

Elevation of Ambient Room Temperature has Differential Effects on MDMA-Induced 5-HT and Dopamine Release in Striatum and Nucleus Accumbens of Rats

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3,4-Methylenedioxymethamphetamine (MDMA) produces acute dopamine and 5-HT release in rat brain and a hyperthermic response, which is dependent on the ambient room temperature in which the animal is housed. We examined the effect of ambient room temperature (20 and 30°C) on MDMA-induced dopamine and 5-HT efflux in the striatum and shell of nucleus accumbens (NAc) of freely moving rats by using microdialysis. Locomotor activity and rectal temperature were also evaluated. In the NAc, MDMA (2.5 or 5 mg/kg, i.p.) produced a substantial increase in extracellular dopamine, which was more marked at 30°C. 5-HT release was also increased by MDMA given at 30°C. In contrast, MDMA-induced extracellular dopamine and 5-HT increases in the striatum were unaffected by ambient temperature. At 20°C room temperature, MDMA did not modify the rectal temperature but at 30°C it produced a rapid and sustained hyperthermia. MDMA at 20°C room temperature produced a two-fold increase in activity compared with saline-treated controls. The MDMA-induced increase in locomotor activity was more marked at 30°C due to a decrease in the activity of the saline-treated controls at this high ambient temperature. These results show that high ambient temperature enhances MDMA-induced locomotor activity and monoamine release in the shell of NAc, a region involved in the incentive motivational properties of drugs of abuse, and suggest that the rewarding effects of MDMA may be more pronounced at high ambient temperature.

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3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy') is a commonly used recreational drug, often ingested at crowded and warm dance clubs and raves. The main adverse effect related to acute MDMA toxicity is hyperthermia, with body temperatures as high as 43°C having been reported (Henry, 1992). The hyperthermic response is responsible for most of the deaths caused by the drug since many of the other toxicological problems that are seen, particularly rhabdomyolysis, disseminated intravenous coagulation, and acute renal failure (Brown and Osterloh, 1987; Henry *et al*, 1992; Scream *et al*, 1992) result from hyperthermia. Hyperthermia is also observed in experimental animals immediately after drug

injection and its magnitude is very dependent on ambient room temperature during drug exposure (eg Green *et al*, 2004).

Systemic MDMA administration has also been shown to increase extracellular dopamine and 5-HT levels in mesolimbic brain areas such as the nucleus accumbens (NAc) (Yamamoto and Spanos, 1988; Marona-Lewicka *et al*, 1996; Kankaanpaa *et al*, 1998). The NAc is a brain area that is responsible for the incentive motivational properties of most drugs of abuse and the rewarding effects of MDMA have been shown using the appropriate paradigms. Thus, rats treated with MDMA developed a positive and dose-dependent response in the conditioned place preference (CPP) test (Marona-Lewicka *et al*, 1996; Bilsky *et al*, 1991; Bilsky and Reid, 1991; Schechter, 1991). Since CPP is believed to be a measure of appetitive behavior where the animal associates contextual cues with either a positive or negative feeling produced by the drug, these results provide direct evidence of the rewarding properties of MDMA in rats. A rewarding effect of MDMA has also been shown in the self-stimulation paradigm in rats (Hubner *et al*, 1988),

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where MDMA lowers the reward threshold of electrical stimulation, and in the drug self-administration test in rats (Schenk *et al*, 2003; Daniela *et al*, 2004) and baboons (Lamb and Griffiths, 1987). The locomotor hyperactivity observed after MDMA injection is also consistent with this drug exerting a positive rewarding effect (Gold and Koob, 1988; Gold *et al*, 1989a). 6-Hydroxydopamine lesions of the NAc attenuated the locomotor response produced by MDMA (Gold *et al*, 1989b), and dopaminergic activity thus appears to be selectively responsible for MDMA- and amphetamine-induced locomotor activity, since blockade of dopamine receptors or 6-OHDA lesions of mesolimbic dopamine fibers did not block caffeine, scopolamine, heroin, or corticotrophin-releasing factor-induced locomotor activation (Swerdlow and Koob, 1985; Vaccarino *et al*, 1986). On the other hand, the fact that 6-OHDA lesions of the NAc attenuated, but did not abolish, the MDMA-induced hyperactivity (Gold *et al*, 1989b) indicates that the MDMA-induced dopamine release in the NAc is not the sole cause of the rewarding effects of MDMA and there is some evidence that serotonergic activity may also be involved.

Recently, it was shown that an elevation of ambient room temperature enhanced the prosocial effects of MDMA and the number of MDMA infusions self-administered by rats (Cornish *et al*, 2003). This suggests that the rewarding effects of MDMA are more pronounced at high ambient temperature and that the enthusiasm of recreational users for consuming the drug in hot environments might not be coincidental. Nevertheless, it is not known whether the neurochemical changes related to the rewarding effects of MDMA are in any way dependent on the ambient room temperature in which the drug is ingested.

Using *in vivo* microdialysis, we have now examined the effect of ambient temperature on MDMA-induced dopamine and 5-HT output and metabolism in the striatum and in the shell of NAc of freely moving rats. Locomotor activity and body temperature during drug exposure was also evaluated at standard (20°C) and high (30°C) room ambient temperature.

MATERIALS AND METHODS

Animals and Drug Administration

Male Dark Agouti rats (175–200 g, Interfauna, Barcelona) were used. They were housed in groups of five, in conditions of constant temperature ($21 \pm 2^\circ\text{C}$) and a 12 h light/dark cycle (lights on: 0700), and given free access to food and water. In order to habituate the animals to the different ambient temperatures studied, on the day of the experiment, rats were maintained at an ambient room temperature between either 19 and 21°C (referred to in the text as 20°C) or 30 and 32°C (referred to as 30°C) for 2.5 h before MDMA (2.5 or 5 mg/kg, *i.p.*) administration and these conditions were maintained for the entire experimental procedure. Rectal temperature data and microdialysate data were obtained from the same animals and separate animals were used for the locomotor activity assessment. Animals were only treated at one dose level and used to study one brain area.

MDMA (NIDA, Research Triangle Park, NC) was dissolved in saline (0.9% NaCl) and given in a volume of 1 ml/kg. Dose is reported in terms of the base.

All experimental procedures were performed in accordance with the guidelines of the Animal Welfare Committee of the Complutense University (following DC86/609/EU).

Measurement of Rectal Temperature

Immediately before and up to 5 h after MDMA injection, temperature was measured every 30 min by use of a digital readout thermocouple (Type K thermometer, Portec, UK) with a resolution of $\pm 0.1^\circ\text{C}$ and accuracy of $\pm 0.2^\circ\text{C}$ attached to a CAC-005 Rodent Sensor, which was inserted 2.5 cm into the rectum of the rat, the animal being lightly restrained by holding in the hand. A steady readout was obtained within 10 s of probe insertion.

