Pergamon

Physiology & Behavior, Vol. 56, No. 1, pp. 73–79, 1994

0031-9384/94 $6.00 + .00

0031-9384(94)E0043-4

Metabolic and Thermoregulatory Responses of the Rat Maintained in Acrylic or Wire-Screen Cages: Implications for Pharmacological Studies[1]

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Received 12 October 1993

GORDON, C. J. AND L. FOGELSON. *Metabolic and thermoregulatory responses of the rat maintained in acrylic or wire-screen cages: Implications for pharmacological studies.* PHYSIOL BEHAV **56**(1) 73–79, 1994.—Because of differences in thermal conductivity, it is likely that a rodent's thermoregulatory requirements and their response to drugs and other stimuli will vary in metal and acrylic cages. To address these issues, thermoregulatory responses were measured in rats housed in an environmental chamber with a floor made of either solid metal (aluminum) or acrylic materials (Plexiglas). Metabolic rate (M), evaporative water loss (E), thermal conductance (C), and tail skin (T_{sk}) and core temperature (T_c) were measured at ambient temperatures (T_a) of 10, 20, 28, 30, 32, and 34°C. These thermoregulatory variables were essentially unaffected by floor type at T_as of 20 and 28°C. The acrylic floor showed greater increases in M, E, T_c, and T_{sk}, but a smaller elevation in C as T_a increased from 28 to 34°C. At a T_a of 10°C, rats on the acrylic floor had a smaller M compared to that measured on the metal floor. Rats were then injected with saline or 30 mg/kg (SC) of 3,4-methylenedioxymethamphetamine (MDMA) and placed in an acrylic cage with wood chip bedding or a wire-screen cage at a T_a of 20°C. The MDMA caused T_c to increase > 2.0°C in rats in the acrylic cage but had no effect on T_c of rats in the wire-screen cage. The marked effect of cage type on basal thermoregulatory processes and thermogenic response to MDMA should be useful in the design and interpretation of many pharmacological studies.

Metabolic rate Evaporative water loss Core temperature Skin temperature Ambient temperature
Thermal conductance 3,4-Methylenedioxymethamphetamine

THE type of cage used to house and study laboratory rodents in pharmacological and toxicological studies is usually dictated by a variety of requirements, such as species, sanitation, breeding requirements, and other experimental factors. One finds that unrestrained rodents are housed and studied in one of two cage types:

1. metal—these cages consist of walls and floor made with wire-screen or related materials, have no bedding material, and provide excellent air circulation
2. acrylic (i.e., plastic) shoe-box type—these cages consist of solid walls and floor made of a synthetic polymers (e.g., polycarbonate, Plexiglas) with low thermal conductivity and usually have a layer of bedding material to absorb urine and odors.

Although acrylic cages are preferred for many rodent studies, metal cages are critical in studies where thorough ventilation is required, such as for gaseous or volatile chemical agent exposure [for review, see (3)].

Most researchers studying metabolism and other thermoregulatory patterns have been aware that the type of cage can be a crucial dependent parameter. Metal cages conduct heat more efficiently, have a better convective heat exchange, and the internal cage temperature is close to that of environmental temperature. Acrylic cages, especially those with filter tops, have poorer air circulation and heat exchange. The air inside these cages can be warmer than the surrounding environment and the bedding material affects thermoregulation differently than that of animals housed in wire-screen cages (3,18). For example, the presence of wood chips or other nesting material can be shown to reduce the metabolic energy demands of rodents subjected to cold environments (10). The type of cage is considered to be an important variable when studying the function of brown adipose tissue (12). Maintaining individual rats on a wire-screen cage at an ambient temperature of 21°C is considered to represent a mild cold stress because metabolic rate is 25% higher than that of animals kept at a thermoneutral ambient temperature (T_a) (2). Clearly, at T_as approximating standard room temperature (e.g., ~22°C), the

[1] This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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metabolic requirements of the rat and other rodents are reduced if housed in an acrylic cage with bedding material.

Given that the thermoregulatory responses of rodents will vary depending on the type of cage used for housing, then it is also likely that an animal's metabolic response to pharmacological and other chemical agents will be markedly affected by cage type. Exposure to neuropharmacological and neurotoxic chemicals will often lead to marked changes in metabolic rate and body temperature in laboratory rodents (9,14). Because the type of cage used for housing can impact on the metabolic rate and body temperature of the rodent, it is thus likely that a species' response to a chemical agent would be affected by the type of cage. These factors are obviously crucial to both basic and applied studies of pharmacology and toxicology in laboratory rodents and should be explored in more detail. The major variable in these cage designs centers on the contact between the animal and the floor of the cage. Conductive heat exchange is likely to be much lower when the animal is placed on a wood chip bedding/acrylic floor compared a solid metal or wire-screen floor.

