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highlighted topics

Molecular Biology of Thermoregulation
Selected Contribution: Ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality

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Romanovsky, Andrej A., Andrei I. Ivanov, and Yury P. Shimansky. Selected Contribution: Ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. *J Appl Physiol* 92: 2667–2679, 2002. First published February 22, 2002; 10.1152/japplphysiol.01173.2001.—There is a misbelief that the same animal has the same thermoneutral zone (TNZ) in different experimental setups. In reality, TNZ strongly depends on the physical environment and varies widely across setups. Current methods for determining TNZ require elaborate equipment and can be applied only to a limited set of experimental conditions. A new, broadly applicable approach that rapidly determines whether given conditions are neutral for a given animal is needed. Consistent with the definition of TNZ [the range of ambient temperature (T_a) at which body core temperature (T_c) regulation is achieved only by control of sensible heat loss], we propose three criteria of thermoneutrality: *1*) the presence of high-magnitude fluctuations in skin temperature (T_{sk}) of body parts serving as specialized heat exchangers with the environment (e.g., rat tail), *2*) the closeness of T_{sk} to the median of its operational range, and *3*) a strong negative correlation between T_{sk} and T_c. Thermocouple thermometry and liquid crystal thermography were performed in five rat strains at 13 T_a. Under the conditions tested (no bedding or filter tops, no group thermoregulation), the T_a range of 29.5–30.5°C satisfied all three TNZ criteria in Wistar, BDIX, Long-Evans, and Zucker lean rats; Zucker fatty rats had a slightly lower TNZ (28.0–29.0°C). Skin thermometry or thermography is a definition-based, simple, and inexpensive technique to determine whether experimental or housing conditions are neutral, subneutral, or supraneutral for a given animal.

thermoneutral zone; Newtonian heat loss; skin vasodilation; skin vasoconstriction; skin temperature; thermography; tail; rat strains

THE OUTCOME OF BIOMEDICAL EXPERIMENTS in the whole animal often depends on the thermal environment. For example, rats do not survive on a protein-poor diet at an ambient temperature (T_a) of 21°C, but they survive and gain body mass on the same diet at a T_a of 5°C (1). During the light phase of the day, rats spend as much as 20% of the time in paradoxical [rapid eye movement (REM)] sleep at a T_a of 29°C, but REM sleep scarcely occurs at a T_a of 34°C (49). A rat's thermoregulatory response to bacterial endotoxin [lipopolysaccharide (LPS)] and the effect of subdiaphragmatic vagotomy on this response are both highly sensitive to T_a (35). At a T_a of 25°C, a high dose of LPS induces hypothermia, which is exaggerated by vagotomy. At a T_a of 30°C, the same dose of LPS causes fever, which is unaffected by vagotomy. The question that then arises is at what T_a should physiological experiments be conducted?

There is no universal answer to this question. Indeed, different physiological functions have different optimal T_a; what is just right for comfortable sleep may be too hot for strenuous exercise. Consequently, experimental protocols to study different functions may require different T_a. Furthermore, certain physiological responses occur only within a particular narrow range of T_a. Thus rodents normally shiver at a low T_a, whereas their thermoregulatory salivation is usually triggered at a high T_a. However, for most biomedical experiments in the whole animal, there is no obvious link between the process studied and the T_a. In these cases, it often makes sense to conduct experiments at a neutral T_a, i.e., within the so-called thermoneutral zone (TNZ). Although definitions of the TNZ are numerous and

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vary substantially in technical details (27), their common denominator is that thermal stress is minimal within the TNZ. This is clearly an advantage of a neutral T_a over sub- and supraneutral T_a. In addition, conducting an experiment in a laboratory animal at a neutral T_a allows for the closest approximation of the results obtained to human physiology because, in a contemporary society, humans (whether healthy or sick) arguably spend most of their time under thermoneutral conditions. The solution then seems to be found: Determine a range of neutral T_a for each species of laboratory animals and always conduct experiments at the T_a established.

Such an approach fails for two reasons. First, within a given species, the TNZ varies widely, depending on a variety of biological factors like health, age, thermal adaptation, and so on. For example, housing rats (46), other mammals, or birds (for review, see Ref. 27) at a low T_a readily shifts their TNZ downward by several degrees. Depending on these biological factors, exposure of the same species or even strain (e.g., Wistar rat) to the same T_a (e.g., 29°C) can be viewed as an exposure to mild heat (cold-adapted, healthy, adult rat), to a neutral T_a (nonadapted, healthy, adult rat), or to moderate cold (malnourished, newborn rat).

Second, the T_a per se is only one of several physical factors determining heat exchange with the environment, which occurs via "dry" (conduction, convection, and irradiation) and "wet" (evaporation) mechanisms (11, 50). In addition to being dependent on the T_a, each mechanism also depends on one or more of the following physical factors: *1*) air humidity, *2*) air velocity, *3*) barometric pressure, *4*) contact with the housing structure (contact area with material other than air and conductive properties of this material), and *5*) effective radiant field. The contribution of some of these factors, especially that of the radiant field, to the overall heat exchange is often underestimated (50). Depending on these factors, an animal's exposure to the exact same T_a can be qualified as exposure to either cold (a single animal in a large, uncovered metal box perfused with humid air at a high velocity) or heat (multiple animals in a covered small plastic box containing excessive amount of bedding material). As a result, a neutral T_a measured in a given experimental setup cannot be used as a standard for experiments conducted in other experimental setups.

If the above arguments are true, the literature can be expected to contain contradictory data on neutral T_a for laboratory animals. And it does. For instance, Herrington (20) and Gordon (13) found that the TNZ for mice is 31–34°C; this range, however, does not even overlap with the range of 26–30°C reported by Oufara et al. (28). Several groups (8, 15, 20, 44, 49) determined the TNZ for the rat to lie between 28 and 34°C, whereas Gwosdow and Besch (18) found that it ranges between 22 and 27°C, and Poole and Stephenson (31) between 18 and 28°C. According to Pace and Rahlman (29), the TNZ is actually a point, not a zone, located at 26.5°C. In the guinea pig, the TNZ was reported to be 14°C wide (20–34°C; Ref. 14) or 0°C wide (29–29°C; Ref. 29) and to center at 25°C (19) or 31°C (20). Such contradictions emphasize that the TNZ for a given species as determined in a particular study is of little help in selecting the T_a for another study with the same species.

A practical solution to this problem is to identify a measurement that instantly determines whether the animal's current environment is thermally neutral, subneutral, or supraneutral. In search of such a measurement, let us consider existing methods for determining the TNZ. The most widely used method is to find the range of T_a in which the metabolic rate is minimal (for detailed discussion of this method and its modifications, see Refs. 16, 27). This method is based on the first edition of the *Glossary of Terms for Thermal Physiology* (6) compiled under the auspice of the Commission for Thermal Physiology of the International Union of Physiological Sciences. It defines TNZ as "The range of T_a within which metabolic rate is at a minimum, and within which temperature regulation is achieved by nonevaporative physical processes alone." However, two subsequent editions of the *Glossary* (8a, 8b) removed the minimal metabolic rate requirement and left only the requirement of the exclusive involvement of nonevaporative heat-loss mechanisms. In addition to not being based on the most recent definition of TNZ, this method (finding the range of T_a in which the metabolic rate is minimal) has several other shortcomings. First, the relationship between the metabolic rate and the T_a is parabola-like, which makes it difficult to robustly define the lower and upper limits of the "flatter" portion of the parabola (27) and often results in an artifactual widening of the TNZ (49). Second, direct determination of the metabolic rate requires an elaborate experimental setup and expensive equipment; it is labor intensive and time consuming. Third and most importantly, measurements of the metabolic rate cannot always be performed in the same physical environment (same setup) as the experiment of interest. The metabolic rate is typically measured in a calorimeter, in which the air velocity, effective radiant field, and contact with the housing structure substantially differ from those of the actual experimental setup (unless the experiment will be performed in the same calorimeter).

