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Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature

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Rudaya, Alla Y., Alexandre A. Steiner, Jared R. Robbins, Alexander S. Dragic, and Andrej A. Romanovsky. Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. *Am J Physiol Regul Integr Comp Physiol* 289: R1244–R1252, 2005. First published August 4, 2005; doi:10.1152/ajpregu.00370.2005.—Most published studies of thermoregulatory responses of mice to LPS involved a stressful injection of LPS, were run at a poorly controlled and often subneutral ambient temperature (T_a), and paid little attention to the dependence of the response on the LPS dose. These pitfalls have been overcome in the present study. Male C57BL/6 mice implanted with jugular vein catheters were kept in an environmental chamber at a tightly controlled T_a. The relationship between the T_as used and the thermoneutral zone of the mice was verified by measuring tail skin temperature, either by infrared thermography or thermocouple thermometry. *Escherichia coli* LPS in a wide dose range (10^0-10^4 μg/kg) was administered through an extension of the jugular catheter from outside the chamber. The responses observed were dose dependent. At a neutral T_a, low (just suprathreshold) doses of LPS (10^0-10^1 μg/kg) caused a monophasic fever. To a slightly higher dose ($10^{1.5}$ μg/kg), the mice responded with a biphasic fever. To even higher doses ($10^{1.75}$-10^4 μg/kg), they responded with a polyphasic fever, of which three distinct phases were identified. The dose dependence and dynamics of LPS fever in the mouse appeared to be remarkably similar to those seen in the rat. However, the thermoregulatory response of mice to LPS in a subthermoneutral environment is remarkably different from that of rats. Although very high doses of LPS (10^4 μg/kg) did cause a late (latency, ~3 h) hypothermic response in mice, the typical early (latency, 10–30 min) hypothermic response seen in rats did not occur. The present investigation identifies experimental conditions to study LPS-induced mono-, bi-, and polyphasic fevers and late hypothermia in mice and provides detailed characteristics of these responses.

body temperature; fever; hypothermia; febrile phases; thermoneutrality; systemic inflammation; stress; mice; rats

IN RAT AND RABBIT MODELS OF bacterial LPS-induced systemic inflammation, the dynamics of the associated thermoregulatory responses have been well characterized and are known to depend on several factors, including ambient temperature (T_a) and LPS dose (for a review, see Ref. 24). At a neutral T_a, intravenous LPS typically causes fever; low, just suprathreshold doses, cause a single monophasic rise in deep body temperature (T_b), whereas moderate to high doses trigger several sequential T_b rises, named febrile phases (19, 26, 35). At least three febrile phases have been identified in the rat (29, 32) and at least two in the rabbit (19). At a subneutral T_a (cool environment), intravenous LPS evokes fever at low doses, mild hypothermia followed by fever at intermediary doses, and deep hypothermia at high, shock-inducing doses (27, 28, 30, 32, 33).

Over the last decade, genetically engineered mice have been increasingly used as a tool for studying the mechanisms of LPS-induced systemic inflammation and its thermoregulatory manifestations (2, 3, 9, 12–14, 18, 21, 37). However, the thermoregulatory responses to LPS have not been fully characterized in this species due to the following potential pitfalls.

First, a stressful, painful procedure of LPS injection was used in the majority, if not all, studies. Typically (for recent examples, see Refs. 3, 6, 13, 16, 21), a mouse that had not been habituated to the injection procedure was removed from its cage and pricked with the injection needle in its abdomen (for an intraperitoneal injection) or tail (for an intravenous injection). Such a stressful procedure resulted in a highly variable, often profound (1–3°C) and long-lasting (30–120 min), elevation in T_b. Because the febrile response starts 10–30 min after LPS administration in several species, including the rat (35) and rabbit (19), the stress hyperthermia was likely to override the initial febrile rise of T_b. That stress hyperthermia masks the first phase of LPS-induced polyphasic fever in the rat has been shown in a direct experiment (27). Not only does needle pricking-induced hyperthermia hide some parts of the thermoregulatory response to LPS, but it also may alter the dynamics of the response at its later stages. For example, Sugimoto et al. (34) have reported that needle pricking transforms the monophasic febrile response of rats to LPS into a qualitatively different, biphasic, response.

The second pitfall is that many experiments in mice were performed at a poorly controlled T_a: it fluctuated within ~2°C in a single study and varied by almost 10°C among studies (6, 13, 16, 21). For example, T_a was 22–25°C in the studies by Li et al. (16) and Oka et al. (21), but it was 29–31°C in the studies by Gourine et al. (6) and Kozak and Kozak (13). Whereas the former range is clearly below the thermoneutral zone of the mouse, the latter range is neutral or near-neutral (5). In several species, especially small rodents such as the rat, the thermoregulatory response to LPS strongly depends on T_a, and a difference of a few degrees centigrade is sufficient to change the response drastically, e.g., from hypothermia to fever (27).

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Hence, poor control of T_a was likely responsible for the overall inconsistency in the T_b patterns observed in mice.

The third pitfall is that the thermoregulatory responses to LPS strongly depend on its dose. As explained above, both rats (27) and rabbits (19) mount qualitatively different responses to different LPS doses. However, with the exception of a few studies (e.g., Refs. 12 and 21), such dependence was largely ignored in the mouse.

The present study was aimed at identifying and characterizing the murine thermoregulatory responses to LPS under experimental conditions free of the three pitfalls described above. In this study, the procedure of LPS injection was painless and nonstressful, T_a was tightly controlled, and a wide range of LPS doses was tested.