Accurate measurements of oxygen consumption and evaporative water loss from animals maintained on wood chip bedding material are difficult using conventional methods. The wood chip bedding interferes with gas equilibration and absorbs moisture. Therefore, to estimate the thermoregulatory responses in these cage types, an alternative approach is to measure these parameters on solid surfaces of either metal or acrylic materials that simulate the thermal environments of wire-screen and wood chip bedding cages, respectively. The present study had two major goals:

1. compare the thermoregulatory patterns of the rat when placed on an acrylic surface with relatively low thermal conductivity to that when placed on a metal surface with a high thermal conductivity, and
2. assess how cage type affects the rodent's thermoregulatory response to the psychoactive drug, 3,4-methylenedioxymethamphetamine (MDMA), which has been shown to have marked effects on thermoregulation in the rat (8,16).

METHOD

Animals used in this study were male rats of the Long–Evans (LE) strain obtained at 60 days of age (Charles River Laboratories). The rats were caged in groups of two in standard polycarbonate cages (length, 47 cm; width, 24 cm; depth, 20 cm) lined with wood shavings. Housing conditions were: 22°C, 50% relative humidity, and a 12:12 L:D photoperiod with lights on at 0600 h. The rats were maintained under these conditions for several weeks before being tested.

Apparatus

An environmental chamber made of stainless steel walls and a solid aluminum lid, used in earlier studies from this laboratory (8), was modified to accommodate a floor made of either Plexiglas or aluminum (Fig. 1). The floor was made of two rectangular sections (12.7 mm thick; 327 mm long; 121 mm wide). They were held together by metal brackets with a 3.2-mm gap running along the center between the two plates. The plates were angled inward to allow urine to fall through the gap and into a tray filled with mineral oil. The mineral oil prevented moisture from interfering with the measurement of evaporative water loss. Grooves (3.2 mm wide) were cut into the floor at 12.7-mm intervals to channel urine away from the rat and help keep its fur dry.

Metabolic rate and evaporative water loss were measured using methods similar to those of previous studies (8). The chamber

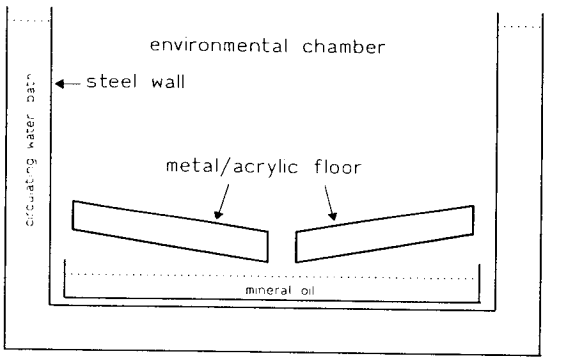

FIG. 1. Cross-sectional view of the environmental chamber and specially designed floor made of acrylic (Plexiglas) or metal (aluminum). Not drawn to scale (see the Method section for additional details).

with the metal and Plexiglas floor allowed for the measurement of these parameters that otherwise would be difficult in standard acrylic cages with wood chip bedding. We have found the wood chip bedding has marked effects on the chamber's moisture content and gas exchange characteristics, making determinations of metabolic rate and evaporative water loss difficult. Briefly, dry air was pushed into the chamber at a flow rate of 3.0 l/min (STP). A fraction of the air stream from the chamber was withdrawn into a dew point hygrometer to measure the rat's evaporative water loss (EWL) and relative humidity of the chamber air. Another fraction was pulled into an oxygen analyzer to measure the change in percent oxygen. The change in oxygen consumption was used to calculate metabolic rate in dimensions of ml O_2/(min $kg^{0.75}$).

The environmental system was also fitted with a Doppler-type system to measure motor activity (8). Although motor activity was monitored during all experiments, it should be noted that the system's performance was affected by the type of cage. The Doppler system generated a larger signal for a given movement on the aluminum floor compared to the Plexiglas floor. It was decided not to use the motor activity data for any quantitative analysis. However, the Doppler output was used in the MDMA studies (discussed below) to provide a qualitative measure of MDMA's effect on motor activity.

Whole-body, dry thermal conductance (C) in dimensions of ml O_2/(min kg °C) was calculated based on the measurement of metabolic rate (M), the oxygen consumption attributed to evaporative water loss (E), colonic temperature (T_c), and T_a (9,15):

$$C = (M - E)/(T_c - T_a). \quad [1]$$

The calculation of E was made assuming a latent heat of vaporization of 2.404 J per mg H_2O evaporated and a respiratory quotient of 0.81 (i.e., 20.1 J of heat per ml O_2).

