Pergamon

Physiology & Behavior, Vol. 58, No. 5, pp. 877–882, 1995

0031-9384/95 $9.50 + .00

0031-9384(95)00136-0

Hyperthermia Following MDMA Administration in Rats: Effects of Ambient Temperature, Water Consumption, and Chronic Dosing

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Received 4 December 1995

DAFTERS, R. I. *Hyperthermia following MDMA administration in rats: Effects of ambient temperature, water consumption, and chronic dosing.* PHYSIOL BEHAV **58**(5) 877–882, 1995.— In two experiments it was found that the hyperthermia which follows MDMA ("Ecstasy") results from an interaction of direct pharmacological effect of the drug and the prevailing environmental conditions in which it is administered. In Experiment 1, rats given fixed doses of either 2.5, 5.0 or 7.5 mg/kg MDMA or saline were injected on different days at ambient temperatures (T_a's) of 11, 24, and 30°C. At each T_a drinking water was freely available following dosing on one session and temporarily unavailable on a second. The hyperthermic and hyperkinetic responses were monitored using remote biotelemetry. Experiment 2 used a between-subject design in which each group of rats received a standard 7.5 mg/kg dose of MDMA administered at only one of the three levels of T_a(24°C) and at only one level of the the water-availability factor. Dosing in some groups was continued for a further 13 days to test for tolerance or sensitization effects. Ambient temperature significantly affected the magnitude of the hyperthermia but not the hyperkinesis. Water deprivation during the drugged period significantly augmented the hyperthermia, but only in the high T_a (30°C.) condition. Chronic dosing produced sensitization of both hyperthermic and hyperkinetic responses. The findings indicate that ambient temperature, water consumption and frequency of drug use affect the hyperthermia which follows MDMA administration.

Hyperthermia Ecstasy Rat Ambient temperature Chronic dosing

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA or "Ecstasy") and other amphetamine related compounds can produce large, and occasionally lethal, elevations in core temperature in the rat and mouse (1–3,12,16). It has been suggested that this hyperthermic effect may be a causal factor in a number of deaths in human recreational MDMA users who have presented for emergency treatment with symptoms including hyperthermia, convulsions, rhabdomyolosis and acute renal failure (10,13,19,22). The toxic syndrome does not appear to be related to overdose as in each case only a few tablets or capsules had been taken, although dosage and purity in such situations is difficult to ascertain. However, in each case, the drug had been consumed in the hot, crowded atmosphere of a dance club or "rave," which suggests that it may reflect a lethal interaction of drug-induced hyperthermia and environmental factors such as high ambient temperature and reduced fluid intake leading to dehydration. This clinically important link between drug and environmental effects has not been fully investigated to date. There is evidence from rat and mice studies that MDMA's hyperthermic response is affected by ambient temperature (1,12,16) but these studies have typically employed very high and sometimes massed doses (e.g., a single 30 mg/kg dose or multiple 20 mg/kg doses at 2-h intervals) which produce high levels of morbidity and neurotoxicity (16,17). In the experiments reported here, a much lower dose range (2.5–7.5 mg/kg) was used which is closer to the estimated typical human recreational dose of 1–4 mg/kg (22) but still capable of inducing significant thermic and kinetic changes. Also, with the exception of the Gordon et al. study (12), which used a biotelemetry procedure similar to our own, there have been few previous attempts to reduce or control for the confounding effects of hyperthermia induced by the restraint and rectal temperature measurement techniques typically employed. In no previous study known to the present author has the effect of availability and consumption of water on MDMA's hyperthermic response been investigated despite the common clinical belief that dehydration is an important factor in the human toxic "heatstroke" response (13,19,22).

In the present experiments, a biotelemetric recording technique was used to continuously monitor the thermic and kinetic responses of rats to a range of doses of MDMA administered at different ambient temperatures and with either restricted or unrestricted access to drinking water. The advantages of biotelemetry

in psychopharmacological research have been discussed previously (8,9) but include: the ability to monitor physiological responses from large numbers of subjects simultaneously (up to 48 rats in our laboratory), to collect data continuously and for an extended period, and to avoid the confounding effects of hyperthermia induced by stressful handling, restraint and rectal temperature measurement. In addition, it facilitates the timing of drug administration coincident with a particular phase of the natural circadian temperature rhythm which can affect the experimental outcome.

