was observed as long as 18 h after 1.0 mg/kg METH and 1.78–2.4 mg/kg MDA, but not after MDMA. Activity levels were only reliably increased by 0.32 mg/kg METH. It is concluded that while all three substituted amphetamines produce hyperthermia in rhesus monkeys, the effects do not depend on elevated locomotor activity and exhibit differences between compounds. The results highlight physiological risks posed both by recreational use of the amphetamines and by current trials for clinical MDMA use. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

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Survey data from the United States show that in 2004 annual prevalence rates for 12th grade students’ illicit use of amphetamines was 10% with 4.6% reporting use in the past 30 days. It is important to establish the thermoregulatory impact of structurally distinct amphetamine compounds from a public health perspective since amphetamine-related fatalities and emergency department (ED) admissions appear to feature significant and malignant elevations in body temperature (Dams et al., 2003; Gilmian, 1997; Green et al., 2003; Kamijo et al., 2002; Koijima et al., 1984; Mallick and Bodenham, 1997; Wallace and Squires, 2000). The Drug Abuse Warning Network estimates 4000 annual ED visits in the US in which (±)3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") is involved and at least 40,000 where (+)-methamphetamine (METH) or amphetamine are involved (Ball et al., 2003, 2004). The hyperthermic response may be a critical determinant of medical emergencies and deaths since many of the toxicological problems that are seen, such as rhabdomyolysis, disseminated i.v. coagulation and acute renal failure (Henry et al., 1992) can result from hyperthermia. The acute thermoregulatory disruption produced by amphetamines is also important beyond acute medical emergency. For example hyperthermia can markedly influence amphetamine-induced neurotoxicity in rodents and nonhuman primates (Bowyer et al., 1992, 1994; Miller and O’Callaghan 1994; Malberg and Seiden 1998; Melega et al., 1998).

It is difficult to determine the relative impact of each of these amphetamines on thermoregulation in humans because recreational users are frequently poly-drug abusers and are often positive for multiple drugs in ED medical situations. Therefore, nonhuman laboratory models are necessary to establish the relative thermoregulatory impact of different amphetamines in order to better understand the clinical implications of amphetamine use and abuse. The present study is focused on the acute effects of MDMA, (±)3,4-methylenedioxymethylamphetamine (MDA) and METH, which are all commonly used in an intermittent pattern in the nightclub/rave party population.

All three of these amphetamines can produce an acute increase in body temperature. MDMA results in an acute elevation of body temperature in human laboratory studies at doses (1.5–2.0 mg/kg, p.o.) within the range of common recreational doses (Freedman et al., 2005; Liechti et al., 2000), but not reliably so at lower doses (Grob et al., 1996; Mas et al., 1999) suggesting a dose-related effect. MDMA (racemic or the S(+) enantiomer) also produces acute hyperthermia in rats (Brown and Kiyatkin, 2004; Dafters, 1994; Malberg and Seiden, 1998), mice (Carvalho et al., 2002; Fantegrossi et al., 2003), guinea pigs (Saadat et al., 2004), pigs (Fiege et
al., 2003; Rosa-Neto et al., 2004), rabbits (Pedersen and Blessing, 2001) and non-human primates (Taffe et al., 2006). METH also increases body temperature in rodents (Bowyer et al., 1994; Brown et al., 2003). Recent studies also suggest that repeated dosing with METH can cause fatal/threatening hyperthermia in at least three nonhuman primate species (Madden et al., 2005; Ricaurte et al., 2002, 2003), and MDA can cause hyperthermia and death in canines (Davis et al., 1987).

