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Research Paper

Effect of physical restraint on the limits of thermoregulation in telemetered rats

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Physical restraint of rodents is needed for nose-only exposure to airborne toxicants and is also used as a means of psychological stress. Hyperthermia is often observed in restrained rats, presumably as a result of impairments in heat dissipation. However, such a hyperthermic response should be dependent on the prevailing ambient conditions. To understand how ambient temperature (T_a) affects the thermoregulatory response to restraint, core temperature (T_c) and heart rate (HR) were monitored by telemetry in rats subjected to 1 h of physical restraint while T_a was maintained at 14–30°C in 2°C increments. The T_c of unrestrained rats was unaffected by T_a. During restraint, T_c was elevated at ambient temperatures with the exception of 14°C, at which the rats became mildly hypothermic. There was an inverse relationship between T_a and HR in both unrestrained and restrained rats; however, HR was significantly elevated in restrained rats at all ambient temperatures except 22 and 24°C. Heat loss from the tail, estimated from T_c and tail skin temperature, was markedly reduced at all but the highest ambient temperatures in restrained rats. The data suggest that the T_a limits of normothermia are narrowed in the restrained rat. That is, between 16 and 20°C, the rat maintains a relatively stable T_c that is slightly elevated above that of the unrestrained rat. At ambient temperatures above or below this range, the rat shows signs of hyperthermia and hypothermia, respectively. In contrast, the limits of normothermia for unrestrained rats range from 14 (or lower) to 30°C. Overall, the ideal T_a for restrained rats appears to be 20°C and no higher than 22°C for the thermoregulatory system to maintain a regulated T_c in rats well adapted to physical restraint.

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Physical restraint of rodents is often used in a variety of physiological and toxicological procedures. Long-term restraint is essential for a variety of air pollutant studies where the nose and mouth of the animal must be maintained in a fixed position within an exposure chamber (Narciso *et al.* 2003; Farraj *et al.* 2009). Long-term restraint may also be required for collecting a variety of physiological measurements and for the parenteral administration of drugs and other agents. In addition, physical restraint is often used as a method to stress rodents and activate their sympathetic nervous system; this is especially important in studying strains of rodents that are more susceptible to hypertension (Johnson *et al.* 2000; McDougall *et al.* 2005).

It has been recognized for many years that the thermoregulatory and cardiovascular systems of rats are compromised during physical restraint (Nagasaka *et al.* 1979; Thornhill *et al.* 1979; Gordon, 1993; Ootsuka *et al.* 2008). When restrained at standard room temperatures (i.e. 22°C), body temperature is typically elevated. The hyperthermic response at standard laboratory temperatures is thought to be a result of the stress-induced increase in metabolic heat production. A transient reduction in tail blood flow may also contribute to the rise in core temperature during restraint (Ootsuka

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et al. 2008). Moreover, restraint stress at a temperature of ∼22°C leads to elevations in heart rate and blood pressure (Chen & Herbert, 1995; Irving *et al.* 1998), largely due to sympathoadrenal system activation. The increase in heart rate was significantly greater in spontaneously hypertensive rats (SHRs) when compared with Wistar–Kyoto rats (Bott-Flügel *et al.* 2011).

Restrained rats are unable to groom saliva onto their fur to dissipate heat and they are considered to be unable to regulate core temperature effectively if restrained at warm temperatures. In contrast, physical restraint of rats at cold ambient temperatures leads to hypothermia, apparently as a result of an inability of the restrained rat to shiver adequately to maintain a sufficient level of heat production (Thornhill *et al.* 1979; Shimada & Stitt, 1983). Interestingly, mice physically restrained at standard room temperature will often become hypothermic (Johnson *et al.* 2000).

While most studies involving physical restraint are typically performed at standard laboratory temperature, the ambient temperature of restraint may vary from season to season depending on the quality of the environmental control system. There is remarkably little information on how ambient temperature affects the ability of the restrained rat to thermoregulate. Little is known on how variations in ambient temperature affect the ability of the restrained rat to control the skin temperature of the tail, which is a crucial site for the control of non-evaporative heat loss (Gordon, 1993). This could be important in a variety of studies where it is assumed that the effects of restraint on thermoregulation are consistent in spite of potential deviations in environmental temperature. Moreover, implementing radiotelemetry in a study of this type is ideal for the following reasons: (i) control animals can be monitored without the typical stress-induced effects of handling and/or tethering needed for conventional physiological monitoring; and (ii) telemetered animals can easily be subjected to restraint-induced stress without the concern of a tether that could affect implementing a protocol of physical restraint (also, see Wright & Katovich, 1996). To this end, the purpose of this study was to assess how ambient temperature affects the thermoregulatory and cardiovascular systems of the physically restrained rat monitored by radiotelemetry.

Methods

Male Sprague–Dawley rats obtained from Charles River Laboratories (Raleigh, NC, USA) were obtained at 60 days of age and housed individually in acrylic cages lined with wood-chip bedding at an ambient temperature of 22°C, 50% relative humidity, and a 12 h–12 h light–dark photoperiod. Food (LabDiet® manufactured by PMI Nutrition International) and water were provided *ad libitum*. All surgical and restraint procedures were approved by our EPA Institutional Animal Care and Use Committee.