Measurement of Locomotor Activity

Animals were placed at either ambient room temperature (20 and 30°C) 2.5 h before treatment and this temperature was maintained for the entire experimental procedure. Rats were treated with MDMA (5 mg/kg, *i.p.*) or saline and immediately placed in a locomotor activity chamber. No habituation to the chamber was performed. The opaque plastic chamber measured $35 \times 37 \times 44$ cm ($w \times l \times h$) with eight infrared beams and photocells distributed in two rows along the length of the chamber. The upper row was raised 10.5 cm from the base of the cage and the lower row 5.5 cm. Spacing between adjacent beams was 7.5 cm. The locomotor activity measurement consisted of the total number of photocell beam breaks (upper and lower beams) recorded in 30 min analyzed by a personal computer. Counting began 10 min after injection and placement of the animals in the chamber area.

Implantation of Microdialysis Probe in the NAc and Striatum

The day before the experiment rats were anaesthetised with pentobarbitone (Euta-Lender, 40 mg/kg) and secured in a Kopf stereotaxic frame with the tooth bar at -3.3 mm below the interaural zero. A guide cannula was implanted in the right side of the brain according to the following coordinates: $+9.4$ mm from the interaural line, -1.0 mm mediolateral, and -5.4 mm below the skull for the NAc, and $+7.9$ mm from the interaural line, -2.5 mm mediolateral, and -4.0 mm below the skull for the striatum (König and Klippel, 1963). Cannulae were secured to the skull as described by Baldwin *et al* (1994). On the day of the experiment, the dialysis probes (membrane length: 2.0 mm \times 500 μm for the NAc and 4.0 mm \times 500 μm for the striatum; CMA/12, Sweden) were inserted in the guide cannulae such that the membrane protruded its full length from the end of the probe.

Measurement of Dopamine, 5-HT and Their Metabolites in the Dialysate

Catechol and indole efflux in the brain *in vivo* was measured by the method described in detail by Colado *et al* (1999).

At 24 h after implantation, probes were perfused with artificial cerebrospinal fluid (aCSF; KCl: 2.5 mM; NaCl: 125 mM; MgCl₂ · 6H₂O: 1.18 mM; CaCl₂ · 2H₂O: 1.26 mM) at a rate of 1 µl/min and samples collected from the freely moving animals at 30 min intervals in tubes containing 5 µl of a solution composed of HClO₄ (0.01 M), cysteine (0.2%), and sodium metabisulfite (0.2%). The first 60 min sample was discarded and the next three 30 min baseline samples collected. After injection, samples were collected every 30 min for 5 h.

Dopamine, 5-HT and the metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA) were measured in the dialysate by HPLC and electrochemical detection. The mobile phase consisted of KH₂PO₄ (0.05 M), octanesulfonic acid (0.4 mM), EDTA (0.1 mM), and methanol (16%) and was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml/min. The HPLC system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 µl, Waters 717 plus Autosampler), a stainless-steel reversed-phase column (Spherisorb ODS2, 5 µm, 150 × 4.6 mm) with a precolumn and a coulometric detector (Coulchem II, Esa, USA). The working electrode potential was set at 400 mV with a gain of 1 µA (for dopamine) and 500 nA (for the remaining compounds). The current produced was monitored by using integration software (Unipoint, Gilson).

Measurement of MDMA Concentration in Striatal Tissue

Brain concentrations of MDMA were determined following a previously described method with minor modifications (Sanchez *et al*, 2001). The striatal tissue was homogenized in ice-cold sodium carbonate–sodium bicarbonate buffer (pH 11.5) using an ultrasonicator. The homogenate was centrifuged at 27 000g for 20 min at 4°C. The supernatant was applied to a 145 mg C8 end-capped SPE light column (International Sorbent Technology, Waters). The column was washed with methanol (2 ml) followed by distilled water (2 ml) before applying the sample (400 µl of supernatant + 350 µl of distilled water). The column was washed with water (2 ml) before selective elution of MDMA with methanol (1 ml).

An aliquot (20 µl) of the resulting eluate was injected into a Waters HPLC system, which consisted of a pump (Waters 510) linked to a manual sample injector (Loop 20 µl, Rheodyne), a stainless-steel column (RP 18, 5 µm, 150 × 4.6 mm, XTerra) fitted with a precolumn (RP 18, 5 µm, 20 × 3.9 mm, XTerra), and a UV/visible detector (Waters 2487). The current produced was monitored using an integrator (Waters M745). The mobile phase consisted of 20 mM potassium dihydrogen phosphate (75%) and acetonitrile (25%), pH 2.5; the flow rate was set to 0.8 ml/min and UV absorption was measured at 235 nm.

Statistics

Data from the locomotor activity experiments were analyzed using one-way ANOVA followed by Tukey's multiple comparison test where significant differences occurred. Data from brain MDMA levels were analyzed by

Student's *t*-test. Statistical analyses of the temperature measurements and dialysis were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, Eire). Data were analyzed by analysis of variance (ANOVA) with repeated measures (program 2V) or, where missing values occurred, an unbalanced repeated measure model (program 5V) was used. Both used treatment as the between subjects factor and time as the repeated measure. To evaluate the effect of ambient temperature, the tests used treatment and ambient temperature as between subjects factors and time as the repeated measure. ANOVA was performed on both pretreatment and post-treatment data. Differences were considered significant at $P < 0.05$. The results of the statistical comparisons are included in the figure legends.

RESULTS

Effect of Ambient Temperature on MDMA-Induced Changes in Rectal Temperature

MDMA (2.5 or 5 mg/kg, i.p.) produced an effect on the rectal temperature of the rats that was dependent on the ambient temperature in which the animals were when it was administered. At an ambient temperature of 20°C, MDMA produced an initial decrease in the rectal temperature of the animals in the first 30 min (Figure 1). Rectal temperature then returned to values similar to those found in saline-treated animals by 1 h and remained so for at least 5 h. However, when administered at an ambient temperature of 30°C, MDMA produced a rapid and marked increase in the rectal temperature of the animals, attaining a peak value of 0.9 or 1.3°C above the saline controls in the first 30–60 min and remaining elevated above controls for 2.5 or 3.5 h (for 2.5 or 5 mg/kg, respectively) (Figure 1).

Effect of Ambient Temperature on MDMA-Induced Changes in Locomotor Activity

When MDMA (5 mg/kg, i.p.) was given to rats housed at 20°C, it doubled the locomotor activity measured in a 30 min period starting 10 min after treatment compared with activity measured in saline-injected rats (Figure 2). Animals housed at 30°C and treated with saline showed a 40% reduction in mean activity compared with saline-treated rats at an ambient temperature of 20°C, although this difference was not significant. MDMA given at high ambient temperature produced a five-fold increase in activity compared with the saline-treated controls at the same ambient temperature; however, their absolute activity was similar to that seen in MDMA-treated rats given the same dose of drug at 20°C.

