Effects of temperature on sleep in the developing rat

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Morrissette, Roger N., and H. Craig Heller. Effects of temperature on sleep in the developing rat. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1087–R1093, 1998.—In altricial species, such as humans and rats, much of the development of autonomic systems occurs postnata- tally. Consequently, vulnerabilities exist early in postnatal development when immature autonomic functions are challenged by external factors such as variations in ambient temperature (Tb). Ta profoundly influences sleep/wake state structure in adult animals and humans, and exposure to excessive warmth has been implicated as a risk factor in sudden infant death syndrome. To better understand the relationship between temperature and sleep during development, we investigated the effect of Ta variation on sleep/wake state structure and sleep intensity in developing rats. In this experiment, sleep intensity was measured by the intensity of slow-wave activity during slow-wave sleep. Neonatal Long-Evans hooded rat pups were surgically prepared for chronic sleep/wake state and brain temperature (Tbr) recording. Two-hour recordings of sleep/wake state and Tbr were obtained from rats on postnatal day 12 (P12), P14, P16, P18, and P20 at a Ta of either 28.0–30.0, 33.0–35.0, or 38.0–40.0°C. Ta significantly influenced sleep/wake state structure but had little, if any, effect on sleep intensity in developing rats.

A CRITICAL FACET of the relationships between temperature and sleep in adult animals is that temperature alters sleep/wake state distribution (17, 36). Increased body temperature (Tb), either by passive heating of ambient temperature (Ta) or through exercise leads to increased non-rapid eye movement (NREM) sleep (19). In humans, brain temperature (Tbr) declines during the first 2–3 h after sleep onset coincident with the peak occurrence of the deepest NREM sleep stages (32, 41). If sleep is extended to 15 h into the early afternoon, when the circadian rhythm for Tb is rising, an increase in NREM sleep is seen (10). It has been theorized that increases in NREM sleep after transient increases in Tb represent an active thermoregulatory response triggered to counter hyperthermia (23, 31).

Sudden infant death syndrome (SIDS) has a strong association with sleep and Tb variation. Infants who succumb to SIDS are almost always found at the end of a sleep period and are often found excessively bundled or close to heating units (30, 35). Likewise, the peak seasonal death rate for SIDS is during the winter months (1, 15, 30, 35). Tb variation could affect sleep/wake state distribution or sleep depth with concomitant increases in arousal threshold, thus preventing infants from properly arousing from life-threatening situations such as prolonged apneas (12).

An equally important facet of the relationships between sleep and temperature in adult animals is that sleep/wake state distribution directly affects thermoregulation (11, 12, 18, 31). At the onset of NREM sleep, hypothalamic thermosensitivity is diminished (18). This coincides with a decrease in heat production, declines in Tb, and an increase in heat dissipation (18). In addition, Tb has a lower level of regulation during NREM sleep than during waking (W), while during rapid eye movement (REM) sleep, thermoregulation is seriously inhibited (11).

During the 2- to 4-mo critical period for SIDS, a normal, healthy infant shows increases in metabolic rate, increases in its body mass-to-surface area ratio, a thickening of the subcutaneous fat layer, and an increase in the effectiveness of its peripheral cold-induced vasomotor response (6). Therefore, these infants have a reduced ability to dissipate heat, making them vulnerable to excessive thermal loads. SIDS victims have been shown to have significantly increased hypothemic and hyperthermic bouts (28), and some infants have shown evidence of excessive sweating and elevated Tb before succumbing to SIDS (15, 28).

Neonatal rats and human infants go through similar postnatal developmental stages. Both are born immature and, whereas rats mature more rapidly than humans, both share a similar temporal order of autonomic system development. Based on similar developmental changes in sleep/wake states (7, 20, 34), thermoregulation (21, 40), circadian rhythms (5, 22, 25), and neural development (42), we hypothesize that rats aged postnatal day 10 (P10) through P20 roughly equate developmentally to human infants over the ages of 2 to 4 mo of age, which is when SIDS is most likely to occur.