Surgery

At an age of approximately 75 days, rats were anaesthetized with isoflurane in 100% oxygen (4.5% initially followed with 2% to maintain a surgical plane). The abdominal area was shaved and prepared for aseptic surgery. A mid-line abdominal incision was made to implant a radiotransmitter to monitor heart rate, core temperature and motor activity (model CTA-F40; Data Sciences International, St Paul, MN, USA). The electrocardiogram leads were tunnelled under the skin and positioned to detect the ECG. The body of the transmitter was sutured to the wall of the abdomen, closed with 4–0 silk. The skin was closed with surgical staples, and rats were administered an analgesic (buprenorphine, 0.03 mg kg^{-1}, s.c.) twice per day for 48 h. Rats were allowed 10 days of recovery prior to handling or testing. For additional details on telemetry surgery, see Gordon (1994).

Restraint protocol

Prior to this experiment, the rats were subjected to a series of restraint procedures, in which half the animals were placed in restrainers for 1 h for four consecutive days while housed in the vivarium. The following week, the remaining four animals were restrained using exactly the same protocol. This 4 day restraint experiment was part of a study on the effect of repeated restraint on adaptation of core temperature and heart rate. The results are to be reported in a separate publication. This protocol served to acclimate the rats to a physical restraint procedure. These rats were then used in the protocol described below.

Heart rate, core temperature and motor activity were monitored in four rats simultaneously while unrestrained at an ambient temperature of 14, 16, 18, 20, 22, 24, 26, 28 or 30°C by housing in an environmental chamber (Sure Temp, Cary, NC, USA). A randomized schedule was followed, in which the rats were first placed in an acrylic cage (i.e. same dimensions as their home cage) with a layer of wood-chip bedding for 90 min at one of the above temperatures. A ventilated acrylic top was placed on the cage, and the temperature of the inside of the cage and the environmental chamber was identical. A thermocouple (copper–constantan) was taped to the tail of the rat about 2 cm from the base prior to the start of the exposure to measure tail skin temperature. The probe was reattached at the end of the 90 min exposure to measure tail skin temperature. Temperatures were recorded with a Physitemp BAT-12R meter (Fort Huron,

NJ, USA). Overall, each rat was subjected to the restraint procedure a total of nine times; once at each of the nine ambient temperatures. The unrestrained exposure was always carried out 14 h prior to the restraint test.

The next day, the same rats were again placed in the cage at the same ambient temperature, with the exception that rats were restrained. The restrainer was placed in the acrylic cage, but there was no bedding or cage top. The restraint device was a nose-inhalation tube (Lab Products, Seaford, DE, USA) constructed of acrylic, with an inner diameter of 7 cm; the length of the tube was reduced from 26.7 to 22.2 cm. These modified inhalation tubes are often used for nose-only inhalation studies in our facility (Farraj *et al.* 2009). The rats were physically restrained and had little ability to move in the tube. A rubber plug placed inside the tube over the back of the animal secured the tail and prevented the rat from turning around in the restrainer. During restraint, a thermocouple was taped to the base of the rat's tail, and tail skin temperature was monitored throughout restraint. The restraint was terminated after 1 h, and rats were returned to their home cages. Telemetry data were collected at 5 min intervals throughout restrained and unrestrained experiments. All eight rats were randomly tested at each of the ambient temperatures described above. An additional 30 min of exposure to the unrestrained conditions was used to assess whether there was further adaptation to the cage and ambient temperature in the freely moving rat. There was at least a 48 h recovery period after a restraint test before a rat was retested.

The heat loss index (HLI) was calculated according to the following formula (Romanovsky & Blatteis, 1996; Gordon *et al.* 2002):

$$\mathrm{HLI} = (T_{\mathrm{sk}} - T_{\mathrm{a}})/(T_{\mathrm{c}} - T_{\mathrm{a}})$$

The HLI is a measure of active, non-evaporative heat exchange from the tail attributed to peripheral vasomotor mechanisms, where T_{sk} is the tail skin temperature, T_{a} the ambient temperature and T_{c} the core temperature. The HLI is has no units and ranges from 0 (fully vasocontricted) to 1 (fully vasodilated). The HLI was calculated following 60 and 90 min in the environmental chamber for restrained and unrestrained rats, respectively.

Statistics

Extraneous values of core temperature and heart rate monitored by telemetry were first clipped using the Data Sciences International analysis program. Any heart rate <200 or >600 beats min^{-1} and temperature data <35 or $>41^{\circ}$C were removed. The mean ± SEM for core temperature and heart rate were calculated for the eight animals as a function of time during the unrestrained and restrained tests. The core temperature and heart rate data recorded at 5 min intervals were subjected to a repeated-measures two-way analysis of variance (RMANOVA) using ambient temperature and time as factors (Sigma Plot, Version 11.0, Point Richmond, CA, USA). In addition, data collected from 50 to 60 min were averaged for restrained and unrestrained groups. These data were then analysed with RMANOVA using restraint treatment and ambient temperature as factors. Significant effects of ambient temperature or restraint were followed up with a Holm–Sidak test, where an ambient temperature of 22°C was used as the point of comparison to the other ambient temperatures to test for significance ($P < 0.05$). The tail skin temperature and HLI data were analysed using RMANOVA, with restraint and ambient temperature as factors.