Effect of Ambient Temperature on the MDMA-Induced Changes in Monoamine Release and Metabolism in NAC and Striatum

Dopamine and metabolites in NAc. Administration of MDMA to rats housed at 20°C produced a slight increase (2.5 mg/kg, i.p.; Figure 3a) or a substantial and rapid increase (5 mg/kg, i.p.; Figure 3b) of the extracellular concentration of dopamine, peaking 60 min after injection

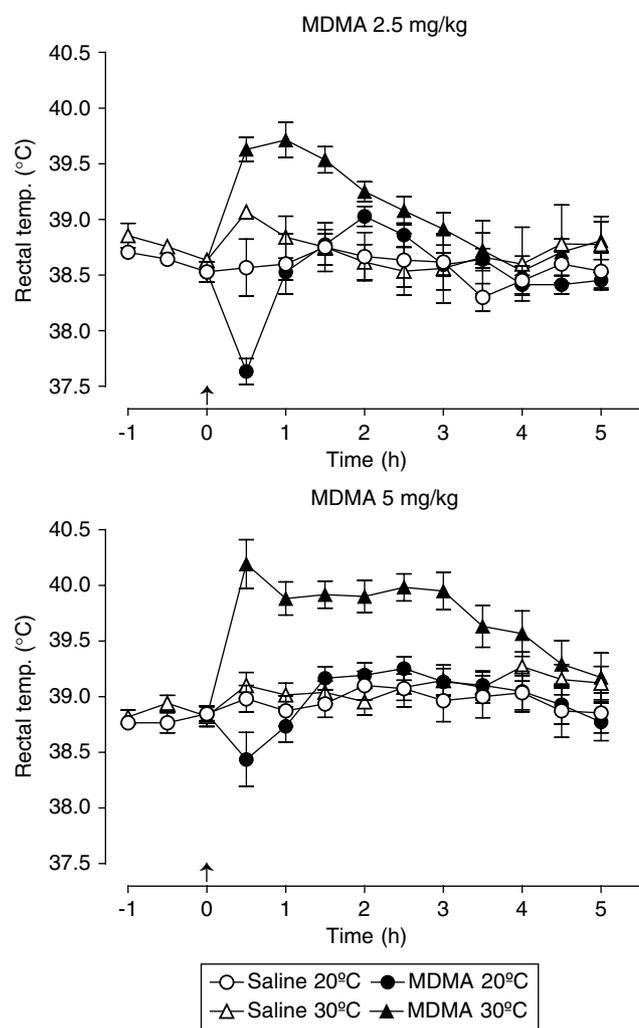


Figure 1 Rectal temperature of rats injected with MDMA (2.5 or 5 mg/kg, i.p.) at different ambient room temperatures. Animals were maintained at a room temperature of 20 or 30°C for 2.5 h before MDMA injection and for 5 h after. At 20°C, MDMA did not significantly modify rectal temperature compared with saline-treated animals (main effect of treatment: $F(1, 19) = 0.099$, NS, for 2.5 mg/kg and $F(1, 24) = 0.0024$, NS, for 5 mg/kg). Nevertheless, when MDMA was given to animals housed at 30°C, a pronounced and sustained hyperthermia compared with saline-treated animals was observed (main effect of treatment: $F(1, 18) = 3.58$, $P < 0.05$, for 2.5 mg/kg; $F(1, 23) = 15.49$, $P < 0.001$, for 5 mg/kg). The interaction of treatment \times ambient temperature was significant at both dosing levels for the interval 0.5–3.5 h (for 2.5 mg/kg: $F(1, 37) = 5.37$, $P < 0.05$; for 5 mg/kg: $F(1, 47) = 13.42$, $P < 0.0001$), indicating that the effect of MDMA was different at the two ambient temperatures. Each value is the mean \pm SEM of 8–15 animals.

and lasting 2 h in the case of the higher dose (Figure 3b). At 30°C room temperature, the MDMA-induced increase in the dopamine concentration of the dialysate was greater than that observed at 20°C and the enhanced efflux was evident for at least 4.5 h after drug injection (Figure 3a, b). The content of dopamine in the dialysate of the saline-treated group maintained at 30°C was also slightly more pronounced than that found in the saline-treated group maintained to 20°C (Figure 3a, b). The absolute basal values of dopamine in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

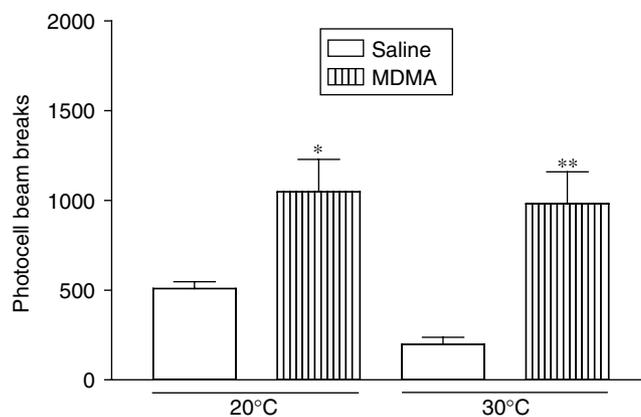


Figure 2 Locomotor activity of rats injected with MDMA (5 mg/kg, i.p.) at different ambient temperatures. Animals were maintained at a room ambient temperature of 20 or 30°C for 2.5 h before MDMA injection and during the entire experimental protocol. Rats were placed in the locomotor activity chamber immediately after MDMA injection and the total number of photocell beam breaks was counted for 30 min starting 10 min after placement of animals in the chambers. Each value is the mean \pm SEM of six animals. Different from the corresponding saline-treated group: * $P < 0.05$, ** $P < 0.01$.

Injection of MDMA induced a sustained decrease in the levels of DOPAC (Figure 3c, d) and HVA (Figure 3e, f) in the dialysate of animals kept at 20°C. Similar effects were observed at 30°C, but the effect on HVA was not significant at the lower MDMA dose (Figure 3e). There was no difference in the levels of DOPAC (Figure 3c, d) and HVA (Figure 3e, f) in the dialysate of saline-treated animals at either ambient temperature. The absolute basal values of DOPAC and HVA in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

5-HT and 5-HIAA in NAc. Administration of MDMA (2.5 or 5 mg/kg, i.p.) to rats at 20°C produced a modest increase in the extracellular concentration of 5-HT only at the higher dose that reached a significant difference *vs* the corresponding saline group between 0.5 and 2.0 h (Figure 4a, b). However, when MDMA was given at 30°C, there was an increase in the 5-HT levels in the dialysate peaking 60 min after injection and lasting for 2–3 h. This effect was significantly different from that observed at 20°C (Figure 4a, b). There was no difference in the levels of 5-HT in the dialysate of saline-treated animals at either temperature (Figure 4a, b). The absolute basal values of 5-HT in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

Injection of MDMA induced a prolonged decrease in 5-HIAA levels in the dialysate of animals at 20°C (Figure 4c, d). When the animals treated with MDMA 5 mg/kg were maintained at 30°C, a biphasic effect was observed, an initial increase in the extracellular 5-HIAA concentration was followed by a sustained and significant decrease. The 5-HIAA curves at both room temperatures were significantly different for the interval 0.5–3.5 h (Figure 4d). No such effect was observed at the lower dose (Figure 4c). There was no difference in the levels of 5-HIAA in the dialysate of saline-treated animals kept at either temperature