The primary aim of the first experiment was to establish whether variations in ambient temperature and the availability of drinking water would have effects on the MDMA-induced hyperthermia that could implicate these factors in the toxic hyperthermia syndrome experienced by some human drug abusers. An additional feature of interest was the interaction of MDMA-induced hyperthermia and hyperkinesis. Simultaneous recording of body temperature and activity levels which biotelemetry allows may rule out certain hypotheses about the drug effect. For example, it is possible that hyperthermia is a secondary response resulting from drug-induced hyperactivity. However, substantial differences in the response profiles of these two measures would be incompatible with this view and would favour the hypothesis that these responses reflect central but independent 5-hydroxytryptaminergic and/or dopaminergic mechanisms (21,23). To examine these issues, rats were administered MDMA or saline injections in "standard," "cool," and "warm" environments and with and without access to drinking water, while their body temperatures and activity levels were continuously monitored. The second experiment sought to strengthen and extend the findings of the first experiment by employing a between-subject design which could rule out an alternative explanation of the data in terms of a sensitization effect. In addition, it examined the nature of the changes to the hyperthermic and hyperkinetic responses to MDMA which occur with chronic dosing.

METHOD

The subjects in the experiments were male Wistar rats (B & K Supplies Ltd. Hull, England). They were housed individually in plastic cages (40 × 25 × 20 cm) with free access to food and water, maintained on a standard 12:12 h light:dark cycle (light on at 07:00) and weighed 250–300 g at the start of the experiment. The temperature of the holding rooms was normally maintained at 24°C. but could be changed when appropriate to any value (± 1°C.) within the range 10–30°C. 3,4-methylenedioxymethamphetamine (MDMA) purchased from Sigma Chemical Co. Poole, Dorset, England was dissolved in 0.9% physiological

