Relationship between Temperature, Dopaminergic Neurotoxicity, and Plasma Drug Concentrations in Methamphetamine-Treated Squirrel Monkeys

Jie Yuan, George Hatzidimitriou, Pranav Suthar, Melanie Mueller, Una McCann, and George Ricaurte

Departments of Neurology (J.Y., G.H., P.S., G.R.) and Psychiatry and Behavioral Sciences (U.M.), The Johns Hopkins University School of Medicine, Baltimore, Maryland; and Department of Experimental and Clinical Toxicology (M.M.), Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, Homburg (Saar), Germany

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ABSTRACT

To examine the relationship between temperature (ambient and core), dopaminergic neurotoxicity, and plasma drug [methamphetamine (METH)] and metabolite [amphetamine (AMPH)] concentrations, two separate groups of squirrel monkeys (n = 4–5 per group) were treated with METH (1.25 mg/kg, given twice, 4 h apart) or vehicle (same schedule) at two different ambient temperatures (26 and 33°C). Core temperatures and plasma drug concentrations were measured during the period of drug exposure; striatal monoaminergic neuronal markers in the same monkeys were determined 1 week later. At the temperature range examined, the higher ambient temperature did not significantly enhance METH-induced hyperthermia or METH-induced dopaminergic neurotoxicity, although there were trends toward increases. Acute METH-induced increases in core temperature correlated highly and directly with subsequent decreases in striatal dopaminergic markers. Squirrel monkeys with the greatest increases in core temperature (and largest dopaminergic deficits) had the highest plasma drug metabolite (AMPH) concentrations. There was substantial interanimal variability, both with regard to elevations in core temperature and plasma drug concentrations. Pharmacokinetic studies in six additional squirrel monkeys revealed comparable individual differences in METH metabolism. These results, which provide the first available data on the within-subject relationship between temperature (ambient and core), plasma concentrations of METH (and AMPH), and subsequent dopaminergic neurotoxic changes, suggest that, as in rodents, core temperature can influence METH neurotoxicity in primates. In addition, they suggest that interanimal differences presently observed in thermal and neurotoxic responses to METH may be related to individual differences in drug metabolism.

Methamphetamine [N-methyl-β-phenylisopropylamine (METH)] is an amphetamine (AMPH) analog with a high potential for abuse (Kalant, 1966; Miller and Hughes, 1994). Indeed, county law enforcement agencies in the United States recently identified METH abuse as their primary drug problem (Kyle and Hansell, 2005; http://www.naco.org). In addition to problems related to its abuse per se, METH has neurotoxic potential toward brain dopamine (DA) and serotonin (5-HT) neurons. In particular, animals given repeated doses of METH develop long-lasting depletions of DA and 5-HT, their major metabolites, their membrane transporters, their rate-limiting biosynthetic enzymes, and their vesicular transporters (Gibb et al., 1994; Lew et al., 1997; Cho and Melega, 2002; McCann and Ricaurte, 2004). Anatomic studies indicate that reductions in presynaptic DA and 5-HT axonal markers are related to destruction of DA and 5-HT axon terminals (Ricaurte et al., 1982, 1984a,b; Sonsalla et al., 1996, Fukumura et al., 1998).

To date, neurotoxic effects of METH have been documented in mice, rats, guinea pigs, cats, vervet monkeys, rhesus monkeys, and baboons (Seiden and Ricaurte, 1987; Lew et al., 1997; Melega et al., 1997; Villemagne et al., 1998). Evidence of possible METH-induced DA neurotoxicity is also available in humans (McCann et al., 1998; Sekine et al., 2001; Volkow et al., 2001). The profile of METH-induced neurotoxic changes varies according to species. In mice, the neurotoxic effects of METH primarily involve DA neurons, with 5-HT neurons generally showing smaller or no lasting effects (Sei-
den and Ricaurte, 1987; O’Callaghan and Miller, 1994; Callahan et al., 1998; Fumagalli et al., 1998; Gluck et al., 2001). In rats, METH-induced neurotoxicity typically involves both DA and 5-HT neurons, with 5-HT neurons typically being more severely affected (Hotchkiss and Gibb, 1980; Ricaurte et al., 1980). In nonhuman primates, as in mice, DA neurons tend to be more affected than 5-HT neurons (Villemagne et al., 1998). The basis for these species differences in neurotoxicity profile is unknown.