Several indirect methods of determining the TNZ also have been proposed. Szymusiak and Satinoff (49) suggested an elegant approach to determine the TNZ as a zone in which the duration of REM sleep is maximal. The underlying assumption is that the animal falls into REM sleep only when internal and external conditions, including thermal, are the most favorable. Furthermore, the thermosensitivity of both heat-production and heat-loss effectors is lowest during REM sleep; hence, the activity of thermoeffectors in this state is lower than it is in wakefulness or slow-wave sleep (12, 30). Unfortunately, this indirect method is applicable only to sleeping animals (the TNZ of awake animals may well differ from that of sleeping animals). This method, which involves electroencephalogram (EEG) recording, is also labor intensive. For adult rats,

maximal REM sleep was reported at a T_a of 29 (26, 49) or 34°C (42).

Another approach is to determine the preferred T_a (thermopreferendum, selected T_a), i.e., the T_a at which the animal spends most of its time when allowed free choice. Thermopreferendum is usually measured in a thermogradient, or thermocline, a device in which different locations have different T_a but are similar otherwise. Not only is it relatively difficult to build a thermogradient (only a few laboratories have the capability of measuring thermopreferendum), but the thermal environment inside the device drastically differs from that in the animal's typical home cage. Usually, a thermogradient is a narrow, tube-like structure made of metal. Inside, the animal stays in contact with thermoconductive floor and walls and is exposed to a unique, highly heterothermal (nonuniform) radiant field. For adult rats, thermopreferendum was reported at 19 (34), 20–25 (9, 15, 43), 27 (25), 24–30 (38), or 30–31°C (33). Although the preferred T_a is usually a neutral T_a, the precise relationship between the two is unclear (16, 48).

Another approach, similar to determining the thermopreferendum, is to find the "inactivity zone" (24). This approach, which has been applied to study environmental preference in fish, is based on an assumption that locomotor activity is minimal when the environment, including thermal, is optimal. (Note that the term TNZ is not applicable to poikilothermic animals; see Refs. 8a, 8b). Interestingly, Poole and Stevenson (31) did not accept the T_a range corresponding to the minimal metabolic rate in rats (28–32°C) as neutral partially on the basis of the argument that motor activity in this range is minimal. The authors considered such inactivity to be a sign of stress rather than of comfort. They suggested a lower range of T_a (18–28°C) as neutral because it was characterized by higher motor activity.

None of the methods of determining the TNZ mentioned above are based on the current definition of the TNZ from the two latest editions of the *Glossary of Terms for Thermal Physiology* (8a, 8b). Both define TNZ as "The range of T_a at which temperature regulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production or evaporative heat loss." Sensible, or Newtonian, heat loss is the total heat loss due to all heat-exchange mechanisms except for evaporation. In practice, the major physiological mechanism of sensible heat loss is cutaneous vasodilation, especially in body parts that serve as heat exchangers with the environment. Such specialized structures are characterized by a high surface-to-volume ratio, the absence of fur, a dense network of blood vessels, and the presence of arteriovenous anastomoses. Examples of such heat exchangers are the human hand, rabbit ear, and rat tail; the latter can conduct 10% of cardiac output and dissipate as much as 40% of the basal metabolic rate (51).

The aim of the present study was twofold. First, we developed three criteria for determining the TNZ on the basis of its current definition. Second, we applied the criteria developed to measure the TNZ of adult rats of five strains in an experimental setup standard for our laboratory. As a corollary, we developed practical guidelines to determine whether the conditions of a given experiment are thermally neutral, subneutral, or supraneutral for the animal under investigation.

Theoretical Groundwork: Criteria Developed

Criterion 1. It is well known that the vasomotor tone of skin in specialized heat exchangers (e.g., rat tail) depends on the T_a. If skin vessels are constantly constricted, an animal likely is exposed to a low (subneutral) T_a. If the vessels are constantly dilated, an animal probably is exposed to a high (supraneutral) T_a. If skin vasomotor tone exhibits substantial fluctuations, frequently changing between vasoconstriction [when body core temperature (T_c) falls below the threshold T_c for skin vasoconstriction] and vasodilation (when T_c rises above the threshold T_c for vasodilation), this can be considered a sign of neither cold nor hot (i.e., neutral) conditions. Indeed, according to the definition of TNZ (8a, 8b), thermoregulation under neutral conditions is achieved by changing skin blood flow. Because technically it is easier to measure tail skin temperature (T_{sk}) than skin blood flow or vasomotor tone, T_{sk} was measured in the present study.

It should be noted, however, that T_{sk} exhibits not only "active" changes (reflecting changes in the vasomotor tone of skin vessels) but also "passive" ones (due to changes in either T_c or T_a, even in the absence of changes in vasomotor tone). To eliminate such passive changes in T_{sk}, Székely (45) introduced the ratio of two temperature gradients, skin-ambient and core-ambient, which we termed the heat loss index (X) (36). X is calculated as

$$X = \frac{T_{sk} - T_a}{T_c - T_a} \quad (1)$$

The physical meaning of X is the fraction of the total Newtonian heat loss from the body "core" to the environment that occurs as a result of nonevaporative heat exchange between the skin and the environment. X changes between 0 (the lower limit corresponding to the maximal possible skin vasoconstriction; $T_{sk} = T_a$) and 1 (the theoretical higher limit corresponding to the maximal possible vasodilation; $T_{sk} = T_c$). X has been used successfully to evaluate the thermoregulatory responses in the rat tail (37), guinea pig ear (36, 47), and human finger (4); in the latter case, a strong correlation between X and blood flow was found.

We propose that *criterion 1* for the TNZ is a wide range of fluctuations of X (ΔX) determined as follows

$$\Delta X = X_{high} - X_{low} \quad (2)$$

where X_{high} and X_{low} are, respectively, the highest and lowest values of X within the epoch recorded at a given T_a. ΔX is a measure of the active variability in T_{sk}.

Criterion 2. Although high values of ΔX mean that skin vasomotor tone is definitely involved in the control of T_c, the opposite statement is not true, i.e., low values of ΔX do not necessarily mean that skin vasomotor tone is not involved. There are two reasons for this. First, ΔX depends on the position of the thermocouple on the tail. As is clear from *Eq. 1*, X approaches 1 when T_{sk} is measured at the tail base ($T_{sk} \approx T_c$), whereas X approaches 0 when T_{sk} is measured at the tip of the tail ($T_{sk} \approx T_a$). In the latter case, even large fluctuations in vasomotor tone would lead to only minimal fluctuations in X. Second, if T_c is regulated tightly (a narrow interthreshold T_c zone between vasoconstriction and vasodilation) and the effector is highly efficient (which it is; see Ref. 51), then high-frequency, low-magnitude changes in vasomotor tone can be expected in the TNZ and lead to low values of ΔX. To address both problems (distal location of the T_{sk} probe and tight control of T_c), we introduce the "middleness" index (Y), which is calculated as

$$Y = (X - X_{min}) \cdot (X_{max} - X) \quad (3)$$

where X_{min} and X_{max} are the minimal and maximal values of X possible for the given location of the probe in animals with similar anatomical and physiological characteristics that determine heat transfer to and from the tail. The procedure of estimating X_{min} and X_{max} for each rat strain used in the present study is described in *criterion 2* in *Data Processing and Analysis*. (Note that X_{min} and X_{max} in *Eq. 3* are generally not the same as X_{low} and X_{high} in *Eq. 2*.) The $Y(X)$ function's graph is a parabola crossing the zero line twice, at X_{min} and X_{max}, with its apex at $X = (X_{min} + X_{max})/2$. The theoretical lower limit of Y is 0. The theoretical upper limit of Y strongly depends on the position of the thermocouple on the tail. Thus, at the base of the tail, where X can reach 1.0, the theoretical upper limit of Y is $(1.0/2)^2 = 0.25$ ($X_{min} = 0$; $X_{max} = 1.0$). However, if the position of the tail skin thermocouple is such that X cannot exceed 0.4 ($X_{min} = 0$; $X_{max} = 0.4$), the upper limit of Y is only $(0.4/2)^2 = 0.04$. Low, near-zero values of Y mean that the animal is either constantly vasoconstricted or constantly vasodilated, which corresponds to a subneutral or supraneutral thermal environment, respectively. High values of Y mean that tail skin vessels are neither constricted nor dilated, and that X is in the middle portion of its operational range; such a situation can occur in the neutral environment. Thus, *criterion 2* for finding a TNZ is a high Y (closeness of X to the median of its operational range).

