Journée du 4 juillet 1969

Première séance

INFORMATION ET SYSTÈMES BIOLOGIQUES

II - ASPECTS THÉORIQUES

PRÉSIDENT S. L. SOBOLEV

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* M. P. Schutzenberger's report has been presented orally. We have not received the manuscript.

De la Physique théorique à la Biologie, C.N.R.S., 1971.

NON-HOLOGRAPHIC ASSOCIATIVE MEMORY

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The remarkable properties of the hologram as an information store have led some people [1, 2] to wonder whether the memory may not work on holographic principles. There are, however, certain difficulties with this hypothesis if the holographic analogy is pressed too far; how could the brain Fourier-analyse the incoming signals with sufficient accuracy, and how could it improve on the rather feeble signal-to-noise ratio [3] of the reconstructed signals? Our purpose here is to show that the most desirable features of holography are manifested by another type of associative memory, which might well have been evolved by the brain. A mathematical investigation of this non-holographic memory shows that in optimal conditions it has a capacity which is not far from the maximum permitted by information theory.

Our point of departure is Gabor's observation [4, 5] that any physical system which can correlate (or for that matter convolve) pairs of patterns can mimic the performance of a Fourier holograph. Such a system, which could be set up in any school physics laboratory, is shown in Figure 1. The apparatus is designed for making "correlograms" between pairs of pinhole patterns, and then using the correlogram and one of the patterns for reconstructing its partner. One of the pinhole patterns is mounted at A, and the other at B. The distance between them equals f, the focal length of the lens L. A viewing screen is placed at C, at a distance f from the lens, and a diffuse light source is mounted behind A. The pattern of bright dots appearing at C is the correlogram between the pattern at A and the pattern at B. Formally, $C = \overline{A} * B$, where the asterisk stands for convolution and A is the result of rotating the pattern A through half a turn round the optical axis. If $\overline{A}$ and B were interchanged, the pattern at C would be $\overline{B} * A = A * \overline{B} = \overline{C}$, so that the correlogram would be inverted. This is clear enough if B is a pinhole, and shows that the order of the patterns is important.

To recover pattern A from pattern B we convert the correlogram into a pattern of pinholes in a black card and place the light source behind it, so that the light shines through C and B on to a viewing screen at A (Fig. 2). A pattern of spots now appears on the viewing screen. All the spots of the original pattern A are present, but a number of spurious spots as well. If the

pinholes were infinitesimal and there were no diffraction effects the reconstructed pattern would be $\overline{C} * B = A * \overline{B} * B$, just as in Fourier holography. If B were a random pattern, one could argue, $\overline{B} * B$ would approximate to a delta function at the origin, so that the reconstructed pattern would look like a slightly bespattered version of the original pattern A. How can we pick out the genuine spots from the others ?

To solve this problem let us simplify the set-up by removing the lens (Fig. 3). Suppose, for example, that A has two holes and B has three. Then the pattern C will consist of six bright spots (barring coincidences). When these spots are converted into pinholes and illuminated from the right, a total of 18 ($= 6 \times 3$) rays will emerge from B and impinge on the screen at A. But we shall not see eighteen spots on this screen, because six of the rays will converge, in sets of three, on to the two points of the original pattern. The other twelve rays will give rise to spurious spots, but (again barring coincidences) these spots will be fainter than the genuine ones. We can therefore expect to be able to pick out the wheat from the chaff with a detector with a threshold slightly less than three units of brightness.

This reasoning applies equally to the " correlograph ", with lens, illustrated in Figs. 1 and 2. So, having found how to get rid of the unwanted background in reconstructing A from B and C, we can now envisage the possibility of constructing multiple correlograms, comprising all the spots present in $C_1 = A_1 * B_1$ or in $C_2 = A_2 * B_2$, and so on. The presentation of B_1 should evoke A_1, presentation of B_2 should evoke A_2, and so on, up to the limite set by the information capacity of the system. But what is this limit ?

To answer this question let us evade the complicated (and basically irrelevant) issues raised by the finite wavelength of light, edge effects and so on, and pose the question in terms of a discrete, and slightly more abstract, model. We suppose A, B and C to be discrete spaces, each containing N points, a_1 to a_N, b_1 to b_N, and c_1 to c_N. The point-pair (a_i, b_j) is mapped on to the point c_k if $i - j = k$ or $k - N$. Conversely, the point-pair (c_k, b_j) is mapped

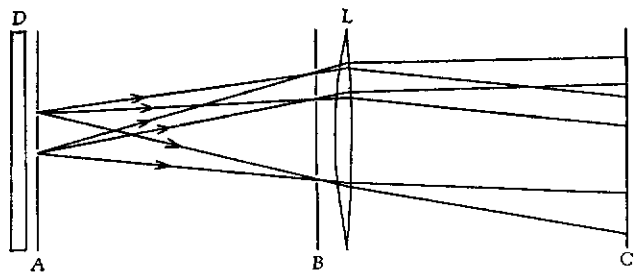

Fig. 1. Constructing a correlogram. D is a diffuse light source, L a lens and C the plane of the correlogram of A with B.

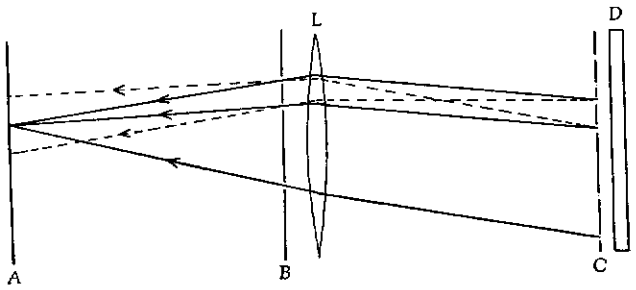

Fig. 2. Reconstructing a pattern. ——, Paths traversed in Fig. 1; - - -, paths not traversed in Fig. 1.

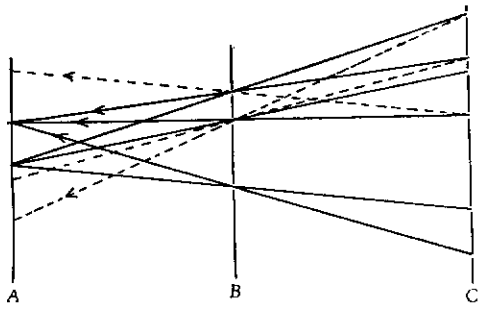

Fig. 3. Showing that original spots are generally brighter.

on to a_i if the same condition is met. Imagine now that we have R pairs of patterns which we wish to associate together, each pair consisting of M points selected from A and another M selected from B. The total number of point-pairs determined by all the pairs of patterns will be RM^2, and we may think of this number of " rays " striking C. If they impinge at random, the probability of any point c_k not being struck will be

$$\exp(-RM^2/N) = 1 - p, \text{ say}$$

The correlogram for the whole set of R pairs will then consist of the remaining pN points of C.

Now consider the reconstruction process. One of the B-patterns, comprising M of the points b_1 to b_N, is selected, and combined with the correlogram to produce pNM " *rays* " impinging on A. Each point of the original A-pattern will receive exactly M rays, so that we should set the threshold of our detector at M if we want to pick up all the original points. Now consider any one of the $N - M$ other points in A. It may receive a ray through any one of the M " holes " in B; the probability that it receives a ray through

a given hole is just p, for this is the chance that the point on C " behind " the hole belongs to the correlogram. The chance of an unwanted point reaching the threshold is thus p^M, and the probable number of spurious points of brightness M is consequently $(N - M)p^M$. If M is a fairly large number, this will be a sensitive function of p, and for given N and M the critical value of p above which spurious points begin to appear may be found from the relation

$$(N - M)\, p^M = 1$$

Alternatively, this may be viewed as a relation which sets a lower limit to the value of M for given values of N and p. A slightly safer estimate is given by

$$\text{(i)} \qquad Np^M = 1, \text{ or } M = -\log N/\log p$$

If M falls below this value, the reconstruction will be marred by spurious points.

Next we enquire about the amount of information stored in the memory when R pairs have been memorized and M satisfies the aforementioned condition for accurate retrieval. We can evoke any one of R A-patterns by presenting the appropriate B-pattern. There are $\binom{N}{M}$ possible A-patterns altogether, so the amount of information needed to store any one of them is $\log \binom{N}{M}$, which is roughly $M \log N$ natural units of information. The total amount of information stored is, therefore, approximately

$$\text{(ii)} \qquad I = RM \log N \text{ natural units}$$

But according to our original calculation of p

$$\text{(iii)} \qquad RM^2 = -N \log (1 - p)$$

and if we are working at the limit of accurate retrieval

$$\text{(iv)} \qquad M = -\log N/\log p \simeq \log_2 N \text{ (see below)}$$

It follows immediately that

$$\text{(v)} \qquad I = N \log p \log (1 - p)$$

As one might have anticipated, this expression has its maximum value when p is 0.5—when the correlogram occupies about half of C.

What is remarkable is the size of I_{max}.

$I_{max} = N (\log 2)^2$ natural units $= N \log 2$ bits. The maximum amount of information that could possibly be stored in C is N bits. So the correlograph, in this discrete realization, stores its information nearly ($\log 2 = 69$ per cent) as densely as a random access store with no associative capability.

As described, the discrete correlograph, like the holograph, will " recognize " displaced patterns. If an A-pattern $\{a_i\}$ and a B-pattern $\{b_j\}$ have

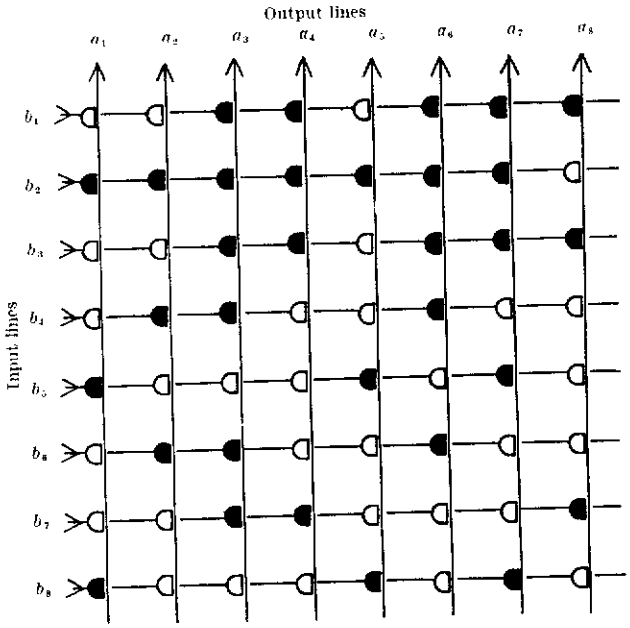

Fig. 4. An associative net.

been associated then presentation of the displaced *B-pattern* $\{b_{j+d}\}$ will evoke the displaced A-pattern $\{a_{i+d}\}$.

But the resemblance does not cease there. Just as in holography, the information to be stored is laid down (i) in parallel, (ii) non-locally and (iii) in such a way that it can survive local damage. In parallel, because each mapping $(a_i, b_j) \rightarrow c_k$ can be effected without reference to any other; the same applies to the reconstructive mappings $(c_k, b_j) \rightarrow a_i$. Non-locally, because the presence of a_i in an A-pattern is registered at M separate points on the correlogram, one for each point of the B-pattern. And robustly, because if the system is not stretched to its theoretical limit it can (as we shall show elsewhere) be used for the accurate reconstruction of A-patterns even when some of the correlogram is " ablated " and/or the B-patterns are inaccurately presented. But it can only be made secure against such contingencies by sacrificing storage capacity—as one would expect.

In our discussion of the process of reconstruction we had occasion to note that a point c_k might owe its presence on the correlogram to the joint occurrence of (a_i, b_j); but that if a pattern were presented containing the point b_{j+d}, the " ray " (c_k, b_{j+d}) would light up the point a_{i+d}, which might never have occurred in any A-pattern. It was this feature which underlay the ability of the system to recognize displaced patterns; but the same feature is a slight embarrassment when one comes to consider how a discrete correlograph, with the reconstructive facility, could be realized in neural tissue. We will not dwell

on this point, except to acknowledge that it was drawn to our attention by Dr F. H. C. Crick, to whom H. C. L.-H. is indebted for provocative comments. But it led us on to a further refinement of our model, in which a given point c_k is admitted to the correlogram only if the particular pair (a_i, b_j) occurs in one of the pairs of patterns, and not otherwise. On this assumption there might be as many as N^2 separate point-pairs to take into account, and a correspondingly large number of points in the space C.

In this form our associative memory model ceases to be a correlograph, having lost the ability to recognize displaced patterns, but its information capacity is now potentially far greater than before. To show this, we will adopt a rather different type of representation, in which the points of A become N_A parallel lines, and those of B become N_B parallel lines. The points of C are the $N_A N_B$ intersections between the lines a_i and the lines b_j.

In this network model, as before, a particular point of C is included in the active set if the pair of lines (a_i, b_j) which pass through it have been called into play in at least one association of an A-pattern with a B-pattern. Let us suppose that R pairs of patterns have been associated in this way, each pair comprising a selection of M_A lines from A and M_B lines from B. Then the chance that a given point of C has not been activated by the recording is

$$\text{(vi)} \qquad \exp(-RM_A M_B/N_C) = 1 - p, \text{ say}$$

where we have written N_C for $N_A N_B$. If B-patterns are being used to recall A-patterns, then there will be a mniimum value of M_B such that if the threshold on the A-lines is set at M_B (so as to detect all the genuine lines) spurious lines will begin to be detected as well. (The argument is just the same as that applied to the correlograph earlier on.) This minimum value of M_B is given by

$$\text{(vii)} \begin{cases} N_A p^{M_B} = 1 \\ \\ \text{or} \quad M_B = -\log N_A / \log p \simeq \log_2 N_A \end{cases}$$

Now the amount of information stored in the memory when R pairs of A-patterns have been memorized is roughly

$$\text{(viii)} \qquad I_A = RM_A \log N_A$$

But from our equation for $1 - p$

$$\text{(ix)} \qquad RM_A M_B = -N_C \log (1 - p)$$

therefore

$$\text{(x)} \qquad I_A = N_C \log p \log (1 - p)$$

showing that, as in the correlograph, the density with which the associative net stores information is 69 per cent of the theoretical maximum value. We may

note, in passing, that I_B, defined as $RM_B \log N_B$, is also equal to $N_C \log p \log (1 - p)$.

An associative network of this kind also operates (i) in parallel (ii) non-locally and (iii) in such a way that local damage or inaccuracy is not necessarily disastrous. We intend to go into the details of (iii) elsewhere. We now succumb to the temptation of indicating how such an associative memory might be realized in neural tissue though, as Brindley has pointed out [6], function need not determine structure uniquely.

The system we have in mind is represented diagrammatically in Fig. 4. The horizontal lines are axons of the N_B input neurones $b_1, b_2, \ldots$, while the vertical lines are dendrites of the N_A output neurones $a_1, a_2, \ldots,$. At the intersection of b_j with a_i is a modifiable synapse c_{ij}. This synapse is initially inactive, but becomes active after a coincidence in wich a_i and b_j are made to fire at the same time by some external stimulus. Such a coincidence is supposed to occur if an A-pattern containing a_i is presented in association with a B-pattern containing b_j. After the activation of c_{ij} (which we regard as a permanent effect) the firing of b_j will locally depolarize the membrane of a_i. The output neurone a_i is then supposed to fire if M_B or more input cells depolarize it simultaneously.

