Therefore, the interference with an equilibrium which exists between afferent influences, some of which originate in the pons and some of which ascend through it, is an important factor in the maintenance of the waking state as indicated by the electroencephalogram.

The suppression of the synchronizing influences affects the cerebral hemispheres bilaterally, while the suppression of the activating influences affects mainly the hemisphere on the side of the lesion.

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References and Notes

1. F. Bremer, *Compt. Rend. Soc. Biol.* **122**, 1235 (1935); G. Moruzzi and H. W. Magoun, *Electroencephalog. Clin. Neurophysiol.* **1**, 455 (1949).
2. J. P. Cordeau and M. Mancia, *Arch. Ital. Biol.* **96**, 374 (1958); C. Batini, G. Moruzzi, M. Palestini, G. F. Rossi, A. Zanchetti, *Arch. Ital. Biol.* **97**, 1 (1959); G. F. Rossi, K. Minobe, O. Candia, *Arch. Ital. Biol.* **101**, 470 (1963).
3. A. Camacho-Evangelista, *Ann. Anat. Pathol.* **9**, 429 (1962).
4. F. Reinoso-Suárez, *Topographischer Hirnatlas der Katze* (Merck, Darmstadt, 1961).
5. This work was supported in part by grants from the U.S. Public Health Service (B-3208 and NB-04312).

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Auditory Nuclei: Distinctive Response Patterns to White Noise and Tones in Unanesthetized Cats

Abstract. *Electrical responses to "white" noise and tonal stimuli were recorded from unanesthetized cats with permanently implanted bipolar electrodes. The cochlear nucleus, inferior colliculus, and medial geniculate each showed distinctive patterns of evoked activity. White noise and tones produced qualitatively different types of response. A decrease in activity characterized the response of the inferior colliculus to tonal stimuli.*

Electrophysiological studies of the auditory pathway have been pursued in hopes of finding differences in "spontaneous" activity and in response characteristics that might underlie some functional localization. In the course of other experiments on the auditory nuclei functional differences were observed that were not anticipated from data reported in studies of single units (*1*, *2*). We found that white noise produced a marked increase in activity in the inferior colliculus, while pure tonal stimuli produced a marked decrease below the spontaneous level. In contrast, in the cochlear nucleus both white noise and tones evoked an increase in activity. This report describes subsequent verification of these observations carried out in a rigorously controlled acoustic environment.

Responses were recorded from five cats with bipolar electrodes chronically implanted in the dorsal and ventral cochlear nucleus, inferior colliculus, and medial geniculate body, pars principalis. All electrode placements were later verified histologically. Electrodes were made of 32- or 36-gauge enameled stainless steel wires with only the cut ends bared and separated by 1 to 2 mm. Muscles of the middle ear were left intact in all animals. The middle ears were examined post mortem and were found to be free from infection. Unanesthetized and unrestrained cats were placed in a wire mesh enclosure (45 by 60 by 45 cm) in the center of an Eckel anechoic chamber (free-field area, 114-cm cube). White noise (flat from 20 to 20,000 cy/sec ± 1 db) and tonal stimuli (from 100 cy/sec to 18,500 cy/sec) were presented through a Quad electrostatic speaker, mounted in the anechoic chamber 1 meter above the animal enclosure. Sound pressure level in the chamber was monitored continuously with a condenser microphone (*3*) (response flat from 20 to 20,000 cy/sec ± 2 db). Purity of the tonal stimuli and whiteness of the noise were checked with a sound and vibration analyzer (*3*). All sound pressure levels (SPL) were referred to 0.0002 dyne/cm^2. The background level of sound in the chamber was 52 db ± 4. Within the enclosure variation in SPL of white noise and tones of 85 db was less than ± 3 db. Responses at each electrode site to three or more presentations of each stimulus were studied on at least four separate days. Electrical activity was photographed on an oscilloscope and averaged with an "integrator" (*4*). Integration was obtained by passing the conventional a-c amplified recording through a full-wave rectifier and smoothing filter; the resulting d-c output was proportional to the amplitude and frequency of the conventional recording. The units of integration were μv-msec.

Figure 1 shows representative simultaneous recordings from the cochlear nucleus and inferior colliculus of an unanesthetized cat, at the onset and offset of white noise (80 db) and 2500-cy/sec tone (80 db). Sustained white noise produced a sustained increase in activity above the spontaneous level and termination of the noise was followed by a depression of spontaneous activity (*4*). The 2500-cy/sec tone produced the same effect as white noise in the cochlear nucleus, but in the inferior colliculus produced a marked decrease in activity below the spontaneous level. The termination of the tone was often followed by an overshoot above the spontaneous level in the inferior colliculus. Figure 2 shows the integrator record of the entire course of these events. Frequency analysis and oscilloscopic monitoring at fast sweep speed showed that the changes in amplitude of the recorded activity were due to changes in the number and/or frequency of individual neuronal discharges, not merely to changes in synchronization.

