Endosperm ribonuclease has been purified, and its specific activity and amino acid composition are known (*9*). The high concentrations in opaque-2 endosperm do not materially contribute to the changed amino acid composition because ribonuclease contributes only 0.05 percent of the total protein. Ribonuclease contains 5.1 percent lysine.

The *opaque-2* mutation offers a unique opportunity for investigation of the possible function of ribonuclease in the control of protein synthesis in maize endosperm.

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Tetrodotoxin Blocks a Graded Sensory Response in the Eye of Limulus

Abstract. *The generalization that tetrodotoxin selectively blocks all-or-none electrical activity of nerve and muscle but has negligible effect upon graded responses of sensory systems does not appear to be valid for the* Limulus *eye. Tetrodotoxin reversibly blocks the graded transient component of this visual response, while the steady-state component of the response is relatively unaffected by the drug.*

The hazards of eating puffer fish have been well documented, and the relatively high concentrations of tetrodotoxin in these fish are more than adequate to account for the observed fatalities (*1*). Tetrodotoxin blocks the all-or-none type of electrical excitability of nerve and muscle (*2*). Experiments (*3*) on crustacean nerve and the giant axon of squid suggest that the drug specifically blocks the membrane processes associated with the initial transient conductance change of the neural impulse. On the other hand, it has been reported that tetrodotoxin has little or no effect upon the graded electrical response of several different sensory systems. The drug has been applied to the crustacean stretch receptor and the mammalian pacinian corpuscle (*4*), the mammalian cochlea (*5*), and several invertebrate sensory systems (*6*). In all cases tetrodotoxin blocked sensory nerve impulses, when observable, but had a relatively insignificant effect, if any, upon the graded sensory response. Data such as we now present indicate that tetrodotoxin has a pronounced effect on the graded visual response of the lateral eye of *Limulus*.

For our experiments the lateral eye of *Limulus* was excised, and a portion was mounted in a flow chamber. The preparation was exposed to a constant flow of sea water, and a glass micropipet electrode was inserted into a visual cell. The micropipet provided a salt bridge from the preparation to reversible electrodes and a d-c recording system. Tetrodotoxin was injected into the flow system through a fine capillary, and duration and rate of injection were controlled with a syringe drive. The test solutions were prepared by dissolving crystalline tetrodotoxin in normal sea water.

The eye was stimulated by a controlled flash program, and a constant stimulus program was maintained for all records of Figs. 1 and 2. In order to achieve a constant state of light adaptation, the eye had been stimulated for at least 1 hour before response 1 was recorded. When the eye was bathed in sea water alone, the visual response consisted of three components: (i) a short initial pulse, especially apparent in records 4 through 6, (ii) a transient component, and (iii) a steady-state component which was maintained for the duration of the stimulus. After application of tetrodotoxin, the transient component was markedly reduced in amplitude, while the amplitudes of the initial pulse and the steady-state component remained almost unchanged. The "noisy" character of these records deserves comment. Tetrodotoxin had been presented to this eye earlier, and its effects had been reversed by washing it out with sea water before the first record. After an eye had been exposed to tetrodotoxin, the responses often were "noisy" even when all other

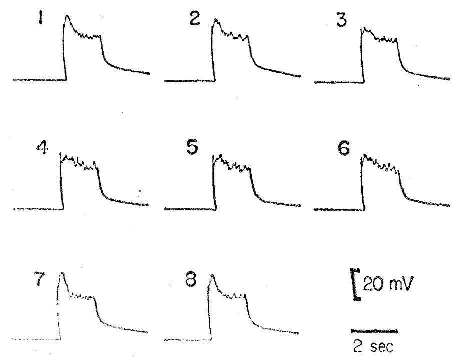

Fig. 1. Effect of tetrodotoxin on the graded response of the *Limulus* eye. Response 1 was a control recorded in sea water. Tetrodotoxin was injected into the bathing solution after record 1. Drug concentration, $< 10^{-5}$ g/ml. Pure sea water was used after record 4 to wash out the drug. All light flashes were of constant intensity and of 2-second duration, and they were repeated every 20 seconds. The curvilinear recording arc is defined for the voltage calibration.

effects of the drug could be reversed in sea water.

In Fig. 2 response amplitudes are plotted against time, the data being derived from the records of Fig. 1. The transient component of the graded response is plotted in the upper curve,

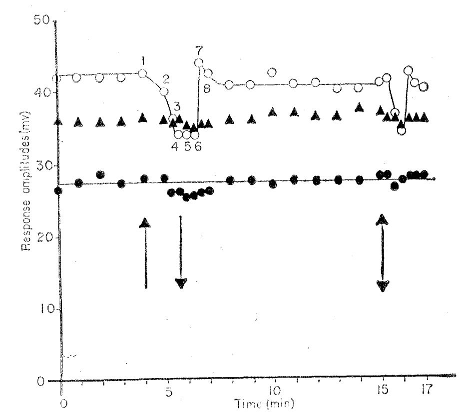

Fig. 2. A plot of response amplitude against time. The data were derived from responses which include the numbered records of Fig. 1. Open circles define the maximum amplitude of the transient component, the triangles define the peak amplitude of the initial pulse, and the solid circles define the amplitude of the steady-state component measured just before the stimulus was switched off. All potentials were measured relative to the resting level of the cell, which defines the zero value of the ordinate. Injection of tetrodotoxin is indicated by the upward arrow, addition of the sea-water wash was initiated at the downward arrow, and the double arrow indicates a brief injection of tetrodotoxin in the presence of the continuous sea-water wash. Drug concentration, $< 10^{-5}$ g/ml. The stimulus program was constant and as specified for Fig. 1.

