Behavioral and Electroencephalographic Arousal to Contrasting Novel Stimulation

Abstract. *Three-second interruptions of continuous auditory stimulation, as well as 3-second periods of stimulation from the same sound source, were effective in producing behavioral arousal and electrocortical desynchronization in sleeping cats. Thus, stimulus offset as well as onset may serve an arousal function. Some differences in behavioral response and electrocortical desynchronization latency were noted.*

Presentation of a novel stimulus to a sleeping animal typically produces behavioral arousal with a concomitant shift in the electroencephalogram from a pattern of sleep, with spindle bursts or high-voltage slow waves, or both, to one of arousal or desynchronization, characterized by low-voltage fast waves. With repetition of the stimulus during sleep, both behavioral and electroencephalographic arousal responses diminish and eventually cease (habituation), but behavioral arousal habituates more rapidly (*1*). Until now, the behavioral and electroencephalographic reactions to a novel stimulus have been studied primarily in relation to the onset of the stimulus (*2*). In this report the behavioral and electroencephalographic arousal responses to the offset of a continuous auditory stimulus, interrupted for brief periods, are contrasted with the presentation or onset of the same stimulus for an equivalent period. Particular attention has been given to differences in behavioral responses and to the latency and duration of electroencephalographic desynchronization after the onset and offset of the stimulus.

Each of four cats participated in four experimental sessions consisting of 16 to 18 hours of sleep deprivation followed by feeding and a 1- to 2-hour period of sleep, during which behavioral observations and electroencephalographic recordings were made. Screw electrodes implanted in the skull over frontal, temporal, and occipital regions were wired to a plug mounted on the top of the head and cemented to the skull by dental acrylic. An overhead cable leading to the electroencephalograph was connected to the animal prior to the test period of sleep and stimulation. It was suspended in such a way that the behavior of the animal was not impaired and movement did not produce severe artifacts.

Sleep deprivation, feeding, and arousal tests during sleep were accomplished with the cat in a sound-proof observation chamber at all times. During the sleep-deprivation period, the cat sat on a brick surrounded by water. Novel stimuli were introduced during the test period only when the cat was in a sleeping posture with eyes closed and the electroencephalogram showed the high-voltage slow waves or spindles, or both, which are characteristic of sleep. A ventilating fan provided a background of ambient noise of approximately 70 db throughout all phases of the study. Two types of auditory stimuli were used: white noise produced by a white-noise generator (*3*), and 500 clicks per second produced by 1-msec square waves from a stimulator (*4*). Both stimuli were 92 db (0.0002 dyne/cm^2) (*5*). These stimuli were presented under two conditions: for 3 seconds, or continuously except for 3-second interruptions. Thus, when stimulus onset conditions were being used, there was either a 3-second burst of white noise or a 3-second train of clicks imposed on the ambient noise background. When stimulus offset was used, the animal had been in the presence of either white noise or the clicks throughout sleep deprivation, feeding, and the sleep-testing period, and these stimuli were interrupted for 3 seconds. Thus, either the onset of stimuli, when only ambient noise was present, or the cessation or offset of these stimuli, when they had been continuously present, constituted a sudden change in the auditory stimulus pattern and intensity. The relatively infrequent presentation and the short duration of both onset and offset stimulus changes therefore constituted novel stimulus conditions against a contrasting background.

The experimental sessions for each cat were separated by intervals of 1 to 5 days. Only one stimulus condition was used during a test session. Twenty trials (presentations or interruptions) were given per test period, the intervals between trials varying from 90 to 300 seconds in an irregular sequence (*6*). Stimuli were presented randomly with respect to ongoing slow-wave sequences or spindle bursts. Thus onset and offset stimuli bore no consistent relation to phase of spindle bursts or location in slow-wave sequences. Novel stimuli were always used in the same sequence for the four experimental sessions for each cat: white noise onset, white noise offset, click offset, click onset.

Behavioral arousal to stimulus onset was characterized by two reactions: (i)