MATERIALS AND METHODS

Animals. Ninety-five C57BL/6 mice (Charles River Laboratories, Wilmington, MA) weighing 27–30 g at the time of the experiment were used. The mice were initially housed two per cage; after surgery, they were housed individually. Standard mice chow (Purina, Richmond, IA) and tap water were available ad libitum. The room was maintained on a 12:12-h light-dark cycle (lights on at 7:00 AM) and had T_a of 27°C. Each mouse was handled daily for 5 days and then systematically habituated (5 training sessions, 3–4 h each) to staying in either a Plexiglas enclosure (length, 15 cm; width, 15 cm; height, 25 cm) or a wire-mesh conical confiner (diameter at base, 3 cm; base-to-vertex distance, 10 cm). The enclosure did not limit the animal's movement; it was used for experiments involving telemetric thermometry. The confiner prevented the animal from turning around without limiting its back-and-forth movements; it was used for experiments involving thermocouple thermometry. All experiments were started between 8:00 AM and 9:00 AM. Each mouse was used in only one experiment and euthanized with pentobarbital sodium (20 mg/kg iv) immediately thereafter. The protocols were approved by the St. Joseph's Hospital Animal Care and Use Committee.

Surgery. Each mouse was subjected to either one or both of the following surgical procedures: chronic jugular vein catheterization and implantation of a temperature transmitter in the peritoneal cavity. These procedures were performed under ketamine-xylazine-acepromazine (42.0, 4.8, and 0.6 mg/kg, respectively, ip) anesthesia and antibiotic (enrofloxacin, 3.75 mg/kg sc) protection. The mouse was kept on a heating pad and periodically (every 5 min) ventilated with oxygen through a custom-made mask. For jugular catheterization, a 5-mm longitudinal incision was made on the ventral surface of the neck, 5 mm right to the trachea. The right jugular vein was exposed, freed from its surrounding connective tissue, and ligated. The vein was then catheterized according to the procedure by Harms and Ojeda (8) with minor modifications. In brief, a silicone catheter (ID, 0.5 mm; OD, 0.9 mm) was attached to the hub end of a bent (L-shaped) 21-gauge injection needle with its hub removed. The needle (with the catheter attached) was thrust into the lumen of the vein and pulled out through the chest muscles. The catheter was then disconnected from the needle, filled with heparinized (50 U/ml) saline, pulled back into the jugular vein, and pushed forward to a premeasured distance. As a result, the tip of the catheter was placed in the superior vena cava. The catheter was fixed in place with ligatures, and its free end was knotted, tunneled under the skin, and exteriorized at the nape. The surgical wound on the neck was sutured. For implantation of a miniature (25 × 8 × 8 mm) temperature transmitter (series 4000 E-Mitter; Mini Mitter, Bend, OR), a midline laparotomy was performed, the probe was inserted in the peritoneal cavity, and the surgical wound was sutured. To prevent postsurgical hypothermia, all mice were allowed to recover from anesthesia at T_a of 32°C. On *day 1* postsurgery, the catheter was flushed with heparinized (10 U/ml) pyrogen-free saline. The mice tolerated the surgical procedures well: they showed only a minor (5–10%) loss of body mass on *day 1* and regained mass on *day 2*. The experiments were performed on *day 3* by using either telemetric or thermocouple thermometry.

Telemetric thermometry. This experimental setup permitted monitoring the abdominal temperature (an index of T_b) of freely moving mice implanted with temperature transmitters. Telemetry receivers (model ER-4000; Mini Mitter, Bend, OR) were positioned inside a climatic chamber (model 3940; Forma Scientific, Marietta, OH) and connected to a computer. The chamber had dimension of 79 × 152 × 69 cm and was equipped with fluorescent lamps that provided regular lighting conditions. Air humidity was set to 50%. According to the manufacturer's specifications, the precision of the temperature control in the chamber is ±0.1°C and the uniformity is ±0.4°C. The home cage of each mouse was placed on top of a receiver, a Plexiglas enclosure was placed inside the cage, and the mouse was left in the enclosure. The jugular catheter was extended with a length of polyethylene-50 (PE-50) tubing filled with the drug of interest (for details, see *LPS preparation and administration* below). The extension was passed through a hanger on the top of the enclosure and then through a wall port of the climatic chamber. The extension was connected to a syringe filled with saline. This telemetry setup (Fig. 1) permitted

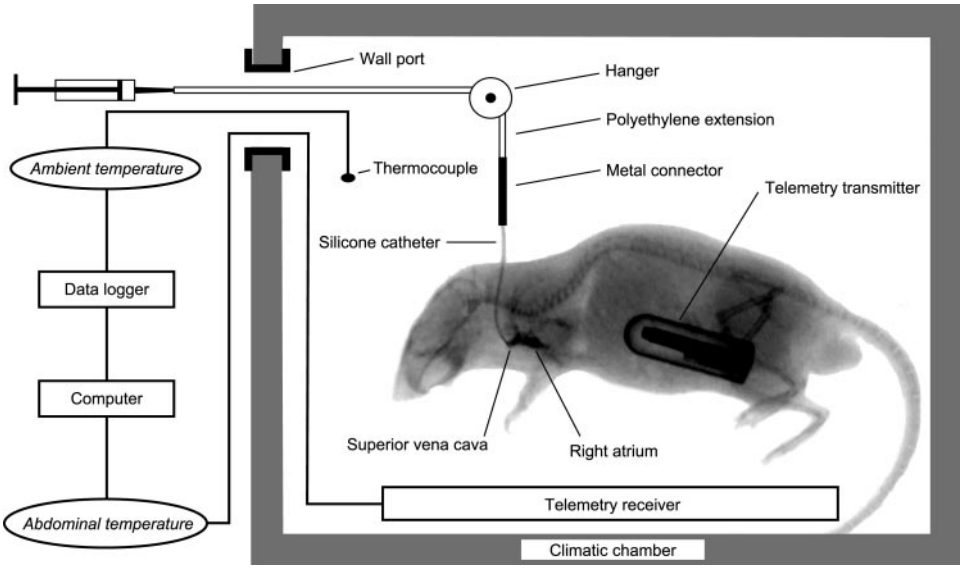

Fig. 1. Schematic of the telemetric thermometry setup. The animal image is a roentgenogram of a mouse chronically implanted with a jugular vein catheter and an intraperitoneal telemetry probe. The mouse was injected with a small amount of a contrasting solution through the catheter.

