AROUSAL FROM SLEEP has often been thought to be an important mechanism to protect an individual from a life-threatening stimulus (24). Arousal from sleep has been studied extensively in adults in response to several respiratory stimuli (3, 4, 14, 26) and in response to acoustic stimuli (6, 9). These previous investigators used an electroencephalogram (EEG) change as indication of arousal from sleep. There is no universally accepted definition of arousal, and it has now been suggested that there is a hierarchy of arousal phenomenon, generated from subcortical as well as cortical sites. Arousal can vary from brief electromyograph increases and increased autonomic activity to sleep stage shifts or full awakenings. Arousing stimuli, for example, obstructive apnea or auditory stimulation, can produce autonomic changes such as increases in heart rate, blood pressure, and ventilation with or without an EEG arousal (9, 26). It has been suggested that these responses represent markers of the activation of brain stem-arousing mechanisms, even in the absence of cortical EEG changes (9).

Arousal responses in infants have also been examined, and it has been suggested that full arousal is not always necessary to protect an infant from an hypoxic stimulus (7, 21, 35). Recently, Lijowska et al. (18) showed that the infant arousal response to an elevated inspired CO₂ level involved a specific sequence of stereotyped behaviors before awakening. These investigators found that the sequence commenced with an augmented breath coupled with a startle, which were then followed by thrashing movements and full arousal. Arousal was determined by these investigators with the use of behavioral criteria. The startle response observed in these infants was viewed as a subcortically mediated defensive response in that it was frequently effective in providing access to fresh air without necessarily involving a full behavioral arousal.

The observations of Lijowska et al. (17) prompted several speculations. First, they suggested that, in the context of arousal from sleep, sighs and startles might be independent brain stem reflexes that are neither cortically mediated or influenced. Second, it was speculated that these responses might be part of a fixed sequence in an arousal pathway that begins in the brain stem and ends in cortical arousal. Finally, indirect evidence and preliminary observations suggested that this pathway might be common to both rapid-eye-movement (REM) and quiet sleep and might be elicited by a variety of stimuli, respiratory as well as nonrespiratory.

Accordingly, the present research was undertaken to evaluate the relationship of subcortical reflexes to cortical arousal and specifically to further test these implied hypotheses arising from the observations of Lijowska and associates (18). We employed EEG monitoring and a nonrespiratory stimulus to induce arousal in healthy sleeping infants. We compared the time of onset of induced respiratory and motor responses to that of cortical responses in both REM and non-NREM (NREM) sleep. Additionally, we used a stimulus that elicits a classic defensive spinal reflex to assess timing and integration, or lack thereof, with brain stem defensive reflexes and cortical arousals.

METHODS

Patients. We studied 15 normal infants (9 girls, 6 boys), with a mean age of 8.9 ± 1.1 wk and a range of 3–17 wk. The infants were recruited from advertisements through the hospital and the local media. All infants were determined to be normal by a clinical examination. Thirteen of the infants were full term; two infants who were preterm had been born at 34 and 36 wk gestation but had no complications associated with their prematurity. The study protocol was approved by the Washington University Human Study Committee, and informed consent was obtained from the parent of each infant before the study.

Each infant was set up for the study, fed, and allowed to fall asleep in a crib in the laboratory. Each study was performed at a time of day that each infant usually slept, and each was...
recorded for between 1 and 3 h, until spontaneous wakefulness.

Polygraphic recordings. All infants underwent a single polysomnographic recording during a daytime nap period. All data were recorded on an eight-channel polygraph recorder (model R611, Beckman Instruments). Sleep state was measured with one channel of centrally placed EOG and one channel of electrooculogram (EOG) to record eye movements. Electrocardiogram (ECG) was also monitored. All EOG, ECG, and ECG electrodes were placed superficially. Airflow was measured from a standard infant resuscitation mask placed over the infant's nose and mouth. The nasal cannula or polyethylene catheter to measure interval pressure at a midpoint in the rubber tube. In the other six infants, airflow was measured from a standard infant resuscitation mask placed over the infant's nose and mouth. The nasal cannula or mask was attached to a differential-pressure transducer (Statham PM15E). The pressure signal was integrated by the mask was attached to a differential-pressure transducer placed over the infant's nose and mouth. The nasal cannula or polyethylene or metal tubing (1.5-mm external diameter), attached to a device that had been calibrated to deliver the stimulus equivalent to grams in weight. Tactile stimuli of various intensities (0–100 g) were applied briefly to the sole of the infant's foot during NREM and REM sleep. The interval between the tactile stimulations was between 5 s and 1 min.