However, given the wide variability of species and the type and doses of amphetamines administered in prior studies, the relative contribution to the thermoregulatory effects of MDMA, METH and MDA is unclear. It is likely that significant thermoregulatory differences between related amphetamines exist. MDMA, MDA and METH are potent indirect monoaminergic agonists, acting to inhibit reuptake mechanisms and to enhance transmitter release, although their relative potencies for releasing serotonin, norepinephrine and dopamine differ from each other within species and these relationships may differ significantly across species (Bataglia and De Souza, 1989; Han and Gu, 2006; Verrico et al., 2005). With respect to human monoamine transporters, MDMA has greater affinity for noradrenergic and serotonin transporters compared with dopamine transporters whereas METH has greater affinity for dopamine transporters. Interpretation of the pharmacology can be complex; for example MDMA has greater affinity than METH for noradrenergic transporters and yet is more potent in stimulating serotonin release in a cell transfection model (Verrico et al., 2005). Such results support the need to compare systemic effects of the amphetamines in the intact organism, ideally one more closely related to humans such as non-human primates.

To date, there are few studies which have systematically measured the thermoregulatory impact of these amphetamines in non-human primates. Recent studies from this laboratory have established that unrestrained rhesus monkeys develop hyperthermia following administration of MDMA without any stimulation of locomotor activity (Taffe et al., 2006; Von Huben et al., 2006). Although much work has been done in rodent species, careful comparisons of the thermoregulatory effects of MDMA, MDA and METH have not been reported within a consistent model. Therefore, the present study was designed to directly compare the acute thermoregulatory effects of MDMA with the effects of the related amphetamines MDA and METH within the same subjects. The goals were to 1) confirm our preliminary finding by testing a wider range of doses of MDMA, 2) determine the relative thermoregulatory disruption of the closely-related MDA (also a metabolite of MDMA) and 3) compare the effects of the more “empathogenic” amphetamines to those of METH as a substituted amphetamine with typically a more classic psychomotor stimulant behavioral profile.

EXPERIMENTAL PROCEDURES

Animals

Six male rhesus monkeys (Macaca mulatta) participated in this study. Animals were 6–10 years of age, weighed 9.0–12.7 kg at the start of the study and exhibited body condition scores (Clingerman and Summers, 2005) of 2.25–3.25 of five at the nearest quarterly examination. Daily chow (LabDiet 5038, PMI Nutrition International, Richmond, IN, USA; 3.22 kcal of metabolizable energy (ME) per gram) allocations were determined by a power function (Taffe, 2004a,b) fit to data provided in a National Research Council recommendation (NRC/NAS 2003) and modified individually by the veterinary weight management plan. Daily chow ranged from 160 to 230 g per day for the animals in this study. The animals’ normal diet was supplemented with fruit or vegetables seven days per week and water was available ad libitum in the home cage at all times. All animals were individually housed throughout the study. Animals on this study had previously been immobilized with ketamine (5–20 mg/kg) no less than semiannually for purposes of routine care and some experimental procedures. Animals also had various acute exposure to scopolamine, raclopride, methylphenidate, SCH23390, Δ²-THC, nicotine and mecamineline in behavioral pharmacological studies and four had been exposed to an oral ethanol induction procedure (Kalter et al., 2004). No experimental drug treatments had been administered for a minimum of one year prior to the start of telemetry studies and thus were not anticipated to have any bearing on the results of the current study. The United States National Institutes of Health guidelines for laboratory animal care (Clark et al., 1996) were followed and all protocols were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute (La Jolla). Efforts were taken in the design and conduct of the study to minimize the number of subjects required as well as potential sources of distress.

The monkeys’ body temperature and activity patterns naturally followed a diurnal pattern of daytime high and nighttime low; however temperature was consistent across the selected injection times (either 10:30 or 13:00 h). These times were carefully selected based on pilot data which indicated both stability of the normal body temperature and a lack of any differential effect of drug challenges across this interval. Activity also follows a circadian cycle as animals do not make many movements across the cage (triggering an activity “count”) when lights are off; however they engage in relatively variable levels of activity, both between animal and across the light period. S.c. body temperature varies about 1.5–2 °C from nighttime low to daytime high which is consistent with reports for i.p. temperature in Japanese (Takasu et al., 2002) macaques or s.c. temperature in cynomolgus (Almirall et al., 2001) or rhesus (Horn et al., 1998) macaques. The s.c. temperature values from Almirall et al. (2001), Horn et al. (1998) and the present study all differ from the i.p. values reported by Takasu et al. (2002) by about −1 to −1.5 °C. In our recent studies we have compared rectal temperatures (obtained under light ketamine anesthesia) with concurrent telemetric temperature values in 17 monkeys; multiple determinations are available for most animals. The s.c. values vary from −1–3 °C lower than rectal temperature across animals but the temperature differential is consistent across determinations within animals.

