Draft Paper 3 March, 1964

ON THE MOLECULAR BASIS OF LONG TERM MEMORY - by Leo Szilard

This paper represents an attempt to give a model that will illustrate what kind of a molecular mechanism ~~that~~ is likely to underlie long term memory. We shall present a detailed mechanism ..... It is not possible at this point to present a detailed mechanism and the details of the mechanisms we shall present are, therefore, based on guesses. It would be rather surprising if all of these guesses would prove to be right and it is much more likely than not that as more facts be known the details will have to be provided. Modification of details need not, however, mean that basic processes upon which memory is based represents a different kind of mechanism from the one here proposed. If the conscious animal is presented by certain stimuli we shall operate a number of simple concepts, one of these is a degree of excitation of a neuron in the cortex. If an animal is exposed to such stimuli we postulate that there are certain neurons in the cortex.....

We define long term memory in the following manner:-

If an animal is presented with certain stimuli a variety of neurons in the cortex will be excited which are not plastic in the sense that they are not altered by the sensory experience. There are cells in the cortex which have remained plastic but these we shall disregard for the moment.

Among the cells of the cortex referred to above which show excitation in reponse to stimulus, there is a third group of cells which will show excitation after a lapse of days, weeks or months. The experience is recalled by the animal. The question which we shall ask ourselves, what might be the molecular basis of the ability of the animal to recall an experience in the sense defined above. The memory stored on a molecular basis might not be present immediately after the sensory experience, rather it might take seconds, months and perhaps as much as one year before the molecular storage of memory to take place in certain cells

which have remained plastic in the cortex and during this period of time we assume that the excitation of some sub-group which were excited by the sensory experience might remain excited. If there were not it would be difficult to explain that memory can be evoked not only hourse, days and weeks, later but also during the first few minutes after the experience. In order to simplify matters we shall disregard for the moment the animals ability to remember not only the . . . . . we shall concern ourselves with explaining the ability of the animal to remember everything . . . . . to remember having been exposed simultaneously to a number of different stimuli; subsequently we shall discuss the ability of the animal to remember a temporal sequence or some other one-dimensional sequence of stimuli which form a pattern. If the animal could only remember simultaneous presence of stimuli it could not distinguish between sequences of letters such as ABC and ACB. Clearly, the way to distinguish between these two involves remembering a one-dimensional sequence. In order to explain the basic phenomena which underlies long term memory . . . . . cannot, of course, by itself explain the great variety of functions that the cortex performs. What the cortex can do depends on the different kind of networks which the cortex contains and only when we know more about the function of the brain then we can begin to explain in detail how the brain in fact manages to remember patterns in general and linear patterns . . . . . the theory which assumes that the laying down of long term memory is essentially the same process which takes place in the process of differentiation that leads to the function . . . that leads to the networks of the brain. The cortex contains plastic cells which are not as yet differentiated and which undergo differentiation as a result of a sensory experience. We assume that during the animal's development and in the early post natal period both deifferentiation and, in our theory, long term memory is based. . . . . is possible.

The cells of the nervous system of a mammal, just as other cells of a mammal, contain DNA sufficient for about one million genes. We assume that each of these genes can make one specific protein which, for the sake of convenience,

3.

In the case of differentiation during animal's development each neuron will have a set of these proteins . . . . . there will occur in each neuron an induction of a particular set of these proteins with the result that these proteins will be produced at a much higher rate than the others and with exception of a third group of nerve cells which remain plastic, the set of lproteins used during the ~~latter~~ differentiation will remain induced.

3 March, 1964

MEMORANDUM ON ANTIBODY FORMATION by Leo Szilard

If the assumption is correct that a cell which has been induced to form an antibody produces thereafter that antibody at a high rate, then we must assume one or the other of the postulates listed:

(a) That the antibody or because of the antibody catalyzes the formation of an inducer -- a small molecule which can combine with the antibody in situ and permit the antibody to detach from the messenger RNA

(b) That for each antibody there is a specific repressor molecule which combines with the antibody in situ and prevents the antibody from detaching from the messenger RNA

(c) That the antibody is its own inducer in some ways and induces its own formation ~~by vitue of~~ not by removing a repressor from the cytoplasm but rather in some other way.

One could, for instance, imagine that the BB dimer combines with the A monomer which is in situ and conversely the AA dimer combines with the B monomer which is in situ and that the A and B monomers thus ~~bind~~ combined with the complimentary dimer can detach from the messenger RNA.

March 6, 1964

MEMORANDUM ON ANTIBODY FORMATION by Leo Szilard

This memorandum is based on two premises:

(a) In the secondary response the specificity of the bulk of the antibody processed is determined by the antigen used for the secondary response.

(b) When the primary response is evoked a number of omnipotential cells are induced to form an antibody to the antigen injected and thereafter these cells will produce this antibody at a high rate. If these premises are correct then one of the three postulates listed under 1, 2, and 3, must be correct.

1. The antibody must catalyze the formation of its inducer -- a small molecule which can combine with the antibody molecule where it is still attached to is messenger RNA and, by exerting an allosteric effect on the antibody molecule "in situ", the inducer must permit the antibody to detach from the messenger RNA.

2. There must be for each antibody a specific repressor molecule - a small molecule which can combine with the antibody which is still attached to its messenger RNA and by exerting an allosteric effect on the antibody molecule in situ prevents the antibody from detaching from its messenger RNA. Further, the antibody molecules in the cytoplasm (accumulated) of the cell must be capable of rather tightly combining their respective repressor molecules.

3. If an antibody does not produce its own inducer and if there are no specific repressors to the antibody which can combine with the free antibody molecules present in the cytoplasm, then the antibody or a precursor of the antibody, presumably a monomer which forms part of the antibody, must be able to induce the formation of the antibody by attaching itself to the same or another monomer which is still attached to its messenger RNA and which is destined to form part of the antibody and by exerting an allosteric effect on this monomer in situ it must facilitate its detachment from its messenger RNA.

Thus, we can, for instance, imagine that ~~the~~ by this mechanism the A gene of an antibody induces the formation of its B gene and vice versa the B gene antibody induces the formation of its A gene.