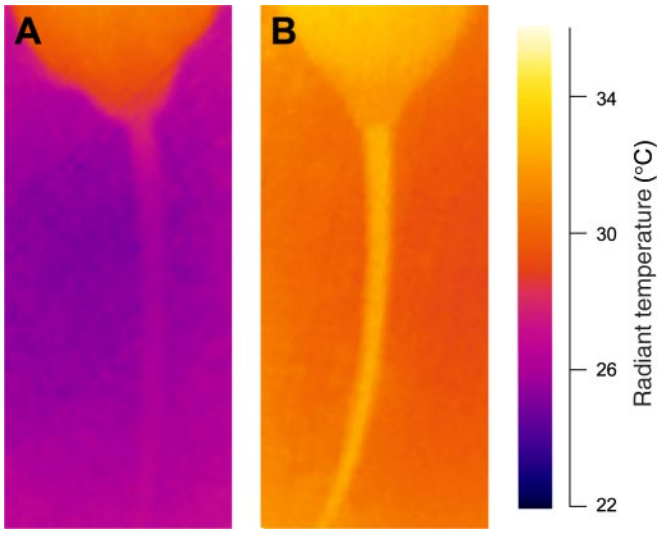

Fig. 2. Thermoneutrality test for mice in the telemetric thermometry setup. The effect of ambient temperature on tail skin temperature (T_{sk}) of mice was determined by infrared thermography. Thermograms were taken at an ambient temperature of 26.0°C (*A*) or 31.0°C (*B*); see *Telemetric thermometry* in MATERIALS AND METHODS for explanations.

intravenous administration of the drug to the mouse from outside the chamber without disturbing the animal.

During an experiment, the mice were exposed to T_a that was set to be either within or below their thermoneutral zone. This zone depends not only on T_a, but also on other physical factors, including air humidity and velocity, effective radiant field, thermal conductivity of the housing structure, and the way the animal's body contacts the housing structure. Hence, the thermoneutral zone often varies among experimental setups (for detailed analysis, see Ref. 25). In this setup, thermoneutrality was determined by measuring radiant tail skin temperature (T_{sk}) by infrared thermography. An infrared camera (ThermoVision A20M; FLIR Systems, N. Billerica, MA) was positioned on top of the enclosure with a mouse freely moving inside the enclosure; the camera was programmed to acquire images every second. When the mice were exposed to a low T_a (e.g., 26°C; Fig. 2*A*), they presented maximal skin vasoconstriction (T_{sk} at a near-T_a level), indicating that this T_a was subneutral (for explanations, see Ref. 25). We then gradually increased T_a in a stepwise fashion (step height, 0.5°C; step duration, >2 h) until moderate tail skin vasodilation (an increase in T_{sk}) was observed, which occurred at 31°C (Fig. 2*B*). Thus T_a of 31°C was found to be neutral for mice in this setup.

Thermocouple thermometry. This experimental setup permitted simultaneous measuring of both colonic temperature (index of T_b) and T_{sk} (index of tail skin vasomotor tone) in loosely confined mice. Each mouse was placed in a confiner and equipped with two copper-constantan thermocouples. The colonic thermocouple was inserted 2 cm beyond the anal sphincter and fixed to the base of the tail with a loop of adhesive tape. The skin thermocouple was positioned on the lateral surface of the tail (at the boundary of the proximal and middle thirds) and insulated from the environment with tape. Thermocouples were plugged into a data logger (Dianachart, Rockaway, NJ) connected to a personal computer. The mouse in its confiner was transferred to a climatic chamber. The jugular catheter was extended with a length of PE-50 tubing filled with the drug of interest (for details, see *LPS preparation and administration* below). The extension was passed through a wall port and connected to a syringe filled with saline. This thermocouple setup (Fig. 3) also permitted intravenous administration of the drug from outside the chamber, without disturbing the mouse.

To determine a neutral T_a in this setup, T_a, T_{sk}, and T_b were monitored with thermocouples while the mice were exposed to different T_as. At T_a of 32°C or lower, T_{sk} was close to T_a and exhibited no fluctuations (Fig. 4), thus indicating maximal tail skin vasoconstriction typical for a subthermoneutral environment. At T_a of 34°C or above, T_{sk} was maintained at a relatively high level without marked fluctuations, which is characteristic of a supraneutral environment. At T_a of 33°C, two signs of thermoneutrality were observed: the average level of T_{sk} was intermediate, and the dynamics of T_{sk} was characterized by large fluctuations (for explanations, see Ref. 25). Hence, T_a of 33°C was found to be neutral for mice in this experimental setup.

LPS preparation and administration. E. coli 0111:B4 LPS was purchased from Sigma-Aldrich (St. Louis, MO). A stock suspension of LPS (10^4 μg/ml) in saline was stored at −20°C. At the time of the experiment, this stock was used either undiluted or diluted to a final concentration of 10^0, 10^1, $10^{1.5}$, $10^{1.75}$, 10^2, or 10^3 μg/ml. For intravenous injection, the PE-50 extension of the jugular catheter was prefilled with 27–30 μl (1 ml/kg) of a suspension of LPS in such a way that the suspension stayed near the mouse end of the extension; the remainder of the extension was filled with saline. The LPS suspension was separated from saline with a ~0.5 μl air bubble. At the time of injection, LPS (10^0–10^4 μg/kg) was flushed into the mouse's circulation by replacing the LPS suspension at the mouse end