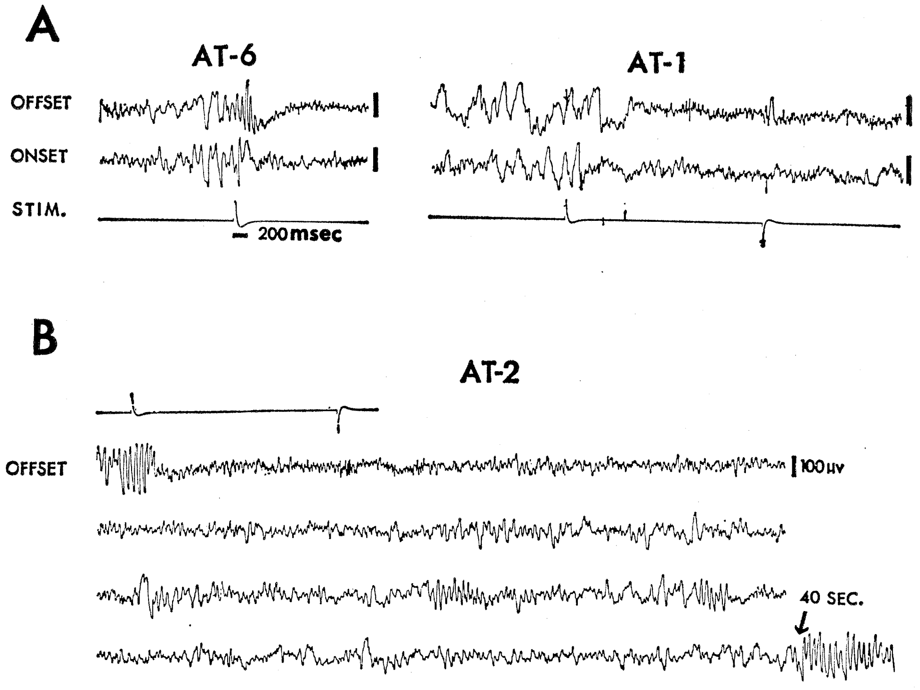

Fig. 1. *A*, Representative tracings from the right frontal-temporal leads for two cats. The stimuli used for these examples were clicks for subject AT-6 and white noise for AT-1. The stimulus marker indicates offset of a continuous stimulus for the top row and stimulus onset for the bottom. *B*, A continuous tracing from the right frontal-temporal leads illustrating the duration of desynchronization to white noise offset for one cat.

Table 1. Median latency of desynchronization. The offset-onset differences were tested by the Mann-Whitney U Test, one-tailed.

Subjects	Clicks					White noise				
	Offset		Onset			Offset		Onset		
	msec	*N*	msec	*N*	Diff.	msec	*N*	msec	*N*	Diff.
AT-1	400	11	266	20	134*	566	15	266	16	300‡
AT-2	349	10	233	11	116†	400	13	218	14	182‡
AT-3	333	10	167	6	166†			233	(15)§	
AT-6	333	15	266	15	67*	383	20	250	20	133‡
Total		46		52			48		50	

* $p < .025$. † $p < .010$. ‡ $p < .001$. § Not included in total.

a sudden lifting of the head, accompanied by opening of the eyes, and (ii) the head turning toward the sound source (orienting response). Orienting no longer occurred after the second or third trial. After several additional trials, only slight head movements or eye responses were observed. In contrast, stimulus offset caused no orienting response but did produce head lifting and eye opening which indicated behavioral arousal. However, a delayed orientation ordinarily occurred in response to the return of the stimulus at the end of the 3-second interruption. This would appear to indicate that prolonged stimulation prior to an interruption or offset had not fatigued or inhibited the mechanism underlying the orienting response. Behavioral habituation to both stimulus onset and offset was generally complete by the tenth trial.

Three measurements or observations were made with respect to electrocortical responses: (i) latency of desynchronization, (ii) duration of desynchronization, and (iii) rate and degree of habituation. Electrocortically, stimulus onset and stimulus offset could be differentiated only in terms of latency of desynchronization. Figure 1*A* shows typical electroencephalogram tracings in which the latency of desynchronization is shorter for stimulus onset than for offset. Table 1 presents median desynchronization latencies for all cats. The median latencies for offset conditions were in all instances considerably longer than those for onset conditions; the differences ranged from 67 to 300 msec. Desynchronization arousal occurred with approximately equal frequency to both stimulus onset and offset, with the exception of cat AT-3, which exhibited neither behavioral nor electroencephalographic arousal to white noise offset, although it was responsive to clicks. Latency or duration of desynchronization bore no relation to the state of spindling or slow waves existing at the time of onset or offset stimuli. While the cause of these latency differences is unknown, it cannot be attributed to echo or speaker reverberation. Measurement of sound level in the chamber following stimulus offset showed maximum decay time to be only 15 msec (*7*). Internal reverberation or neural aftereffects are not unknown but cannot be specified for the stimulus conditions utilized here.

The duration of desynchronization was measured to the first 1-second interruption of this pattern by spindles or slow waves, or both. Figure 1*B* presents a representative continuous record. The latency and duration of desynchronization as a function of trials for both onset and offset of white noise for cat AT-2 are given in Fig. 2. The results for the other cats were essentially similar. Although very long latencies occurred occasionally, there was no trend toward increasing latencies as a function of trials, as reported by Sharpless and Jasper (*1*). There were no significant differences between durations of desynchronization for onset and offset conditions; both were long on the initial trial, but decreased markedly by the second or third trial, and were ordinarily brief thereafter. The rate of decrease of the duration of desynchronization (habituation) is similar for both onset and offset conditions.

