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.

DAVID GITLIN
TERUO SASAKI
Department of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania

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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

(CAR) through a floor grid (*6*). Flicker was presented from an overhead light source and imposed a moderate fluctuation in the illumination in the cage, which was always lit by overhead bulbs. To avoid effects related to fortuitous selection of frequencies, three pairs of frequencies were used. For each pair of frequencies, two or more animals were trained so that the frequency which was the food signal for some was the avoidance signal for others, and vice versa.

After acquisition of the two discriminative behaviors, each animal received several months of overtraining. Then each animal was subjected to a series of test sessions in which a novel test stimulus (V_3) was occasionally interspersed between presentations of the actual conditioned stimulus. Test stimulus V_3 was a light flickering at a frequency midway between V_1 and V_2. The three different stimuli were in random sequence. Thus, the animal was constantly shifting from side to side of the cage, providing appreciable variability in eye and head position as well as body orientation. Other work has provided no indication that the influence of position or movement could account for these wave-shape differences (*7*).

Stimulus V_3 sometimes elicited performance of the approach response and sometimes elicited the avoidance response. Average evoked potentials and variances were computed separately for trials in which V_3 elicited CR (V_3CR) and for trials resulting in CAR (V_3CAR). The significance of differences between these averages was assessed by the *t*-test. Comparisons between these averages, and between each of the averages and waves from V_1CR and V_2CAR trials, were made by calculating correlation coefficients (*8*).

Data are given separately according to two methods of data selection. First, an arbitrary time period of 4 seconds preceding the behavioral performance was used. Second, wave-shape sequences toward the end of the behavioral trials were selected which seemed characteristic of the behavioral outcome. Average evoked responses from the last 4 seconds of each trial are presented in Fig. 1. The averages of selected sequences of evoked potentials which occurred late in these same trials are presented in Fig. 2.

A set of data is presented from the visual cortex or the lateral geniculate body of each experimental animal. In each set, the first and second wave shapes, from V_1CR and V_3CR, are strikingly similar. The third and fourth wave shapes, from V_3CAR and V_2CAR, are also similar. The difference between V_3CR and V_3CAR is shown by the dissimilarity between the second and third wave shapes.