In Fig. 4 we indicate what the state of the network would be atfer it had learned to associate the following pairs of patterns :

(xi)

B-pattern	A-pattern
1,2,3	4,6,7
2,5,8	1,5,7
2,4,6	2,3,6
1,3,7	3,4,8

The synapses indicated by solid semicircles would be active, those indicated by open semicircles being still inactive. In this particular example, N_A and N_B are both 8, and M_A ($\simeq \log_2 N_B$) and M_B ($\simeq \log_1 N_A$) are both 3. R, the number of pairs of patterns associated, has been chosen so as to make p, the proportion of synapses active, close to 0.5; in fact p equals 0.5 exactly. These various numbers illustrate the system working near its maximum capacity. The reader may verify that every B-pattern except the first evokes the correct A-pattern at a threshold of 3; the only mistake the system makes is that when supplied with the B-pattern 1, 2, 3 it responds with an A-pattern 3, 4, 6,7 containing four elements.

To summarize, we have attempted to distil from holography the features which comment it as a model of associative memory, and have found that the performance of a holograph can be mimicked and actually improved on by discrete non-linear models, namely the correlograph and the associative net just describde. Quite possibly there is no system in the brain which corresponds

exactly to our hypothetical neural network; but we do attach importance to the principle on which it works and the quantitative relations which we have shown must hold if such a system is to perform, as it can, with high efficiency.

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(Reprinted from *Nature*, **222,** 960 (1969)).

De la Physique théorique à la Biologie, C.N.R.S., 1971.

DO SPECIFIC BIOCHEMICAL CORRELATES TO LEARNING PROCESSES EXIST IN BRAIN CELLS ? *

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The present paper will deal mainly with the biochemical changes observed in neurons during three different learning experiments. First, however, some experiments showing differences in the protein composition of nerve and glia cells with be described. Of the learning experiments, the first is a case of instrumental learning in rats, in which changes in the synthesis of three acidic neuronal proteins and in the RNA base composition of neurons occurred; the arguments that these changes are specifically related to the training and that they are an expression of increased gene activation will be presented. In the next study, the protein changes observed in brain cells during simple sensory conditioning in rats will be described, and it will be argued that these are due to an increased level of attention rather than to learning, *per se*. Finally, some RNA data on neurons from monkeys performing a visual discrimination test will be reported.

Neuronal and glial proteins

Four years ago, Moore and collaborators [1, 2] described a brain-specific protein, called S100, because it is soluble in saturated ammonium sulfate. It is an acidic protein, has a molecular weight of around 20,000 constitutes 0.1 % of the brain proteins and moves close to the anodal front in electrophoresis. It develops after 12 days postnatally in the rat and is present only in nervous tissue. Thirty per 100 moles of its amino acids are acidic. It contains 30 % glutamic acid and no tryptophane. S100 can be further separated into at least 3 fractions, of which 2 have a high turnover and react immunologically with antiserum against S100 [3]. The S100 protein is not linked to carbohydrates (Fig. 1).

Hydén and McEwen [4] have shown by antiserum precipitation reactions supported by the Coons'technique [5] that S 100 is mainly a glial, protein which

* The main content of this paper has also been published in *Proceed. Sympos. on Biology of Memory*, Sept. 1, 1969, Tihany, Ed. G. Adám.

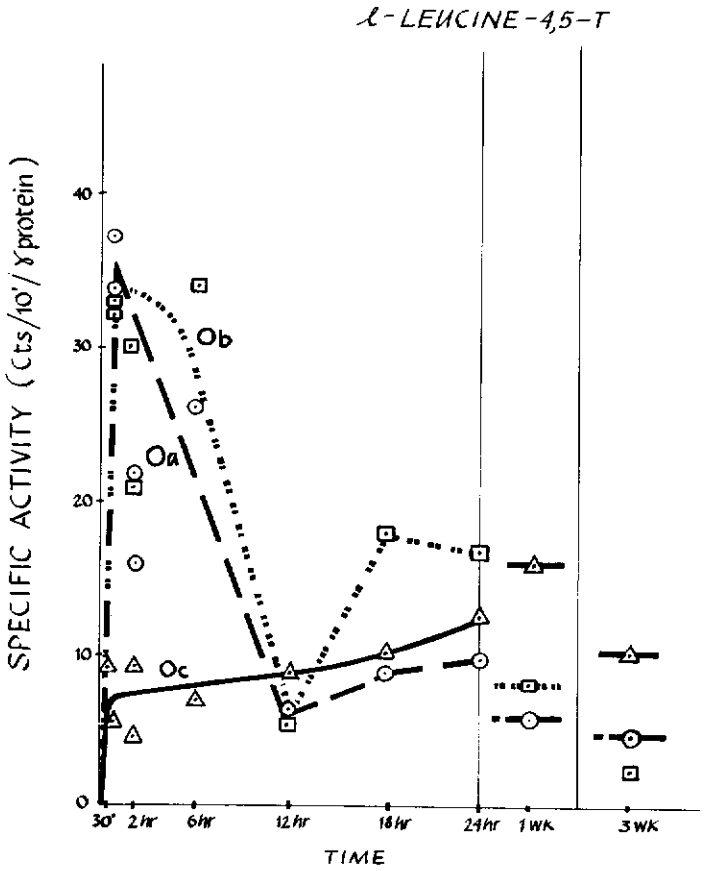

Fig. 1. Specific radioactivity of bands Oa, Ob, and Oc separated on 11,2 % polyacrylamide gels as a function of time between isotope injection and sacrifice. Radioactivity was determined after combustion of slices of the polyacrylamide gels by liquid scintillation counting. Isotope : 1-leucine-4,5 T.

in nerve cells is found only in the nuclei. Recently, Benda and collaborators [6] confirmed its presence in glia and showed its 10-fold growth in a clonal strain of glial tumors. Perez and Moore [7] have also presented evidence that S100 is mainly a glial protein. Moore and Perez [8, 9] have described another brain-specific protein which seems to be localized exclusively to the nerve cells and which has been named the 14-3-2 protein.

There is evidence for the existence of still other brain-specific soluble proteins. MacPherson [10] has described one in the β-globulin range, Kosinski [11] has described five soluble proteins, and Warecka and Bauer [12, 13] recently described an α-glycoprotein rich in neuraminic acid, which develops three months after birth in man and is probably derived from glia.

Bennett and Edelman [14] have purified and characterized still another acidic brain-specific protein.

An immunological study of Deiters' nucleus

We have examined the properties of antibodies prepared against neurons and glia [15] obtained from Deiters' nucleus in the continuing attempt to identify brain-specific proteins in them. The antigens in brain cells presumably number in the order of hundreds; Huneeus-Cox [16, 17], for instance, successfully prepared antisera against eleven antigens in preparations of squid axoplasm that did not include the external membranes. In our study antigen consisted of glial material dissected from the Deiters' nucleus of the rabbit by the free-hand technique previously described [18]. The dissection was carried out at 4 °C, with careful removal of capillaries and nerve cell bodies and processes; in this way, 3.2 mg of Deiters' nucleus glia was collected from 40 rabbits. The other antigen consisted of 1.3 gm of whole Deiters' nucleus, containing both neurons and glia, dissected from 100 rabbits.

Each of these antigens was homogenized and mixed with both complete and incomplete Freund's adjuvant. A group 6 six rhesus monkeys weighing 3-3.5 kg was injected intramuscularly with 0.6 ml of one or the other emulsion once a week for 4 weeks. None ever showed neurological symptoms, or signs of tuberculosis. The animals were bled after one week. On day 44 each monkey received a booster injection of 0.2 ml of its antigen emulsion precipitated with $Al_2(SO_4)_3$, and was bled one week later. These sera were tested on Ouchterlony

TABLE I

A. *Gel precipitation reactions* (+) *between anti nucleus Deiters' antiserum* (1:512) *and and a homogenate of nucleus Deiters.*

Antigen (μg/μl)	Reaction	Antigen	Reaction	Antigen	Reaction	Antigen	Reaction
8.20	-	0.80	+	0.30	+	0.05	-
4.10	-	0.50	+	0.20	+	0.02	-
2.10	-	0.40	+	0.10	+	0.01	-
1.00	-						

B. *Gel precipitation reactions* (+) *between anti Deiters' glia antiserum* (1:512) *and an antigen homogenate of Deiters' glia.*

Antigen (μg/μl)	Reaction	Antigen	Reaction
0.67	+	0.08	-
0.60	+	0.04	-
0.16	+	0.02	-

TABLE II

Gel precipitation reaction (+) between anti Deiter's glia antiserum and 0.9 g of protein extracted from nerve and glia cells dissected from Deiters' nucleus. Normal serum controls negative in each case.

Antiserum Dilution	Protein from	
	Nerve cell	Glia cell
1 : 64	–	+
1 : 128	–	+
1 : 256	–	+
1 : 512	–	+
1 : 1024	–	–
1 : 2048	–	–

plates against extracts of glia and of Deiters' nucleus, and their precipitation activities against sucrose-Triton X-100 extracts of both glia and of Deiters' nucleus material were also evaluated. In addition, the micromethod for double diffusion in one dimension in glass capillaries previously described [4] was used as an assay system, the Coons' [5] multiple layer indirect method for immunofluorescence applied to cryostat sections through the Deiters' nucleus, with evaluation of the specific fluorescence appearing in the nerve and glia cells was

TABLE III

Precipitation reaction between anti Deiters' glia antiserum (1:512) and homogenates homogenates of Deiters' nerve cells and corresponding volumes of glia. Neuronal protein estimates based on 12,000 µg of protein per cell. Glial protein per unit volume estimated at 50 % neuronal.

Deiters' neurons			Deiters' glia (same volume as nerve cells)	
Number of nerve cells	Calcul. protein in 10^{-6} g*	Precip.	Calcul. protein in 10^{-6} g*	Precip.
300	3.6	–	1.8	+ 2 ppt
150	1.8	–	0.9	+ 2 ppt
70	0.9	–	0.45	+ 2 ppt
60	0.72	–	0.36	+ 1 ppt
30	0.36	–	0.18	+ 1 ppt
15	0.18	–	0.09	–
6	0.09	–	0.045	–
3	0.045	–	0.022	–

also used. Some samples of the antisera were absorbed in two or three steps with sucrose-Triton X-100 homogenates of glia and of rabbit spleen, while others were twice absorbed with rabbit spleen and then absorbed with glia.

Tables I to IV summarize some results of these studies. Both the anti-Deiters' nucleus and the anti-glia sera formed well-defined precipitates with μg/μl amounts of their respective antigens (Table I). Table II shows that the antiglia serum formed precipitates with the glia but not with nerve cells obtained from Deiters' nucleus, and that no precipitates formed when normal rabbit serum was used against these antigens.

Table III shows the results of an antigen dilution study : homogenates of isolated nerve cells and of the same volumes of glial cells were tested against the antiglia antiserum in the dilution 1 : 512. Even when 300 isolated nerve cells were used no precipitation was obtained, but glial homogenates gave well-defined precipitates.

Precipitates were obtained when the anti-Deiters' glia antiserum was tested against glia dissected from other parts of the brain, e.g. from the hypoglossal nucleus and from the spinal cord and cerebral cortex, but none appeared against homogenates of motor neurons, pyramidal nerve cells of the hippocampus and granular cells from the cerebellum, all containing from 3.5 to 0.01 μg of protein per microliter.

Antiserum against the whole Deiters' nucleus gave two precipitation lines with both glia and nerve cells as antigens. However, when this antiserum was absorbed with glia or with spleen, only the nerve cell homogenates gave precipitates (Table IV).

TABLE IV

Number of precipitation lines after absorption of anti Deiters' nucleus antiserum. Antigen : homogenates from 120 *isolated nerve cells and corresponding amount of glia containing* 1.6 μg *used in each case. All dilutions tested* (1:2, 1:4, 1:8, 1:16) *gave the same result.*

	Protein form	
	Nerve cells	Glia cells
Unabsorbed	2	2
Absorbed with glia	1	0
Absorbed with spleen	1	0

The results with the fluorescence technique matched those obtained with the immunodiffusion technique as summarized in these Tables. Experiments were carried out according to the multiple layer method of Coons [5, 19].

Five μ thick cryostat sections through the lateral vestibular nucleus were first dried (sometimes left overnight in the refrigerator at + 4 °C) and subsequently fixed in cold acetone for 30 sec. After being washed for 5 min in the buffered saline, the sections were covered with the antiserum to be investigated for 30 min. After thorough washing (3 × 5 min) in a cold pH 7.1 phosphate buffered saline, a goat-antimonkey globulin-gamma-globulin conjugated with fluorescein isothiocyanate (Difco product) was applied to the sections for 30 min and the excess removed by repeated washing (again 3 × 5 min) in the buffer. Control sections heated with normal monkey serum and with conjugated gammaglobulin only were regularly used with each experimental series. The sections were finally mounted in a small drop of buffered glycerol (9 parts of glycerol, 1 part of buffered saline) under a coverslip and immediately observed in a Zeiss fluorescence microscope. After the photographs were taken using the high speed Ektachrome film with exposures varying from 1 to 5 sec, the sections were restained with Ehrlich's hematoxylin eosin. The fields previously photographed were identified under the light microscope, and rephotographed in black and white, thus enabling comparison of conventional microscopical appearances of the structural details with fluorescent pictures.

Antiserum to whole Deiters' nucleus when absorbed with glia, or with spleen, or with both, gave no fluorescence in glial cells, but did so in nerve cells; this fluorescence was localized to the outer rim of the cell body and to the dendritic processes, which could be traced through the section by their brilliant fluorescence, suggesting that the antigens were localized in the plasma membranes. The reaction was positive furthermore in the nerve cell nucleus, but not at the site of the nucleolus.

From these observations the following conclusions can be made. Neurons and glia differ with respect to antigen composition. This is an interesting finding from the point of view that both types of cells develop from the same type of ectodermal stem cell. The question is then whether the antigens are specific for the type of cell in which they occur. Judged by the absorption experiments, the neuronal antigens seem to be specific for that type of cell. It should be noted that the neuron specific antigens were concentrated to the processes, to the outer-most part of the cell and to the nucleus, and especially to the nuclear membrane.

It seemed on the other hand clear that the antigens in the glia were not glia-specific. They were localized all over in the cell body, but not in the nucleus. If the immunological organ specificity is considered, it seems to be due to the presence of antigens in the neurons.

On the other hand, glial cells possess protein which is confined only to the nerve tissue and which they share with neurons, namely the acidic S100 protein. The presence of this antigen cannot be demonstrated by the method used in this study to prepare immune sera [20].

Altered protein synthesis during training

Experiment 1: handedness transfer

A) *Incorporation of ^{3}H-leucine into the acidic protein fractions 4 and 5*

If a narrow glass tube is arranged a few centimeters from the floor, filled in its lower third with protein pills, 4 mm diameter and slightly tilted downward at its lower end, rats will reach down into the tube to retrieve the pills, one by one. They generally use either the left or right hand as they perform this task, and they can be induced to transfer this handedness [21]. When tested in free-choice reachings, all the rats in the present study showed clear preference for the left or right hand in 23 out of 25 reaches. A wall was then placed parallel to the glass tube so as to prevent use of the preferred paw; the rats began to retrieve the food pills with the non-preferred paw. When given two training periods of 25 minutes per day in this situation their performance, measured as the number of successful reaches per 25 minutes, increased linearly up to day 8. Performance curves were obtained on all rats used in

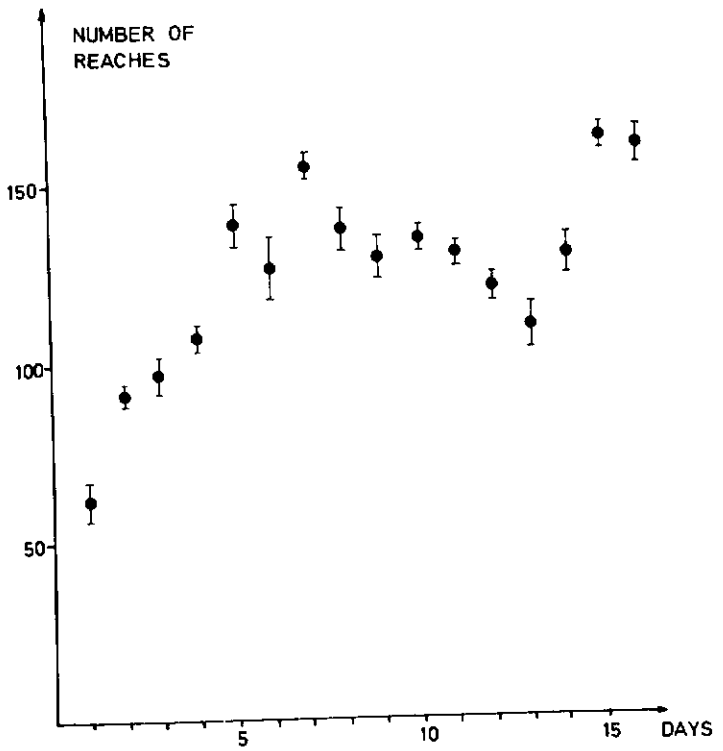

Fig. 2. Performance at reversal of handedness as the average number of successful reaches for 10 rats trained 2 × 25 minutes per day during 16 days.

our experiments and were similar to that shown in Fig. 2 which demonstrates the performance curve of a separate set of 12 rats during 16 days.
Once learned, this new behavior is retained for a long time. Since no stress (surgical, mechanical or shock) is applied to induce the new behavior, this procedure has distinct advantages over other behavioral experiments used in rats.

