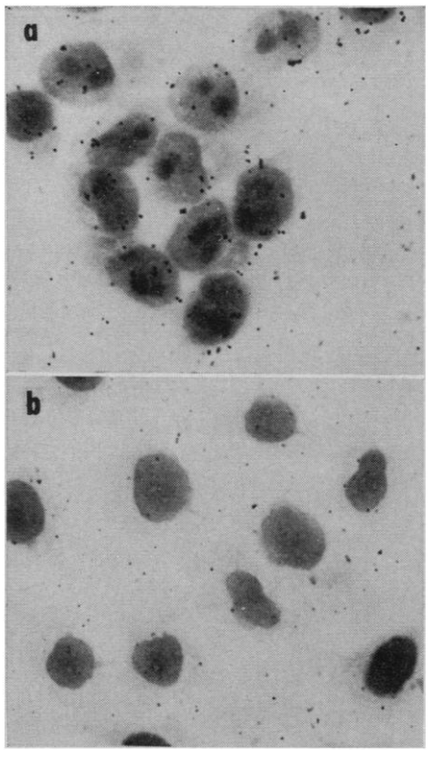

Fig. 3. Radioautographs of T-1 cells labeled with I^{125}-L-thyroxine (2 μc/ml) for 30 minutes and exposed for 3 days ($\times$ 900). (*a*) Cells pretreated 30 minutes with 4.45 $\times$ $10^{-6}M$ of L-thyroxine. Note high localization by nuclei, especially by perinuclear zone. (*b*) Cells pretreated with actinomycin D (0.05 μg/ml) for 8 hours. Virtually no grains appear over nuclei.

in production of nuclear RNA was the first evoked in this way and was detectable after treating for a fraction of an hour. These findings are consonant with those of Widnell and Tata (*11*), which indicate that exogenous thyroid hormones in vivo affected RNA polymerase activity prior to the augmentation of protein synthesis. Nevertheless, the possibility of direct action by these hormones on protein production independent of RNA messenger intervention must be entertained, especially in view of such findings with cell-free rat liver preparations made by Sokoloff, Francis, and Campbell (*12*), who used 6.5 $\times$ $10^{-5}M$ to 6.5 $\times$ $10^{-4}M$ L-thyroxine. Since these superphysiological and toxic concentrations of thyroxine were ineffective or inhibitory in influencing proliferation and plating efficiency of T-1 cells (*3*), the relation of their observations to in vivo response patterns should be explored further. Similar reservations may hold for the more recent studies of the inability of L-thyroxine to alter the DNA melting profile (*13*).

The loci within peripheral cells which concentrate thyroid hormones have not yet been detected, despite intensive search. In a detailed and painstaking study, Tata, Ernster, and Suranyi (*14*) determined the subcellular distribution in liver and skeletal muscle of endogenously labeled thyroid hormone as well as of I^{131}-labeled T_4 and T_3 added to tissue homogenates; no specific subcellular sites of localization were found. It would seem that hunting with radioautography for such centers has the overriding merit of analysis being conducted on intact cells, particularly when coupled to a cultured homogeneous population capable of hormonal response.

While it appears that thyroid hormones mediate their effects by way of the nucleus and participating messenger RNA, the precise mechanism for this interaction remains to be established. The I^{125} radioautographs suggest the genetic matter and the nuclear envelope as possible primary foci of thyroid hormonal activity. If the latter is involved, a conceivable hormonal role would be regulation of the transfer of synthesized messenger RNA and of its precursors across the nuclear-cytoplasmic interface. Interaction of hormone and DNA could be the mode by which the milieu triggers genetically predetermined latent behavior and could account for the differential specificity of cellular response (*15*). Distinguishing among these and other possible mechanisms must await attack by higher resolution techniques, perhaps by electron microscope radioautography of these cultured cells. In any event, to the older ideas of hormonal action, namely, control of enzymatic action (*16*) and regulation of membrane permeability (*17*), must now be added hormonal control of genic expression (*18*).