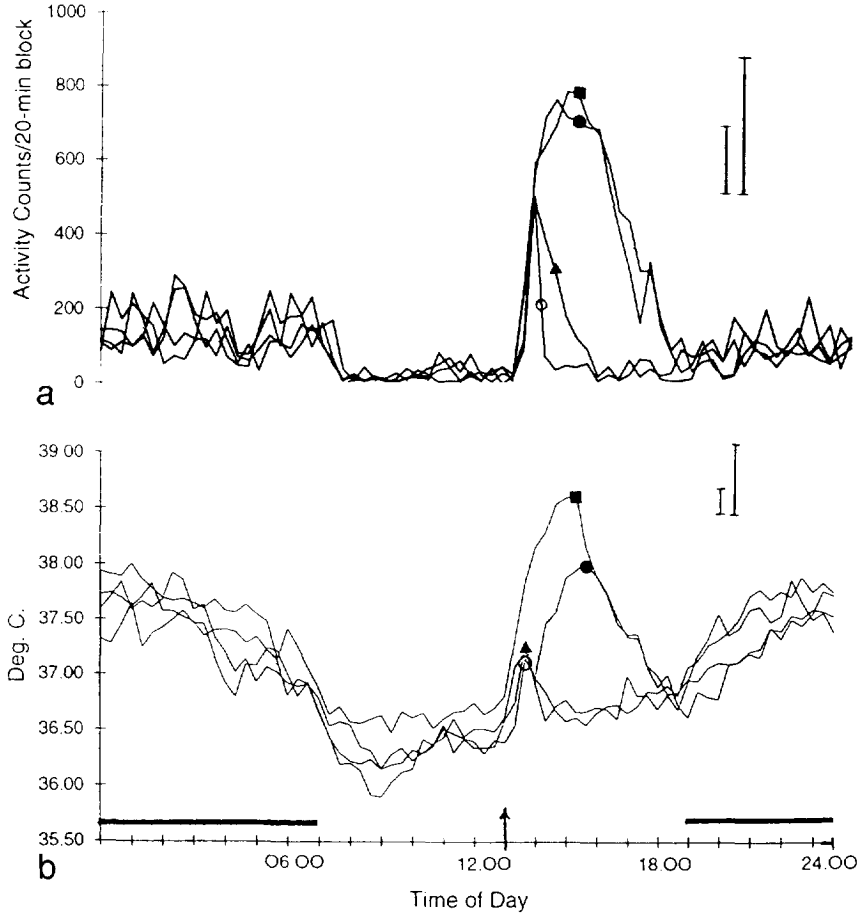

FIG. 1. (a) Mean activity counts and (b) body temperatures recorded on the first of 6 drug test sessions in Experiment 1. Temperatures and activity counts are plotted at 20-min intervals over the period 00.00–23.40 h. The arrow marks the time of drug or saline injection which was administered during the light phase (unfilled bar) of the 12:12 h light:dark cycle. Ambient temperature was 24deg C. and drinking water was freely available. ■, 7.5 mg/kg; ●, 5.0 mg/kg; ▲, 2.5 mg/kg; , saline (N = 6, in each case). SEM's have been omitted from the graphs for clarity but maximum and minimum values are indicated by the vertical lines.

saline and injected subcutaneously in the neck. Saline injections (1.0 ml/kg) were administered by the same route. Temperature and activity were monitored continuously by means of a computer-controlled biotelemetry system (Dataquest III, Mini-Mitter Co Inc, Oregon, USA). This system has been described fully elsewhere (6) but briefly, wax-coated transmitters (Mini-Mitter model VM-FH), implanted under anaesthesia (Hypnorm/Hypnovel) in the rats abdominal cavity, emit radio-frequency pulses (range 300–600 Hz) whose frequency varies linearly with temperature in the physiological range. Receivers located beneath each rat cage transmit the frequency information to a computer which records and analyses the data. The receivers also detect moment-to-moment changes in position and orientation of the transmitters caused by body movements which are translated into activity counts. In the present experiments the system was configured for 24 h per day monitoring with recording of temperature and activity counts every 20-min.

Fourteen days prior to drug treatments, transmitters were surgically implanted and temperature and activity levels were subsequently monitored at 20-min intervals, 24 h per day to establish normal circadian rhythms. During this familiarisation phase the rats were routinely weighed, handled for a few minutes and given a single saline injection each day. On the 15th postoperative day of Experiment 1, the rats were arbitrarily assigned to one of four groups ($N = 6$) which received a single injection of Saline or 2.5, 5.0, or 7.5 mg/kg MDMA on each of 6 consecutive test days. The rats were injected at 13:00 h, midway through the low (light) phase of their activity and temperature cycles. The tests were given in the order 24 +, 24-, 11 +, 11-, 30 +, 30- where in each case the number refers to the prevailing ambient temperature and the sign to the availability of water condition. Where appropriate, the adjustment of the thermostatic room heaters and ventilators responsible for changing ambient temperature commenced 15 min prior to injection to ensure that the ambient temperature had stabilised at the correct level when the drug was administered. Recalibration to the standard 24°C. temperature was carried out 3 h after the injection. On the water deprivation days, the water bottles were removed from the rat cages 2 h prior to the injection, and were replaced 3 h after the injection.

Because Experiment 1 used a within-subject design with each rat tested under each treatment condition it is possible that the effects of manipulating ambient temperature and water-availability conditions might be confounded with an unrelated change in responsiveness to repeated dosing. In fact, there is evidence that both sensitization (increasing effectiveness with repeated doses) and tolerance (decreasing effectiveness with repeated doses) can develop to chronic MDMA administration (4,21). To rule out this potentially confounding factor, therefore, Experiment 2 employed a between-subjects design in which each rat was injected with a dose of 7.5 mg/kg MDMA and tested at only one level of ambient temperature and one level of water-availability. The 7.5 mg/kg dose was chosen because this dose was found to be the most responsive to the environmental manipulations in Experiment 1. In addition, since sensitization and/or tolerance to MDMA is potentially a clinically important factor in habitual drug use it was studied directly in this experiment by continuing to administer MDMA for a further thirteen sessions while monitoring thermic and kinetic responses. Finally, water consumption, which was not monitored in the first experiment, was directly measured in Experiment 2.