The precise mechanism by which METH produces a distal axotomy of brain DA/5-HT neurons remains to be elucidated. However, studies over the last decade have demonstrated that temperature (ambient and core) can markedly influence METH-induced DA/5-HT neurotoxicity in rodents (mice and rats). Specifically, higher temperatures enhance neurotoxicity, whereas lower temperatures typically afford neuroprotection (Bowyer et al., 1992, 1994; Ali et al., 1994; Miller and O’Callaghan, 1994, 2003; O’Callaghan and Miller, 1994; Albers and Sonsalla, 1995; Farfel and Seiden, 1995; Callahan et al., 1998). In contrast to the sizeable literature in rodents, only one study has evaluated the influence of temperature on METH-induced neurotoxicity in nonhuman primates (Melega et al., 1998). Based on the observation that hypothermia (secondary to MK-801) did not afford protection against METH-induced DA neurotoxicity in vervet monkeys, these authors concluded that rodents and primates might possess different regulatory factors with regard to temperature and METH neurotoxicity (Melega et al., 1998).

Little is presently known about the relationship between temperature, neurotoxicity, and plasma METH concentrations. In fact, to our knowledge, there are no studies that have measured each of these parameters in the same animal. As such studies could yield insight into the mechanisms of METH neurotoxicity, the present study was undertaken to evaluate the relationship among temperature (ambient and core), plasma concentrations of METH and its metabolite AMPH during the period of drug exposure, and measures of dopaminergic axon terminal integrity 1 week later. In a separate group of squirrel monkeys, we also characterized the pharmacokinetic profile of METH and its metabolite (AMPH).

Materials and Methods

Animals. Adult squirrel monkeys (Saimiri sciureus) of both genders, ranging in weight from 0.69 to 1.3 kg, were used. Animals were housed in standard steel cages in a colony room maintained at an ambient temperature of 26 ± 1°C and 20 to 40% humidity, with free access to food (New World Primate Diet) and water. The colony room was maintained on a 14:10 cycle (14-h light/10-h dark), with lights on at 7:00 AM and off at 9:00 PM. The facilities for housing and care of the animals are accredited by the American Association for the Assessment and Accreditation of Laboratory Animal Care. Animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee at The Johns Hopkins University School of Medicine. The committee recognized that an ambient temperature of 33°C exceeded the maximum temperature recommended for nonhuman primates by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, 1985) but approved an explicit exception for this study. Only animals on the relevant protocol were exposed to an ambient temperature of 33°C.

Drugs and Chemicals. d-Methamphetamine was obtained through the National Institute on Drug Abuse drug supply program (Bethesda, MD), and its authenticity was confirmed by means of gas chromatography (GC/MS determinations, racemic amphetamine and methamphetamine were obtained from Lipomed (Cambridge, MA); racemic d₃-amphetamine (AMPH-d₃), and racemic d₄-methamphetamine (METH-d₄) were purchased from Cerelliant (Round Rock, TX); and heptafluorobutyric anhydride was purchased from Alltech Associates (Deerfield, IL). Clean Screen with Clean Thru tips solid-phase extraction columns (6 ml) were obtained from United Chemical Technologies (Bristol, PA). [3H]WIN55,288 was purchased from New England Nuclear (Boston, MA). [3H]Dihydroxybutazone was obtained from Amershams Life Sciences (Buckinghamshire, UK). Other drugs and chemicals were obtained from the following source: dopamine hydrochloride, dihydroxyphylacetic acid, 5-hydroxytryptamine creatinine sulfate complex, 5-hydroxyindoleacetic acid, dicyclohexylammonium salt, sodium octyl sulfate, and EDTA (Sigma-Aldrich, St. Louis, MO) and sodium phosphate, citric acid, phosphoric acid, and perchloric acid (J. T. Baker, Phillipsburg, NJ).

Drug Administration. d-Methamphetamine (or vehicle) was administered orally. Oral administration was accomplished by orogastric gavage, with the animal gently restrained in a Plexiglas chair during the procedure. Dose selection was based upon pilot studies and published reports indicating that two doses of METH given hours apart have the potential to produce lasting effects on brain DA neurons in nonhuman primates (Melega et al., 1998). We ultimately selected a dose of 1.25 mg/kg, given twice at a 4-h interval, because pilot studies showed that this dose regimen is well tolerated and produced a modest reduction in striatal DA neuronal markers 1 week later, thus leaving room for a possible enhancement by a higher ambient temperature (33°C instead of 26°C). Drug (or vehicle) administration was performed at 11:00 AM and 3:00 PM, so that assessment of effects on core temperature took place during a relatively stable period of the circadian temperature cycle (see Results).