It would be tempting to assume that the B gene induces the formation of the A gene only in the presence of the antigen and the same holds for the induction of the B gene by the A gene. It would be further tempting to say that if the determining group of the antigen is slightly altered the same B gene will induce a slightly different A gene and vice versa, the same A gene will induce a slightly different B gene. It would be tempting to assume this because it would then take a much smaller number of cistrons producing A and B genes to account for the high specificity of the antibody for the antigen which induces it but if the antibodies were to owe the high specificity for the antigen to a mechanism of this sort, then it would be difficult to explain how the antigen evoking the secondary response could lead to the production of an antibody which fits the antigen used in the primary ionization area within the antigen evoking the secondary response.

March 17, 1964

Introduction

It is conceivable that the ability of the CNS to percieve a geschtalt and to recall it on the right occasion rests on the same basic biological phenomena which takes place only in the nerve cells and of which we have at present no inkling. If this were the case any attempt to guess what the molecular basis of long term memory might be would be bound to fail.

At the risk that the attempt may be premature, I propose to describe here a hypothetical molecular process which, if it actually occurs in neurons, would be capable of constituting an efficient system for recording sensory experiences and of recalling these experiences in the right circumstances that given a neural network which is adequate for the purposes, it is claimed that the particular solution to the problem of memory here described is unique. Rather, the particular model we have chosen represents a guess and we cannot expect correctly to guess every detail yet, with luck, our guess can very well correctly describe the general nature of the molecular processes which take place in the central nervous system when a sensory experience is recorded and when it is recalled.

According to the notions here adopted, the neurons in the cortex involved in recording a pattern or geschtalt and in evoking the memory of this pattern on the right occasion, fall into two broad classes. To one of these belong all neurons which full "differentiate" during embrionic development or during the early post natal period and thus achieve the final "chemical specificity" by the end of the early post natal period. To all of these we shall refer as the congenitally-determined neurons.

To the other broad class of neurons belong those which are still plastic at birth and which may acquire their final chemical specificity during the lifetime of the individual by "differentiating" under the influence of congenitally-determined neurons on occasions when certain "congenitally-determined" neurons are activated by suitable outside stimuli. It is necessary to say first of all what I mean when I speak here of chemical specificity of the congenitally-determined neurons.

March 13, 1964

Similarly, the level of the enzyme β galactosidase can be raised about one thousand-fold by lactose which is a natural inducer of this enzyme. The differentiation might consist of the elevation of one set or another of certain specific proteins brought about by a change in concentration of a certain kind of chemical agent which is specific for and conceivably identical with these proteins since one such protein is elevated ..... since once the level of such a protein is raised, it appears raised thereafter, we must either assume the proteins produced their inducer . . . . . that these proteins raised the concentration of their own inducer or decreased the concentration of their own repressor and moreover conceivably these proteins could be their own inducers. In this regard, differentiation is different from enzyme induction bacteria for the enzyme induced in bacteria does not maintain this high concentration when the external inducer is removed.

In the case of the differentiation of the nervous system with which we are here concerned, it would be simplest to assume that the neuro-specific proteins are their own inducers and that the neuro-specific proteins which are elevated in the three congenitally-determined neurons diffuses into the memory neuron when such a neuron is simultaneously exposed to volleys fired by the three congenitally-determined neurons is thereby temporarily rendered permeable for these neuro-specific proteins. Alternatively, we might consider that the specific proteins which become elevated in differentiation do not act as their own inducer ..... do not remain elevated because they act as their own inducer but rather because they chemically bind their own repressor and thereby reduce the free concentration of their own repressor. In this case we would have to say in our case that when a memory neuron is simultaneously exposed to volleys fired by the three congenitally-determined neurons, the cell membrane of the memory neuron is temporarily rendered permeable for these neuro-specific proteins. Alternatively, we might thus assume that the specific proteins which become elevated in differentiation do not act as their own inducer . . .

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do not remain elevated because they act as their own inducer but rather because they chemically bind their own repressor and thereby reduce the free concentration of their own repressor. In this case we would have to say, in our case, that when a memory neuron is simultaneously exposed to volleys fired by the three congenitally-determined neurons the cell membrane of the memory neuron is temporarily rendered permeable to all repressor molecules and the repressor molecules which correspond to the neuro-specific proteins that are elevated in the three congenitally-determined neurons diffuse from the memory neuron where their concentration is high, into those of the three congenitally-determined neurons where their concentration is low.

We have mentioned so far ..... between neurons only to the extent that they are mediated with their axons. Neurons connect with others, however, also through dendrides which originate from the cell body of one neuron and end in a bouton on the cell body of other neurons. All that we have said about the effect of volleys of nerve impulses passing down the axons and reaching other neurons through fibres of the axon which end in a bouton might hold also for depolarization waves which pass along the dendridge towards the bouton located at the end of the dendride. According to the notions here adopted . . . . Let us for the sake of concreteness of discussion assume that a neuron of class A when it responds to certain well defined visual stimulus by sending volleys along its axon towards the memory cell which we have singled out for attention. Let us more specifically assume that this neuron specifically responds to change of illumination in certain well defined portions of the visual space provided that the colour of the light employed is red, and, according to the notions here adopted, the chemical specificity of this neuron is determined by a set of n neuro-specific proteins. According to the notions here adopted this particular neuron, which shares a sub set of this set of n neuro-specific proteins with all other neurons which register a change of illumination in this particular part of the visual space, it will share any subset with all neurons which respond to a strong change in light intensity and so neurons of the visual space which respond to a stimuli which have something in common with each other that can be verbalized in a simple manner, share a sub set of the neuro-specific neurons, i.e. there is a smaller or larger overlap between them.

12. (revised)

We have so far discussed only interconnections between neurons which are connected through other axons. Neurons interconnected with each other also through dendrides which originate from the cell body of one neuron and which end in boutons touching the cell bodies of other neurons.

It would seem reasonable to assume that what we have said about the effect of volleys of nerve impulses passing down the axon of a neuron and reaching another neuron through a fibre of its axon which ends in a bouton on the cell body of another neuron, would hold also for depolarization waves which pass along a dendride from the cell body of the neuron which ends in a bouton that touches the cell body of another neuron.