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Tetrodotoxin Does Not Block Excitation from Inside the Nerve Membrane

Abstract. *Tetrodotoxin does not block the action potential or membrane sodium current when internally perfused through the giant axon of a squid at much higher concentrations than those required for blocking by external application. It is suggested that the gate for the sodium channel is located on the exterior surface of the axon, because tetrodotoxin is not lipid soluble.*

Tetrodotoxin, the active component of the puffer fish poison, has now become a very popular and important tool for electrophysiological studies of excitable tissues. This is primarily because of its unique ability, at very low concentrations, to selectively block the voltage-dependent mechanism for increase in sodium conductance, the mechanism that is responsible for the excitation in axons. The action is quite distinct from that of procaine or cocaine, which block the mechanism for increase in potassium conductance as well (*1*). The selective blocking action of tetrodotoxin was first suggested in frog muscle fibers (*2*), and then definitely confirmed by voltage-clamp experiments with giant axons of lobsters

and squids (*3*, *4*). Extended studies further differentiated its specific action from that of procaine (*5*).

In the meantime, intracellular perfusion techniques were developed for giant axons of squids (*6*), enabling us to study symmetrical or asymmetrical properties of the nerve membrane with respect to the actions of various ions and drugs (*7*). Preliminary experiments revealed that internally perfused tetrodotoxin has no effect on the sodium conductance mechanism of squid axons at a concentration ($6 \times 10^{-8} M$) that was effective when applied from outside the nerve membrane (*8*). However, Nakamura, Nakajima, and Grundfest (*4*) were able to block action potentials by injection of tetrodotoxin at an estimated concentration of $3 \times 10^{-7} M$. Further experiments have therefore been performed by means of current-clamp and sucrose-gap voltage-clamp techniques with internally perfused squid giant axons 400 to 500 μ in diameter.

For current-clamp experiments, the internal potential was measured by means of a glass capillary of about 100 μ outer diameter, filled with 0.6*M* KCl, and having a 25-μ silver wire inside to reduce direct current resistance (*9*). A 50-μ silver wire, with the insulation removed, and silver chloride-coated for a length of about 10 mm from the tip, was used as current electrode. This was twisted around the capillary potential electrode in such a way as to locate the tip of the latter at the middle of the 10-mm Ag-AgCl region. The junction potentials of the capillary electrode were measured in each external and internal solution with a calomel electrode for reference, and substracted from the apparent values for the resting potential. The internal solution contained (in millimoles): K, 400; glutamate, 370; sucrose, 333; H_2PO_4, 15; the *p*H was 7.3. Natural sea water was used as external bathing medium. Current-clamp experiments were performed at a room temperature of 20° to 22°C.

The results of the experiments were quite unequivocal. An example of a series of action potentials is illustrated in Fig. 1. Internally perfused tetrodotoxin at a concentration of $1 \times 10^{-6} M$ had no effect on the resting potential, the magnitude and the maximum rate of rise of the action potential, and the threshold membrane potential for a period of up to 37 minutes. Internal perfusion was also tried with $1 \times 10^{-5} M$

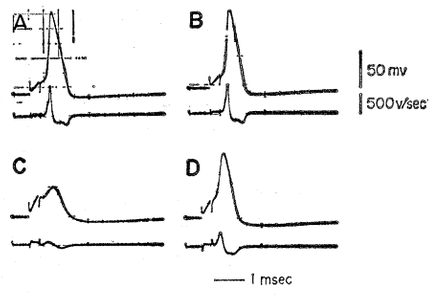

Fig. 1. Action potentials of an internally perfused giant axon of a squid. (*A*) Normal internal and external solutions; (*B*) after *internal* perfusion with $1 \times 10^{-6} M$ tetrodotoxin for 20 minutes; (*C*) after *external* perfusion with $1 \times 10^{-7} M$ tetrodotoxin for 6 minutes; (*D*) after washing with normal external solution for 13 minutes.

tetrodotoxin for 17 minutes with no change in these parameters whatsoever. External perfusion of tetrodotoxin at a concentration of $1 \times 10^{-7} M$ blocked the action potential in 3 to 6 minutes without changing the resting potential. Partial to complete recovery occurred after washing with normal sea water for 10 to 15 minutes.

