sponse, is abolished by blinding and by superior cervical ganglionectomy (*8*). Since exogenous melatonin inhibits the gonadal effects of constant light (*12*), it is likely that these effects are controlled, at least in part, by the pineal (*8*). This is supported by a recent experiment in which we found that the changes in estrous activity, ovarian weight, and pineal HIOMT activity induced in rats by continuous illumination are also abolished by bilateral lateral hypothalamic lesions that section the inferior accessory optic tracts in the medial forebrain bundle (*13*). These findings appear, then, to establish a function for the inferior accessory optic tracts, separate from that of other retinal projections, in the control of several neuroendocrine responses to environmental light.

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Averaged Neural Electrical Activity and Arousal

Abstract: *Multiple-unit activity from many brain sites of the unrestrained cat was recorded by means of an averaging technique during all phases of sleep and waking. All thalamic and medial reticular sites showed activity levels closely correlated with the animal's observed behavior even under conditions when the electroencephalogram appeared dissociated from behavior.*

Since the description of the ascending reticular activating system by Moruzzi and Magoun (*1*), many investigators have demonstrated that electric stimulation of this system produces behavioral arousal and electroencephalographic desynchronization. On the basis of electric stimulation of the brain it has also been suggested that the thalamic nuclei may regulate the "state of consciousness" (*2*). Several factors of cerebral physiology vary with the level of arousal, including electrocorticograms, d-c potentials, impedance, temperature, and cerebral blood flow (*3*). However, the spontaneous activity of the reticular and thalamic structures has not yet been correlated with the behavior of the awake, unrestrained animal. In this report the relationship between spontaneous neural activity and arousal is investigated.

Data were collected from 26 unanesthetized and unrestrained male and female adult cats. Records were obtained from more than 200 brain sites, including cortical, thalamic, reticular, and lower sensory nuclei, as well as from nonsensory structures. Placements of electrodes were verified histologically. All recordings, whether subcortical or cortical, were made with Teflon-coated stainless steel electrodes led to a Winchester connector plug that was mounted over the animals' frontal sinus. The electrodes were 100 or 200 μ in diameter and they were arranged side by side. The bare tips were separated 0.5 to 1.0 mm. Electromyographic (EMG) activity was recorded by bipolar stainless steel wire electrodes that were implanted into the deep musculature of the neck.

Electric signals generated by both neural and muscular activity were led through a-c coupled amplifiers to an oscilloscope and an "averaging" circuit; this averaging circuit ("integrator") has been used extensively and has been described (*4*). The input was averaged by passing the amplified a-c signal through a full-wave rectifier and a resistor-capacitor circuit smoothing filter with a 3-second time constant. The resulting d-c voltage output, which was displayed by Esterline Angus penwriters, is directly proportional to the amplitude and the frequency of the activity recorded. The band pass of the amplifiers was set at 80 to 10,000 cycle/sec in order to eliminate slow waves in the electroencephalogram (EEG) frequency range and to record only fast activity. This recording of fast activity is derived from multiple units (*5*). The output was expressed in microvolt-milliseconds and was calibrated by passing a 1-msec square wave at 1000 cycle/sec through the circuit.

All recordings were obtained by continuous monitoring of the EEG and EMG; the animals were also observed through a one-way window. The following characteristics were used to define certain behavioral states: (i) arousal (walking, grooming, orienting to external stimuli, and desynchronization of EEG); (ii) quiet alert (sitting or lying, head erect, eyes open, and desynchronization of EEG); (iii) drowsy (head falling, eyes closing, and spindles in EEG); (iv) slow-wave sleep (lying, head down, eyes closed and slow-wave EEG); and (v) paradoxical sleep (lying, intermittent twitching, atonia of neck muscle, and desynchronization of EEG).