Recordings from all five electrodes implanted in the cochlear nucleus showed an increase in activity in response to tones between 300 and 3000 cy/sec. The largest responses were evoked by tones of 1000 to 2000 cy/sec. These responses were of greater amplitude than the response to white noise of the same intensity. Tones above 3000 cy/sec produced small decreases in activity at two of the electrodes in the cochlear nucleus and no response at the other three. Frequency-following (bursts of activity phase-locked to the stimulus cycle) was seen up to 4000 cy/sec.

Recordings from all seven electrodes implanted in the inferior colliculus showed decreased activity in response to tones above 2000 cy/sec. Recordings from three electrodes showed no increase for any tones and a marked decrease from 200 to 18,500 cy/sec. Recordings from the other four electrodes showed an increase in activity from 300 to 1500 cy/sec and a marked decrease for higher frequencies. These two patterns of response could not be related to the position of the electrode tips within the nucleus; for example, anterior as opposed to posterior, dorsal as opposed to ventral, or in proximity to incoming lemniscal fibers instead of in the main body of the nucleus. High amplitude frequency-following accompanied all increases in activity but was also occasionally observed at low amplitude in response to tones which produced a decrease in activity.

Recordings from the three electrodes implanted in the medial geniculate body showed a slight decrease in activity in

response to tones of 100 to 18,500 cy/sec.

The amplitude of the response in all three nuclei changed nonlinearly with changes in sound intensity. This corresponds to the findings in studies of single units (*1*, *2*). The nonlinearity in some cases was nonmonotonic (*2*) and varied with stimulus frequency.

The extreme variability in the amplitudes of the responses from the inferior colliculus and the cochlear nucleus deserves special emphasis. In unanesthetized animals it was common to find variations of 50 percent in the amplitude of the response to a constant sustained white noise or tonal stimulus from trial to trial, or even during a single presentation of the stimulus lasting only a few minutes. Amplitude variations could be seen when the animal was not moving. During such variations, recordings from the round window often showed no change in activity at the sensory receptor, which rules out dependence of these variations upon the nonacoustic contractions of the middle ear muscles reported by Starr (*5*) and Carmel and Starr (*6*). Thus, it was not possible to report a single value for response amplitude at each frequency, and the results reported here are based on recordings made on several days with many presentations of each stimulus.

Three animals were also studied under pentobarbital anesthesia. If anesthesia were light enough to permit some spontaneous activity in the inferior colliculus, then tonal stimulation produced the same response pattern as in the awake animal. With deep anesthesia there was no response or only a slight increase in activity. In recordings from the cochlear nucleus the response pattern to tones was the same whether the animals were anesthetized or awake, but responses to white noise were of greater amplitude under anesthesia.

These auditory nuclei can thus be distinguished by their responses to white noise and tonal stimuli recorded with gross electrodes. A decrease in activity characterizes the response of the inferior colliculus to tones. Perhaps this has not been emphasized in studies of single units because anesthetized or decerebrate preparations have too little spontaneous activity to demonstrate the effect. This marked decrease in total activity in the inferior colliculus produced by tones could conceal an increase in activity in small populations of units tonotopically distributed, each responding to a characteristic frequency. Such an organization would fit well into the model suggested by Allanson and Whitfield (*7*). The increased activity produced by white noise is consistent with this formulation.

The surprisingly small response of the medial geniculate body to sustained white noise has been noted (*4*). Sustained tonal stimuli produced a decrease in activity, similar to but much smaller than the response of the in-