However, there still remained the possibility that equal partial blockage of both sodium and potassium conductance mechanisms could have occurred without a significant change in the configuration of the action potential (*10*). For this reason, and also to confirm the above-mentioned results in terms of the sodium and potassium conductance mechanisms, voltage-clamp experiments were done with internal perfusion of tetrodotoxin.

For voltage-clamp experiments, a perfused giant axon was mounted in a sucrose-gap chamber similar to that

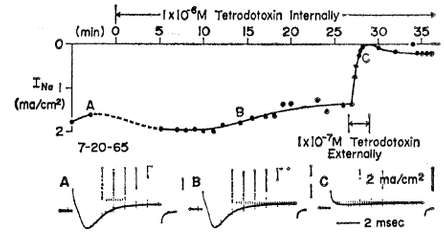

Fig. 2. Early transient sodium currents associated with a step depolarization from the holding membrane potential of —90 mv to 0 mv in an internally perfused giant axon of a squid, as a function of time. (*A*) Control record of membrane current; (*B*) $1 \times 10^{-6} M$ tetrodotoxin inside for 13 minutes; (*C*) $1 \times 10^{-7} M$ tetrodotoxin outside for 1 minute in the continuous presence of $1 \times 10^{-6} M$ tetrodotoxin inside.

described previously (*11*). Two sucrose streams separated the axon into three portions; the central portion was small (about 200 μ wide), composing an artificial node, and measurements were made on this node. During the voltage-clamp run, the membrane was held at a hyperpolarized level to remove sodium inactivation. Sodium was added to the internal solution to define the equilibrium potential for sodium, and fluoride was used in place of glutamate as anion to give better excitability. Thus the internal solution contained (in millimoles): K, 400; Na, 10; F, 380; sucrose, 316; H_2PO_4, 15; *p*H 7.3; or K, 400; Na, 50; F, 420; sucrose, 250; H_2PO_4, 15; *p*H 7.3. Voltage-clamp experiments were performed at a temperature of about 5°C.

Beyond the usual slow temporal deterioration of the conductance mechanisms in perfused axons, no change in the initial sodium current and in the delayed potassium current was observed at a tetrodotoxin concentration of $1 \times 10^{-6} M$ for a period of 25 to 30 minutes (Fig. 2). External application of $1 \times 10^{-7} M$ tetrodotoxin, in the presence or absence of $1 \times 10^{-6} M$ tetrodotoxin inside, blocked the sodium current in 2 to 3 minutes without affecting the potassium current (Fig. 2). No change was observed in the time to peak sodium current during internal perfusion of tetrodotoxin or during the course of blockage following external application of tetrodotoxin.

The present results confirmed and extended those reported previously (*8*). It is not immediately clear why the injection of tetrodotoxin by Nakamura, Nakajima, and Grundfest (*4*) caused a blockage of action potential. However, the inability of tetrodotoxin to block excitability from inside of the nerve membrane may be taken as well established, because tetrodotoxin was certainly in contact with the inner surface of the nerve membrane at a constant concentration for a long period of time.

Tetrodotoxin is insoluble in most organic solvents (*12*) and hence most probably so in lipids. A corollary of this property and of the present experimental results is that the site of action of tetrodotoxin is not located at the inner edge of the nerve membrane. It is reasonable to assume that the site is located at the outer surface. Therefore, the site of action can be visualized at the gate of the sodium channel in the nerve membrane. The

fact that very low concentrations of tetrodotoxin are enough to block the sodium conductance increase makes us think that these sites are sparsely distributed on the membrane surface. It is known that guanidine can pass through the squid nerve membrane and produce the action potential under certain conditions as sodium does (*13*), and that the tetrodotoxin molecule has a guanidinium group (*12*). It appears possible that the guanidinium of tetrodotoxin becomes lodged in the gate of the sodium channel on the surface of the nerve membrane, thereby blocking the movement of sodium ions as suggested for saxitoxin (*14*).

The situation might be quite different for lipid-soluble blocking agents such as procaine and alcohols which can diffuse into the phospholipid layer of the nerve membrane, thereby requiring much higher concentrations to become effective in blocking excitability (*1, 10, 15*).