Criterion 3. On the basis of the *Glossary* definition (8a, 8b), the TNZ should satisfy *criteria 1* and *2*. However, both criteria are necessary but not sufficient because they suggest that skin vasomotor tone is involved in the regulation of T_c, but they do not show whether this effector's contribution is predominant. To address this issue, we propose a third criterion, which is based on the correlation between T_c and T_{sk}. Many authors have observed strong negative correlations between T_c and T_{sk} in a thermoneutral environment (for illustrations, see Refs. 23, 51). Indeed, at a constant T_a, when T_c increases and reaches the threshold T_c for skin vasodilation, blood vessels in heat exchangers become dilated, Newtonian heat loss increases, and T_c starts decreasing. When T_c decreases and reaches the threshold for skin vasoconstriction, the opposite processes occur. Such a negative correlation must be strongest when the vasomotor tone of the tail skin vasculature is the only thermoeffector mechanism used because, in this case, all active changes in T_c would reflect changes in tail skin vasomotion. When several effectors are involved, the relationship between T_{sk} and T_c is "contaminated" by the contribution of other thermoeffectors, and a weaker correlation is expected. When the skin vasomotor tone is not continuously adjusted to the current thermoregulatory needs (i.e., when incessant vasoconstriction or vasodilation is exhibited), there is only "passive" heat exchange between the body's core and the tail. Under such circumstances, T_{sk} either correlates positively with T_c ($X \approx 1$) or loses its dependence on T_c ($X \approx 0$). The passive relationships between T_{sk}, T_c, and T_a become clear if *Eq. 1* is modified as follows

$$T_{sk} = X \cdot T_c + (1 - X) \cdot T_a \quad (4)$$

Thus either a positive correlation or no correlation between T_{sk} and T_c can be expected under deep subneutral and high supraneutral conditions, whereas a strong negative correlation between the two temperatures (*criterion 3*) is expected within the TNZ.

List of Symbols

T_a	Ambient temperature, °C
T_c	Body core (e.g., colonic) temperature, °C
T_{sk}	Tail skin temperature, °C
X	Heat loss index; a ratio of two temperature gradients ($T_{sk} - T_a$) and ($T_c - T_a$); dimensionless
X_{high}	Highest value of X recorded within a given epoch; dimensionless
X_{low}	Lowest value of X recorded within a given epoch; dimensionless
ΔX	Difference between X_{high} and X_{low}; dimensionless
X_{max}	Theoretical upper limit of X for a given location of the skin thermocouple; dimensionless
X_{min}	Theoretical lower limit of X for a given location of the skin thermocouple; dimensionless
Y	Middleness index; product of ($X_{max} - X$) and ($X - X_{min}$); dimensionless
z	Coefficient of correlation between T_c and T_{sk} in a given subject for a given condition; dimensionless
Z	Mean coefficient of correlation between T_c and T_{sk} for a given condition; calculated as a weighted mean of the coefficients z; dimensionless

METHODS

Animals

Ninety three male rats were purchased from Charles River Laboratories (Wilmington, MA): 32 Wistar [Crl(WI)BR], 23 Long-Evans [Crl(LE)BR], 17 BDIX (BDIX/CrCrlBR), 10 Zucker fatty [Crl:(Zuk)-*fa*/*fa*BR], and 11 Zucker lean [Crl:(Zuk)-*Fa*/*?*BR]. At the time of the experiments, all animals were 7–9 wk old. Rats were housed three per standard acrylic cage. The room was on a light-dark cycle of 12:12 h (lights on at 7:00 AM); T_a was maintained at 22°C. Food (Teklad Rodent Diet "W" 8604, Harlan Teklad, Madison, WI) and water were available ad libitum. The animals were handled and weighed regularly. They were also habituated (five 3- to 4-h training sessions) to cylindrical confiners made of stainless steel wire. The same confiners were used later in the experiments. The confiners limited the animals' back-and-forth movements and prevented them from turning around. These confiners have been used extensively in our laboratory (22, 37–39). Rats easily adapt to them and often prefer them to the open space of their home cages. Neither a stress fever (7) nor any other signs of stress have been found in well-adapted, restrained rats. In fact, the confined rats always have the same body temperature as their freely moving counterparts would have at the same T_a (for detailed discussion, see *Thermometry in Restrained Rats: A Special Consideration*, Ref. 39). All experiments began at 9:00 AM. The protocols have been approved by the Legacy Health System (Portland, OR) and St. Joseph's Hospital and Barrow Neurological Institute (Phoenix, AZ) Institutional Animal Care and Use Committees.

Instrumentation

For an experiment, each rat was instrumented with cooper-constantan thermocouples to record its colonic temperature (a measure of T_c) and tail T_{sk}. The colonic thermocouple was inserted ~9 cm beyond the anal sphincter and fixed to the base of the tail with tape. The skin thermocouple was positioned on the lateral surface of the tail approximately at the border of its middle and distal thirds. The thermocouple was affixed to the tail and shielded from heat loss to the ambient air with a loop of insulating tape. The thermocouples were connected to a data logger (model AI-24, Dianachart, Rockaway, NJ) and a personal computer. The rat was then placed in the confiner (described in *Animals*) and transferred to a climatic chamber (Forma Scientific, Marietta, OH).

Experimental Protocol

Initially, T_a in the climatic chamber was maintained at a level randomly selected from the following set of 13 T_a: 27.0, 27.5, 28.0, 28.5, 29.0, 29.5, 30.0, 30.5, 31.0, 31.5, 32.0, 32.5, or 33.0°C. (The two highest T_a were used only in experiments in BDIX rats.) Relative humidity was maintained at 50%. After a stabilization period (~90 min), measurements were begun, and T_c, T_{sk}, and T_a were sampled every 20 s for 45 min. Thereafter, T_a in the chamber was changed to a different one, randomly chosen from the same set of 13 T_a. After another stabilization period (~90 min), T_c, T_{sk}, and T_a were measured again for 45 min. Finally, one more T_a was randomly chosen, and the measurements were repeated as described above. Typically, three (and no more than three) T_a were tested in each experiment. Each animal was subjected to no more than four experiments with at least 2 days of rest between experiments. On average, each rat was studied at five T_a.

Experiment with Thermosensitive Liquid Crystal Paint

In one experiment in four Wistar rats, no thermometry was performed, but the rats' tails were coated with temperature-sensitive liquid crystals and photographed. Rats were placed in stocks and then in the climatic chamber. Their tails were threaded through holes in a white cardboard screen, which was later used as a background for photographs. The tails were covered with two coats of black "backing" paint followed by two coats of R25C5W thermal liquid crystal paint (Daigger, Lincolnshire, IL). T_a was initially set at 27.0°C and then changed, first to 29.0 and later to 32.0°C. At each T_a, the tails were photographed with a digital camera.

Data Processing and Analysis

Data preprocessing. To reduce the variability of T_{sk} due to the possible difference in positioning of the tail skin thermocouple, data were preprocessed as follows. First, the median value of T_{sk} was determined for each rat (subject) under each T_a (condition). Second, for each condition, median values were averaged across subjects. Third, for each subject under each condition, a correction coefficient was calculated by dividing the intersubject mean T_{sk} by the subject's median T_{sk}. Finally, each value of T_{sk} of each subject under each condition was multiplied by this correction coefficient.