Thus, in Experiment 2, following the 14-day handling and injection familiarization phase, the rats were arbitrarily assigned to six groups ($N = 8$) each of which received a single injection of 7.5 mg/kg of MDMA. Groups 11 +, 24 + and 30 + were tested at the indicated ambient temperatures and with water freely available; groups 11-, 24- and 30- were tested with water bottles removed. Details of diurnal time of injections and water-bottle removal were as described for Experiment 1. Drinking water (at 24°C) was made available in 200 ml glass bottles fitted with leak-free metal spouts, and water consumption in the water-available groups was measured by weighing the bottles at the time of injection and at 3 h postinjection. Ad libitum food and water was provided on the subsequent 13 days when all rats from the 3 water-available groups continued to receive a single daily 7.5 mg/kg dose, administered at the same point in the diurnal rhythm (i.e., at 13:00 h).

In both experiments, statistical analysis was carried out on the peak thermic and kinetic response data (see Fig. 2 legend). In each case an initial multivariate analysis of variance with Dose (Experiment 1 only), Ambient temperature and Water availability as factors was followed by post hoc Scheffé tests of individual group comparisons.

RESULTS

Figure 1, which plots mean body temperature and activity levels on the first of the test sessions in Experiment 1, with an

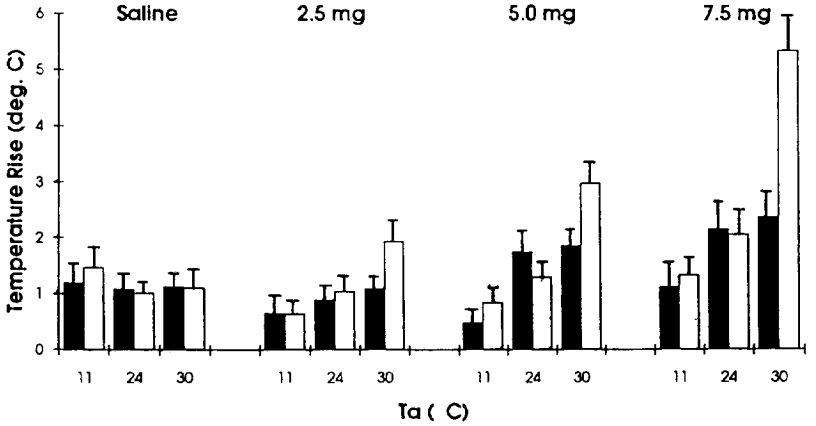

FIG. 2. Mean peak hyperthermic response on 6 consecutive test sessions in Experiment 1. Column heights are the group means ($N = 6$) of the differences between the maximum body temperature recorded in the 5-h postinjection period and the last preinjection temperature of individual rats (+SEM). Numbers on X-axis refer to the ambient temperature (T_a) in °C at which the injections were administered. Filled and unfilled columns refer to the drinking water available and nonavailable conditions, respectively.

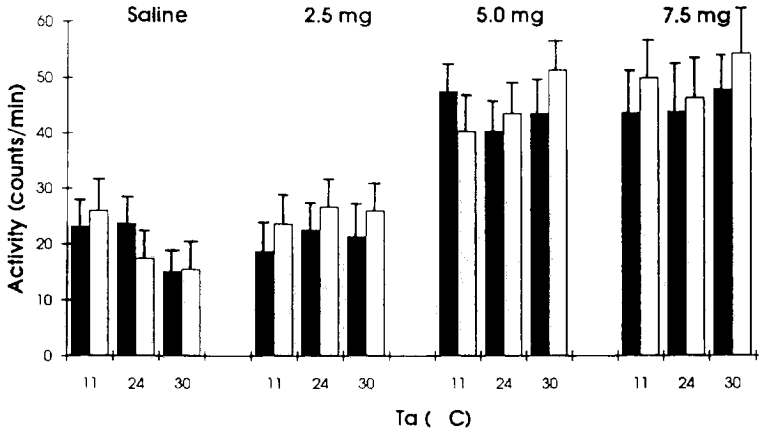

FIG. 3. Mean peak hyperkinetic responses on the 6 test sessions in Experiment 1. Column heights are post–pre injection difference scores of activity counts calculated as in previous figure (+ SEM). Other details are as in the legend to Fig. 2.

ambient temperature of 24°C. and drinking water available, shows the form and time-course of the hyperthermic and hyperkinetic responses to MDMA and their relationship to the diurnal temperature rhythm.