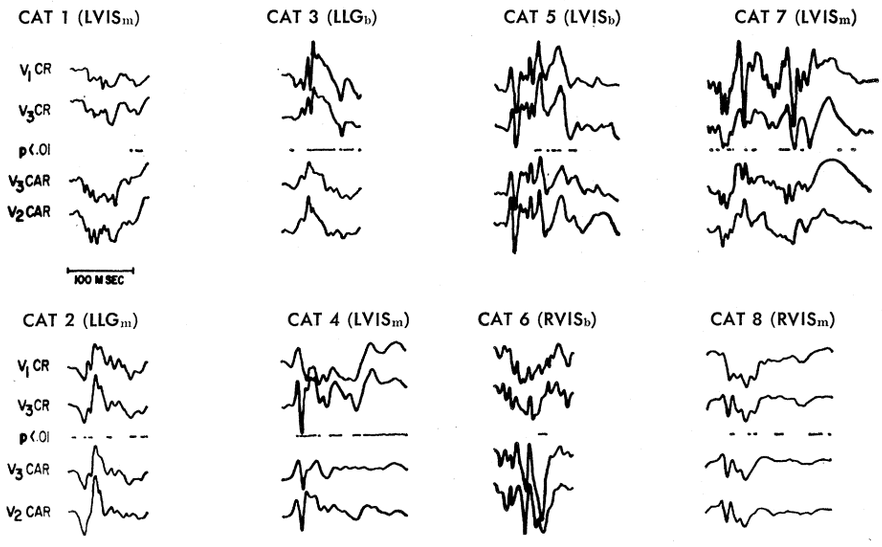

Fig. 1. Average response wave shapes recorded from one structure in each experimental animal, computed from last 4 seconds of trials resulting in correct performance of a lever-press for food during presentation of flicker at frequency 1 (V_1CR), correct performance of a lever-press to avoid shock during presentation of flicker at frequency 2 (V_2CAR), and during presentations of flicker at an intermediate frequency 3 resulting in generalized approach (V_3CR) or avoidance (V_3CAR). Interrupted line between the second and third wave shape in each group indicates those intervals during which the significance of the difference between V_3CR and V_3CAR exceeds the 0.1 level. *LLG*, Left lateral geniculate body; *LVIS*, left visual cortex; subscript *m* denotes monopolar derivation versus a frontal reference; subscript *b* indicates bipolar derivation taken between two electrodes 1 mm apart. Frequencies were as follows: Cats 1 and 2, $V_1 = 7.7$, $V_2 = 3.1$, $V_3 = 5.0$; cat 3, $V_1 = 3.1$, $V_2 = 7.7$, $V_3 = 5.0$; cats 4, 5, and 8, $V_1 = 5$, $V_2 = 2$, $V_3 = 3.1$; cat 6, $V_1 = 2.5$, $V_2 = 1.0$, $V_3 = 1.75$; cat 7, $V_1 = 1.0$, $V_2 = 2.5$, $V_3 = 1.75$. Sample size was variable, with an average of 56.

The interrupted lines between V_3CR and V_3CAR indicate intervals within which these differences are statistically significant ($P < .01$). For all eight animals in this study, the average response wave shape during V_3CR was significantly different from that observed during V_3CAR. In a ninth

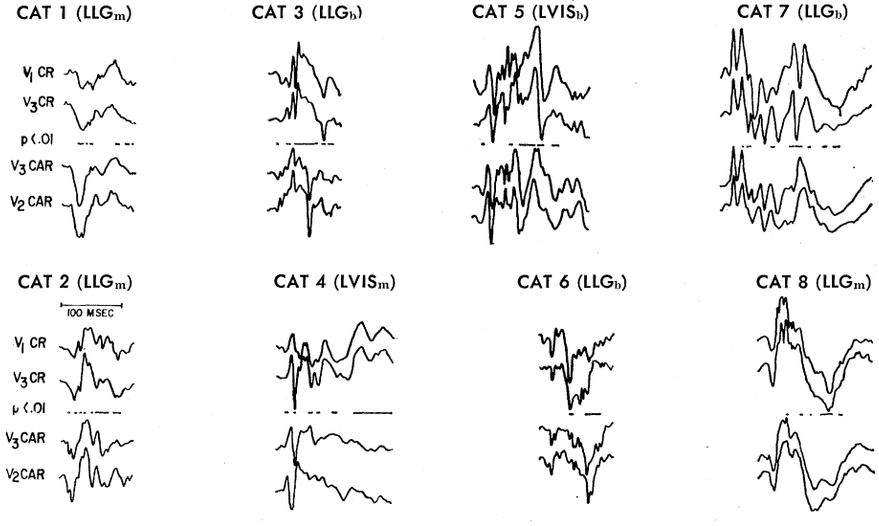

Fig. 2. Response wave shapes, as in Fig. 1, but with averages based upon sequences of evoked potentials selected by the experimenter from the last 4 seconds of multiple behavioral trials. Average sample size, 15.

Table 1. The coefficients in the top half of the table were computed from averages of the evoked responses during the last 4 seconds of each of several trials (shown in Fig. 1). The coefficients in the bottom half were computed from averages based on characteristic wave-shape sequences selected by the experimenters from the last portion of the same several trials (shown in Fig. 2).

Cat	$V_1CR \times V_2CAR$	$V_3CR \times V_3CAR$	$V_3CAR \times V_2CAR$	$V_3CAR \times V_1CR$	$V_3CR \times V_1CR$	$V_3CR \times V_2CAR$
			Last 4 seconds			
1	.35	.72	.88	.32	.74	.71
2	.83	.98	.92	.94	.94	.87
3	.50	.79	.93	.61	.90	.69
4	−.13	.58	.12	.21	.54	.44
5	.64	.75	.80	.72	.88	.66
6	.14	.62	.82	.39	.64	.45
7	.42	.63	.76	.53	.67	.55
8	.68	.94	.85	.76	.81	.78
			Selected sequences			
1	.65	.69	.92	.63	.83	.72
2	.49	.65	.91	.59	.91	.53
3	.21	.43	.88	.32	.89	.40
4	−.47	.02	.54	−.41	.78	−.17
5	.50	.60	.96	.57	.85	.51
6	.15	.14	.81	.17	.59	.19
7	.70	.75	.92	.78	.74	.69
8	.86	.96	.97	.91	.95	.92