In adult rats, there are three sleep/wake states: W, REM sleep, and NREM sleep. W is characterized by desynchronous electroencephalographic (EEG) activity and enhanced electromyographic (EMG) activity. REM sleep has similar desynchronous activity in the EEG, but the EMG is significantly reduced due to the presence of muscle atonia (20). In humans, REM sleep is also characterized by irregularity of heart rate and respiration and phasic occurrences of myogenic jerks, rapid eye movements, and pontogeniculooccipital waves (33, 34). Similar characteristics have been found for rats and other mammals (7, 13, 20). NREM sleep is distinguished from REM and W by the presence of synchronized activity, called slow waves, in the EEG. Hence, NREM sleep is also referred to as slow-wave sleep (SWS). NREM sleep is also characterized by a decrease in EMG activity to a level below W but above REM sleep (7, 13, 20, 33, 34).

Before the appearance of differential EEG patterns in neonates, neonatal arousal states are divided into either W, active sleep (AS), or quiet sleep (QS) based on behavioral observations of body movement. Distinct EEG patterns begin to appear at P10–P11 in the rat (3,
control the T_{a} inside the recording chamber. The recording

16

libitum. During data collection, animals were placed into

12:12-h light-dark cycle. Food and water were available ad

all not of the phasic features of adult REM sleep are present by P20
(7). For this reason we chose to call this REM-like state

“AS/REM,” because it contains characteristics of both

and adult REM sleep. The term SWS will be used to
define the QS/NREM-like state, because the presence

of slow waves in the EEG was the main defining criteria
of this state.

Through spectral analysis of the EEG, the intensity of
slow waves or slow-wave activity (SWA) can be
quantified. SWA within NREM sleep has been shown to
increase as a function of prior waking and is therefore
believed to reflect a homeostatic sleep restorative pro-

cess (39). Likewise, SWA can be considered a measure of
NREM sleep intensity or depth of sleep (39). This
experiment was designed to determine how 3 h of a
specific chronic T_{a} exposure affects sleep/wake state
structure and intensity in neonatal rat pups age P12–
P20.

METHODS

Housing conditions. All Long-Evans hooded male rats were

housed in the colony room with one dam and litter per cage.
The animals were maintained at a T_{a} of 22 ± 0.5°C on a

12:12-h light-dark cycle. Food and water were available ad

libitum. During data collection, animals were placed into
double-walled recording chambers made of clear acrylic (22 ×

16 × 18 cm). The walls were perfused with heated water to

control the T_{a} inside the recording chamber. The recording

chamber floor was made of a taut piece of neoprene. The T_{a}

and T_{br} thermocouples and cable commutators were secured
to the top of the recording chambers.

Surgery. A total of 29 male Long-Evans hooded rat pups were

chronically implanted for sleep/wake state and T_{br}
recording at P10. Animals were anesthetized with methoxyflu-
orane (Metofane; Pitman-Moore). A dorsal midline incision in

the skin was made on the top of the skull. The skull was then
cleaned with hydrogen peroxide solution (3%). To record the
frontal-parietal EEG, four miniature stainless steel screws
were soldered to Teflon-coated stainless steel wire and im-
planted into the skull (0.0 mm anterior and ±2.0 mm lateral
to bregma, 0.0 mm anterior and ±2.0 mm lateral to lambda). Three Teflon-coated stainless steel stranded wire electrodes were
inserted bilaterally and at midline into the dorsal

nuchal muscle to record EMG activity. To record T_{br}, a sealed
stainless steel guide cannula or reentrant tube (0.65 mm OD),
was inserted into the skull (0.5 mm anterior, 0.5 mm lateral,
and 4.0 mm ventral to bregma) to allow thermocouple place-
ment into the brain. All electrodes were soldered to a seven-

pin gold connector (MicroTech) that was affixed to the skull
along with the reentrant tube via dental acrylic (Hygienic).
Immediately after surgery, animals were returned to their
home cages with their nursing dams and littermates. All
animals were allowed at least 2 days to recover from surgery.