Note that the magnitude of the diurnal variation in body temperature is commensurate with that induced by a 5 mg/kg dose of MDMA. It has previously been shown that drug-induced hyperthermia may be significantly masked if the drug is administered during the high or ascending limb of the circadian oscillation (8) which is why the injections were given during the low phase of the temperature and activity rhythms.

Peak thermic and kinetic responses on the 6 test days in Experiment 1 are shown in Figs. 2 and 3, respectively.

The results clearly indicate profound effects of the environmental factors on the drug-induced hyperthermic response. The main effect of Dose was highly significant $F(3, 20) = 15.4$, $P < 0.001$, confirming the positive relationship between dose and hyperthermia suggested in the figure. There was also a highly significant main effect of ambient temperature $F(2, 40) = 33.2$, $P < 0.001$. The interaction of Dose and ambient temperature factors was also significant $F(6, 40) = 7.8$, $P < 0.001$, and post hoc tests showed that the high room temperature of 30°C. significantly increased the hyperthermic effect of MDMA, while the low room temperature of 11°C. significantly decreased the hyperthermic effect, relative to the standard 24°C. condition ($p < 0.05$ in each case). Access to drinking water was also a significant factor $F(1, 20) = 21.1$, $P < 0.001$, as was the interaction of Dose and Water-Access $F(3, 20) = 4.6$, $P < 0.05$. Posthoc tests of the interactions showed that removal of the water bottles caused a dramatic increase in body temperature response only in the 7.5

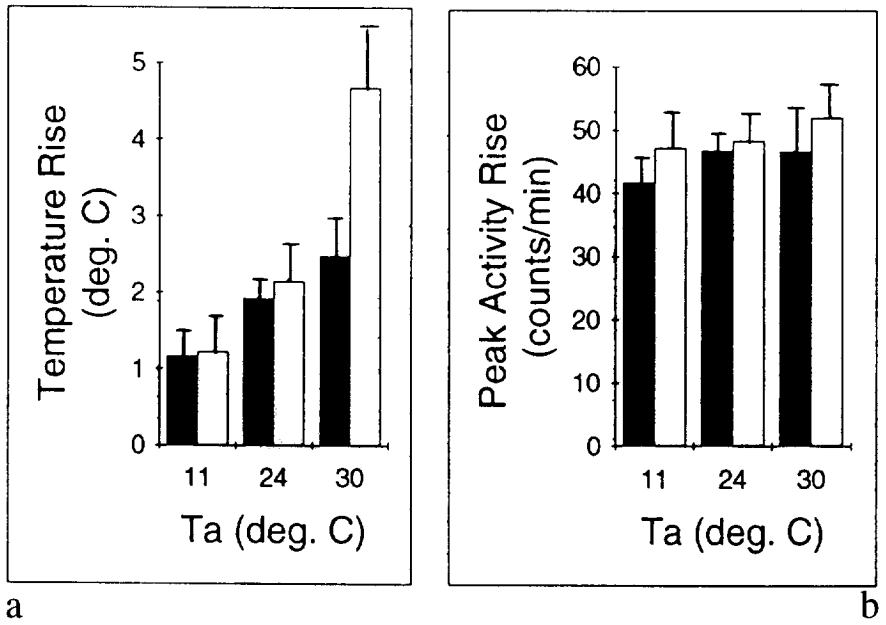

FIG. 4. Experiment 2. (a) Mean peak hyperthermic (b) and hyperkinetic responses (+ SE mean) in groups receiving a single dose of 7.5 mg/kg MDMA at one of three ambient temperatures. Responses are calculated as described previously (see Fig. 2 legend). Filled and unfilled columns refer to the drinking water available and nonavailable conditions, respectively. $N = 8$, in each case.