Criterion 1. To estimate the variability of X, ΔX was calculated according to *Eq. 2*. First, the estimates of X_{high} and X_{low} were found as the highest and lowest values of X for each subject under each condition, and the median ΔX was determined across subjects for each condition. The $\Delta X(T_a)$ curves obtained resembled the Greek letter Ω, either in its normal (vertical) position or slightly rotated to the right or to the left. The horizontal segments of the Ω were assumed to belong to the same straight line representing the passive (i.e., in the absence of any changes in the control of skin vasomotion) dependence of ΔX on T_a. The middle, bell-shaped segment of the Ω was assumed to be due to active changes in the vasomotor control in the TNZ. Next, the T_a range corresponding to the higher values of ΔX was determined by best-fitting each $\Delta X(T_a)$ curve with a combination of a parabola (for the middle segment) and straight line (for the two marginal segments) and finding their intersection points.

Criterion 2. To calculate Y, we first determined X_{min} and X_{max} (*Eq. 3*). The preliminary analysis of the data collected indicated that T_{sk} approached T_a at low T_a, meaning that $X_{min} \approx 0$ (see *Eq. 1*). It was also observed that T_{sk} never approached T_c under the T_a tested, meaning that, for the position of the skin thermocouple used, X_{max} did not reach its theoretical limit of 1. To estimate X_{max}, two assumptions were made, viz., that X_{high} approaches X_{max} and that ΔX approaches 0 at high T_a. On the basis of these assumptions, the following procedures were performed. First, the experimentally obtained curves $X_{high}(T_a)$ and $\Delta X(T_a)$ were linearly approximated over the range of the three highest values of T_a used. Second, the curve $\Delta X(T_a)$ was extrapolated to find the value of T_a corresponding to $\Delta X = 0$. Next, we estimated X_{max} for each rat strain as the value of X_{high} at the T_a corresponding to 0 value of ΔX and found Y (see *Eq. 3*). Finally, the T_a range corresponding to the higher values of Y was determined by best fitting each $Y(T_a)$ curve with a combination of a parabola (the middle segment) and straight line (the two marginal segments) and finding their intersection points, as was done to find the T_a range corresponding to the highest values of ΔX (see *Criterion 1* above).

Criterion 3. Prior to the correlation analysis, T_c and T_{sk} records were preprocessed by subtracting the corresponding temporal trend determined on the basis of second-order poly-

nomial fitting. Then, for each subject under each condition, the coefficient z of correlation between T_c and T_{sk} was found, and the mean coefficient Z was calculated for each condition as a weighted mean of the coefficients z. The weights were normalized so that their sum was equal to 1, and each weight was calculated as proportional to the inversed variance of the corresponding z (i.e., larger weights were assigned to correlation coefficients with a higher level of statistical significance). Next, in each curve $Z(T_a)$, a 1°C-wide segment of the highest negative and a 1°C-wide segment of the highest positive steepness were found. The steepness was measured on the basis of best fitting a straight line across three adjacent points on the curve. Then, all points between the two steepest segments (including the segments' end points) were used to best fit a straight horizontal line. The margins of the TNZ were determined as the intersection points of this horizontal line with the two slopes.

RESULTS

Phenomena Recorded

Typical temporal dynamics of T_{sk} and T_c at "low" (27.0°C), "intermediate" (29.5°C), and "high" (32.0°C) T_a are shown by individual curves obtained in Zucker lean rats (Fig. 1). Normally, T_{sk} exhibited no oscillations at 27.0°C and no or low-magnitude (<1°C) oscillations at 32.0°C. However, T_{sk} fluctuated markedly at 29.5°C, often by several degrees Celsius. Not only the spectra of T_{sk} fluctuations but also the relationship between T_{sk} and its minimal and maximal values differed drastically at different T_a (Fig. 2). In Fig. 2, T_{sk} is expressed as X, and values of X are shown relative to

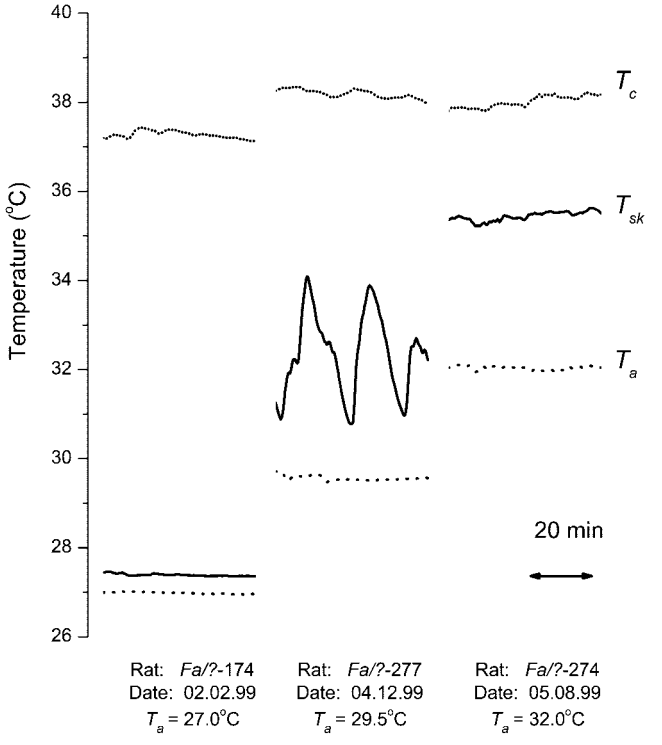

Fig. 1. Typical records of core temperature (T_c), tail skin temperature (T_{sk}), and ambient temperature (T_a) obtained in 3 experiments conducted in 3 different Zucker lean rats at 3 different T_a: 27°C (*left*), 29.5°C (*middle*), or 32°C (*right*).

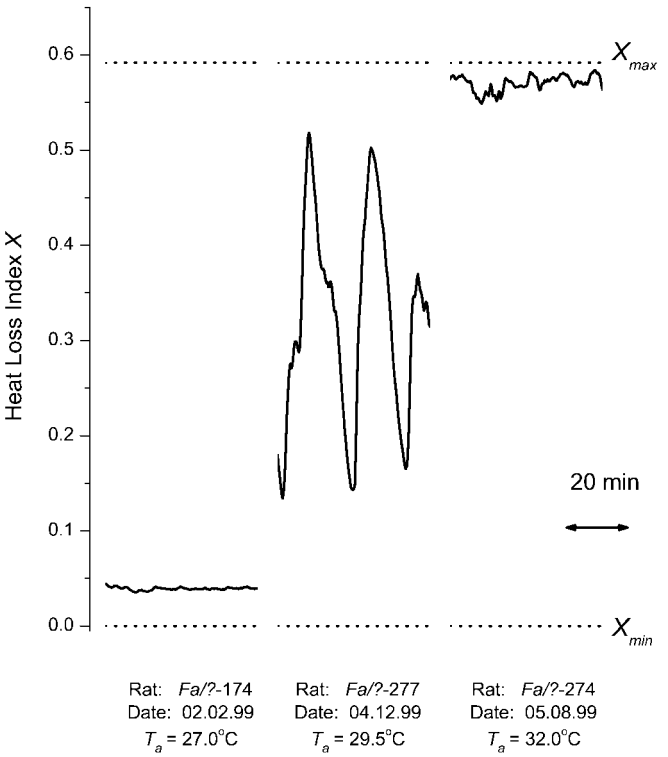

Fig. 2. Values of tail T_{sk}, expressed as the heat loss index (X; see *Criterion 1* under *Theoretical Groundwork: Criteria Developed*), are shown relative to the lower (X_{min}) and upper (X_{max}) limits of the operational range of X for three different Zucker lean rats at three different T_a. Data presented are the same as in Fig. 1.