To trace protein synthesis during this learning the rats, under fluothane anesthesia, received 60 μCi of ^{3}H-leucine in 60 μl intraventricularly in both hemispheres half an hour before their final training period. Hippocampal nerve cell samples were then taken for analysis 15 minutes after the last training period. Nerve cells of the hippocampus were selected because 1) several clinical

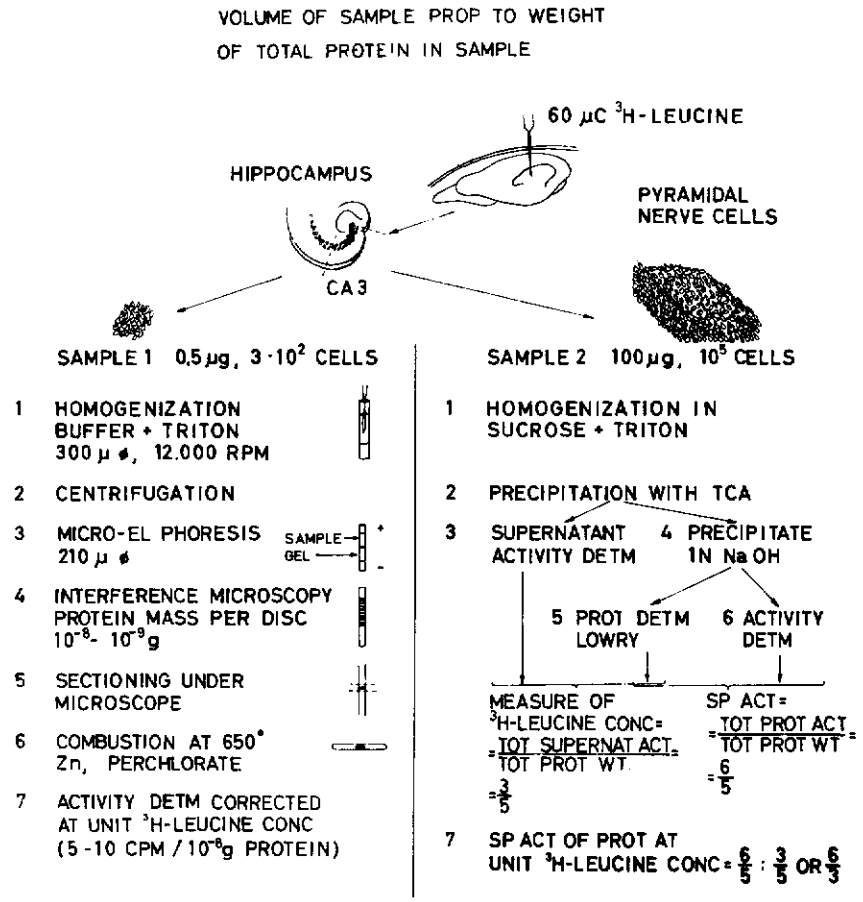

Fig. 3. Outline of the microdisc electrophoresis procedure for separation of 10^{-7} to 10^{-9} gm of protein and evaluation of incorporation of radioactive amino acid into the individual fractions. Volume of sample is proportional to weight of total protein in sample.

and behavioral studies have shown the importance of this structure for the formation of long-term memory [see e.g. 22, 23, 24, 25, 26]; 2) its bilateral destruction results in severe defects in learning and formation of memory [22, 23]; 3) during attentative learning, impedance changes occur in the hippocampus [26]; and 4) no memory is formed if protein synthesis in the hippocampus is inhibited by 90 % [27, 28].

The micromethod used for protein analysis was as follows. About 300 pyramidal cells from the CA3 region of the hippocampus, separately dissected out freehand on a cooling table, were analyzed for protein by a technique already described [29] *. An outline of this procedure is given in Fig. 3. The left side of the scheme gives the various steps leading to the value of the specific activities per amount of protein in each protein microfraction.

Since these specific activity values vary, because of variation in the local concentration of leucine-^{3}H, the correction procedure shown on the right side of Fig. 3 was applied in order to allow a comparison of values from identical parts of both hemispheres or from different animals. This was accomplished in a separate experiment, where the relation between the uncorrected specific activities and the concentration of the free leucine-^{3}H in the hippocampal nerve cells was determined and found to be linear. Dividing the specific activity values obtained by the values of the leucine-^{3}H concentration determined locally allowed all specific activities to be compared at uniform free leucine-^{3}H concentration.

In an earlier study [30], the incorporation of leucine-^{3}H in the CA3 nerve cell protein fractions 4 and 5 (Fig. 4) was evaluated on the fifth day of training, i.e. on the linear, increasing part of the performance curve. The specific activities of these protein fractions were significantly greater in trained rats compared to control rats of the same age ($P < 0.005$), and there was some evidence for higher incorporation in the hippocampus contralateral to the training paw.

Protein fractions 4 and 5 presumably each contain several species of proteins, and there is no reason as yet to believe that the qualitative characteristics of the protein formed during training is specific for the process since no data as to the composition of these proteins exist. Nevertheless, it is pertinent to ask whether the increased synthesis of fractions 4 and 5 is specific for the training.

This we attempted to do in the present study by measuring fractions 4 and 5 in rats given 5, 7, and 10 training sessions according to the following schedule. A group of 24 rats was given 5 days of training. Five of these

* One may ask why it seems necessary to struggle with such minute amounts of material and with the dissection of such small areas within the brain. As an answer, we would like to advocate the view that altered synthesis, if any, is more likely to be found in a uniform cell population from an area that is clearly involved functionally. In a mixed cell population from a whole brain such changes easily disappear in the background noise.

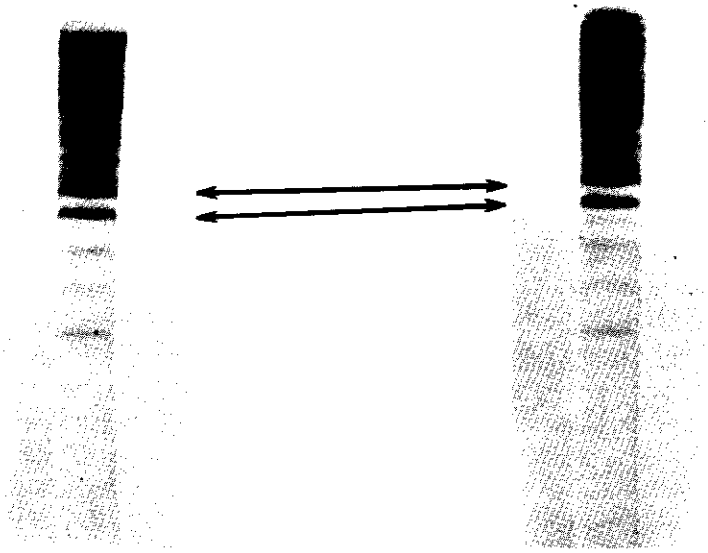

Fig. 4. Protein of pyramidal nerve cells of the hippocampus, CA3 region, separated on 400 μ diameter polyacrylamide gels, and stained with amido black. Fractions 4 and 5 from the anodal front are indicated by arrows.

(Group I) received leucine-^{3}H prior to the last training session and the CA3 hippocampal nerve cell material was taken for analysis as described above. The remaining animals were placed in cages, and given food and water *ad libitum*. After fourteen days, they were all subjected to two training periods of 25 minutes each; five of these animals (Group II) were given leucine-^{3}H, and the CA3 nerve cell material was taken for analysis. The remaining rats (Group III) were returned to their cages for fourteen additional days, then trained for three days with two training periods per day (each of 25 minutes), and, after leucine-^{3}H injection, their hippocampal brain cells were taken for analysis. The controls were untrained rats of the same age of which 50 % were littermates of the experimental animals.

TABLE V

Average number of successful reaches per day for rats using the nonpreferred paw to retrieve food pills from a narrow glass tube.

	Number of rats	Reaches
Group I (Performance on day 5)	24	100
Group II (Performance on day 14)	19	90
Group III (performance on day 30)	14	90

The performance of the rats in the three groups are shown in Table V. Table VI demonstrates that the specific activities of protein fractions 4 and 5 were singificantly increased after 5 and 7 training days but *not* after 10. The corrected specific activities (counts per minute per microgram) of protein fractions 4 and 5 differ from the corresponding values in a paper recently published [30]; this is due to a more refined separation technique which allowed a better separation of smaller amounts of the protein sample. The values found for the unseparated protein were, of course, not affected. The unseparated protein of the CA3 pyramidal nerve cells behaved like that of fractions 4 and 5 in showing higher incorporation values in Groups I and II, but not in Group III (Table VI).

TABLE VI

Corrected specific activities of hippocampal CA3 nerve cell proteins, both the unseparated and fractions 4 and 5.

Corrected specific activity refers to counts per minute per microgram ± standard error of the mean.

	Fractions 4 and 5			Unseparated protein	
	No of rats	No of gels	Corrected spec. act.	No of samples	Corrected spec. act.
Group I (training 5 days)	5	10	3.3 ± 0.40	10	14.20 ± 1.90
Group II (resumed training day 14)	5	10	3.9 ± 0.48	10	15.50 ± 1.90
Group IIA (half training time)	2	5	13.0 ± 0.60		
Group III (resumed training day 30)	14	35	1.8 ± 0.17	28	5.10 ± 0.58
Control	10	24	1.5 ± 0.16	20	6.00 ± 0.92

Following a chance observation, we made a study of the incorporation of leucine-^{3}H by two rats which were subjected to half the initial training time allowed the other rats. Whereas Group I had 10 training sessions (2×25 minutes $\times$ 5 days), these 2 rats had 5 (2×25 minutes for 2 days, 1×25 minutes on day 3), and on day 14, they ware given only a single 25 minute training session before being killed for analysis. On training day 3 they had made 120 reaches, and in the final session 100 reaches. These animals, Group IIA in Table VI, gave a greater protein synthesis response than those receiving the longer training.

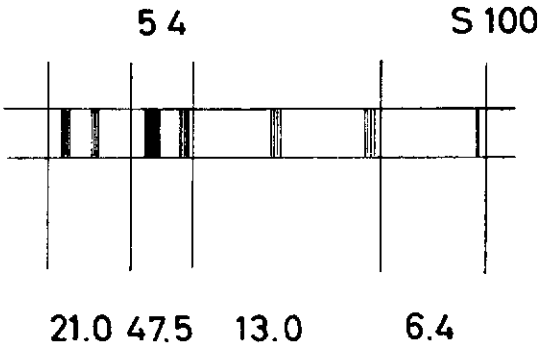

Fig. 5. Gels 400 μ in diameter, containing separated ^{3}H-labeled pyramidal nerve cell protein from the CA3 region, were cut in four pieces and the radioactivity was determined as counts per minute after combustion. Note that the radioactivity in protein fractions 4 and 5 is relatively great.

Since the incorporation of ^{3}H-leucine into the 4 and 5 protein fractions increased significantly during the training, it became important to know the relation of these fractions to the rest of the nerve cell protein in terms of ^{3}H-leucine incorporation. Protein of the CA3 nerve cells from Group I rats (trained for 5 days) was therefore separated on polyacrylamide gels, divided in four parts, and the radioactivity was determined in each part; as can be seen from Fig. 5 the radioactivity of protein fractions 4 and 5 is relatively high.

B) *Increased synthesis of* S 100 *protein*

Both the electrophoretic pattern of the soluble CA3 nerve cell protein

(a) (b)

Fig. 6. Photographs of nerve cell protein of the hippocampus, CA3 region, separated on polyacrylmaide gels 400 μ in diameter and stained by amido black. a) From control rat, b) from rat on day 5 of training with the nonpreferred paw. The acidic proteins migrate toward the bottom of the gel.

isolated in the experiment just described and micro-densitometer recordings (Fig. 7) made of 75 protein separations stained with brilliant blue showed two protein bands at the front in the trained rats compared to only one in the controls (Fig. 6, Fig. 7, Table VII).

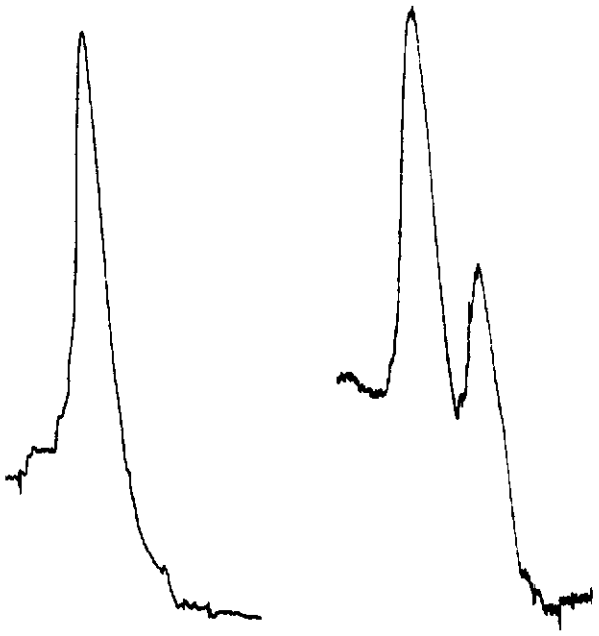

Fig. 7. Microdensitometric recording of the anodal front protein fractions shown in Fig. 6 a, control (left) and in Fig. 6 b, trained animal (right).

TABLE VII

Frequency of single and double front anodal protein fractions in the electrophoretic pattern of 75 polyacrylamide gels from 23 rats (7 controls, 4 resumed training on 14th day, 12 resumed training on 14th day and on 30th day)

	Number of protein bands	
	One	Two
Control	20	0
Group II	5	10
Group III	20	20

This protein fraction of the controls gave a positive immunological reaction when treated with antiserum against the S100 protein. Fig. 7 shows that the amount of protein contained in the two anodal bands of trained rats was greater

than the amount of protein contained in the one band of controls. Furthermore, when gel cylinders from experimental rats wih two anodal front bands were immersed in saturated $(NH_4)_2SO_4$ solution for 20 minutes, the band closest to the anode disappeared, identifying it as S100. These facts — the electrophoretic localization of the new protein fraction, its disappearance in saturated $(NH_4)_2SO_4$ and the increased amount of front protein in the trained rats — suggest that brain-specific S100 protein increased in amount during training. This S100 protein was presumably localized in the nuclei of the hippocampal neurons.

