Cold stimulates the behavioral response to hypoxia in newborn mice

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Cold stimulates the behavioral response to hypoxia in newborn mice. Am J Physiol Regul Integr Comp Physiol 296: R1503–R1511, 2009. First published March 18, 2009; doi:10.1152/ajpregu.90582.2008.—In newborns, hypoxia elicits increased ventilation, arousal followed by defensive movements, and cries. Cold is known to affect the ventilatory response to hypoxia, but whether it affects the arousal response remains unknown. The aim of the present study was to assess the effects of cold on the ventilatory and arousal responses to hypoxia in newborn mice. We designed an original platform measuring noninvasively and simultaneously the breathing pattern by whole body plethysmography, body temperature by infrared thermography, as well as motor and ultrasonic vocal (USV) responses. Six-day-old mice were exposed twice to 10% O2 for 3 min at either cold temperature (26°C) or thermoneutrality (33°C). At 33°C, hypoxia elicited a marked increase in ventilation followed by a small ventilatory decline, small motor response, and almost no USVs. Body temperature was not influenced by hypoxia, and oxygen consumption (V̇O2) displayed minimal changes. At 26°C, hypoxia elicited a slight increase in ventilation with a large ventilatory decline and a large drop of V̇O2. This response was accompanied by marked USV and motor responses. Hypoxia elicited a small decrease in temperature after the return to normoxia, thus precluding any causal influence on the motor and USV responses to hypoxia. In conclusion, cold stimulated arousal and stress responses to hypoxia, while depressing hypoxic hyperpnea. Arousal is an important defense mechanism against sleep-disordered breathing. The dissociation between ventilatory and behavioral responses to hypoxia suggests that deficits in the arousal response associated with sleep breathing disorders cannot be attributed to a depressed hypoxic response.

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METHODS

Animals. Six-day-old mouse pups were obtained from outbred Swiss female mice (IFAA-CREDO, L’Arbresle, France), housed at 24°C with a 12:12-h light-dark cycle, and fed ad libitum. In the main study, we used 34 litters with a mean of 10.12 ± 2.37 pups per litter. Five additional litters were used for complementary studies. Table 1 shows the number of pups in each of the four groups of the main study, as well as the mean body weight and body temperature before the start of the tests in each group. The absence of previous studies on the ultrasonic response to hypoxia in newborn rodents precluded power analysis. Experimental protocols were approved by the Institutional Review Committee, met the guidelines of the Institut National de la Santé et de la Recherche Médicale, and were carried out in accordance with the Guide for the Care and use of Laboratory Animals (National Institutes of Health). All efforts were made to minimize animal suffering, especially by developing fully noninvasive functional tests.

Plhysomography. Breathing variables were measured noninvasively using a battery of four whole body flow barometric plethysmographs (6, 13, 31, 40), which allowed simultaneous measurement of breathing variables and USVs (Fig. 1A). Plethysmograph chambers were immersed in a thermoregulated water bath that maintained their temperature at 26°C or 33°C. Thermographic studies in newborn rats indicate that 26°C corresponds to maximal thermogenesis by brown adipose tissue and can therefore be considered cold (4). The limitations of the plethysmographic method in newborn mice has been discussed elsewhere (29). While not free from interpretational difficulties (16, 37), whole body plethysmography is the only method that provides semiquantitative measurements of tidal volume (V̇T) while allowing valid measurements of breath duration (ṪTOT) and apneas in freely moving animals. In particular, the breathing-related pressure...
changes may be influenced by compression and rarefaction of external gases in the measurement chamber, caused by airway resistance during inspiration and expiration, respectively (16,37). Because of the limitations of flow barometric plethysmography, the absolute values of VT and ventilation (VE) are indicative only, whereas TTOT absolute values and apnea durations are reliable.

**Breathing variables.** TTOT (s), VT (μl·g⁻¹·BTPS), and minute ventilation (VE, calculated as VT·TTOT⁻¹ and expressed in μl·s⁻¹·g⁻¹·BTPS, and temperature pressure saturated) were calculated on apnea-free periods (see apnea determination below). Breath-by-breath values for VE, VT, and TTOT were averaged over consecutive 30-s periods. The baseline levels for these variables were calculated as the mean value over the 3 min of air breathing preceding the first hypoxic period. The baseline levels for these variables were calculated as the mean value over the 3 min of air breathing preceding the first hypoxic period.

