late gases over the surface of these cells was observed. The L cells were grown in monolayer cultures on Leighton cover slips in medium NCTC-109 with 10-percent horse serum, and HeLa cells in Eagle's balanced salt solution with added L-glutamine and 10-percent horse serum. No antibiotics were added. About 48 to 72 hours after preparation, the cover slips were put into Rose perfusion chambers, filled with fresh medium, and mounted on a special carrier on the stage of a microscope. A camera attached to the microscope permitted continual observation of any changes.

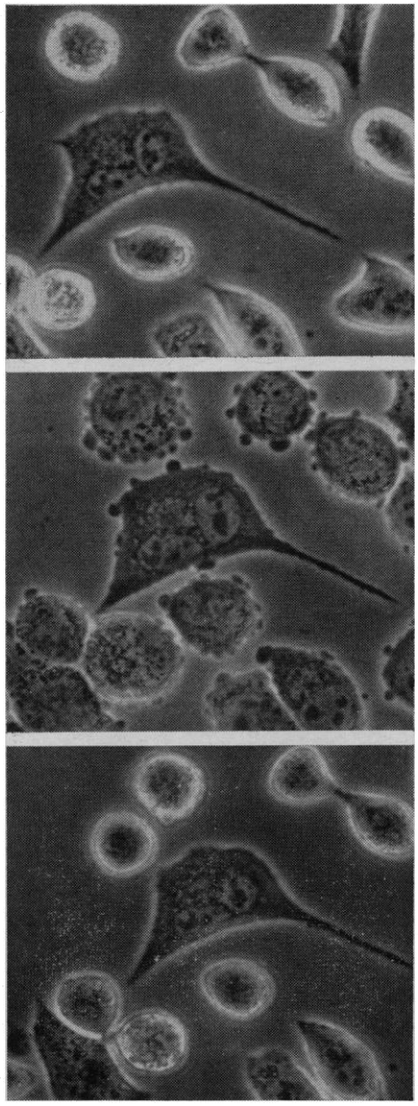

Fig. 1. Changes in L cells caused by exposure to illuminating gas. (Top) Before exposure to gas. (Middle) Immediately after injection of gas, cells show swelling, increased granularity, and surface "blebbing." (Bottom) Cells appear to have returned to their original state, 2 minutes after removal of gas bubbles by change of media. (about × 1000)

Smoke from the combustion of cigarettes, of cigarette tobacco only, of cigarette paper, and of onion skin paper and non-smoke gases such as carbon monoxide, hydrogen sulfide, illuminating gas, oxygen, and helium were injected in volumes of 0.2 to 0.3 cm^3. The gases were obtained and injected under controlled conditions.

Immediately after the injection of all gases tested there is a very rapid increase of the surface area of the cell as well as increased granularity. Most evident, however, is "blebbing" of the surface of the cells. Lewis (*1*) described the formation of "blebs" along the edge of tissue cultures exposed to alkali and Buchsbaum and Kuntz (*2*) noted "blebs" after the injection of certain drugs into a perfusion chamber. Dornfeld and Owczarzak (*3*) demonstrated that ethylenediaminetetraacetic acid produces surface bubbling. Landau (*4*) described surface "blebs" in cells following release of high hydrostatic pressure.

We found that "blebbing" is most pronounced immediately after bubbles of gas move over the surface of the cells. It is most marked near the center of the bubble. The reaction occurs on cell surfaces that are free and exposed and is not seen on the surfaces of neighboring cells. The "blebbing" persists as long as bubbles of gas remain in the chamber. If the contaminated medium is replaced by fresh medium within 10 to 15 minutes after contamination, the cells are promptly restored to their original appearance. If "blebbing" is allowed to continue for more extended periods, cellular activity gradually decreases and the cells die.

Further observations showed that "blebbing" does not occur when cells in a chamber without medium are exposed to air or medium alone, under varying degrees of increased pressure. The pressure in the chamber was increased by occluding the outlet needle while the gas or medium was being injected. Cells grown on a Leighton cover slip were observed microscopically while the cover slip, moistened with medium, was exposed to air and the medium was allowed to evaporate. "Blebbing" did not develop.