and apparently tetrodotoxin did not entirely eliminate the transient response in this preparation. However, in the records of Fig. 1 the steady-state component declines rather rapidly during the stimulus interval—in fact rather more rapidly than is typical—and the amplitude of the steady-state component was measured at the end of the stimulus period. If the amplitude of the steady-state component had been measured earlier in the stimulus period, the lower curve would be displaced upward to approach the minimum value of the transient curve. The argument is not crucial, because in several preparations tetrodotoxin abolished the transient component altogether for a variety of stimulus conditions and response amplitudes. This particular preparation (Fig. 1) was selected because the effects of the drug could be established and reversed very quickly. The double arrow in Fig. 2 indicates a brief injection of tetrodotoxin as the sea water was continuously exchanged. The drug inhibited the transient component of the graded response, and its effects were completely reversed in less than 60 seconds from the time that the drug was injected into the flow system. Solution exchange alone required intervals of this order of magnitude, since at least 60 seconds was necessary to clear the flow system when a dye was injected through the capillary.

Tetrodotoxin has been used to dissect the graded visual response (*7*), and it acted characteristically in blocking impulses in optic nerve fibers. The data suggested that the drug acts specifically on the neural processes rather than by uncoupling the neural system from the graded response. Tetrodotoxin appeared to discriminate between graded and neural processes on a quantitative basis. If *C* was the minimum concentration required to eliminate neural impulses, approximately 100*C* was required to abolish the transient component of the graded response. In general, concentration *C* had no measurable effect on the graded response.

The dosage characteristics of the drug varied considerably. The variation correlated perfectly with the use of three different batches of crystalline tetrodotoxin (Sankyo Corporation). The most potent batch was studied most carefully, and the drug blocked the transient component of the graded response at concentrations of about 10^{-7} g/ml. The intermediate batch blocked the transient graded response at 10^{-5} g/ml. The third batch eliminated the neural response at concentrations of 10^{-5} g/ml, but it did not block the transient graded response at maximum practical concentrations of 5×10^{-5} g/ml. Perhaps the variation between batches could be explained by differences in potency of several closely related chemical derivatives of tetrodotoxin (*1*).

The receptor potential of the pacinian corpuscle gradually declines in magnitude when subjected to relatively high concentrations of tetrodotoxin for prolonged periods (*8*). Occasionally a decline in the magnitude of the steady state component of the visual response of the *Limulus* eye was observed after prolonged exposure to tetrodotoxin. When the tetrodotoxin solution was replaced with normal sea water, the steady-state component stabilized at the lower amplitude but the effect on the transient response was reversed. That is, the difference between the maximum response amplitude and steady-state amplitude returned to its original value. For example, in a curve similar to that of Fig. 2, the difference between the open circles and the solid circles would be the same before and after application of the drug, while the solid circles would be displaced downward in an irreversible fashion after exposure to the drug (*7*, fig. 5).

Although unusual in its sensitivity to tetrodotoxin, the graded transient response of the *Limulus* eye exhibits two other properties which seem to be somewhat unusual among sensory systems. First the transient response can reverse the resting potential level of the sensory cell (*9*). Secondly, while graded over much of its range, the transient component exhibits regenerative properties over a portion of this range under a set of well-defined conditions (*10*).

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Voluntary Control of Microsaccades during Maintained Monocular Fixation

Abstract. *A contact-lens technique was used to record eye movements made by two subjects instructed either to "fixate" stationary white-light targets or to "hold" their eyes in position in the presence of the same targets. A marked reduction in saccade rate, frequently reaching zero throughout 9.8-second trials, was observed under the "hold" instruction.*

Microsaccades (very small, high-velocity eye movements) occur once or twice each second while subjects maintain fixation of a stationary target. These movements are commonly described as "involuntary" because they are observed after experienced subjects have been instructed to "fixate." The instruction to "fixate" has been considered to be equivalent to an instruction to hold the eye *still* once the image of a fixation target has been brought to some preferred position on the retina (*1*).

Microsaccades may serve an important visual function. Cornsweet, for example, showed that they return the retinal image of the fixation target object to some "optimal locus" from which it has drifted during intersaccadic intervals (*2*). This "optimal locus" is assumed to be the center of best vision. It seems possible, then, that microsaccades are executed in order to produce the best visual detail in the target image; and, therefore, the conventional instruction, "fixate," may, in fact, be different from an explicit instruction to "hold" one's eye still in the presence of a visible fixation target. If a subject chooses to ignore detail in the fixation target under "hold" instructions, microsaccades should be eliminated or reduced appreciably.

Eye movements under "fixate" and "hold" instructions were recorded by a contact-lens technique incorporating