**Hypoxic tests.** Groups of four pups were taken at random from the litter and assigned alternately to the group receiving two hypoxic tests or to the normoxic control group (hereafter designated as hypoxic and normoxic groups). In the hypoxic group, after 20-min normoxia, hypoxia was achieved by switching the airflow to 10% O₂ by 10.220.32.247 on October 1, 2017 http://ajpregu.physiology.org/ Downloaded from

**USV measurement.** USVs were recorded using an ultrasound bat detector D230 (Pettersson Elektronik, Uppsala, Sweden). The frequency range of the detector (10 to 120 kHz) included the frequency range of USVs in newborn mice (40–90 kHz). The microphone was placed inside the animal chamber and connected to the electronic processing device via a hermetic pass-through connection (Fig. 1). Sounds were digitized at 200 kHz (16 bits, model PCI 6143; National Instruments, Austin, Texas) and stored on a disk by using a homemade LabView program. We carefully avoided contamination of USV recordings by parasite sound signals. The water bath isolated the measurement chamber from external noises. The pups were free from contact stimuli, which are potent triggers of USVs in newborn rodents (7,8). The harmonic structures and bandwidth of ultrasound recordings (40–90 kHz) were typical for USVs, indicating that contamination by parasite sounds caused by friction against the walls of the chamber did not occur. Total USV durations were calculated over consecutive 30-s periods. The USV response to 10% O₂ was compared.
puted as the difference between air and the peak USV response determined individually over the entire time exposed to hypoxia (ΔUSV). USVs did not contaminate ventilatory measures.

**Body temperature.** We measured the emission of IR radiations from the skin surface of the interscapular region using an IR camera (model Thermovision A20; FLIR Systems, Boston, MA) according to a method previously developed in newborn rats (45). The interscapular region contains the heat-generating brown adipose cells and is the area of highest skin temperature (4). The camera was connected to the computer via the firewire interface and images were processed using the FLIR Web Server functionalities, which controlled the camera and image acquisition and allowed the automatic online determination of the peak temperature value of the interscapular region. IR measurement was done through a special window of the plethysmograph chamber made of zinc selenide, to ensure permeability to IR emission.

To examine the consistency of IR measurement with other estimations of core temperature, we compared the IR interscapular temperature and esophageal temperature in four 6-day-old pups that were placed at 21°C for 10 min and showed progressive cooling. Esophageal temperatures were measured by inserting a temperature probe connected to a digital thermometer (K-type chromel-alumel thermocouple; Hanna Instruments, Bedfordshire, UK) 2–3 mm in the mouth of the restrained pup. We found a very high correlation between interscapular and esophageal temperatures (r² correlation coefficients: 0.96, 0.98, 0.95, and 0.98, respectively). These results confirmed previous measurements in newborn rats showing a very high correlation between interscapular and esophageal temperatures by thermocouples attached to the skin over ambient temperatures ranging from 22.5°C to 37°C (46).

Finally, we checked that the zinc selenide window did not influence body temperature measurements. To this aim, we measured IR temperature measurements with and without the window in 6-day-old pups, 10 min after acclimation to three ambient temperatures: 26, 30 and 33°C (4 pups per temperatures). The order of the two measurements (with or without the window) was counterbalanced across the pups. The differences were negligible (<0.1°C) and not statistically significant.

**O₂ consumption.** In a separate study, we measured the time course of O₂ consumption (V˙O₂) in 6-day-old mice. A plethysmograph chamber was equipped with a fan (model 252; ebm-papst, Obernai, France) to stir the gas and ensure a constant washout kinetics, whatever the position of the pup (this disrupted the ventilatory signal and USV recording). The fan was connected to 12-V DC power supply through the wall of the chamber and was separated from the pup by a metallic mesh (1 × 1 mm meshwork). Water vapor was removed from the outflow circuit using calcium sulfate (Drierite; Fischer Scientific, Illkirch) and scrubbed of CO₂ using sodium hydroxide (Ascarite, Fischer Scientific). The fractional content of O₂ was then measured continuously (Oxzilla II O₂ Analyzer; Sable Systems, Las Vegas, NV) (root mean squared noise: < 2 ppm; peak-to-peak noise: < 8 ppm). The cell of the analyzer was temperature controlled at any temperature between 15°C and 50°C (and therefore insensitive to the present changes in ambient temperature). First, the changes in O₂ content due to washout of the chamber after transitions from air to hypoxia and from hypoxia to air were measured with no pup in the chamber. To do this, an inert object of equivalent shape and volume of 6-day-old pups (about 5 ml) was placed in the chamber. The time lag of the analyzer response to the switch in gas mixtures was < 30 s. Each pup then underwent the protocol as defined in the main study. V˙O₂ was calculated as the product of the flow through the chamber (100 ml/min) by the instantaneous difference between O₂ content with and without the pup in the chamber. V˙O₂ was divided by body weight. The analyzer was calibrated before each recording session. A second reference curve was established after each test to check for possible drifts in O₂ measurements. Drifts were not observed. For each temperature condition, the pups were taken at random from the litter and assigned alternately to the hypoxic and the normoxic group. The V˙O₂ response to hypoxia was expressed as the percentage V˙O₂ change relative to baseline V˙O₂, using the formula 100-(peak V˙O₂ − baseline V˙O₂)/baseline V˙O₂.