Analysis. The sleep state was determined by EEG and EOG criteria, on the basis of established criteria for neonates and infants (1, 25). Sleep was staged as either awake, NREM sleep, or REM sleep. Each infant was studied for at least one cycle of sleep, until they woke spontaneously from their nap. In total, 578 min of NREM sleep and 229 min of REM sleep were recorded; three infants did not have any REM sleep recorded during their nap.

The video from each study was also examined and correlated with the events on the polygraphic recording. Each tactile stimulation was examined carefully for any response. Events within 3 s of stimulus presentation were scored as being associated with the stimulus. Any spinal, respiratory, startle, and cortical responses were recorded. The video recording was advanced frame by frame to identify the point on the polygraph that the stimulus was applied and the time that motor activity commenced. A cortical arousal was defined as an abrupt change in the EEG pattern, with frequencies corresponding to that of alpha (8–13 Hz) or frequencies >16 Hz for a minimum of 1 s.

The number of spinal, respiratory, startle, and cortical responses for all infants were calculated according to sleep state, and the association between each of the responses was examined. The number of each response was totaled for each infant, expressed as a percentage of total responses, and then averaged for all infants. The frequency of each response and its occurrence with other responses were examined. The time between the stimulus and each of the responses was measured and averaged according to sleep state for the infants. In the timing of the cortical responses, examples of cortical arousal that had large movement artifacts (that is, pen-blocking artifact) where the point of EEG frequency shift could not be identified were excluded from the analysis. The number of augmented breaths, startles, and arousals occurring spontaneously during NREM and REM sleep was also recorded. A total of 1,274 and 349 responses to tactile tests were examined during NREM and REM sleep, respectively. In addition, a total of 201 and 171 spontaneous responses were examined during NREM and REM sleep, respectively.

The differences in the frequency of arousal responses between different sleep states were analyzed by using $\chi^2$ analysis, and the differences in timing between different responses and sleep states were analyzed by using the t-test. All data are expressed as means ± SE. A P value of $<0.05$ was considered significant.

RESULTS

Responses to tactile stimulation. The events and behaviors associated with tactile stimulation were spinal, respiratory, startle, and cortical responses. The most common responses were spinal responses alone, that is, foot-twitch and leg-withdrawal reflex. Respiratory responses were of two types, either an augmented breath, characterized by a large biphasic inspiration (Fig. 1), or a transient acceleration of respiratory frequency for two to three breaths immediately after the stimulus and followed by a return to the prestimulation respiratory pattern (Fig. 2). There was a similar occurrence of the two respiratory responses between sleep states; 55.3 and 44.6% of respiratory responses were augmented breaths during NREM and REM sleep, respectively (P > 0.05, $\chi^2$). Startles observed were scored as an abrupt change in the EEG pattern, with frequencies >16 Hz for a minimum of 1 s.

Arousal sequence to tactile stimuli. The cortical response to tactile stimulation occurred as a sequence of responses that always commenced with the spinal response, was followed by a respiratory response in most cases and then by a startle, and ended with cortical arousal (Fig. 3). All cortical responses occurred after spinal and startle responses; however, respiratory responses were occasionally absent in the arousal sequence in four of the infants. The infants who had atypical responses were aged 6, 7, 9, and 17 wk. The dissociation of respiratory responses in the arousal sequence was more common during NREM sleep, occurring in 4.9 ± 2.6% of cortical responses, compared with occurring in 12.1 ± 1.2% of cortical responses during REM sleep, but this was not statistically different (P > 0.05, $\chi^2$).

Incomplete or partial arousal sequences also occurred, and although, respiratory responses were occasionally absent, the sequence of arousal responses always occurred in the same order, starting with a spinal response. Respiratory responses were not associated with the tactile stimulation without following a spinal response. Similarly, startle responses associated with the stimulus always followed a spinal response.
and, most of the time, a respiratory response. Spinal responses alone, spinal with respiratory responses, and spinal with respiratory and startle responses were elicited with tactile stimuli without resulting in cortical arousal (Fig. 4, Table 1). The individual responses observed in these incomplete sequences were not different to that observed in sequences ending in cortical arousal.

Timing of the arousal sequence. In tactile stimulation tests that elicited the arousal sequence from spinal to cortical responses, the time from stimulus onset to the spinal response was significantly shorter than the time to the startle response for both sleep states. Similarly, the time from stimulus onset to the startle response was significantly shorter than the time to cortical arousal during NREM and REM sleep ($P < 0.05$, $t$-test).

