the $LH_2$-AMP in dimethyl sulfide we have inhibited hydrolysis by chemical means even at high *p*H values, effectively accomplishing what the enzyme can do at neutral *p*H. As might be expected D-$LH_2$-AMP and L-$LH_2$-AMP do not show the stereospecificity in nonenyzmatic chemiluminescence which was previously reported for the enzymatic reaction (*9*).

H. H. SELIGER
W. D. MCELROY
*McCollum-Pratt Institute* and
*Department of Biology,*
*Johns Hopkins University,*
*Baltimore, Maryland*

### References

1. W. D. McElroy and A. Green, *Arch. Biochem. Biophys.* **64**, 257 (1956); W. D. McElroy and H. H. Seliger, in *Light and Life*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1961), p. 219.
2. W. C. Rhodes and W. D. McElroy, *J. Biol. Chem.* **233**, 1528 (1958).
3. This work was supported in part by the Atomic Energy Commission, the National Institutes of Health, and the National Science Foundation.
4. E. H. White, F. McCapra, G. F. Field, W. D. McElroy, *J. Am. Chem. Soc.* **83**, 2402 (1961).
5. E. White, in *Light and Life*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1961), p. 183.
6. H. G. Khorana, *J. Am. Chem. Soc.* **76**, 3517 (1954).
7. H. H. Seliger and W. D. McElroy, *Arch. Biochem. Biophys.* **88**, 136 (1960).
8. H. H. Seliger, *Anal. Biochem.* **1**, 60 (1960).
9. ———, W. D. McElroy, E. H. White, G. F. Field, *Proc. Natl. Acad. Sci. U.S.* **47**, 1129 (1961).

21 June 1962

## Evoked Responses to Clicks and Electroencephalographic Stages of Sleep in Man

*Abstract.* The form of the average evoked response to clicks is highly correlated with the background electroencephalogram. However, the response during the emergent low-voltage "dreaming" stage is different from that seen during the low-voltage phase at the beginning of sleep. The results provide additional evidence that the emergent low-voltage stage is a neurophysiologically unique phase.

When recorded from the human scalp, the amplitude of specific electrical events evoked in the brain by sensory stimuli is usually smaller than that of the complex background activity. Since Dawson (*1*) described an electronic averaging technique for discriminating these signals from background noise, several such methods have been developed which are based on the assumption that the evoked electrical response bears some definite time relation to the stimulus (*2*). The present study used a digital system to average evoked responses to clicks both during waking and sleep in human subjects.

As a human subject sleeps, electroencephalographic (EEG) potentials shift continuously through several characteristic patterns. At the onset of sleep a low-voltage pattern with irregular frequency is seen, stage 1 (*3*). There is then a progression through stages 2 and 3 which is characterized by 14-cy/sec spindling and slower activity, to stage 4 where trains of delta waves dominate the record. Stage 4 gives way to stages 3 and 2, and then a low-voltage, irregular pattern appears which is similar to the pattern at the beginning of sleep. Rapid eye movements accompany this emergent low-voltage phase, however, and if awakened from this stage, subjects are likely to report dreaming (*3*). The low-voltage pattern at the onset of sleep is usually not accompanied by rapid eye movements or dream recall (*3*). During a normal night of sleep the cycle described above is repeated about every 90 to 120 minutes.

Generally, thresholds for arousal are lowest during low-voltage and highest during high-voltage stages, but classification of the emergent irregular, low-amplitude phase is controversial. Dement and Kleitman found that the threshold for awakening was considerably higher in this phase than at the beginning of sleep (*3*), and Jouvet and others have shown that in the cat a similar low-voltage pattern of sleep is associated with raised thresholds for arousal (*4*). To distinguish this emergent low-voltage phase from the similar pattern at the beginning of sleep, we will call it stage $1_{rem}$.

For this study three young adult male subjects slept in an electrically shielded chamber where the ambient noise level was constant at approximately 60 decibels (db), relative to 0.0002 dyne/cm$^2$. The click stimulus, with an intensity of 85 db, was the amplified gate output pulse from a type 162 Tektronix wave-form generator which was fed to an 8-inch cone speaker located about 1 foot above the subject's head. A click was presented every 2 seconds throughout the night. Potentials recorded from bipolar electrodes which were placed on the vertex at $c_z$ and in the left occipital region at $o_1$ were amplified and recorded on magnetic tape (*5*). For each stimulus the recorded activity was sampled every 6 msec through a period of 1536 msec from stimulus onset. The voltage of each of these 256 ordinates was fed to a Packard Bell 250 general-purpose digital computer which was programmed to compute the mean on each ordinate after the data from 100 stimuli were received. These averages of the individual ordinates were graphically displayed by an *x-y* plotter.

