agar (*7*). This medium covered the bottom of a small plastic petri dish. The imbedded skin fragments were then covered with 1 ml of a fluid medium consisting of 60 percent Puck's medium (*6*), 25 percent fetal bovine serum (*6*), 15 percent rabbit serum (*4*), and 100 units of penicillin and streptomycin per milliliter. Then 0.1 ml of virus suspension was added to the culture. Uninfected fragments were grown for controls.

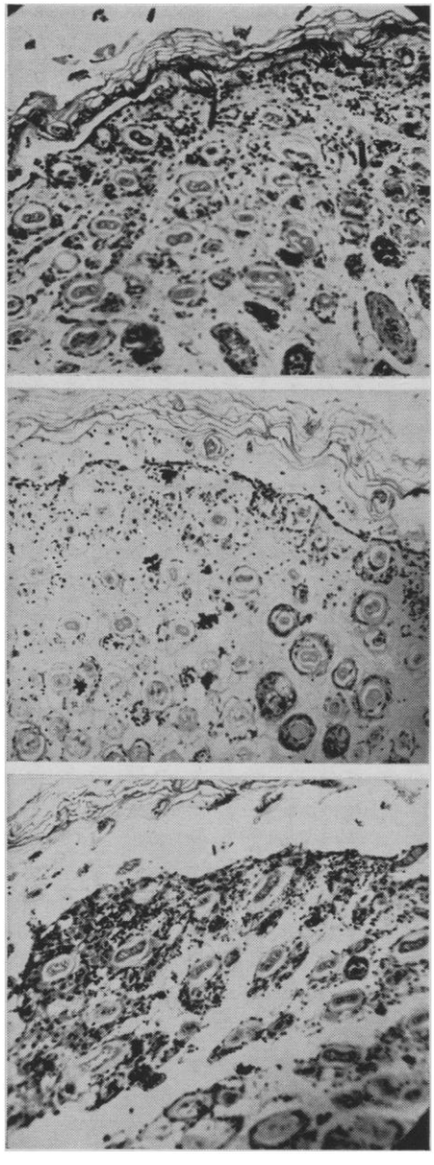

Fig. 1. Sections of the cultures at various stages. Hematoxylin and eosin, about × 90. (Top) Control culture after 5 days. The aspect is still very much that of noncultured rabbit skin, except for the separation between the keratin and the basal layer. At this stage the virus-infected cultures have the same aspect as the control cultures. (Middle) Control culture after 20 days. A single layer of epidermal cells covers the surface of the fragment. Note also atrophy of the epidermal elements of the hairfollicles. (Bottom) Virus infected culture after 20 days. The epidermal cells have invaded the whole fragment.

All cultures were grown for 30 days at 37°C in a humidified atmosphere of 5 percent CO_2 in air. The fluid phase of the medium was changed every 4 days, but the fragments of skin were left imbedded. At intervals of 3 or 4 days, some control and virus-treated fragments were taken out for histological examination. Tissues were fixed with buffered formalin and stained with hematoxylin and eosin. Serial sections were then made of each fragment examined.

Three series of experiments were run, each time with virus from different papillomas. The evolution of both control and virus-infected cultures was the same until about the 10th day, and the number of epidermal cells gradually declined. In control cultures only a single layer of epidermal cells remained at the end of 30 days. These cells covered the upper surface of the fragment or, rarely, encased the whole fragment. The epidermal elements of the hair follicles also decreased, and none were found outside the follicles (Fig. 1, top and middle). In the virus-treated cultures, from the 10th day on, there was a marked proliferation of the epidermal cells, starting on the upper side of the fragment and gradually invading the whole fragment (Fig. 1, bottom). It was impossible to determine whether the proliferation had its origin in the basal layer, in the hair follicles, or in both. Nearly all virus-treated fragments were invaded by epidermal elements. In another experiment with adult rabbit skin, proliferation was not consistent, occurring only occasionally in some virus-infected cultures.