Results

There were marked differences in the time course of heart rate and core temperature depending on whether the rats were unrestrained or restrained. When placed in the environmental chamber unrestrained, core temperature increased transiently, starting in the low 37.0°C range, peaking at approximately 37.5–37.8°C after 15–20 min, then quickly subsiding within 60 min (Fig. 1*A*). Although there was a significant interaction between time and ambient temperature on core temperature of unrestrained rats, the responses were very similar, and when the data were collapsed across time there was no significant effect of ambient temperature. Heart rate of the unrestrained rats was initially ∼275 beats min^{-1} and increased markedly within 5 min to over 350 beats min^{-1} after placement in the chamber. Ambient temperature had a significant effect on heart rate; exposure to cold temperatures led to an elevation in heart rate (Fig. 2*A*). The highest increase in heart rate was seen at an ambient temperature of 14°C, at which mean heart rate (averaged over the 90 min period) was 75 beats min^{-1} higher than that of rats maintained at 30°C. There were generally some slight reductions in heart rate and core temperature from 60 to 90 min of unrestrained exposure; however, the additional 30 min of unrestrained exposure had no significant effects.

The transient elevation in core temperature when first placed in the chamber was absent when the rats were restrained (Fig. 1*B*). Core temperature was initially 37.0°C and then rose gradually over time of restraint for rats exposed to ambient temperatures >24°C. At the cooler ambient temperatures, core temperature was relatively stable for the 60 min period, whereas at the coldest ambient temperature of 14°C, there was a significant decrease.

Compared with unrestrained rats, the heart rate of restrained rats was significantly higher and their heart rate remained elevated throughout the period of restraint, an

effect especially prominent at cooler ambient temperatures of 14 and 16°C. There was a significant effect of ambient temperature on heart rate during restraint ($P < 0.001$; Fig. 2*B*). When all ambient temperatures tested were averaged, the overall heart rate of restrained rats was 42 beats min^{-1} higher compared with the unrestrained rats ($P < 0.001$).

Ambient temperature and restraint treatment had significant effects on tail skin temperature (Fig. 3*A* and *B*). In unrestrained rats, tail skin temperature measured before placement in the chamber was 26–28°C. After 90 min, tail skin temperature was significantly elevated at ambient temperatures ≥26°C and reduced at temperatures ≤20°C (Fig. 3*A*). Tail temperature initially measured in restrained

