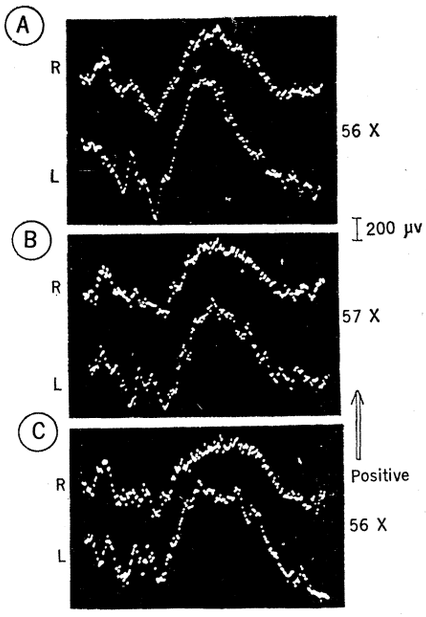

Fig. 3. Three successive summated cortical responses to click in one subject. The biological invariance of the short latency initial inflection on the right side is shown.

is on a compressed time scale. As a control, in nearly all tests the conditions of the experiment were maintained, except that no noise or specific sound was used to trigger the CAT sweep. At these times the general picture of Fig. 1D was obtained.

The potential patterns of four randomly chosen subjects to whom click stimuli were presented are shown in Fig. 2; this figure demonstrates the almost invariant character of the initial, approximately 14 msec inflection from the right brain. It is observed that the secondary oscillations and the positive peaks vary considerably.

The stability of the right-sided initial response to clicks in three successive summated responses in a single individual is shown in Fig. 3. Although the initial responses from the left brain vary, the 14 msec right brain responses remain biologically invariant.

Four subjects in this series were predominantly left-handed; their summated cortical evoked responses were similar in all ways to the preponderantly right-handed persons.

If it is allowed that the amplitude of summated evoked cortical responses is directly related to the site of predominant processing of the auditory signals, then it seems indisputable from these physiological data that noises (clicks) are initially processed primarily in the right brain. Again, if the above reasonable assumption is accepted, single-syllable words are processed in each cerebral hemisphere equally in approximately half of the subjects studied; in the other half of the subjects, however, the left brain shows a dominance for verbal processing.

From studies in the visual system (*3*) it would appear that the wave-form differences observed between clicks and words might be the consequence of the variable primary stimuli used; but the consistent differences in amplitudes and contours over the two hemispheres, as seen in this auditory work, were never evident in full field illumination, irrespective of the quality of visual stimuli employed.

Such differential cerebral processing of the auditory input as described is not unique. Recently it has been shown that in the visual system (*4*) there is a difference in right brain and left brain processing of language and nonlanguage data as determined by material derived from averaged evoked responses from each occiput.

An obvious extension of this auditory work for operational clinical purposes will be the introduction of precision sources of phonetic input.

This physiological study generally confirms, and somewhat furthers, the psychophysiological work of Kimura.

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5. The amplitude calibrations were made by impressing a known voltage square wave through the entire system, and recording the single sweep output from the CAT face. This method obviates the variance effect of the subjects' successive inputs. The primary biological signals were calculated to be in the range of 3.5 to 3.75 μv.

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Acetylcholine Liberation from Cerebral Cortex during Paradoxical (REM) Sleep

Abstract. *The rate of liberation of free acetylcholine from the surface of prostigmin-treated cerebral cortex in the freely moving cat has been determined in states of slow wave sleep, paradoxical or activated sleep, and waking. The average rate during slow wave sleep (1.2 nanograms per minute per square centimeter of cortical surface) increased during paradoxical sleep (2.2 nanograms per minute) and during waking (2.1 nanograms per minute). The rate of acetylcholine release is thus related to the electroencephalogram pattern of desynchronized activation of the cortex rather than to the behavioral responsiveness of the animals.*

The rate of liberation of acetylcholine (ACh) from the surface of the cerebral cortex treated with an anticholinesterase may be increased two to three times when an animal is awakened from natural slow wave sleep by normal sensory stimulation or by electrical stimulation of the midbrain reticular system (*1*). These observations, confirmed in other laboratories (*2*), have led to the conclusion that the desynchronized activation of the cerebral cortex which characterizes the electroencephalogram (EEG) during states of alertness or arousal from slow wave sleep (SWS) may be cholinergic at the cortical level, even though other neurochemical transmitter substances may be involved as well in the mediation of states of sleep and wakefulness at subcortical levels, such as the monoamines (*3*).

There is, however, another state of deep sleep which is characterized by desynchronized activation of the electrical activity of the cerebral cortex similar to that seen in waking and alertness. This has been called paradoxical sleep (PS) because the animal (or man) appears to be deeply asleep and even more relaxed than in SWS, whereas the EEG resembles that of a state of alertness. There are also characteristic rapid eye movements which suggested the designation REM sleep. There remains to be determined whether the rate of ACh liberation from cerebral cortex is increased during PS, as in wakefulness, after the appearance of the EEG, or whether it

remains at a low level corresponding to the behavior of the animal.