Cellular changes in control, experimental, and recovery phases, after injection of illuminating gas, are shown in Fig. 1. The effects of the other gases are similar.

It appears from our observations that the "blebbing" results from a disturbance of the sol-gel relationship of the cell surfaces and apparently can occur at a gas-membrane interface. Further studies now in progress may reveal some of the factors involved in this phenomenon.

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18 August 1961

Spontaneous Discharge of Single Neurons during Sleep and Waking

Abstract. Populations of single neurons in visual cortex, middle suprasylvian association cortex, and brain stem of cat show greater variance of spontaneous discharge rates during waking than during sleep; this change in variance occurs in the absence of significant changes in mean discharge rates. Neurons which discharge rapidly during sleep tend to discharge even more rapidly during waking, whereas neurons with relatively low rates of discharge during sleep tend to have reduced spontaneous activity during waking.

Recent studies of the effects of sleep and waking on spontaneous discharge of single neurons have indicated that neurons may show either increases or decreases of spontaneous activity as a result of arousal (*1–5*). The proportion of units showing one or the other of these responses to arousal varies both as a function of the cerebral area from which recordings are obtained and as a function of the behavior of the experimental animal during the waking state (*2*). Our observations provide information about a characteristic of unit discharge, during sleep, which is associated with the direction of change of discharge rate which occurs with waking.

Experiments were carried out in unrestrained cats (*3*, *4*). Microelectrodes of a variety of types were employed. Units in visual cortex were recorded exclusively with tungsten microelectrodes (*6*). Brain stem units were recorded with tungsten or steel (*7*) microelectrodes. Initially, steel electrodes were used to facilitate identifica-

tion of electrode position (*7*); later, it was found that generally more satisfactory recordings were obtained with tungsten electrodes, whose positions were determined as described by Hubel (*3*). Units in suprasylvian cortex were recorded with glass-insulated platinum microelectrodes (*8*). Each unit included in the study was observed during both sleep and waking. The majority of units in visual cortex and brain stem, and all units in suprasylvian gyrus, were observed during two periods of sleep interrupted by waking, or during two periods of waking interrupted by sleep. The effects of sleep and waking were independent of the order in which sleep and waking were recorded. Sleep was identified by the appearance of large slow waves in the electrocorticogram and by the behavior of the animal (*9*). Waking was produced by noises or tactile stimuli; the waking state was identified by the usual electroencephalographic and behavioral criteria. The waking state studied here was one in which sensory input was quite restricted, and in which novel sensory stimuli were almost entirely excluded. Such conditions were maintained in an attempt to have sensory input during waking differ as little as possible from the low level of sensory input and almost complete absence of novel stimuli during sleep.

Table 1 shows that in all three cerebral areas, a majority of units (153 out of 242) had lower rates of spontaneous discharge during waking than during sleep; there was not, however, a statistically significant change in mean discharge rates between sleep and waking. The median discharge rates were greater during sleep than during waking in each of the three groups. Table 1 also shows that, in suprasylvian and visual units, there was a statistically significant relationship between the discharge rate during sleep and the direction of change of discharge rate as a result of waking. Units with discharge rates during sleep below the median had a greater tendency to decrease with waking than did units above the median. A similar relationship was present in brain stem units, which becomes more evident when only units with discharge rates above 10 per second and below 2 per second are analyzed. In the lower part of Table 1, units have been classified according to whether their discharge rates during sleep were greater than 10 per second or less than 2 per second. Table 1 shows that, for each of the three areas, units which had rates below 2 per second for sleeping had a greater tendency to show reduction of activity with waking than did units which had rates above 10 per second for sleeping. In the visual area and in the brain stem, units with rates above 10 per second showed a preponderance of increases in activity with arousal.