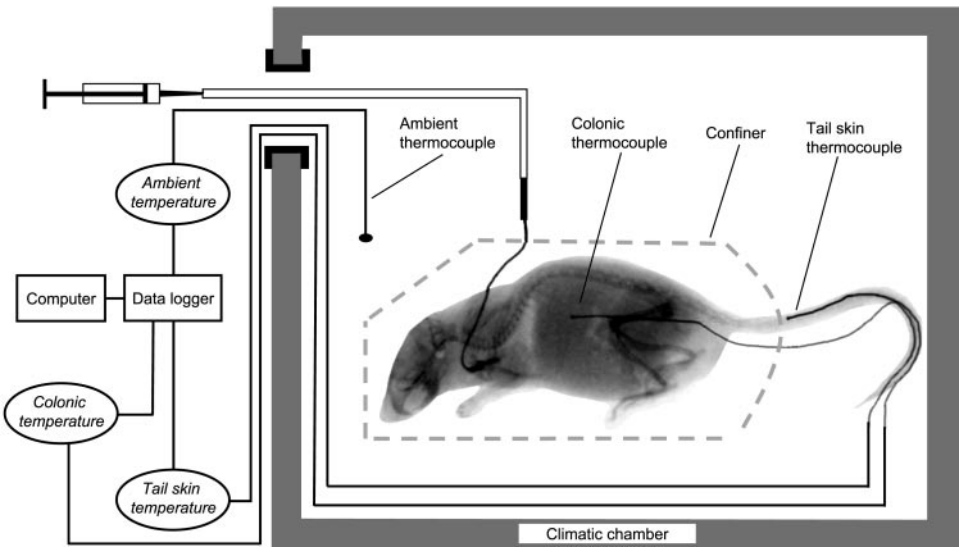

Fig. 3. Schematic of the thermocouple thermometry setup. The animal image is a roentgenogram of a mouse chronically implanted with a jugular catheter, instrumented with colonic and tail skin thermocouples, and injected with a small amount of a contrasting solution through the catheter.

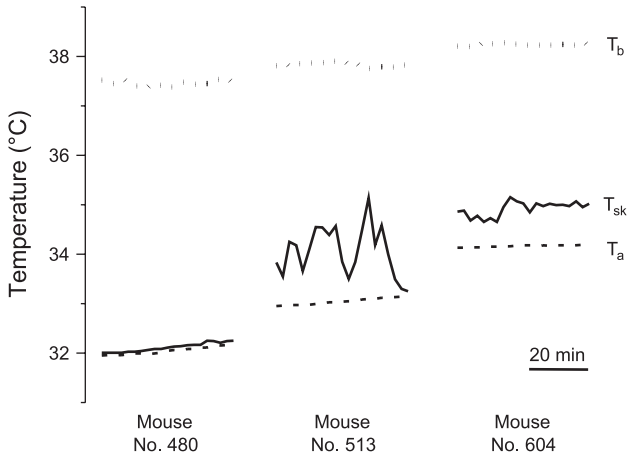

Fig. 4. Thermoneutrality test for mice in the thermocouple thermometry setup. The effect of ambient temperature (T_a) on deep body temperature (T_b) and T_{sk} of mice was determined by thermocouple thermometry. Recordings were made at T_a of 32, 33, or 34°C; see *Thermocouple thermometry* in MATERIALS AND METHODS for explanations.

of the extension with 0.3 ml of saline injected from the syringe end. In a control, the extension was prefilled with saline only, and the injection was performed by the same procedure. This procedure was executed without touching the animal or causing pain or stress. In a separate experiment, LPS (10 ml/kg; 10^3–10^4 μg/kg) or saline was injected intraperitoneally by a common, stressful procedure that involved pricking the abdominal wall with a needle.

Data processing and analysis. The absolute values of T_b and, when available, T_{sk} were used to evaluate the thermoregulatory responses to LPS. In most cases, the responses were compared across treatments and time points by a two-way ANOVA, and F and P values (for the entire observation period/response duration) were reported. When the effect of treatment was evaluated on two parameters simultaneously (the effect of LPS dose on the latency and amplitude of the first phase of polyphasic fever and the effect of LPS on T_b and T_{sk} in the thermocouple experiment), one-way MANOVA was used, and R and P values were reported. The analyses were performed using Statistica AX'99 (StatSoft, Tulsa, OK). Compared responses were considered significantly different at $P < 5.0 \times 10^{-2}$. Results are reported as means ± SE.

RESULTS

At T_a of 31°C (which was neutral for mice in the telemetry setup), intravenous saline caused a slight (~0.5°C) decrease in T_b over the course of the experiment (Fig. 5*A*). Such a decrease often occurs in untreated or saline-treated mice during the light phase of the day at a subneutral or neutral T_a, presumably reflecting the circadian rhythm of T_b (15, 20, 21, 31, 36). Compared with saline, all doses of LPS (10^0–10^4 μg/kg) induced a significant rise in T_b (10^0 μg/kg: $F_{1,429} = 1.6 \times 10^1$, $P = 9.4 \times 10^{-5}$; 10^1 μg/kg: $F_{1,390} = 1.3 \times 10^2$, $P = 1.0 \times 10^{-7}$; $10^{1.5}$ μg/kg: $F_{1,429} = 7.2 \times 10^1$, $P = 1.0 \times 10^{-7}$; $10^{1.75}$ μg/kg: $F_{1,507} = 3.6 \times 10^2$, $P = 1.0 \times 10^{-7}$; 10^2 μg/kg: $F_{1,507} = 1.1 \times 10^3$, $P = 1.0 \times 10^{-7}$; 10^3 μg/kg: $F_{1,507} = 7.3 \times 10^3$, $P = 1.0 \times 10^{-7}$; 10^4 μg/kg: $F_{1,468} = 9.5 \times 10^3$, $P = 1.0 \times 10^{-7}$). The dynamics of the response was dose dependent. LPS induced a monophasic fever at 10^0–10^1 μg/kg (Fig. 5, *B* and *C*) and a biphasic fever at $10^{1.5}$ μg/kg (Fig. 5*D*). Similar to what occurs in rats at a narrow dose range around 10^1 μg/kg (24), there was a drastic change in the response of the mice when the dose increased from $10^{1.5}$ to $10^{1.75}$–10^2 μg/kg: the biphasic fever was replaced by a qualitatively different polyphasic fever (Fig. 5, *E* and *F*). Three distinct febrile phases were observed over the time of the experiment; the third phase was the most pronounced (Fig. 6, *A* and *C*). When the data were plotted in a phase plane (rate of T_b change vs. T_b; for further explanations, see Ref. 29), these three phases were visualized as three separate loops (Fig. 6, *B* and *D*). As the dose of LPS was further increased (10^3–10^4 μg/kg), the second and third phases merged together and became indistinguishable (Fig. 5, *G* and *H*). Similar to LPS fever in the rat (26, 27, 30, 35), both the latency and amplitude ($R_{6,38} = 1.3 \times 10^1$; $P = 5.9 \times 10^{-8}$) of the first phase of polyphasic fever in the mouse decreased as the LPS dose increased (Fig. 7).