At P21, rats were given a lethal overdose with 4% halothane
gas, their implants were removed, and the surgical area was
inspected for pathology.

Recording procedure. At 1 h after lights-on in the colony
room, pups were removed from their home cages and given a
light dose of methoxyfluorane anesthesia to facilitate attach-
ment to recording cables. The pups were then placed in
recording chambers with T_{a} preset to either 28.0–30.0, 33.0–
35.0, or 38.0–40.0°C. Animals were allowed 1 h of chamber
acclimation and recovery from anesthesia. Starting at 2 h
after lights-on, sleep/wake state and T_{br} data were collected
for the next 2 h. Animals were then returned to their home
cages with their nursing dams and littersmates.

Data analysis. The T_{a} and T_{br} thermocouple signals were
amplified to a 0.1 V/1.0°C signal and stored by computer in
10-s epochs. The differential output between frontal-parietal
EEG electrodes was amplified to a −5.0- to +5.0-V signal band
filtered between a 0.3-Hz high-pass and 30-Hz low-pass
filter (Grass Instruments). The amplified signal was then
digitized by a data acquisition computer system (Data Trans-
lation) at 100 Hz. A fast Hartley transform was used to
transform the digitized input in the frequency domain of
consecutive 10-s epochs. EEG power spectra from 0 to 20 Hz
were then calculated and stored. The EMG signal was
collected by amplifying the differential output between two of
the three EMG electrodes to a −5.0- to +5.0-V signal band
filtered between a 3.0-Hz high-pass and 75-Hz low-pass filter
(Grass Instruments). The EMG signal was integrated per
epoch so that a single quantified EMG value could be as-
signed to each epoch. EEG power spectra were then averaged
for delta (0.75–4.0 Hz), theta (6.0–9.0 Hz), and sigma (10.0–
14.0 Hz) frequency ranges as previously described (2).

Sleep/wake states were scored algorithmically using inte-
grated EMG values and EEG power spectra values (2).
Waking was first separated from the two sleep states by
generating a scatterplot using sigma × theta by integrated
EMG values for each 10-s epoch. A high integrated EMG
value and a low sigma × theta value would represent waking
epochs. Second, to determine SWS from REM sleep, a scatter-
plot using the integrated EMG and delta values was used.
Low EMG values and low delta activity would indicate REM
epochs, whereas low EMG and high delta activity would
indicate SWS epochs. State scoring confirmation and artifact
removal was done by visually reviewing each epoch of data.
Age-dependent changes in power spectra did not affect the
state scoring criteria.

The amount of time an animal spent in each arousal state
(W, AS/REM, SWS) is expressed as the percentage of total
recording time (%TRT). The %TRT, number of bouts of each
state, mean bout length, number of brief arousals (nBA), and
SWS SWA were determined for each animal. Because rat
pups can change from sleep to wake and back to sleep in <10
s, a 10-s minimum was used to define a bout length. Likewise,
a brief arousal was scored as any waking state that lasted
<20 s. Due to wide variance in absolute EEG power values
between animals, SWS SWA scores were standardized to z
scores and then to T scores before analysis across develop-
mental age. For analysis across T_{a} condition, SWS SWA
was normalized to SWS total EEG power to control for changes
in EEG power across development. Data were statistically ana-
lyzed by a two-way ANOVA repeated-measures test with
Fisher’s test applied for post hoc pairwise comparisons.

RESULTS

Effect of T_{a} on T_{br}. As expected, when data were
pooled across age, T_{a} had a highly significant effect on
This is evident by the fact that T_{br} did not drop below normal ranges in the coolest T_{a} but was elevated to hyperthermic levels during the warmest T_{a} condition (37, 38).