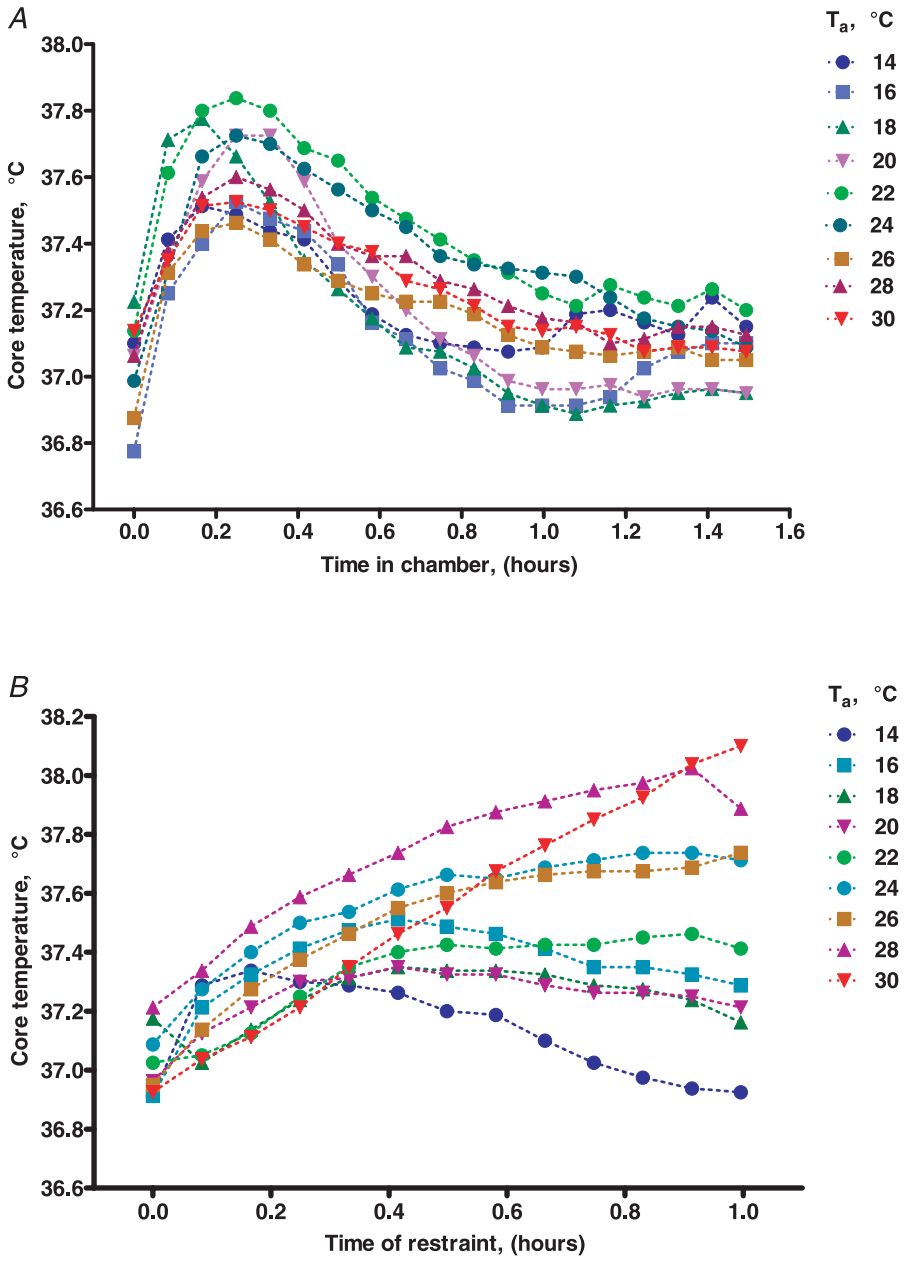

Figure 1. Time course of core temperature in unrestrained (*A*) and restrained rats (*B*) when maintained at ambient temperatures (T_a) of 14–30°C

$n = 8$ per ambient temperature. For the sake of clarity, error bars have been omitted from the figure. Overall, the standard error for all time points ranged from 0.1 to 0.2°C. Results of repeated-measures analysis of variance (RMANOVA) are as follows: restrained, T_a, $F(8,56) = 4.48$, $P < 0.001$; restrained, time, $F(12,84) = 11.43$, $P < 0.001$; restrained, $T_a \times$ time, $F(96,672) = 7.70$, $P < 0.001$; unrestrained, T_a, $F(8,56) = 2.05$, $P < 0.056$; unrestrained, time, $F(12,84) = 75.66$, $P < 0.001$; unrestrained, $T_a \times$ time, $F(96,672) = 1.98$, $P < 0.001$.

rats as they were being placed in the environmental chamber was more varied compared with the unrestrained animals (Fig. 3*B*). Their initial temperatures were lower than the 26–28°C range observed in the unrestrained rats. After 60 min restraint, tail skin temperature was significantly elevated at ambient temperatures ≥28°C and reduced at temperatures ≤22°C. It is interesting to note that at the coldest ambient temperature of 14°C, tail temperature of the restrained rats was 14°C after 60 min while that of the unrestrained rats was 18°C after 90 min.

Calculation of HLI illustrates interactions between restraint and ambient temperature (Fig. 4). In unrestrained animals, the HLI increased slightly from the coldest to warmest ambient temperatures, ranging from 0.2 to 0.38. The HLI of restrained animals was less than 0.1 over an ambient temperature range of 14–26°C. There was an abrupt rise in HLI as temperature was raised from 26 to 30°C. The HLI of restrained and unrestrained rats at 30°C was nearly equal.