Because a low-amplitude first phase is often overlooked (for review, see Ref. 24), it was important to confirm its existence in the mouse by measuring the underlying effector activity. At neutral and supraneutral T_as, the first febrile phase in rats (29, 35) and rabbits (19) is brought about, at least partially, by skin vasoconstriction (revealed as a decrease in T_{sk}). T_b and T_{sk} were simultaneously monitored by thermocouple thermometry in LPS-treated mice at T_a of 33°C, which was neutral for mice in the thermocouple setup (see MATERIALS AND METHODS). The dose of $10^{1.75}$ μg/kg was selected for this experiment because it caused the most pronounced first phase in the telemetry setup (Fig. 5*E*). A similarly pronounced first febrile phase was caused by this dose in the thermocouple setup (Fig. 8*A*). The T_b rise was associated with a significant decrease in T_{sk} from ~34°C to a near-T_a value ($R_{16,52} = 3.3 \times 10^0$, $P = 6.0 \times 10^{-4}$; Fig. 8*B*), thus indicating tail skin vasoconstriction.

The thermoregulatory responses of mice to LPS (10^0, $10^{1.75}$, or 10^4 μg/kg) or saline were also studied at T_a of 26°C, which was subneutral for this species in the telemetry setup. The mice injected intravenously with saline showed a gradual, slight (~0.5°C) decrease in T_b over the course of the experiment (Fig. 9*A*). Compared with saline, every dose of LPS tested induced significant changes in T_b (10^0 μg/kg: $F_{1,468} = 2.2 \times 10^2$, $P = 1.0 \times 10^{-7}$; $10^{1.75}$ μg/kg: $F_{1,507} = 2.1 \times 10^2$, $P = 1.0 \times 10^{-7}$; 10^4 μg/kg, fever: $F_{1,299} = 1.2 \times 10^2$, $P = 1.0 \times 10^{-7}$; 10^4 μg/kg, hypothermia: $F_{1,221} = 9.2 \times 10^2$, $P = 1.0 \times 10^{-7}$). These changes differed from those observed at a neutral T_a in two ways. First, the animals appeared more sensitive to LPS in a cool environment. Indeed, the first febrile phase caused by low doses (10^0 and $10^{1.75}$ μg/kg) had a substantially shorter (by ~20 min) latency at a subneutral T_a (Fig. 9, *B* and *C*) than at a neutral T_a (Fig. 5, *B* and *E*). Furthermore, the response to 10^0 μg/kg was transformed from a monophasic to biphasic fever (compare Figs. 5*B* and 9*B*). Second, the febrile response in a cool environment was followed by a late decrease in T_b. At higher doses (>10^2 μg/kg), this decrease led to hypothermia, which started at ~180 min postinjection and was pronounced (~3°C at 10^4 μg/kg; Fig. 9*D*). At lower doses (e.g., $10^{1.75}$ μg/kg), this decrease resulted in the disappearance of the otherwise prominent third febrile phase (compare Figs. 5*E* and 9*C*).

Under no circumstances did the mice exposed to a subneutral T_a respond to a stress-free intravenous injection of LPS with early (latency, ~30 min) hypothermia, although such hypothermia reportedly (4, 7, 21, 23) occurs in the same species in response to a stressful injection of moderate-to-high doses of LPS. The mice in the present study failed to develop early hypothermia even when high doses of LPS (10^3–10^4