Apparatus

Radio telemetric transmitters (TA10TA-D70; Transoma/Data Sciences International, St. Paul, MN, USA) were implanted s.c. in the flank. The surgical protocol was adapted from the manufacturer’s surgical manual and implantation was conducted by, or under supervision of, the TSRI veterinary staff using sterile techniques under isoflurane anesthesia. Temperature and gross locomotor activity recordings were obtained continuously from the telemetric transmitters implanted in the monkey via an in-cage receiver.
Fig. 1. The mean (N=6, bars indicate S.E.M.) s.c. temperature values following acute challenge with doses of MDMA, MDA and METH are presented. Breaks in the series indicate the time of injection. The statistical analysis included the interval –10–240 min after injection and a significant change from the –10 min time point is indicated by the open symbol for each treatment condition. The * and # indicate time points in which all four (*) or three of four (#) active dose conditions differed significantly from the vehicle temperature; see text for additional effects determined to be statistically reliable.
(RMC-1; Transoma/Data Sciences International). Data were recorded on a 5 min sample interval basis by the controlling computer and represented as a moving average of three samples (−5 min, current, +5 min) for each 10 min. Occasional missing data points were replaced with a linear interpolation of adjacent points. Ambient room temperature was also recorded by the system via a thermometer mounted near the top of the housing room.

**Drug challenge studies**

For these studies doses of (±)3,4-methylenedioxymethamphetamine HCl (MDMA; 0.56, 1.0, 1.78, 2.4 mg/kg), (±)3,4-methylenedioxymphetamine HCl (MDA; 0.56, 1.0, 1.78, 2.4 mg/kg) and (+)-methamphetamine HCl (METH; 0.1, 0.32, 0.56, 1.0 mg/kg) were administered intramuscularly in a volume of 0.1 ml/kg saline. Drugs were provided by the National Institute on Drug Abuse (Bethesda, MD, USA). Treatment order was pseudorandomized within compound to the extent possible with the small sample size to minimize the impact of any potential order effects. Generally, the MDMA studies were conducted first, MDA second and the METH last; however, there was some degree of overlap of the schedule across compounds. Dose ranges were originally based on pill-content analyses suggesting 75–125 mg MDMA per ec-

![Fig. 2](image_url)

**Fig. 2.** The mean (N=6) s.c. temperature values in the 20 h following acute challenge with doses of MDMA, MDA and METH are presented. Error bars (S.E.M.) are selectively presented for visual clarity. The statistical analysis included the interval 1–18 h after injection and the open symbols indicate a significant difference from the vehicle condition at a given time point. A significant increase from the time point preceding injection is indicated by *, however significant decreases from baseline are not depicted, see Results.
MDMA significantly increased body temperature within 10–15 min of drug administration (Fig. 1). The 10-min sample analysis confirmed significant main effects of drug condition \([F_{4,25} = 4.33; P < 0.05]\), time post-injection \([F_{25,125} = 11.78; P < 0.0001]\) and an interaction of factors \([F_{100,500} = 1.80; P < 0.0001}\). The post hoc test confirmed a significant temperature increase over baseline 20–30 min after vehicle, 20–90 min after the 0.56 dose, 20–100 min after the 1.0 dose, 20–100 and 140 min after the 1.78 mg/kg dose and 20–60 min after the 2.4 mg/kg dose. The post hoc test also confirmed that temperature was significantly higher than the respective vehicle time points 40–110 and 140 min after 0.56 mg/kg, 30–150 after 1.0 mg/kg, 30–160 min after 1.78 mg/kg and 10–160 min after 2.4 mg/kg.