Overall Design. Two different groups (n = 4–5 per group) of drug-naive animals were used to test the effects of increased ambient temperature on METH-induced changes in core temperature and subsequent DA neurotoxicity (i.e., five monkeys received vehicle and METH at 26°C and four different monkeys received vehicle and METH at 33°C) (Table 1). The rationale for the temperatures selected (26 and 33°C) was as follows. An ambient temperature of 26°C was selected because it is considered to be in the thermoneutral range for the squirrel monkey (Stitt and Hardy, 1971; Robinson and Fuller, 1999); an ambient temperature of 33°C was used as a “warm” environment because it is toward the high end of the thermoneural range for the squirrel monkey (Stitt and Hardy, 1971; Robinson et al., 1993) and because studies in rodents indicate that a 7°C increase in ambient temperature increases METH neurotoxicity (Callahan et al., 1998).

First, 24-h baseline core temperatures for each monkey were collected at the ambient temperature in which animals would subse-
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core temperature measurements were sensitive to the quality of than 1°C in a 1-min period were excluded from the analysis because were used for analysis. Average 1-min values that changed by more A.R.T., version 2.3; Transoma Medical, St. Paul, MN). Core temper- manipulation or data collection. Core temperature data were col- days to recover from surgery before undergoing any experimental were anesthetized with isoflurane. The sterile transmitter was in- transmitter (model TA10TA-F40) was implanted in the peritoneal rameters from conscious, freely moving animals. In brief, a small (Pharsight, Mountain View, CA). Data were fitted to a noncompart- times. Core temperatures of squirrel monkeys were pre- vented from exceeding 41°C (by means of ventilation in a cooler environment). Immediately before the second dose and 2 h after the second dose of METH, blood samples were collected for subsequent determination of plasma drug concentrations. On day 9 (i.e., 1 week after METH treatment), the animals were sacrificed for measurement of monoamine neuronal markers. Thus, for each animal, we collected data on the acute temperature response, plasma drug con- centration, and subsequent regional brain DA/5-HT neuronal mark- ers. As detailed in Table 1, because of technical issues, we were unable to collect core temperature data in one animal treated at 26°C and drug plasma concentration data in a different animal treated at 33°C.

Pharmacokinetic Study. For this experiment, designed to as- sess interanimal differences and characterize the pharmacokinetics of METH (and its metabolite, AMPH) in squirrel monkeys, six different squirrel monkeys were used. All of these animals had partic- ipated in prior studies involving stimulant drug administration; however, none had received any other drug for at least 4 weeks before this study (as previously noted, all other experiments reported in this article used drug-naïve animals). METH was administered at a dose of 1.25 mg/kg p.o. Blood samples were collected at 5, 15, 30, 60, 120, 180, 240, 300, and 360 min after METH administration. At each time point, the animal was briefly anesthetized with isoflurane to facilitate blood sampling. Plasma concentrations of both METH and AMPH were determined. Pharmacokinetic parameters (peak plasma concentrations \(C_{\text{peak}}\), times of peak plasma concentration \(T_{\text{max}}\), area under the concentration-time curve, and elimination half-lives \(t_{1/2}\)) were determined using the software program WinNonlin (Pharsight, Mountain View, CA). Data were fitted to a noncompart- mental model with first-order input and elimination.

Core Temperature Measurement. Core temperature was mea- sured noninvasively using a telemetry system (Data Sciences Interna- tional Inc., St. Paul, MN) designed to monitor physiological pa- rameters from conscious, freely moving animals. In brief, a small transmitter (model TA10TA-F40) was implanted in the peritoneal cavity. This device permits continuous, on-line monitoring of core temperature. For implantation of the transmitter, squirrel monkeys were anesthetized with isoflurane. The sterile transmitter was in- serted into the peritoneum. Monkeys were allowed a minimum of 7 days to recover from surgery before undergoing any experimental manipulation or data collection. Core temperature data were col- lected using a commercially available software package (Dataquest A.R.T., version 2.3; Transoma Medical, St. Paul, MN). Core temper- ature was sampled every 1.5 s (40 times/min), but 30-min means were used for analysis. Average 1-min values that changed by more than 1°C in a 1-min period were excluded from the analysis because core temperature measurements were sensitive to the quality of radiofrequency.