At this point it seems appropriate to comment on the protein changes during the intermittent training over a period of one month.

The rats performed well both on the 5th, 14th, and 30 th day, i.e. when they had received training for 5, 7, and 10 days. If the increased synthesis in the hippocampal nerve cells had been an expression of increased and sustained neural function, then the increased incorporation values on the 5th day of initial training and on the 14th day of resumed training had presumably been found also when the rats were subjected to 3 days of resumed training 30 days after the initial training sessions. The fact that the incorporation values did not at that last differ from those of the controls is a strong indication that the observed increase in synthesis is correlated to learning processes occurring during the training. We would like to suggest the interpretation that when the novelty of the task has passed, the hippocampal nerve cells cease to respond with increased synthesis of this type of protein. A response may well occur in other parts of the brain.

It is even more striking that the S100 protein increased in amount during the learning to reserve hand since it is a brain-specific protein and thus can be expected to mediate specific brain functions.

Our interpretation of the result given above is that the increase of the S100 protein during reversal of handedness specifically relates the S 100 protein to the learning processes. However, as we pointed out above, training involves several factors not related to learning *per se*. In the reversal of handedness experiments, the unspecific factors have been eliminated or reduced to a minimum. The motor and sensory activity, attention, motivation, and reward are equated between the experimental and control animals, and the stress involved in reversal of handedness is minimal. In view of these considerations, we used a technique which specifically related the S100 proteni during reversal of handedness to learning *per se*.

A group of 8 rats were trained during 2×25 minutes per day for three days. Between the first and second training session of the fourth day, half of the rats were injected intraventricularly on both sides with 2×25 μg of antiserum against S100 in 2×25 μl. The other half of the group was similarly injected with the same amount and volume of antiserum against rat γ-globulin. The

rats were slightly anesthetized with Fluothane. The animals were trained a second session on the fourth day 45 minutes after the injections. After this treatment, the rats were trained for three further days with 2×25 minutes per day. The results are presented in Fig. 8. The number of reaches is plotted against the number of training days. Before injection of antisera, all rats followed an identical performance curve. After the injection of antiserum against rat γ-globulin, these rats followed a performance curve which was an extrapolation of the performance curve before the injection. The same was the case with a rat which was injected with the same volume of physiological NaCl.

By contrast, the rats injected with antiserum against S100 protein did not

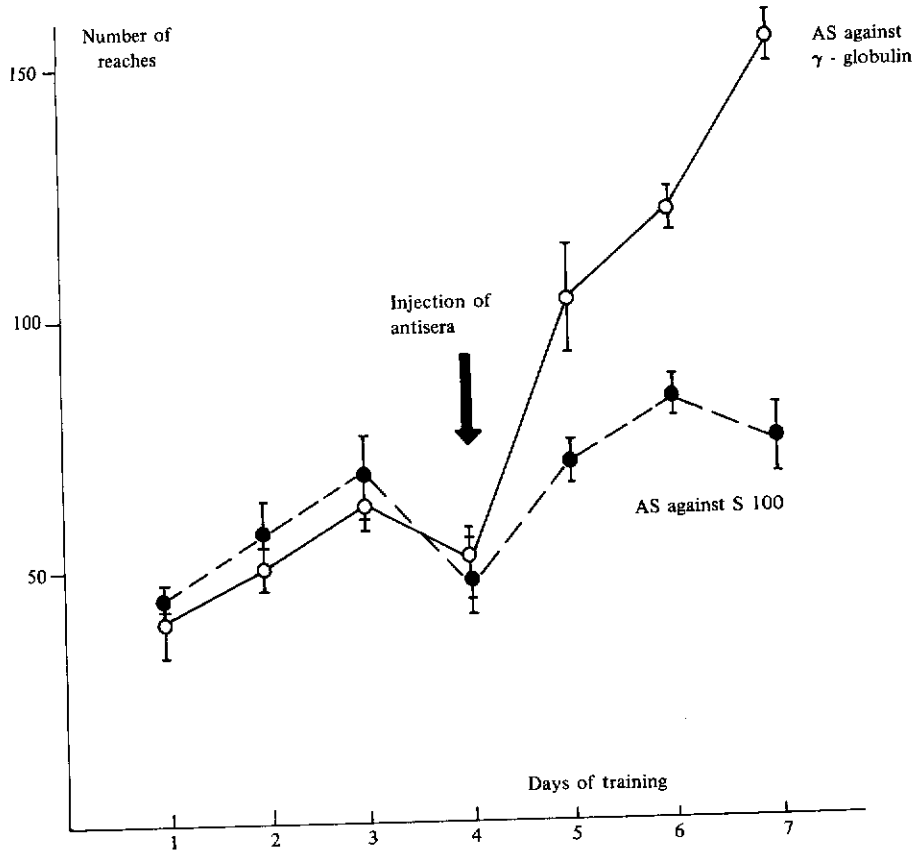

Fig. 8. Performance curves of two groups of rats, 6 experimental, 5 controls. One group injected with antiserum against S100, the other with antiserum against rat gamma-globulin on the fourth day of training.

increase in performance, i.e. the number of reaches per day remained at the same values as those immediately before the injection. As is seen from the curves in Fig. 8, the difference in number of reaches between the two groups of injected rats is clearly significant.

Another way to present the results is the following. For each rat, the sum of reaches for the first three training days is calculated as is also the sum of reaches for the last three training days. The number of reaches during the day of injection are thus not included in these sums. The ratio between the first and second sum is determined. The averages of this ratio are 0.73 ± 0.053 for the rats injected with S100 antiserum (6 rats), and 0.42 ± 0.033 for the control rats (5 rats). The difference between these ratios is highly significant ($P < 0.001$). It is obvious that the experimental rats show a decrease in learning capacity.

If instead the difference between the second and the first sum is calculated, you obtain for the rats injected with S100 antiserum 78 ± 18, and for the control rats 206 ± 19 reaches. The difference between these numbers (128) is highly significant ($P < 0.001$) leading to the same conclusion as above that the experimental rats show a decrease in learning capacity. Thus, the S100 protein is specifically correlated to learning processes.

Experiment 2: sensory conditioning test in rats: incorporation of ^{3}H-leucine into neuronal protein

This experiment was designated to limit participation of such factors as motivation, motor activity and reinforcement in an experiment involving change of behavior [31]. For that purpose we measured protein synthesis in rats subjected to paired and unpaired tone and light stimuli, and to light stimulus alone. A total of 80 rats were used in pilot tests, behavioral checks and for final experiments. The rat was placed in a cage with a wired floor in a sound absorbing dimly lit room for 20 minutes prior to the experiment. One group of rats recived an acoustic signal of 1000 Hz (conditioning stimulus) followed by a visual (unconditioned) stimulus. The tone and light stimuli both lasted 0.2 sec and were presented automatically at a frequency of 6/minute. Another group of rats was used as behavioral controls. In this group the tone and light stimuli were followed by an electric shock; in ten trials the rats learned to jump up to a shelf to escape the electrir shock (criterion 8 out of 10) when the tone-light stimuli were presented. This test, a type of sensory conditioning described and discussed by Morrell [32], demonstrated that a linkage had been formed between the two sensory areas. A third group of rats received tone and light stimuli distributed at random. A fourth group received only light stimulus at a frequency of 6/minute.

The experimental rats were first bilaterally injected with ^{3}H-leucine intraventricularly during light fluothane anesthesia, then given sound-light or sound-

TABLE VIII

Tone-Light Conditioning in Rats.
Incorporation of ^{3}H-leucine into protein isolated from hippocampal nerve cell and visual cortex of rats.
Data in cpm total protein/cpm total supernate.

Stimuli	Number of samples	Incorporation of ^{3}H-leucine	
		Visual cortex	Hippocampus
Paired	8	3.49 ± 0.23	3.43 ± 0.41
Unpaired	8	3.50 ± 0.23	4.45 ± 0.56
Light only	12	3.48 ± 0.18	
Control	8	4.46 ± 0.41	2.86 ± 0.41

light-at random stimulation for 15 minutes. The time lapse from the last injection to sampling of brain material was 40 minutes.

Both types of stimulation *increased* the incorporation of ^{3}H-leucine into the neuronal protein of the hippocampus but *decreased* it in the visual cortex (Table VIII). Control animals given light stimulus alone showed decreased incorporation in the visual cortex.

There are two findings in this sensory conditioning experiment which seem to exclude the possibility that the protein changes were correlated with learning processes during the conditioning. The first is the fact that light stimulus alone gave the same incorporation values of the visual cortex as did the paired and unpaired tone-light stimuli. The second circumstance is the fact that the tone-light stimuli distributed at random gave the highest incorporation of ^{3}H-leucine into the protein of the hippocampal nerve cells. Therefore, the conclusion is that the protein changes observed during the conditioning presumably are expression of increased attention or orientation reflexes. The finding that the incorporation values for the cells of the visual cortex in all three types of sensory experiments were lower than those of the controls, agrees with electrophysiological observations.

Discussion

The aim of the studies reported here has been to correlate protein changes in nerve cells (and glial cells) in particular parts of the brain with learning processes which occur during training. It seems evident that mapping the areas that respond with defined changes in protein fractions during behavioral experiments is prerequisite for a comprehensive theory on the mechanisms relating macro-molecules in brain cells to storage and retrieval of information.

The observations relating behavioral responses to synthesis and composition of RNA in nerve and glial cells [33, 34, 21, 35, 36], taken in conjunction with the observations on protein reported here, may be considered as a beginning which may eventually form the basis of such a theory.

It is interesting that the immunological study reported here brought out such clear differences in the antigen compositions of neurons and glia. This finding brings into question the matter of transfer of RNA from glia to neuron for which view there exists some evidence [37]. Such transfer could still take place even if the neuronal protein programmed by the glial RNA was not antigenic for rabbits challenged by our technique. S100 protein for instance, is not antigenic unless injected under special circumstances.

This S100 protein seems, however, to be definitely linked to learning, as is demonstrated especially well in the experiment where antiserum against S100 impaired learning while that against γ-globulin did not (Fig. 8). All factors including that of stress were identical for the control and experimental rats in this study. Before and after the antisera injections all were subjected to the same training program and the injections into the brain ventricles were carried out under identical conditions. Additional food was supplied to rats receiving S100 protein antiserum to compensate for the different amounts of reinforcement obtained. The result showing that only the S 100 protein antiserum inhibited further learning seems clearly to link this brain-specific protein, S 100, to learning processes occurring during training.

As for the nerve cell proteins 4 and 5, these are acidic even though their composition is still unknown. Their response during intermittent training spread over one month seems significant and is pertinent for the interpretation that the synthetic response was linked to learning processes within the training. The fact that the hippocampal nerve cells did not respond with increased synthesis of these proteins after the last training sessions (a month after the initial training) excludes the possibility that the increased protein synthesis during the two previous training sessions was merely an expression of incresed motor activity, sensory activity, attention, or change in age.

The protein changes in the transfer of handedness experiment, a case of instrumental learning, can be compared with those in the sensory-sensory experiments, a case of classical conditioning. The instrumental case is a complicated type involving i.a. motor-sensory activities, motivation and attention. The acidic — including S100 — protein in hippocampal nerve cells rises during acquisition of behavior in this case, but during sensory conditioning no systematic change related to learning can be seen in either the hippocampal or the cortical cells. The protein synthetic response of hippocampal cells in this type of classical conditioning cannot therefore be equated with that taking place during instrumental learning. In sensory conditioning, the direction and magnitude of hippocampal protein synthesis changes seem only to follow the response

of the cells to the sensory input, and to have no relation to the learning factors involved.

Thus both the light-tone and the at-random stimuli gave high incorporation values of ^{3}H-leucine into the hippocampal nerve cell protein. At visual cortex the incorporation values were equal and lower compared to the controls for all conditions of stimulation. It may therefore be tentatively concluded that in hippocampal cells the biochemical mechanisms taking place during instrumental learning differ from those during classical sensory conditioning.

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DISCUSSIONS

S.L. SOBOLEV : Je voudrais vous conter une anecdote. Un homme sortant d'un restaurant a perdu sa montre. Il va un peu plus loin où se trouve une lanterne et commence à chercher. Son ami lui demande : « Pourquoi cherches-tu ici, es-tu sûr que tu as fait tomber ta montre ici ? » « non, mais ici il y a la lumière, on peut chercher; là où elle est tombée, on ne peut rien chercher ».

Commençons la discussion générale. Professeur Kandel.

E. KANDEL : I would like to introduce into the discussions a transitional system between the model system we have considered this morning and the more complex systems of learning we will be considering this afternoon. Essentially this involves the use of invertebrate animals for the study of learning. Now one might argue that this is really a very poor strategy because the most interesting kinds of learning occur only in higher forms. However,

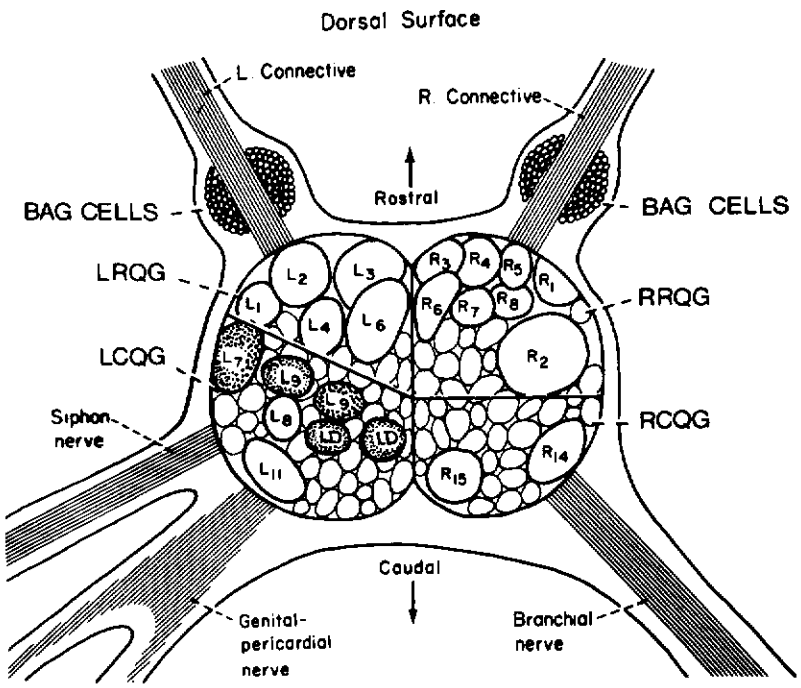

Fig. 1. *Dorsal surface of the abdominal ganglion.* The identified cells (shaded) which are motoneurons to the organs of the mantle cavity are located in the left caudal quarter ganglion. (From Kupfermann and Kandel 1969).

I would argue that this is really not the issue at hand. The more relevant question is, are certain kinds of learning so general as to be found in a variety of animals. The answer to that is clear. Learning processes such as habituation, dishabituation, classical and operant conditioning are quite general and are found in lower as well as in higher forms. These can therefore be well approached in simple preparations.

As is illustrated in Slide 1, these preparations offer a number of technical advantages. This is a diagram of the abdominal ganglion of *Aplysia,* a marine mollusc, that was introduced into neuro-physiology by Mme Arvanitaki and is probably known to the neurophysiologists among you. Its great advantage is that its cells are extraordinarily large, ranging in size from 50 microns to almost a millimeter, so that it is very easy to record from individual cells with extracellular micro-electrodes. For example, one can put separate electrodes into different cells and see whether the cells are interconnected. One can also apply acetylcholine, or other putative transmitter substances iontophoretically to the outside of the cell. In addition, the ganglion can be isolated from the animal, put in an experimental chamber, and perfused with artificial solution. As a result one can control the ionic environment of the ganglion fairly precisely. In addition, individual cells can be dissected out for biochemical analysis as Giller and Schwartz have recently done. Another feature of this ganglion is that some cells are so distinctive that they can be identified as unique elements which can be studied repeatedly from preparation to preparation. There are now 30 cells and several cell clusters that can be so idientified. In some of these cells one can also specify the connections that the cell makes with other cells in the ganglion.