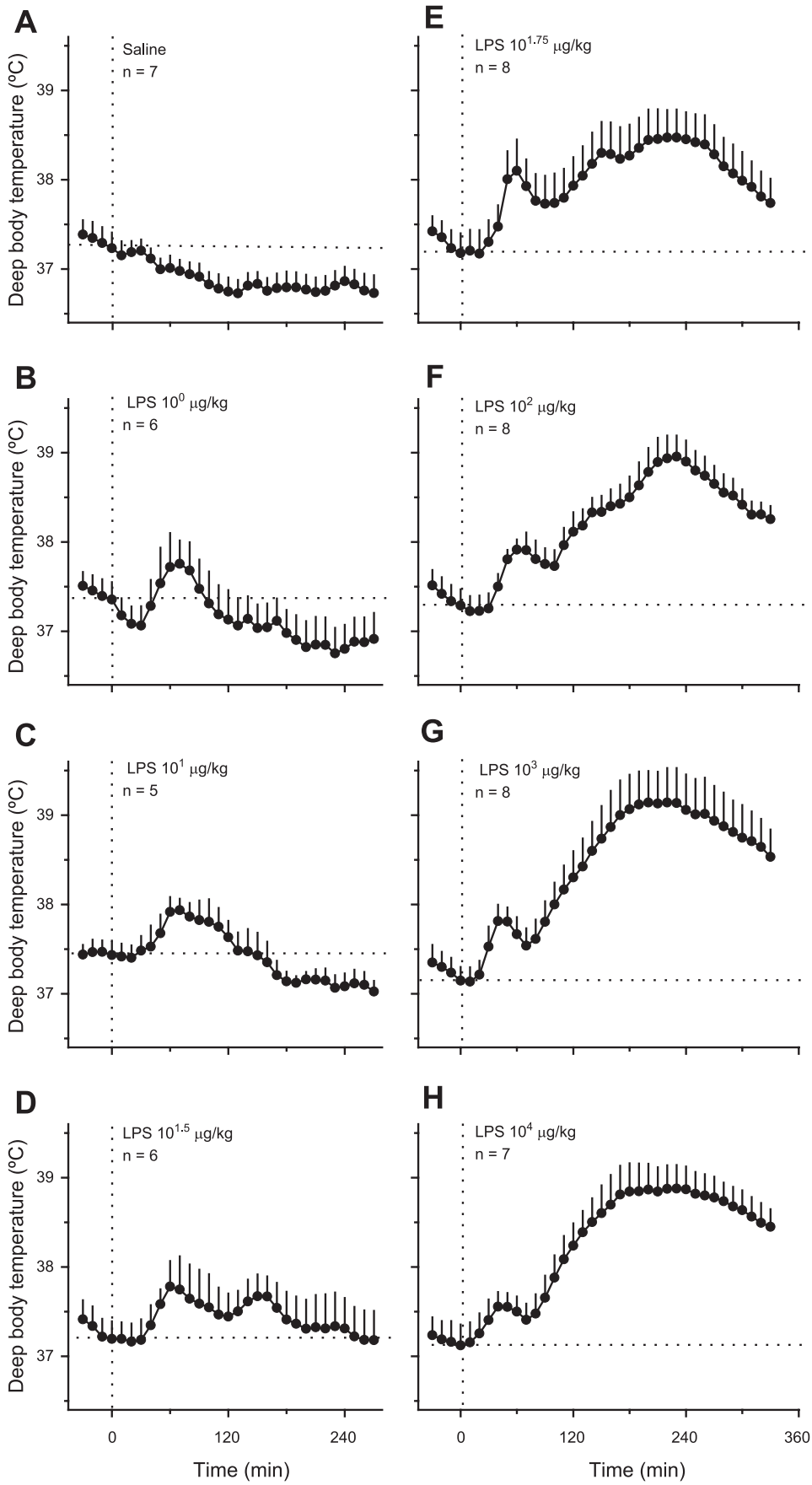

Fig. 5. Effects of intravenous LPS (doses indicated) or saline on the T_b of mice at thermoneutrality. T_b was monitored by telemetric thermometry at a neutral T_a of 31°C. The vertical dotted lines show the time of injection. The horizontal dotted lines show the T_b at the time of injection. Here and in Figs. 8–10, the number of animals (*n*) is indicated.

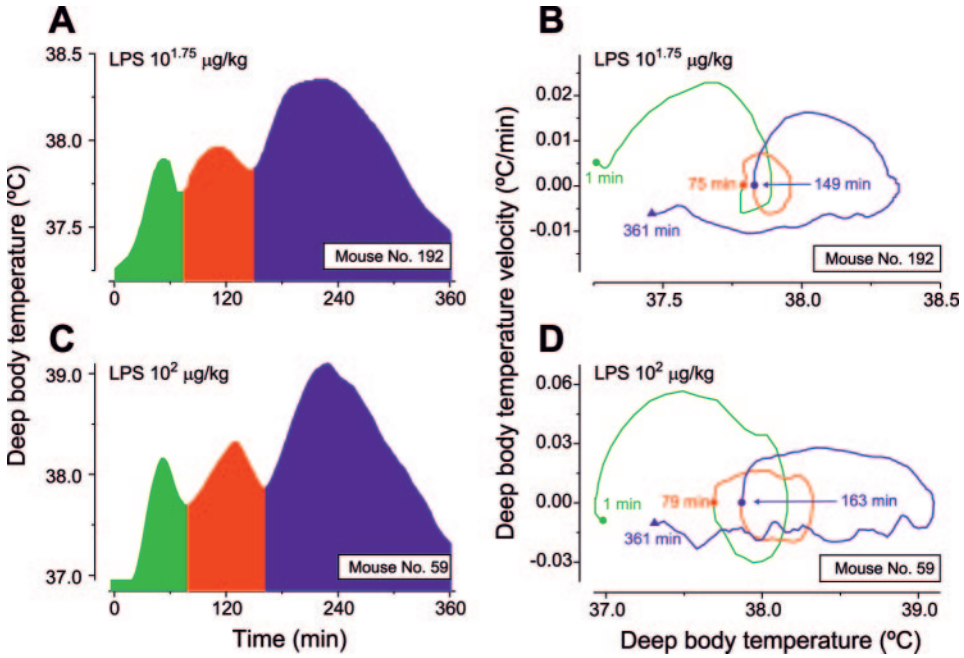

Fig. 6. The polyphasic febrile responses of individual mice to intravenous LPS (doses indicated) at thermoneutrality. *A* and *C*: plots of T_b vs. time (time plots). *B* and *D*: plots of the rate of change in T_b (temperature velocity) vs. T_b (phase-plane plots). In the phase-plane plots, the onset of each phase is indicated with a circle symbol, and the end of the recording is shown with a triangle. The time elapsed after the injection (in minutes) is shown next to each symbol. The 3 febrile phases can be identified as 3 increases in the abdominal temperature (*A* and *C*) and 3 complete loops (shown in different colors) in the phase plane (*B* and *D*). To eliminate a high-frequency noise, the curves were smoothed by the Savitzky-Golay method (Origin 6.0; Microcal Software, Northampton, MA).

µg/kg) were injected at T_as as low as 20, 18, or even 16°C (data not shown). It was, therefore, important to test whether a stressful intraperitoneal injection of LPS would cause early hypothermia in the setup used. At a subneutral T_a of 26°C (telemetry setup), the mice were injected with intraperitoneal LPS (10^3 or 10^4 µg/kg) or saline via a common procedure involving animal handling and needle pricking. When injected this way, saline caused an immediate small increase in T_b

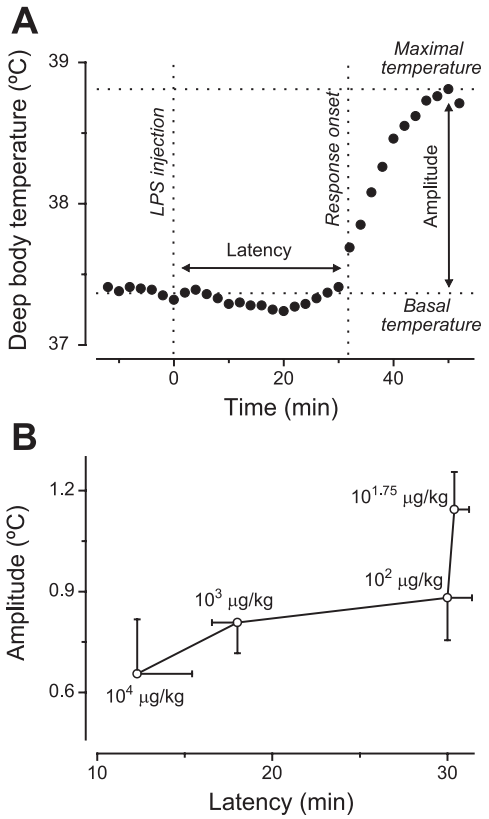

Fig. 7. Relationship between the amplitude of the first phase of polyphasic fever and the latency to its onset. *A*: an individual plot is used to show how the amplitude and latency were determined. *B*: a plot of the means is used to reveal the effect of LPS dose (shown next to each symbol).