**Statistical analysis.** We analyzed baseline breathing variables and the ventilatory, USV, and motor responses to hypoxia. For all variables, the responses to hypoxia (absolute values and percentage V˙E changes) were subjected to analyses of variance (StatView 5; Abacus Concepts, Berkeley, CA), with group (hypoxic vs. normoxic) and temperature (33°C vs. 26°C) as between-subjects factors and test (two hypoxic tests) as the within-subject factor. Litter had no significant effect, neither as a main between-factor nor in interaction with other factors of the analysis, and will not be mentioned further. Values are means ± standard deviation (SD) in the text and table and means ± SE in the figures.

**RESULTS**

**Baseline normoxic breathing.** Illustrative examples of ventilatory, motor, and USV recordings are shown in Fig. 2. Large proportions of the 40-min recordings were free of movement artifacts at 33°C (hypoxic group: 81% (SD 7); normoxic group: 83% (SD 8)) and at 26°C (hypoxic group: 72% (SD 9); normoxic group: 73% (SD 8)). The temperature-related differences in movement durations are analyzed below (MVT).

At 33°C, V˙E decreased progressively in both the hypoxic and the normoxic group during 5–6 min following introduction in the plethysmograph, probably as a result of habituation, then leveled off (Fig. 3A). This initial V˙E decrease was mainly due to a progressive TTOT increase, since changes in VT were minimal and not significant (Fig. 4A). Most pups showed apneas, which represented a small proportion of the ventilatory recordings (Fig. 4A).

At 26°C, V˙E levels remained high throughout the initial normoxic period in both the hypoxic and the normoxic group, without any habituation effect (Fig. 3B). Baseline normoxic V˙E was significantly higher at 26°C than at 33°C in both groups (hypoxic group: P < 0.0001; normoxic group: P < 0.0001; Table 1 and Fig. 3). This difference was due mainly to smaller TTOT values (hypoxic: P < 0.0001; normoxic: P < 0.0001) and, to a lesser extent, to larger VT values at 26°C than at 33°C (hypoxic: P < 0.0001; normoxic: P < 0.001; Table 1 and Fig. 4). Apneas were nearly abolished at 26°C and mainly restricted to posthypoxic periods (hypoxic: P < 0.0001; normoxic: P = 0.0008; Table 1 and Fig. 4B).

**Ventilatory response to hypoxia.** At 33°C, hypoxia caused a sharp increase in V˙E (Fig. 3A). The initial V˙E increase was a result of a decrease in TTOT and an increase in VT; it was accompanied with a marked decrease in apnea duration (Fig. 4A). The mean latency to peak V˙E in the hypoxic group at 33°C was 137 s (SD 28) in test 1 and 139 s (SD 27) in test 2 (Fig. 2A). After hypoxia, V˙E decreased to baseline levels (Fig. 3A).

At 26°C, hypoxia caused a small V˙E increase, rapidly followed by a large ventilatory decline (Fig. 3B). The small initial V˙E increase was chiefly ascribable to a decrease in TTOT, since VT showed only a minimal increase (Fig. 4B). The mean latency to peak V˙E was 72 s (SD 44) in test 1 and 85 s (SD 41) in test 2 (difference: not significant). V˙E decreased rapidly and remained below prehypoxic baseline levels throughout the posthypoxic period (Fig. 3B). However, despite different baseline V˙E levels, the absolute peak V˙E during hypoxia was highly similar at 33°C and at 26°C (Fig. 3).