These results indicate that stimulus cessation (offset) is readily capable of eliciting electroencephalographic and behavioral arousal, with consequences very similar to those of stimulus onset. Thus it appears that a sudden change in stimulus conditions, rather than stimulus onset in itself, is responsible for both behavioral and electroencephalographic arousal. It is well known that the reticular formation is influenced by input from all sense modalities and that the ascending reticular activating system reflects this influence by the degree of electrocortical desynchronization and behavioral arousal it provokes (*2*). Elsewhere, it has been suggested that the reticular formation may adjust or adapt to the amount of sensory influx imposed upon it, and that it may serve as a monitor of sensory input and as a regulator of electrocortical activity and behavioral arousal (*8*). Our re-

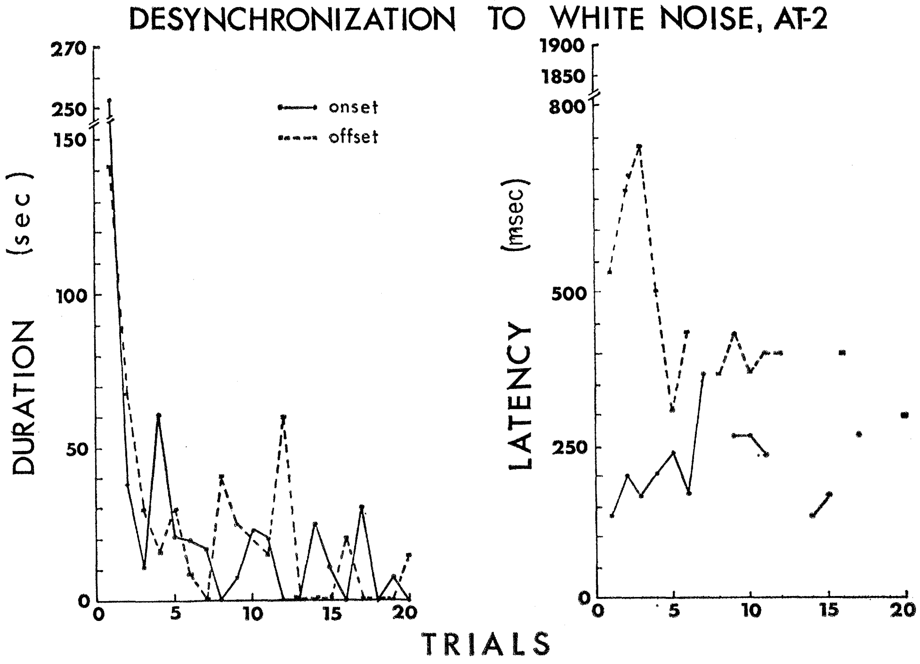

Fig. 2. The duration and latency of desynchronization as a function of trials for cat AT-2. The absence of plotted points for several trials on the latency graph indicates that no desynchronization occurred.

sults are consistent with the position that change in the adaptation level of the reticular formation, either by increase or decrease of sensory stimulation, is capable of effecting arousal in a sleeping animal.

NORMAN M. WEINBERGER
DONALD B. LINDSLEY
Departments of Psychology and Physiology, and *Brain Research Institute, University of California, Los Angeles*

References and Notes

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2. D. B. Lindsley, in *Neurophysiology*, Sec. 1 of *Handbook of Physiology*, J. Field, Ed. (American Physiological Soc., Washington, D.C., 1960), vol. 3, pp. 1553–1594.
3. Grayson-Stadler.
4. Grass P-4.
5. Measured by a General Radio sound level meter.
6. Trials were not given during episodes of low-voltage sleep—that is, "paradoxical sleep." On these infrequent occasions, intervals between trials were as long as 10 minutes, while the experimenter delayed trial onset until a high-voltage pattern returned.
7. Stimulus decay was measured by means of an oscilloscope, a Shure No. 245 microphone being used as transducer.
8. D. B. Lindsley, in *Sensory Deprivation*, P. Solomon *et al.*, Eds. (Harvard Univ. Press, Cambridge, Mass., 1961); and in *Nebraska Symposium on Motivation*, M. R. Jones, Ed. (Univ. of Nebraska Press, Lincoln, 1957).
9. Supported by ONR contract Nonr-233 (32). One of us (N.M.W.) is a National Institute of Mental Health postdoctoral fellow, No. 2-F2-MH-10,327.