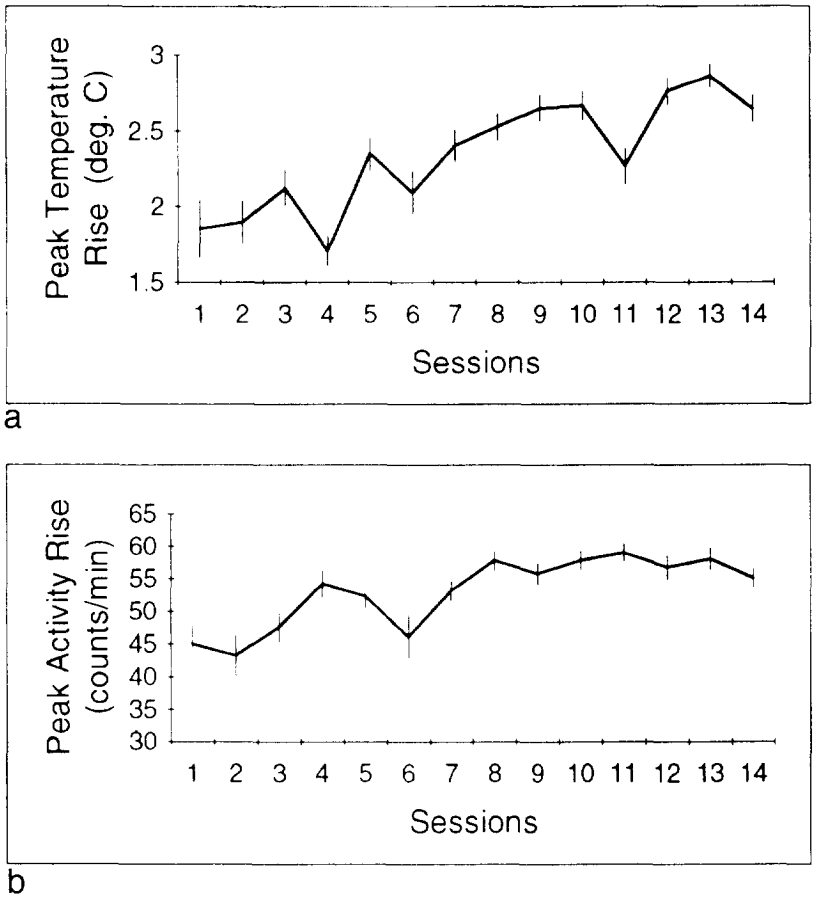

FIG. 5. Experiment 2. (a) Mean peak hyperthermic and (b) hyperkinetic responses to a single daily dose (7.5 mg/kg MDMA) administered on 14 consecutive days ($N = 24$). Response measures calculated as previously described (see Fig. 2 legend). Ambient temperature was 24°C and water was freely available.

mg/kg dose group and in the 30°C. environment ($p < 0.05$), but had no significant effect at the lower doses and ambient temperatures. The time-course of hyperactivity was similar to that of hyperthermia but there were marked differences in the overall pattern of responding (see Fig. 3). Again, activity increases were positively dose-related $F(3, 20) = 87.7$, $P < 0.001$ but were not affected by either ambient temperature or water deprivation condition ($p > 0.05$ in each case).

Figure 4 shows the peak hyperthermic and hyperkinetic response to the 7.5 mg/kg test dose of MDMA administered at the different ambient temperatures and water-availability levels in Experiment 2. The effects of ambient temperature and water-availability are similar to those observed in the equivalent dose group (7.5 mg/kg) of the first experiment. In the case of the hyperthermic response, analysis of variance revealed significant main effects of ambient temperature ($F(2, 42) = 40.75$, $p < .001$) and water-availability ($F(1, 42) = 14.2$, $p < .001$) and their interaction ($F(2, 42) = 9.75$, $p < .001$), and posthoc Scheffé tests showed that the hyperthermia induced at 11°C was significantly attenuated ($p < .025$) and that at 30°C was significantly augmented ($p < .001$) relative to the standard 24°C condition. As before, the increased hyperthermia due to temporary water deprivation was significant only in the 30° ambient temperature condition ($p < .001$). Analysis of the hyperkinetic response showed that, as before, kinesis was not significantly affected by either T_a ($F(2, 42) = .417$, $p = .66$) or water consumption ($F(1, 42) = .844$, $p = .36$). As expected the increasing importance of water availability with increasing ambient temperature was reflected in the quantity of water consumed in the different water-available groups. Mean water intake (gms) during the 3-h measurement period was 2.47 (SEM 0.19), 2.79 (SEM 0.28), and 4.88 (SEM 0.36) in groups 11 +, 24 + and 30 +, respectively. Consumption in group 30 + was significantly different from the other two groups combined [P(two-tailed t) < .001] which did not differ from each other ($P > .15$).