The observations were confirmed by ANOVAs of the ventilatory response to hypoxia. Peak V˙E, expressed as the per-
percentage change from baseline \( V \dot{E} \), was significantly lower at 26°C than at 33°C in both tests (test 1: group by temperature interaction: \( P < 0.0001 \); partial comparisons between 26°C and 33°C: \( P < 0.0001 \), Fig. 5). The two tests yielded identical results (test 2 not shown). This difference in peak \( V \dot{E} \) can be attributed to smaller percentage changes in \( T_{TOT} \) (group by temperature interaction: \( P < 0.0001 \); partial comparisons between 26°C and 33°C: \( P < 0.0001 \)) and \( V_T \) (group by temperature interaction: \( P < 0.0001 \), and partial comparisons between 26°C and 33°C: \( P < 0.0001 \), Fig. 5) during exposure to cold.

Motor response to hypoxia. At 33°C, movement duration decreased progressively in both the hypoxic and the normoxic groups when the pup was placed in the plethysmograph chamber (Fig. 3A). This decrease was consistent with the \( V \dot{E} \) decline ascribed to habituation (Fig. 3A). During the initial normoxic period, movement duration was longer at 26°C than at 33°C, in both groups (\( P < 0.0001 \) in both groups, Fig. 3 and Table 1).

At 33°C, movement duration during hypoxia (in the hypoxic group) was short (Fig. 3A). At 26°C, movements lasted considerably longer and displayed a clear-cut biphasic pattern, which covaried with the \( V \dot{E} \) and USV hypoxic responses (Figs. 3B and 4B). The \( \Delta MVT \) was significantly stronger at 26°C than at 33°C (group by temperature interaction, \( P < 0.0001 \), and partial comparison shown in Fig. 5). In the hypoxic group, \( \Delta MVT \) was larger in test 2 (\( P < 0.0002 \), not shown) than in test 1, but this difference was probably due to the prehypoxic movement depression following test 1. Comparisons between 26°C and 33°C yielded the same statistical results as for test 1.

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Fig. 2. Examples of respiratory recordings at 26°C. A: ventilatory signal. Hypoxia elicited a defense-alerting response characterized by movements, which are detected as sharp disturbances of the respiratory signal. Note the apnea during the posthypoxic ventilatory depression. B: raw sound signal; USVs were produced in the 30–90 kHz range. C: USV frequencies. At 33°C, USV duration remained close to zero throughout the recording (not shown).

Fig. 3. Ventilatory, USV, and motor responses to hypoxia (MVT) in 6-day-old mice (●, 33°C: \( n = 87 \), 26°C: \( n = 81 \)) and normoxic control pups (○, 33°C: \( n = 92 \), 26°C: \( n = 84 \)) at 33°C (A) and 26°C (B). Hypoxia (3-min 10% \( O_2 \)) is represented by shaded areas. Cold increased normoxic ventilation, depressed the hyperpneic response to hypoxia, and increased the motor and USV responses to hypoxia. Ventilation (\( V \dot{E} \)) was averaged over consecutive 30-s periods. Total USV and MVT durations were calculated over successive 30-s periods. \( T \), body temperature. Values are group means (error bars are means ± SE; most are smaller than the symbols). See RESULTS for statistical analyses.
USV response to hypoxia. At 33°C, USV duration remained close to zero in both the hypoxic and the normoxic group throughout the recording (Fig. 3A, and Table 1). During the initial normoxic period, USV durations were longer at 26°C than at 33°C, in both groups (P < 0.0001 in both groups, Fig. 3 and Table 1).

At 26°C, USV displayed a biphasic pattern similar to that of V˙E (Fig. 3B). Hypoxia elicited a considerably stronger USV response (ΔUSV) at 26°C than at 33°C (group by temperature interaction: P < 0.0001, and partial comparison shown in Fig. 5). In the hypoxic group, ΔUSV was smaller in test 2 (P < 0.0001, not shown) than in test 1, but the comparisons between 26°C and 33°C yielded the same statistical results as for test 1.

Effects of prehypoxic behavioral state. In the hypoxic groups, the proportions of movement time during the 3-min period preceding the first hypoxic test (i.e., after habituation) were larger at 26°C than at 33°C [33°C: 10% (SD 12); 26°C: 28% (SD 23), P < 0.0001]. Similarly, the percentages of time with USVs before the first hypoxic test were small at both temperatures but larger at 26°C than at 33°C [33°C: 0.0% (SD 0.0); 26°C: 1.8% (SD 3); P < 0.0001].

