in conductance during the muscarinic ACh and the NE actions reflects a real difference in membrane response from that during the nicotinic ACh actions (*16, 17*). In addition, the effect of initial depolarization on the NE response (rabbit cells) and of initial hyperpolarization on the muscarinic ACh response (frog cells) are opposite in direction to those expected from the ionic hypothesis for electrogenesis of such responses (see *3*). We conclude, therefore, that neither the hyperpolarizing action of NE nor the muscarinic depolarizing action of ACh on sympathetic ganglion cells is mediated by the generation of membrane currents that are due to ions moving down their electrochemical gradients.

Activation of an electrogenic Na-K pump could provide one alternative possibility for generating the hyperpolarizing response to NE without an increase in membrane conductance (*2, 12, 17*). However, we found that ouabain ($10^{-5}M$), a specific inhibitor of such an active transport mechanism for Na coupled to K intake (*18*), did not block the response to NE at a time when ouabain had already depressed the sodium pump; depression of the sodium pump was shown by changes in the cell's action potential due to accumulation of Na^+ (*19*). This evidence argues against at least the ouabain-sensitive type of sodium pump as the electrogenic source of either the NE response or, by implication, the slow IPSP in these cells (*17*).

The electrogenic mechanisms for both the NE and the muscarinic ACh responses are still to be worked out. However, the findings of the unique similarities of the NE and muscarinic ACh mechanisms to those mediating the slow IPSP and slow EPSP, respectively, provide strong additional support for the proposals that NE or a closely related substance is the synaptic mediator for the slow IPSP (*1, 4*), and that ACh is the mediator for the slow EPSP in these cells (*4, 5*). Furthermore, NE and ACh, both compounds of physiological and pharmacological importance, can elicit postsynaptic responses by physicochemical mechanisms different in principle from those which mediate the better-known synaptic transmitter actions (*3*).

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17. H. Pinsker and E. R. Kandel [*Science* **163**, 931 (1969)] have recently reported that neurons in *Aplysia* ganglia can exhibit a cholinergically mediated slow IPSP with no change in membrane conductance; however, this response, whether elicited by neural action or applied ACh, was selectively blocked by ouabain.
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Immunoglobulins G, A, and M Determined in Single Cells from Human Tonsil

Abstract. *The analysis of specific proteins in single cells is described and applied to the determination of immunoglobulins G, A, and M (γG, γA, and γM) in lymphoid cells of the human tonsil. The number of cells containing γA or γM was much less than the number of cells containing γG, but the average cell content of γA or γM was much greater than the average cell content of γG. Similarly, in one child where relatively few cells containing γG were found, the average cell content of γG in those cells was higher than that in the other children. The limit of sensitivity of the method for each of the three immunoglobulins with the antiserums used was less than 10^{-14} gram of the specific protein.*

A general method for the determination of specific proteins in single cells forms the basis for the estimation of immunoglobulins G, A, and M (γG, γA, and γM) in lymphoid cells obtained from tonsillar tissue at tonsillectomy of otherwise normal children aged 2½ to 8 years. Cells were teased from the fresh tissue and washed four times in Alsever's solution (cells washed from two to five times gave the same results); virtually all of the nucleated cells in these preparations were lymphoid. A given number of cells in 20 μl of Alsever's solution, or approximately 5×10^5 lymphoid cells, was spread over the surface of a mixture of antiserum and agar which had been poured to a depth of approximately 2 mm between two glass strips placed 25 mm apart on a microscope slide (2.5 × 7.5 cm) (*1*); the glass retainers were 10 by 25 mm strips cut from other microscope slides, and the agar surface formed between them was a square about 25 mm on a side. The antiserum-agar mixture contained, per 100 ml of mixture, 1.5 g of agar, 0.1*M* sodium borate buffer of *p*H 8.6, 0.8 g of sodium deoxycholate, and a given amount of rabbit antiserum specific for either γG, γA, or γM. The deoxycholate was essential for eventual lysis of all cells coming to rest on the agar as the suspending solution evaporated and for the subsequent release of cellular immunoglobulin (*2*); simple lysis of cells by freezing and thawing or by sonic vibration did not free immunoglobulin from the cells. The slides were kept in a humid chamber for 6 days, after which the agar was covered with a square of cellulose acetate mem-

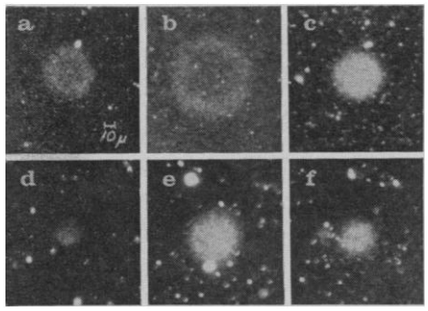

Fig. 1. Representative precipitation rings; (a–d) γG, (e) γA, and (f) γM. All at same magnification; 10 μ indicated in (a).

brane and then dried at 37°C; the cellulose acetate was removed from the agar by immersing the slide in 0.15*M* NaCl for 1 to 2 minutes. The slides were then examined with a darkfield microscope. Both the number and the diameters of the rings of specific precipitate (Fig. 1) that had formed between the immunoglobulin released from the cells and the antiserum in the agar were recorded. Either the sizes of the precipitation rings were measured directly with an eyepiece micrometer, or the rings were photographed on 35-mm Tri-X film and measurements were taken of the projected negative images.