Procedure

The T_a of the environmental chamber was regulated at 10, 20, 28, 30, 32, and 34°C. Individual rats (mean body weight = 441 ± 5.6 g) were placed in the chamber for 60 min while dew point temperature and percent oxygen data were printed at 2-min intervals on a digital-analog recorder (Dianachart). Colonic temperature was measured at the end of each experiment by inserting a thermocouple probe (Physitemp, RET-2) 5–6 cm beyond the anal sphincter. Tail skin temperature was also measured at the same time using a noncontact, infrared thermometer (Biotherm, model C600-M). This was positioned approximately 2 cm from

the base of the tail and gave an instantaneous reading of tail skin temperature. After testing, the rat was returned to its home cage and was not used again for at least 24 h. All rats were given one habituation session in one of the cage types prior to testing and were studied at a single T_a in both cage types before being tested at another T_a.

Drug Experiment

To evaluate the potential effect of cage type on thermoregulatory response to pharmacological intervention, a series of experiments was designed using the chemical agent 3,4-methylenedioxymethamphetamine (MDMA) as a prototypical compound. The MDMA was selected for several reasons: (i) it has been shown to induce marked hyperthermia in the rat, an effect that is dependent on T_a (8,16); and (ii) it is purported to be a neurotoxicant and has been shown to cause long-term changes to serotonergic pathways (4,13). It is thought that many of MDMA's neurotoxic effects are related to its effects on body and brain temperature (11). Two experiments were performed with MDMA.

1. New sets of naive LE rats were used for the MDMA studies. In the first study (mean body weight = 388 ± 5.5 g) an environmental chamber (described above) was modified to hold either an acrylic cage with wood chips or a metal wire-screen cage. The inner dimensions of both cages were nearly identical (17 cm wide by 32 cm long by 19 cm high). The wire screen cage was made from stainless steel wire screen (8 cm mesh) and the edges of the cage were reinforced with metal rods. This cage was positioned over a pan containing mineral oil to collect urine and fecal material. The acrylic cage was modelled to conform to the dimensions of typical cages used in rodent husbandry and to fit inside the environmental chamber. It was made of 0.6-cm thick Plexiglas and had an open top. To facilitate the circulation of air through the cage, 70 1.0-cm holes were evenly spaced on the upper portion of the four sides. The lower 7 cm of the walls and bottom of the cage were solid. The bottom was lined with a 2.5-cm thick layer of clean wood shavings, identical to that used in the animal colony. A thermocouple in a protective metal tube was positioned at the level of the rat and was used to monitor air temperature.

The rats were injected with saline or MDMA at a dose of 30 mg/kg (SC) and immediately placed in the chamber that was set at 18–20°C (mean air temperature = 21.2–22.5°C). This cooler temperature was selected because we have previously found that this dose of MDMA has no effect on core temperature of LE rats in a wire-mesh cage at 20°C. It was thought that the acrylic cage would exacerbate the thermal effects of MDMA under these ambient conditions. One hour after injection, core and tail skin temperature were measured as described above.

2. Additional MDMA studies were performed on naive rats using the metal and Plexiglas floors as described. This permitted the measurement of additional thermoregulatory parameters as previously described. In one study the environmental chamber was maintained at 20°C and the thermoregulatory responses to saline or 30 mg/kg MDMA were determined. Rats (mean body weight = 385 ± 5 g) were injected on 1 day with saline while the aforementioned metabolic parameters were determined. The following day the same animals were injected with MDMA and the same parameters were determined. In another study, the environmental chamber was set at 30°C and the rats (mean body weight = 385 ± 6 g) were given saline on 1 day followed the next day by 10 mg/kg MDMA. The smaller dose of MDMA was used at the warmer T_a because the rat's sensitivity to MDMA is accentuated at warmer temperatures (8).

Data Analysis

Metabolic rate, evaporative water loss, and thermal conductance were calculated by averaging the percent oxygen and dew point temperature data over the last 10 min of the 60-min testing period in the environmental chamber. The effect of T_a and cage type on these data were assessed using a repeated-measures two-way analysis of variance (ANOVA) (GB-STAT, Bethesda, MD). If the factors T_a, cage type, or T_a–cage type interaction were significant ($p < 0.05$), then the data at each T_a were further evaluated for statistical significance using a Tukey's protected t-test. The MDMA core and skin temperature data collected in the wire-screen and acrylic cages were analyzed using a two-way ANOVA followed with a Tukey's protected t-test if significant effects of drug or cage treatment were detected. The MDMA data collected from animals exposed on acrylic and metal floors at T_as of 20 and 30°C were analyzed using a three-way repeated-measures ANOVA with the saline and MDMA treatments designated as the repeated measure.