We have developed a technique for the determination of the rate of ACh liberation from the surface of the cortex in the intact, freely moving cat maintained in a restraining box in a sound-resistant electrically shielded room. Observations of the activity and posture of the animal, together with continuous records of the cortical EEG, the electrical activity of the neck muscles, and an electrical record of eye movements made it possible to select periods of time (5 to 10 minutes) during which the animal remained in a given state of sleep (SWS, PS) or wakefulness (W). Sustained periods of wakefulness were maintained by opening the door of the soundproof room and administering mild sensory stimuli to the animal (auditory and tactile).

Sampling of the exudate from the pial surface of the cortex was carried out by means of a perspex chamber sealed into the skull with dental cement so that it rested lightly on the pial surface of the anterior suprasylvian and postcruciate cortex after removal of the dura. The chamber was screwed into a trephine hole which had been threaded during an operation conducted under surgical barbiturate anesthesia. Stainless steel tubes were inserted into the chamber and connected to polyethylene tubing so that Elliott's solution that contains prostigmin could be injected into the chamber (over the cortex) or withdrawn from the chamber at any time throughout the experiment. Each sample was taken in 1 ml of Elliott's solution containing prostigmin (50 μg/ml). At the beginning of a certain stage of sleep or wakefulness, this solution was withdrawn and a fresh solution (1 ml) was injected into the chamber. At the termination of the state of sleep or waking, the fluid was rapidly withdrawn by vacuum into a test tube that was maintained at 0°C in crushed ice.

Also under anesthesia, stainless steel recording electrodes were inserted through the skull over the intact dura to record the electrical activity of the cortex. Two electrodes were placed on either side of one eye, one on the nasal margin, another on the temporal margin, in order to record the electrical accompaniment of eye movements. Finally, a pair of electrodes was inserted into the occipital neck muscles to monitor their tonic activity in order to help identify paradoxical sleep, which occurs when these muscles become completely relaxed. Experiments were begun 2 to 3 days after the operation.

Table 1. Rate of liberation of acetylcholine from cerebral cortex during slow wave sleep (SWS), paradoxical sleep (PS), and waking (W). Expressed in nanograms per minute per square centimeter of cortical surface.

Exp.	Acetylcholine liberated		
	SWS	PS	W
1	1.3	3.2	
2	2.0		3.5
3	1.7	3.1	3.3
4	1.2	3.1	2.5
5	0.4	0.9	0.7
6	1.1	2.0	1.9
7	1.0	1.5	1.2
8	1.1	1.9	1.9
9	1.0		1.9
Mean	1.2	2.2	2.1
S.D.	0.4	0.8	0.9

Acetylcholine concentration in the superfusate was estimated by bioassay on the rectus abdominus muscle of the frog (*4*). Control studies showed that the presence of noncholinergic substances that might cause muscle contraction was negligible.

Because of the small amounts of acetylcholine that are liberated from the cortical surface according to our techniques, it was necessary to obtain perfusate for a period of at least 5 minutes, and preferably for 10 minutes, in a given state of consciousness. Consequently, the samples that we have assayed were selected only from those which represented at least 5 minutes in a clear period of sleep or wakefulness according to the EEG and behavioral observations.

The results of nine experiments in seven cats in which satisfactory samples (10 to 20 seconds in duration) were obtained during a given state of wakefulness or slow wave sleep are shown in Table 1. The values given are presented in amount of acetylcholine as bioassayed against known standards on the frog's rectus muscle and expressed in nanograms per minute per square centimeter of cortical surface. The rate of liberation of acetylcholine found in the long-term, freely moving, unanesthetized cat preparation shows an average of about 1 ng/min per square centimeter in the state of slow wave sleep with a little over 2 ng/min in the state of wakefulness. This corresponds very closely to our previous results that (*1*) used a somewhat similar technique in natural sleep and wakefulness. The average amount of ACh liberated during paradoxical sleep in seven determinations in which satisfactory periods of paradoxical sleep could be obtained was 2.2 ng/min per square centimeter. This is approximately the same as was observed during wakefulness (2.1 ng/min).

These results would suggest that there is an increase in rate of liberation of ACh from cerebral cortex that accompanies the desynchronized activation of paradoxical sleep, as in arousal and alertness. It would appear, therefore, that the rate of cortical ACh liberation is related to the desynchronized activation of the EEG rather than to the behavioral responsiveness of the animal. These results add another parameter to the paradoxical nature of that curious state of sleep during which the cerebral cortex appears to be activated similar to that of the waking or alert animal and during which a comparable increase in rate of liberation of ACh occurs.

Other physiologically active excitatory or inhibitory compounds such as amino acids [particularly glutamic acid and gamma aminobutyric acid (GABA)] are liberated from the cortical surface in relation to the activation of the EEG in different states of consciousness (*5*), although relationships to paradoxical sleep have not been determined. It seems likely that the action of physiologically active amino acids in addition to a better understanding of the action of the monoamines (noradrenaline, dopamine, and serotonin) at the cortical level will be necessary before the true nature of the chemical mediation of different states of consciousness is fully understood.

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