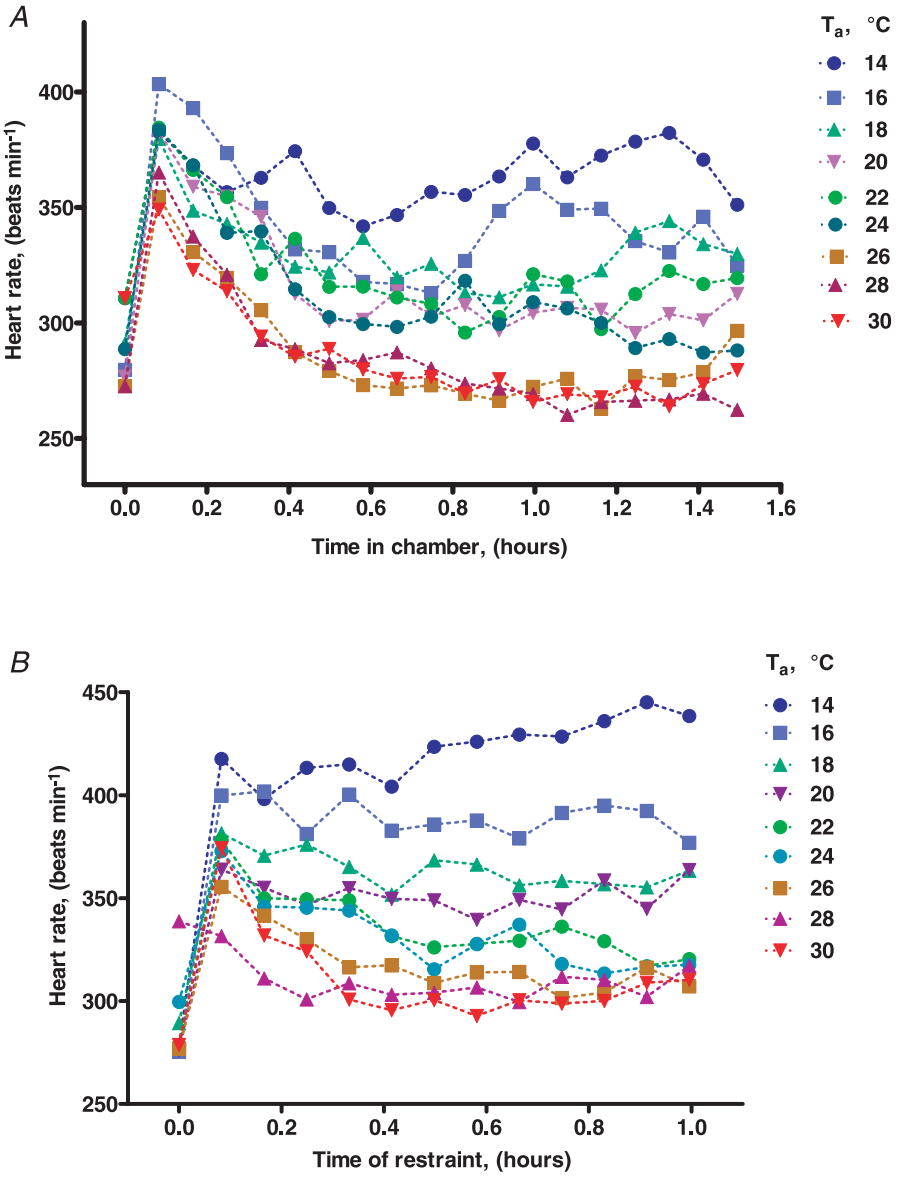

Figure 2. Time course of heart rate in unrestrained (*A*) and restrained rats (*B*) when maintained at ambient temperatures of 14–30°C

$n = 8$ per ambient temperature. For sake of clarity, error bars have been omitted from the figure. Overall, the standard error for all time points ranged from 5 to 15 beats min^{-1}. RMANOVA results are as follows: restrained, T_a, $F(8,56) = 28.63$, $P < 0.001$; restrained, time, $F(12,84) = 51.51$, $P < 0.001$; restrained, $T_a \times$ time, $F(96,672) = 8.22$, $P < 0.001$; unrestrained, T_a, $F(8,56) = 31.71$, $P < 0.001$; unrestrained, time, $F(12,84) = 34.84$, $P < 0.001$; unrestrained, $T_a \times$ time, $F(96,672) = 2.42$, $P < 0.001$.

The overall effect of ambient temperature on the core temperature and heart rate following 60 min of restraint is comparable to 90 min of unrestrained exposure (Fig. 5*A* and *B*). Comparing all data with an ambient temperature of 22°C, representing the standard temperature of housing, restraint had significant effects on core temperature at ambient temperatures of 14, 28 and 30°C. It is also important to note the trend for an increasing core temperature as ambient temperature was raised above 22°C. The heart rate of restrained rats was statistically unchanged from an ambient temperature of 22 to 30°C in restrained rats, whereas the heart rate of unrestrained rats was significantly reduced at ambient temperatures ≥26°C and elevated at temperatures ≤16°C (Fig. 5*B*).

Discussion

We define the ambient limits of normothermia (LIN) as the range of ambient temperatures over which the core temperature of a homeotherm is regulated at the same level (Gordon, 1993). Following recovery from the transient rise in core temperature when first placed in the environment chamber, the LIN of the unrestrained rat is defined as 14–30°C. Ambient temperature had no effect on core temperature of unrestrained rats after 60 or 90 min (Fig. 5*A*). These limits for the present study are indeed similar to that of the unrestrained Long–Evans rats maintained at different ambient temperatures for 24 h (Yang & Gordon, 1996). The LIN of the unrestrained rat is achieved by a combination of vasomotor control of heat loss from the tail and other surfaces over an ambient temperature range of approximately 26–30°C and the ability to raise heat production as ambient temperature is lowered below 26°C (Gordon, 1993).

Restraint clearly narrows the range of thermoregulatory control (Fig. 6*A*). In this figure, the data from Fig. 5*A* have been replotted, and the dashed line (fitted by eye) illustrates our estimate of the LIN for the rat after 60 min of restraint. We estimate the LIN of the restrained rat to be 16–20°C. Within this range, core temperature is maintained at a slightly elevated level ($\Delta T \approx 0.2$°C) compared with the unrestrained rat. Overall, the impact of restraint stress on thermoregulatory control may best be summarized by calculating the range of the LIN, which is at least 16°C in the unrestrained rat but is reduced to approximately 4°C by restraint.