X_{min} (corresponds to maximal vasoconstriction) and X_{max} (corresponds to maximal vasodilation) for the given rat strain (Zucker lean). T_{sk} was always near the lower limit of its operational range throughout the

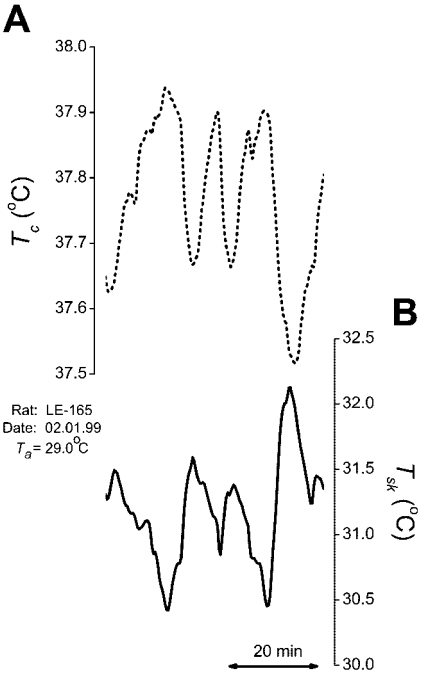

Fig. 3. Simultaneous records of T_c (*A*) and T_{sk} (*B*) obtained from a Long-Evans rat at a T_a of ~29°C.

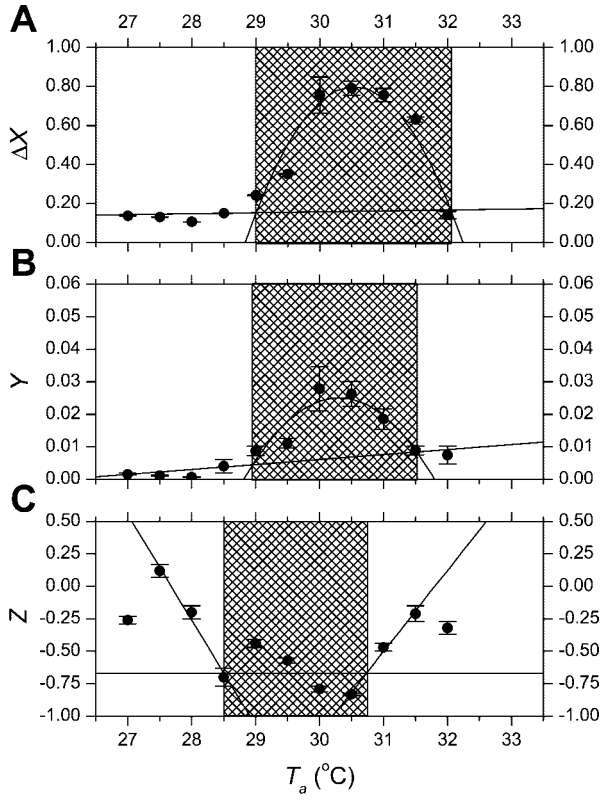

Fig. 4. Dependence of three T_{sk}-derived indexes on T_a for Wistar rats. The indexes shown are the magnitude of changes in X (ΔX), the middleness index (Y), and the mean correlation coefficient (Z) between T_c and T_{sk} (for details, see *Data Processing and Analysis*). Each index defines a TNZ (shaded area) as follows: range of T_a corresponding to a high magnitude of T_{sk} fluctuations (high values of ΔX); the range of T_a in which T_{sk} is close to the median of its operational range (high values of Y); and the range of T_a corresponding to a strong negative correlation between T_c and T_{sk} (high negative values of Z). These three ranges correspond to the three criteria of the TNZ (see *Theoretical Groundwork: Criteria Developed* in METHODS). Data are best fitted by a combination of parabolas and straight lines (see *Data Processing and Analysis*). Data are presented as means ± SE.

experiment conducted at the low T_a and always near the higher limit of its operational range at the high T_a. At the intermediate T_a, T_{sk} fluctuated in the middle portion of the range. When fluctuations in T_{sk} were large, T_c and T_{sk} frequently changed in a coordinated fashion, i.e., an increase in T_c was accompanied by a decrease in T_{sk} (tail skin vasoconstriction), whereas a decrease in T_c was accompanied by an increase in T_{sk} (vasodilation). Such coordinated changes are illustrated by temperature records obtained in a Long-Evans rat at 29.0°C (Fig. 3). Visual inspection of the raw data revealed no marked interstrain differences, except that BDIX and Wistar rats tended to develop high-magnitude fluctuations in T_{sk} at slightly higher T_a than Long-Evans and Zucker rats. This inspection also suggested the following for a narrow zone of T_a around 29–30°C: Fluctuations in T_{sk} are large (*criterion 1* for the TNZ; Fig. 1), the value of T_{sk} is near the middle of its operational range (*criterion 2*; Fig. 2), and the correlation between T_{sk} and T_c is strongly negative (*criterion 3*; Fig. 3).

Criteria Applied

To define the TNZ, we applied *criteria 1*, *2*, and *3* (see *Theoretical Groundwork: Criteria Developed*) to each rat strain and found three ranges of T_a, viz., a range characterized by large fluctuations in T_{sk} (*criterion 1*), a range in which the value of T_{sk} is near the median of its operational span (*criterion 2*), and a range of strong negative correlation between T_{sk} and T_c (*criterion 3*). These three ranges were determined as having high values of ΔX, high values of Y, and high negative values of Z, respectively (see *Data Processing and Analysis*). The results are presented as graphs of $\Delta X(T_a)$, $Y(T_a)$, and $Z(T_a)$ for Wistar (Fig. 4), BDIX (Fig. 5), Long-Evans (Fig. 6), Zucker lean (Fig. 7), and Zucker fatty (Fig. 8) rats. All strains showed prominent, bell-shaped increases in both ΔX and Y (Fig. 7). For each strain, the two bell shapes were positioned in a similar range of T_a and peaked at almost the same T_a. In all strains, the dependence of Z on T_a also was similar. A strong negative correlation between T_c and T_{sk} (Z often approaching −1) was observed in the middle portion of the T_a range investigated (e.g., Figs. 6 and 8). However, Z rapidly increased and often crossed the zero line at T_a above and below the negative correlation zone (e.g., Figs. 5 and 8). The borders of the TNZ for each strain, as defined by the three criteria applied, are shown in Fig. 9. For all strains, the TNZ, as determined by any of the three criteria, lies within the T_a range of 27.4–32.5°C. Figure 9 also shows the

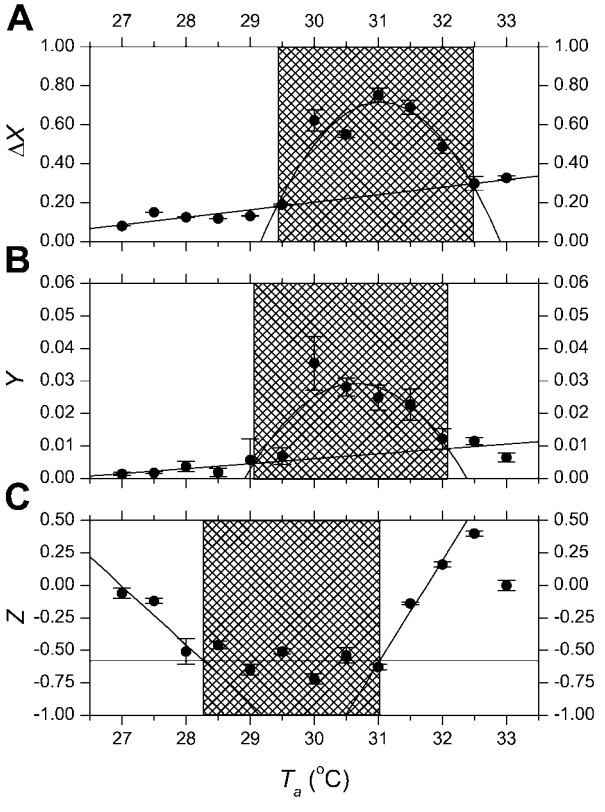

Fig. 5. Dependence of three T_{sk}-derived indexes, i.e., ΔX, Y, and Z (*A, B,* and *C,* respectively), on T_a for BDIX rats.