Apart from Coman, who found stimulation of cell growth in roller tube cultures of rabbit epithelial cells (*8*), there are, to my knowledge, no reports of any clear-cut effect of the rabbit papilloma virus *in vitro*. It is therefore significant that this virus, which has never shown any cytopathic effect in regular tissue culture, caused cell proliferation when applied to organ cultures. There may indeed be other tumor viruses which, when tested on monolayers of trypsinized cells, either do not show any effect and thus escape notice or cause mainly a cell destruction and hence have been classified as nontumor viruses. The results obtained by Dawe and Law with the polyoma virus, the findings of Lasfargues with the mouse mammary tumor virus, and our results with the rabbit papilloma agent show that, for the study of tumor agents, organ cultures offer an interesting alternative to monolayers of trypsinized cells. More especially, organ cultures of human tissues may offer an approach to investigate the possible neoplastic abilities of extracts from human tumor material, or agents already isolated from such material.

EDWARD DE MAEYER*
Rockefeller Institute, New York,
and *Rega Institute, Louvain, Belgium*

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* Fulbright scholar. Present address: Rega Institute, Louvain, Belgium.

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Effects of Increments and Decrements of Light on Neural Discharge Rate

Abstract. Neurons in the lateral geniculate nucleus of the monkey discharge impulses periodically under conditions of both dark and light adaptation. Brief intensity changes in one direction produce decreases in firing rate roughly proportional to the logarithm of the intensity; intensity changes in the other direction produce corresponding increases in firing in the same neuron.

The presence of a maintained neural discharge in cells in the visual system, regardless of whether the eye is being stimulated with light or not, has led to the view that the response to light must be a modulation of this maintained firing rate rather than the firing of a certain number of spikes (*1*). Nevertheless, the generally accepted view is that it is only the increase in firing rate from the maintained level which is carrying significant information about the stimulus conditions.

We have argued, on the basis of finding cells in the lateral geniculate nucleus of the monkey which show graded increases and decreases of firing rate depending on the wavelength of the light (*2*), that a single cell can carry information about two different (and

opposite) conditions through modulation of the maintained rate in the direction either of increased or of decreased firing rate.

The present study is an examination of the responses of single cells to intensity changes in opposite directions to determine whether the same relationships might hold. Almost all studies of the visual system have concentrated on responses of cells to flashes of light, that is, to increases in light intensity. It is clear, however, that the detection of decreases in light intensity from the general adaptation level is as important for the normal functioning of the visual system as the detection of increases.

In this experiment the responses of single cells in the macaque lateral geniculate nucleus (the 4th-order neurons in the visual system of the macaque) were recorded with microelectrodes. A conventional amplifying and monitoring system was used; the nerve spikes, shown on an oscilloscope, were photographed with moving film. When a unit was isolated its resting discharge rate was recorded for several seconds while the animal was in the dark-adapted state. An adapting light of a certain low intensity was then turned on and left on. The rate of discharge was recorded when the light was turned on and periodically thereafter for several minutes.

When the response rate had stabilized, the responses to 1-second increases and decreases of light from this adaptation level were recorded. Several different levels of increase and decrease (from 1 to 10 db) were introduced by moving neutral step wedges at a focal point in the optical system. After the effects of light and dark pulses of various amounts around this light level had been determined, the whole procedure was repeated at higher adaptation levels.

Cells in the lateral geniculate nucleus discharge spikes periodically when the animal is in complete darkness. When an adaptation light is turned on there is an initial increase or decrease in firing rate, depending on the type of cell. If the light is left on, however, the discharge rate returns, usually to approximately the same value as in dark adaptation. The maintained discharge, then, is present in dark and light adaptation and is often essentially independent of the light intensity when the eye is adapted to that intensity.

Many investigators, recording from cells which increase their rate of firing in response to a flash of light, have shown that the amount of increase in discharge rate is roughly proportional to the logarithm of the intensity (*3*). We find that cells which decrease their firing rate in response to flashes of light also show graded decreases proportional to the logarithm of the intensity increase. Furthermore, cells of each of these types, in the monkey, show both graded increases and decreases in firing rate in response to increments and decrements of light.