Effects of MDMA on temperature did not last beyond the first three hours after dosing (Fig. 2). The analysis of hourly time points after dosing confirmed a significant main effect of time post-injection \([F_{18,90} = 41.93; P < 0.0001}\) and an interaction of factors \([F_{21,360} = 1.81; P < 0.001}\). The post hoc test confirmed that temperature was significantly elevated for 1 h after 0.56 and 2.4 mg/kg, and for 2 h after 1.0 or 1.78 mg/kg of MDMA. In addition, temperature was significantly elevated above vehicle for two hours after 0.56 and 1.0 mg/kg and for three hours after 1.78 or 2.4 mg/kg. Consistent with the usual nighttime cooling, temperature was significantly below baseline 6–18 h after 1.0 and 2.4 mg/kg doses, 7–18 h after the 1.78 mg/kg dose and 8–18 h after vehicle or 0.56 mg/kg MDMA.
Activity was also significantly reduced in the few hours after injection as confirmed by significant effects of time post-injection ($F_{25,125} = 2.11; P < 0.01$) and an interaction of factors ($F_{100,500} = 1.54; P < 0.01$); see Fig. 3. The post hoc test confirmed that activity was significantly reduced from the baseline 30 and 50 min after 1.7 mg/kg dose and 20–50, 120, 160–180 and 220 min after 2.4 mg/kg MDMA.

The hourly activity counts (Fig. 4) were also significantly affected by time post-injection ($F_{18,90} = 7.19; P < 0.0001$) and an interaction of factors ($F_{72,360} = 1.38; P < 0.05$). The post hoc test confirmed that activity was significantly lower than baseline 3 h after 0.56 mg/kg, 2 h after 1 mg/kg and 1 and 3 h after 2.4 mg/kg of MDMA. Activity was also consistent with the usual circadian pattern as it was significantly lower than baseline 6–18 h after vehicle or 1.0 mg/kg, 6–17 h after 0.56 mg/kg, 7–17 h after 1.78 mg/kg and 5–17 h after 2.4 mg/kg MDMA. Finally, activity was significantly higher than corresponding vehicle time points 4 and 18 h after 1.78 mg/kg MDMA.

Fig. 4. The mean (N=6) activity values in the 20 h following acute challenge with doses of MDMA, MDA and METH are presented. Error bars (S.E.M.) are selectively presented for visual clarity. The statistical analysis included the interval 1–18 h after injection and the open symbols indicate a significant difference from the pre-injection baseline and a significant increase from the vehicle conditions is indicated by *.
MDA

MDA also significantly increased body temperature (Fig. 1). The analysis confirmed significant main effects of drug condition \( [F_{4,20} = 5.60; P < 0.01] \) and time post-injection \( [F_{25,125} = 5.00; P < 0.0001] \); however, a trend for an interaction of factors was not statistically reliable \( [F_{100,500} = 1.24; P = 0.077] \). The post hoc test confirmed a significant temperature increase over baseline 20–60 min after the 0.56, 1.78 and 2.4 mg/kg doses and 40–70 min after the 1.0 dose. The post hoc test also confirmed that temperature was significantly higher than the respective vehicle time points 40–70 after 0.56 mg/kg, 60–70 after 1.0 mg/kg, 40–70, 90–100 and 140–160 min after 1.78 mg/kg and 20–120 min after 2.4 mg/kg of MDA. An apparent difference in temperature after the 1.0 mg/kg dose compared with other active doses was not statistically reliable.