Effect of age and T_{a} on state percentages (%TRT). State percentages changed significantly with age. %SWS significantly increased with age [F(4, 68) = 7.596, P < 0.0001, n = 15], whereas %AS/REM significantly decreased [F(4, 68) = 7.336, P < 0.0001, n = 15]. %W showed no significant change across age [F(4, 68) = 1.034, P = 0.3964, n = 15]. Figure 2A displays the changes in sleep/wake state percentages across age.

State percentages were significantly affected by T_{a}. %SWS was significantly higher in the two warmest T_{a} conditions [F(2, 68) = 10.928, P < 0.0001, n = 26], whereas %AS/REM was significantly higher at 33.0–35.0°C [F(2, 68) = 15.422, P < 0.0001, n = 26]. %W was significantly lowest at 33.0–35.0°C and significantly highest at 28.0–30.0°C [F(2, 68) = 18.805, P < 0.0001, n = 26]. Figure 2B shows changes in sleep/wake state percentages across the three different T_{a} conditions.

Effect of T_{br} on state percentages. T_{br} was positively correlated with %SWS (r = 0.478, P < 0.0001, n = 83) and negatively correlated with %AS/REM (r = −0.258, P < 0.05, n = 83). Figure 3A displays the scatterplot for T_{br} and %SWS while Fig. 3B shows the scatterplot for T_{br} and %AS/REM.

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Fig. 1. Mean ± SE brain temperatures (T_{br}) at different postnatal ages (postnatal day 1 (P1), etc.) and ambient temperature (T_{a}).

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Fig. 2. A: mean ± SE state percentages by age pooled across T_{a} condition. abc: Significantly different (P < 0.05) from P12, P14, and P16, respectively. B: Mean ± SE state percentages by T_{a} condition pooled across age. ef: Significantly different (P < 0.05) 28–30 or 33–35°C, respectively.

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Fig. 3. A: scatterplot with regression line (±SE) showing the relationship between mean T_{br} and percentage slow-wave sleep (%SWS). B: scatterplot showing the relationship between mean T_{br} and percentage active sleep/rapid eye movement sleep (%AS/REM). Both scatterplots pool T_{a} conditions and ages. * Significant difference (P < 0.05).
The significant positive correlation between Tbr and %SWS is both Ta condition dependent and age dependent. Individual analyses revealed a significant correlation at a Ta of 33–35°C (r = 0.517, P < 0.01, n = 27) and at age P14 (r = 0.591, P < 0.005, n = 20) and P16 (r = 0.715, P < 0.005, n = 17). No other significant Ta-specific or age-specific correlations were found between Tbr and %SWS.

The significant negative correlation between Tbr and %AS/REM is Ta condition dependent but shows no significant age-dependent relationship. Individual analyses revealed a significant correlation at a Ta of 33–35°C (r = −0.587, P < 0.005, n = 27) and 38–40°C (r = −0.646, P < 0.0005, n = 30).

Effect of age and Ta on bout number. There was a significant age-dependent effect on state bout number when pooled across all Ta conditions. Both SWS [F(4,68) = 3.617, P = 0.0099, n = 15] and AS/REM [F(4,68) = 4.047, P = 0.0053, n = 15] showed a significant decrease in the number of bouts from P12 to P14 and P16. Waking showed no significant age effects [F(4,68) = 1.175, P = 0.3298, n = 15]. Figure 4A displays the changes in state bout number with age.

Ta significantly affected state bout number. All three states, AS/REM [F(2,68) = 47.453, P < 0.0001, n = 26], SWS [F(2,68) = 65.464, P < 0.0001, n = 26], and W [F(2,68) = 37.575, P < 0.0001, n = 26], showed significant increases in bout number at 38.0–40.0°C compared with the other two Ta conditions. AS/REM bout number also showed a significant increase at 33.0–35.0°C compared with 28.0–30.0°C. A significant interaction between developmental age and Ta was found for AS/REM bout number [F(8,68) = 2.968, P = 0.0066], so interpretations of main treatment effects should be made with caution. Figure 4B shows changes in state bout number across Ta condition.