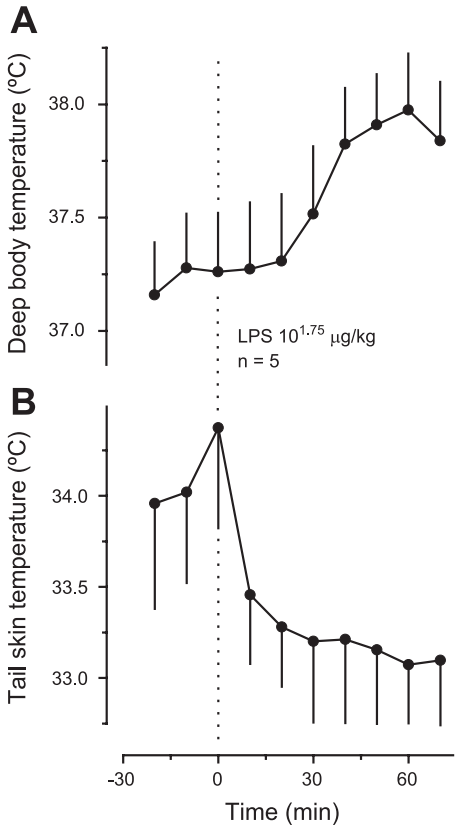

Fig. 8. T_{sk} response to intravenous LPS (dose indicated) during the initiation of fever at thermoneutrality. T_b (*A*) and T_{sk} (*B*) were monitored by thermocouple thermometry at a neutral T_a of 33°C. The vertical dotted line shows the time of injection.

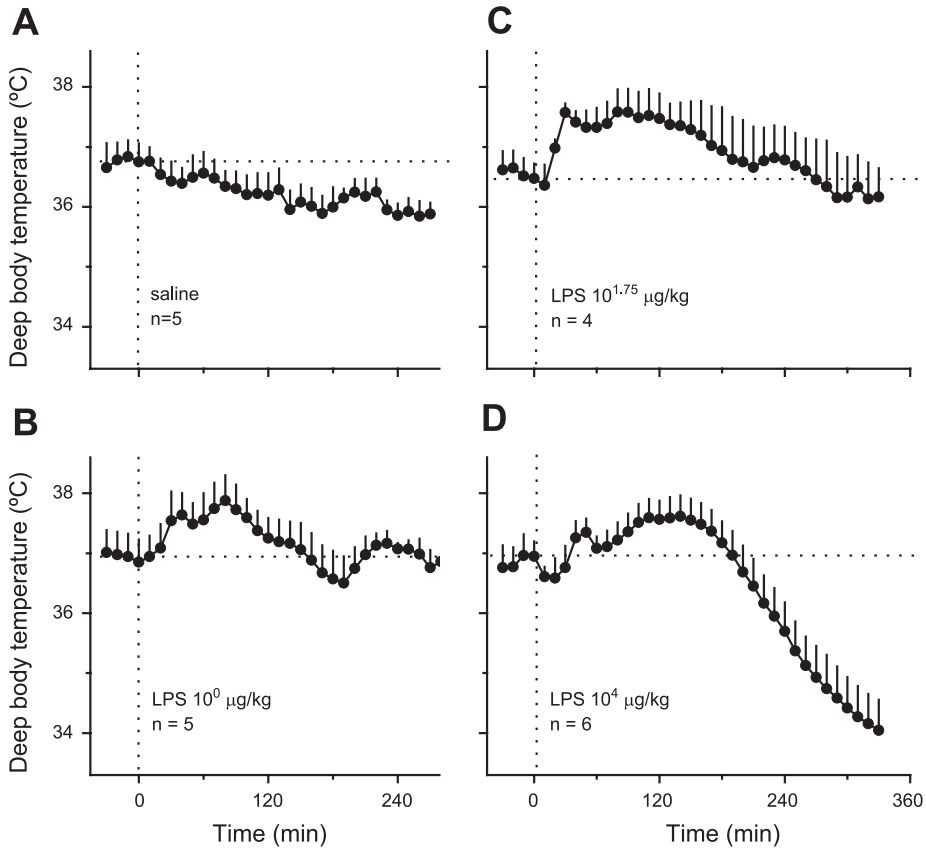

Fig. 9. Effects of intravenous LPS (doses indicated) or saline on T_b of mice in a cool environment. T_b was monitored by telemetric thermometry at a subneutral T_a of 26°C. The vertical dotted lines show the time of injection. The horizontal dotted lines show T_b at the time of injection.

(stress hyperthermia) followed by a slow decline in T_b. In response to LPS, the mice developed a similar small increase in T_b followed by hypothermia; compared with saline, hypothermia was significant only in response to the highest dose of LPS ($F_{1,96} = 8.7 \times 10^0$, $P = 4.1 \times 10^{-3}$; Fig. 10). In contrast to the late hypothermic response to stress-free intravenous administration of LPS (Fig. 9), the response to stressful intraperitoneal administration was early (latency, ~30 min).