RESULTS

Effect of Acrylic and Metal Floor on Thermoregulatory Responses

The type of floor had marked effects on the rat's thermoregulatory responses as a function of T_a (Fig. 2). In most cases, the two-way ANOVA revealed significant effects of floor type, T_a, and their interaction on each thermoregulatory variable. Metabolic rate was not significantly affected by floor type, but there was a significant T_a effect ($p < 0.0001$) and a $T_a \times$ floor type interaction ($p = 0.005$). Metabolic rate was quite stable for both floor types over a T_a range of 28–32°C (Fig. 1). At a T_a of 34°C there was a significant elevation in metabolic rate of rats kept on the acrylic floor, whereas rats on the metal floor were unaffected. Metabolic rate was elevated at T_as of 20 and 10°C. Rats on the metal floor at a T_a of 10°C had a significantly higher metabolic rate over that of animals housed on the acrylic floor.

Evaporative water loss was significantly affected by the floor type ($p = 0.005$), T_a ($p = 0.0002$), and by a floor type $\times$ T_a interaction ($p < 0.0001$). Evaporative water loss was stable and minimal in both groups over a T_a range of 20–30°C. As with metabolic rate, increasing T_a to 34°C led to a marked elevation in evaporative water loss in animals maintained on the acrylic floor. At a T_a of 10°C there was a notable increase in EWL in rats kept on the metal floor, but the difference was not statistically significant.

Colonic temperature was significantly affected by floor type ($p = 0.0006$), T_a ($p < 0.0001$), and by a floor type $\times$ T_a interaction ($p < 0.0001$). Colonic temperature on both floor types was relatively stable between T_as of 20–32°C. Rats on the acrylic floor showed a marked elevation in core temperature when exposed to a T_a of 34°C. It is of interest to note that both groups showed an elevation in core temperature as T_a was reduced to 10°C. Tail skin temperature was significantly affected by floor type ($p = 0.002$) and T_a ($p < 0.0001$), but there was no floor type $\times$ T_a interaction. There was a near linear relationship between T_a and tail skin temperature on both the metal and acrylic floors. Tail skin temperature was significantly warmer in rats kept on the acrylic floor at T_as of 30 and 32°C.

Thermal conductance was significantly affected by floor type ($p = 0.006$), T_a ($p < 0.0001$), and by a floor type $\times$ T_a interaction ($p < 0.0001$). This variable was minimal in both groups at a T_a of 10°C. Thermal conductance increased gradually with rising T_a and then rose more sharply as T_a increased from 28 to 34°C. Thermal conductance of rats on the metal floor increased sharply

