The significance of the increased activity of CPK and aldolase in the serum of acutely psychotic patients regardless of diagnosis, as well as in patients receiving psychotomimetic drugs, remains to be determined. It probably represents increased permeability of the cell membrane of some tissue to these enzymes, but it could also be due to decreased clearance of these enzymes from serum (*16*). It is difficult to conceive of any direct relevance of these changes to the psychotic process without an implication of a change in brain function. There is a possibility that an increased cell permeability or decreased clearance from serum is the peripheral manifestation of a pathologic process occurring in the central nervous system, just as, in tetanus, tetanus toxin increases muscle cell permeability to CPK and aldolase and blocks specific inhibitory neurons (*17*). The finding that an increase in serum of muscle-type CPK occurs in the absence of gross muscle damage in a variety of disturbances of central nervous functioning, including some cases of brain trauma, brain tumor, meningitis, encephalitis, epilepsy, or cerebral vascular insufficiency (*1*, *9*, *10*, *18*), suggests that the acute psychotic syndromes could be much more closely related to organic brain disease than they were previously believed to be, and that some endogenous agent or process, activated or initiated by disruption of cerebral integrity, could affect the activity of CPK and aldolase in the serum. Investigation of the cause of increased enzyme permeability or decreased clearance of enzymes from serum in human tissues which may be available for direct study, or in animal models which could be developed, may permit some approach to central nervous system events which could occur in the acute psychotic state.

Our data, in agreement with clinical experience, suggest that in acute psychoses the attempt to meet a stressful life situation disrupts the functional integrity of the central nervous system and leads both to mental symptoms and the endogenous agent or process which produces the increased activity of CPK and aldolase. A common specific mechanism leading to the release of muscle enzymes in the acute psychoses, the drug-induced "model" psychoses, and perhaps in other altered states of consciousness, such as those subsequent to sensory deprivation and sleep deprivation, indicates a fundamental biological relation between these states which was previously suspected but not proved. The data indicate that determination of CPK and aldolase activity in the serum might be of value as a chemical test for acute psychosis as well as in the prediction of incipient decompensation in patients being studied over a period of time.

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## Protein Synthesis in the Hippocampal Pyramidal Cells of Rats during a Behavioral Test

Abstract. *Protein synthesis was studied in the pyramidal nerve cells of the CA3 region in the hippocampus of rats during a behavioral test involving transfer of handedness. A new electrophoretic technique was used for separation of $10^{-7}$ to $10^{-9}$ gram of protein and radiometric determination of the various protein fractions. After interventricular administration of $^{3}$H-leucine, protein synthesis of two fast-moving fractions was significantly higher bilaterally in the hippocampus of the trained rats. There was also a trend to lateralization of the highest protein synthesis to the learning side.*

The hippocampus modulates and integrates reticular patterns of activation and thalamic patterns of projection during learning. It seems to integrate patterns of ascending activation in subcortical regions with cortical activity. The emotional part involved in the establishment of new behavior seems to be functionally integrated by the hippocampo-limbic region. The stimulatory level is low in the hippocampus, and a burst of activity in it easily spreads to other parts of the limbic system and affects various emotional modalities, such as aggression and regulation of the hormonal outflow from the hypothalamus. Impulses from viscera and those sensed as pain seem to reach the hippocampus via caudal parts of the reticular formation. Bilateral destruction of the hippocampus in man results in severe memory defects, inability to learn, and dysfunction of the mechanisms for thought processes (*1*). Experiments with electrodes implanted in the hippocampus have shown definite changes in the electrical pattern during learning (*2–4*). It also seems that when some new element is introduced into a learning situation, the electrical response in the hippocampus is increased. As the new element becomes familiar, there occurs a decrease of response which is obvious in the hippocampus (*2*, *3*). Penfield (*5*) has concluded that in man the recording of current experience is impossible without the hippocampus. Flexner and collaborators (*6*) have correlated behavior in mice with protein synthesis which was inhibited to various degree by puromycin. Destruction of long-term memory was found when protein synthesis was inhibited by 80 percent in the hippocampus, the tem-

poral cortex, and part of the remaining neocortex. Recent memory was destroyed when protein synthesis was inhibited to the same degree in only the hippocampus and temporal lobe (*7*).