The activity recorded from a thalamic site during changing behavioral states is shown in Fig. 1. The multiple-unit activity is indicated by the top trace; the third trace shows the simultaneous average of the multiple-unit activity. There is a marked increase in neural activity at the thalamic recording site during arousal and this is accompanied by a tonic increase in neck EMG activity and a typical EEG desynchronization pattern. Spontaneous rises in thalamic activity often occur several seconds before any overt or EMG signs of arousal. Both multiple-unit and averaged activity at the thalamic site gradually decline as the animal becomes less alert; this activity continues to decrease as spindles appear in the EEG. Averaged thalamic activity reaches its lowest level during slow-wave sleep. In the transition from slow-wave to paradoxical sleep, there is an initially slow, and then rapid, rise in thalamic activity. This characteristic change may occur as much as 10 seconds in advance of changes in the EMG. Thalamic activity in paradoxical sleep is as high or higher than that of the aroused state. Decreases in thalamic activity often precede either a decline in arousal or a spontaneous termination of paradoxical sleep. Marked increases in neural activity were observed when there was no overt behavioral change and only slight EMG indications of orientation; an example is shown in Fig. 1, where novel white noise was presented through overhead speakers.

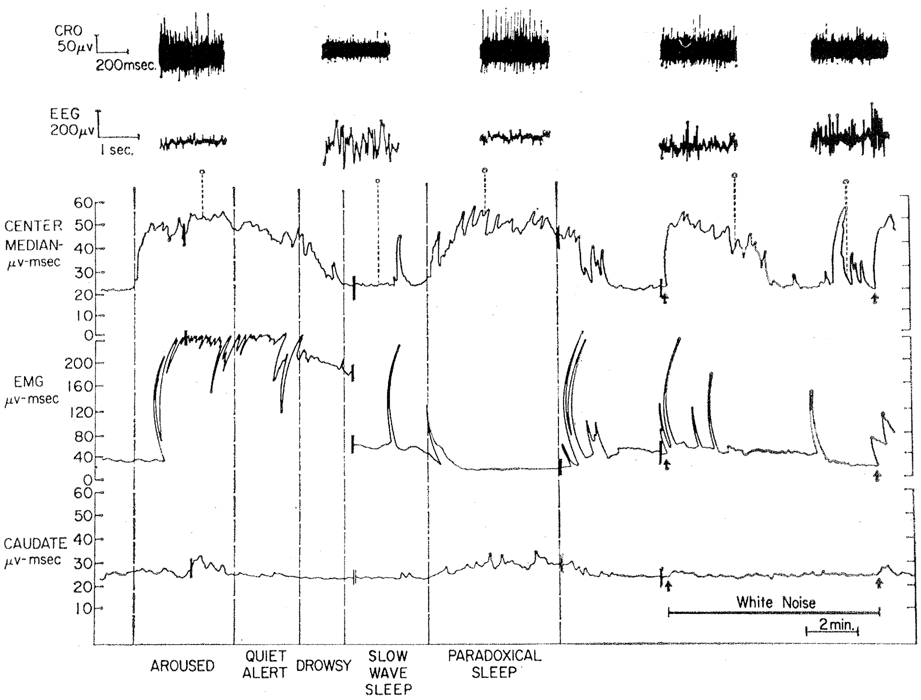

Fig. 1. Recordings during changing behavioral states. (Top trace) Oscilloscope (*CRO*) display of thalamic multiple-unit activity, which is then presented to the averaging circuit. (Second trace) Cortical EEG. (Third trace) Averaged thalamic activity. The thalamic multiple-unit activity and the EEG are recorded simultaneously at a point indicated on this trace. (Fourth trace) Averaged neck musculature activity. (Fifth trace) Averaged caudate activity.