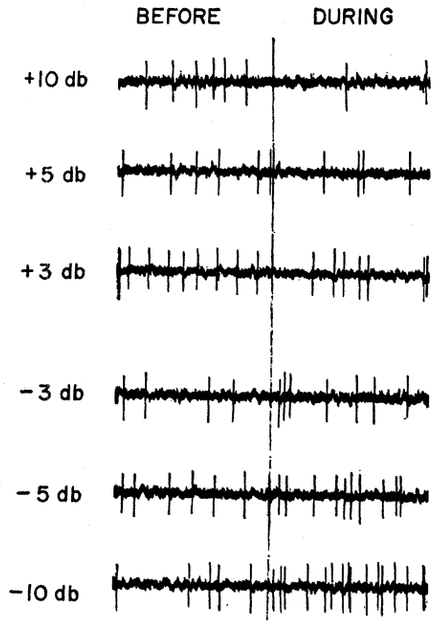

Fig. 1. Superimposed records of the responses of a single cell to various increments and decrements in light intensity. Each record shows responses during 700 msec before the intensity change (to the left of the vertical line) and responses during the first 700 msec after the intensity change (to the right).

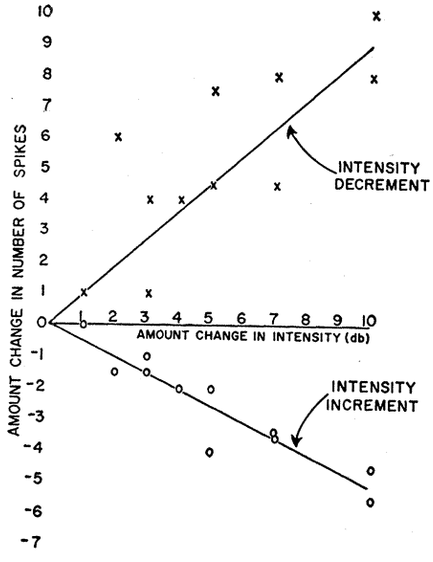

Fig. 2. Plot of the number of spikes during the first 700 msec of the change in intensity minus the number of spikes during the preceding 700 msec. *X*, decrement in intensity; *O*, increment in intensity.

Figure 1 shows records of a typical cell which decreases its rate of firing to a flash of light. The responses of this cell to various increments and decrements of intensity from a moderate light level are presented. It can be seen that the greater the light increment the greater the decrease in firing, and the greater the light decrement the more the firing rate increases. The results from this cell are plotted in Fig. 2, where each point is an average of two determinations made a few seconds apart; the points were obtained and replicated in a random order.

The slopes of the increment and decrement curves vary from cell to cell. Some cells show somewhat greater responses to increments than to decrements; others behave in the reverse manner. The constant features are the changes in opposite directions to increments and decrements in light intensity in each cell, and the graded character of the changes in firing rate, usually logarithmic over the intensity range studied.

These results tend to support the notion that the "spontaneous activity" of sensory neurons serves as a sort of carrier frequency around which signals are modulated, with decreases from this frequency as well as increases carrying information about the stimulus.

Arden and Söderberg (*4*) have reported that the maintained discharge rates of cells of the lateral geniculate nucleus of rabbit are largely determined by extraoptic inputs, perhaps from the reticular formation arousal system. We have also found that the activity rate decreases when pentobarbital is intravenously injected into an unanesthetized animal while recordings were being obtained from a single cell. Since a higher activity rate would allow for greater information to be transmitted through the lateral geniculate nucleus, if our speculations here are correct, this might provide a mechanism by which the input to the central nervous system from the eye is regulated (*5*).

RUSSELL L. DE VALOIS
GERALD H. JACOBS
ARTHUR E. JONES
Department of Psychology,
Indiana University, Bloomington

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Vacuum Filtration of Large Volumes in the Laboratory

Abstract. Laboratory vacuum filtration of large volumes of liquids may be safely, easily, and economically accomplished without the use of pumps and without the necessity for placing receivers under vacuum. In the method described the lowered pressure is generated by the filtrate as it falls down a vertical tube.

Filtering liquid volumes of tens or hundreds of liters in the laboratory is frequently a difficult task. Normal laboratory procedures are generally limited to small-scale operations, and large-scale equipment is rarely available, and then only at high cost. In a previous publication a method was described whereby vacuum filtration of large volumes with Büchner funnels could be speeded up greatly (*1*). We now extend the method (*2*) by providing a procedure for filtering such volumes without the necessity for putting a large receiver under vacuum (which, because of the danger of implosion, becomes increasingly dangerous with increasingly large receivers) and without using a pump (which may result in contamination or heating of the liquid that is being pumped).