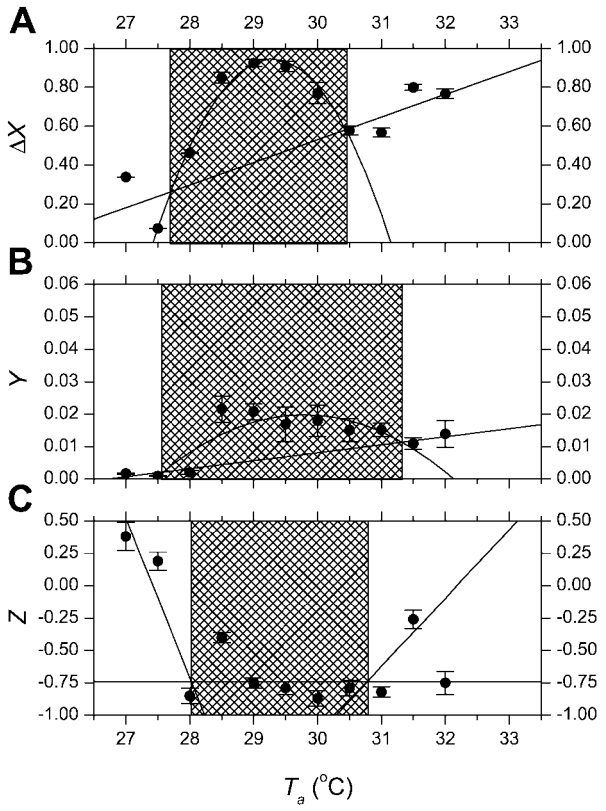

Fig. 6. Dependence of three T_{sk}-derived indexes, i.e., ΔX, Y, and Z (*A, B,* and *C,* respectively), on T_a for Long-Evans rats.

TNZ satisfying all three criteria for each strain. The low border of this "conservative" TNZ is the same as the highest one among the three lower borders determined by the three individual criteria; the high border is the lowest determined by the three individual criteria. Except for Zucker fatty rats, the range of 29.4–30.4°C seems to satisfy all criteria for all rat strains tested under our conditions. The design of our study, i.e., determination of the TNZ in a rat strain and not in individual rats, did not permit statistical comparison across strains.

Thermography

In an experiment in four Wistar rats, the rats' tails were coated with a temperature-sensitive liquid crystal suspension. Rats were exposed for 2 h to each of three T_a (viz., ~27.0, 29.0, and 32.0°C), and photographs were taken at the end of each exposure period (Fig. 10). At a T_a of 27°C, the entire tail surface was brown-black (T_{sk} of <30°C), with almost no intra- and intersubject variation. This finding indicates that no substantial vasodilation occurred at this T_a during the time of observation. At a T_a of 32°C, the entire tail surface was homogenously dark blue (T_{sk} of ≥35°C), with no intra- or intersubject variation. This indicates that all rats exhibited tail skin vasodilation at the time the photograph was taken. At a T_a of 29°C, different portions of the tail skin in different animals were brown or black (T_{sk} of <30°C), green ($T_{sk} \approx 30$°C), light blue (30°C < T_{sk} < 35°C), or dark blue (T_{sk} of ≥35°C), with large inter- and intrasubject variations. This means that the skin vessels of different animals had different vasomotor tone, varying from marked vasoconstriction (T_{sk} of <30°C; the far right animal) to intermediate states (30°C < T_{sk} < 35°C; two rats on the left) to marked vasodilation ($T_{sk} \geq 35$°C; second rat from the right). Interestingly, Fig. 10 also illustrates the fact that T_{sk} strongly depends on where along the tail it is measured (see *Theoretical Grounds: Criteria Developed*), and that a shift from vasoconstriction to dilation is equivalent to moving the thermocouple toward the tail base (see Fig. 10, two images on *left*; T_a of 29°C).

DISCUSSION

TNZ in the Rat: Criteria Applied

It has long been known that T_{sk} (or blood flow) exhibits a high variability and/or strong negative correlation with T_c in the TNZ (3, 32, 41, 51), but these phenomena have not been used to determine the TNZ. On the basis of the current definition for TNZ (8a, 8b), we developed three criteria of thermoneutrality (*Theoretical Grounds: Criteria Developed*): a high magnitude of T_{sk} fluctuations (*criterion 1*), closeness of T_{sk} to the median of its operational range (*criterion 2*), and a strong negative correlation between T_c and T_{sk} (*criterion 3*). We applied these three criteria to five rat strains and, for each strain, found three corresponding

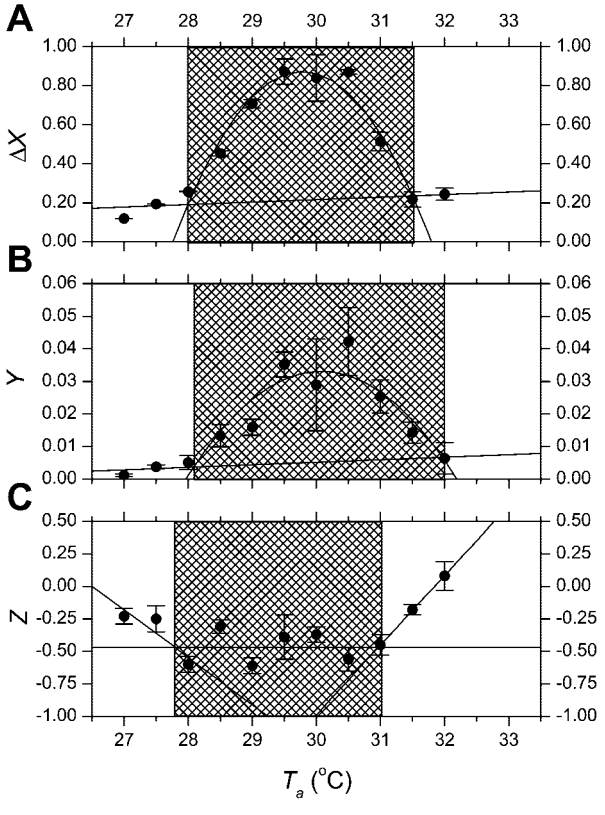

Fig. 7. Dependence of three T_{sk}-derived indexes, i.e., ΔX, Y, and Z (*A, B,* and *C,* respectively), on T_a for Zucker lean rats.

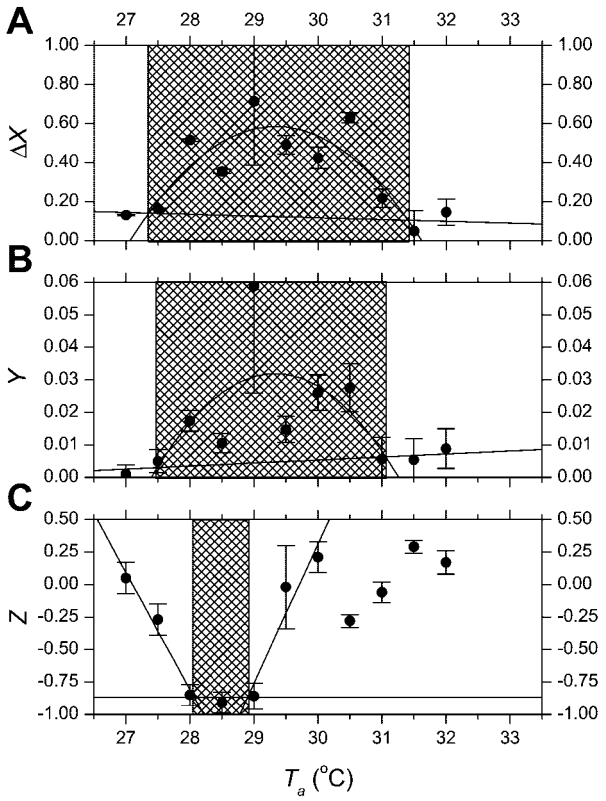

Fig. 8. Dependence of three T_{sk}-derived indexes, i.e., ΔX, Y, and Z (*A, B,* and *C,* respectively), on T_a for Zucker fatty rats.

ranges of neutral T_a. For each rat strain, we also found a TNZ satisfying all three criteria, the conservative TNZ. To the nearest half degree, this conservative TNZ was 29.0–30.5°C for Wistar, 29.5–31.0°C for BDIX, 28.0–30.5°C for Long-Evans, 28.0–31.0°C for Zucker lean, and 28.0–29.0°C for Zucker fatty rats.