Let us now consider a particular neuron of Class A which will, for example, respond to a change of light intensity by sending volleys along its axon, provided that the colour of the light employed is red, provided tha t the change in illumination is located in one particular region of the visual space and provided the change in illumination is strong rather than weak.

As we have postulated,the chemical specificity of such a neuron is determined by a set of n neuro-specific proteins. According to the notions here adopted, this particular neuron will share a subset of this set n neuro-specific proteins with all other neurons which respond to a change of illumination in red. It will share another subset of these neuro-specific proteins with all other neurons which respond to a change in illumination localized in the same area of the visual space and it will share a third subset of neurons with all other neurons which respond to a change in the light intensity, provided the change is strong rather than weak. What we have just said is meant to be a particular example of our general notion. . . . . .
As a general rule neurons which respond to a change in light intensity share a subset of neuro-specific proteins if they respond to visual stimuli having something in common with each other that can be verbalized in a simple manner.

..... change in illumination localized in the same area of the visual space and it will share another subset with all other neurons which respond to a change of light intensity in the red region of the spectrum and it will share a third subset with all other neurons which respond to a change in light intensity when the change is strong rather than weak.

This is meant to be a particular example of our notion that as a general rule congenitally-determined neurons which respond to visual stimuli share a subset with their neuro-specific proteins (i.e. that there is a smaller or larger overlap between he settof n neuro-specific proteins which are elevated) provided that they both respond to visual stimuli having something in common with each other that can be verbalized in a comparably simple manner such as, for instance, localization in the visual space, colour of the light signal, its intensity, direction, etc.

The same general notion would hold true for classes of congenitally-determined neurons which respond to sensory stimuli other than light.

Introduction

It is conceivable that the ability of the CNS to remember patters (gestalt) and to evoke and to recall the memory is based on performances of the cell of which we have at present no inkling. Any guess we might make concerning the molecular basis of long term memory might be would be bound to fail.

The purpose of this paper is to show that it is possible to describe a molecular process which might be capable of accounting for the known ability of the CNS to rember a pattern . . . to recall a sensory experience without having to ascribe to the living cell performances that are dissimilar to the performances that we have come to ascribe to them on the basis of experiments which have nothing to do with the CNS. The purpose of the present paper is to answer the question whether it is possible to describe a hypothetical molecular process which in combination with suitable chosen networks would be capable of constituting an efficient system that would be capable of recording a sensory experience and of recalling the memory of that xperience in the right circumstances. I intend to show that it is indeed possible to describe such a moleuclar process without contributing to neurons performances which are too dissimilar. . . . . for which there are no analogies in living cells outside the nervous system. The particular model here described represents a guess and it would be too much to expect for a guess to be correct in all of its details but with luck o our guess could correctly describe the general nature of the molecular processes which take place in the CNS when a sensory experience is recorded and when its memory is evoked.

14.

If, following the example of Pavlov, one sets up a response in a dog, say in response to a specific light stimulus which is specifically recording localisation of the light signal in the visual space, recording the colour of the light and the intensity of the signal, without training the dog to discriminate between the specific light signal and other light signals which have with the specific signal in common either the localization in the visual space or the colour of the signal or intensity of the signal, then such a dog may respond not only to a specific light signal but also

14. (continued)

to light signals which have one of the above mentioned attributes in common with it. One would interpret this on the basis of the notions set forth above by saying that a dog which was exposed to a specific signal once and was rewarded with food, may remember no more than one single pattern or gestalt which in principle could be recorded in a single memory neuron and there is no need to assume that a dog stores in separate neurons the memory of the signal having come from a particular part in a visual space, that the colour of the signal or its intensity or direction, however, when the dog is taught to discriminate with this particular signal and other related signals then something might be called into play in the CNS which goes beyond what we have discussed so far.

Insert in Introduction

According to the notions here adopted, there are two kinds of neurons in the cortex which are involved in a remembering a pattern or gestalt and in evoking the memory and this pattern on the right occasion.

Transcribed posthumously

While the neuro-specific proteins whose production has not been induced will remain at a low level of concentration, in order to simplify our discussion we shall postulate that in each fully-differentiated congenitally-determined neuron of the CNS there is a set of smaller neuro-specific proteins which have been induced where n is the same for all congenitally-determined neurons.

Accordingly the number of congenitally-determined neurons which differ from each other by virtue of their chemical specificity would be given by the binomial coefficient:

For $N = 10^4$ and for $n = 10$ this binomial coefficient would be of the order of magnitude of ---- 10. This is 10,000 times larger than the number of neurons of the CNS of man which is usually quoted to be about 10 ---- . As we shall see later, however, there may be reason to believe that n is considerably larger than 10.

We are now ready to formulate our first postulate which is illustrated in Fig. 1. In this figure a plastic neuron is schematically represented with this axon. As the figure indicates branch fibres of a number of different neurons contact this plastic neuron, each through its own bouton. We have singled out for particular attention a fibre from a neuron of Class (C1), a fibre from a neuron of Class (A_i), and a fibre from a neuron of Class (B_k). We assume that a neuron from Class A responds to a specific sensory stimulus, say an optical stimulus. Further, we assume that neurons of Class B are stimulated. . . . . We further assume that the stimulation of neurons of Class B evokes in the animal the sensation of pleasure or some other effect and, finally, we assume that when the animal experiences pleasure or some other effect the level of excitation of neurons of Class C is raised and that all other circumstances being equal, some neuron of Class C at random is caused to send volleys of nerve impulses along its axon. In our particular case we shall assume that the neuron of Class C firing will be the neuron C_1, which has a fibre with a bouton ending on the plastic neuron which we have singled out for schematic representation in Fig. 1. We shall designate the set of neuro-specific proteins of neurons Ai with ai, the set of neuro-specific proteins elevated in neuron Bk, with bk, and the set of neuro-specific proteins elevated in neuron C1 with C1 and our

first postulate may be illustrated by saying that if the plastic neuron depicted in Fig. 1 receives nerve impulses simultaneously from the neuron Ai, Bk and Cl, sufficient to excite a neuron to fire volleys of nerve impulses along its axon, then for a period of time of the order of several minutes, while the activities of neurons AI, BK and CL may be ascertained through some suitable auxiliary network, these three neurons will induce the differentiation of the plstic neuron in the sense that the three sets of neuro-specific proteins AI, BK and CL will be induced and each neuro-specific protein contained in any one of these three sets will thereafter be maintained in the memory neuron at a high level. The plastic neuron would thus, within a period of time of minutes, achieve its full chemical specificity and, thereafter, not be induced to further differentiation. The number of neuro-specific proteins elevated in the memory neuron can be considerably larger than n -- the number we have postulated for congenitally-determined neurons In the example given above, the neuro-specific proteins elevated in the memory neuron would be given by 3n.