The particular study I would like to describe concerns a group of cells in the left lower portion of the ganglion. These cells control the defensive withdrawal of the gill, the siphon, and the mantle shelf, a behavior that Vincent Castellucci, Irving Kupfermann, Harold Pinsker and I have been studying for the past two years. This withdrawal response is similar to the defensive escape and withdrawal responses found in higher forms. An interesting feature of this behavior is that it is modifiable.

The second slide shows what this behavior looks like. This is a diagram of the whole *Aplysia* with its parapodia and mantle shelf drawn back in order to expose the gill which is this animal's respiratory apparatus. If you touch the siphon or the mantle shelf there is a withdrawal of the siphon, the mantle shelf and the gill (Part B). Also indicated in Part C of this slide is the receptive field of this reflex, the area on the skin which on stimulation elicits this response.

The third slide indicates the several levels on which we have studied this behavior. First we examined the whole animal in a chamber which was aerated and perfused in order to study the intect animal from an experimental

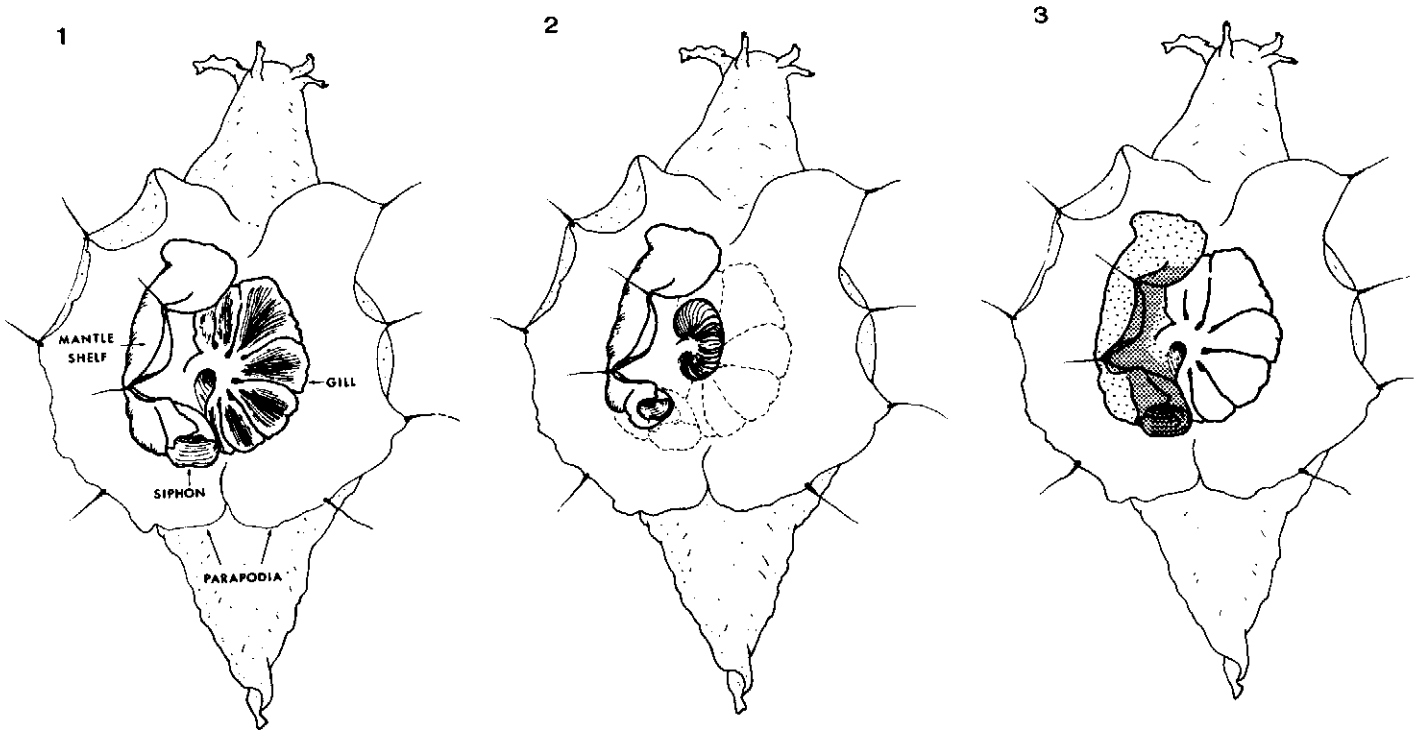

Fig. 2. (A) *Dorsal view of an intact animal illustrating the gill withdrawal reflex.* The parapodia and mantle shelf have been retracted to reveal the gill. (A1) Position of organs in unstimulated condition. (A2) Position of organs during withdrawal reflex following tactile stimulation within receptive field. (A3) Tactile receptive field for withdrawal reflex. The area which produces strong effects is heavily stippled. The surrounding area (dotted) produces weaker effects. It was also possible to get weak effects by very strong stimulation outside of the main receptive field. (From Kupfermann and Kandel 1969).

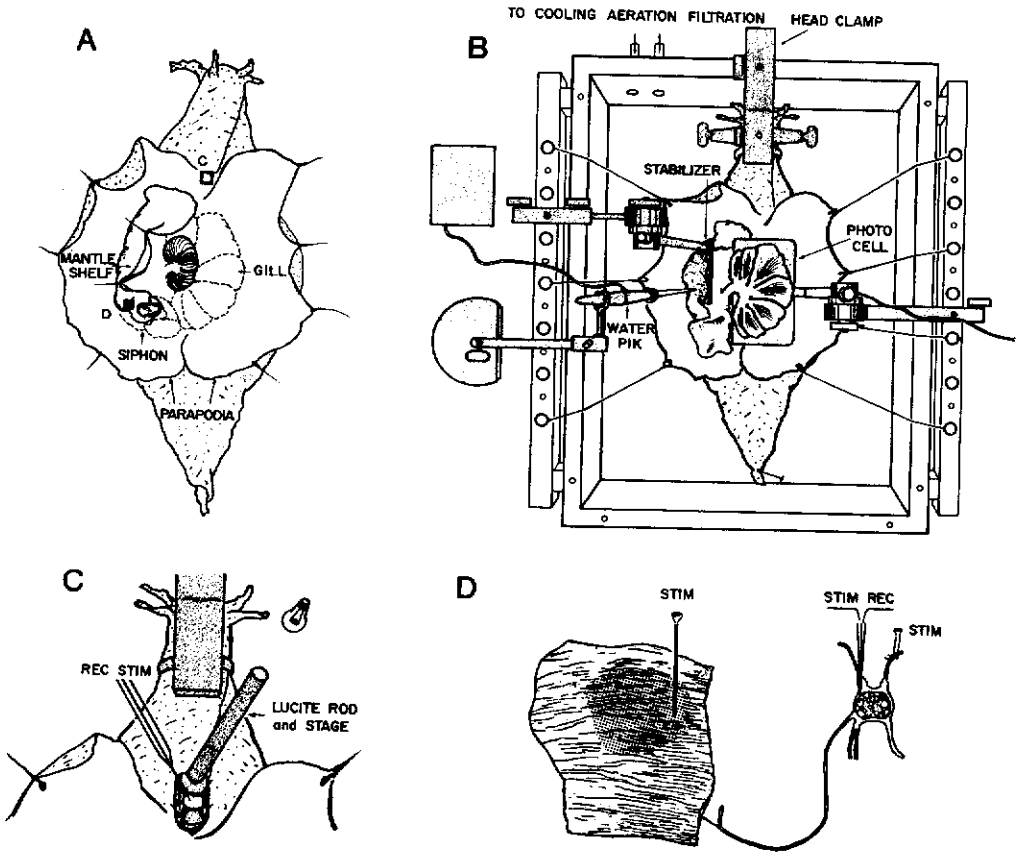

Fig. 3. *Three different preparations used to study habituation and dishabituation of the gill withdrawal reflex.* Part A, a top view of an Aplysia (as in Fig. 1).

Part B: In the intact behavioral preparation the animal was immobilized in a small aquarium. A constant and quatifiable tactile stimulus was provided by a jet of sea water from a Water Pic. The behavioral response was monitored by means of a photocell placed under the gill.

Part C: In the semi-intact preparation the animal was set up in the same way as the intact preparation, except that a small hole was made just anterior to the mantle region. The abdominal ganglion with its connectives and peripheral nerves attached was lifted out and pinned to an illuminated stage. Identified motoneurons were impaled with double barrel microelectrodes for recording and direct stimulation.

Part D: The isolated ganglion preparation. A small piece of skin from a part of the receptive field was removed with its afferent nerve to the ganglion intact. A double electrode was inserted into one of the motoneurons for recording and for continually measuring the membrane resistance. A weak constant current electrical stimulus was applied to the skin or to isolated strands of nerve.

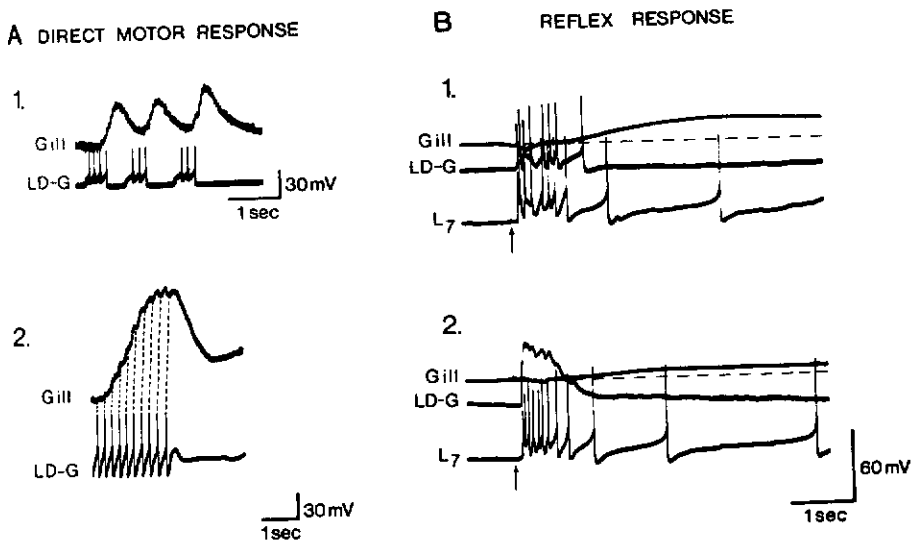

Fig. 4. *Gill responses.* Upper traces in Parts A, B, and C represent the output of a photocell placed under the gill. Lower traces are intracellular recordings from motoneurons causing gill contraction. (A1) Smooth contraction produced by small number of spikes in cell LD-G. (A2) Individual twitches produced by LD-G after it was first rapidly fired to potentiate its effects. (B) Reflex response of gill and intracellular record from two motor neurons of the gill. In Parts B1 and B2, an identical tactile stimulus was applied to the siphon (arrow). Reduction in motor response after removal of cell LD-G from the reflex path by directly hyperpolarizing the cell. The large EPSPs that ordinarily underlie the spiking in Cell LD-G are unmasked by this procedure. (From Kupfermann and Kandel 1969).

psychological point of view, with no damage at all to its central nervous system. To study the neural controls of this behavior we used a second preparation in which the ganglion was externalized by making a small hole in the neck of the animal. A stage was placed underneath the ganglion so that individual cells could be impaled with microelectrodes. Finally, the ganglion was taken out of the animal and placed in a chamber and with just a piece of the skin from part of the reflex receptive and the nerve connecting it with the ganglion.

We first tried to establish the neural circuit of the reflex (Slide 4). We attempted to define the motor components of the reflex by stimulating individual cells and recording the responses of the gill with a photo cell. Stimulation of individual motor cells proved quite effective, two or three action potentials in a motor neuron produced fairly significant contractions of the gill and individual action potentials in one of the two major neurons often produced individual one-for-one twitches of the gill. We next mapped the sensory receptive field of these cells. Here were recorded simultaneously from the motor cells and the gill. Stimulating part of a receptive field produced a large

excitatory post synaptic potential in the motor neurons causing them to fire and this, in turn, produced contractions of the gill.

With these types of experiments one can specify both the motor and the sensory components of the reflex. The fifth slide illustrates the simplified partial circuit diagram of the reflex. The sensory component consists of mechanoreceptors in the skin of the siphon and the mantle shelf which respond to light touch and activate at least five motor neurons that we have so far identified. Some of the motor neurons are very effective, indicated by the large triangles, and some less effective. The sensory input is mediated by two pathways, one is a monosynaptic pathway and the other is more complex consisting of one or more interneurons.

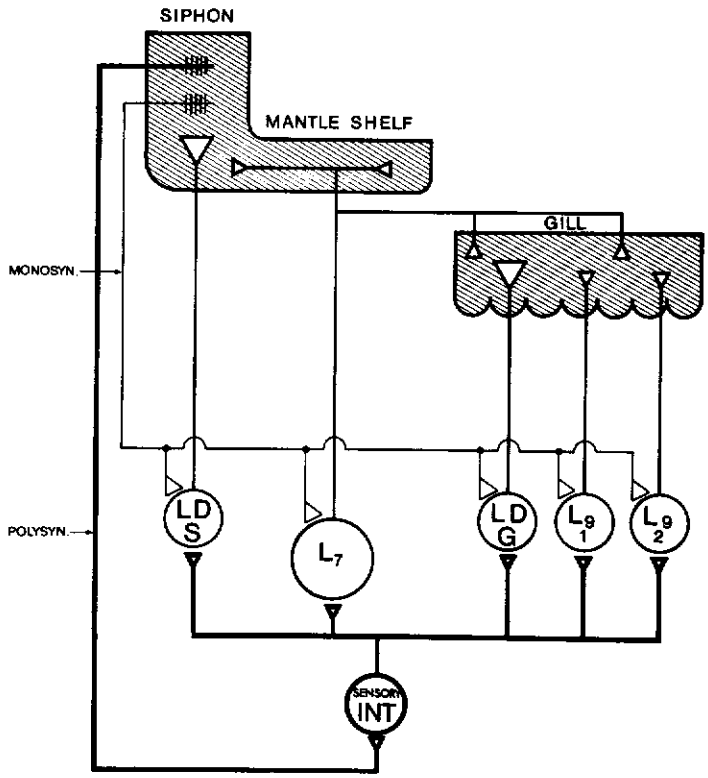

Fig. 5. *Proposed schematic diagram of circuit controlling defensive withdrawal reflex of the gill.* All of the motor neurons receive excitatory input from tactile receptors in the skin, presumably via both mono- and polysynaptic pathways. Except for the monosynaptic input to L7 which has been directly demonstrated (6), all the other indicated mono- and polysynaptic inputs have been inferred from less direct data. The diagram illustrates the total withdrawal reflex of siphon, mantle shelf and gill. In subsequent experiments we examined only the gill component of the total withdrawal reflex. (Modified from Kupfermann and Kandel 1969).