The amount of either γG, γA, or γM in an additional portion of the cell suspension was determined by radial immunodiffusion (*3*); the same antiserum-agar mixture was used, that is, the same dilution of the same lot of antiserum in the same concentrations of borate buffer and deoxycholate. The cells were first mixed with deoxycholate, the final concentration of the latter being 0.8 g per 100 ml; portions (~ 15 μl) of the cell mixture, containing ~ 6×10^7 lymphoid cells, were then placed in wells in the agar. Known amounts of γG, γA, or γM in 15-μl volumes were used to obtain the relation between precipitation ring diameter D and the amount of antigen A. When the ring diameter exceeded two to three times the diameter of the antigen well, the ring diameter could be expressed as a linear function of the amount of antigen: $D = m \log A + \log b$, where b was a constant and m was the slope of the line.

With the information thus obtained, the diameters of the precipitation rings found for immunoglobulins in single cells were converted into absolute amounts of specific immunoglobulin, or antigen, per cell as follows. A given diameter of a precipitation ring was arbitrarily assigned a value of 1.00 unit of specific immunoglobulin and plotted on semilogarithmic paper, the ordinate being the logarithm u of the amount of immunoglobulin per cell in arbitrary units and the abscissa being the diameter d of the precipitation ring. A line with a slope m was then drawn through the point thus plotted; m was obtained earlier for the specific antiserum by radial immunodiffusion. From this linear relation, the individual precipitation rings were converted into arbitrary units of antigen u. The average amount of antigen per cell $\bar{u}$, in arbitrary units, was then calculated from $\bar{u} = \Sigma u/N$, where N was the number of precipitation rings. To convert arbitrary units into absolute units of antigen, the average amount of antigen $\bar{A}$ (in picograms) per cell was calculated from $\bar{A} = A \cdot n/C \cdot c$, where A was the amount of specific immunoglobulin released from a known number of cells C, as determined earlier by radial immunodiffusion; n was the number of precipitation rings for a given number of cells c, as found by microscopic count. The average amount of antigen per cell was $k\bar{u}$, where k was a constant equal to $\bar{A}/\bar{u}$. Log k added to log u along the ordinate of the precipitation graph converted arbitrary units into picograms of specific immunoglobulin. Once established for a given antiserum, the calibration graph thus constructed could be used for all cell determinations made with this antiserum. Three separate calibration curves for a single antiserum specific for γG, with different numbers of cells from a different individual for the construction of each graph, differed by less than 7 percent in the amounts of γG estimated for a given diameter of the cell precipitation ring. The limit of sensitivity of the method

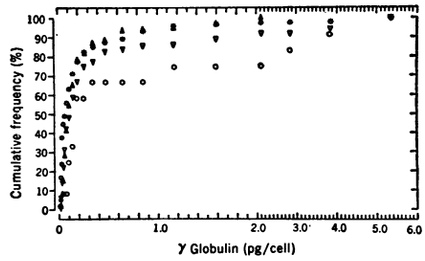

Fig. 2. Cumulative frequency of cellular γG content as a percentage of those cells containing γG. Different symbols represent different children.

for γG, γA, or γM under our conditions was less than 10^{-14} g of the specific protein.

When the diameter of the precipitation ring obtained by radial immunodiffusion was less than two to three times the diameter of the antigen well, the slope of the curve relating D to log A tended to increase with decreasing amounts of antigen (*3*). Although few-

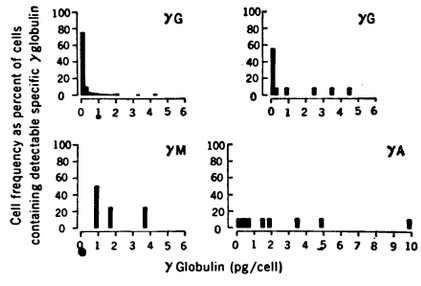

Fig. 3. Cell distribution of specific immunoglobulin content as a percentage of those cells containing that immunoglobulin. (Top left) Average of the γG cells represented in Fig. 1 by the triangles and starred symbols; (top right) γG cells from tonsil with fewest relative number of γG cells (patient L.P., represented by circles in Fig. 2); (bottom left) γM cells; and (bottom right) average of γA cells from two children.

Table 1. Number of cells in the human tonsil which contain a specific immunoglobulin and the average amount of that immunoglobulin in those cells.