The analysis of hourly time points after dosing (Fig. 2) confirmed a significant main effect of drug condition \( [F_{4,20} = 11.39; P < 0.0001] \), time post-injection \( [F_{18,90} = 33.77; P < 0.0001] \) and an interaction of factors \( [F_{72,360} = 1.50; P < 0.01] \). The post hoc test confirmed that temperature was significantly elevated from baseline 1 h after 0.56 or 2.4 mg/kg, and for 2 h after 1.78 mg/kg of MDA. In addition, temperature was significantly elevated above vehicle 1 h after 0.56 mg/kg and 1–2 h after 1.78 or 2.4 mg/kg of MDA. Consistent with the usual nighttime cooling, temperature was significantly below baseline 6–18 h after 0.56 mg/kg, 7–18 h after the 1.0 mg/kg dose and 8–18 h after vehicle and 1.78 or 2.4 mg/kg MDA. The effects of the higher doses lasted overnight as temperature was significantly higher than the respective vehicle time points 10, 12–17 h after 1.78 mg/kg and 10–17 h after 2.4 mg/kg MDA. The 1.78 mg/kg dose also significantly elevated temperature compared with similar time points after 0.56 mg/kg (10–11, 13–15 h post-injection) and 1.0 mg/kg (13 h post-injection) doses of MDA. Similarly, the 2.4 mg/kg dose also significantly elevated temperature compared with similar time points after 0.56 mg/kg (7, 10–15 h post-injection) and 1.0 mg/kg (11 h post-injection) doses of MDA.

Activity was decreased in the few hours after injection (Fig. 3) as confirmed by a significant main effect of time post-injection \( [F_{72,360} = 7.32; P < 0.0001] \). The post hoc test confirmed that activity was significantly lower than baseline 20–80 and 120 min after 1.0 mg/kg MDA, 30, 40, 70 and 100 min after 1.78 mg/kg and 40–120 min after 2.4 mg/kg MDA. The hourly activity counts (Fig. 4) were also significantly reduced as was confirmed by a significant main effect of time post-injection \( [F_{18,90} = 7.90; P < 0.0001] \). The post hoc test confirmed that activity counts were lower than baseline after vehicle (2, 4, 6–18 h) as well as the 0.56 mg/kg (4–18 h), 1.0 mg/kg (1–2, 6–17 h), 1.78 mg/kg (1–3, 6–17 h) and 2.4 mg/kg (2, 6–17 h) doses of MDA.

METH

METH also significantly increased body temperature in the first few hours after administration, however the time course differed notably (Fig. 1). The analysis confirmed significant main effects of drug condition \( [F_{4,20} = 15.71; P < 0.0001] \), time post-injection \( [F_{25,125} = 6.44; P < 0.0001] \) and of the interaction of factors \( [F_{100,500} = 2.82; P < 0.0001] \). The post hoc test confirmed a significant temperature increase over baseline 30–60 min after vehicle, 30–190 min after the 0.1 mg/kg dose, 20–240 min after the 0.32 mg/kg dose, 10–240 min after the 0.56 mg/kg dose and 20–110 and 140–240 min after 1.0 mg/kg of METH. The post hoc test also confirmed that temperature was significantly higher than the respective vehicle time points 110–200 min after 0.1 mg/kg, 20–240 min after 0.32 mg/kg, 40–240 min after 0.56 mg/kg and 20–100 and 130–240 min after 2.4 mg/kg of METH. No significant effects on activity were confirmed for the first four hours after administration in this analysis.

METH also significantly disrupted temperature during nighttime hours (Fig. 2). Analysis of the hourly time averages confirmed a main effect of drug condition \( [F_{4,20} = 16.73; P < 0.0001] \), time post-injection \( [F_{18,90} = 69.19; P < 0.0001] \) and of the interaction of factors \( [F_{72,360} = 3.28; P < 0.0001] \). The post hoc test confirmed that temperature was significantly elevated over baseline 2–3 h after 0.1 mg/kg, 1–3 h after 0.32 mg/kg, 1–5 h after 0.56 mg/kg and 1–6 h after 1.0 mg/kg METH. Similarly, temperature was significantly higher than the respective vehicle time points 2–5 h after 0.32 mg/kg, 2–8 h after 0.56 mg/kg and 3–17 h after 1.0 mg/kg METH. The 1.0 mg/kg dose also significantly elevated temperature 4–17 h post-injection relative to the 0.1 mg/kg dose, 6–17 h post-injection relative to the 0.32 mg/kg dose and 9–16 h post-injection relative to the 0.56 mg/kg dose. The post hoc test also confirmed significant circadian cooling with temperatures reliably lower than baseline 8–18 h after Vehicle, 0.1 or 0.32 mg/kg conditions, 9–18 h after 0.56 mg/kg METH and 14–18 h after 1.0 mg/kg METH.