Waking is associated with an increase in population variance of spontaneous discharge rates, neurons tending to move away from the mean during waking. The occurrence of this tendency in three different cerebral areas, together with the finding of Creutzfeldt and Jung (*5*) that such a relationship also obtains in the motor cortex, would indicate that this difference between sleep and waking may occur rather generally. It seems not unlikely, as suggested by Phillips (*10*), that during sleep, units tend to discharge at some preferred mean rate which is in part a function of local membrane characteristics, whereas during waking an increase in excitatory or inhibitory influences, or both, tends to cause divergence of discharge rates from this preferred frequency.

The results indicate that, in the cerebral regions investigated, waking (with minimal stimulation from the environment) and sleep differ with respect to organization of activity within the neuronal population rather than with respect to total amount of discharge. The waking state involves greater differentiation of discharge rates, whereas the population is more homogeneous during sleep. When waking is associated with presentation of stimuli which are adequate to excite neuronal discharge, there then results a major increase in mean discharge rates in the neuronal population as a whole. Thus, we have observed that the mean discharge rate of neurons in the visual cortex is considerably greater during visually guided behavior than during either sleep with slow electroencephalographic waves or waking in the absence of visual stimulation.

It therefore appears that in both a primary receiving and an associative

Table 1. Relation of effects of waking to median discharge rate during sleep, and relation of effects of waking to high and low rates of discharge during sleep. Abbreviations: S, spontaneous discharge rate during sleep; W, spontaneous discharge rate during waking; M, median discharge rate of population during sleep. Units are classified according to whether their spontaneous discharge increased (W > S) or decreased (W < S) with waking, and according to whether they were above (S > M) or below (S < M) the median discharge rate of the population during sleep.

Brain region	Number of units		Spikes per second	
	W > S	W < S	S	W
Analysis by median discharge rate				
Visual cortex				
S > M	27	18		
S < M	15	30		
$\chi^2 = 6.43$	$p < .02$			
Mean			7.30	7.95
Median			5.20	3.56
Brain stem				
S > M	9	16		
S < M	5	20		
$\chi^2 = 1.59$	$p < .30$			
Mean			8.08	8.82
Median			3.10	1.65
Suprasylvian cortex				
S > M	26	26		
S < M	7	43		
$\chi^2 = 15.09$	$p < .001$			
Mean			5.02	5.05
Median			1.88	0.68
Analysis by high and low discharge rates				
Visual cortex:				
S > 10 per sec	18	9		
S < 2 per sec	10	17		
$\chi^2 = 4.75$	$p < .05$			
Brain stem				
S > 10 per sec	6	1		
S < 2 per sec	3	14		
$\chi^2 = 7.11$	$p < .01$			
Suprasylvian cortex				
S > 10 per sec	7	10		
S < 2 per sec	7	44		
$\chi^2 = 5.88$	$p < .02$			

area of the cortex, and in the medial brain stem, the physiological correlate of waking with respect to neuronal discharge is a change in pattern rather than an increase in total amount of activity.

The change in pattern of unit discharge supports the concept of Bremer (*11*) that "wakefulness should also permit the differentiation of cortical receptions which is necessary for perceptual integration."

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28 September 1961

Spatial Discontiguity in Monkeys with Lesions of the Frontal Cortex

Abstract. In learning a black-white discrimination response, the efficiency of rhesus monkeys with lesions of the anterior frontal cortex varies inversely with the spatial separation of the discriminative stimuli from the places of response and reward. Though there is a similar relationship in intact animals, impairment of learning due to discontiguity is greater in the animals with brain lesions.

Contiguity in space and time among elements to be associated has long been regarded as an effective condition for learning. This principle had recently been demonstrated experimentally for spatial contiguity in discriminative learning (*1*). Moreover, McClearn and Harlow (*2*) have shown that for normal rhesus monkeys, the probability of correct reaction decreases as the distance between relevant visual cues and the loci of reactions and of their outcomes increases.