The larger amounts of movements and USVs during prehypoxic normoxia at 26°C compared with 33°C indicated a larger number of awake periods at 26°C. We examined whether these differences in prehypoxic states accounted for the increased motor and USV responses at 26°C. We matched a subsample of pups at 26°C (n = 14) with a subsample at 33°C (n = 13) for movement and USV levels over the 3-min preceding hypoxia (Fig. 6). This was done by selecting two subsamples of pups with reduced motor and USV activity at both tempera-

Fig. 4. Tidal volume (V˙E), breath duration (T_TOT), and apnea duration in response to 3-min 10% O2 exposure (shaded areas) in 6-day-old mice (○, 33°C: n = 87, 26°C: n = 81) and normoxic control pups (●, 33°C: n = 92, 26°C: n = 84) at 33°C (A) and 26°C (B). Cold abolished the hypoxic increase in V˙E and strongly depressed the T_TOT response. Apneas were nearly abolished throughout the recording. V˙E and T_TOT were averaged over consecutive 30-s periods. Total apnea duration was calculated over successive 30-s periods. Values are group means (error bars are means ± SE; many are smaller than the symbols). See RESULTS for statistical analyses.

Fig. 5. V˙E, MVT, and USV responses to hypoxia. Values are expressed as percentages (V˙E, V˙I, and T_TOT) or absolute differences (USVs, apneas, and movements) relative to baseline levels calculated over the 3 min of normoxia preceding hypoxia. Only the first test is shown (the second test yielded similar results). Cold depressed the ventilatory response and increased the defense-alerting response to hypoxia. Partial comparisons between hypoxic and normoxic groups were significant (except for ΔUSV at 33°C) and are not indicated, for the sake of clarity. Values are group means (error bars are means ± SE). ***P < 0.0001.
Fig. 6. Ventilatory, USV, and MVT responses to 3-min 10% O₂ exposure (shaded area) in 6-day-old mice. Data from two subsamples of hypoxic groups (at 33°C and at 26°C) matched for USV and movement levels over the 3 min preceding hypoxia (■, 26°C; n = 14; ▲, 33°C; n = 13). The normoxic groups are not shown. Cold depressed the ventilatory response and increased the defense-alerting response to hypoxia. These effects were not caused by cold-induced differences in prehypoxic behavioral states. Values are group means (error bars are means ± SE).

Fig. 7. Top: infrared thermographs of the dorsal skin surface at 26°C of ambient temperature. The peak body temperature (spot) was obtained in the interscapular region, that contains thermogenic brown adipose tissues. Bottom: interscapular temperature (Tis) in response to 3-min 10% O₂ exposure (shaded areas) in 6-day-old mice at 33°C (▲, hypoxic group, n = 4; ●, normoxic group, n = 4) and at 26°C (●, hypoxic group, n = 14; ○, normoxic group, n = 15). At 33°C, hypoxia had no effect on body temperature. At 26°C, hypoxic elicited a small drop (<0.6°C) in body temperature that appeared after return to normoxia. Values are group means of peak body temperatures (error bars means ± SE).

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study with concomitant measures of IR body temperature in an independent sample of pups (33°C: n = 4, in both hypoxic and normoxic groups; 26°C: n = 15 in the hypoxic group, and n = 14 in the normoxic group). Body temperature was determined as the peak value of the IR thermograph (Fig. 7A).

At 33°C, body temperature increased progressively and was not influenced by hypoxia (Fig. 7B). At 26°C, body temperature decreased progressively, and both hypoxic exposures elicited a small decrease in temperature (<0.6°C, Fig. 7B). Temperature decreases reached significant levels after the return to normoxia (difference between 3-min posthypoxia and 3-min prehypoxia, P < 0.0001 and P = 0.043, for the two hypoxic tests, respectively, Fig. 7). Furthermore, at the time of onset of the motor and USV responses (i.e., 30 s after the onset of hypoxia) body temperature in normoxic and hypoxic groups were indiscernible (Fig. 7). Thus, the motor and USV responses to hypoxia at 26°C were not secondary to the decrease in body temperature.

O₂ consumption. To explain the cause of cold-induced changes in breathing pattern and body temperature, we replicated the protocol of the main study with continuous measurement of V˙O₂ in an independent sample of pups (33°C: n = 7 in the hypoxic group, and n = 8 in the normoxic group; 26°C: n = 7 in both hypoxic and normoxic groups).