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Electroencephalographic Data: Reduction by Wave-Width Analysis

Abstract. *While frequency analysis of the electroencephalogram is not unusual, relatively little examination has been performed in the time domain. Separation of the waves into the intervals of their duration above a baseline, irrespective of wave shape, has allowed a simple, numerical distinction to be made between the "eyes-open" and "eyes-closed" electroencephalogram.*

During investigations in our laboratory in which the electroencephalographic record was used to study and identify arousal of the cat from the sleeping state, visual examination was considered sufficient to identify the amount of synchronous activity (*1*). While this technique was entirely adequate for identification of well-defined (extreme) equilibrium states of sleep and arousal, for evaluation of the transitional periods, which were much more indefinite, some type of data reduction system was indicated. Two factors were considered important in the selection of such a system. First, since synchrony and asynchrony appeared to be of equal importance, equal weight should be given to waves of both large and small amplitude. Second, in the event short time periods were critical, analysis in the time domain would be more adaptable than frequency domain analysis.

For these reasons attention was given to reducing the electroencephalographic amplitude time-series with respect to the period (half wave length) between successive baseline crossings. The basic unit of period is similar to that suggested by Stein, Goodwin, and Garwin (*2*), Prast and Noell in 1949 (*3*), and Burch, Saltzberg, and associates (*4*). By this technique, rectangular waves of equal height are produced with duration determined by the time the input discriminator is exceeded and the point at which the amplitude drops below the discriminator value.

The actual electroencephalographic data are recorded on frequency-modulated tape with a bandwidth from 0 to 250 cy/sec. The analysis through a separate system is shown schematically in Fig. 1. The basic discriminator for conversion of the individual waves to shaped rectangular pulses is a modified, transistorized Schmitt discriminator. While this discriminator may be placed arbitrarily at any higher setting, the minimal setting practical at the present time is equivalent to 5-μv input. Any lower setting of the discriminator (that is, closer approach to the theoretical baseline) is at this time prohibited by the inherent noise in the system. Since most of the electroencephalographic recording falls within a dynamic range of 25, this circuit must exhibit no overload characteristics within such a range.

The duration of the shaped rectangular pulse is measured by gating a clock-pulse generator by the leading and trailing edges of the shaped pulse. After differentiation of the rectangular pulse the leading edge initiates the gating pulse for the clock generator. The clock generator continues to run until the trailing edge terminates the gate. The number of clock pulses generated is then proportional to the frequency of the clock and duration of the gating pulse. In the example shown, a frequency of 1000 pulses per second is utilized; thus for a 14-msec wave, 14 pulses are produced. In practice a 100 kilocycle crystal-controlled oscillator is used as the master clock with a variable scaler reducing the effective frequency to the time resolution desired. Channeling of the burst of clock pulses into storage bins related to the number of counts in the burst has been accomplished by the address scaler and storage units of a Victoreen 120 pulse-height analyzer. In this unit, two decade Burroughs switching tubes are utilized to form an address scaler with channels of 1 to 100. Twenty storage channels are available to tabulate any 20 of these channels at one time. The present techniques have tabulated pulses of 1-msec duration. This resolution, however, appears to be in excess of what is needed, and in the following histograms of pulse-width distributions bins of 4-msec increments have been recorded.

In a first investigation with this system, histograms of pulse-width distribution were obtained from frontal-occipital and parietal-occipital leads of subjects with eyes alternately open and closed. The subjects were young adult male and female volunteers with no known psychiatric or neurological history and were selected without special criteria regarding their electroencephalograms.

Repetitive trials showed the predominant alpha-waves present during the

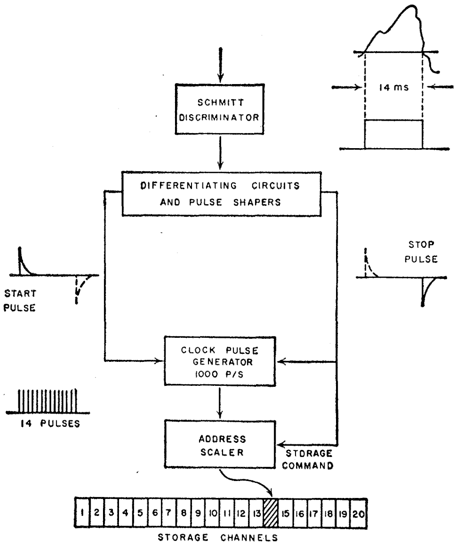

Fig. 1. Schema of electronic technique of pulse-width analysis showing a single theoretical electroencephalographic wave of 14-msec duration with subsequent shaping and relations necessary for analysis and storage.