Fig. 1 shows typical samples from the computed record of one subject. An upward deflection represents positivity at $c_z$. Note that several prominent components of the response are labeled,

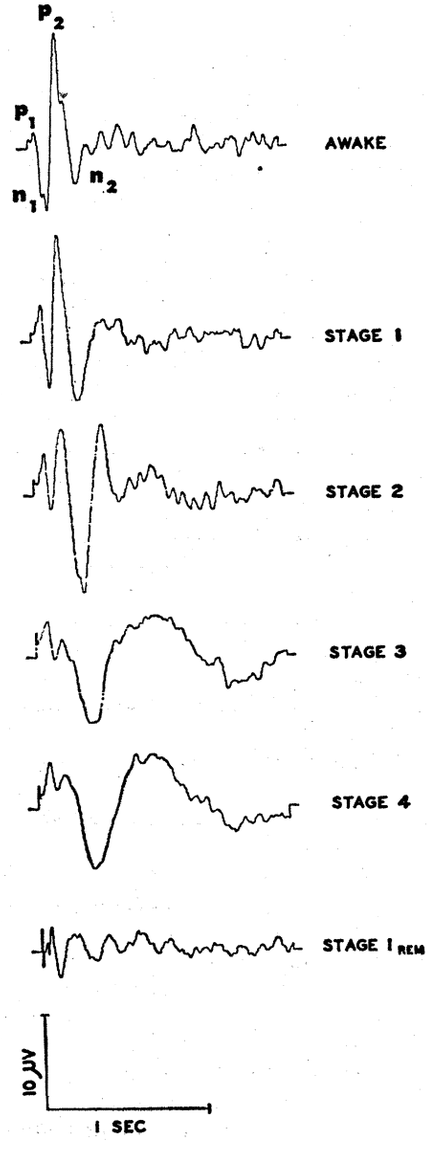


Fig. 1. Average responses to clicks as a function of the EEG stage of sleep (subject B.D.). Upward deflection denotes positivity at $c_z$.

Table 1. Means ($\bar{X}$); standard deviations ($\bar{s}$), and $\eta^2$ values for components of the average evoked response in various EEG Stages. Scores are in microvolts.

| Item | Score for component of evoked response | | | |
|---|---|---|---|---|
| | $p_1$ | $n_1$ | $p_2$ | $p_2 - n_2$ |
| | | *Stage A* | | |
| $\bar{X}$ | 1.4 | −4.0 | 6.9 | 10.6 |
| $\bar{s}$ | 1.6 | 2.0 | 2.4 | 2.4 |
| | | *Stage 1* | | |
| $\bar{X}$ | 2.7 | −2.7 | 4.8 | 9.1 |
| $\bar{s}$ | 1.3 | 2.3 | 2.2 | 3.2 |
| | | *Stage 2* | | |
| $\bar{X}$ | 3.5 | −1.0 | 1.7 | 6.1 |
| $\bar{s}$ | .9 | .9 | 1.4 | 2.3 |
| | | *Stage 3* | | |
| $\bar{X}$ | 4.3 | −1.1 | 2.1 | 6.5 |
| $\bar{s}$ | .9 | 1.1 | 1.2 | 1.7 |
| | | *Stage 4* | | |
| $\bar{X}$ | 4.5 | −.3 | 1.4 | 7.0 |
| $\bar{s}$ | 1.4 | 1.3 | 1.5 | 1.6 |
| | | *Stage* $1_{rem}$ | | |
| $\bar{X}$ | 2.7 | −2.4 | 2.5 | 4.0 |
| $\bar{s}$ | .8 | 1.5 | 1.1 | .3 |
| | | $\eta^2$ | | |
| | .51 | .45 | .54 | .25 |

$p_1$ (first positive event), $p_2$, and so on. As the EEG shifts from the waking pattern through stages 2 and 3 to stage 4, there is a striking and consistent change in the wave form. The amplitude of $p_1$ increases, the amplitudes of $n_1$ (first negative event) and $p_2$ decrease, and the amplitude of $n_2$ increases markedly. A third positive wave with a period of one-half to one second appears first in stage 2, and reaches relatively high amplitude in stages 3 and 4. The form of the evoked response in stage $1_{rem}$ is similar to that seen during waking, and at the beginning of sleep, but the average amplitude of the several components is small, particularly for $p_2$ and $n_2$.

Table 1 shows the mean amplitude in microvolts, the average standard deviations, and the $\eta^2$ values for the three subjects on $p_1$, $n_1$, $p_2$, and $p_2 - n_2$. The general increase in $p_1$ and decrease in $n_1$ and $p_2$ can be seen as the background EEG shifts from the waking pattern to stage 4. In stage $1_{rem}$ the average amplitudes of $p_1$ and $n_1$ are very close to those in stage 1, but the amplitude of $p_2$ is considerably smaller than in stage 1. The average peak-to-peak amplitude ($p_2 - n_2$) is smaller in stage $1_{rem}$ than in any other stage. Using the stage of sleep score as the treatment variable, one-way analyses of variance were computed on each amplitude measure for each man. There were about 70 average evoked responses during a single night; these were distributed somewhat unevenly through waking and the five stages of sleep. Within each subject all of the resulting $F$ ratios were significant at $p < .001$. The effect of stage of sleep on these measures, therefore, was very reliable. The average $\eta^2$ values for the three men indicate that stage of sleep accounted for 25 to 50 percent of the variance in these measures of amplitude.