The hourly activity counts (Fig. 4) were also significantly lower as was confirmed by a significant main effect of time post-injection \( [F_{18,90} = 7.19; P < 0.0001] \). The post hoc test confirmed that activity was significantly lower than baseline 7–17 h after vehicle, 7–16 h after 0.32 mg/kg, 9–11 and 13–17 h after 0.56 mg/kg and 9–12 h after the 1.0 mg/kg dose of METH. Activity was increased over the vehicle condition 2 h after 0.32 mg/kg METH.

DISCUSSION

The results of the present study establish that rhesus monkeys develop elevated body temperature following an i.m. injection of a range of doses of each of three substituted amphetamines. These data support and extend our initial report (Taffe et al., 2006) in confirming that monkeys’ hyperthermic responses to these compounds are similar to humans and not hypothermic, which contrasts with one prior report on the effects of \( (\pm) \)MDMA in rhesus monkeys (Bowyer et al., 2003). The study also shows that the immediate temperature response in ~4 h after the administration of MDA is quite similar to the MDMA response at identical doses and that doses of METH elevate temperature over a more protracted time course. The immediate temperature response to all three amphetamines was not strongly dose-dependent across the tested ranges al-
though dose-dependent elevations of nighttime temperature were observed after MDA and METH. The temperature responses did not appear to depend on significant increases in locomotor activity following any of the compounds. This latter finding may indicate important differences between nonhuman primate and rodent responses to the amphetamines.

The magnitude of the acute hyperthermic response subsequent to amphetamine exposure (maximum change in the 4 h post-injection: 2.4 mg/kg MDMA, 0.71 °C; 2.4 mg/kg MDA, 0.65 °C; 1.0 mg/kg METH, 0.94 °C) is consistent with prior reports of drug-induced hyperthermia. For example, Freedman and colleagues (2005) reported that an oral dose of 2.0 mg/kg MDMA elevates human temperature by ~0.3–0.6 °C. Yuan et al. (2006) found that the highest mean temperature increase observed in squirrel monkeys after a single oral 1.25 mg/kg dose of METH reached ~0.6 °C over the pre-injection baseline and ~0.8 °C over the vehicle condition at a similar time point. Furthermore, racemic MDMA results in significant hyperthermia of ~0.7–1.0 °C in rhesus monkeys under normal ambient temperature conditions (Taffe et al., 2006; Von Huben et al., 2006). The lack of a strong dose dependency of the immediate hyperthermic response confirms a prior observation on the effects of MDMA (Von Huben et al., 2006) and was consistent across compounds in this study. This outcome suggests perhaps that thermoregulatory mechanisms in the rhesus monkey which are triggered upon temperature elevations of about 0.7–1.0 °C may not be affected by the amphetamines at these doses.

Major differences emerged in the duration of the amphetamine-induced hyperthermia. The temperature responses to MDMA and MDA peaked sharply ~60–90 min post-injection but thereafter declined steadily. This pattern is consistent with reports that plasma MDMA levels peak within 60 min after administration in rhesus and squirrel monkeys (Bowyer et al., 2003; Mechan et al., 2006). In contrast, the temperature response to METH initially peaked ~60 min post-injection but was sustained at high levels for 180–300 min post-injection depending on the dose. This finding is consistent with a report that while plasma METH levels reach a peak within 60 min after administration and then rapidly decline in squirrel monkeys, the metabolite amphetamine reaches plasma levels which approximate the early METH peak and persists 60–180 min post administration (Yuan et al., 2006). In total, the acute temperature responses in the present study correspond quite well to reported pharmacokinetic data assuming that amphetamine is an active metabolite of METH with respect to thermoregulation.