Effect of age and Ta on mean bout length. Developmental age had a significant effect on mean bout length. Both SWS [F(4,68) = 3.951, P = 0.0061, n = 15] and AS/REM [F(4,68) = 4.411, P = 0.0031, n = 15] showed a significant increase in mean bout length with specific ages, P14 and P16 for SWS and P14 for AS/REM. Waking showed no significant differences in mean bout length across age [F(4,68) = 0.724, P = 0.5787, n = 15]. Figure 4C demonstrates the changes in state mean bout length across age. Ta had a significant effect on mean bout length. As with number of bouts, all three states, SWS [F(2,68) = 13.985, P < 0.0001, n = 26], AS/REM [F(2,68) = 20.822, P < 0.0001, n = 26], and W [F(2,68) = 21.114, P < 0.0001, n = 26], showed significant effects at 38.0–40.0°C. In this case, all three state mean bout lengths were decreased in the 38.0–40.0°C Ta condition. Waking mean bout length was also significantly decreased at 33.0–35.0°C relative to 28.0–30.0°C. See Fig. 4D for relative changes in state mean bout length across Ta.

Effect of age and Ta on nBA. Although nBA was not affected by age [F(4,68) = 1.290, P = 0.2827, n = 15] significant increases in nBA [F(2,68) = 33.42, P < 0.0001, n = 26] were seen with increases in Ta. Figure 5A and B demonstrates these relationships.

Effect of age and Ta on SWS SWA. SWS SWA showed no significant effect across the Ta conditions [F(2,68) = 0.655, P = 0.5229, n = 26] but showed a significant

![Fig. 4](http://ajpregu.physiology.org/)
effect across age. SWS SWA significantly increased with age \[F(4,40) = 138.752, P < 0.0001, n = 11\] and showed significant differences at each age group. Figure 6, A and B, demonstrates these relationships.

DISCUSSION

This study was designed to determine how 3 h of a specific \(T_a\) exposure affects sleep/wake state structure and SWS intensity in the developing rat. \(T_a\) variations had a significant effect on sleep/wake state structure. %AS/REM peaked at 33.0–35.0°C, whereas %SWS was high at both 33.0–35.0 and 38.0–40.0°C. It has been shown in adult rats that the amount of time an animal spends in REM sleep is maximal within the \(T_a\) range at which metabolic rate is not elevated by energy expenditure for thermoregulation, the thermoneutral zone (TNZ; Ref. 36). Because %AS/REM peaks at the 33–35°C \(T_a\) condition, this suggests that the TNZ for these developing rats may fall within this \(T_a\) range. These results agree with other estimates of the TNZ in neonatal rats (37, 38). There was also a significant increase in the number of AS/REM bouts at this same \(T_a\). In adult rats, as \(T_a\) is moved toward the TNZ, there are more transitions into REM sleep (18). Likewise, as \(T_a\) deviates away from the TNZ, NREM sleep accounts for a larger percentage of total sleep time (TST) than REM sleep (18). This is seen at 38.0–40.0°C, where %AS/REM is significantly less than at 33.0–35.0°C, whereas %SWS remains high. Although both \(T_a\) condi-

tions of 33.0–35.0 and 38.0–40.0°C suppress %W, the former does so by promoting both AS/REM and SWS, whereas the latter does so by promoting SWS alone. Both of these effects are similar to responses seen in adult rats (27, 36).