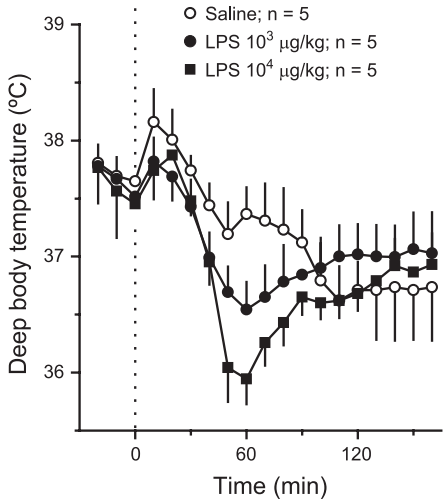

Fig. 10. Effects of intraperitoneal LPS (doses indicated) or saline on the T_b of mice in a cool environment. T_b was monitored by telemetric thermometry at a subneutral T_a of 26°C. The vertical dotted line shows the time of injection.

DISCUSSION

Murine thermoregulatory responses to LPS at thermoneutrality. Although it has been repeatedly shown that mice can develop fever in response to LPS, the results obtained were often contaminated by stress hyperthermia (due to a painful injection procedure) and by a thermoregulatory response to external cooling (due to a poorly controlled, subneutral T_a). As a result, many authors (e.g., Refs. 3, 11, 15, 17, 18, 38) have described a response that consisted of rapid hyperthermia of variable magnitude and duration followed by either fluctuations of T_b without a clear pattern or by a steady decline of T_b to a hypothermic level. Importantly, the responses to the same or a similar dose of LPS differed drastically among studies and sometimes even within a study. The present research identified the dynamics of the thermoregulatory responses of mice to LPS administered in a stress-free fashion via a chronically implanted intravenous catheter. The mice were kept in an environmental chamber with tight control of T_a, and the relationship between T_as used and the thermoneutral zone was verified.

We have found that mice exposed to a neutral T_a respond to LPS with fever in a dose-dependent fashion: as the dose is increased from 10^0 to 10^2 μg/kg, the fever is transformed from monophasic to biphasic to polyphasic. The polyphasic character of the febrile response of mice to LPS has been recognized by Oka et al. (21). By assessing thermoeffector activity (vasomotor tone of the tail skin) and using several modes of data presentation, including the phase-plane plot, the present study has identified and characterized three phases of the polyphasic response. The present study has also established that many

characteristics of LPS fever in mice are similar to those reported for rats. For example, the second and third phases of the febrile response in mice (Fig. 5) and rats (27) become less distinct and fuse when the dose of LPS approaches 10^3–10^4 μg/kg. Another similarity is that both the amplitude and the latency of the first phase of polyphasic fever in mice (Fig. 7) and rats (26, 35) are inversely proportional to the LPS dose. Whereas it is not surprising that the latency of the response decreases as the dose of LPS increases, the fact that the amplitude of the first phase in both species decreases with an increase in the dose is counterintuitive and remains unexplained.

Thermoregulatory responses of mice to LPS in a cool environment. The present study has found that exposure of mice to a cool environment changes the thermoregulatory responses to LPS in two ways. First, it has a sensitizing effect on the febrile response. Indeed, responses to low (10^0 μg/kg) and intermediate ($10^{1.75}$ μg/kg) doses of LPS start earlier in a cold environment. Furthermore, what is a monophasic fever in a neutral environment turns into a biphasic fever in the cold. Such a paradoxical, sensitizing effect has not been described before. Second, the febrile response of mice in a cool environment is followed by a late (starts at ~180 min postinjection) decrease in T_b. At lower doses of LPS (e.g., $10^{1.75}$ μg/kg), this decrease is seen as the disappearance of the otherwise prominent third febrile phase. At higher doses (>10^2 μg/kg), this decrease results in a pronounced (~3°C) hypothermic response. In other words, T_b-decreasing mechanisms are turned on at later stages of the response to LPS in a cool environment. These mechanisms seem to compete with T_b-increasing mechanisms, and such a competition may result in the disappearance of late febrile phases and/or in the development of hypothermia. A late (latency > 120 min) hypothermic response to LPS in mice has been previously reported (1, 12, 21, 22). Such a response may last for up to 48 h and seems to be associated with ongoing inflammation, as evident from the sensitivity of late hypothermia to both dexamethasone and inhibitors of eicosanoid biosynthesis (22).

The most surprising observation of the present study is that mice do not respond to LPS with an early hypothermia, whereas, in rats, the early (10–30 min, latency; ~90 min, nadir) hypothermia is a highly reproducible and pronounced (up to several degrees centigrade) response to moderate or high doses of intravenous LPS in a subthermoneutral environment (27, 28, 30, 32, 33). The present study shows that C57BL/6 mice do not develop early hypothermia even in response to very high doses of LPS (up to 10^4 μg/kg) at T_as as low as 16°C. Furthermore, another strain of mice (wild-type mice with a mixed B6;129P2 background; Taconic, Germantown, NY) has also been found to be incapable of responding with early hypothermia to LPS (10^3–10^4 μg/kg iv) at a subneutral T_a of 26°C (A. A. Steiner, A. Y. Rudaya, and A. A. Romanovsky, unpublished observations). However, the early hypothermic response did occur in C57BL/6 mice in the present study when LPS was administered by using a common, stressful procedure of intraperitoneal injection. Such a response has also been observed by others in different mouse strains when LPS was administered by a stressful procedure involving either pricking the abdomen (21, 23) or tail (4, 7) with an injection needle for intraperitoneal or intravenous administration, respectively. That early hypothermia occurs in the mouse exclusively under stressful conditions suggests that this is not an intrinsic response to LPS in this species. This finding may be another example of how stress interferes with thermoregulatory responses (10, 27, 34).

In summary, the present investigation identifies experimental conditions to study LPS-induced mono-, bi-, and polyphasic fevers and late hypothermia in mice and provides detailed characteristics of these responses. It also highlights the importance of methodological issues in thermoregulation research. While studying the febrile and hypothermic responses of mice to LPS, caution should be taken to administer LPS in a stress-free fashion, to account for the intricate dependence of the response on LPS dose and to run experiments at a controlled T_a.

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