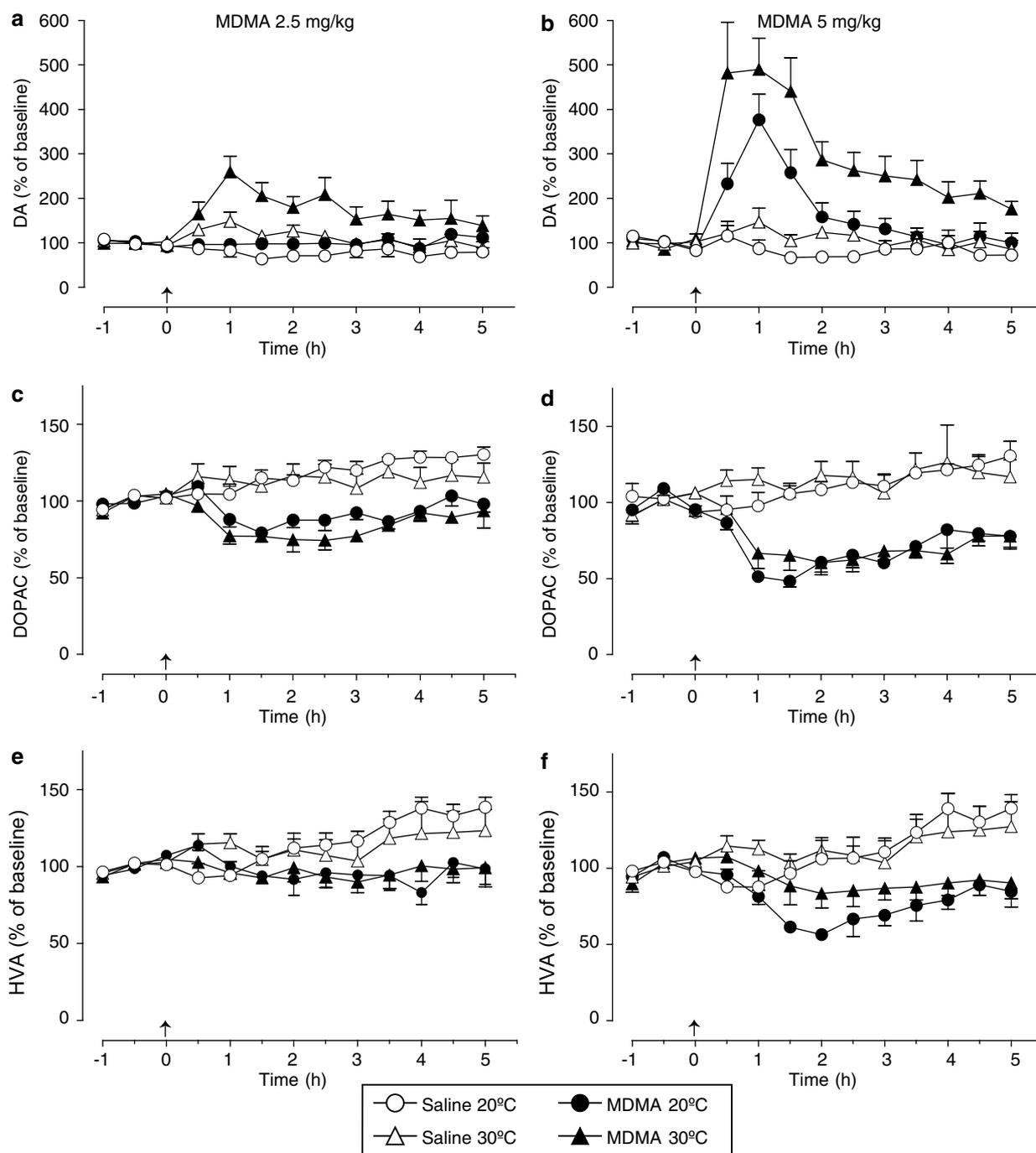


Figure 3 Changes in the extracellular levels of dopamine (DA; a, b), DOPAC (c, d), and HVA (e, f) in the NAc of rats following administration of MDMA (2.5 or 5 mg/kg, i.p.) at different ambient temperatures. Rats were maintained at a room temperature of 20 or 30°C for 2.5 h before MDMA injection and for 5 h after. At both room temperatures (20 and 30°C), MDMA produced an increase in the content of DA compared with saline-treated animals (main effect of treatment: for 2.5 mg/kg: $F(1, 12) = 5.29, P < 0.05$; $F(1, 13) = 8.28, P < 0.01$, respectively; for 5 mg/kg: $F(1, 11) = 9.34, P < 0.001$; $F(1, 8) = 65.02, P < 0.001$, respectively) and a decrease in the levels of HVA (main effect of treatment: for 2.5 mg/kg at 20°C only: $F(1, 14) = 7.93, P < 0.01$; for 5 mg/kg: $F(1, 10) = 17.99, P < 0.001$; $F(1, 10) = 5.45, P < 0.05$, respectively), and DOPAC (main effect of treatment: for 2.5 mg: $F(1, 13) = 44.02, P < 0.001$; $F(1, 13) = 10.76, P < 0.001$, respectively; for 5 mg/kg: $F(1, 11) = 39.34, P < 0.001$; $F(1, 10) = 26.54, P < 0.001$, respectively). The interaction of treatment \times ambient temperature was significant for dopamine at both dosing levels (for 2.5 mg/kg: $F(1, 25) = 3.41, P < 0.05$ and for 5 mg/kg: $F(1, 19) = 7.63, P < 0.01$) indicating that the effect of MDMA was different at the two ambient temperatures. Animals maintained at 30°C and injected with saline showed a modest increase in DA levels compared with those at 20°C (main effect of ambient temperature: for (a) $F(1, 14) = 14.39, P < 0.001$; for (b) $F(1, 9) = 5.26, P < 0.05$). Values are expressed as a percentage of the mean of three measurements before drug administration. Each value is the mean \pm SEM of 5–9 animals. Basal concentrations in saline-treated rats are shown in Table 1.

(Figure 4c, d). The absolute basal values of 5-HIAA in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

Dopamine and metabolites in striatum. Administration of MDMA (2.5 or 5 mg/kg, i.p.) to rats housed at 20°C produced a substantial and rapid increase of the extra-

Table 1 Basal Values for Extracellular Dopamine (DA), 5-HT, and Corresponding Metabolites in Saline-Treated Rats and Statistical Comparison of the Effect of Temperature