Sleep consolidation is disrupted at 38.0–40.0°C compared with the other two \(T_a\) conditions. The number of bouts for AS/REM, SWS, and W are significantly increased at 38.0–40.0°C, whereas mean bout lengths are significantly decreased for all three states. The number of brief arousals is another measure of sleep consolidation or fragmentation of sleep. As with number of bouts, number of brief arousals is significantly higher at 38.0–40.0°C than at either 28.0–30.0 or 33.0–35.0°C. Although a \(T_a\) of both 33.0–35.0 and 38.0–40.0°C increases TST in developing rat pups, these temperatures also increase the fragmentation of sleep and so disrupt sleep/wake state structure.

Sleep intensity or depth of sleep was measured by SWS SWA. No significant \(T_a\) effects were seen for sleep intensity, as measured by SWS SWA. These results do not support our hypothesis that an increase in \(T_a\) may increase sleep depth or intensity and run contradictory to the increases in SWS SWA seen after hypothalamic warming in adult rats (24). One factor that may be behind these results is the fact that we waited 1 h after the initial \(T_a\) condition exposure to record SWA. This

Fig. 5. A: mean ± SE number of brief arousals across age. B: mean ± SE number of brief arousals by \(T_a\) condition. Significantly different from 28–30 or 33–35°C, respectively.

Fig. 6. A: mean ± SE SWS slow-wave activity (SWA) across age. SWS SWA values are standardized and converted to T scores. B: mean ± SE SWS SWA by \(T_a\) condition. SWS SWA values are normalized and presented as a ratio of absolute SWS SWA to SWS total power. abSignificantly different from P12, P14, P16, and P18, respectively.
was necessary to allow for anesthesia recovery but may have masked the true response of SWS SWA to an increase in $T_a$. It may be that the greatest increase in SWS SWA occurs after the initial exposure to an increase in $T_a$. After sleep deprivation in adult rats, the first 4 h of recovery results in enhanced SWS SWA and a subsequent reduction in nBA (9). This inverse relationship between SWS SWA and nBA in adults was not seen in our rat pups and suggests that the two elements, SWS SWA and nBA do not have the same relationship during development as they do in adulthood.

Developmental changes in sleep/wake state percentages are similar to those found by other authors (20, 26). There was a steady increase in %SWS coincident with a decrease in %AS/REM. Likewise, SWS SWA showed a steady increase coincident with brain maturation in developing rats (13). No significant effects of number of brief arousals were found across developmental age, but state bout number and mean bout length showed age-dependent effects. SWS showed significant decreases in bout number, with significant increases in mean bout length at P14 and P16, whereas AS/REM mean bout length was significantly increased at P14 only. Recent data suggest that, in neonatal rats P20 and younger, SWS SWA may not be influenced by sleep deprivation, as is seen in adults (8). This means that SWS SWA may not be regulated at these ages. Age-specific increases in state bout length may prove to be a better measure of sleep intensity or depth of sleep than SWS SWA. Arousal threshold experiments across varied state bout lengths need to be conducted to answer this question.

Perspectives

Sleep/wake state characteristics have been collected from infants that have been rescued from a SIDS event. In these near-miss infants, increases from controls are seen for %AS/TST (14, 29), AS mean bout length (29), QS mean bout length (29), and %TST (4, 14, 29). Near-miss infants show a decrease in %QS/TST (14, 16) and in the number of awakenings from sleep (4). These data suggest that near-miss SIDS infants spend more time sleeping, with increased mean bout lengths of both AS and QS, and thus awaken less frequently (4, 14, 29). Our developing rats age P14 and P16 also show an increase in %AS/REM, AS/REM and SWS mean bout length, and %TST when exposed to a $T_a$ of 33–35°C. The results from this study suggest that rats age P14–P16 exposed to a $T_a$ of 33–35°C share similar sleep/wake state characteristics as SIDS near-miss infants. It is concluded that subtle changes in $T_a$ variation can significantly affect sleep/wake state structure, specifically state percentages and sleep consolidation in developing rats. Manipulations of $T_a$ at specific ages in the developing rat can mimic the sleep/wake state structure changes seen in infants at risk for SIDS.

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