Figure 5 shows the hyperthermic and hyperkinetic responses over the course of the 14 sessions of chronic administration in Experiment 2. The augmentation of both responses across sessions is indicative of a sensitization effect. A paired-sample t-test comparing the mean of sessions 1–7 with that of sessions 8–14 was highly significant for both measures [P(two tailed) < .001, in each case]. That this trend was not an artifact arising from a drift in the baseline body temperature and activity levels over the sessions was shown by comparing the mean predrug baseline scores of sessions 1–7 with those of sessions 8–14. These did not differ [P(two-tailed) > .05].

DISCUSSION

Taken together the results of the two experiments reported here clearly implicate the importance of factors other than drug dose in determining MDMA's hyperthermic response. Both ex-

periments have confirmed previous findings of the effects of ambient temperature levels on the magnitude of hyperthermia (1,12,16). The observed differences in patterns of thermic and kinetic responses makes it very unlikely that the hyperthermia is a direct result of increased activity levels. The experiments also showed that the availability of drinking water affects the hyperthermic response and in a manner which interacts with ambient temperature and drug dose. This was apparent from the highly significant augmentation of hyperthermia under water deprivation conditions which occurred in the high dose/high ambient temperature groups in both experiments. The results of chronic dosing with MDMA show that there is an augmentation of both hyperthermia and hyperkinesis with repeated exposure to the drug. However, this sensitization is not responsible for the pattern of increasing hyperthermia and hyperkinesis across the series of six test sessions of Experiment 1, since an identical pattern of results was observed in the test phase of Experiment 2 where each subject was exposed to only a single dose. Despite the important clinical implications there appears to have been little systematic investigation of the hyperthermic response to chronic as opposed to acute MDMA administration, but hyperkinetic effects have variously been reported to show sensitization (21), tolerance (4), or no change (11) with repeated administration. In addition, it has been reported that conditioned locomotor responses can occur to distinctive environmental stimuli which have been repeatedly associated with MDMA administration (11), a phenomenon which has been postulated to play an important part in the development of tolerance and sensitization to drugs of abuse (7,15,20). Clearly, the development of tolerance and/or sensitization to MDMA's physiological and behavioural effects, including the role of drug conditioning, is something which requires further investigation.It is likely that the thermic and kinetic effects of MDMA, which have a similar response profile (see Fig. 1), both reflect the drug's activating effect on serotonergic pathways. For example, MDMA causes release of serotonin from rat brain slices in vitro (14) and elicits a pattern of behavioural responses, including hyperkinesis, which is known as the serotonin syndrome in vivo (21). Drugs which act to prevent the release of 5-HT block both the hyperkinetic effect (5) and hyperthermic effect (18) of MDMA. Understanding the mechanism of MDMA's hyperthermic response and the environmental and other factors which influence it is important because of recent evidence linking the drug's hyperthermic response to its neurotoxicity. For example, recent studies have shown that drug and physical treatments which reduce MDMA's hyperthermic response also reduce the level of drug-induced neurotoxicity in the striatum of the mouse (16) and in the striatum, hippocampus and cortex of the rat (18).