Spinal responses occurred at an average of 97.2 ± 5.5 and 133.3 ± 8.7 ms after the tactile stimulus during NREM and REM sleep, respectively (Fig. 5). The time from the stimulus to the spinal response was significantly longer during REM sleep ($P < 0.05$, $t$-test).

The onset of startle occurred an average of 515.7 ± 26.3 and 427.2 ± 45.3 ms after the withdrawal reflex during NREM and REM sleep, respectively. Contrary to the timing of the spinal response, the time from the stimulus to the startle response was significantly longer during NREM sleep than during REM sleep ($P < 0.05$, $t$-test).

Cortical responses occurred either simultaneously with the startle response or immediately after the startle response. The cortical response occurred an average of 106.6 ± 16.0 and 180.3 ± 36.6 ms after the startle response for NREM and REM sleep, respectively. The total time from the stimulus to the cortical response was similar during both sleep states ($P > 0.05$, $t$-test).

Spontaneous activity. Spontaneous arousal sequences also occurred during sleep, and the majority occurred as an augmented breath coupled with a startle, which were then followed by a cortical response (Table 1). Atypical spontaneous arousal responses were also observed. Occasional arousal sequences without an obvious augmented breath were observed in five infants.
aged 3, 7, 8, and 12 wk. In another infant, aged 6 wk, cortical arousal followed an augmented breath and movement, but there was not a typical startle response. Augmented breaths alone as well as augmented breaths coupled with a startle also occurred independent of cortical arousal during both NREM and REM sleep. The augmented breaths, startles, and cortical responses that occurred spontaneously were similar in appearance to those in response to tactile stimuli.

**DISCUSSION**

Currently, there is no universally accepted definition of arousal, the minimum change in the EEG, and the criteria to indicate an arousal remain arbitrary. It has been suggested that at least 3 s of high-frequency EEG activity are necessary to establish arousal (30). There is no evidence, however, that EEG changes <3 s are not important, and the point at which minor EEG changes become physiologically significant is unclear. For the present study, an EEG change for a minimum of 1 s was used to optimize sensitivity. This is similar to arousal criteria used by other investigators (9, 21, 26).

The results of the present study confirm and extend the findings of Lijowska and colleagues (18). These investigators demonstrated that there was a specific arousal sequence in response to an elevated inspired CO₂ level that involved presumptive brain stem responses before full arousal. The sequence always commenced with an augmented breath coupled with a startle and then ended with thrashing and a behavioral arousal. Our results extend the previous work in infants by including EEG criteria to determine the timing of cortical arousal, whereas Lijowska et al. relied on behavioral criteria to indicate arousal. In other circum-

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Fig. 3. Sequential photograph of frames of videotape of a sleeping infant during a stimulus sequence. Top: infant sleeping just before a tactile stimulation test. Note investigator's hand and stimulus probe in corner at top left just before touching infant's foot. Middle: immediately after tactile stimuli; arrow indicates that infant's left leg has been withdrawn from stimulus. Bottom: startle response after spinal response; arrows indicate sudden movement of arms and extension of the legs that are part of the characteristic startle. Photographs retouched to increase contrast.
stances, sigh and startle have been shown to be reflexes with afferent and efferent connections localized to brain stem nuclei (5, 22). In the present case, startles, sighs, and trachypneic responses were confined to a narrow time period after spinal reflexes and before cortical responses. This observation is supporting evidence for a brain stem origin of startles and associated respiratory responses to stimuli in sleeping infants.

We found that a tactile stimulus usually resulted in an arousal sequence similar to that previously observed in response to increased inspired CO2. The similarity of response to two different stimuli suggests a common neural pathway for infant arousals independent of the nature of the arousing stimuli. However, contrary to findings from experiments in which CO2 was used as a stimulus, we found occasional arousal sequences that did not include respiratory responses. Although Lijowska et al. (18) found that augmented breaths were usually clearly present in infant arousal sequences caused by hypercapnia, in ~5–10% of cases this was less certain because their recording technique did not allow definitive assessment of respiration. We employed flowmeters for recording respiration and found that the association among startles, sighs, and related respiratory events is somewhat looser than previously envisioned by Lijowska et al. Our findings clearly show that a sigh is not a necessary precursor to a startle in the arousal neural pathway.