Correlations between the peak latencies of the components of the evoked response and the EEG stage of sleep scores were low. For example, for $p_1$ there was a slight increase in the average peak latency (from about 40 to 50 msec) as the subjects shifted from waking to stage 1, but no further increase occurred with the onset of deeper stages of sleep. Succeeding peaks, $n_1$, $p_2$, and $n_2$ have average peak latencies of about 97, 173, and 276 msec. These latencies are similar to those described by Davis *et al.* for the *K* complex, and can be identified as the *K* components of the evoked response (*6*). These data, then, confirm Geisler's report that the *K* component of the evoked response changes as the subject goes to sleep (*7*). In addition, these results indicate that the wave form continues to change as the subject shifts into deeper stages of sleep, and that the earlier portions of the response also participate in this change. In general, the entire wave form of the evoked response appears to be closely associated with the stage of sleep reflected in the background EEG pattern.

The shape and amplitude of the average response in stage $1_{rem}$ are difficult to classify on a light-deep continuum of sleep. Clearly the evoked response is not identical to that seen at the beginning of sleep. Other evidence has accumulated in studies of humans that stage $1_{rem}$ is not simply a light phase of sleep. Thresholds for awakening are raised (*3*), muscle tonus is decreased (*9*), skin resistance is increased (*10*), and body movement is reduced (*3*). The conclusion that stage $1_{rem}$ is similar to the low-voltage phase of sleep has been justified by experiments in the cat by Dement (*11*), Jouvet (*4*), and others. This stage, termed by Jouvet the rhombencephalic phase, is characterized by low-voltage fast EEG activity, disappearance of muscle tonus, and the presence of rapid eye movements. The threshold for awakening in this phase is considerably higher than in the slow-wave phase. We cannot conclude, on the other hand, that the low-voltage phase is simply a deep stage of sleep, for both Huttenlocher and Evarts showed that while evoked responses in the reticular formation and cortex of cats were practically absent, there was a remarkably high level of spontaneous activity in the same neural units during this stage (*12*). These investigators each concluded that external stimuli were being occluded during this highly activated phase of sleep.

The low average amplitude of the evoked responses seen in our records during stage $1_{rem}$ is consistent with the idea that events from an external stimulus tend to be occluded during the "dreaming" phase of sleep, and the data support the contention of Hawkins *et al.* (*10*) that stage $1_{rem}$ should be treated as a neurophysiologically unique phase of sleep.

HAROLD L. WILLIAMS
DONALD I. TEPAS*
HENRY C. MORLOCK, JR.
*Walter Reed Army Institute of Research, Washington 12, D.C.*

**References and Notes**

1. G. D. Dawson, *Electroencephalog. Clin. Neurophysiol.* **6**, 65 (1954).
2. J. S. Barlow, *ibid.* **9**, 340 (1957); J. C. Armington, D. I. Tepas, W. J. Kropfl, W. Hengst, *J. Opt. Soc. Am.* **51**, 877 (1961); W. A. Clark, M. J. Goldstein, Jr., R. M. Brown, D. F. O'Brien, H. E. Zieman, *Trans. Inst. Radio Eng. BME-8* (1961), p. 46.
3. W. Dement and N. Kleitman, *Electroencephalog. and Clin. Neurophysiol.* **9**, 673 (1957).
4. M. Jouvet, in *Symposium on the Nature of Sleep*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1961), p. 188; Z. P. Horowitz and M. Chow, *Science* **134**, 945 (1961).
5. H. H. Jasper, *Electroencephalog. Clin. Neurophysiol.* **10**, 370 (1958). The symbols $c_z$ and $o_1$ refer to positions in the 10–20 electrode system recommended by the International Federation for Electroencephalography and Clinical Neurophysiology.
6. H. Davis, P. A. Davis, A. L. Loomis, E. N. Harvery, G. Hobart, *J. Neurophysiol.* **2**, 500 (1939).
7. C. D. Geisler, *Armed Services Technical Information Agency, Technical Report 380, No. AD 264724* (1960).
8. M. Roth, J. Shaw, J. Green, *Electroencephalog. Clin. Neurophysiol.* **8**, 385 (1956).
9. R. J. Berger, *Science* **134**, 840 (1961).
10. D. R. Hawkins, H. B. Puryear, C. D. Wallace, W. B. Deal, E. S. Thomas, *ibid.* **136**, 321 (1962).
11. W. Dement, *Electroencephalog. Clin. Neurophysiol.* **10**, 291 (1958).
12. P. R. Huttenlocher, *J. Neurophysiol.* **24**, 451 (1961); E. V. Evarts, in *Symposium on the Nature of Sleep*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1961), p. 171.

* Present address: Minneapolis-Honeywell Regulator Company, Minneapolis, Minnesota.

4 September 1962.