action with the ACh-receptor at the junction of this preparation. If the action of ACh is due to its effect on a specific receptor protein, one would expect that the active site of this protein is similar in all excitable membranes. If this is the case, O, S, and Se isologs should exhibit relative differences of potency in axons similar to those observed at junctions, thus indicating the similarity of the macromolecules reacting with these isologs and their essentially similar functions.

The effects of some O, S, and Se isologs of the tertiary analog of benzoylcholine on the action potential of squid giant axons have been recently reported (*13*). We have extended these studies (Table 1), and isologs of benzoylcholine, in which the carbonyl oxygen has been replaced by sulfur (*14*), have been included (Table 2). Substitution of O by S and further substitution by Se in a series of isologs of the tertiary analog of benzoylcholine results in a progressive increase of their potency (Table 1). At the *p*H of these experiments, the compounds are partly in their charged and partly in their uncharged form. The charged form would not be expected to readily penetrate the squid-axon membrane (*1*, *9*, *15*), while even the uncharged molecule might not be completely permeable. Partial disruption of the permeability barriers by treatment of the axon with cottonmouth-moccasin venom increased the potency of these isologs (Table 1). This concentration of venom has no effect on the height of the conducted action potential (*9*). The two isologs containing Se caused an irreversible block of conduction, whereas the effects of the other compounds were reversible. In view of the very high tendency of selenolesters, compared to thiolesters, to acylate certain nucleophiles (*16*), selenolesters related to ACh might prove useful tools for labeling the receptor protein. The last compound listed in Table 1 (Se, S) is of special interest since it produced repetitive or spontaneous firing, which continued for up to 1½ hours after the axon was returned to normal sea water. This compound might be especially suitable for analysis of drug-binding to and of induced activation of the ACh-receptor.

The quaternary analogs of the compounds shown in Table 1 were also tested (Table 2). Because of their lipid insolubility, these compounds would not be expected to penetrate readily to the membrane of the squid axon. Five out of the six compounds were inactive on control squid axons (Table 2), whereas after the axons are treated with venom their order of potency is similar to that previously shown for the tertiary compounds (Table 1), although the concentrations required are greater. The isolog containing Se and S was most potent and caused repetitive firing. There is at least a hundredfold range in potency in the series of compounds tested (Tables 1 and 2). It appears that, although these compounds are isosteric, they differ markedly in their abilities to bind to receptor sites or to induce conformational changes of the ACh-receptor, which, in turn, may be related to differences in their electronic distribution. Similarly, the pharmacological effects are greatly modified when the ether oxygen of acetylcholine is replaced by S or Se (*17*). Our results are in almost exact agreement with observations made on the junction of the isolated electroplax, where potency also increased as O was substituted by S and Se (*12*).

Our observations support the assumption that the specific sites of reaction of the ACh-receptor are similar in both axons and junctions, since subtle change of electron distribution of a molecule, without a change of its shape, induces similar modification of the biological action in both excitable membranes.

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Single Fibers of Cat Optic Nerve: "Thresholds" to Light

Abstract. *Absolute thresholds of 39 single fibers of the optic nerves of 20 cats were determined by inspection of post-stimulus time histograms, each computed from the responses to 60 to 100 identical flashes of white light. The values found—from 1.1 × 10^{-7} to 6.8 × 10^{-6} candella per square meter (nits)—agree well with psychophysical thresholds found in previous investigations.*

Preparations tested electrophysiologically appear to be considerably less sensitive than man and animals tested by behavioral methods (*1*). This discrepancy has led to investigations, with microelectrodes, of the absolute threshold in visual neurons of the cat (*2*, *3*). Results of these studies are about one log unit higher than the mean threshold found by behavioral methods (*4*, *5*). These discrepancies could be due to the nonphysiological state of the preparations, or to insufficient sensitivity of the data-processing techniques used to

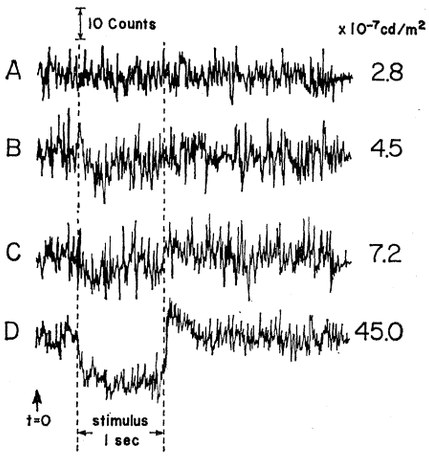

Fig. 1. The traces are PST histograms of the activity of a single fiber at four luminance intensities displayed in such a manner as to emphasize stimulus-locked activity. In histograms B, C, and D, a response is clearly detectable. Histogram A is the control histogram for this series. Each histogram is composed of 400 bins, each of which represents 10 msec.

distinguish between responses to light and so-called reference activity.