At 33°C, during normoxia, V˙O₂ transiently increased during 1–2 min following the introduction in the plethysmograph in both the hypoxic and the normoxic group (Fig. 8). This effect was possibly due to early changes in movement duration (Fig. 3A). Then V˙O₂ decreased progressively and was very slightly affected by hypoxia in the hypoxic group (Fig. 8). The percentage decrease of V˙O₂ for the two hypoxic tests were −14% (SD 15) and −11% (SD 10), respectively, in the hypoxic group. Similarly, previous results in 3-day-old mice exposed to 10% O₂ for 10 min showed a 12% drop in V˙O₂ (39). Larger drops in V˙O₂ during hypoxia were reported (2), but differences in the duration and intensity of the hypoxic stimuli, and in mouse strains make direct comparison difficult. At 26°C, V˙O₂ increased progressively during normoxia in both the hypoxic and the normoxic group, in keeping with body temperature decreases.
Effects of cold on normoxic breathing. In normoxia, cold stimulated \( V_{\dot{E}} \), chiefly by increasing the breathing rate, and abolished apneas. The \( V_{\dot{E}} \) increase was caused by the rise in production by brown adipose tissue (44). Similarly, in full-term human infants within 6 days after birth, cold (25–28°C) increased normoxic \( V_{\dot{E}} \), by ~40%, compared with values measured at 32–34°C (9) and in preterm infants, decreasing ambient temperature two degrees below thermoneutrality increased \( V_{\dot{E}} \) by 8% in quiet sleep and 20% in active sleep (10). Thus, our finding that cold increased \( V_{\dot{E}} \) in newborn mice extends previous observations in other species, including humans.

Effects of cold on the ventilatory response to hypoxia. Our study provides the first data about the effect of cold on the hypoxic ventilatory response in newborn mice. Cold drastically depressed the initial hyperpneic component of the hypoxic response, while it increased the subsequent ventilatory decline. However, the \( V_{\dot{E}} \) peak and the trough values observed during and after hypoxemia did not differ in both temperature conditions. This result is in line with previous data from newborn piglets (32) and humans (36). In newborn mammals, the ventilatory decline that follows the initial hyperpneic response to hypoxia is due, at least in part, to a decrease in metabolic rate (38). At cold temperatures, the need for increased thermogenesis magnifies the effects of hypoxia-induced metabolic depression, which may account for the increased hypoxic depression (36). The present data clearly indicated a drastic drop in \( V_{\dot{O}_2} \) during hypoxia in cold conditions. In human infants, the increase in ventilation caused by hypoxia (12% \( O_2 \) for 3 min) was abolished when ambient temperature was lowered from 32–34°C to 25–28°C (9).

Motor and alerting USV responses to hypoxia. Our study is the first to involve simultaneous analysis of breathing patterns, motor response, and USVs in newborn rodents. Cold is a primary stimulus for eliciting USVs in many mammalian species (7). As expected, during normoxia, the pups produced more movements and USVs at 26°C than at 33°C. Because movements and USVs elicit maternal attention and care, they are thought to play a communicative role (7, 11, 34), although this view is debated (3). In our study, hypoxia was associated with USV production only under cold conditions. One possible explanation is that hypoxia is more harmful when the metabolic rate is increased as a result of cold exposure than under thermoneutral conditions.

The role of ventilatory efforts in the arousal response to hypoxia. Cold deeply depressed the \( V_{\dot{E}} \) hypoxic response (expressed as the percentage of prehypoxic levels). Baseline ventilation was higher at 26°C, hypoxia caused a slight increase and a large ventilatory decline. In contrast, cold sharply enhanced the motor response and elicited USV production. This suggested that cold decreased the threshold of motor and USV productions, independently from the \( V_{\dot{E}} \) response. Consistent with this dissociation between the ventilatory and behavioral responses to hypoxia, we previously found that the motor response to hypoxia in P0-P2 mice was substantially delayed compared with the peak \( V_{\dot{E}} \) response (12). We also reported that intermittent hypoxia increased the motor-response delay, while decreasing the time-to-peak \( V_{\dot{E}} \) in 4-day-old mice (15). Taken together, these previous results suggest, in line with the results reported here, that mechanisms other than ventilatory effort may contribute to trigger the arousal response to hypoxia.