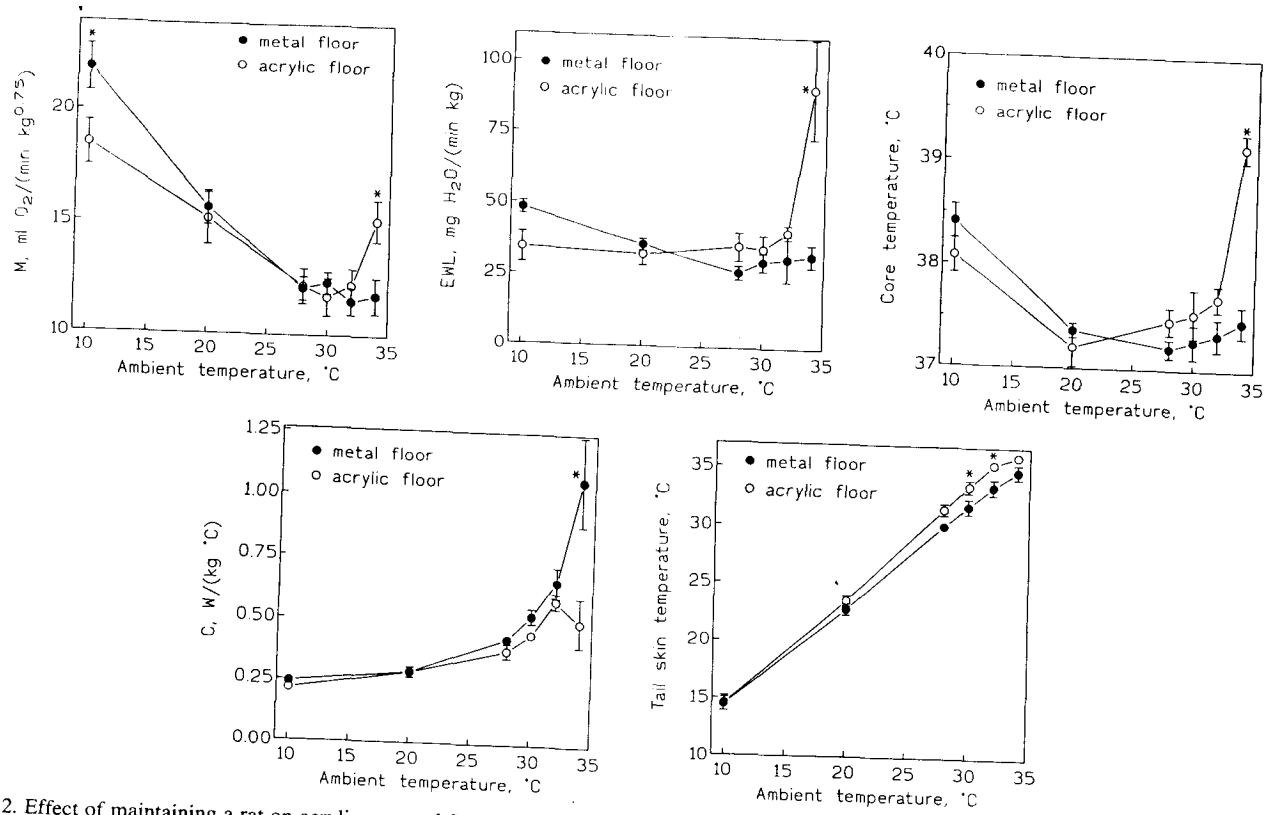

FIG. 2. Effect of maintaining a rat on acrylic or metal floor on the thermoregulatory response to changes in ambient temperature. Thermoregulatory responses include metabolic rate (M), evaporative water loss (EWL), whole-body thermal conductance (C), and colonic and tail skin temperature. n = 5–6 at each T_a and floor type. Data plotted as mean ± SE. Asterisks indicate significant difference in floor type at a given T_a.

at the warmest T_a of 34°C, whereas thermal conductance on the acrylic floor peaked at 32°C and then decreased.

Interaction Between Cage Type and Response to MDMA

Housing rats in the acrylic or wire-screen cage had a marked effect on the thermoregulatory response to MDMA (Fig. 3). The MDMA had no effect on core temperature in rats placed in the wire-screen cage, whereas rats in the acrylic cage underwent a >2.0°C increase in core temperature in 60 min. The two-way ANOVA indicated significant effects of MDMA ($F = 17.4$, $p = 0.0005$), cage type ($F = 9.5$, $p = 0.0047$), and a MDMA × cage type interaction ($F = 12.8$, $p = 0.0016$). Tail skin temperature was also significantly affected by the cage type ($F = 31.2$, $p < 0.0001$), but there were no MDMA or MDMA × cage type interactions. Tail skin temperature was significantly higher in animals kept in the acrylic cages. In spite of the insignificant MDMA response determined by the two-way ANOVA, it should be noted that tail skin temperature of MDMA-treated rats in the wire-screen cage was 1.3°C below that of the saline-treated animals and was found to be statistically significant using a two-tailed Student's t-test ($t = 2.36$, $p < 0.025$).

The thermoregulatory response to MDMA was also affected by housing the animals on either an acrylic or metal floor at T_as of 20 and 30°C (Table 1). The three-way ANOVA revealed a variety of significant effects of floor type, T_a, and MDMA treatment, as well as their interactions. A summary of the statistical analysis of each thermoregulatory variable is given.

Metabolic rate. This variable was significantly increased by MDMA at T_as of 20 and 30°C ($p < 0.0001$). Floor type and T_a did not have a significant effect; however, there was a significant interaction between T_a and MDMA treatment ($p = 0.004$) and a marginal interaction between floor type and MDMA treatment ($p = 0.06$).

Colonic temperature. The MDMA elicited a precipitous elevation in core temperature. There were highly significant effects of T_a ($p = 0.001$) and MDMA treatment ($p < 0.0001$) on core temperature, but no effect of floor type. There also was a significant interaction between T_a and MDMA treatment ($p = 0.004$).

Tail skin temperature. MDMA elicited a significant reduction in tail skin temperature, an effect that was also seen in the animals exposed in the acrylic and wire-screen cages (see above). This variable was significantly affected by floor type ($p = 0.003$), T_a ($p < 0.0001$), and MDMA treatment ($p < 0.0001$). There were also significant interactions between floor type and MDMA treatment ($p = 0.01$) and between T_a and MDMA treatment ($p = 0.0008$).