Plasma Sampling. For collection of blood samples used in the determination of plasma drug concentrations, animals were removed temporarily from their home cages and anesthetized briefly with isoflurane, as below. While under isoflurane anesthesia, animals were placed in a supine position on a heating pad maintained at 37°C by circulating warm water via a heat therapy pump (Gaymar Industries Inc., Orchard Park, NY), on a steel surgical table. Blood samples were collected immediately before and 2 h after the second METH dose (i.e., 4 and 6 h after the first dose of METH). These time points were chosen based on the pharmacokinetics of METH in the squirrel monkey, with an eye toward obtaining estimates of peak plasma drug concentrations of METH and AMPH after the second dose of METH. At each time point, approximately 0.5 to 1.0 ml of blood was withdrawn from the femoral vein using a disposable, sterile 3-ml syringe and 23-gauge 1-inch needle. Blood samples were dispensed into 4-ml Vacutainer Plus hematometry (lavender) tubes, containing 7.2 mg of K₂EDTA solution (Becton Dickinson, Franklin Lakes, NJ), and stored on ice for up to 30 min until centrifuged. Samples were centrifuged at 1100 g for 10 min at 4°C (Sorvall RC5B Plus; Kendro Laboratory Products, Newtown, CT), and plasma was withdrawn using a 5/8-inch Pasteur pipette and decanted into a 1.5-ml microcentrifuge tube. Sodium metabisulphite (250 mM) was added at a volume of 30 μl/ml plasma to minimize oxidation of the compounds of interest. Samples were vortexed for 10 s and then stored at −20°C until further processed.

Determination of Plasma METH and AMPH Concentra- tions. Sample preparation and derivatization were carried out as described previously by Peters et al. (2003b), with minor changes. A volume of 0.1 ml of internal standard containing 1.5 μg/ml each of METH-d₈ and AMPH-d₈ was added to 0.1 ml of plasma diluted with 2 ml of purified water. The analytes were eluted with 1.2 ml of a mixture of methylene chloride, 2-propanol, and ammonium hydrox- ide (80:20:2 by volume), and derivatization was carried out under heat (60°C) for 30 min (instead of using microwave irradiation). Analysis was performed using a model 6890N gas chromatograph system combined with a model 5973 inert electron impact mass spectrometer and ChemStation software version Rev.D.01.00 (Agili- cent Technologies, Palo Alto, CA). The GC conditions were as follows: spitless injection mode; column, HP-5MS (30 m × 0.25 mm i.d.), 5% phenylmethylsiloxane, 250 nm film thickness; injection port temperature, 250°C; carrier gas, helium; flow rate, 1.0 ml/min; column temperature, initially 80°C, increased to 180°C at 12°C/min; final temperature, 1 min. The MS conditions were as follows: transfer line heater, 280°C; source temperature, 230°C; electron ionization mode; ioniza- tion energy, 70 eV; and selected-ion monitoring with the following program: solvent delay 3 min; time window A, 3.00 to 5.50 min, 118, 210, 254 (target ion) for METH; time window B, start at 5.50 min, 91, 118, 240 (target ion) for AMPH; time window C, start at 5.50 min, 112, 123, 261 (target ion) for METH-d₈ and 118, 210, 254 (target ion) for AMPH. Quantification was carried out by comparison of peak area ratios (analyte versus internal standard) with calibration curves in which peak area ratios of spiked calibrators were plotted against their concentrations. The concentrations of the calibration curves ranged from 5 to 400 ng/ml.

Brain Dissection. One week after drug treatment, animals were sacrificed under deep sodium pentobarbital anesthesia (60 mg/kg i.p.), and the brain was removed from the skull in a cold room (4°C). Regional dissection of the brain was performed at 4°C using the Emmers and Akert (1963) atlas as a guide. Brain regions of interest were isolated from coronally cut sections (approximately 4–5 mm thick) by means of free dissection over ice. Brain tissue was wrapped in aluminum foil and then stored in liquid nitrogen until assay.

High Performance Liquid Chromatography Monoamine Assays. Concentrations of DA and 5-HT and their major metabolites were measured by reverse phase high performance liquid chroma- tography coupled with electrochemical detection as described previ- ously (Yuan et al., 2001).