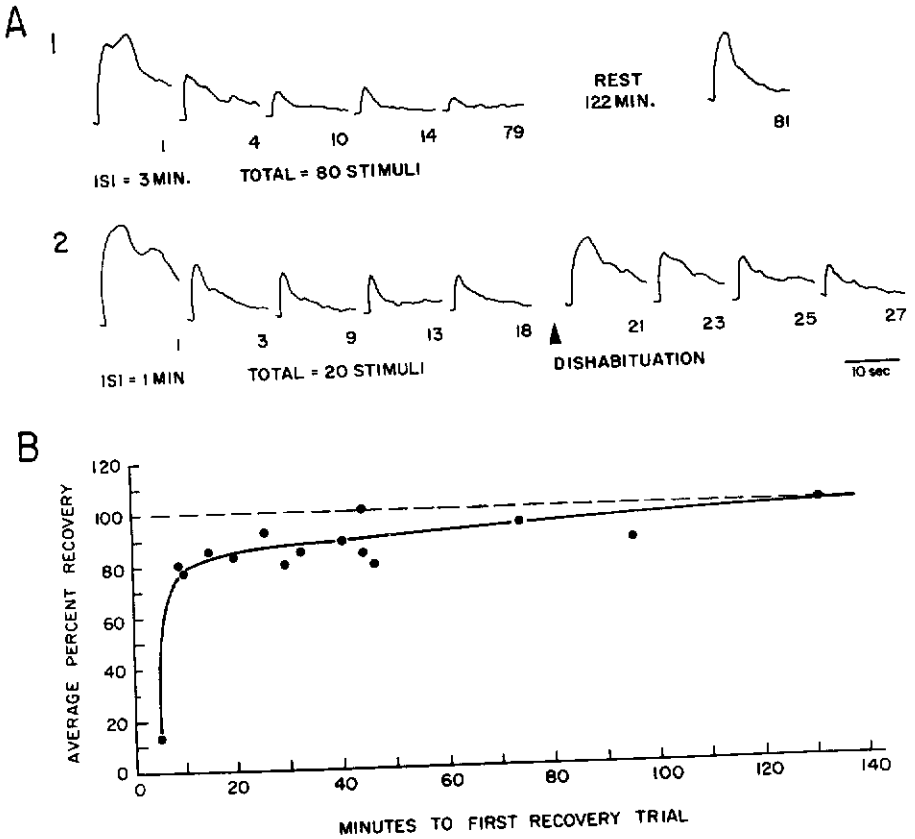

Fig. 6. *Habituation, spontaneous recovery and dishabituation of the gill withdrawal reflex.* Part A. Sample records from two separate habituation runs in a single experiment. The interstimulus interval (ISI) and total number of habituatory stimuli are indicated for each run. Part 1 shows decrement of the response with repetition of the stimulus. Following 122 min. rest the response was almost fully recovered. Part 2 shows a later run from the same experiment. After rehabituation of the response a dishabituatory stimulus consisting of a strong and prolonged tactile stimulus to the neck was presented at the arrow. Successive responses were facilitated for several minutes.

Part B. The time course of recovery was estimated by habituating individual animals with repeated stimuli and testing for the percent of recovery by presenting a single stimulus after different intervals of rest. The curve is based on 44 separate habituation and recovery runs in 27 different animals. Each point is the average of three measures (last point based on only two) taken at roughly the same interval. In longer experiments, later responses would often recover beyond the initial control level (e.g. compare the first respone in Fig. 2A1 with that in 2A2). For the purpose of this figure all responses equal to or greater than the control response for that run were assigned a value of 100 %. The shortest time in which full recovery occurred was 10 minutes whereas the longest time in which the response was not fully recovered was 122 minutes. (From Pinsker, Kupfermann, Castellucci and Kandel).

We now asked ourselves : given a partial wiring diagram for this behavior what kind of changes occur in this circuit when behavior is modified ? We therefore next examined the capability of the reflex for behavioral modifications and found that it shows habituation and dishabituation. In these studies we reduced the task of analysis by focussing only on the gill component of the withdrawal reflex.

Slide 6 illustrates that if the gill withdrawal response is repeatedly elicited the response becomes progressively smaller and finally may disappear. Once habituated response restoration can occur in one of two ways : 1) spontaneously, by not stimulating for periods ranging from 15 minutes to one or more hours, or 2) by dishabituation, that is by the presentation of strong stimulus to another point on the animal's body.

We next wanted to know : where does this change take place ? and what are the mechanisms for the habituation and the dishabituation ? We therefore addressed ourselves to the *locus* of the plastic change. We found that the change did not involve the afferent input or the motor output. The critical change occurred centrally and at least part of it involved a set of synapses between the afferent fibers and the motor neurons. This could be shown in the following way. By recording simultaneously from the motor neuron and from the gill response (Slide 7A) we found that repeated stimulation

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Fig. 7. *Correlation of contraction of the gill and responses of motor neuron (L7).* Part A. Gill contractions (top traces of each line) and simultaneous intracellular recordings from an identified motor neuron, L7 (bottom traces). Sample records are all from the same preparation. Tactile stimuli (500 msec in duration) were presented to the mantle shelf every 90 seconds. Top row : Habituation run. Stimuli were presented over a period of 21 minutes. Number of spikes in one second interval following the first evoked spike in each trace : 9, 6, 6, 4. Middle row : Partial recovery (after a 9 minute rest) and subsequent rehabituation of the reflex. Number of spikes : 7, 6, 5, 3. Bottom row : Dishabituation. Following the last habituation trial shown in the first trace a strong stimulus was applied to the siphon. The discharge of the motor neuron and the amplitude of the gill contraction progressively increased during the first three stimuli following the dishabituatory stimulus, and remained elevated for several minutes. Number of spikes 4, 5, 7, 5.

Part B. Gill responses produced by intracellular stimulation of same motor neuron illustrated in Part A, before habituation (B1) and during maximal habituation of the reflex (B2).

Part C. Magnitude of gill contraction produced by motor neuron (L7). Same experiment as in parts A and B. The abscissa indicates the number of spikes produced by depolarizing pulses, one second in duration and of different intensities. The ordinate indicates in arbitrary units the amplitude of the gill contraction as measured by the output of a photocell placed under the gill. The amplitude of the gill contraction was linearly related to the amount of photocell uncovered as the gill contracted. (From Kupfermann, Castellucci, Pinsker and Kandel).

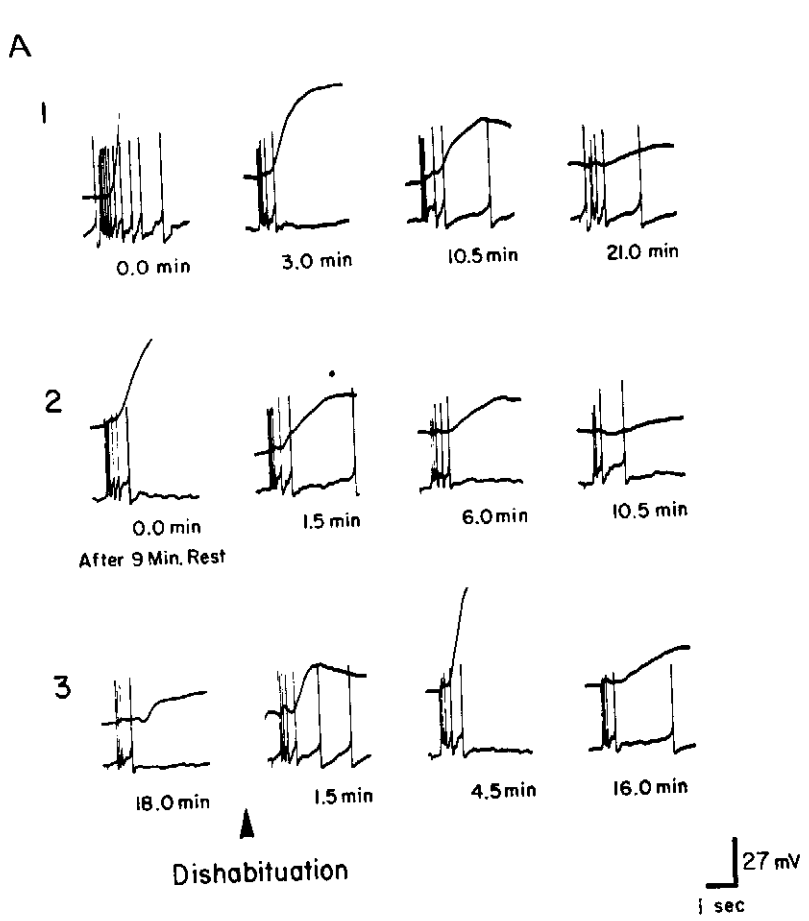

A
1
0.0 min
3.0 min
10.5 min
21.0 min
2
0.0 min
After 9 Min. Rest
1.5 min
6.0 min
10.5 min
3
18.0 min
1.5 min
4.5 min
16.0 min
Dishabituation
27 mV
1 sec
B
1
2
54 mV
1 sec
C
Response Amplitude
15
10
5
0
5
10
15
20
Number of Spikes

Fig. 7

led to a decrease of response of the motor neurons. Following repeated stimulation there was a smaller synaptic potential and fewer spikes in the gill motor neuron and a weaker gill withdrawal response than during control. With rest, recovery occurred and both the number of action potential and the amplitude of the gill response increased. With repeated stimulation the responses decremented again. Once rehabituated a stimulus to the head dishabituated the gill response and this was paralleled by an increase in the response of the motor neuron. Direct stimulation of the motor neuron produced a contraction of the gill which was unaffected by habituation. This showed that the motor neuron *per se* was unaffected (Slide 7B).

The eighth slide shows the final step in our analyses. We stimulated single receptors coming from the periphery to the motor neuron. This provided a monosynaptic test pathwat. Using this simple pathway we found that the changes in the synaptic potential associated with repeated stimulation paralleled the habituation and dishabituation. With repeated stimulation the elementary excitatory synaptic potential declined. Following a rest of 20 minutes the synaptic response recovered. Repeated stimulation again led to decrement and a stimulus to another pathway produced dishabituation.

These findings are summarized in Slide 9. This slide shows a motor neuron, L7, receiving a monosynaptic connection from the periphery. This is the cell on which most of our studies were done and we have used the monosynaptic connection on this motor neuron as a model for examining the whole reflex. In this elementary system we can localize the change to the single synapse between the sensory fibers and the motor neuron. Repeated stimulation of these pre-existant synapses leads to a striking decrease in their effectiveness.

Fig. 8. *Response decrement and facilitation of elementary presumably monosynaptic EPSPs.* The tops of the spikes have been cut off in the first two traces of Parts A, B and C.

Part A. Decrement of an EPSP in L7 produced by electrical stimulation of the skin (30 second or 10 second interstimulus intervals (ISI). After 19 minutes the ISI was shortened from 30 seconds to 10 seconds.

Part B. Spontaneous recovery (after rest of 20 minutes) and subsequent decrement.

Part C. Facilitation of the decremented EPSP following a train of stimuli to the left connective (9/sec for 2 seconds).

Part D. Decrement and facilitation of a unitary EPSP produced by a single spike elicited by intracellular stimulation of a sensory neuron (interstimulus interval 10 sec.). Top trace of each pair is from the motor neuron (L7), lower trace from the sensory neuron. First three pairs of traces illustrate consecutive stimuli. Following the third evoked EPSP a strong stimulus was applied to the left connective (7/sec for 5 seconds). The fourth pair of traces shows the facilitated EPSP 30 seconds after connective stimulation. (From Castellucci, Pinsker, Kupfermann and Kandel).

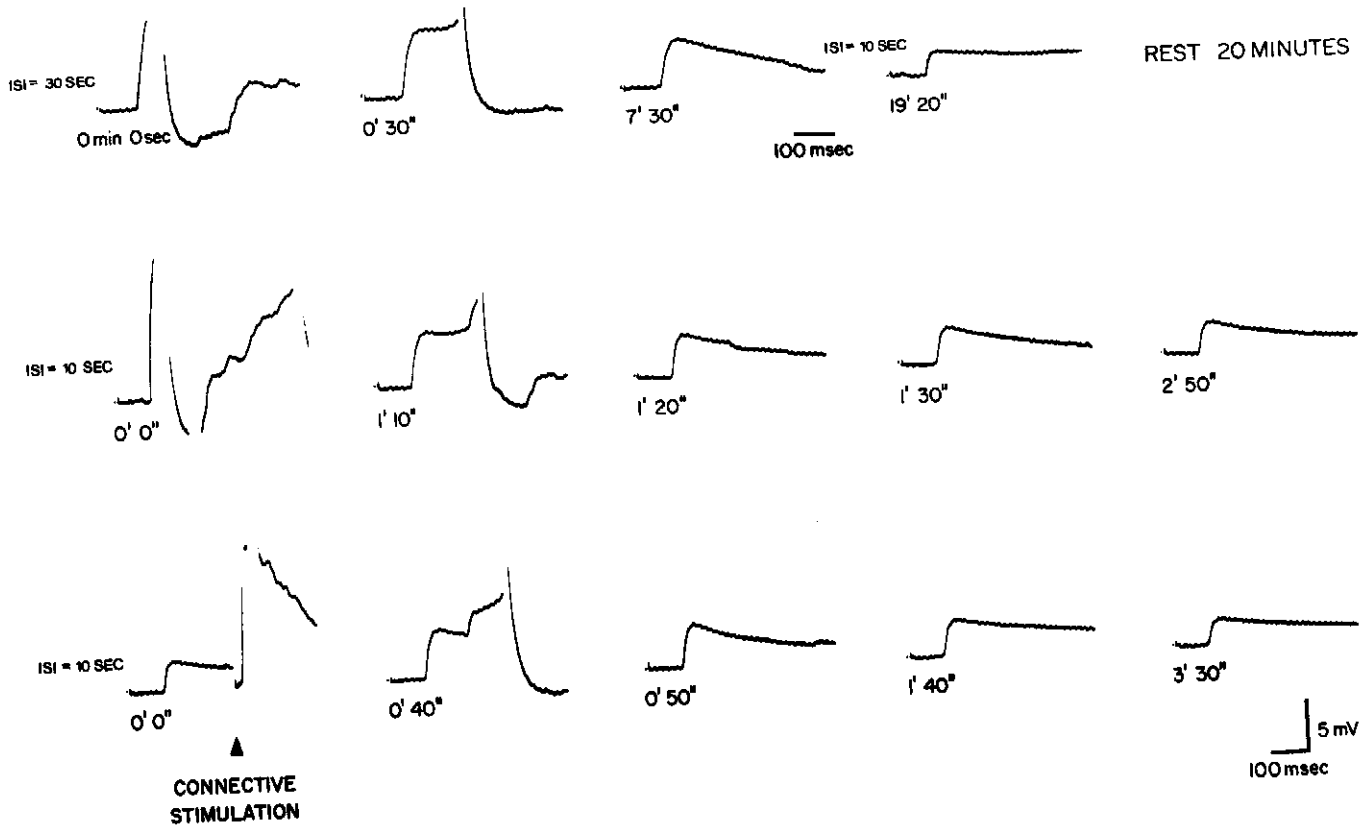

ISI = 30 SEC
0 min 0 sec
0' 30"
7' 30"
ISI = 10 SEC
19' 20"
100 msec
REST 20 MINUTES
ISI = 10 SEC
0' 0"
1' 10"
1' 20"
1' 30"
2' 50"
ISI = 10 SEC
0' 0"
0' 40"
0' 50"
1' 40"
3' 30"
5 mV
100 msec
CONNECTIVE
STIMULATION

Fig. 8

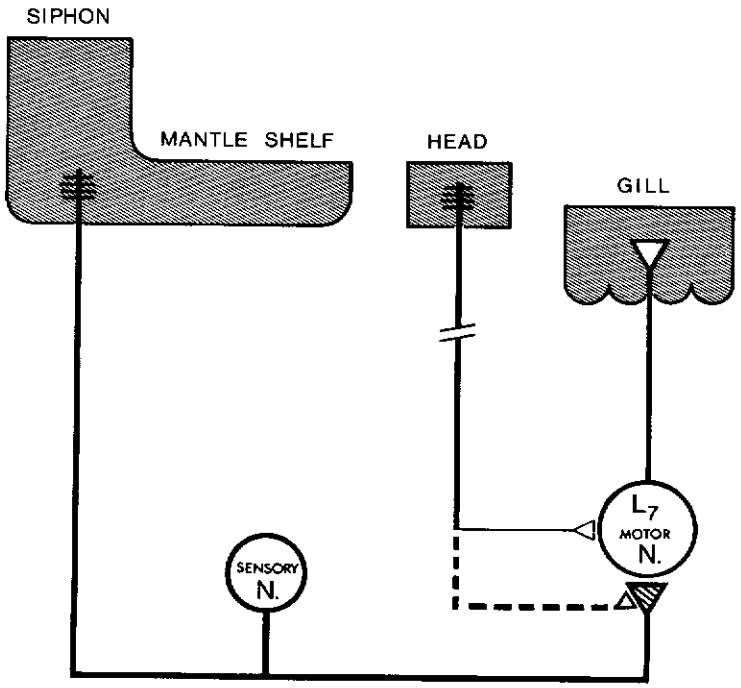


Fig. 9. *Schematic . .iring diagram to indicate the locus of the postulated plastic changes underlying habituation and dishabituation of the gill withdrawal reflex.* Habituation is due to a decrement in excitatory transmission at the crosshatched synapse. Dishabituation is due to a heterosynaptic facilitation of the same synapse. The dashed line indicates a hypothetical pathway which synapses on the presynaptic terminals of the sensory fibers and mediattes the proposed presynaptic facilitation. The dishabituatory stimulus also produces an excitatory input to the motor neuron. Dishabituation can be produced by a strong stimulus to most parts of the animal's body surface although only the head is indicated in the diagram. The exact neural pathway from the head, indicated by the interrupted line, has not yet been worked out.
(From Castelllucci, Pinsker, Kupfermann and Kandel).