animal, similar results have been obtained with an approach-approach discrimination. Thus, the wave shape released by V_3 during generalization cannot be determined by such unspecified factors as arousal, attention, intention to move, or type or amount of motivation, but depends upon the specific CR which is subsequently performed. These differences could frequently be observed in nonaveraged recordings. In several animals, they were sufficiently consistent to permit prediction of the outcome of the generalization trials.

Wave shapes were quantitatively compared by computation of the product moment correlation coefficient between pairs of responses. Although cross-correlation provides an objective measure of wave shape similarity, the procedure has shortcomings. In some cases, wave shapes which were obviously different both by visual inspection and by *t*-test yielded high correlation coefficients. In general, this was due to the fact that the differences consisted of fast components which accounted for but a small part of the total energy of the wave shape. This was particularly true for cat No. 8.

Table 1 contains the correlation coefficients between the four different kinds of wave shapes for each set of data illustrated in Figs. 1 and 2. The low cross-correlations between V_1CR and V_2CAR indicate that the wave shapes elicited by the two discriminated stimuli were markedly different. The wave shape released by the novel stimulus during generalization was not fully determined by the stimulus characteristics, since $V_3CR \times V_3CAR$ is relatively low in most cases. The wave shape elicited by V_3 during differential generalization resembles the usual response to V_1 when CR occurs ($V_3CR \times V_1CR > V_3CR \times V_2CAR$), but resembles the response to V_2 when CAR occurs ($V_3CAR \times V_2CAR > V_3CAR \times V_1CR$). The exact probability of obtaining the distribution of values in columns 3 to 6 of the upper part of Table 1 by chance is less than .002, whereas the probability of the distribution obtained for the selected samples is less than .0001. Thus, our conclusions are supported by the results of an arbitrary and objective computation as well as by the procedure based upon selected data, although the latter method yields more striking results.

Data from electrodes in the visual system have been presented because they were particularly striking and consistent. However, the phenomena were also observed in many other brain regions. Of the total of 96 placements in which unselected data were studied in eight cats, for 64 regions the wave shape observed during V_3CAR resembled the response to V_2 during CAR more than the response to V_1 during CR. For 65 regions, the wave shape during V_3CR resembled the response to V_1 during CR more than the response to V_2 during CAR. The chi square for this distribution of results equaled 22.6 ($P<<<.001$). The V_3CR and V_3CAR wave shapes became markedly different for all cats at latencies which ranged from 30 to 50 msec. In other studies (*9*) a burst of neuronal discharge beginning at a latency of about 35 msec has been observed in the lateral geniculate body during generalization. Further, the shape of the multiple unit poststimulus histogram during generalization closely reproduced the usual response to the familiar conditioned stimulus. Thus, phenomena comparable to those reported here have been observed on the unit level of analysis.

We conclude that the shape of the evoked potential released by a novel stimulus during generalization is not solely determined by the actual physical stimulus, but contains an endogenous component which varies depending upon the meaning attributed by the animal to the signal. Second, the two different endogenous processes released during differential generalization correspond well to the wave shapes normally elicited by the discriminated stimuli which were used to establish the differentiated behavioral responses. Third, release of these stored patterns involves discharge of a widely distributed set of neurons which begins at about 35 msec.

These findings constitute evidence that during conditioning a specific temporal pattern of neuronal activity or engram is established in the nervous system. Activation of this memory by a neutral stimulus during generalization is accompanied by the appearance of characteristic endogenous wave shapes in various regions of the brain. Those regions presumably mediate remembering of the appropriate behavior.

E. R. JOHN
M. SHIMOKOCHI*
F. BARTLETT

Brain Research Laboratories,
Department of Psychiatry,
New York Medical College,
New York 10029

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* Visiting scientist from Osaka University, Japan.

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