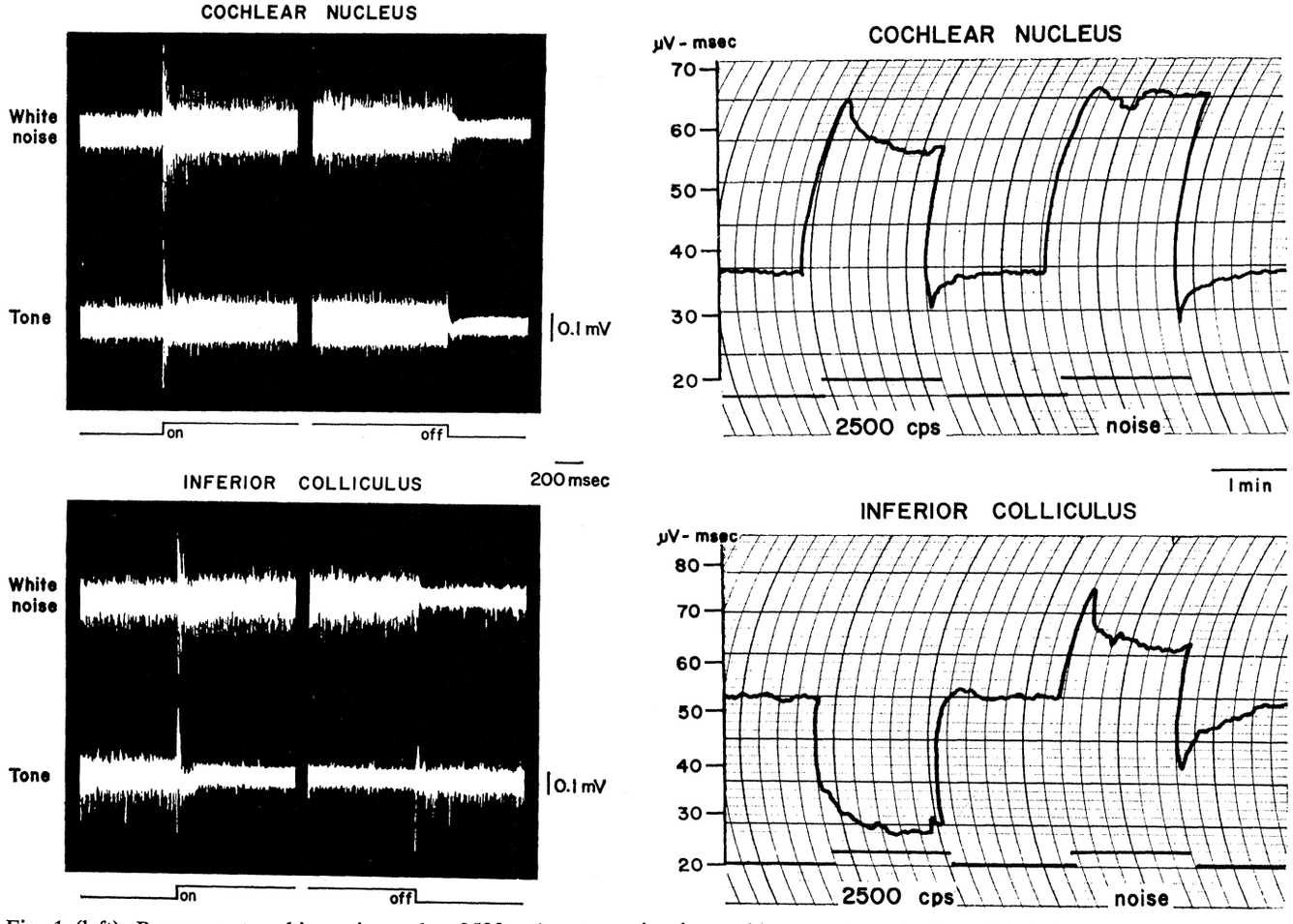

Fig. 1 (left). Responses to white noise and a 2500-cy/sec tone, in the cochlear nucleus (top) and inferior colliculus (bottom). Simultaneous oscilloscopic recordings. Responses are similar to white noise and different to tonal stimuli. Fig. 2 (right). Integrator recordings from cochlear nucleus (top) and inferior colliculus (bottom). Responses to a 2500 cy/sec tone and white noise. These recordings illustrate the entire time course of the events shown in Fig. 1.

ferior colliculus. Although the change was again small it was greater than the change produced by white noise.

It should not have been surprising that the nature of the sound stimulus used is critical in electrophysiological studies of the auditory system. Starr and Livingston (*4*) have pointed out the profound differences in distribution of activity evoked by clicks or other transients, as compared with the distribution of activity evoked by sustained white noise. The data reported here emphasize the differences between "white" noise and tonal stimuli, and the distinctiveness of the response patterns of the auditory nuclei.

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References and Notes

1. Y. Katsuki, T. Sumi, H. Uchiyaman, T. Watanabe, *J. Neurophysiol.* **21**, 569 (1958); Y. Katsuki, T. Watanabe, N. Maruyama, *ibid.* **22**, 343 (1959); J. E. Rose, R. Galambos, J. R. Hughes, *Bull. Johns Hopkins Hosp.* **104**, 211 (1959); J. E. Hind, J. M. Goldberg, D. D. Greenwood, J. E. Rose, *J. Neurophysiol.* **26**, 321 (1963 → S. D. Erulkar, *Proc. Roy. Soc. London Ser. B* **150**, 336 (1959); S. Hilali and I. C. Whitfield, *J. Physiol.* **122**, 158 (1963); R. Galambos, *J. Neurophysiol.* **15**, 381 (1952); R. Galambos, J. E. Rose, R. B. Bromiley, J. R. Hughes, *ibid.*, p. 381; J. E. Rose and R. Galambos, *ibid.*, p. 343.
2. J. E. Rose, D. D. Greenwood, J. M. Goldberg, J. E. Hind, *J. Neurophysiol.* **26**, 294 (1963).
3. Condenser microphone and sound vibration analyzer (type 1554 A) from General Radio.
4. A. Starr and R. B. Livingston, *J. Neurophysiol.* **26**, 416 (1963).
5. A. Starr, *Exptl. Neurol.*, in press.
6. P. Carmel and A. Starr, *J. Neurophysiol.* **26**, 598 (1963).
7. J. T. Allanson and I. C. Whitfield, in *Symposium on Information Theory, London, 1955*, C. Cherry, Ed. (Academic Press, New York, 1956), p. 269.