REFERENCES

1. Bowyer, J. F.; Tank, A. W.; Newport, G. D.; Slikker, W., Jr.; Ali, S. F.; Holson, R. R. The influence of environmental temperature on the transient effects of metamphetamine on dopamine levels and dopamine release in rat striatum. J. Pharmacol. Exp. Ther. 260:817–824; 1992.
2. Bowyer, J. F.; Gough, B.; Slikker, W., Jr.; Lipe, G. W.; Newport, G. D.; Holson R. R. Effects of a cold environment or age on methamphetamine-induced dopamine release in the caudate putamen of female rats. Pharmacol. Biochem. Behav. 44:87–98; 1993.
3. Bowyer, J. F.; Davies, D. L.; Schmued, L, Broening, H. W.; Newport, G. D.; Slikker, W., Jr.; Holson, R. R. Further studies of the role of hyperthermia in metamphetamine neurotoxicity. J. Pharmacol. Exp. Ther. 268:1571–1580; 1994.
4. Callaway, C. W.; Geyer, M. A. Tolerance and cross-tolerance to the activating effects of 3,4-methylenedioxymethamphetamine and a 5-hydroxytryptamine$_{1B}$ agonist. J. Pharmacol. Exp. Ther. 263:318–326; 1992.
5. Calloway, C. W.; Wing, L.; Geyer, M. A. Serotonin release contributes to the stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J. Pharmacol. Exp. Ther. 254:456–464; 1990.
6. Cunningham, C. L.; Peris, J. A microcomputer system for temperature biotelemetry. Behav. Res. Meth. Instrument. 6:598–603; 1983.
7. Dafters, R. I.; Bach, L. Absence of environment-specificity in morphine tolerance acquired in nondistinctive environments: Habituation or stimulus overshadowing? Psychopharmacology 87:101–106.
8. Dafters, R. I.; Taggart, P. Interaction of circadian rhythm and opiate-induced thermic and kinetic responses:a biotelemetric investigation. Life. Sci. 47:2155–2161; 1990.
9. Dafters, R. I.; Taggart, P. A biotelemetric investigation of morphine's thermic and kinetic effects. Psychopharmacology 106:195–201; 1992.
10. Dowling, G. P.; Mcdonough, E. T.; Bost, R. O. "Eve" and "ecstasy": A report of five deaths associated with the use of MDEA and MDMA. J. Am. Med. Assoc. 257:1615 – 1617; 1987.
11. Gold, L. H.; Koob, G. F. MDMA produces stimulant-like conditioned locomotor activity. Psychopharmacology 99:352–356; 1989.
12. Gordon, C. J.; Watkinson, W. P.; O'callaghan, J. P.; Miller, D. B. Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol. Biochem. Behav. 38:339–344; 1991.
13. Henry, J. A.; Jeffreys K. J.; Dawling, S. Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). Lancet 340:384–387; 1992.
14. Johnson, M. P.; Hoffman, A. J.; Nichols, D. E. Effects of the enantiomers of MDA, MDMA and related analogues on [H3]dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132:269–276; 1986.
15. Mansfield, J. G.; Cunningham, C. L. Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. J. Comp. Physiol. Psychol. 94:962–969.
16. Miller, D. B.; O'Callaghan, J. P. Environment-, drug-, and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. J. Pharmacol. Exp. Ther. 270:752–760; 1994.
17. O'Callaghan, J. P.; Miller, B. B. Neurotoxity profiles of substituted amphetamines in the C57BL/6J mouse. J. Pharmacol. Exp. Ther. 270:741–751; 1994.
18. Schmidt, C. J.; Black, C. K.; Abbate, G. M.; Taylor, V. L. Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT$_2$ receptors. Brain Res. 529:85–90; 1990.
19. Screaton, G. R.; Cairns, H. S.; Sarner, M.; Singer, M.; Thrasher, A.; Cohen, S.L. Hyperpyrexia and rhabdomyolosis after MDMA ("ecstasy") abuse. Lancet 339:677–678; 1992.
20. Siegel, S. Tolerance to the hyperthermic effect of morphine in the rat is a learned response. J. Comp. Physiol. Psychol. 92:1137–1149.
21. Spanos, L. J.; Yamamoto, B. K. Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. Pharmacol. Biochem. Behav. 32:835–840; 1989.
22. Tehan, B.; Hardern, R.; Bodenham, A. Hyperthermia associated with 3,4- methylenedioxyethamphetamine ("Eve"). Anaesthesia. 48:507–510; 1993.
23. Yamamoto, B. K.; Spanos, L. J. The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. Eur. J. Pharmacol. 148:195–203; 1988.