Monkeys with anterior frontal lobe lesions are deficient in delayed-reaction responses (*3*) required by tasks in which critical cues are *temporally* separated from reactions and outcomes. Mishkin and Weiskrantz (*4*) have reported that the discriminative learning of monkeys with frontal lesions is also impaired (relative to that of control animals) by temporal discontiguities. The experiment reported here tested the effect of *spatial* separations on monkeys with frontal brain lesions. The testing procedure of the McClearn and Harlow experiment was replicated.

The four monkeys used in the present study and McClearn and Harlow's four monkeys had very similar histories. Both groups consisted of adult animals that had been tested continually for over 3 years. Both had had extensive training on discrimination learning set, delayed reaction, and a variety of other problems. Thus, both groups were well adapted to the general test situation. The surgery for the animals in this study (*5*) involved bilateral removal of the cortex rostral to the premotor area and frontal eye fields, similar to that described by French (*6*) for "Area 9" monkeys, but it also included the removal of cortex along the dorsal margin of the longitudinal fissure. One month after the operations, the monkeys were tested on 5-second direct, spatial delayed reactions, and they performed no better than would be expected by chance. This was in marked contrast with their nearly perfect preoperative scores on identical problems. Immediately after these preliminary tests, the monkeys were tested as described below.

The upright spatial contiguity board (*2*, Fig. 1) was mounted on the movable carriage of a Wisconsin General Test Apparatus. Two vertical channels, 2 inches wide, were symmetrically located on the board, 15 inches from center to center. At the bottom of each channel a wooden block with a 2- by 2-inch face could be pushed back by a subject to expose an underlying foodwell. The discriminanda (discriminative stimuli), also blocks with 2- by 2-inch faces, could be fixed in the channels at varying distances above the manipulanda (response blocks). The face of one discriminandum was painted black and the other white, while the rest of the display was a uniform gray.

Raisins were given as rewards for reactions to the manipulandum below the positive discriminandum; reactions to the other manipulandum produced no reward. Within a series of four noncorrection trials, the bottom edges of the two discriminanda were kept at the same distance (0, 1, 2, or 4 inches) above the top edges of the manipulanda. From series to series, the vertical separations were changed, with the restriction that each condition should appear once in each group of four consecutive series. The lateral position of the positive discriminandum was varied independently according to a Gellerman

Table 1. Total correct responses of four monkeys with frontal brain lesions during 20 days of discrimination testing. Each monkey was given a total of 320 trials for each condition.

Condition	Monkey			
	C	G	S	Z
0 inch	272	295	273	304
1 inch	245	275	218	274
2 inches	226	264	183	256
4 inches	194	263	179	214

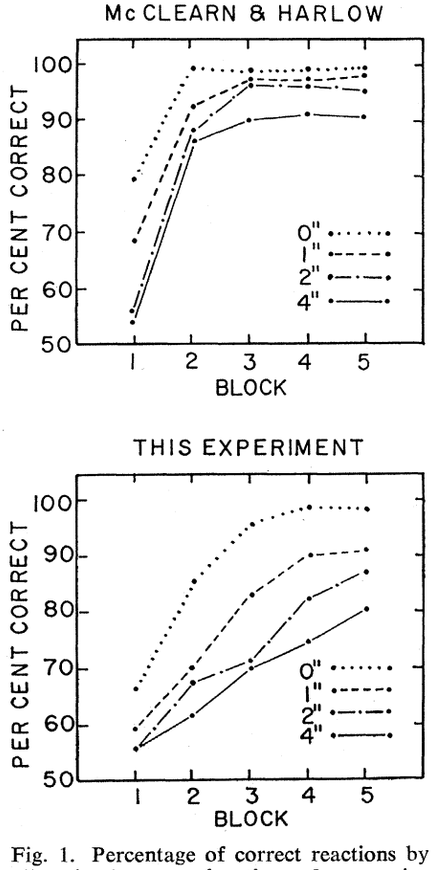

Fig. 1. Percentage of correct reactions by all animals as a function of successive 4-day periods (blocks) of testing during which each animal was given 64 trials for each condition. In both experiments four monkeys were used.