The stress from restraint leads to sweeping elevations in heart rate and probablly elevation in metabolic rate (Nagasaka *et al.* 1979) along with a reduction in heat loss (i.e. HLI) across a wide range of ambient temperatures. This places additional demands on the thermoregulatory system of the rat; that is, vasomotor control of heat loss is likely to be restricted in these conditions, leading to a narrowing of the LIN. Wright & Katovitch (1996) used radiotelemetry to monitor tail skin and core temperature and found a reduction in tail skin temperature during physical restraint. Only when ambient temperature increases above 26°C is the restrained rat apparently able to initiate an increase in heat loss from the

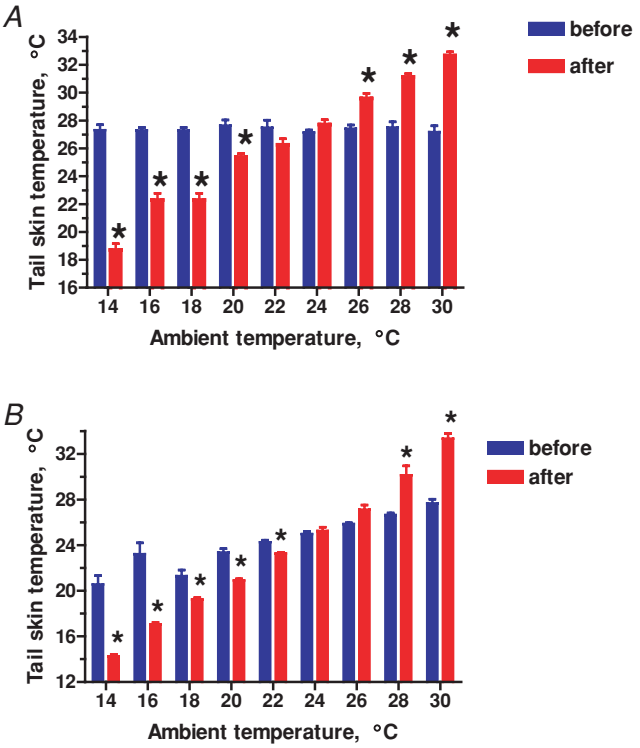

Figure 3. Effect of restraint on tail skin temperature
A, tail skin temperatures as a function of ambient temperature in unrestrained animals before and after a 90 min test session. *B*, tail skin temperatures as a function of ambient temperature in restrained animals before and after a test session. *$P < 0.05$ different from the corresponding temperature before the test session at a given T_a.

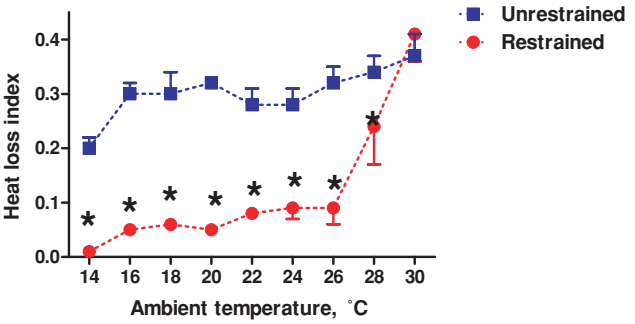

Figure 4. Relationship between ambient temperature and heat loss index (HLI) in restrained and unrestrained rats over an ambient temperature range of 14–30°C
RMANOVA results are as follows: restraint, $F(1,7) = 42.1$, $P < 0.001$; T_a, $F(8,56) = 15.3$, $P < .001$; restraint × T_a, $F(8,56) = 6.5$, $P < 0.001$. * Significant differences ($P < 0.05$) between restrained and unrestrained animals at a given T_a.

tail. Ootsuka *et al.* (2008) made continuous measurements of tail blood flow in rats subjected to 30 min of physical restraint and found a transient reduction when first placed in the restrainer, followed by recovery and elevation in blood flow after about 20 min of restraint. We cannot reconcile a rise in total tail blood flow and reduction in the HLI from the tail observed in the present study. The vasculature of the rat tail is complex, and it is possible that tail blood flow is maintained while heat loss from the skin is reduced (for review, see Gordon, 1993).

It is well known that physical restraint leads to an elevation in heart rate of rats (McDougall *et al.* 2005); however, little is known about how ambient temperature affects this response. As heart rate generally reflects the metabolic demands of the rat (Gleeson & Baldwin, 1981; Gonzalez *et al.* 1998), we expected interactions between restraint stress-induced tachycardia and ambient temperature because metabolic demand increases as ambient temperature decreases (Yang & Gordon, 1996). Heart rate during restraint was significantly elevated at all ambient temperatures tested with the exceptions of 22 and 24°C (Fig. 2*B*). It is interesting to note that the heart rate of restrained rats changed little over temperatures of 22–30°C. The heart rate of unrestrained rats dropped abruptly as ambient temperature increased from 26 to 28°C (Fig. 5*B*). It would appear that the restrained rats subjected to a wide range of ambient temperatures maintained control over their heart rate. We conclude that the overall elevation in heart rate in this group reflects the impact of the psychological stress on the cardiovascular system. Moreover, the marked reduction in the HLI of the restrained rats maintained at ambient temperatures of 14–26°C is also assumed to reflect the impact of the stress of the physical restraint on the mechanisms of peripheral vasomotor control. The data suggest that the restrained animals were peripherally vasoconstricted until ambient temperature exceeded 26°C, whereupon there was a marked rise in HLI, reflecting peripheral vasodilatation and an increase in skin blood flow to increase heat dissipation. It is concluded that the