Another stimulus sent in from another region of the body acts on this synapse to increase its effectiveness thereby producing dishabituation. It should be emphasized that we have used the monosynaptic pathway between the mechanoreceptor neurons and L7, one of the two major neurons of the reflex, as a model for studying the total reflex. Comparable experiments with monosynaptic inputs need to be done on other gill motor neurons, particularly on LD-G, the other major motor neuron, and on the polysynaptic pathway.

To briefly summarize, our study shows that the essential feature for studying behavior is to work out the wiring diagram of the behavioral system. Once the wiring diagram is known the analysis of the locus and the mechanism of the plastic change becomes simplified. From our results it appears that

at least for this component of a short term behavioral modification one does not have to postulate the development of new connections or the growth of new neurons. What occurs is a change of the functional expression of pre-existing synaptic connections. Studies of development and regeneration in a number of systems indicate that many and perhaps all the connections are anatomically specified at birth. What seems not to be specified is the functional effectiveness of each synapse and it is this variable that can change with environmental manipulations such as learning.

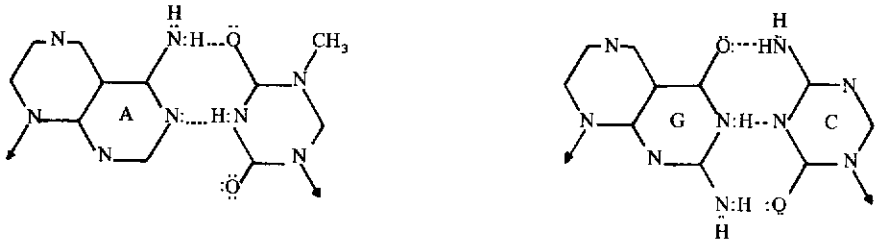
Another point that emerges from these studies is the locus for one type of information storage. In this instance it is localized to the synapse. Moreover, we have some indirect evidence suggesting that the change is localized to the presynaptic terminals. Using this type of an approach one might be able to examine how neural information is stored in a variety of more complex learning situations.

P. O. LÖWDIN : I would like to make a general comment. Depending on the nature of our conference, we have a great deal of biology and theoretical physics, and perhaps a little bit of mathematics. To at least some of us there is a missing link, namely chemistry. Actually chemistry, at least to us " physicists ", seems to be a great deal of unnecessary knowledge, a great deal of trivial names which we do not want to learn. Many of us hope that there would be a short cut from theoretical physics over to biology (laughter), some of us even hope that there would be a short cut from mathematics directly over to biology.

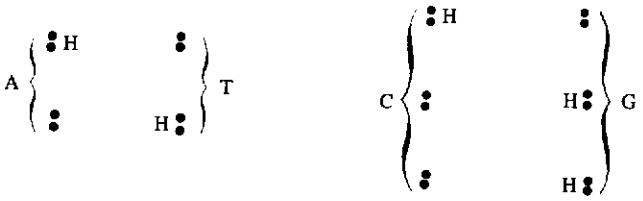
Let me take one example : in the theory of genetics, one has 64 codons and only 20 amino-acids, and you remember all that in 1954 Gamow in a beautiful picture showed how these 64 codons by means of a diamond code could immediately be reduced to twenty. However, then the biologist came along and showed that the whole thing did not agree with experimental experience. Then the biologists themselves took up the problem in 1957 from the point of view of mathematics, and Crick, Griffith, and Orgel constructed a model which in my opinion is one of the most beautiful achievements in this area, namely the " comma-less code " which reduced the 64 codons to 20 possibilities in a remarkable way. And then later in 1961, Crick and his coworkers showed that even this very nice idea was meaningless. So perhaps one should, from these examples take a little warning, that it is very difficult to go directly from physics and mathematics over to biology.

Chemistry has, at least in principle, been reduced to physics, and one of the questions we are here to discuss is to what an extent biochemistry and biology may also be reduced to physics. As a typical example, I will comment on the character of the genetic code and the long-time memory in biology. We know today, with a great deal of certainty, that the genetic information

is stored in the DNA-molecule as a linear sequence of four nitrogen bases (A, T, G, C), and that the double helix consists of two complementary sequences which are held together by hydrogen bonds. In this Watson-Crick model, there are two hydrogen bonds in the A-T pair and three hydrogen bonds in the G-C pair, and the fitting of these hydrogen bonds forms the basis for the complementarity, i.e. for the genetic code itself :



The hydrogen bonds were introduced in 1919 by Huggins, and, in the language of Lewis, a hydrogen bond is a proton shared between two electron-pairs. The memory device in the nitrogen bases is hence a pattern of protons and electron-pairs, and the bases are characterized by the following genetic templates :



The memory unit is hence either an empty electronpair or a pair with a proton :

To the delight of the mathematician, this is a typical flip-flop device corresponding to the symbols 0 and 1 in a standard digital computer using binary numbers.

From the point of view of the physicist, the electrons and protons are wave packets obeying the laws of modern quantum mechanics, and it is then possible, at least in principle, to calculate the stability of the genetic code at a given temperature on this basis. This means that there ought to be a field of " quantum genetics " dealing with problems of this type as a part of " quantum biology " in general.

There has been a great deal of chemistry involved in this discussion, and I have a feeling that, if we physicists do not look at biology in terms of

biochemistry and molecular and submolecular biology, we are not going to find the general principles which are linking theoretical physics and biology. The example chosen shows that, even in the complexities of biochemistry, there may be some very simple fundamental principles.

One more thing, so that I shall not be misunderstood. Professor Hydén has said this morning that perhaps also the short-time memory associated with learning is established in the arrangement of nucleotides in a specific way, and this may be a very good idea. But then we meet the fantastic problems discussed by Longuet-Higgins, namely the relation between the input signal and the storage record — for instance, the problem how an electromagnetic signal received by our eyes can guide the formation of a nucleic acid somewhere in our brain. Even here I personally believe that it is impossible to try to bypass biochemistry and molecular biology in order to establish the relations between theoretical physics and biology. In a coming conference, it may hence be worthwhile to put some emphasis also on the chemical points of view.

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Per-Olov Löwdin, " Proton Tunneling in DNA and its Biological Implications ". *Revs. Mod. Phys.*, **35**, 724 (1963).

Per-Olov Löwdin, " Some Aspects of Quantum Biology ", *Biopolymers Symp.*, **1**, 293 (1964).

H. HAKEN : At the last meeting two years ago, I suggested that lasers might offer useful models for biological action with respect to coherence, stability, and self control. Now, my first comment is that what you propose in the holophone could also be verified by lasers, namely the filter is a Fabry-Perot resonator and the gain is achieved by the active medium. Now comes my remark : when, in your case, the active medium is above threshold, a new phenomenon occurs which is called frequency locking. It could have some bearing on the recognition of patterns. Because if the pattern which you want to recognize is not completely exact to the original pattern, the frequency locking makes a drift of the frequencies, so that the not quite correct pattern is put in complete analogy to the correct image. I don't know if this is of any use but, perhaps I should mention this. Would you like to comment ?

H. C. LONGUET-HIGGINS : I want to make it plain that I distinguish between the holophone, which is the analogue of holography in the time dimension and, on the other hand, the associative net which is essentially a digital system. All it has in common with the holophone is that both devices record correlations.

I agree that if one were thinking about the possible application of holophonic principles to the brain, one wouldn't be content to let the matter rest

with a simple linear filter bank. One would want to consider non-linear and threshold phenomena and, in that way, perhaps, one could overcome some of the difficulties. Could I make an independent remark in relation to Pr. Lowdin's intervention ? I agree that, of course, one cannot hope to interpret biology in terms of theoretical physics without going through the intervening discipline of chemistry, but, I think that it's nevertheless possible to discuss biological problems from a mathematical point of view, without necessarily paying attention to chemical details. This, after all, was done by Mendel when he produced the concept of heredity; and one does not usually criticise Mendel for not having paid due attention to chemistry. I think that one has to converge on an understanding of biological problems from two different directions. One is the attempt to define the phenomena in an organizational manner and this, I think, is a job for mathematicians. I believe myself that the computing scientist can be very helpful here, as Pr. Schutzenberger implied in the first part of his talk this morning. The other approach is trying to interpret higher-level generalizations in terms of principles at a lower level. Here I think it is that the intervening disciplines (chemistry and physical chemistry, solid state-physics, and so forth) become essential.

A. FESSARD : Certainly the transition from lower to higher levels, from molecules, or unitary neuroelectric signals (spikes), to memory — in its mental or behavioural aspects — is not easily described or understood. However, it seems to me that as far as information representation or coding is concerned an intermediate domain has too often been overlooked, intermediate between the molecular and molar levels and offering operational properties. It is that of the so-called neuro-architectonics of the CNS tissue.

In relation to Dr. Higgins' hypothesis, I would emphasize the fact that in many places within the brain neuronal networks are such as suggesting for them a role of matrices of switchboards. Cerebellum, hippocampus, and even specific areas of the cerebral cortex represent such framework-like arrangements with their parallel axonal fields at right angle with ranks or columns of regularly spaced neurons. There traces of activity would seem to find a way to be orderly stored. Such bi- or tridimensional cartesianlike networks are capable of so many different patterns of activity — almost an infinite number indeed — that the risk of superimposition and confusion of traces with any new complex afferent message is very small by chance, and can most often result in rare and not significant overlaps, except when there is a purposeful functional analogy between the previously stored pattern and the spatio-temporal structure of the new message. Long-term memory traces may thus be preserved and approximately protected against deterioration in spite of their underlying sets of neurons being unceasingly — or almost so — submitted to an information inflow in the course of the whole life.

Finally, I would again emphasize the role that fine topological structures in the CNS seem to play in such operations as those of " pattern recognition " which are so often at work within the brain tissue as a basis for perception, memory recall, decision processes, etc... at least according to my interpretation of these phenomena.

E. Roy John : It is possible that the argument which I think Doctor Fessard was making is so, that, namely, there are enough connections and interconnections in the brain so that a subset is preserved and set aside for the storage of information about any particular event.

There is an alternative which I think we should consider and that is that some set of neurons, whether that set be the whole brain or a region therein, is involved in the mediation of many, if not all, memories, and that in this kind of a situation, the neuron can play many different roles. One need not think in terms of segregating a certain number of elements or a certain number of connections for a particular item of information to be represented. One can think of the representation of information by the distribution of activity in a set of components which can enter many different states. I would very much like to hear Professor Longuet-Higgins discuss whether the kind of model described in the work he discussed today can be extended so that the same set of representational materials can represent a series of sequential associations without interference. To me the critical question to answer is the one Professor Fessard raises : how was interference prevented ? But to answer it by assuming it is prevented by segregating a particular set of cells is unfounded. It need not be that way at all, that's a very grave assumption to make.

H. C. Longuet-Higgins : I'm very glad Professor Roy John has asked that question because I realise that I didn't deal it properly in what I said before. First of all, the question of the same material being used for all the individual jobs that have to be done. If you have an associative net, with 10^{12} intersections, then the optimal number of pulses to put in along either of the two input cables is something of the order of 40 (that's log 10^{12}). You can store approximately 5×10^8 pairs of such patterns, all in this one network without them getting in one another's way.

I think there are probably two quite different kinds of temporal memory, or process, which have to be considered. First of all there are processes which have to occur at exactly the right tempo. I believe there are probably such processes, as you yourself also believe. One can of course extend the concept of the associative net to include a dimension of time as well as of space.

Secondly, if you have a time-keeping device of some kind which will run down one side of the network, putting in pulses according to when they occur, you can in fact to exactly the same sort of thing as I described

for spatial patterns. So, I don't think there's any difficulty in principle.

The difficulties, of course, all arise in practice; I mean how do we tell where such things are in the brain, and how do we identify the exact way in which they work, and on that I'm afraid I must be entirely agnostic.

J. POLONSKY : My remark concerns Professor Lowdin's statement.
It seems to me useful to link together the four methods of approach :

— Mathematical, Physical, Chemical and Biological.

Actually, the mathematical model of a biological system keeps a very general character and only introduces logical constraints. By using a physical model of the same system, we add new constraints drawn from physical laws. The chemical model adds new restrictions and finally the biological model drives us toward a supplementary reduction of the degrees of freedom on the system.

Obviously, if the biological model perfectly describes the system, no further information is needed.

Generally, however the biological model is not complete and the cross-correlations with other models can be illuminating.

Each of the four families of constraints can supply non redundant information for describing the different functions of the structure.

A. D. MC LACHLAN : I would like to ask about this problem of the interference in the memory; it seems to me one might be able to distinguish two possible ways in which the memory is recorded.

One would be analogous to a photographic plate or film; that is, once you expose a film to light and develope the image, then that film is used up. You cannot now take a second picture on it. So, if you take this model, then you might say that a synapse, once modified, can never be modified again in its life. This is the first class. Now the second possibility would be something like a magnetic tape which we can record on. If you now try to record something again you erase the original message. So, I would like to ask, is it known if the synapses in the nervous system are modified irreversibly, or is it possible to do a second modification which completely erases the first one ?

Pr. KANDEL : Actually this is one of the great problems in neurophysiology. One can demonstrate quite clearly that synapses can undergo functional change; but the changes that have been demonstrated following nerve stimulation last only several hours. In no single case has one shown permanent changes in synaptic function following synaptic stimulation. This may be because the appropriate kind of experiment has not been done yet. The only clue that one has to more prolonged synaptic changes is that there are certain kinds of trophic influences that nerve cells convey to other cells that they innervate. For example,

a neuron may specify whether the muscle it innervates will be slowly contracting or rapidly contracting. In that instance, this represents a constantly maintained state of differentiation, which can be reversed. So there is reason to believe, at least from those analogies, that you can get long lasting changes, and that they are further modifiable as time goes on.

S. BENNETT : In our discussion of complex logic and information handling systems, we have alternated between looking broadly at the performance of the system, and more narrowly at the specifications of individual components. For example, we have talked of nucleic acids as conveyors of genetic information. And Professor Löwdin has suggested a model for memory in which a hydrogen is transferred between two hydrogen-bonded base pairs in polymer structure such as a nucleic acid. I wish to raise a question about components of nucleic acids.