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Light: Evidence for Its Direct Effect on Hypothalamic Neurons

Abstract. *Continuous light impinging on the suprachiasmatic region of enucleate rats resulted in a constant estrous-like vaginal cycle. The lowest precentage of cornified cells was obtained from exposure of the arcuate or mammillary neurons to light. Ovarian and pituitary weight increased significantly after exposure of the arcuate region to continuous light.*

Environmental lighting profoundly affects pituitary-gonadal function in many vertebrates. A number of workers have shown that the estrous cycle of the female rat is extremely sensitive to the lighting regime (*1*). The problem is thus a search for the receptors.

Benoit and co-workers (*2*) in a series of investigations on the duck have indicated that, even after optic enucleation, light focused on the pituitary, hypothalamus, or rhinencephalon was effective in stimulating the gonads. Recently Ganong and co-workers (*3*) have shown that sunlight can penetrate the hypothalamus in the brains of sheep, dog, rabbit, and rat. Our experiments are an attempt to determine whether light can alter the estrous rhythm in the rat, if the light impinges directly on the hypothalamus.

We observed the following effects, presumably due to light being transmitted directly, by way of glass rods (optic fibers), onto hypothalamic neurons. When light was directed to the suprachiasmatic region of enucleate rats, there was a significant increase in the number of days during which cornified cells were found in the vaginal washings. The data obtained from the vaginal washings are graphically presented in Fig. 1.

These data were analyzed by the chi-square test and are based on the cell types present in the vaginal smear during the last 20 days of the 6-week experimental period. The analysis showed that there was no change in the number of days on which cornified material was present when cycles of light were presented to the animal by way of the eyes or optic fibers in the suprachiasmatic region. Under constant light a similar relation held for these two groups. However, under constant light, cornified cells were present in over 80 percent of the smears, compared with 40 percent under cyclic light. The only other animals showing a significant change from the animals that were only blind or in experimental control groups were those to which light was transmitted by way of optic fibers onto either the arcuate neurons or the mammillary nuclei. In both instances the chi-square test indicated a highly significant decrease in the number of days with cornified material; cornified cells being found on 45 percent of the days in both these experimental groups, compared to 60 percent in the animals that were only blind or those with fiber implants in other regions of the hypothalamus.

Ovarian weight did not change as a result of constant light on the suprachiasmatic region. However, application of this light by way of the eyes resulted in an average decrease of 42 percent in ovarian weight. Ovaries from the animals with eyes intact contained mostly large follicles, whereas after light had been administered by way of the optic fibers some corpora lutea were also found.

The ovarian weight increased 33 percent when light was directed to the arcuate region. The controls showed no change, nor did the ovarian weight change for any other experimental

Table 1. Effect of light, directed to the hypothalamic neurons, on the weight (wet) of pituitaries and ovaries of rats. The significance of the results are shown by *t*-tests. Figures after weights represent the standard errors of the mean. S, Suprachiasmatic; A, arcuate; M, mammillary; exp, exposed; cov, covered.

Treatment		Animals (No.)	Weight		*t*-test	
Blinded	Placement of rod		Pituitary (mg)	Ovary (mg)	Pituitary	Ovary
		Group I. Cyclic light (14:10)				
−	None	8	13.4 ± 0.88	81.7 ± 3.95		
+	None	5	13.6 ± 0.41	73.2 ± 5.05		
+	S	5	13.3 ± 0.40	98.4 ± 9.18		
		Group II. Constant light				
−	None	6	13.7 ± 0.55	46.6 ± 2.47		
−	S	9	14.4 ± 0.26	46.9 ± 2.49	.001	.001
+	S exp	9	12.2 ± 0.39	79.5 ± 2.01		
+	S cov	8	12.3 ± 0.44	88.4 ± 3.23	−	−
+	None	6	12.1 ± 0.22	77.6 ± 2.48	−	−
+	A exp	5	14.3 ± 0.81	106.6 ± 6.49	.01	.001
+	A cov	8	12.6 ± 0.56	79.5 ± 2.37	.01	
+	A cov + tape	5	11.6 ± 0.59	81.1 ± 2.32		
+	M exp	7	11.7 ± 0.30	87.7 ± 3.46	.01	
+	M cov	6	13.0 ± 0.48	90.8 ± 2.93		