The data obtained are internally consistent and agree well with several earlier studies, thus confirming the legitimacy of the criteria established. First, for each rat strain tested, each criterion readily defined a narrow TNZ, and the zones determined on the basis of different criteria were reasonably close to each other (Fig. 9). The sufficient *criterion 3* determined a slightly narrower TNZ than either of the two necessary criteria. Second, except for Zucker fatty rats, the range 29.5–30.5°C appeared to satisfy all three criteria for the TNZ in all rat strains studied under our conditions. This range is within the TNZ as determined by the minimal metabolic rate for several rat strains: Wistar (28–32°C; Ref. 31), Holtzman (28–33°C; Ref. 8), a nonspecified strain of laboratory rats (29–31°C; Ref. 20), Long-Evans, Sprague-Dawley, and Fischer-344 (all 28–32°C; Ref. 15). Third, the maximal REM-sleep time, which is considered a marker of thermoneutrality, occurs in Long-Evans rats at 28–30°C (49). This range is almost identical to the conservative TNZ for Long-Evans rats found in the present study (28.0–30.5°). Fourth, neither the minimal metabolic rate (15) nor our approach reveals substantial differences in the TNZ among the regular strains of laboratory rats. Fifth, Zucker fatty rats possess some, although minor, thermoregulatory peculiarities compared with lean rats (2, 10, 22). For the same T_a, the rates of heat production and heat loss are both slightly higher in obese animals, whereas their T_c and metabolic responses to cooling are slightly lower (2, 10). TNZ (as determined by maximal time of REM sleep) has been reported to be similar, if not the same, for obese and lean rats (26). Likewise, the present results show that Zucker obese rats have an overlapping (yet slightly lower and slightly narrower) TNZ than the other strains tested.

Neutral T_a or Neutral Operative T_a?

Most reported data (8, 15, 20, 26, 31, 44, 49) as well as the present results suggest that the TNZ for the rat is narrow (2–4°C) and centered at 29–30°C. Yet some authors have found a much wider and lower zone (22–27°C or 18–28°C; Refs. 18, 31) or a much narrower and lower zone (26.5–26.5°C; Ref. 29). Such divergence is expected because the TNZ depends on many biological (age, thermal adaptation, etc.) and physical (air humidity, air velocity, barometric pressure, contact with the housing structure, effective radiant field) factors and hence varies widely (see *introduction*).

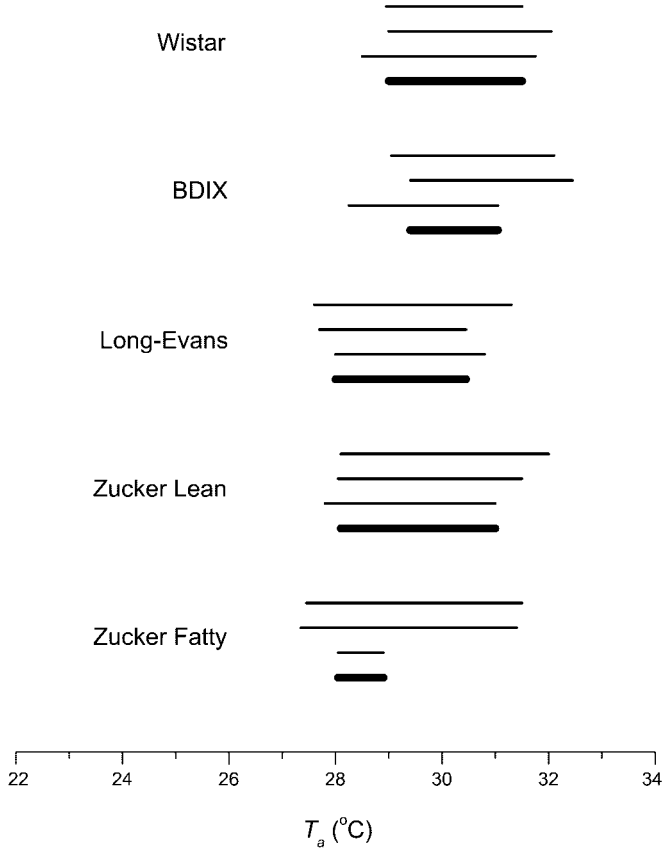

Fig. 9. Four TNZs are shown for each of the five rat strains listed: *1*) TNZ defined on the basis of *criterion 1* (first thin line from the top); *2*) TNZ defined on the basis of *criterion 2* (second thin line); *3*) TNZ defined on the basis of *criterion 3* (third thin line); *4*) the "conservative" TNZ that satisfies all three criteria (thick line). See also *Criteria Applied* under RESULTS.

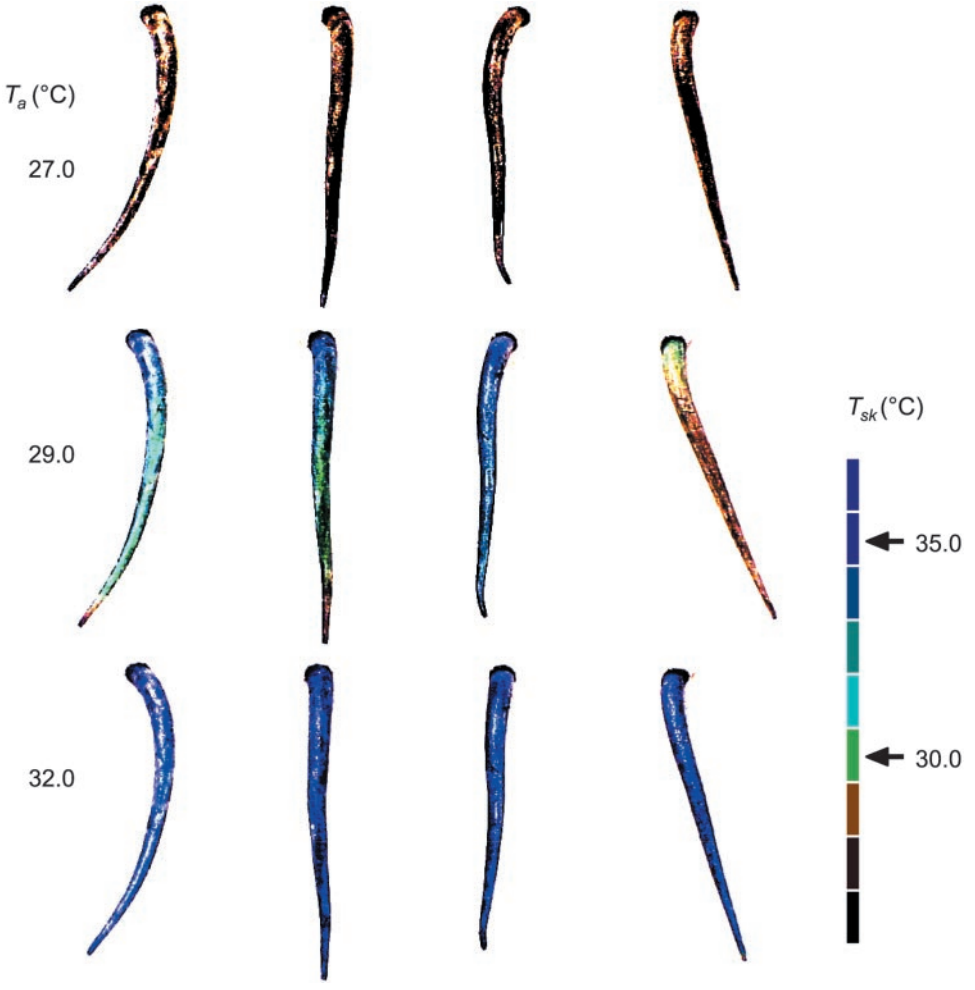

Fig. 10. Tails of four Wistar rats coated with temperature-sensitive liquid crystals. Photographs were taken at a T_a of 27°C (*top*), 29°C (*middle*), or 32°C (*bottom*). An approximate scale of T_{sk} is shown at *right*.