One may ask at this point through what mechanism in the three congenitally-determined neurons AI, BK, CL, induce the differentiation -- in the sense of the term described above of the memory neuron which we have singled out for our attention.

At the present time we know very little about differentiation of cells during embryon development in general. There is a general belief that the differentiation of cells within a tissue may be induced in some way by cells of another tissue provided the cells of this different tissue are close proximity and in physical contact with each other. There is also an assumption that when an antigen is injected into a rabbit for the first time it may induce certain cells of the lymphatic system of the rabbit to differentiate in the sense that the antigen will induce a cell to raise the level of antibody and that such a cell will thereafter maintain that particular antibody at a high level of concentration. If this were correct the antibody-forming cells of the rabbit would remember having once been exposed to a given antigen long after that antigen has disappeared. In a paper which appeared in 1960 I postulated that the ability of the rabbit to respond four weeks after the first injection with

a copious production of antibody to that antigen may be based on such a differentiation induced by the antigen in a certain number of cells of the lymphatic system at the time when the antigen is injected into the rabbit and I have described one particular hypothetical process through which such differentiation could be brought about by the antigen. In postulating such a hypothetical mechanism I allowed myself to be guided by what we know about the phonomenon of enzyme induction in bacteria.

In bacteria we know, for instance, that the enzymes involved in the synthesis of argonin can be repressed about a thousandfold by adding argonin to the gross medium and in the circumstances there is reason to believe that argonin is a natural repressor of a number of enzymes lying in the biochemical path of the synthesis argonin.

Similarly, the level of the enzyme -- Galactozidase, can be raised about one thousandfold by lactose which, in the circumstances, may be regarded as a natural inducer of this enzyme.

However, assuming that the induction of antibody by an antigen raises the production of antibody in the lymphatic cells by a mechanism similar to enzyme induction, there is still an essential difference between this kind of differentiation and enzyme induction in bacteria. If the rate of production of an enzyme is repressed in a growing bacteria by adding the growth medium or if the rate of the production of enzyme is enhanced by adding an inducer to the growth medium, the rate of enzyme production will remain high or low only as long as the repressor or inducer are present in the growth medium. As soon as the external repressor or inducer is removed in bacteria the rate of enzyme production reverts to normal.

When we apply the term "differentiation" we mean a permanent change which remains even if the external agent disappears which brought about the change, i.e. differentiation involves some sort of a locking mechanism through which the specific protein molecule which has been raised to a high level of concentration can maintain its rate of production at a high level, i.e. the specific protein molecule must be able to act as its own inducer directly or in a loose sense of the term in some indirect manner.

The particular mechanism which I postulated in my below-quoted paper assumed that there is a repressor for each antibody molecule and that this repressor can tightly combine with the antibody molecule, raising the concentration of the antibody molecule beyond a certain threshhold , would then . . . . . that free concentration of the repressor to such a low value with the rate of antibody production would then no longer be limited by the repressor concentration but by some other factor. There is no evidence to show that it is this particular mechanism through which an antibody forming cell differentiates to produce thereafter one specific antibody nor is there so far conclusive evidence that when the antigen is injected for the first time into a rabbit it induces differentiation of the lymphatic cells in the sense mentioned above, rather the possibility is not ruled out so far that all the antigen does is to induce proliferation in lymphatic cells which, so to speak, by chance, are specifically producing the antibody which is specific for the antigin.

On the basis of the discussion which I put forward in my paper, H.S. Anker suggested in a Note to Nature that a similar biochemical mechanism might account for memory in the CNS*.

If it should turn out that nature did not in fact avail itself of the principle of differentiation in antibody production then it might still turn out that the considerations relating to antibody production have served as a crutch on which to lean in considering possible biochemical mechanisms for memory.

* Nature, 188, 938, 1960.

"New Page 8"

Further we assume that the production of an antibody is brought about by antigen in a cell of the lymphatic system through a mechanism similar to enzyme induction..... there would still remain a significant difference between the hypothetical differentiation brought about by the antigen and the enzyme induction bacteria. When the rate of production of an enzyme is repressed in a bacterial culture by adding a repressor to the growth medium or when the rate of the production of enzyme is enhanced by adding an inducer to the growth medium the changed rate of enzyme production will persist only as long as the repressor or inducer receiving remain in the growth medium. As soon as the repressor or inducer is removed the rate of enzyme production reverts to normal. In the case of the "differentiation" that we postulated takes place when an antibody is formed in the primary response the change that takes place remains even after the external agent disappears which had caused the change to take place. In order to account for this memory we must postulate some sort of locking mechanism through which a specific protein molecule, once its concentration has been raised to a high level maintains thereafter its rate of production at a high level. This means that the specific protein molecule must be able to act as its own inducer either directly or, in a loose sense of the term, in some indirect manner. Taking his departure from these considerations, H.A. Anker, in his Letter to Nature, said that a similar biochemical mechanism might also. . . . . .

As an example for such a locking mechanism I discussed in my paper the possibility that the rate of production of each antibody is controlled by a specific repressor molecule and that such a repressor molecule can tightly bind. ..... combine..... with the antibody molecule for which it is specific. Raising the concentration of the antibody molecule beyond a certain threshhold would then reduce the concentration of the free repressor to a low value at which the rate of antibody production would be limited by some factor other than the concentration of the repressor .

Taking his departure from these considerations, H. Anker, in his letter to Nature, said that a similar biochemical mechanism might also account, not only for the memory laid down in the primary response to an antigen, but it might also account for memory in the CNS.