DA Transporter and Vesicular Monoamine Transporter Binding. The density of the DAT and vesicular monoamine trans- porter-type 2 binding sites in regional brain homogenates was de- termined using previously described methods (Villemagne et al., 1998).

Statistics. Temperature data were analyzed using analysis of variance for repeated measures. Neurochemical data were evaluated by means of one-way analysis of variance. When statistical differ- ences were observed, post hoc comparisons were performed using the
least significant difference multiple range test. Pearson product moment correlations were calculated to evaluate the relationship among drug plasma concentrations, temperature responses, and subsequent DA deficits. Significance was set at $P < 0.05$. Data analysis was performed using the Statistical Program for the Social Sciences (SPSS for Windows, release 10.5).

Results

In keeping with previous reports (Fuller et al., 1979; Robinson and Fuller, 1999), squirrel monkeys exhibited diurnal variation in their core temperature (Fig. 1). On average, core temperature was higher when lights were on (7:00 AM to 9:00 PM) than when lights were off (9:00 PM to 7:00 AM). Under the conditions of our study, the 24-h mean core temperature was 37.9°C, with a standard deviation of 0.93°C. Core temperature was relatively stable between 8:00 AM and 7:00 PM.

Having identified a relatively stable period of the circadian core temperature cycle in our squirrel monkeys (8:00 AM to 7:00 PM), we proceeded to assess the effect of increased ambient temperature on METH-induced hyperthermia. This was done at and 33°C, using separate groups ($n = 4–5$ per group) of animals to test the effects of METH at each ambient temperature. As shown in Fig. 2, METH increased core temperature at both 26 and 33°C. Mean core temperature increases appeared greater at 33°C than at 26°C, but the difference did not achieve statistical significance. As can be seen in the figure, there was substantial interanimal variability in thermal response, both in monkeys treated with METH at 26°C and in those treated at 33°C. Per the predetermined protocol (see Materials and Methods), two of the four monkeys treated at 33°C were moved to a cooler environment (as a precautionary measure) when they reached a core temperature of 41°C. Both of these animals looked perfectly fine despite their elevated core temperature.

Next, we examined the effect of increased ambient temperature on METH-induced DA neurotoxic changes. Dopaminergic neuronal markers were determined 1 week after METH treatment. On average, monkeys treated at 33°C had larger dopaminergic deficits than those treated at 26°C (Fig. 3). However, like elevations in core temperatures, dopaminergic deficits induced by METH at the two ambient temperatures (26 and 33°C) were not significantly different. Again, substantial interanimal variability was noted at both ambient temperatures. Indeed, as shown in Fig. 3, one monkey treated at 26°C (unfortunately the animal in which core temperature data are not available, see Materials and Methods and Table 1) showed >90% deficits in DA neuronal markers 1 week after METH treatment. Regional brain 5-HT and 5-hydroxyindoleacetic acid were not significantly affected by prior treatment with METH, at either 26 or 33°C, even in animals with large dopaminergic deficits (not shown).

Analysis of the relationship between acute increases in core temperature and subsequent decreases in dopaminergic neuronal markers showed that the two variables were highly correlated (Fig. 4). Similarly high correlations were observed when the area under the concentration-time curve, rather than the $T_{\text{max}}$, of the temperature response was considered. Thus, regardless of the ambient temperature during METH treatment (26 or 33°C), squirrel monkeys with the greatest increases in core temperature developed the largest DA deficits.

To begin to identify the basis for the substantial interanimal variability described above, we examined the relationship among drug plasma concentrations, acute increases in core temperature and subsequent decreases in dopaminergic markers. Given the study design, this was feasible in each animal. Plasma concentrations of METH, determined 2 h after the second dose (i.e., 6 h after the first dose of METH),
did not correlate with either METH-induced hyperthermia or METH-induced DA neurotoxic changes (not shown). In contrast, plasma concentrations of METH's metabolite, AMPH, correlated highly and directly with acute increases in core temperature (Fig. 5). In addition, there were significant correlations between plasma AMPH concentrations and dopaminergic neuronal markers (Fig. 6).