Evaporative water loss. This variable was also increased by MDMA treatment. There was no significant effect of floor type, but there were highly significant effects of T_a ($p < 0.0001$) and MDMA treatment ($p < 0.0001$). There was also a significant interaction between floor type and MDMA treatment ($p = 0.038$) and T_a and MDMA treatment ($p < 0.0001$).

Thermal conductance. This variable was unaffected by floor type, but there was a highly significant effect of T_a and a marginal effect of MDMA treatment ($p = 0.06$). There was also a significant interaction between T_a and MDMA treatment ($p = 0.002$).

Motor activity. Motor activity was not analyzed because of problems with the acrylic and metal floors on the monitoring device (see the Method section). However, it can be seen that MDMA led to marked elevations in this variable at both T_as of 20 and 30°C (Table 1).

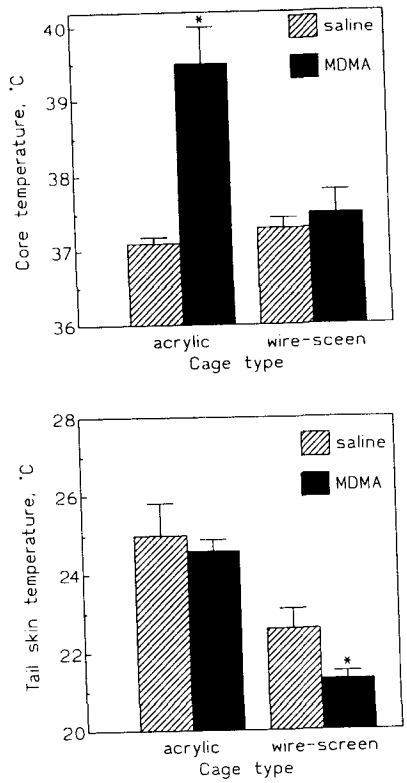

FIG. 3. Effect of housing in acrylic or wire-screen cage on the colonic and tail skin temperature measured 60 min after treatment with 30 mg/kg MDMA (SC) at a mean air temperature of 21.2–22.5°C. Asterisks indicate significant difference between saline and MDMA treatment. n = 8 per treatment group.

The effects of the various cages on thermoregulation was further illustrated by comparing the heat loss of a "phantom rat" using a 1.0-l water bottle made of high-density polyethylene (Table 2). The bottle was filled with warm water (42°C) and two thermocouples were placed near the center of the fluid at each end of the bottle. The average temperature decrease of the water in the bottle was recorded over a 60-min period and then analyzed with linear regression. The correlation coefficient of the time vs. temperature data points was greater than 0.99 in all experiments. Heat loss in watts was calculated based on the cooling function (Table 2). Not surprisingly, the greatest heat loss was observed in the wire-screen and metal floor cages. The smallest heat loss occurred when the bottle was placed in the acrylic-wood chip cage. It is interesting to note that the difference in heat loss between the acrylic cage with wood chip bedding and the wire-screen cage was 18%, whereas the difference for the metal and acrylic floors was only 12%.

DISCUSSION

Substituting a floor made of aluminum with one made of acrylic in the environmental chamber illustrates the importance of conductive heat exchange between the rat and its cage. That is, with all other conditions being identical in the environmental chamber, the data show that rats on acrylic or metal floors had notable thermoregulatory differences when exposed to warm and cold T_as. The thermoregulatory response to acute MDMA administration also can be modulated by housing in wire-screen or acrylic cages. Evaluation of MDMA's effects in animals exposed on the acrylic or metal floor also suggests that conductive heat exchange between the animal and cage floor is an important factor in understanding MDMA's thermal effects.

The most prominent effects occurred with metabolic rate, evaporative water loss, and colonic temperature when T_a was elevated from 32 to 34°C. Rats on a metal floor remained normothermic with minimal changes in metabolic rate and evaporative water loss. On the other hand, rats on the acrylic floor exhibited a sharp elevation in core temperature and metabolic rate. The rise in evaporative water loss in this group represents a response to increase heat loss in the face of rising body temperature. The inability of rats on the acrylic floor to increase thermal conductance at a T_a of 34°C is undoubtedly critical in accounting for the inability to maintain a normal core temperature. There were no effects of metal and acrylic floors on the thermoregulatory parameters measured at T_as of 20–32°C. At the coldest T_a tested (10°C), rats on the metal floor exhibited a significant elevation in metabolic rate, but colonic and tail skin temperature were unchanged. It would appear that the elevated metabolic rate reflects the increased conductive heat transfer to the metal floor.