In our study, averaging methods were used to extract responses of single units from the reference activity. The post-stimulus time (PST) histograms were computed (*6*). The time marked as 0 is a point on the abscissa located ½ second before the onset of the light flash. Each light flash lasts 1 second. When the processed data is displayed in this manner one can easily distinguish activity associated only with the stimulus (stimulus-locked) from reference activity that precedes and follows it.

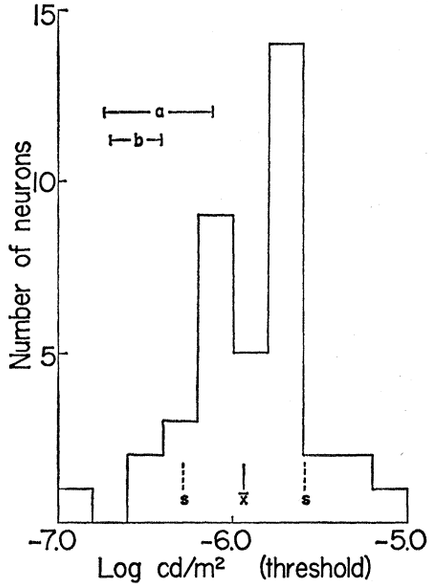

Fig. 2. Distribution of the "thresholds" of 39 neurons. $\bar{x}$, Mean value; *s*, standard deviation. The range of behavioral thresholds found by Bridgeman and Smith (*5*) is designated by *a*; that found by Gunter (*4*), by *b*.

The activity of single fibers of the cat's optic nerve was recorded with equipment and surgical methods similar to those used in previous investigations (*7*). Stimulation with white light at different intensities of luminance was obtained with a glow-modulator tube and Wratten neutral-density filters. Flashes occurring at 5-second intervals were projected on a diffusing screen set before the cat's eye so as to produce a large-field stimulus. For each intensity, set in increments of 0.2 log units, averaged responses to 60 to 100 flashes are displayed as a PST histogram.

The lowest intensity at which there is, by visual comparison with the PST control histogram (*8*), a significant amount of stimulus-locked activity constituted our closest approximation to a "threshold." Trace A was the control histogram for the series in Fig. 1. After an animal had been adapted to dark for 1 hour, the experimental series was started with a light intensity below or in the region of that of the threshold. The luminance was increased in steps of 0.2 log units until "threshold" was clearly exceeded. A more pronounced response was obtained for higher light intensities.

Responses due to the light stimulation were detected visually in the PST histogram at stimulus levels lower than those at which responses are detectable in the unaveraged record. Hence, it is unlikely that simple inspections of the firing pattern following a single flash would yield visual thresholds comparable to psychophysical thresholds. Thus, computation in the form of PST histograms yields thresholds that are lower than the values for electrophysiological thresholds previously reported.

The range of threshold values for 39 neurons from 20 cats was 1.1×10^{-7} to 6.8×10^{-6} cd/m^2 (screen luminance) or -7.0 to -5.4 log cd/m^2. The mean value of these 39 thresholds was 1.6×10^{-6} cd/m^2, with a standard deviation of 1.8×10^{-3} cd/m^2. For comparison with the results of other investigations, the mean of the log values was -5.9 log cd/m^2 with a standard deviation of log values equal to 0.35 log units. In 14 other neurons, an exact determination of the thresholds was not possible owing to insufficient data, but for these units luminances between 1.7 and 7.8×10^{-6} cd/m^2 produced definite responses in the PST histograms. Of the 39 neurons with well-determined thresholds, 21 were on-units (ones in which the firing rate increases as the light intensity increases) and 18 were off-units (units in which the firing rate decreases as the light intensity increases). The on-units showed thresholds between 2.7×10^{-7} and 6.8×10^{-6} cd/m^2; the off-units had values between 1.1×10^{-7} and 2.6×10^{-6} cd/m^2.

Using a conditioning test Gunter (*4*) found thresholds of 2.0 to 3.9×10^{-7} cd/m^2 for white light in the intact cat. The threshold values for white light reported by Bridgeman and Smith (*5*) for behavioral methods range between 1.8 and 7.6×10^{-7} cd/m^2. Previous electrophysiological thresholds in the feline visual system (*2*, *3*) lie considerably above these values. The values reported by Barlow *et al.* (*2*) for retinal ganglion cells are about 18 to 110 times the thresholds calculated from Gunter's results, and the thresholds measured by Marriott *et al.* (*3*) for the lateral geniculate body are 3 to 720 times greater than Gunter's results.

The thresholds of 15 neurons of this investigation are in the range of values given by Bridgeman and Smith. Two neurons show lower thresholds than the mean value given by Gunter, and the highest threshold of the reported neurons is 20 times the mean value according to Gunter. All of the thresholds of this investigation are within a range of 1.8 log units.

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