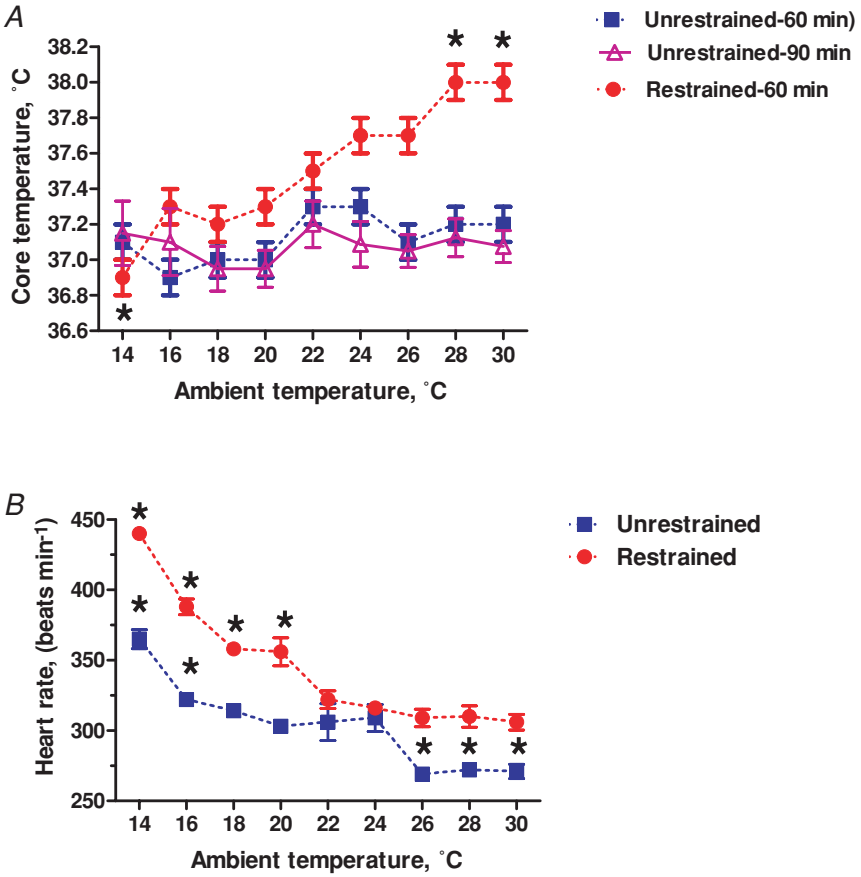

Figure 5. Relationship between ambient temperature and core temperature (*A*) and heart rate (*B*) averaged during the last 10 min of restraint; also plotted is the response of unrestrained animals
* Significant difference ($P < 0.05$) when compared with animals tested at 22°C. RMANOVA results are as follows: core temperature, restraint, $F(1,7) = 1.3$, $P = 0.008$; core temperature, T_a, $F(8,56) = 8.6$, $P < 0.001$; core temperature, restraint × T_a, $F(8,56) = 10.0$, $P < 0.001$; heart rate, restraint, $F(1,7) = 86.8$, $P < 0.0001$; heart rate, T_a, $F(8,56) = 39.1$, $P < 0.001$; heart rate, restraint × T_a, $F(8,56) = 2.85$, $P = 0.01$.

restrained rat, while under significant psychological stress, is nonetheless capable of activating a vasodilatory response that minimizes an additional rise in core temperature.

Measuring total body heat production and heat loss of restrained rats placed in a calorimeter at an ambient temperature of 25°C, Nagasaka *et al.* (1979) showed an initial period where heat production was increased while heat loss remained the same. Thus, heat storage was positive and core temperature increased during restraint. As restraint continued for 140 min, heat loss and production balanced, but the rat remained hyperthermic. Following release from restraint, heat loss increased above heat production and core temperature slowly recovered. Restraint in rodents clearly elicits an adrenergic, hypermetabolic response (Nagasaka *et al.* 1979). Ootsuka *et al.* (2008) found a marked rise in temperature of the interscapular brown adipose tissue during physical restraint. This would suggest that restraint-induced activation of thermogenesis in brown adipose tissue contributes to the overall rise in core temperature. In addition, the observation of an elevation in heart rate with restraint in warm and cool environments is evidence of a hypermetabolic response brought on by restraint.