The MDMA experiment illustrates the marked interaction between thermoregulatory responses in different environmental settings and the potential efficacy of a chemical agent. In the present study, MDMA's hyperthermic effect was exacerbated by housing rats in the acrylic cage with wood chips compared to animals maintained in wire-screen cages. MDMA's principal acute effect is thought to be attributed to the release of 5-HT in the central nervous system. This elicits a general serotonergic syndrome characterized by low body posture, head weaving and increased motor activity, piloerection, salivation, and hyperthermia (8,17). However, the hyperthermic response of MDMA is closely dependent on environmental temperature. Rats maintained at a T_a of 30°C in a wire-screen type cage display a doubling of metabolic rate and a core temperature of over 41°C in just 60 min after a 30 mg/kg dose of MDMA. On the other hand, at a T_a of 10°C, the same dose of MDMA has no effect on metabolic rate and leads to a 2.0°C reduction in core temperature (8). MDMA's hyperthermic action may play a key role in its neurotoxicity. For example, MDMA-induced reduction in serotonin content of the cortex, hippocampus, and striatum is markedly attenuated by preventing rats from becoming hyperthermic (16). The data of the present study suggest that neurotoxic variability to compounds such as MDMA would be strongly dependent upon the type of cage. It behooves investigators to document the type of cage when involved in rodent studies where a metabolic and/or thermoregulatory end point may be directly or indirectly involved.

The MDMA studies in rats housed on acrylic or metal floors further illustrates the importance of conductive heat transfer. There were a variety of significant differences in thermoregulatory response as well as significant interactions between floor type, T_a, and MDMA treatment. The effects of MDMA on core temperature in animals on the acrylic and metal floors were not as distinct when compared to that of animals exposed in the acrylic and wire-screen cages (see Fig. 3). One possible explanation is that the acrylic cage with wood chip bedding provided more insulation than the acrylic floor itself. Indeed, the water bottle experiment (Table 2) indicates that heat loss on the acrylic floor is 9.4% greater than on the acrylic floor with wood chips. However, it is not clear if this reduction in heat transfer explains the marked difference in core temperature following MDMA treatment. Overall, the MDMA data collected from animals on the metal and acrylic floors indicates that conductive heat exchange between the surfaces of the rat that make contact with the floor of its cage can affect the response to drugs such as MDMA.

TABLE 1

THERMOREGULATORY RESPONSES TO SALINE AND MDMA IN RATS ON AN ACRYLIC OR METAL FLOOR

Parameter	Drug Treatment/Floor Type			
	Saline		MDMA	
	Acrylic	Metal	Acrylic	Metal
	T_a = 20°C			
M [ml/(min kg$^{0.75}$)]	13.7 ± 0.4	16.6 ± 1.0	27.1 ± 1.9	24.2 ± 1.1
T_{col} (°C)	37.1 ± 0.1	37.3 ± 0.2	38.4 ± 0.7	37.6 ± 0.6
T_{sk} (°C)	23.6 ± 0.3	22.8 ± 0.7	22.5 ± 0.4	22.2 ± 0.5
EWL [mg/(min kg)]	33.8 ± 3.1	51.1 ± 7.2	145.7 ± 11.8	123.5 ± 6.7
C [W/(kg °C)]	0.26 ± .01	0.28 ± .02	0.31 ± .03	0.29 ± .01
MA (% of saline)	100 ± 6.2	100 ± 12.2	218.6 ± 11.0	168.4 ± 4.5
	T_a = 30°C			
M [ml/(min kg)]	15.6 ± 0.8	12.6 ± 0.6	30.8 ± 1.3	28.2 ± 1.9
T_{col} (°C)	37.4 ± 0.1	37.1 ± 0.07	40.6 ± 0.4	39.7 ± 0.3
T_{sk} (°C)	33.9 ± 0.2	31.4 ± 0.3	30.1 ± 0.07	29.7 ± 0.2
EWL [mg/(min kg)]	41.0 ± 2.1	38.4 ± 3.3	205.6 ± 6.1	190.6 ± 14.3
C [W/(kg °C)]	0.67 ± 0.01	0.54 ± 0.02	0.44 ± 0.05	0.45 ± 0.04
MA (% of saline)	100 ± 4.0	100 ± 9.8	254.0 ± 8.1	231.1 ± 11.3

MDMA dose: 20°C, 30 mg/kg, SC; 30°C, 10 mg/kg, SC. n = 6 per treatment group.
M = metabolic rate; T_{col} = colonic temperature; T_{sk} = tail skin temperature; EWL = evaporative water loss; C = thermal conductance; MA = motor activity.