Immunoglobulin	Number of cells				Average Immunoglobulin per cell (pg)
	Examined	Containing immunoglobulin		Lymphoid cells per immunoglobulin cell	
		Total number	Number per 10^5 lymphoid cells		
γG	D.S.D. 3.3×10^5	90	27	3.7×10^3	0.42
	T.L. 6.3×10^5	131	21	4.8×10^3	0.24
	F.S. 8.0×10^5	68	8.5	1.2×10^4	0.22
	L.P. 8.0×10^5	13	1.6	6.1×10^4	0.97
γA	D.D. 7.5×10^5	4	0.5	1.9×10^5	2.2
	J.M. 2.4×10^5	6	2.5	4.0×10^4	3.0
γM	D.D. 7.5×10^5	4	0.5	1.9×10^5	1.7

er than 1 percent of the cells gave precipitation rings less than 30 μ in diameter, or approximately three times the size of the large lymphoid cells, a linear relation was assumed for precipitation rings less than 30 μ. Consequently, the amount of antigen in cells with rings less than 30 μ in diameter may have been overestimated. A nonlinear relation in the calibration curve was largely avoided by proper dilution of antiserum, the diameter of the precipitation ring formed by a given amount of antigen being proportional to the dilution of the antiserum (*1*).

The method thus described differs in several important aspects from that reported (*1*) for determination of soluble proteins in single cells. (i) In the previous method, it was necessary to rely on the presence of relatively large numbers of cells containing the antigen, since both the average and median diameters of the precipitation rings had to be calculated to convert the ring diameters into absolute quantities of specific protein. These factors no longer need be determined, and the method can be used (Table 1) when less than one cell per 10^5 cells contains the antigen. (ii) In the earlier method, the diameters had to be determined with at least two different dilutions of a given antiserum; only one dilution is necessary now. (iii) In the previous method only proteins which are diffusible in agar at the time of cell lysis could be estimated; in the present method, proteins already synthesized by the ribosomes but still bound in a form not diffusible in agar are measured as well, since deoxycholate may be used to release the bound protein. Our new method is less complicated mathematically, requires fewer manipulations and less antiserum, and can be used where the latter cannot.

Cells from the tonsils of four children were studied for γG content, cells from the tonsils of two other children were examined for γA, and cells from the tonsils of one of the latter children were studied for γM (Table 1 and Figs. 2 and 3). The number of cells containing γM or γA were fewer than the number containing γG. Of the four γG surveys, an average of one cell per 6900 lymphoid cells contained detectable γG; but of the two γA surveys, an average of one cell per 67,000 lymphoid cells contained γA; and the γM survey revealed only one cell containing γM per 190,000 lymphoid cells, Thus, for a given number of lymphoid cells in the tonsils, there were about 1/10th as many cells containing γA cells on the average as there were γG cells, and 1/28th as many γM cells as there were γG cells. On the other hand, the average amount of γG per cell was only 0.46 pg compared to an average of 2.6 pg for γA and 1.7 pg for γM; thus the average γA content was 5.6 times that for γG, and the average γM content was 3.7 times that for γG. The average amount of cellular γA in the tonsils studied was $1/10 \times 5.6$ times that of γG; and the average amount of γM was $1/28 \times 3.7$ times that of γG. In the normal child over 2 years of age, the average amount of γA synthesized per day is approximately 0.5 times that of γG, and the average amount of γM synthesized per day is about 0.2 times that of γG (*4*). Thus, the relative number of cells containing a specific immunoglobulin multiplied by the average cell content of the immunoglobulin reflected the relative average total body synthesis of that immunoglobulin. The method may be applied in studies of cell population kinetics.

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Neural Readout from Memory during Generalization

Abstract. *Eight cats with implanted electrodes were trained to obtain food on presentation of one flicker frequency and to avoid shock on presentation of a second flicker frequency. A third flicker frequency, midway between the first and the second, was then presented. Differential generalization ensued, in which either the food response or the avoidance response was performed. Average evoked potentials from generalization trials with different outcomes were significantly different. The wave shape elicited by the stimulus for generalization closely resembled the usual response to the appropriate signal for the behavior which was displayed. This constitutes evidence for release of a neural process representing previous experience. The release of this process begins about 35 milliseconds after stimulation.*

We have described features of the average evoked potential elicited when a previously trained animal performs a conditioned response upon presentation of a novel test stimulus (*1–5*). We have identified such behavior as generalization, arguing that it must require release of both an endogenous pattern of neuronal activity and a behavior established to the conditioned stimulus during training. The potential evoked by the test stimulus in trials resulting in generalization is markedly different from the evoked potential caused by the same stimulus when generalization failed to occur (*3*). The wave shape of the evoked potential caused by the test stimulus in trials resulting in generalization closely resembled that usually elicited during correct behavioral response to the conditioned stimulus. Thus, the endogenous activity closely reproduced the usual temporal pattern of potential evoked by the conditioned stimulus.

Such findings might mean that a pattern of activity specific to the meaning of a particular conditioned stimulus was stored in the nervous system and could be released by an appropriate trigger. Conversely, the released pattern might be unspecific, merely reflecting arousal, attention, the intention to move, or fluctuations in level of motivation, and bearing no relationship to the informational significance attributed to the ambiguous test signal. We now report results of an experiment which shows that the released endogenous pattern reflects readout of specific information.

After implantation of 34 electrodes in each animal, eight cats were trained to discriminate between two frequencies of flicker. One frequency (V_1) was the cue to press the right lever on a work panel in order to obtain food (CR). The second frequency (V_2) was the signal to press the left lever on the work panel to avoid electric shock