Effects of temperature on sleep in the developing rat

ROGER N. MORRISSETTE1,2 AND H. CRAIG HELLER1
1Department of Biological Sciences, Stanford University, Stanford 94305; and
2Program in Neuroscience, University of California at Los Angeles, California 90025

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 postnatally. Consequently, vulnerabilities exist early in postnatal development when immature autonomic functions are challenged by external factors such as variations in ambient temperature (T_a). T_a 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 T_a 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 (T_br) recording. Two-hour recordings of sleep/wake state and T_br were obtained from rats on postnatal day 12 (P12), P14, P16, P18, and P20 at a T_a of either 28.0–30.0, 33.0–35.0, or 38.0–40.0°C. T_a 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 (T_b), either by passive heating of ambient temperature (T_a) or through exercise leads to increased non-rapid eye movement (NREM) sleep (19). In humans, brain temperature (T_br) 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 T_b is rising, an increase in NREM sleep is seen (10). It has been theorized that increases in NREM sleep after transient increases in T_b represent an active thermoregulatory response triggered to counter hyperthermia (23, 31).

Sudden infant death syndrome (SIDS) has a strong association with sleep and T_a 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). T_a 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 T_b, and an increase in heat dissipation (18). In addition, T_b 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 shown to have significantly increased hypothemic and hyperthermic bouts (28), and some infants have shown evidence of excessive sweating and elevated T_b 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 myoclonic 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\textsubscript{a} inside the recording chamber. The recording chambers were double-walled made of clear acrylic (22°C ± 0.5°C on a light-dark cycle). Food and water were available ad libitum. During data collection, animals were placed into recording chambers with T\textsubscript{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\textsubscript{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\textsubscript{sa} and T\textsubscript{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 Translation) 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 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 assigned 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 integrated 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 scatterplot 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\textsubscript{a} scores before analysis across developmental age. For analysis across T\textsubscript{a} condition, SWS SWA was normalized to SWS total EEG power to control for changes in EEG power across development. Data were statistically analyzed by a two-way ANOVA repeated-measures test with Fisher’s test applied for post hoc pairwise comparisons. RESULTS Effect of T\textsubscript{a} on T\textsubscript{br}. As expected, when data were pooled across age, T\textsubscript{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.
The significant positive correlation between $T_{br}$ and %SWS is both $T_a$ condition dependent and age dependent. Individual analyses revealed a significant correlation at a $T_a$ of 33–35°C ($r = 0.517, P < 0.01, n = 27$) and at age $P_{14}$ ($r = 0.591, P < 0.005, n = 20$) and $P_{16}$ ($r = 0.715, P < 0.005, n = 17$). No other significant $T_a$-specific or age-specific correlations were found between $T_{br}$ and %SWS.

The significant negative correlation between $T_{br}$ and %AS/REM is $T_a$ condition dependent but shows no significant age-dependent relationship. Individual analyses revealed a significant correlation at a $T_a$ 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 $T_a$ on bout number. There was a significant age-dependent effect on state bout number when pooled across all $T_a$ 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 $P_{12}$ to $P_{16}$. Waking showed no significant age effects $[F(4,68) = 1.75, P = 0.3298, n = 15]$. Figure 4A displays the changes in state bout number with age.

$T_a$ 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 $T_a$ 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 $T_a$ 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 $T_a$ condition.

Effect of age and $T_a$ 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, $P_{14}$ and $P_{16}$ for SWS and $P_{14}$ 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.

$T_a$ 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 $T_a$ condition. Waking mean bout length also decreased significantly 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 $T_a$.

Effect of age and $T_a$ 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 $T_a$. Figure 5, A and B, demonstrates these relationships.

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

---

Fig. 4. A: mean ± SE state bout number by age pooled across $T_a$ condition. B: mean ± SE state bout number by $T_a$ condition pooled across age. C: mean ± SE bout length by $T_a$ condition pooled across age. ABC significantly different from P12, P14, P16, respectively. DE significantly different from 28–30 or 33–35°C, respectively.
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-

![Fig. 5. A: mean ± SE number of brief arousals across age. B: mean ± SE number of brief arousals by \( T_a \) condition. \(^{\text{ef}} \)Significantly different from 28–30 or 33–35°C, respectively.](image)

![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. \(^{\text{abcd}} \)Significantly different from P12, P14, P16, and P18, respectively.](image)

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.
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.

The authors thank Joel Benington for assistance with software. This research was supported by a National Research Service Award predoctoral fellowship from the National Institute of Child Health and Human Development (NICHD) to R. Morrisette (5 F31 HD-07895–02) and by an NICHD Perinatal Emphasis Research Career Development Grant (5 P50 HD-20970). Address for reprint requests: R. N. Morrisette, Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305–5020. Received 17 April 1997; accepted in final form 7 January 1998.

REFERENCES