Understanding how cage construction or floor type governs the thermoregulatory effects of drug such as MDMA can be expressed using a modified heat balance equation (7,9,15). In steady-state conditions (i.e., Δ heat storage = 0), total heat loss (H_t) is equal to metabolic heat production (M), which is equal to the sum of heat loss from evaporation (E), radiation (R), convection (C), and conduction (K):

$$H_t = M = E \pm R \pm C \pm K. \quad [2]$$

Each of the heat loss terms is complex and dependent on core, surface, and ambient temperature. For purposes of this discussion, a simplified form of the heat balance equation can be utilized:

TABLE 2

HEAT LOSS OF WATER BOTTLE PLACED IN ENVIRONMENTAL CHAMBER SET AT A T_a OF 20°C

Cage type	Heat Loss (W)
Wire-screen	7.78
Acrylic with wood chips	6.61
Metal floor	7.94
Acrylic floor	7.06

Bottle was placed in each of the four cage types that were used to house rats during testing. The decrease in temperature of the water over period of 60 min was measured to calculate total heat loss in watts (W) from the bottle using the equation: W = (°C/s) (g) (c), where g is weight of water in grams and c is specific heat of water (4.18 J/g/°C). Note: 1.0 J/s = 1.0 W.

$$M = C(T_c - T_a) + E \quad [3]$$

where C = thermal conductance and takes into account the heat dissipated by radiation, convection, and conduction; E = evaporative heat loss (i.e., rate of water loss multiplied by latent heat of vaporization). It should be noted that eq. (3) is a rearrangement of the terms used to calculate whole-body thermal conductance [eq. (1)] (15). This relationship can also be used to illustrate the impact of cage type on the thermoregulatory demands of the rat by solving eq. (3) for T_c:

$$T_c = T_a + (M - E)/C. \quad [4]$$

Thus, at a fixed T_a, when M increases as would occur from the administration of MDMA, thermal homeostasis can only be maintained if C and/or E increase. If there are limitations on the ability to increase thermal conductance, which appears to be the case when rats are housed in acrylic cages or kept on a floor made of insulative materials, then there will be a greater requirement to dissipate heat by evaporation. Evaporative water loss in rats is normally not activated until ambient heat stress becomes relatively excessive (i.e., at T_as ≥ thermoneutrality) (7,9). A significant limitation on evaporation at warm T_as will lead to an elevation in body temperature. Thus, using a simplified heat balance equation, it can be shown how an acrylic cage with wood chip bedding exacerbates the hyperthermic effects of a thermogenic compound like MDMA. Furthermore, as T_a is increased to 34°C, rats in an acrylic floor incur a greater heat load because thermal conductance does not increase as effectively when compared to rats kept in wire-screen cages. It is also seen from the relationship in eq. (4) that a higher thermal conductance in resting animals in acrylic cages will lessen the metabolic requirements for thermal homeostasis at relatively cool T_as. This explains the lower metabolic rates of rats kept in acrylic cages at 10°C.

The water bottle experiment is useful for demonstrating the potential impact of cage type on heat loss. Although the heat loss from the bottle is an over simplification of the rat's thermoregulatory response, the results nonetheless corroborate the MDMA studies. The smallest heat loss observed in the acrylic-wood chip cage may explain the acute rise in core temperature of the rats injected with MDMA and placed in this environment compared to the wire-screen and metal floor environments. Likewise, the small heat loss measured on the acrylic floor compared to the metal floor further indicates the difficulty for the rat to dissipate heat at warm T_as when housed on the acrylic floor.

To summarize, using exposure periods of 60 min, it was shown that the thermoregulatory requirements of the rat can be markedly affected by being housed on a highly conductive metal floor or insulative acrylic floor. The measurements of thermoregulatory responses made on the acrylic and metal floors should lead to a better understanding of thermoregulatory responses in animals housed in different types of cages. Dry heat loss is impeded on the acrylic floor as T_a increases, creating a greater strain on the animal's thermoregulatory system. Overall, stress on the thermoregulatory system is minimized by housing in acrylic cages with wood chip bedding when T_a is relatively cool, whereas at relatively warm T_as, stress is minimized by housing in wire-mesh cages. The studies using metal and acrylic floors suggests an important role for heat conduction from the ventral surface of the rat during heat and cold stress. Moreover, because of the reduced heat loss, the hyperthermic efficacy of MDMA is also exacerbated in animals placed in acrylic cages with wood chip bedding or on a floor made of acrylic material. A simplified version of the heat balance equation is useful for understanding the impact of cage type on thermoregulatory requirements. The relationships developed in the present study should be of use to those concerned with metabolic and thermoregulatory responses in rodents subjected to pharmacological and other chemical treatments.

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