The procedure consists of attaching a long tube to the outlet of the Büchner funnel and allowing the filtrate to fall down the tube into an open container. In this manner a Torricellian vacuum is generated beneath the porcelain plate of the funnel. The appropriate length when filtering aqueous solutions is a minimum of 34 feet, since this will yield the maximum vacuum.

We placed a large table-type Büchner funnel on the sixth floor of the building and ran a length of 8-mm glass and Tygon tubing (supported by a wooden rod) down an open stair well to the third floor. We thus were able to lower the pressure at the filter surface to 30 mm and to filter successfully large quantities of lake water through either paper filters or Millipore membranes (pore diameter, 0.45 μ).

The general arrangement is shown in Fig. 1. A large-bore stopcock, *2*, is connected to the outlet of the Büchner funnel, *1*. The other end of the stopcock is joined to a T-tube, *3*, which has a funnel, *4*, connected to it by a plastic tube, *5*, which can be clamped shut. The third arm of the T is connected to the long vertical tube, *6*. To begin operation the system must be primed as follows:

The stopcock is closed and the clamp at 5 is opened. Distilled water is poured rapidly into the funnel until a substantial part of the vertical tube is filled. Then the clamp is replaced quickly. Next, the Büchner funnel is filled with the liquid to be filtered and the stopcock is opened. Filtration then proceeds until the filter clogs. The stopcock may be used to control the rate of filtration, but it should not be closed completely until the filter is allowed to run dry for changing. Otherwise the filter will float to the surface and particulate matter will escape to the filtrate. Also, as the rate of filtration slows there is a tendency for air to enter the bottom end of the tube. This can be alleviated by immersing the end of the tube in the filtrate.

Aside from being inexpensive, simple, and foolproof, the method has the advantage that, because the receiver is not under vacuum, volatile substances are not pumped off and can be recovered for further concentration by a method such as freezing-out (*3*). Although bubbles form in the liquid because of the low pressure near the top of the long vertical tube, these bubbles dissolve during their passage down the tube as the pressure increases toward the ambient atmospheric pressure. Similarly, highly volatile solvents may be filtered without loss. In cases where it is desirable to limit pressure difference across the filter to avoid rupturing cells, it is necessary only to shorten the vertical tube to the appropriate fraction of 34 feet. In this way it is impossible for the pressure difference to increase accidentally.

The general approach outlined here may also be used with pressure type filters, simply by placing the filter at the bottom of a long tube containing the liquid to be filtered (*3*).

JOSEPH SHAPIRO
HANS RURAINSKI

Department of Sanitary Engineering and Water Resources, Johns Hopkins University, Baltimore 18, Maryland

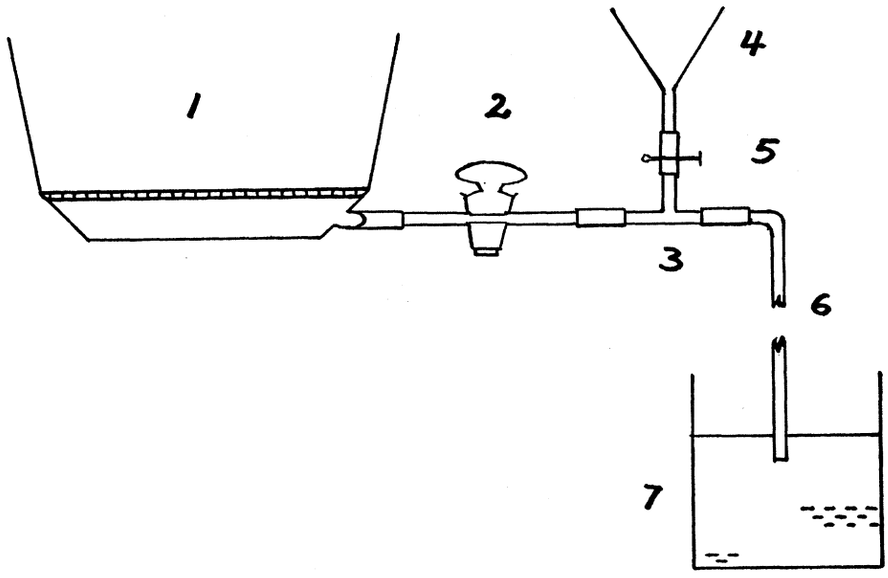

Fig. 1. Arrangement of equipment for large-scale vacuum filtration without pumps.

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