Today, four years later, there is still no conclusive evidence to show that when an antigen is injected for the first time into a rabbit it induces differentiation of the lymphatic cells in the meaning of the term discussed above and so far the possibility has not been ruled out that all that the antigen does is to induce proliferation in the lymphatic cells which happened to be producing the antibody which is specific for the antigen. But, while there is still no conclusive evidence that memory established through differentiation, plays a role in the antibody response, memory undoubtedly plays a role in the CNS and we propose, following Anker's suggestion, to assume that memory in the CNS is laid down in a molecular process in which a specific protein is induced and thereafter maintains its high level of concentration through some sort of locking mechanism.

This means that a specific protein molecule must be able to act as its own inducer either directly or, in a loose sense of the term, in some indirect manner. The specific protein molecule could, for example, reduce the concentration of a molecule which acts as its repressor or the specific protein could enhance the production of a molecule which acts as its inducer, or else it could be its own inducer, or else it could be composed of two components which induce each other.

Just what the mechanism of the induction process involved in laying down the memory is, we cannot say with any reasonable assurance at this time. All we shall say at this point is as follows: the differentiation which takes place during the embryon development in the nervous system, which we have assumed leads to the presence of a set of neuro-specific proteins in the congenitally-determined neurons, presupposes the operation of some sort of locking process through which the chemical specificity of the congenitally-determined neuron is maintained once it has been established. We shall assume that the same process operates in the differentiation which, according to our assumptions, a memory neuron which we have singled out for attention undergoes when it is induced to undergo differentiation by the three congenitally-determined neurons A_i, B_j and C_k.

Introduction:

The subject matter of this paper is the capability of the CNS to recall and record an experience and the molecular processes on which such a capability could conceivably be based. The chances are that we do not know enough about the biological processes that may occur in the living cell to hazard a guess regarding the nature of the molecular process involved in the CNS in the cause of recording and recalling an experience. Nevertheless it may make sense to ask whether it is possible to think of a hypothetical molecular process which could conceivably take place in the cortex and on which an efficient system of recording and recalling an experience could be based - in nerve networks which are attached for the purpose.

If the average number of neurons which a memory will occupy is comparatively small and if the networks which are adequate for the recall of an experience in the right circumstances require a comparatively small number of neurons then the system may be regarded as efficient.

I propose to describe here a hypothetical molecular process which appears to be efficient in the meaning of the term just defined. And in these circumstances there is hope that if worse came to worst the final verdict would be"si non é vero é ben travato."

The particular model given belo is not meant to represent a unique solution to the problem of memory. The choice of the particul model here described is based on a guess and we cannot expect correctly to guess all the details yet, with luck, our guess might correctly describe the general nature of the molecular processes which take place in the CNS when the experience is recorded and when it is recalled.

There is also the notion that when an antigen is injected into a rabbit for the first time it may induce certain cells of the lymphatic system of the rabbit to differentiate in the sense that the antigen may induce a cell to raise the level of concentration of antibody which is specific for it and that thereafter this cell will maintain this particular antibody at a high level of concentration. If this is correct then we may say that the antibody forming cells of the rabbit, which have been once exposed to a given antigen will thereafter, by virtue of the antibody which they will forever maintain at a high concentration, remember having been exposed to this antigen.

If the same antigen is then injected into the rabbit after about 4 weeks it will induce proliferation in the cells which contain the antibody at a high concentration. This accounts for the copious production of the antibody in the socalled secondary response.

When I postulated that a memory of this sort plays a role in antibody formation in 1960 I was guided by what we know about the phenomenon of enzyme production in bacteria. We know, for instance, that in bacteria the enzyme involved in the synthesis arginin can be repressed about 100-fold by adding arginin to the growth medium and there is reason to think that arginin is a natural repressor of a number of enzymes lying on the biochemical pathway of the synthesis of arginin. Similarly, we know that in bacteria the level of the enzyme β-galactosidase can be raised about 1000-fold by adding lactose to the growth medium and there is reason to think that lactose is a natural inducer of this enzyme. The change rate of enzyme production will persist, however, as long as a repressor or inducer remains in the growth medium. As soon as a repressor or inducer is removed the rate of enzyme production reverts to normal, i.e. in a growing bacterial culture, the bacteria do not remember having been exposed to a repressor or an inducer. In the case of differentiation, however, once the level of concentration of a specific protein molecule is raised the level of this protein molecule remains high even though the somatic cell undergoes many divisions and in order to account for this phenomenon, one would have to assume that once the concentration of a specific protein molecule is raised above a certain threshhold thereafter the rate of production of this protein remains high.

In contrast to this, if a somatic cell undergoes differentiation during embryon development and the new set of specific proteins appears in the cell in high concentration, this will thereafter contain these proteins at a high concentration even thought it may undergo many cell divisions.

In this respect the differentiation of somatic cells is different from enzyme repression and induction in bacteria. When a somatic cell undergoes differentiation during embryon development there may appear in the cell a number of new specific proteins raised to a high level of concentration. The differentiation might have been initiated by some external causative agent but if, subsequently, the differentiated somatic cell divides in the absence of any such external agent the descendent of the cells will continue to contain these new specific proteins at a high concentration. Similarly, if we wish to interpret the formation of an antibody in respnse to the first injection of the antigen as differentiation of certain cells of the lymphatic system induced by the antigen, then we would assume that once the concentration of antibody is raised above a certain threshhold in the lymphatic cell thereafter that cell maintains the production of that antiboy at a high level if such a cell divides its descendents will then continue to produce the antibody at the same high level.

This implies some sort of a locking device and one can think of a number of different models to account for the phenonenon of locking. For instance, if one assumes that the rate of production of each antibody is controlled by a specific repressor molecule which tightly combines with the antibody molecule for which it is specific, raising the concentration of the antibody molecule beyond a certain threshhold would then raise the concentration of the free repressor to a low value and correspondingly the antibody would then be produced at a high rate. Taking his departure from these considerations H. Anker suggested that the biochemical mechanism which accounts for the memory recorded in the response to the first injection of the antigen might also account for the memory which operates in the performance of the CNS.

We propose to assume here, folloiwng Anker's suggestion that memory in the CNS is laid down in the molecular processes in which a specific protein is induced and is thereafter maintained at a high level of concentration.