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Corticosteroid Responses to Limbic Stimulation in Man: Localization of Stimulus Sites

Abstract. *Corticosteroids in human plasma and urine increase after amygdala stimulation, and plasma corticosteroids decrease after hippocampus stimulation. Five subjects underwent unilateral temporal lobectomy, and histopathologic localization of electrode sites was attempted. Localization was successful for six sites: three in basolateral amygdala and three in hippocampus.*

The effects of electrical stimulation of limbic structures upon plasma corticosteroid levels in man have been reported (*1*). Four patients with severe temporal-lobe epilepsy had chronic implantations of electrodes in these areas as part of a study to localize seizure focuses prior to attempts at control by surgical removal of one anterior temporal lobe (*2*). Three of the patients underwent stimulation of the amygdala, one patient bilaterally, and in each instance the 17-hydroxycorticosteroid level in plasma rose significantly within the 1st hour after stimulation. Three of the patients underwent stimulation of the hippocampus, one patient bilaterally, and in each instance 17-hydroxycorticosteroid level in plasma rose significantly within the 1st hour after stimulation. Maximal responses occurred after 5 to 30 minutes in the two patients who were sampled several times during the hour.

The location of each electrode was ascertained at the time of implantation by x-ray measurements and stereotaxic-atlas verification. Marker lesions were made before explantation. Since the first report, all four patients have undergone unilateral temporal lobectomy, and exact histopathologic localization of the electrodes has been attempted (Fig. 1)—successfully for all electrode tracts examined except for two in the fourth patient; the sites verified are listed in Table 1.

Also since the first report a fifth patient has undergone stimulation of both amygdalas, in alternating fashion, urinary 17 - hydroxycorticosteroids [Porter-Silber chromagens (*3*)] being measured hourly, both on a control day with mock stimulation and following the stimulus train. For this patient, stimulus parameters were bidirectional square-wave pulses (10 volts, ten per second, 1-msec duration) for the first 20 seconds of each minute. The left and right amygdalas were stimulated

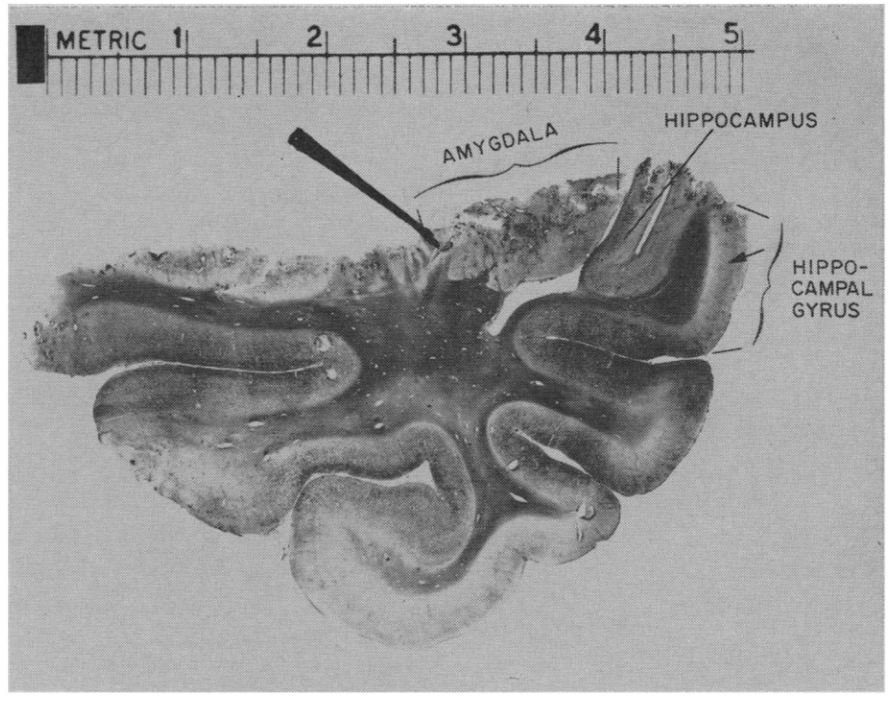

Fig. 1. Section of right temporal lobe, showing marker lesion (electrode tip) in basolateral amygdala. Lesion is the darker area at the immediate right of the pointer tip; Weil stain.