The highest dose of METH resulted in a disruption of temperature regulation that lasted overnight until the following morning. Similar effects of a smaller magnitude were observed following the highest two doses of MDA; however, MDMA did not result in overnight disruption at these doses. The mechanism of this extended elevated temperature response is unknown but might theoretically be related to pharmacokinetics since MDA has been reported to have a significantly longer half-life than MDMA in humans and monkeys (Bowyer et al., 2003; de la Torre et al., 2004; Kraemer and Maurer, 2002). Still it should be appreciated that most published reports on the pharmacokinetics of MDA derive from investigations of MDMA as a metabolite of administered MDMA. MDA is, however, only a minor metabolite of MDMA with peak plasma levels of about 10% of the administered MDMA dose reported (Bowyer et al., 2003; de la Torre et al., 2004; Kraemer and Maurer, 2002). Given that MDA levels rise slowly to a peak some 7 h after an MDMA injection in monkeys (Bowyer et al., 2003), it seems unlikely that (metabolite) MDA contributed much to the effects of MDMA observed in this study. In contrast, the plasma levels of the METH metabolite amphetamine are equivalent to the administered METH dose at peak and remain significantly elevated at least 6 h after a 1.25 mg/kg oral dose of METH in squirrel monkeys (Yuan et al., 2006). In addition, the current locomotor activity results suggest that some significant disruption of sleep may have occurred following METH (Fig. 4). These results also suggest that it is important to consider drug effects that may be related to the timing of administration within the diurnal cycle and/or qualitative effects on the sleep cycle.

Although not directly addressed in this report, the data may also highlight different thermoregulatory patterns produced by amphetamines which differ in effect on serotonin, dopamine and noradrenaline signaling. The CNS mechanisms involved in amphetamine-induced thermodynamics include all three of these monoamines, which all interact with all three monoamine transporters and release transmitters, albeit with varying potencies. Administration of 5-HT2A and 5-HT2C receptor antagonists can block MDMA hyperthermia in rodents (Fantegrossi et al., 2003; Herin et al., 2005; Mechan et al., 2002), as can depletion of pre-synaptic serotonin stores (Fantegrossi et al., 2005; Saadat et al., 2005). Conversely, administration of an MAO-A inhibitor (Freezer et al., 2005) or 5-HT1A receptor antagonist can prolong MDMA-induced hyperthermia (Saadat et al., 2004). Dopaminergic contributions to hyperthermia appear to be primarily mediated by the D1-like receptors since the D1-like antagonist SCH23390 blocks MDMA or METH hyperthermia where D2-like antagonists are less effective (Broening et al., 2005; Mechan et al., 2002). The α1- and β-adrenergic receptors also contribute to these effects, see (Sprague et al., 2005) for review. Significant differences are reported for relative potency of a given amphetamine to interact with SERT, DAT and NET derived from humans and rodents (Han and Gu, 2006; Verrico et al., 2005). These findings suggest that additional exploration of specific monoaminergic contributions to amphetamine-induced hyperthermia in nonhuman primate species is warranted.

The activity data also support and extend our prior finding that MDMA, administered in doses similar to human recreational use, does not stimulate significant locomotor activity in rhesus monkeys under normal laboratory housing conditions in the first few hours after dosing (Taffe et al., 2006; Von Huben et al., 2006). Animals were observed by either direct observation or via video feed for 2.5 h after dosing in each condition and the activity data generated from the radiotelemetry devices are highly consistent with
direct observation. The present data show that this effect is consistent across a range of relevant doses and MDA appears to have a similar profile. This finding is important because it demonstrates that the immediate hyperthermic effects of MDMA and MDA are not exclusively due to increased activity. In fact, in the case of MDMA and MDA the higher doses produced a marked reduction in activity 1–2 h after dosing and a slight increase in activity (over vehicle) 4–5 h after dosing, a pattern which contrasts with the temperature response. Importantly, all animals were consistently immobile in the period 1–2 h after MDMA or MDA without evidence of repetitive movements (stereotypy); however they tended to react with appropriate, if blunted, ocular/head movements and facial expressions to the behavior of other animals in the room and/or investigators entering briefly for direct observation. The effect of METH was different in that it did not consistently decrease locomotor activity in the first few hours. The effect of METH appeared to conform somewhat to a classic “inverted U” dose effect pattern common with many behavioral effects of stimulants. That is, individual animals tended to exhibit increased activity at one of the middle doses and lowered activity at the higher doses; individual differences in this pattern led to effects where are apparent but did not reach statistical significance, save for 2 h after 0.32 mg/kg (Fig. 4). A modest amount of repetitive movement was observed in some animals after METH however these effects were not consistent across all individuals. Thus, our current findings provide further support that the nonhuman primate may be a closely matched analog of human laboratory findings, and even fatalities, in which individuals did not engage in substantial locomotor activity (Freedman et al., 2005; Liechti et al., 2000; Patel et al., 2005). These findings may also point to particular differences in the response of primates versus rodents to the substituted amphetamines of “empathogenic” character which appear to produce typical psychomotor-stimulant patterns of increased locomotion in rodents.