A number of different models could be postulated in order to explain how a neuro-specific protein once its concentration is raised above a threshhold level, may thereafter maintain its concentration at a high level. Among the various possibilities are that the neuro-specific protein, if present at a high concentration, lowers the concentration of the free repressor either produces it own inducer or acts as its own inducer. Alternatively, if there are specific repressors for neuro-specific proteins one might postulate a locking mechanism of the kind I have discussed above in connection with the formation of antibodies. Just what the mechanism of the induced differentiation of a memory cell might be cannot be stated with any reasonable assurance at this time but all that we need to say at this point is as follows.

The differentiation which takes place during embryon development of nervous system which leads to the presence of a set of neuro-specific proteins in the congenitally-determined neurons presupposes the operation of some sort of locking process through which the chemical specificity of the congenitally-determined neuron is maintained once it has been established. We may assume the same locking process would operate also in the memory neuron which we have singled out for attention, after its differentiation has been induced by the three congenitally-determined neurons A_i, B_j and C_k.

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Differentiation might have something in common with enzyme induction in the sense that in both cases a gene which was potentially capable of producing an enzyme either does not produce an enzyme or produces an enzyme at a very low rate until something happens that induces the formation of the enzyme. Thus, we know, for instance, that in bacteria the level of the enzyme Galactosidase can be raised about a thousandfold by adding lactose to eh growth medium and there is reason to think that lactose is a natural inducer of this enzyme. The change rate of enzyme production will persist in the growing bacteria culture, however, only as long as the inducer remains in the growth medium. As soon as the inducer is removed the rate of enzyme production reverts to normal, i.e. in a bacterial culture the bacteria do not remember for long having been exposed to the inducer. While the lactose is present the gene which is specific for the β-Galactosidase produces a specific RNA molecule - the messenger RNA, which is specific for the β-Galactosidase and this messenger molecule produces the polypeptide chain with the base sequence of the messenger chain determining the amino sequence of the polypeptide chain. Four of these polypeptide chains combine to form the enzyme β-Galactosidase.

This messenger RNA is not formed in the absence of the inducer lactose.

Differentiation of somatic cells is at least in one respect different from enzyme induction bacteria.

In contrast to this, when a somatic cell undergoes differentiation during embryon development there appears in the cell a number of new proteins, raised to a high level of concentration and thereafter the cell, as well as its descendents, continue to contain these proteins at a high concentration. In order to account for this phenomenon of persistence - which is absent in enzyme induction in bacteria - one may assume that once the concentration of a protein molecule is raised above a certain threshhold, thereafter, the rate of the production of this protein molecule remains high, i.e. the phenomenon of persistence seems to imply the operation of some sort of locking mechanism. It seems to imply that a specific protein molecule must act as its own inducer either directly or in some indirect manner. How a memory neuron which we have singled out for attention may be induced to differentiate under the influence of the three congenitally-determined neurons A_i, B_j, C_k, we may take it for granted that after such a memory neuron acquires its final chemical specificity this chemical specificity will persist virtually the same locking mechanism which operates in the congenitally-determined neurons which attain their final chemical specificity during embryon development. This, however, does not tell us in what manner the congenitally-determined neurons can induce differentiation in a memory neuron, nor does the phenomenon of enzyme induction provide us with any real clue, still we know, in the case of enzyme induction in bacteria at least this much.

While lactose is present in the growth medium, the gene which is specific for the β-Galactosidase produces an RNA molecule - a messenger RNA which is specific for β- Galactosidase. The base sequence of this messenger RNA determines the amino acid sequence of the polypeptide chain and four of these polypeptide chains combine to form the enzyme Galactosidase.

It has been established that in the absence of the inducer the gene which is specific for β Galactosidase is shut off and the corresponding messenger RNA is not formed.

In a congenitally-determined neuron we may assume that in addition to a set of n neuro-specific proteins which are present at a high concentration, there are also present at a high concentrtion the corresponding messenger RNAs and perhaps some third class of compounds which are specific for the set of proteins that are maintained at a high

concentration and have something to do with maintaining these proteins at a high concentration. It is impossible to imagine that if such a neuron were in physical contact with a memory neuron and if, under certain circumstances, the membranes separating the two neurons from each other become permeable, either for a neuro-specific protein or for the messenger RNAs of neuro-specific proteins, are the class of compounds which are responsible for the phenomenon of persistence.

On this basis we may assume that any congenitally-determined neuron in which a set of n neuro-specific proteins is maintained at a high concentration, the corresponding messenger RNA molecules are also produced at a high rate. We do not know what role the neuro-specific proteins or the other messenger RNA will play in the locking mechanism which is responsible for persistence. It is even conceivable that a third class of compounds which are specific for these proteins which are present at a high concentration ~~and~~ are responsible for the persistence of these proteins. In order to account for the differentiation of the memory neuron which we have singled out for attention, under the influence of the three congenitally-determined neurons, we shall postulate that when a congenitally-determined neuron fires the membrane covering the boutons located at the end of branch fibres of such a neuron becomes permeable and similarly, we shall postulate that if, under the influence of volleys reaching the cell body of the memory neuron through branch fibres of congenitally-determined neurons A_i, B_l and C_k, the memory neuron is excited to the level where it begins to fire then the membrane covering the cell body of the memory neuron also becomes permeable.

Under these circumstances, the best we can do is to assume there is a class of compounds which plays a key role in the differentiation of the congenitally-determined neurons. We may assume that each key compound is specific for one neuro-specific protein but we cannot say whether the key compound is an inducer or a repressor, whether it is a protein molecule or an RNA molecule and we cannot even exclude the possibility that the key compound might be the neuro-specific protein itself.

In order to account for the induction of differentiation by the three congenitally-determined neurons of the memory neuron, which we have singled out for attention, we postulate the following:

Whenever a congenitally-determined neuron fires the membrane covering the boutons at the end of the branch fibres of its axon become permeable for the class of key compounds. We further postulate that if under the influence of volleys reaching the cell body of a neuron through a branch fibre of the congenitally-determined neurons A_i, B_l and C_k the memory neuron is excited to the level where it fires then the membrane covering the cell body of that memory neuron becomes permeable for the entire class of key compounds. In these circumstances if the memory neuron fires under the influence of the activity of the congenitally-determined neurons A_i, B_l and C_k key compounds can freely diffuse from these congenitally-determined neurons ~~in which~~ into the memory neuron and vice versa.