To determine whether the interanimal differences detailed above might, at least in part, be related to individual differences in METH metabolism, we examined the pharmacokinetic profile of METH and its metabolite (AMPH) in a separate group of squirrel monkeys (n = 6). As shown in Fig. 7 and Table 2, individual differences in METH metabolism are substantial, with wide ranges in various pharmacokinetic parameters including Cmax and t1/2 values. Brief anesthesia with isoflurane, used to facilitate blood sampling, had a tendency to transiently decrease core temperature (10–15 min) in the squirrel monkey (not shown).

**Discussion**

The present study provides the first available data on the within-subject relationship between temperature (ambient and core), plasma concentrations of METH (and AMPH), and subsequent dopaminergic neurotoxicity. Results indicate that plasma concentrations of AMPH, acute increases in core temperature, and subsequent decreases in dopaminergic neuronal markers are highly correlated. In addition, they reveal substantial interanimal variability in both the thermal and neurotoxic responses to METH and suggest that, at least in part, these response differences may be related to individual differences in METH metabolism. Each of these findings is discussed, in turn, below.

The indication that core temperature can influence METH-induced DA neurotoxicity in nonhuman primates comes from the highly significant correlation presently observed between increases in core temperature and decreases in striatal dopaminergic markers (Fig. 4). Virtually identical observations have been made in rodents (Bowyer et al., 1994; Albers and Sonsalla, 1995). Thus, in both rodents and nonhuman primates, increases in core temperature are associated with augmented METH-induced DA neurotoxicity. At first glance,
this conclusion may seem at odds with that of Melega et al. (1998) stating that the effects of temperature on METH neurotoxicity might be species-dependent (see Introduction). However, these authors based their conclusion on the observation that decreased core temperature (secondary to MK-801-induced anesthesia) did not afford protection against METH-induced DA neurotoxicity in vervet monkeys. However, a similar effect has been reported in rodents. Specifically, Albers and Sonsalla (1995) found that decreased core temperature (secondary to reserpine) did not afford protection against METH-induced DA neurotoxicity in mice. Thus, in both rodents and primates, drug-induced decreases in core temperature do not always afford protection against METH-induced DA neurotoxicity. The fact that increases in core temperature are not essential for the expression of METH-induced DA neurotoxicity (either in rodents or primates) does not detract from the fact that, in general, increases in core temperature are associated with increased METH neurotoxicity. Indeed, these seemingly discrepant facts serve as a reminder that the mechanism by which core temperature influences METH neurotoxicity is unknown. That said, it is clear that core temperature can influence METH neurotoxicity and that the expression of DA neurotoxicity after amphetamines (METH and AMPH) is critically dependent upon the function of the DAT (Ricaurte et al., 1984b; Marek et al., 1990; Pu et al., 1994; Fumagalli et al., 1998). As we have previously shown that increases in temperature lead to increases in DAT function (Xie et al., 2000), it is possible that, at least in part, increased core temperature enhances METH-induced DA neurotoxicity by amplifying a DAT-dependent neurotoxic cascade (Callahan et al., 2001). Alternatively, elevations in core temperature could enhance neurotoxicity via alternate mechanisms. For example, core temperature elevations could lead to increased formation of reactive oxidative species (Cubells et al., 1994; Giovanni et al., 1995; Yamamoto and Zhu, 1998) or amplify other molecular events thought to underlie the neurotoxic mechanism of action of METH and related drugs (see Bowyer and Holson, 1995; Seiden and Sabol, 1996).

Under the present experimental conditions, increased ambient temperature did not produce significant effects on thermal or neurotoxic responses to METH in squirrel monkeys.

This contrasts with previous observations in rodents (see Introduction). However, factors that (alone or in combination) may have contributed to the lack of a significant effect of ambient temperature in squirrel monkeys include the limited range of ambient temperatures tested (26–33°C), the high degree of interanimal variability (see below), and the relatively small sample size necessarily used. In addition, our decision (for safety reasons) not to allow any monkey to exceed a core temperature of 41°C may have played a role, because two of the monkeys treated at an ambient temperature of 33°C (whereas none of those treated at 26°C) reached a core temperature of 41°C and had to be moved to a cooler
environment. Thus, in effect, our study design may have inadvertently introduced a ceiling effect that disproportionately affected the animals treated at the warmer ambient temperature. Finally, there may be innate differences between rodents and primates in thermoregulation.