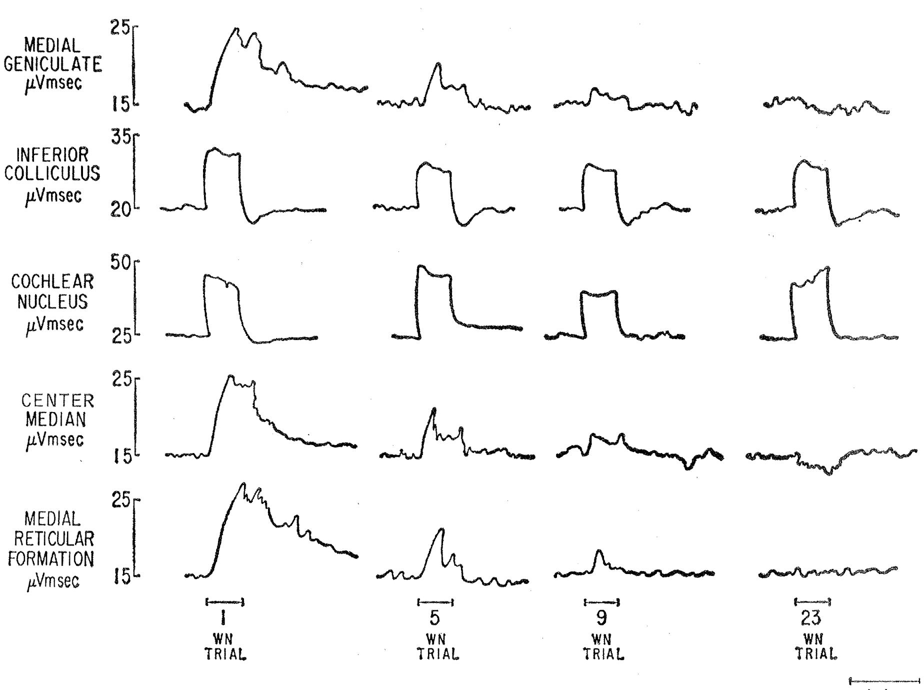

Fig. 2. Habituation of the arousal response with simultaneous recordings of averaged activity. The responses in thalamic and medial reticular structures are similar and decrease with habituation. Sustained responses in lower auditory nuclei are maintained throughout.

The changes in thalamic activity shown in Fig. 1 are representative of 108 thalamic recording sites. The number of placements for each of the thalamic nuclei studied are indicated by the numbers in parentheses: center median (26), central lateral (6), dorsomedial (2), lateral ventral (4), posterolateral ventral (12), pulvinar (8), medial geniculate (28), and lateral geniculate (22). Simultaneous averaged unit recordings were made from multiple thalamic sites during spontaneously changing behavioral states. The patterns of averaged thalamic activity were consistently identical. In addition, during arousal that was elicited by external stimuli (light flash, white noise, and touch) the recorded patterns of increased activity were identical in specific and nonspecific thalamic nuclei.

Multiple-unit recordings were obtained from electrodes located in the medial aspect of the reticular formation (36 placements within 3 mm of midline, from the posterior limit of the inferior colliculus to the anterior limit of the superior colliculus) during changing behavioral states. The patterns of averaged activity obtained from these reticular sites were not distinguishable from those of the thalamus. All reticular and thalamic sites showed their greatest activity during arousal and paradoxical sleep, and their lowest activity was observed during slow-wave sleep.

Changes in averaged caudate activity (Fig. 1) were representative of several other brain sites, including hippocampus (8 placements), amygdala (3), putamen (20), caudate (4), cochlear nucleus (12), inferior colliculus (14), and superior colliculus (8). Both the pattern and magnitude of averaged activity were different from those seen in the reticular and thalamic sites. Small rises in activity were occasionally seen with arousal and they tended to be associated with movement. Small sustained rises were also seen in paradoxical sleep (less than a 25-percent increase from slow-wave sleep). In contrast, averaged activity from reticular and thalamic sites showed constant and large (>75 percent) increases from slow-wave sleep to either arousal or paradoxical sleep.