Brain area	Compound	20°C			30°C			F-value
		-60 min	-30 min	0 min	-60 min	-30 min	0 min	
NAc	DA	0.46 ± 0.09	0.48 ± 0.09	0.36 ± 0.06	0.47 ± 0.09	0.41 ± 0.08	0.45 ± 0.17	F(1,21) = 0.001, P = 0.97
	DOPAC	204 ± 23	219 ± 23	195 ± 22	165 ± 20	182 ± 21	188 ± 20	F(1,23) = 0.90, P = 0.35
	HVA	112 ± 12	125 ± 15	117 ± 14	102 ± 11	112 ± 12	115 ± 12	F(1,22) = 0.07, P = 0.79
	5-HT	0.70 ± 0.16	0.56 ± 0.08	0.53 ± 0.07	0.57 ± 0.21	0.56 ± 0.20	0.41 ± 0.08	F(1,20) = 0.17, P = 0.66
	5-HIAA	49 ± 5	52 ± 5	48 ± 5	49 ± 4	52 ± 4	53 ± 5	F(1,22) = 0.11, P = 0.74
Striatum	DA	0.63 ± 0.07	0.63 ± 0.08	0.70 ± 0.09	0.54 ± 0.06	0.46 ± 0.05	0.47 ± 0.05	F(1,21) = 3.21, P = 0.09
	DOPAC	338 ± 44	399 ± 55	435 ± 63	279 ± 38	361 ± 49	440 ± 58	F(1,23) = 0.07, P = 0.79
	HVA	296 ± 38	339 ± 41	375 ± 48	254 ± 33	313 ± 39	390 ± 49	F(1,22) = 0.01, P = 0.92
	5-HT	0.65 ± 0.08	0.64 ± 0.08	0.61 ± 0.07	1.00 ± 0.15	0.90 ± 0.11	0.76 ± 0.12	F(1,22) = 3.54, P = 0.07
	5-HIAA	95 ± 6	100 ± 7	100 ± 7	111 ± 13	123 ± 14	131 ± 17	F(1,22) = 2.57, P = 0.12

Three 30 min baseline samples were collected immediately before injecting MDMA. Values are mean (pg/μl) ± SEM of 22–25 animals.

cellular concentration of striatal dopamine, peaking at 60 min and lasting 3–4 h. At 30°C, the changes induced by MDMA on the dialysate dopamine concentration were similar in magnitude and duration to those observed at 20°C (Figure 5a, b). There was no difference in the extracellular concentration of dopamine of saline-treated animals kept at either temperature (Figure 5a, b). The absolute basal values of dopamine in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

Injection of MDMA induced a sustained decrease in the levels of DOPAC (Figure 5c, d) and HVA (Figure 5e, f) in the striatal dialysate of animals kept at either ambient temperature. There was no difference in the levels of DOPAC (Figure 5c, d) or HVA (Figure 5e, f) in the dialysate of saline-treated animals at either temperature. The absolute basal values of DOPAC and HVA in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

5-HT and 5-HIAA in striatum. Administration of MDMA (2.5 or 5 mg/kg, i.p.) to animals housed at 20°C produced a substantial and rapid increase in the extracellular concentration of 5-HT in the striatum peaking 60 min after injection and lasting 2–3 h (Figure 6a, b). Similar changes were seen in animals housed at 30°C (Figure 6a, b). There was no difference in the levels of 5-HT in the dialysate of saline-treated animals at either temperature (Figure 6a, b). The absolute basal values of 5-HT in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

Low-dose MDMA (2.5 mg/kg, i.p.) produced a sustained decrease in the extracellular levels of 5-HIAA, which was not modified by an increase in ambient temperature (Figure 6c). While injection of MDMA at the higher dose to animals at 20°C also induced a sustained decrease in dialysate 5-HIAA levels, in animals kept at 30°C, it induced

a biphasic response consisting of an initial rise followed by a decrease (Figure 6d). Nevertheless, 5-HIAA curves at both room temperatures were not significantly different (Figure 6d). There was no difference in the levels of 5-HIAA in the dialysate of saline-treated animals kept at different temperatures (Figure 6c, d). The absolute basal values of 5-HIAA in the dialysates of saline-treated animals maintained at 30°C were similar to those observed in the group at 20°C (Table 1).

Effect of Ambient Temperature on Striatal Levels of MDMA

In order to investigate the possible effect of ambient temperature on the concentration of MDMA in the brain, rats were given MDMA (5 mg/kg, i.p.) and killed 1 h later. This time point was chosen because the cerebral concentration of MDMA normally peaks 60 min after MDMA injection (Esteban *et al*, 2001) and it was the time point at which neurotransmitter release peaked. There were no differences between the MDMA levels found in the striatum of rats treated with the drug at 20°C (22.9 ± 3.4 nmol/g tissue, *n* = 4) and those treated at 30°C (22.6 ± 0.2 nmol/g tissue, *n* = 4).

DISCUSSION

Using intracerebral microdialysis in freely moving rats, this study has shown that elevation of ambient temperature enhances the effect induced by low and medium doses of MDMA on dopamine release in the shell of the NAc but not in the striatum. The output of 5-HT is also enhanced in the NAc, but not striatum, by high ambient temperature conditions.

Although MDMA increased both dopamine and 5-HT release in the NAc of animals at 30°C, the magnitude of the MDMA effect on extracellular dopamine levels (260 and