Lethal thresholds for amphetamines have not been well described in nonhuman primate models; however, evidence from studies suggests that lethality involving hyperthermia is indeed possible. One available report shows that the 24-h LD₉₀ in rhesus monkeys is 22 mg/kg (95% C.I. 17–28) MDMA, i.v., and 6 mg/kg (95% C.I. 5–9) MDA, i.v. (Hardman et al., 1973), although little information on correlates of fatality such as hyperthermia were described. More recent studies suggest that fatal hyperthermia can result from repeated dosing with METH in three species of nonhuman primates (Madden et al., 2005; Ricautre et al., 2002, 2003). The effects of MDA on thermoregulation have been less studied in nonhuman primates but it can cause fatal hyperthermia (−4.5 °C) in canines (Davis et al., 1987). Davis et al. (1987) also reviewed available information on human fatalities associated with MDA in which the pathology appears to be quite similar to recent MDMA-associated fatality reports (Dams et al., 2003; Gillman, 1997; Greene et al., 2003; Mallick and Bodenheim, 1997). Lethality has also been reported after single day repeated MDMA dosing (cumulative dose of 25.8 mg/kg, i.g.) in squirrel monkeys; however, the role of hyperthermia was not discussed (Mechan et al., 2006). Finally, we have recently observed two cases in which rhesus monkeys required emergency intervention following 10 mg/kg racemic MDMA, i.m., and exhibited a peak colonic temperature of 42.2 °C and 43.2 °C prior to emergency cooling and stabilization.

The findings from this study are also relevant to evidence that three of the more common constituents of street ecstasy pills (Baggott et al., 2000; Tanner-Smith, 2006) all disrupt thermoregulation, producing hyperthermia in monkeys under normal ambient temperatures. Over the past decade, studies have increasingly shown that hyperthermia can markedly influence the severity of neurotoxicity observed after MDMA and related amphetamines in rodent models. The relative thermoregulatory contribution of each agent is therefore important to identify risks posed by real world ecstasy use, given that many ecstasy pills are contaminated with non-MDMA compounds and that some ecstasy consumers may explicitly seek non-MDMA pill constituents (i.e. of reputed “speedy” vs. “dopey” subjective properties (Levy et al., 2005). The behavioral and cognitive implications of the hyperthermic and neurotoxic effects are not fully known; however, cognitive deficits in ecstasy users have been described repeatedly; see (Gouzoulis-Mayfrank and Daumann, 2006; Morgan, 2000; Parrott 2000, 2001) for review. An initial series of nonhuman primate behavioral studies failed to establish a clear relationship between MDMA-induced serotonin depletions and behavioral disruption (Frederick et al., 1998; Taffe et al., 2001, 2002; Winsauer et al., 2002). Such studies were quite limited in size (treatment groups N = 3); however, there was an indication in one of the studies that a treated individual with the most severe serotonin depletion and post-treatment behavioral impairment was the only one to become clearly hyperthermic (Bowyer et al., 2003). The broader importance of the present work is the establishment of a reliable and repeatable monkey model of amphetamine hyperthermia with which to investigate critical factors, individual, environmental or dose-related, which may contribute to unregulated and threatening temperature disruption.

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