The best we can do in these circumstances is to assume that there is a class of compounds which plays a "key role" in the differentiation and persistence of the chemical specificity of the congenitall-determined neurons, that each key compound is speciric for one specific protein and that a key compound is present at a high concentration in those congenitally-determined neurons in which corresponding neuro-specific proteins are maintained at a high concentration. We cannot say, however, whether the key compound is a protein molecule, a RNA molecule or some other kind of molecule, nor can we exclude the possibility that the key compound might be the neuro-specific protein itself.

In order to account for the induction of differentiation of the memory neuron by

the three congenitally-determined neurons specified above, we may now postulate the following. Whenever one of the congenitally-determined neurons fires the membrane covering the bouton at the end of the branch fibres of its axon become permeable for all key compounds and further that if, under the influence of volleys reaching the cell body of the memory neuron through branch fibres of axons of the congenitally-determined neurons Ai, A_1, and C_k, the memory neuron is excited to the level where it fires where the membrane covering the cell body of the memory neuron also becomes permeable for all key compounds, in the sense that such key compounds can diffuse from the boutons of the neurons A_i, B_1 and C_k into the cell body of the memory neuron.

When the concentration of the key compounds which diffuse into the memory neuron from the congenitally determined neurons A_i, B_1 and C_k reaches the threshhold level from then on the hypothetical locking mechanism goes into effect with the result that from then on the three sets of neuro-specific proteins which are elevated in the congenitally determined neurons Ai, B_1 and C_k are raised to a high level of concentration and are kept at that high level of concentration thereafter in the memory neuron. We assume that the memory neuron then has attained its final chemical specificity and will cease to be plastic. About the only significant way in which it will thereafter differ from a congenitally-determined neuron is that a number of neuro-specific proteins elevated is larger than n . In our particular case, for example, this number would be 3 n.

We assume that it may take a number of minutes for the key compounds to diffuse into the memory neuron and in sufficient quantity to reach threshhold concentration and the membranes involved remain permeable for such a period of time. We assume in particular that the excitation of the congenitally-determined neurons Ai, B_j and C_k is in one way or another maintained for a few minutes and that as long as they remain excited the relevant membranes remain permeable for the key compounds. Protoplasm within the axons of congenitally-determined neurons A_i, B_j and C_k plays an important part in facilitating the diffusion in the key compounds from the boutons of the congenitally determined neurons into the cell body of the memory neuron.

We assume that a memory neuron will thereafter cease to be plastic. It will still be distinguishable from a congenitally-determined neuron, however, in as much as the number of neuro-specific proteins elevated in a memory neuron can be considerably larger than n; for example in our particular case this number would be 3 n.

Having described how a memory neuron may under a permanent change under the influence of sensory stimulus it still leaves open the question of how this sensory experience may be recalled. Let us now consider neurons whether they be congenitally-determined neurons or memory neur9ns which have ceased to be plastic and have reached their final chemical specificity through differentiation induced by certain congenitally-determined neurons. Let us now consider a number of such neurons and assume that a fibre of the axon of each one ends with a bouton which touches the cell body of the neuron as illustrated schematically in Fig. 2. According to our notions, the contribution to the excitation of the neuron by branch fibres which end in a bouton on the surface of the cell body of the neuron will be proportional to the concentration of some transmitter substance which shall call "acetylcholine" which is asymptotically approached when a volley of nerve impulses having some frequency travels down the branch fibre into the bouton. We shall assume that the rate of production of acetylcholine is proportional to some universal function of the frequency of these nerve impulses . We shall further assume that the rate of concentration reached by acetylcholine for any given rate of production will be universally proportional to the concentration of an enzyme which we shall call cholinesterase. We Shall further assume that cholinesterase is produced in all boutons at the same concentration.

Let us now consider a neuron which has a branch fibre of its axon which ends in a bouton on the surface of the cell body of the neuron represented in Figure 2, and which, if it fires, makes a positive contribution to the excitation level of the neuron of Fig. 2. For the purpose of this discussion we shall assume that the contribution made to the excitation of the neuron of Fig. 2 will be proportionate to the concentration of some transmitter substance, to be designated as acetylcholine, which is asymptotically approached with nerve impulses travelling down the branch fibre into the bouton.

We shall assume that the rate of production of acetylcholine is proportionate to some function ..... to some function ..... of the frequency ..... of this nerve impulse and we shall further assume that the rate of destruction of acetylcholine in the bouton will be proportionate to the concentration of an enzyme, to be designated cholinesterase. Finally, we shall assume that the enzyme cholinesterase is produced in all boutons at the same rate but that it is inactivated at the bouton neuron interface at some rate ..... which depends on the chemical specificity of the bouton and the chemical specificity of the neuron in a manner that we shall describe below.

If from some point in time on acetylcholine is produced at a rate proportionate to x and if it is destroyed at a rate which is proportionate to z, then it follows that the concentration s of acetylcholine in the bouton will asymptotically approach ..... that acetylcholine will approach in the bouton is proportionate to ..... and inversely proportionate to the cholinesterase concentration z in the bouton.

Since we have assumed that cholinesterase is produced in all boutons at the same rate but destroyed at different rates in the different boutons ..... x it follows that cholinesterase concentration z is inversely proportionate to x and it further follows that the acetylcholine level which is asymptotically reached in any bouton that receives nerve impulses at the frequency nu is proportionate to the product .....

For the sake of simplicity of discussion we shall assume that each neuro specific protein which is maintained at an elevated concentration in a neuron is maintained at the same concentration. On the basis of these simplifying assumptions we shall now present a biological model which leads us to our second postulate. This postulate shows that x in the above given formula is given by the overlap of the set of neuro-specific proteins elevated in the bouton and the set of neuro-specific proteins elevated in the neuron. x is defined by the number of neuro-specific proteins which these two sets have in common. If the neuron is one which is congenitally-determined and if the bouton ~~belongs~~ comes from a congenitally-determined neuron then the overlap x can be anything between zero and n. The same holds true if the neuron is congenitally-determined but the bouton belongs to a memory neuron or fice versa. However, if the neuron is a memory neuron and the bouton also belongs to a memory neuron, then the overlap x can be considerably larger than n.