At both ambient temperatures tested (26 and 33°C), we observed substantial interanimal variability, both with respect to thermal and neurotoxic responses to METH. The basis for these individual differences is unknown. However, it may be relevant that monkeys with the greatest increases in core temperature (and largest DA deficits) had the highest plasma drug concentrations. This observation raises the possibility that increased plasma drug concentrations lead to increased core temperature and that these factors, individually or in combination, act to increase dopaminergic neurotoxicity. Alternatively, increases in core temperature may somehow influence drug metabolism or clearance of drug and thereby lead to higher plasma drug (and metabolite) concentrations (and more pronounced neurotoxic effects). However, to our knowledge, there is no evidence that increased core temperature, per se, inhibits METH or AMPH metabolism, clearance, or cytochrome P450 activity. Indeed, Clausing et al. (1995) have shown that hypothermia (rather than hyperthermia) leads to higher amphetamine concentrations. Thus, in light of these considerations, we presently favor the view that drug concentrations (at relevant sites of action) are the principal determinants of increases in core temperature and neurotoxicity and that the interanimal variability presently observed may be related, at least in part, to individual differences in drug metabolism. In a preliminary way, this view is supported by our demonstration that there are substantial interanimal differences in the pharmacokinetic parameters of METH and AMPH in squirrel monkeys (Fig. 7; Table 2). To our knowledge, these are the first such data reported.

Although there was a highly significant correlation between plasma concentrations of AMPH and increases in core temperature (as well as significant or near significant correlations between plasma AMPH concentrations and decreases in various DA neuronal markers), there was no correlation between plasma concentrations of METH and the above-mentioned outcome measures (core temperature and dopaminergic deficits). The better predictive value of plasma AMPH, as compared with METH, may be related to the fact that, in the squirrel monkey, METH is rapidly converted to AMPH in the periphery (Fig. 6), thus minimizing the amount of METH reaching the central compartment (brain). As the neurotoxic effects of AMPH are largely restricted to brain DA neurons, whereas those of METH involve both DA and 5-HT neurons (see Introduction), rapid conversion of METH to AMPH in the periphery may account for the absence of brain 5-HT deficits in the present study.

The observation that two oral doses of METH, on the order of those used by some humans (Cho and Melega, 2002), are sufficient to produce DA neurotoxic changes in squirrel monkeys is in keeping with our own earlier findings in baboons (Villemagne et al., 1998), as well as those of Melega et al. (1998) in vervet monkeys. Of note, however, these studies did not allow for the development of tolerance, which can diminish METH-induced neurotoxic changes (Schmidt et al., 1985; Gygi et al., 1996; Stephans and Yamamoto, 1996; Riddle et al., 2002; Johnson-Davis et al., 2003). This issue notwithstanding, the present results indicate that multiple high doses of METH are not always necessary for the production of dopaminergic neurotoxicity and demonstrate that plasma levels of AMPH associated with dopaminergic neurotoxicity in nonhuman primates are on the order of those in some METH and AMPH users (Nakashima et al., 2003; Peters et al., 2003a).

Several limitations of the current study should be mentioned. First, the range of ambient temperatures studied was limited and may not have included a transition point at which the influence of ambient temperature would have been significant. Second, the sample size was small (n = 4–5 per group), a necessary feature of primate studies. A larger sample size might have resulted in improved power to draw conclusions regarding potential effects of ambient temperature. Third, there is a possibility that isoflurane anesthesia, used to facilitate plasma sampling, might have influenced the effect of altered core temperatures. However, previous studies suggest that isoflurane does not influence the degree of neurotoxic injury produced following treatment with other amphetamine neurotoxins (Mechan et al., 2005), and, indeed, in the current study, significant toxicity was observed despite the transient hypothermic effect of anesthesia. Finally, the
In conclusion, results from the present study, the first to explore within-subject relationships among temperature (ambient and core), plasma drug concentrations, and dopaminergic neurotoxicity, underscore the fundamental importance of core temperature in METH neurotoxicity across species (rodents and primates). In addition, they show that there are substantial interanimal differences in thermal and neurotoxic responses to METH and suggest that such differences may, at least in part, be related to individual differences in METH metabolism. As similar interindividuall differences have been noted in humans (Cook et al., 1992, 1993; Schepers et al., 2003), additional studies are needed to identify their basis and potential clinical consequences.

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Address correspondence to: Dr. George A. Ricaurte, Department of Neurology, Johns Hopkins Medical Institutions, 5501 Hopkins Bayview Circle, Rm. 5B.71E, Baltimore, MD 21224. E-mail: Ricaurte@jhmi.edu