Considering the marked reduction in HLI and elevation in heart rate at nearly all ambient temperatures, the restrained rats in this study were undergoing a marked stress response in spite of the fact that they appeared to be well adapted to the restraint device. Within the ambient temperature range of 16–20°C, the core temperature of the restrained rats is only 0.2°C above that of the unrestrained rats, but it is evident that their thermoregulatory system is in a stressed state because a shift in ambient temperature above or below this zone leads to the beginnings of hyperthermia and hypothermia, respectively. From the study of Nagasaka *et al.* (1979), we conclude that control of heat production and heat loss in the restrained rats is markedly sensitive to ambient temperature, resulting in the relationship seen in the restrained rats in Fig. 6*A*. We suspect that the ability of the restrained rats to regulate heat loss is hampered at ambient temperatures that are normally not stressful. The marked differences in HLI response in unrestrained and restrained rats suggest that

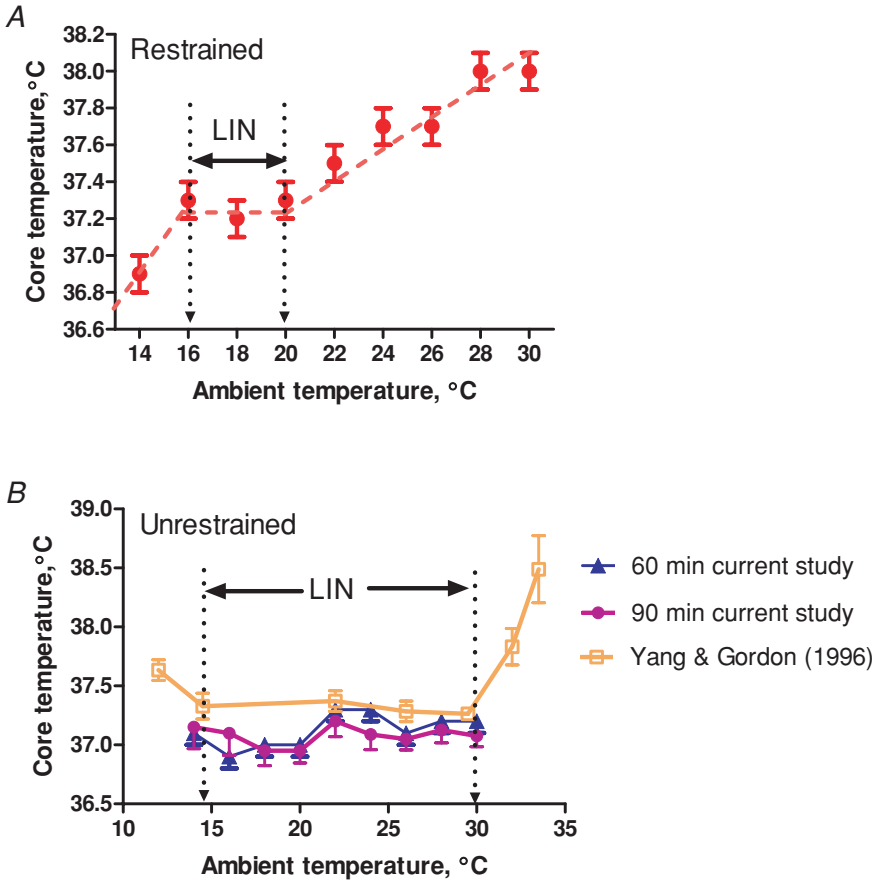

Figure 6. Effect of restraint on limits of normothermia (LIN)
A, estimated limits of normothermia (LIN) for the restrained rat as based on its core temperature following 1 h of restraint. *B*, relationship between T_a and core temperature following 60 and 90 min of exposure in unrestrained rats. Also plotted are data from Yang & Gordon (1996) for 12 h mean daytime core temperatures of Long–Evans rats and estimated LIN.

the stress impairs the normal regulation of heat loss from the tail and possibly other bare surfaces involved in heat exchange (e.g. paws).

Overall, the rats in this study were assumed to be well adapted to the restraining device but still showed a narrowing of the LIN. The responses of naive rats placed under restraint are expected to be markedly exaggerated. In addition, we do not know how restraint limits thermoregulation when it is carried out for a long period of time. Some restraint protocols for inhalation procedures may last for 6 h. To avoid thermal stress in the restrained rat, ambient temperature should be maintained between 18 and 20°C, with 22°C being a maximal temperature of exposure. Interestingly, 22°C is the typical standard room temperature for most laboratories, but temperature can decrease or increase around this average temperature. During restraint conditions, an increase in temperature above 22°C is predicted to be an additional stress to the rat thermoregulatory system. However, additional studies should be performed to evaluate factors such as adaptation to the restraint and length of time of restraint to further evaluate the LIN in the restrained rat. In addition, it would be important to assess the relationship between the LIN and the thermoneutral zone in the restrained rat. In the unrestrained rat, the upper limits of the thermoneutral zone (i.e. the range of ambient temperatures over which metabolic rate is minimal or basal) corresponds closely with the upper limits of the LIN (i.e. 30°C; see Gordon, 1993). There is little, if any, information on the thermoneutral zone of the restrained rat. If the LIN is narrowed during restraint, as shown in the present study, then one would expect a marked shift in the relationship between ambient temperature and metabolic rate, including a shift in the zone of thermoneutrality.

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