Chemically, nucleic acids are special examples of a larger class of organic compounds called nucleotides. In nucleotides, one or more phosphate groups, a five-carbon sugar, and an organic base (such as flavine, a purine or a pyrimidine, are joined covalently in a linear sequence, with the sugar in the middle. Nucleotides occur in biological systems, not only in polymer form, as in nucleic acids, but also in monomer and in dimer form. Examples of monomer nucleotides are linear and cyclic adenylic acids, ADP, ATP, and guanilic acid. ATP functions vary widely in biological systems as a specific carrier of energy and of phosphate groups. Cyclic adenylic acid (CAMP) serves as an intermediary in many cellular control reactions. Of the dimers, several serve as coenzymes or as hydrogen or electron acceptors and donors in biological oxidation-reduction exchanges. In polymer form, nucleotides form nucleic acids which, as already discussed extensively here, serve in protein synthesis, in genetic coding and in other control mechanisms in cell physiology, and perhaps as just suggested by Professor Löwdin, in memory as well.

The functions of nucleotides as recognized so far are very diverse, and are not well understood in terms of the behavior of the bonding electrons in the molecules. What is it about the properties of an unusual type of chemical unit, comprised of a phosphate, a pentose sugar, and an aromatic organic base (or cation), the whole unit called a nucleotide, and appearing in monomer, dimer and polymer form, which makes it very important in biological systems, serving a variety of functions, appearing again and again in different roles ? We have asked and have partially answered the question, what is the role of nucleic acids in biological systems ? We can ask a more general, and perhaps a more profound question : "What is the general role of nucleotides in biological systems ?".

Each linear nucleotide is a dipole at physiological pH, with one or more anionic phosphate groups at one end and a cationic organic nitrogen at the

other. These are separated by an insulating portion residing in the sugar residue. Thus each nucleotide is a miniature molecular electrical capacitor. The cationic portion is a flat heterocyclic aromatic ring structure with π electron clouds on each side and a tunnelling pathway presenting a relatively low energy barrier between the two π clouds at the ring nitrogens. These π electron orbitals have their own harmonics and resonances, specified as to frequency and geometry by the configurations of the molecules.

What is there about this combination of phosphate, sugar and base, which makes the nucleotide configuration a characteristic and essential component of all living things, a participant in a great variety of control mechanisms and a medium for storing and transmitting energy and information ? Our recognition of the role of the sequence of nucleotides in nucleic acids in coding for protein amino acid sequences is a splendid triumph, but does not address itself to the question of the general molecular physiological role which all nucleotides share in common, and which must be executed by the bonding electrons in the configurations. What is there in common between the role of the nucleotide in nucleic acids, in ATP, in CAMP, and in the dinucleotide coenzymes ? An understanding of the answer to this question would convey a way to a much more penetrative grasp of the role of physical theories in biology.

P.O. LÖWDIN : I would like to say that I wholeheartedly agree, but I would also like to tell you that I have a female colleague in Stockholm, by the name of Barbro Grabe who has devoted a great deal of time and research to the study of ATP and the energy relations, and I feel that her work is very important.

J. DUCHESNE : Je voudrais faire une remarque très générale qui se rapporte à la classification usuelle des sciences, qui mène des mathématiques jusqu'à la biologie, en passant par la physique et la chimie. Il me paraît qu'une classification de cette sorte ne peut, en effet, à l'heure actuelle, entraîner que de la confusion dans les esprits. Cette situation résulte de ce qu'il devient de moins en moins souhaitable de tenter de distinguer physique et chimie, puisque les phénomènes ne se prêtent généralement pas, quant à eux, à une telle séparation. C'est ainsi, à titre d'exemple, que si l'on décrivait la distribution de charge dans une molécule compliquée, comme le font les chimistes théoriciens, on ferait sans doute de la chimie. Mais si, d'autre part, on se proposait légitimement de vérifier le résultat des calculs en faisant usage d'une méthode expérimentale, comme la résonance quadripolaire nucléaire, ainsi que nous en avons montré récemment la possibilité, on serait contraint de dire que l'on fait de la physique. Pour éviter de tels paradoxes, je crois qu'il serait bien plus simple et utile d'aborder les phénomènes selon différentes échelles, macroscopiques, microscopiques ou submicroscopiques. A ce dernier niveau, on peut considérer deux dimensions : la dimension électronique (de l'ordre de 10^{-8} cm)

et la dimension nucléaire (de l'ordre de 10^{-13} cm). Je dois dire un mot ici sur ce dernier aspect qui n'a guère été considéré jusqu'ici et qui dépasse les bornes de la biologie moléculaire. Il s'agit de ce que j'appellerais la biologie nucléaire. Je voudrais donc attirer de nouveau l'attention sur une intervention que j'ai faite au cours de ces dernières séances, selon laquelle les animaux supérieurs, au cours des phénomènes respiratoires, exhalent un anhydride carbonique variable avec la classe. Aucune différence cependant n'est relevée à l'échelle moléculaire, puisqu'aussi bien ce sont les rapports isotopiques C^{12}/C^{13} qui sont affectés. Cela me paraît avoir une grande importance et il faudra expliquer ce phénomène qui suppose l'explication des mécanismes fins suivant lesquels les enzymes, qui participent à la décarboxylation des acides du cycle de Krebs, sont capables de faire une distinction entre les différents isotopes. Au total donc, il me semble que la vision, qui consiste à approcher les phénomènes suivant les échelles, assure la possibilité de synthèses bien supérieures et aussi une stimulation bien plus puissante. Se placer dans une classification rigide, par contre, conduit à restreindre presque volontairement le champ de vision.

S.L. Sobolev : Je voudrais faire moi-même deux ou trois remarques générales avant de donner la parole aux autres participants. Il y a, en tout cas, des problèmes qui me semblent tout à fait mathématiques, qui n'ont pas été touchés dans cette discussion générale, lesquels ? Quand nous avons quelquefois formé le programme pour une calculatrice, nous savons bien que ce programme doit être écrit par exemple par une mise de commande et pas plus, car un homme ne peut pas effectivement écrire les commandes qui comprennent, qui contiennent, quelques milliards d'opérations. Mais quand la calculatrice marche elle fait 7 milliards d'opérations en développant le grand programme suivant des règles qui ont été données par un homme qui écrivait ce premier programme type. Maintenant, dans toutes les discussions que nous avons eues aujourd'hui et hier, nous n'avons pas touché à ce problème, ce second problème, comment peut-on en sortant de ce petit programme, excusez moi, peut être une mise peut être beaucoup plus, mais en sortant du programme qui pouvait être écrit dans une cellule unique, peut se développer dans ces milliards de cellules que contient le corps humain, par exemple. Ce n'est pas une exagération. Il faut connaître le mécanisme suivant lequel on le fait, et bien, comment a-t-on trouvé la déchiffration, les codes de biologie et d'embryologie, de replication, etc. Ayant fait d'abord un modèle mathématique qui contient de façon tout à fait simple, on peut donner un programme écrit par les chiffres de 0 à 3, c'est-à-dire le système de base 4. Je sais très bien que c'était un grand effort et un grand succès de deviner comment l'on peut faire cette codation. En tout cas, il fallait avoir une idée qui paraissait être banale, il fallait avoir une idée, maintenant nous n'avons aucune idée sur le procédé de développement

de ce grand programme qu'une cellule effectue et de tous les autres programmes qui sont dans le contrôle et l'automation de tout ce qui se fait dans un organisme assez développé qui est le cerveau qui le centre. Cet exemple duquel je parle peut être donne une idée de ce qu'il ne faut pas réellement séparer les branches différentes de la science qui entrent dans la biologie maintenant. Je suis absolument d'accord sur cette classification. Voici les mathématiques, la physique, la chimie, c'est inutile, peut être cela a-t-il quelque sens, mais c'est toujours absolument inutile. Si vous divisez, vous posez alors les limites sur lesquelles vous n'avez pas le droit de passer. Alors, il ne faut pas se réunir pour résoudre le problème de la biologie. La biologie n'ira jamais plus loin quand vous séparez ses pieds. Mais, au contraire, quand on travaille sur la question, il faut toujours faire des choses particulièrement importantes, et des choses partielles qui doivent être faites par des spécialistes dans cette discipline des mathématiques, de la physique, etc. Voici, c'est une petite remarque qui, je pense, peut faire un résumé de la discussion générale. Eh bien, je vous donne la parole.

Question : I want to ask a question about the other one. Dr. Kandel, is it true that every synapse you've observed is either an inhibitory or excititory, or can they be switched from the one to the other ?

E. KANDEL : Most synapses are either purely inhibitory or purely excitatory. There is one type of synapses that Wachtel and I have encountered between two identified cells in the abdominal ganglion of *Aplysia* in which the synaptic response is a function of frequency of stimulation. At low frequencies of stimulation the synapse responds in an excitatory manner and at high frequencies of stimulation it responds in an inhibitory manner. But this synapse is the exception rather than the rule.

A. LICHNEROWICZ : Juste une question qui concerne à la fois l'intervention de notre Président et celle du Professeur Longuet-Higgins. Parmi énormément de choses intéressantes, l'une des choses qui me paraît essentielle dans l'approche du Pr. Longuet-Higgins est le caractère non local, le caractère global du système qu'on envisage. Non local veut toujours dire non local *à une certaine échelle*. Peut-on imaginer qu'un système global, qui est stable pour de petites perturbations, bascule brusquement d'un état à un autre. Peut-on imaginer qu'un tel mécanisme joue à travers des hiérarchies; nous aurions ainsi d'autres systèmes déterminés par ces états je dirais globaux, macroscopiques; peut-on avoir une *hiérarchie* de systèmes analogues à ceux que vous avez décrits ?

H. C. LONGUET-HIGGINS : My understanding of French is not very good. I hope I've got your question correctly. Were you asking whether one could imagine a

system in which the storage was fully non-local and in which one could have resistance to damage ?

A. LICHNEROWICZ : Perfect. That is non local, non local is always with respect to a certain type of dynamics. I argue what you may consider for instance a hierarchy of different system of this ?

H. C. LONGUET-HIGGINS : I would say that obviously one could. It would be very interesting to design such systems and to study their properties in detail. Perhaps I could ask a question to Dr. Kandel, whose contributions interest me very much. I wonder, has anybody found in the Aplysia preparation evidence for heterosynaptic facilitation or inhibition ?

E. KANDEL : I believe prolonged heterosynaptic facilitation was first described in *Aplysia* by Tauc and myself. Subsequently, Tauc described heterosynaptic inhibition, having a similar long time course. Previously we had only a catalogue of plastic change. As a result of recent knowledge on the functional organization of the ganglion we can now examine which plastic changes are involved in which particular learning situation. What emerges from this analysis is that low frequency synaptic depression is the mechanism for habitation, and heterosynaptic facilitation is the mechanism of dishabituation.

B.W. AGRANOFF : I would like to go back a little bit, and discuss what the behaviour at changes are and whether or not known synapic phenomena are adequate to explain them. I believe that most behaviourists feel that there are certain types of memory which are temporary, equivalent to a man's remembering a phone number until he can dial it. There is no evidence for formation of a permanent trace. When long term memory is formed, that is, of several day's or weeks duration, and is then lost, the question is : has it been interfered with by some other competing memory, or has it actually been destroyed ? This is a question for which, at the present time, there is no answer. While I have the microphone, I would like to ask Pr. Hydén about the proteins that were separated on acrylamide gel. Does he have any idea what percentage of the total protein of the cell actually migrates from the sample gel ? Under usual circumstances, without the use of detergents, etc., much of the protein is insoluble and therefore not easily examined by this technique. Also a large amount of protein remains in the sample gel under many conditions. It would be useful to know, what actual fraction of the total proteins in the cell is involved.

L. ROSENFELD : My overall impression from listening to the discussions has been rather different from that of our chairman and of some others. I became

even more convinced than I was at the beginning that what physicists — whether they label themselves physicists or mathematicians or chemists — can bring to biology is, at the moment, extremely limited. First of all I notice that the basic physical, mathematical and logical aspects of this wonderful analysis of the molecular processes active in biology has been done by the biologists themselves, without any help from the physicists. On the other hand, the worst thing anybody can do, whether he is a mathematician or a chemist or a physicist or even a biologist, is to ask about an explanation of " life ", or " memory ", or any such big concept. My impression is that the only meeting point at which some collaboration may be fruitfully developed is some concrete problem of limited scope and of course of the simplest possible kind. In this conference I have been struck most by what I learnt from Dr. Spiegelman about getting smaller and smaller biological molecules and reducing the system, perhaps, to a form which may be subjected without excessive simplification to a physical analysis. And as another example, we have just heard from Pr. Kandel about this very simple system of nervous communication and nervous reactions which might perhaps also be amenable to more quantitative analysis.

J. SAUVAN : Je vais essayer de donner une réponse à la question de notre Président, et peut-être que, pour une fois, la biologie pourra servir aux mathématiques et à la physique. Le problème que vous avez posé est celui-ci : comment se fait-il que l'on puisse déclancher à l'intérieur d'un ensemble, d'un réseau, de plusieurs milliards de neurones, une telle suite d'actions alors que l'excitation n'est constituée que par une suite extrêmement courte de commandes.

Ce problème est débattu depuis longtemps en biologie, il s'agit de la querelle de l' " épigénèse " et de la " préformation ". Cette dernière postule que pour déclancher une suite d'actions extrêmement complexes (l'embryogénèse d'un homme par exemple), la description de cette énorme suite d'actions doit être préfigurée dans l'ovule ou le spermatozoïde, donc que cette genèse était " programmée ". L' " épigénèse " au contraire, et elle n'est plus discutée depuis longtemps, estime qu'à chaque étape du développement interviennent des facteurs distincts qui concourrent à l'édification de nouveaux mécanismes qui deviennent à leur tour " facteurs " des développements ultérieurs. Il en est certainement de même pour le système nerveux dans lequel l'arrivée de quelques informations nouvelles se combine avec celles qui sont déjà engrammées, créant ainsi des assemblages originaux d'informations qui se confrontent à leur tour au contenu de mémoire et agissent en quelque sorte comme le ferait un apport extérieur de nouveaux messages. Il y a un phénomène de foisonnement " logique " qui peut, peu à peu, envahir une grande portion du cerveau.

Je voudrais en profiter pour répondre également à la question de Monsieur Lichnerowicz, car les deux choses se tiennent. Avant de m'occuper de

modèles de mémoire cérébrale ,j'ai construit des systèmes qui possédaient plusieurs zones de stabilité, plusieurs zones d'équilibre dynamique. La caractéristique de ces systèmes était qu'ils déterminaient eux-mêmes la zone de stabilité qui constituait leur référence et n'en changeaient que lorsque les messages reçus de l'environnement étaient tellement perturbants qu'il n'était plus possible de respecter cette zone de stabilité. L'essentiel de ces mécanismes est constitué par leur bouclage interne sur les valeurs de consigne de leurs propres servo-mécanismes. En outre ces systèmes ne cherchent pas un " état de repos " et ne sont pas conduits par l'entropie, parce que l'approche de l'état d'équilibre constitue un facteur déséquilibrant, ce qui est facile à réaliser. Dans de tels systèmes on voit apparaître le comportement que j'ai décrit plus haut; en particulier sous l'influence d'une information extrêmement courte et de peu d'ampleur, ils adoptent soit des comportements cycliques très complexes répétés un certain nombre de fois pour être suivis de comportements cycliques différents soit des comportements acycliques très longs. Il ne s'agit jamais de comportements aléatoires ,ils sont toujours relatifs à des régulations internes bien définies, mais nombreuses.

Je pense avoir ainsi donné quelques éléments de réponse à la question comment un nombre tellement important de neurones peut-il se mettre en branle à partir d'un nombre d'informations extrêmement faible.