The contribution of the radiant field to the overall heat exchange is often underestimated (50) and so is the contribution of the animal's contact with the housing structure (contact area and conductive properties of the material). Gordon et al. (17) measured the cooling rate of a "phantom mouse" (small aluminum cylinder) inside a standard acrylic cage and calculated the operative T_a. Operative T_a is the T_a of a uniform (isothermal) "black" enclosure with which the body of the mouse would have the same rate of Newtonian heat exchange as with the actual nonuniform environment of the cage (8a). Adding a filter top to the cage increased operative T_a by ~2.0°C; adding wood-shaving bedding led to an additional increase of ~6.3°C; and burying the mouse in the shavings resulted in a further increase in operative T_a of ~2.5°C (17). In another experiment (same paper), increasing the number of mice in an aluminum enclosure (thermogradient) from one to four decreased the preferred T_a by 1.0–1.5°C, presumably reflecting an equal increase in the operative T_a. Thus adding bedding and a filter top to the cage changes the operative T_a of a single mouse from 19.2 to 30.5°C (at a room T_a of 22°C), and switching to group housing should further increase operative T_a to ~32°C. These results by Gordon et al. (17) clearly demonstrate that even small changes in experimental conditions can strongly affect heat exchange between the animal and environment and, therefore, change the animal's neutral T_a.

Although a range of neutral T_a determined in one experimental setup cannot necessarily be applied to another setup, it can be used as a rough estimate of the TNZ under similar experimental conditions. Thus, based on the present study and studies by others (8, 15, 20, 26, 31, 44, 49), a T_a of ~30°C is neutral for adult rats of common strains in calorimeters, environmental chambers, and similar setups (no bedding, no filter tops, minimal behavioral thermoregulation, no group thermoregulation). For rats maintained in their home cages (with bedding and/or filter tops and/or under group housing), the neutral T_a is likely to be a few degrees lower. Indeed, a recent report by Hosono et al. (21) demonstrates that rats under conditions similar to those in their home cages show the largest fluctuations in T_{sk} at a T_a of ~25°C.

The major problem with the TNZ is the following. When it is expressed in terms of actual T_a (traditionally defined TNZ), it is applicable only to the set of environmental conditions in which it was measured; when physical conditions change, the TNZ changes. To

standardize all experimental conditions (so that the traditionally measured TNZ becomes a standard applicable to a variety of experimental setups) is unrealistic. On the other hand, the TNZ can be expressed in terms of operational T_a. In this case, it does not depend on experimental conditions and becomes universal. Unfortunately, measuring operative T_a is technically challenging (17), which makes this approach impractical. Rather than establishing a norm for the TNZ, whether expressed as a range of T_a or operative T_a, a better practical approach would be to use criteria (such as those presented here) to determine whether given experimental conditions are thermally neutral, supraneutral, or subneutral for the particular animal.

Practical Recommendations and Limitations

The present study demonstrates that skin thermometry (Fig. 1) or thermography (Fig. 10) of the specialized heat-exchange organs can be used to determine whether given experimental conditions are neutral. Thermocouple thermometry and/or temperature-sensitive paint (both used in the present study) can be recommended as inexpensive and simple techniques. (Although costly, other techniques for measuring T_{sk} or skin blood flow, such as infrared thermography, T_{sk} telemetry, or laser Doppler flowmetry, also may be used.) For practical purposes, environmental conditions should be regarded as subneutral if T_{sk} (measured by any of these methods) is constantly low (close to T_a) and shows no substantial variation, either in time or across animals. Conditions should be regarded as supraneutral if T_{sk} is constantly high (relatively close to T_c) and exhibits no substantial variation. Conditions should be regarded as neutral if T_{sk} is somewhere between T_a and T_c and highly variable across time and/or animals. If more sophisticated physiological and data processing techniques are plausible, a correlation analysis between T_{sk} and T_c can be performed. A strong negative correlation should be interpreted as an indicator of thermoneutrality; its absence is an indicator of nonneutral thermal conditions.

Each technique described above has its limitations and shortcomings. Some (e.g., thermocouple thermometry) typically require animal restraint, others (e.g., T_{sk} telemetry; see Ref. 21) involve surgical intervention (probe implantation), and still others (e.g., thermography) can be conducted only when the heat-exchange organ is fully visible. Some techniques (infrared thermography) are artifact free, whereas others (skin painting) can affect local heat exchange by themselves. Some procedures (e.g., correlation analysis between T_c and T_{sk}) can determine the TNZ with a high resolution (~0.1°), whereas others (painting with liquid crystals) can be used for rough estimations only. Some study protocols (exposures of a large number of animals to a large number of T_a at different times of day in a random order) can be designed to determine the TNZ for the study population as a whole (as it was done in the thermometry experiment of the present study), whereas other protocols (exposures of the same animal to a small number of T_a within a relatively short time period) can be designed to estimate the individual TNZ (as was done in the thermography experiment). However, the large number of techniques available assures that a reasonably good solution can be found to any particular problem.

In contrast to any specific technique or experimental design, the proposed general approach is applicable to a wide variety of animal species and experimental setups. It has only a few limitations. The study subject must possess a specialized heat-exchange organ (e.g., rat tail, rabbit ear, or human finger). At the time of the study, there must be no strong nonthermoregulatory influences (competitive homeostatic demands, pharmacological treatments, etc.) on vasomotor tone in the heat-exchange organ. The subject should be in thermal equilibrium with its environment (steady state). Finally, T_a studied can range from a low subneutral to a high supraneutral, but it must not be noxious. At extreme, noxious T_a, the paradoxical phenomena of cold-induced vasodilation (5) and heat-induced vasoconstriction (40) can occur and render the proposed approach inapplicable. However, such extreme T_a are not neutral a priori and are, therefore, outside the focus of this study.

Conclusions

We have developed three new T_{sk}-based criteria of thermoneutrality: *1*) a high magnitude of T_{sk} fluctuations, *2*) closeness of T_{sk} to the median of its operational range, and *3*) a strong negative correlation between T_c and T_{sk}. These criteria are derived from the current definition of TNZ (8a, 8b). We applied these three criteria to five rat strains and obtained internally consistent data that are also in agreement with several earlier studies.

There is a widely spread misbelief that the same animal should have the same TNZ under different experimental conditions. TNZ expressed as a range of T_a (not as a range of operative T_a) strongly depends on physical environment and readily changes from one set of environmental conditions to another. As a rough estimation, the TNZ of adult, healthy rats of common strains in experimental setups involving no bedding or filter tops and disallowing for group thermoregulation is likely to be ~30°C. In home cages (with bedding and/or filter tops and/or under group housing), the conditions are expected to be neutral when T_a is a few degrees lower, perhaps in the mid-twenties.

To make sure that the T_a in a particular experimental setup is within the TNZ for a particular animal, measurements should be performed in this particular animal and in the same experimental setup. Skin thermometry (or thermography) is the most direct (definition based), simple, and inexpensive technique to determine whether given experimental or housing conditions are neutral, subneutral, or supraneutral.

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