The motor and USV responses to hypoxia were not determined by the prehypoxic behavioral state. The durations of gross body movements and USV production shed light on behavioral states. In newborn rodents, sleep-wake states cannot
be determined by electroencephalogram or electrooculogram (27). They can be assessed, however, using behavioral criteria, such as gross body movements, nuchal EMG, motor twitches (27), and USVs (8). Body movements and USVs were used as markers for behavioral states in the present study. Before the first hypoxic test, movement and USV times were longer at 26°C than at 33°C, suggesting that cold may have reduced sleep time. A similar effect was found in 1-wk-old rats exposed to an ambient temperature drop from 35°C to 27°C (43). We addressed the hypothesis that the cold-induced decrease in behavioral sleep time preceding hypoxia accounted for the stronger motor and USV responses to hypoxia at 26°C compared with 33°C. This hypothesis was refuted by our analysis of the motor and USV responses in subsamples of pups matched for prehypoxic USV and motor activity levels. Cold enhanced the motor and USV responses in these matched subsamples. In addition, the second hypoxic test at 26°C was preceded by marked depression of USVs and movements, very similar to that observed at 33°C, yet was associated with a strong USV response.

The USV response to hypoxia was not determined by body temperature. Using a newly designed system to measure body temperature by IR thermography in unrestrained pups inside the plethysmograph chamber, we found that, at 33°C, body temperature increased progressively and was not influenced by hypoxia. In contrast, at 26°C, body temperature decreased progressively, and both hypoxic exposures elicited a small decrease in temperature (<0.6°C, this had minor impact on VT calculation), suggesting a transient drop in heat production by brown adipose cells (35). When oxygen consumption is increased by cold exposure, hypoxia can cause a significant hypometabolism, accompanied by a decrease in body temperature, especially in newborns (35). Body temperature changes most often appeared after the return to normoxia, thus precluding any causal influence on the motor and USV responses to hypoxia.

Our study does not identify the mechanism of the motor and USV responses to hypoxia in cold conditions, but it ruled out ventilatory efforts and body temperature changes as crucial contributing factors. Rather, the present study suggests that these responses rely on central nervous centers involved in arousal, such as the locus coeruleus. The involvement of central processes is further supported by a recent study showing that newborn mice produced USVs, not only in response to hypoxia, but also in response to artificial odors previously paired with hypoxia (5). Thus, the arousal responses to hypoxia may be produced in anticipation of actual hypoxic stimuli, as a result of learning processes.

Clinical implications. Because arousal is an important defense mechanism against sleep-disordered breathing, the role of the ventilatory response to hypoxia in arousal mechanisms is clinically relevant. Cold produces similar effects on breathing in newborn mice and human infants, including increased normoxic ventilation and a decreased hypoxic hyperpneic response. The motor and USV responses to hypoxia in newborn mice resemble movements and crying responses to hypoxia in human newborns (17, 22, 33, 48, 51), although the exact chronology of these two responses could not be determined with accuracy, due to technical limitations. These similarities provide a basis for investigating disorders of the arousal response to hypoxia using mouse models, although caution is in order when extrapolating the results of mouse studies to infants.

The dissociation between the ventilatory and the arousal responses to hypoxia found in the present and previous studies (12) goes against the hypothesis that arousal deficits are mediated by impaired ventilatory responses to hypoxia.

The present study suggests that central processes, rather than ventilatory and temperature responses accounted for the arousal response to hypoxia. Our results do not support the hypothesis that cold may depress the arousal response to hypoxia. In fact, the arousal response was increased by cold. The present results may also suggest that the combination of warming and hypoxia depresses arousability to hypoxia in infants. Impaired arousal responses to hypoxia have been consistently implicated in SIDS (50). However, the relevance of our data with respect to SIDS may be questioned due to the discrepancy between the age of peak occurrence of SIDS in infants (2–4 mo) and the age of mouse pups in the present study (postnatal day 6), which corresponds to prematurity.

Perspectives and Significance

We examined the ventilatory and arousal responses to hypoxia in newborn mice, using a fully integrated and noninvasive approach in newborn mice. We found that the sequence of ventilatory and behavioral responses to hypoxia in mouse pups closely resembled the sequence of responses to hypoxia described in human infants. Furthermore, we found that cold stimulated defensive movements and elicited alerting USVs, while markedly depressing the hyperpneic response to hypoxia. The dissociation between ventilatory and behavioral responses to hypoxia suggests that deficits in the arousal response associated with sleep-breathing disorders are not ascribable to a depressed hypoxic response.

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REFERENCES