Spinal responses have not previously been described as a component of the infant arousal pathway. Spinal withdrawal reflexes in humans are well described as a defensive response elicited by tactile, pressure, and pain stimuli (28). The observation of spinal reflexes as the initial response to an arousal-producing stimulus conforms to Sherrington's concept (28) of "spreading" spinal reflexes, in which a sufficiently strong stimulus can induce a simple reflex that then may be followed sequentially by allied or "chained" reflexes as the stimulus effects spread up or down the spinal cord. It should be noted that such a model for stimulus-induced arousal behaviors is essentially different from that suggested by Sullivan et al. (31) to explain the interrelationships among a laryngeal stimulus, cough, and cortical arousal in sleeping dogs. Their observations suggested that cortical arousal occurred before cough

Table 1. Tactile and spontaneous arousal sequences during NREM and REM sleep

<table>
<thead>
<tr>
<th></th>
<th>NREM Sleep</th>
<th>REM Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elicited complete arousal responses, %total responses ending in a cortical arousal</td>
<td>11.2 ± 1.9</td>
<td>21.2 ± 7.2</td>
</tr>
<tr>
<td>Elicited partial arousal responses, %total responses</td>
<td>74.9 ± 4.3</td>
<td>69.1 ± 9.0</td>
</tr>
<tr>
<td>Spinal only</td>
<td>7.1 ± 1.8</td>
<td>6.6 ± 2.4</td>
</tr>
<tr>
<td>Spinal → respiratory</td>
<td>6.8 ± 2.0</td>
<td>3.1 ± 1.7</td>
</tr>
<tr>
<td>Spontaneous arousal sequences, %total cortical responses</td>
<td>12.3 ± 5.0</td>
<td>4.9 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of complete arousal responses to tactile stimuli ending in cortical and partial sequences ending in spinal, respiratory, and startle responses during non-rapid-eye-movement (NREM) and rapid-eye-movement (REM) sleep. Spontaneous arousal activity is also included.

Fig. 4. Polygraphic example of a spinal, respiratory, and startle response without any EEG change. Note transient increase in end-expiratory volume of rapid breaths as in Fig. 1 but without any increase in tidal volume.

Fig. 5. Timing of spinal, startle, and cortical responses. Solid line, timing during non-rapid-eye-movement (NREM) sleep; dashed line, timing during rapid-eye-movement (REM) sleep.
have produced an increased VT, end-expiratory volume typical of an augmented breath. Although the increase in VT of 20% of the baseline respiration value and a ventilation in sleeping adult subjects, with an increase in the latter respiration by using a nonrespiratory afferent (6), who found that it is possible to significantly modulate respiratory responses in the arousal sequence included both typical augmented breaths and brief tachypnea. These are likely related responses. Augmented breaths in infants vary greatly in amplitude and may be biphasic or multiphasic (8, 32). In either case, a series of closely spaced inspirations accounts for the increased VT and end-expiratory volume typical of an augmented breath. Although the series of rapid breaths that we often observed may not have produced an increased VT, end-expiratory volume usually increased, suggesting a similarity between this tachypneic response and augmented breaths. Augmented breaths in the previous study (18) were viewed as functionally appropriate responses to a respiratory stimulus. In the present study, the respiratory responses were elicited by using a nonrespiratory stimulus, suggesting that respiratory responses are an integral part of the arousal process itself. Aside from strictly respiratory functions, it may be that augmented breaths have other general functions such as facilitating venous blood return, which thereby prepares the infant’s cardiovascular system for motor increased activity during arousals.

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The present findings agree with those of Carley et al. (6), who found that it is possible to significantly modulate respiration by using a nonrespiratory afferent stimulus to produce arousal. Similar to their findings, we found that the respiratory responses occurred in the absence of, or before, the cortical response. Carley and colleagues showed that an arousal stimulus augmented ventilation in sleeping adult subjects, with an increase in VT of 20% of the baseline respiration value and a decrease in inspiratory time of ~10%. These differences could reflect distinct maturational differences of arousal between infants and adults.

The sequence of events involved in the infant arousal response was similar during the different sleep states. The pattern and the type of responses elicited from tactile stimulation were the same during both NREM and REM sleep. There were differences, however, in the latency of the individual responses of the sequence between the two sleep states. Spinal responses occurred more rapidly during NREM sleep than during REM sleep. Spinal responses may have been slower during REM sleep as a consequence of the decreased tone in postural muscles that is experienced during REM sleep (23). Monosynaptic reflexes have been demonstrated to be delayed during sleep compared with wakefulness, and the reflexes are depressed in REM sleep compared with NREM sleep (33).