We are led to the postulate by assuming that the neuro-specific proteins of the neuron are located in the membrane covering the cell body of the neuron and the neuro-specific proteins of the neuron to which the bouton belongs are located in the membrane which covers the bouton.

We are now ready to present a biological model which relates to the rate of destruction of the enzyme cholinesterase at the bouton cell body interface. This biochemical model is based on the assumption that molecules of the set of neuro-specific prteins which characterize the neuron to which the bouton belongs are embedded in the membrane that covers the bouton and that molecules of the set of the neuro-specific proteins which characterize the neuron are embedded in the membrane which covers the cell body of the neuron. We assume that such a neuro-specific protein in the membrane covering the neuron can form a dimer at the interface with a molecule of the same neuro-specific protein provided that molecules of the same neuro-specific protein are contained in the membrane of the bouton. We assume that when such a dimer is formed the neuro-specific protein contained in the bouton undergoes an allosteric transition and that a molecule which is so dimerized can ~~combine~~ bind and not only bind but also

inactivate cholinesterase. A rather similar phenomenon is known in the case of antibody molecules.

Fig. 2 shows schematically four differently shaped neuro-specific proteins contained in the bouton embedded in the membrane covering the bouton. Each of these is dimerized with a molecule of its own kind contained in the neuron. In addition, the figure shows three different neuro-specific proteins contained in the neuron for which there is no counterpart in the bouton. This is just a rough illustration. We shall assume here that every neuro-specific protein embedded in the membrane covering the bouton inactivates cholinesterase at the same rate, i.e. the number of cholinesterase molecules destroyed is proportionate to the cholinesterase concentration in the bouton and the number of dimers at the interface of the neuron and the bouton. Further, for the sake of simplicity we shall here assume that each neuro-specific protein each belongs to the set that characterizes the bouton and that each neuro-specific protein which belongs to the set which characterizes the neuron is represented by the same number of molecules at the bouton neuron interface. With this simplified assumptions one might then say that the rate at which cholinesterase is inactivated in the bouton is proportionate to its concentration and is for any given conentration proportionate to the overlap number of the sets of neuro-specific proteins characterizing the bouton and the neuron depicted in Fig 2. respectively. The overlap x is defined as a number of neuro-specific proteins which these two sets have in common.

If the neuron is one which is congenitally-determined and if the bouton also comes from a congenitally-determined neuron, then the overlap x is a number between zero and n. The same holds true if the neuron is congenitally-determined but the bouton belongs to the memory neuron or vice versa. However, if the neuron is a memory neuron that was induced to differentiate and if the bouton also belongs to a memory neuron that has been induced to differentiate then the overlap x can be considerably larger than n.

There is a class of dreams further described by Freud and discussed by him in great detail which is centered on what Freud calls the "traumgedanke". According to the notions adopted here one traumgedanke would just about tie down one nueron which is capable of recording a memory. This means that if an individual were given information contained in a simple sentence, composed of subject, predicate and object, every few seconds, 24 hours a day, under conditions where he would be able later on to recall in the right circumstances what he was told, and if this went on for a hundred years then the memory that he would store would irreversibly tie down just about 10^9 neurons. This is 10 times less than the total number of neurons in the cortex of man, which is usually quoted to be ..... I t may be seen from this that models concocted which are less efficient than our model by a factor of say 1000 would not be able to account for the mental capacity of man.

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We shall consider a number of memory cells, each of which remains plastic until it is transprinted. Each of these memory cells has an input coming from a number of A type neurons which differ in their chemical specificity but have an overlap with A_i. Further all these neurons have one input from one neuron of class B.

Further, all these plastic neurons have a non-transprinting input from a neuron of Class C. We shall make now the following assumptions: None of these plastic neurons can be transprinted except if they receive an input from the neuron of Class C and further a neuron of Class fires only when either B fires or at least one of the plastic neurons that has been transprinted with the character of B fires. The neuron of Class C will also fire if the neuron B does not fire but one of the plastic neurons that has been transprinted with the character of B fires. Neuron C will not fire if both the neuron B and at least one of the plastic neurons which have been transprinted with the character B fires.

Now let us then assume that the neuron AI is excited and that shortly thereafter the neuron B is excited and one of the plstic neurons which receives nerve impulses simultaneously from Ai, B, and C fires and while it fires gets transprinted and adopts the character of Ai and B. We shall call this the first fully transprinted neuron of the group we are considering.

Let us assume that what happens next is that a neuron Ai + δ gets excited but B does not. Assuming, say 50% overlap between A + δ and Ai we shall assume that the neuron No. 1 will fire and because neuron B does not fire, neuron C will fire. The group of neurons we are now considering will now receive nerve impulses from C and Ai + δ and one of them, we shall assume, will be induced to fire and while it fires it will be transprinted with the character AI + δ but not B. We shall call this neuron the anti-neuron No. 1. We shall postulate that every neuron of the group receives inhibitory fibres from all other neurons of the group.

Because of the overlap between Ai and Ai + δ, neuron No. 1 will fire and because neuron B did not fire, neuron C will fire.

Let us now assume that neuron No. 1 has been transprinted and thus a conditioned response has been established which in our terminology means that a conditioned response has been established. Let us now see what has happened if neuron Ai is fired again and neuron B is fired also. The transprinted neuron No. 1 will in this case fire and accordingly the neuron C will not fire but if neuron C does not fire, none of the plastic neurons in the group will fire either and thus, in conformity with our first postulate the reinforcement of the conditioned response which is already established does not lead in our system to to any recording of memory.

We will now consider the process of extinguishing. Let us, therefore, assume that neuron Ai fires but neuron B does not fire. In this case the transprinted neuron No. 1 will fire and accordingly the neuron C will fire. As a result of the combined firing of neuron Ai and C any plastic neuron may now fire and in the process of firing be transprinted with Ai but not with B.