It has been repeatedly demonstrated that the cortical EEG can be dissociated from observed animal behavior by lesions, states of fatigue, rewards, and drugs (*6*). In this report atropine (4 mg/kg intraperitoneally) produced the expected prolonged hypersynchronous slow-wave activity in the EEG. In contrast, averaged thalamic activity continued to fluctuate in a manner consistent with the observed behavior of the animal. The usual high levels of activity were seen during arousal, and the lowest levels of activity were seen during sleep even though the character of the EEG slow waves remained unchanged. In addition, the normal animal occasionally demonstrated slow waves and spindles in the EEG during grooming and eating while thalamic activity remained at high levels consistent with the alert behavioral state of the animal.

Another example of the relationship of thalamic and reticular activity to arousal is seen during habituation of the arousal response. White-noise stimuli of 30-second duration were repeatedly presented to the animal whenever the averaged thalamic activity reached its lowest level for that animal (Fig. 2). Averaged activity recorded from the inferior colliculus and cochlear nucleus increased immediately after the onset of white noise and was sustained for the duration of the stimulus. In previous studies this characteristic sustained response occurred specifically along the classical auditory pathway (*4*). In the present study sustained responses were seen at the level of the medial geniculate only when the stimuli aroused the animal. The animal was fully habituated at approximately the 20th trial; that is, additional stimulus failed to elicit any sign of EEG desynchronization. At the point of habituation there may be either no change in activity or even a drop in activity below the base line (Fig. 2, trial 23). In contrast, the rise in averaged activity remains sustained during all presentations of noise in auditory structures below the thalamus. The averaged activity within the thalamus declined during the course of stimulus presentation and provided a quantitative index of the degree of habituation. This type of decrease in response was seen in all records obtained from thalamic nuclei and medial reticular sites (Fig. 2). The thalamic and reticular responses are clearly not determined by the physical properties of the stimulus but are related to the level of arousal elicited by that stimulus.

This study demonstrates a close correlation between arousal and the multiple-unit recordings from reticular and thalamic structures. The averaging technique can describe changes in the activity of a limited number of neural elements at the electrode site. The increased activity in a cell population during alert behavior does not necessarily mean that individual units show increased activity. The averaging of multiple-unit activity could mask the various changes in single-unit patterns, such as those that have been described for sleeping and waking (*8*). However, studies of reticular and thalamic single-unit activity in unanesthetized animals are consistent with the averaged unit recordings reported here (*9*).

Schlag and Balvin (*5*) used an averaging technique with paralyzed animals to demonstrate a relationship between reticular "background" activity and the cortical EEG. Our results demonstrate a similar relationship. In addition, they extend the anatomic range of this relationship to include all thalamic sites and relate the averaged activity to observed behavior.

The similar pattern of averaged activity observed in records obtained from the medical reticular formation and the thalamic nuclei might have been suggested by previous observations. Ascending and descending long tracts have been described between the medial aspect of the reticular substance and the midline thalamus (*10*). The apparent homogeneity of spontaneous electrical activity within the thalamus may be explained by the numerous interconnections demonstrated between the intralaminar nuclei and the rest of the thalamus in anatomical and physiological studies (*11*). Our work demonstrates a characteristic thalamic responsiveness to stimuli of any modality capable of eliciting arousal, and this is consistent with observations of single-unit activity. Arden and Soderberg (*12*) found that the resting spontaneous discharge of many cells in the lateral geniculate correlated with the changes in the EEG; the activity was greater during desynchronization of the EEG, and less during synchronization. Although there was no direct response to sound stimuli, any sound that caused EEG desynchronization also caused some cells to increase their activity. Therefore, Arden and Soderberg postulated a "second input" from the reticular activating system which is capable of maintaining the resting discharge and modifying the evoked cellular activity.

The fact that averaged multiple-unit recordings vary consistently with observed behavior suggests that this approach will be of great value in analyses of the relationship of arousal to more complex behavior.

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