By means of a watch spiral placed on the first axle a convenient tension is applied to the muscle (maximum 250 mg). The beam of light is reflected by the mirror onto a centimeter-graduated semi-circular screen, placed at a distance of 1 m and equidistant at every point from the mirror. Under these conditions, the system provides an amplification of about 1000 times. The responses are measured by reading directly on the screen, in cm, the course of the beam of light. Doses of acetylcholine were generally used which produced readings of about 100 cm.

For the dosage of acetylcholine, a small longitudinal strip of the rectum abdominal muscle of the toad (*Bufo arenarum* Hensel) was employed. This was obtained from the zone above the abdominal vein. In most cases this zone is no more than 2–3 mm wide, and is easily distinguished from the rest. Relaxation is rapid, allowing for a dose to be tried every 5–10 min. In this muscle the response was proportional to the logarithm of the dose, and the effects were clearly different when tried in two points with doses whose relation was 3:4 or 4:5. The responses were reproducible and in 10 experiments the index of precision gave a mean value for λ of 0.047 ± 0.007. The useful doses for this muscle oscillated between 8–15 ng in 0.05 ml.

Résumé. Une modification à la technique du microbain pour le dosage de matériel biologique est décrite. La contraction musculaire, amplifiée mécaniquement, est mesurée par le trajet d'un rayon de lumière sur un écran.

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STUDIORUM PROGRESSUS

Arousal Threshold in the Cat as a Function of Sleep Phase and Stimulus Significance

Recently, a number of investigators, notably DEMENT and KLEITMAN[1] and JOUVET et al.[2], have differentiated in both man and animals between two types of naturally occurring sleep. The predominant phase of sleep is referred to as *high voltage sleep*; the other, which is associated with dreaming in humans, is variously called *paradoxical sleep, activated sleep, rhombencephalic sleep*, and *REM sleep*. In addition to its low voltage EEG characteristics, this latter stage of sleep has a number of other distinguishing features. The one which is most relevant to this paper is that arousal thresholds are reported to be higher in paradoxical sleep than in high voltage sleep[2–4]. However, assessment of arousal thresholds was based primarily on electrical stimulation of the brain stem reticular formation[2,3] and on responses to neutral auditory stimuli[4]. Those studies which have presented meaningful stimuli to subjects during high voltage and paradoxical sleep were primarily concerned with investigating learning and discrimination during the sleeping state as evidenced by differential responsiveness while still asleep[5]. In addition, these investigators established stimulus significance by employing an aversive situation.

The experiment reported here directly investigated differences of arousal to significant and non-significant stimuli during high voltage and paradoxical sleep and used an appetitive situation to establish stimulus significance (the delivery of milk to food and liquid deprived cats).

Two adult cats were intensively investigated. Each animal was prepared with permanent recording electrodes on the cerebral cortex, in the dorsal hippocampus, and in the dorsal neck muscles. After recovery from surgery, each animal was adapted to a training compartment equipped with a loudspeaker, an observation window, and a dish connected to an automatic milk delivery device. Stimulus significance was manipulated by a four-stage program of training with awake subjects which consisted of: (a) adaptation to trains of clicks of various durations and intensities; (b) conditioning to associate the termination of a train of clicks with delivery of milk (the click intensity was 81 db SPL for one subject and 85 db SPL for the other); (c) extinction training which consisted of presentation of the clicks alone; and (d) reconditioning which was effected in the same manner as the original conditioning.

During all conditioning and testing, the subjects were maintained on an 18 to 20 h food and liquid deprivation schedule. A short duration conditioning stimulus was used initially, and then gradually lengthened to 100 sec (the situation was that of a Pavlovian delayed conditioning paradigm). Test trials, which were presented only during sleep, consisted of a 50 sec train of clicks alone. As soon as clicks presented during sleep resulted in behavioral arousal, the clicks were terminated by the experimenter. Cortical and hippocampal EEG and dorsal neck EMG were monitored on a Grass polygraph in order to distinguish between high voltage and paradoxical sleep.

Figure 1 represents the percentage of awakenings for each cat from high voltage sleep to clicks during each of the four stages of the experiment: (A) before conditioning, (B) after conditioning, (C) after extinction, and (D) after reconditioning. Statistical analysis indicates a significant difference in the percentage of arousals between each successive pair of experimental conditions, i.e. between (A) and (B), (B) and (C), and between (C) and (D). The results of test trials after extinction were essentially the same as the results of test trials before conditioning, indicating that the clicks during these two stages of the experiment had approximately the same arousal effects. It should be

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[1] W. DEMENT and N. KLEITMAN, EEG clin. Neurophysiol. *9*, 673 (1957).

[2] M. JOUVET, F. MICHEL, and J. COURJON, C. r. Soc. Biol., Paris *153*, 101 (1959).

[3] O. BENOIT and H. BLOCH, J. Physiol., Paris *52*, 17 (1960).

[4] W. DEMENT, EEG clin. Neurophysiol. *10*, 291 (1958).

[5] N. BUENDIA, M. GOODE, G. SIERRA, and J. P. SEGUNDO, Exper. *19*, 208 (1963).

noted that the high probability of awakening to test trials after conditioning (B) and reconditioning (D) were with click intensities of 81 db and 85 db for the two animals, respectively; whereas only 3–4% awakenings occurred before conditioning (A) and after extinction (C) with click intensities of about 97 db. When intensities of 81 db and 85 db were presented before conditioning and after extinction, there were 0% awakenings.

Figure 2 represents the percentage of awakenings from paradoxical sleep during each stage of the experiment. After conditioning (B) the probability of awakening was not significantly greater than before conditioning (A).