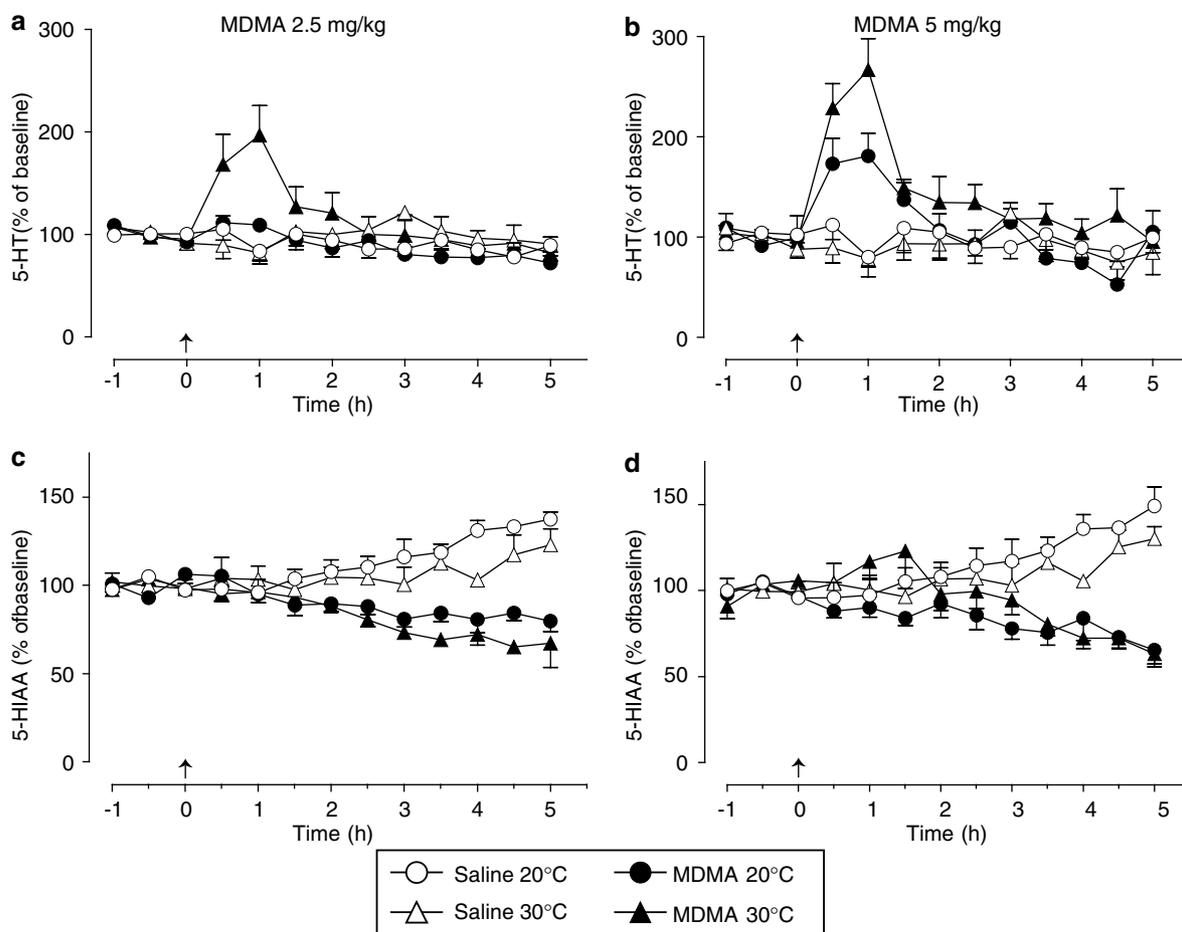


Figure 4 Changes in the extracellular levels of 5-HT (a, b) and 5-HIAA (c, d) in the NAC of rats following administration of MDMA (2.5 or 5 mg/kg, i.p.) at different ambient temperatures. Rats were maintained at a room temperature of 20 or 30°C for 2.5 h before MDMA injection and for 5 h after. At 20°C MDMA (5 mg/kg, i.p.) reduced 5-HIAA levels compared with saline-treated animals (main effect of treatment: $F(1, 12) = 21.21, P < 0.001$) and produced a significant increase in 5-HT concentration vs the corresponding saline from 0.5–2.0 h (main effect of treatment: $F(1, 9) = 3.97, P < 0.05$). At 2.5 mg/kg, MDMA only reduced 5-HIAA levels (main effect of treatment: $F(1, 11) = 30.58, P < 0.001$). When MDMA was given at 30°C, it produced a pronounced and prolonged increase in 5-HT concentration compared with saline-treated controls (main effect of treatment: for 2.5 mg/kg between 0.5 and 2.0 h: $F(1, 13) = 5.86, P < 0.01$; for 5 mg/kg between 0.5 and 5 h: $F(1, 9) = 18.49, P < 0.001$). The interaction of treatment \times ambient temperature was significant for 5-HT at both dosing levels (for 2.5 mg/kg between 0.5 and 2.0 h: $F(1, 26) = 4.08, P < 0.05$; for 5 mg/kg between 0.5 and 5 h: $F(1, 18) = 4.24, P < 0.05$), indicating that the effect of MDMA was different at the two ambient temperatures. MDMA also produced a reduction in 5-HIAA levels (main effect of treatment: for 2.5 mg/kg: $F(1, 8) = 8.51, P < 0.01$; for 5 mg/kg: $F(1, 8) = 6.06, P < 0.01$). The interaction of treatment \times ambient temperature was not significant for 5-HIAA at either dosing level (for 2.5 mg/kg: $F(1, 19) = 0.00, NS$; for 5 mg/kg: $F(1, 20) = 2.27, NS$), indicating that the effect of MDMA was the same at the two ambient temperatures, excepting for the interval 0.5–3.5 h at the higher dose ($F(1, 20) = 3.80, P < 0.05$). Values are expressed as a percentage of the mean of three measurements before drug administration. Each value is the mean \pm SEM of 5–8 animals. Basal concentrations in saline-treated rats are shown in Table 1.

490% increase for 2.5 and 5 mg/kg at 20°C) was larger than that elicited on 5-HT concentration (197 and 265%). This more pronounced effect of MDMA on dopamine release is not a consequence of the high ambient temperature since White *et al* (1994) observed a similar difference at standard room temperature. Nor can the difference in increases in the levels of DA and 5-HT in the NAC following MDMA be attributed to a different affinity of MDMA for monoamine transporters or to its different potency for inducing [3 H]-DA or [3 H]-5-HT release *in vitro*. Using hippocampal and striatal synaptosomal preparations, it has been shown that MDMA is only slightly more potent at inhibiting [3 H]-5-HT than [3 H]-dopamine uptake (1.7 and 3.2 times, in each area, respectively) and that it is 10 times more potent at increasing [3 H]-5-HT than [3 H]-dopamine release (Crespi

et al, 1997). Nevertheless, in NAC, there is a greater density of dopamine than 5-HT terminals and this morphologic difference may account for the relatively larger MDMA-induced increase in extracellular dopamine levels compared with 5-HT (Dewar *et al*, 1991; Mennicken *et al*, 1992). In addition, although 5-HT axon density is higher in the shell of NAC than in the dorsal striatum (Deutch and Cameron, 1992) few axons in the area express 5-HT transporters. It is worth mentioning that MDMA penetrates into 5-HT nerve terminals by means of 5-HT uptake system (Sanchez *et al*, 2004) and increases 5-HT release through the 5-HT transporter operating in reverse (Rudnick and Wall, 1992).

In addition to increasing dopamine release, MDMA also decreased the extracellular concentrations of DOPAC, HVA, and 5-HIAA in the NAC at both ambient temperatures. This