The startle response, in contrast, occurred more rapidly during REM sleep than during NREM sleep. Startles during wakefulness are considered to be generated from the brain stem and are believed to involve the bulbo-pontine reticular formation. It has been suggested that, because this center receives inputs from subcortical and cortical levels, it is possible that under differing pathological circumstances, it may generate reflex responses of different latencies (5). The differing states of neural activity that occur in the different sleep states possibly influenced the latency to startle. The level of cortical activity during REM sleep is more closely related to the activity in wakefulness than to the activity during NREM sleep (13). It is possible that the REM brain stem functions are also more closely related to awake functions than to NREM sleep functions, and therefore a shorter latency may be expected.

The latency from the tactile stimulus to the spinal response is similar to that previously recorded in awake adult humans (10). The latency to the startle response in NREM and REM sleep, however, is longer than would be expected in awake adults. It is possible that the effect of sleep or the type of stimulus used may have resulted in a delay of the startle response in infants. Another possibility is that a difference in the brain stem conduction time could account for the longer startle latency during sleep in infants. Brain stem conduction time has been demonstrated to be longer in the immature brain stem and decreases with increasing maturation (12). The total time from the stimulus to cortical arousal during both sleep states, however, was <1 s, which is similar to that recorded in adults in response to acoustic stimulation (6).

The responses to tactile stimulation could occur either as a complete sequence ending in a cortical arousal or as sets of incomplete sequences but always occurred in the order of spinal to brain stem to cortical responses. The spinal response or the spinal and brain stem responses were largely unaffected by the presence or absence of a cortical arousal from sleep. These findings are consistent with previous research that found that arousing stimuli, such as acoustic stimulation or obstructive sleep apnea, can induce autonomic brain stem responses in adult humans without causing EEG arousal and that the brain stem responses are of a similar pattern to those occurring with EEG changes (2, 6, 9, 15, 21, 26, 35). In an animal model of arousal, peripheral components, including orienting and startle responses, are involved in the arousal function of cats and would occur in the absence or presence of cortical components (27).

The results of the present study confirm the hypothesis suggested by McGinty et al. (19) on descending inhibition of arousal pathways. McGinty and colleagues described a sequence of distinct behaviors in sleeping kittens and hypothesized that the terminally
occurring behaviors would be suppressed by greater neural inhibition than the initial behavior and therefore favor early termination of the sequence. The minimum response observed to any stimulus in the present study was a spinal response that could occur alone or be followed by other arousal responses. It could then be inferred that the spinal response threshold is lower than the brain stem responses and, similarly, that the brain stem threshold is likely lower than cortical response threshold.

Spontaneous augmented breaths, startles, and cortical arousals also occurred. Similar to the tactile-induced arousals, the arousal responses involved a specific sequence of brain stem followed by cortical responses. The brain stem and cortical responses that occurred spontaneously were similar in appearance to the tactile-elicited arousal sequences and are possibly generated from the same neural pathway. Spontaneously occurring augmented breaths and startles during sleep in infants have been described, and their incidence is age dependent. There is possibly an endogenous rhythm of spontaneous brain stem and cortical activities in the infants studied, similar to that described by other investigators (16, 19, 20, 34).

Failure to arouse to a life-threatening stimulus has long been suggested as a potential mechanism of sudden infant death syndrome (SIDS). Pathological abnormalities in brain stem areas that are believed to be involved in the control of respiration and arousal have also been described in SIDS victims (11). The present studies extend the concept of arousal in infants to include arousal-related behavior elsewhere in the nervous system occurring independently of cortical arousal. Inasmuch as a number of these noncortical reflexes may have important protective and life-supporting functions, such observations more clearly suggest ways in which failure of the arousal pathway could lead to lethal respiratory or cardiovascular failure in SIDS.

We conclude that arousal in infants to a tactile stimulus involves a progression of central nervous system activation from the spinal to cortical levels. Arousal sequences elicited by tactile stimulation, as well as spontaneously occurring arousals, involve a specific pathway of responses that occur in association with the classically defined EEG arousal response. The entire pathway or part of it in the order of spinal to cortical responses could be elicited by tactile stimuli. The different components of the arousal pathway may be important for an infant to respond appropriately to stimuli during sleep without necessarily disturbing sleep. Interruption or a depression of arousal responses may have implications in the response to a life-threatening stimulus.

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