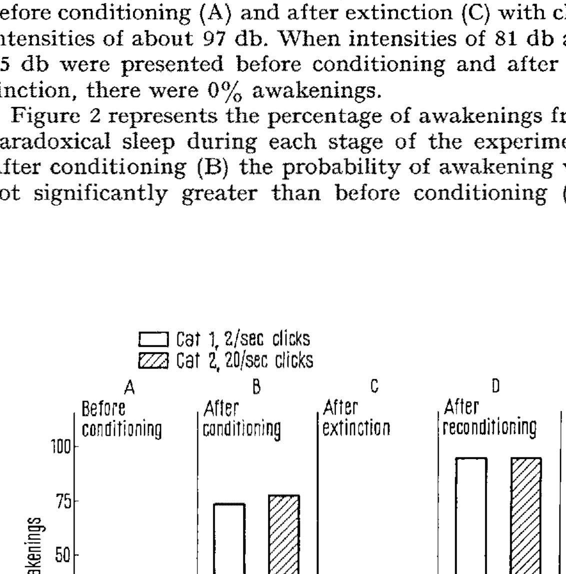

Fig. 1. % awakenings from high voltage sleep at different stages of conditioning. A bar over the decibel level indicates a mean value, with the range presented in parentheses below.

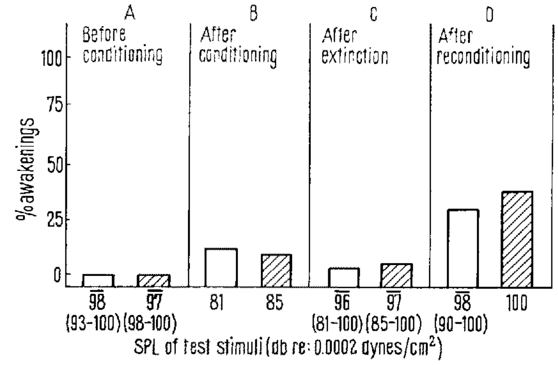

Fig. 2. % awakenings from paradoxical sleep at different stages of conditioning.

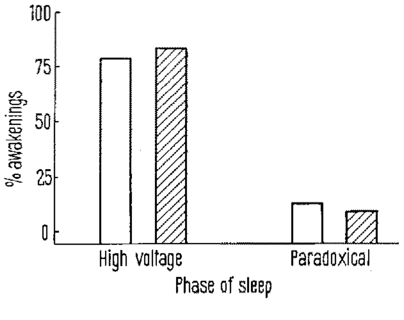

Fig. 3. % awakenings from high voltage sleep and paradoxical sleep after conditioning. The test stimulus intensity was the same during both phases of sleep (for cat 1 it was 81 db; for cat 2 it was 85 db).

This indicates that stimuli of intensities which did arouse from high voltage sleep were relatively ineffective in arousing from paradoxical sleep. In order to produce a significantly greater percentage of arousals during paradoxical sleep to meaningful as compared to non-meaningful stimuli, click intensity had to be increased to 100 db. It should be noted that these intensities after reconditioning (D) were within the range used before conditioning (A) and after extinction (C), so that intensity alone was not the significant factor in producing arousals.

Figure 3 directly compares arousals after conditioning from high voltage and paradoxical sleep to stimuli of the same intensity in order to illustrate the difference in sensitivity to arousal between these states.

It is concluded that assignment of significance to a stimulus by positive reinforcement increases its effectiveness of arousing from both high voltage and paradoxical sleep, but that arousal probability from paradoxical sleep is much lower than from high voltage sleep, as is the case with neutral auditory stimuli, electrical stimulation of the brain stem reticular formation, and with stimuli associated with negative reinforcement. The mechanism of attention, as investigated by HERNANDEZ-PEON[6], may have relevance for understanding these data. When an organism is occupied with processing one form of information, corticofugal and reticulofugal influences modulate other sensory inputs at a subcortical level (at the level of the first sensory relay nucleus or more peripherally). If paradoxical sleep does reflect a state of information processing, as is suggested by more active neuronal activity during paradoxical sleep in the cat[7,8] and by dreaming in the human[1], then this attentional mechanism may account for the increased threshold of awakening from paradoxical sleep than from high voltage sleep.

The fact that the meaningfulness of a stimulus can be effective in arousing an animal during high voltage sleep when the nervous system is relatively inefficient for information processing implies that there is, in a sense, a sensory filter which selectively attenuates inputs except those which are novel or have significance for the animal. But these inputs, transmitted to a nervous system in high voltage sleep, have only the expedient effect of arousing the animal, i.e. they act as electrical stimulation of the brain stem reticular formation to produce an 'arousal' of higher centers. The aroused organism can then bring the full information processing facilities of these higher centers to bear on the situation[9].

Résumé. Chez le Chat éveillé après conditionnement au moyen de clicks et de lait, les clicks déclenchent le réveil de manière plus efficace qu'en l'absence de conditionnement. Le seuil d'éveil s'est avéré plus bas lors d'un sommeil accompagné d'ondes lentes de haut voltage que pendant le sommeil paradoxal.

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[6] R. HERNANDEZ-PEON, in *Sensory Communication* (W. A. ROSENBLITH, Ed.; M.I.T. Press, Wiley, New York 1962), p. 497.

[7] E. EVARTS, in *Ciba Foundation Symposium on the Nature of Sleep* (G. E. W. WOLSTENHOLME and M. O'CONNER; Eds. J. and A. Churchill Ltd., London 1961), p. 171.

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[9] Supported by NSF Grant No. GB 1965.