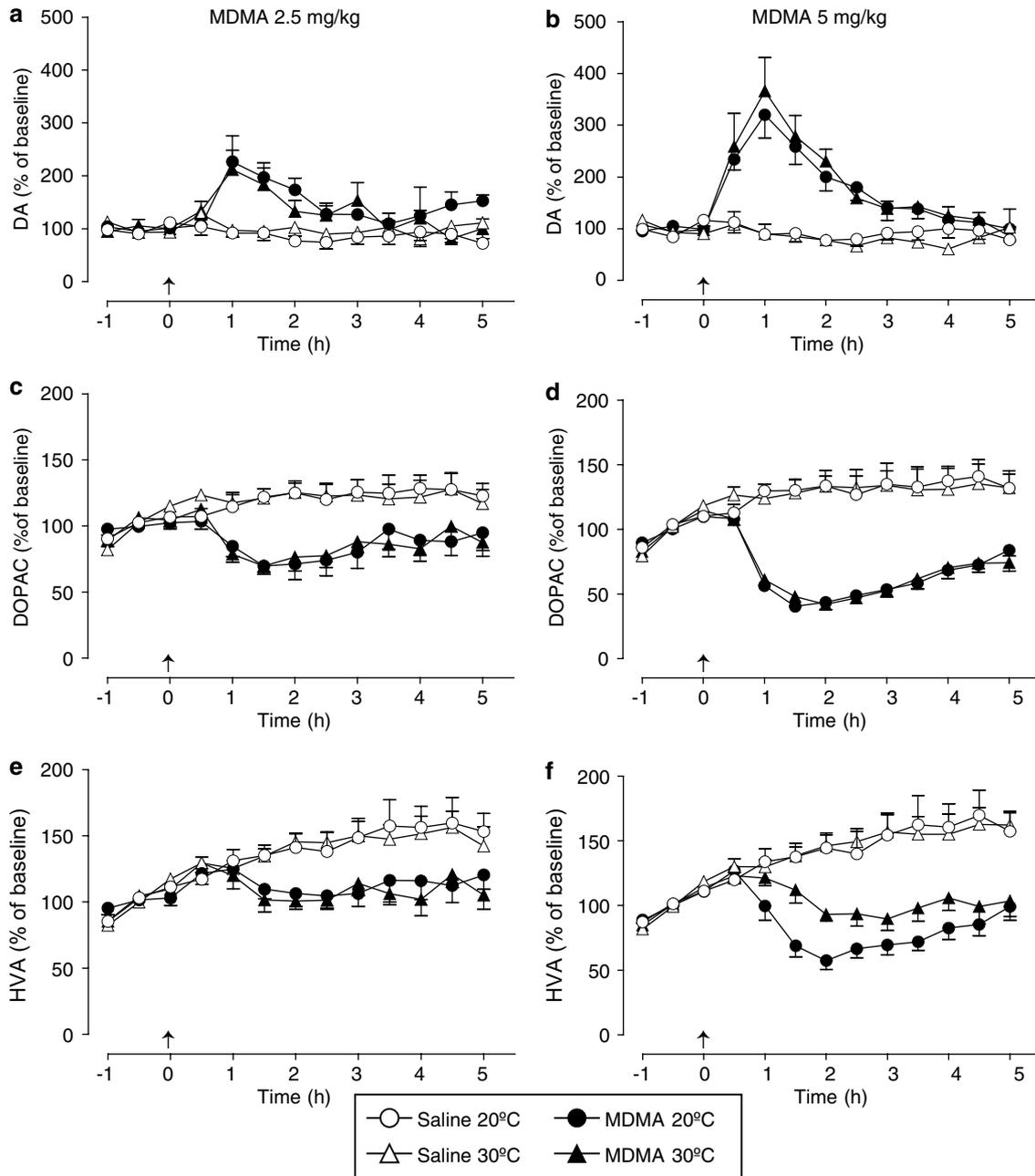


Figure 5 Changes in the extracellular levels of dopamine (DA; a, b), DOPAC (c, d) and HVA (e, f) in the striatum of rats following administration of MDMA (2.5 or 5 mg/kg, i.p.) at different ambient temperatures. Rats were maintained at a room temperature of 20 or 30°C for 2.5 h before MDMA injection and for 5 h after. At both room temperatures (20 and 30°C), MDMA produced an increase in the content of DA compared with saline-treated animals (main effect of treatment: for 2.5 mg/kg: $F(1, 11) = 14.61$, $P < 0.001$; $F(1, 11) = 3.51$, $P < 0.05$, respectively; for 5 mg/kg: $F(1, 11) = 22.53$, $P < 0.001$; $F(1, 8) = 12.74$, $P < 0.001$, respectively) and a decrease in the levels of HVA (main effect of treatment: for 2.5 mg/kg: $F(1, 14) = 6.79$, $P < 0.01$; $F(1, 14) = 17.85$, $P < 0.001$, respectively; for 5 mg/kg: $F(1, 11) = 26.61$, $P < 0.001$; $F(1, 9) = 23.12$, $P < 0.001$, respectively) and DOPAC (main effect of treatment: for 2.5 mg/kg: $F(1, 14) = 19.26$, $P < 0.001$; $F(1, 15) = 12.01$, $P < 0.001$, respectively; for 5 mg/kg: $F(1, 11) = 81.67$, $P < 0.001$; $F(1, 10) = 45.63$, $P < 0.001$ respectively). Values are expressed as a percentage of the mean of three measurements before drug administration. Each value is the mean \pm SEM of 4–9 animals. Basal concentrations in saline-treated rats are shown in Table 1.

result could reflect a decrease in the metabolism of the monoamines as a consequence of the ability of MDMA to inhibit MAO activity. The MDMA-induced reduction in 5-HIAA levels at the dose of 5 mg/kg was less pronounced when MDMA was given to rats kept at 30°C. A reduction in extracellular 5-HT metabolism due to an increased MAO inhibition could be responsible for this effect, but it seems

more likely that the high levels of 5-HIAA are reflecting a higher 5-HT release into the extracellular space.

One further possibility that could explain the changes induced by MDMA at high ambient temperature on monoamine release and metabolism in the NAc is enhanced penetration of MDMA through the blood–brain barrier. However, alterations in the pharmacokinetic properties of

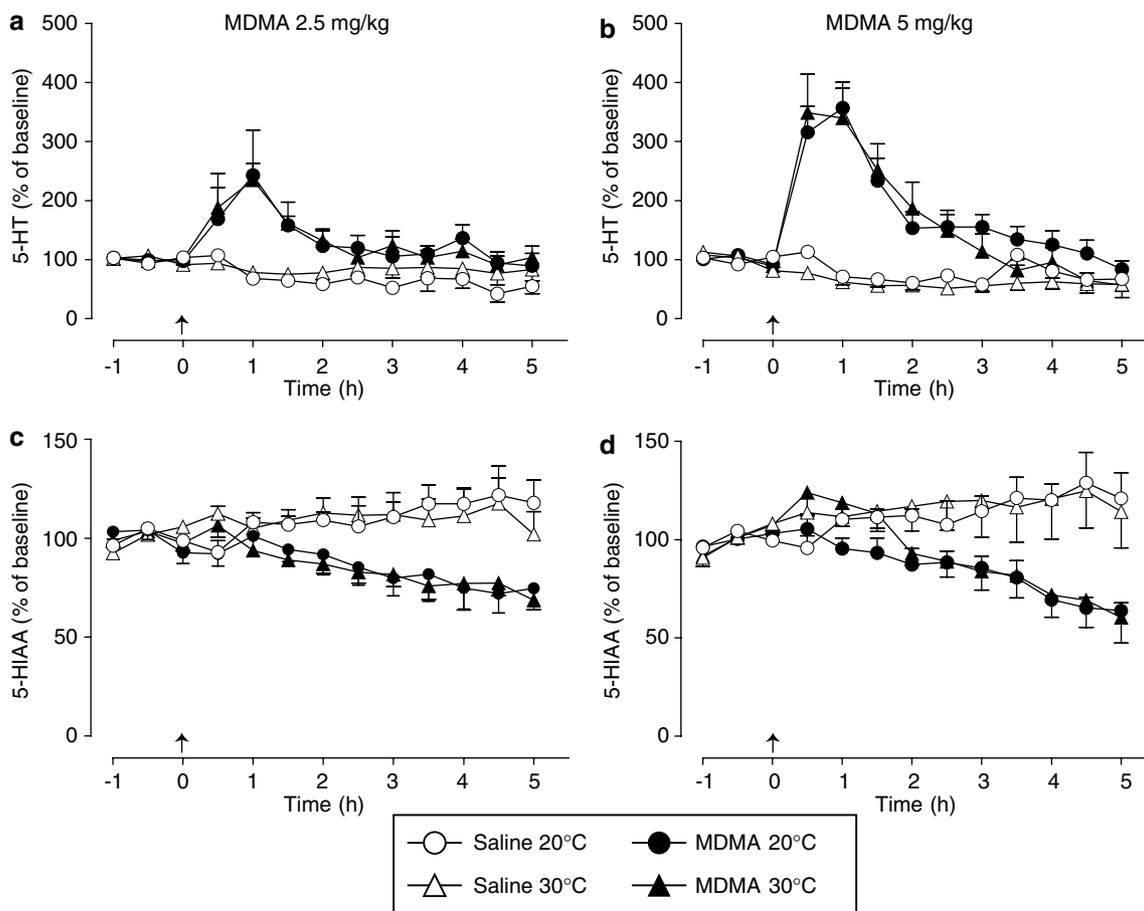


Figure 6 Changes in the extracellular levels of 5-HT (a, b) and 5-HIAA (c, d) in the striatum of rats following administration of MDMA (2.5 or 5 mg/kg, i.p.) at different ambient temperatures. Rats were maintained at a room temperature of 20 or 30°C for 2.5 h before MDMA injection and for 5 h after. At both room temperatures (20 and 30°C), MDMA produced an increase in the content of 5-HT compared with saline-treated animals (main effect of treatment: for 2.5 mg/kg: $F(1, 11) = 4.19, P < 0.05$; $F(1, 14) = 9.35, P < 0.01$, respectively; for 5 mg/kg: $F(1, 11) = 10.95, P < 0.001$; $F(1, 9) = 10.46, P < 0.001$, respectively) and a decrease in the levels of 5-HIAA (main effect of treatment: for 2.5 mg/kg: $F(1, 14) = 7.50, P < 0.01$; $F(1, 14) = 7.77, P < 0.01$, respectively; for 5 mg/kg: $F(1, 12) = 18.97, P < 0.001$; $F(1, 8) = 3.37, P < 0.05$, respectively). The effects of MDMA on 5-HT and 5-HIAA levels were similar at both ambient temperatures. Values are expressed as a percentage of the mean of three measurements before drug administration. Each value is the mean \pm SEM of 4–9 animals. Basal concentrations in saline-treated rats are shown in Table 1.

the drug at elevated temperature can be discarded since MDMA concentrations in the brain of rats at 30°C were similar to those obtained at 20°C room temperature.

Administration of MDMA (2.5 or 5 mg/kg) had little effect on rectal temperature, other than a transient hypothermia, when the drug was given to rats housed at 20°C, but it produced a sustained and marked hyperthermia when administered to rats housed at 30°C, confirming previous observations (Green *et al*, 2004). We have previously shown that the initial hyperthermia that follows MDMA injection appears to be due to dopamine release acting on D₁ receptors and inhibition of normal heat loss mechanisms that are normally occurring, such as tail vasodilation (Mechan *et al*, 2002). Since heat loss through this mechanism is presumably more difficult to achieve when the animal is in a high ambient temperature, a clear hyperthermia will occur and is unlikely to be due to greater dopamine efflux and function, rather being due to the environmental conditions. However, we and others, have also found that increased 5-HT function is necessary to enhance heat loss mechanisms when animals are in high

ambient temperature conditions. Decreasing cerebral 5-HT content by administration of the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine or a neurotoxic dose of MDMA or administration of the 5-HT antagonist methysergide results in heat-exposed rats having impaired thermoregulation (Giacchino *et al*, 1983; Green *et al*, 2004; Saadat *et al*, 2005). The fact that there is a greater 5-HT efflux in the NAc at high ambient temperature is therefore of interest, not necessarily because this region is responsible for the heat loss mechanism *per se*, but rather as a reflection that such a change does occur in specific brain regions and could be involved in initiating heat loss mechanisms.

It seems clear that the enhanced locomotor activity following MDMA is not the cause of the hyperthermic response since MDMA produced a significant increase in locomotor activity in rats housed at 20°C but rectal temperature did not increase. Furthermore, the absolute locomotor response was no greater following MDMA in rats housed at 30°C than those housed at 20°C. However, the former group also experienced a marked hyperthermic response. All these data argue against a relationship

between locomotor activity and MDMA-induced hyperthermia, a conclusion also made by Dafters (1995) following his studies on hyperkinesia and hyperthermia following MDMA administration.

Locomotor activity following administration of amphetamines is generally considered to result from increased dopaminergic activity in the mesolimbic region, while stereotypy is associated with dopamine release in the striatum (Kelly *et al*, 1975; Fibiger and Phillips, 1974), and therefore the greater increase in extracellular dopamine in the NAc may account for the greater increase in locomotor activity, above control values, in animals housed at high ambient room temperature.

What is particularly interesting with regard to the enhanced dopamine release in the NAc in rats housed at high room temperature is its possible relationship to the rewarding action of MDMA. The mesolimbic dopamine system has long been suggested to be involved in the rewarding properties of recreational drugs (Fibiger and Phillips, 1974; Wise and Bozarth, 1982), including MDMA (Beardsley *et al*, 1986). Low-dose MDMA has been shown to have rewarding properties when given at standard room temperature as it induces a positive effect in the CPP test (Marona-Lewicka *et al*, 1996). Recently, Cornish *et al* (2003) reported greater MDMA-induced social interaction behavior in rats housed at 30°C compared with 20°C and also greater self-administration of MDMA at high temperature. It is tempting to suggest that the enhanced mesolimbic dopamine release seen at high temperature is responsible for the behavioral changes seen by Cornish *et al* (2003).

In addition, evidence suggests that the 5-HT system may also be involved in mediating the rewarding and stimulant properties of MDMA either by direct or indirect mechanisms. Dopamine release in the ventral tegmental area may be facilitated by 5-HT, possibly acting indirectly through a GABAergic mechanism (Kalivas, 1993; Prisco *et al*, 1994; Trifunovic and Brodie, 1996). Furthermore, various 5-HT receptor subtypes have been shown to modulate dopamine release in the NAc and the striatum (Benloucif and Galloway, 1991; Chen *et al*, 1991; Benloucif *et al*, 1993; Parsons and Justice, 1993; Lucas *et al*, 1997). Moreover, it appears that 5-HT release and subsequent 5-HT_{1B} receptor (and possibly 5-HT_{2A} receptor) stimulation is required for MDMA-induced hyperlocomotion to develop (Callaway *et al*, 1990; Kehne *et al*, 1996; McCreary *et al*, 1999). In particular, since dopamine release in the NAc is positively controlled by 5-HT_{1B} (Boulenguez *et al*, 1996) and 5-HT_{2A} receptors (De Deurwaerdere and Spampinato, 1999), it may be that the increase in locomotor activity induced by MDMA is a consequence of an increase in dopaminergic transmission mediated by 5-HT in the NAc (McCreary *et al*, 1999).

Therefore, the facilitatory effect of MDMA on dopamine and 5-HT efflux in the NAc suggests that the rewarding properties of MDMA could be more pronounced at high ambient temperature.

Human recreational users of MDMA may experience greater psychoactive effects of MDMA when taking it in warm crowded conditions. The corollary of this, however, is that high ambient temperature produces a greater hyperthermic response to the same MDMA dose, which is associated with greater long-term neurotoxicity in rat

models (Broening *et al*, 1995; Malberg and Seiden, 1998; O'Shea *et al*, 1998; Sanchez *et al*, 2004). This may suggest that human recreational users put themselves at greater risk by ingesting the drug in hot room conditions.

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