We have studied protein synthesis of the pyramidal nerve cells of a defined, small part (CA3) of the hippocampus in relation to the performance and behavior of rats in transfer of handedness.

Protein synthesis has been judged by the incorporation of $^3$H-leucine in the cellular protein. The CA3 area was chosen partly because functionally it is one of the most reactive areas of this region and partly because biochemical changes, if any, are more likely to be found in small defined areas of the brain and in uniform cell populations within this area, than in a mixed population from, for example, half a hemisphere. In the latter case, biochemical changes in a defined group of nerve cells can disappear in the averaging analytical macroprocedure (*8*). We used Sprague-Dawley rats (around 150 g) in a test for transfer of handedness. The originally described experimental setup (*8*, *9*) was modified. A simple practical arrangement (*10*) induced the rat to transfer to the nonpreferred paw in retrieving food; that is, gave an adaptive change in the rat's behavior as the result of experience. The rats had to reach far down in the narrow glass tube to grasp each food pill, which is an important feature of the experimental setup. Once learned, the new behavior is retained for a long period of time, up to 9 months (*10*). The performance is linear up to the 8th day after which the reaches for food are constant with the paw that is not preferred. The rats were therefore taken on the 4th or 5th day.

One hour and a half before the rats were killed, they were anesthetized with sodium evipan (7 mg/100 g of body weight), and 120 $\mu$l of $^3$H-leucine [1 $\mu$c/$\mu$l (New England Nuclear Corp.)] was injected into each lateral ventricle. One hour later the animals performed in the behavioral test. They were killed immediately afterwards. The hippocampus was rapidly dissected out, and the CA3 region was removed and placed on a cooling stage of a stereomicroscope. Pyramidal nerve cell bodies were dissected out with stainless steel microtools (*11*).

Three hundred pyramidal nerve cells were isolated and used for extraction of protein and further separation by disc electrophoresis (*12*) (Fig. 1). A larger piece containing pyramidal nerve cell bodies, approximately $10^5$ cells, was sampled for determination of the $^3$H-leucine concentration in the CA3 region (and its immediate neighborhood). The latter information was used to correct the specific activity of the various protein fractions for variation in the amount of free and available $^3$H-leucine (see below). This variation must be considered since the concentrations of precursor amino acid in the left and right sides of the hemisphere or from animal to animal may differ depending on differences in circulation, dilution effects, and so forth.

The technique is briefly as follows. The cells are homogenized in 0.25*M* sucrose or in buffer with the addition of 0.5 percent Triton X-100. A loop of 28-$\mu$ stainless steel fastened to a high-speed dental drill (12,000 rev/min) is used to homogenize the solution in a 400-$\mu$ capillary. The cells are homogenized for 2 minutes and centrifuged. A sample (0.5 $\mu$l) of the homogenate is placed on the upper gel in a 200-$\mu$ capillary containing the polyacrylamide gel.

The capillaries are carefully cleaned and coated with methyl cellulose to prevent electroendosmosis. The polyacrylamide gel is prepared from recrystallized monomers and contains 0.5 percent hydantoin. After polymerization, this gel in a 25 percent concentration has a stability and pore size which is suitable for separation of brain protein. The electrophoresis is performed on five gels at a time. Two of these are used for interferometric determinations of protein amounts in the fractions and two for radiometry. Each capillary contains sample (0.5 $\mu$l), 5 percent upper gel (0.07 $\mu$l), and running gel (0.5 $\mu$l). The electrophoresis is performed at 60 volts for 25 minutes, after which the gel is pushed out into a 80 percent ethanol solution and the protein is precipitated.

The amount of protein per fraction in the gel is determined by interference microscopy after the gel is embedded in a mixture of glycerol and benzyl alcohol with a refractive index of 1.482 ± 0.005, which is that of the gel.

The tritium activity of the fractions is determined by combusting the dissected-out fractions together with Zn and $KClO_4$ at 650°C in a glass capil-

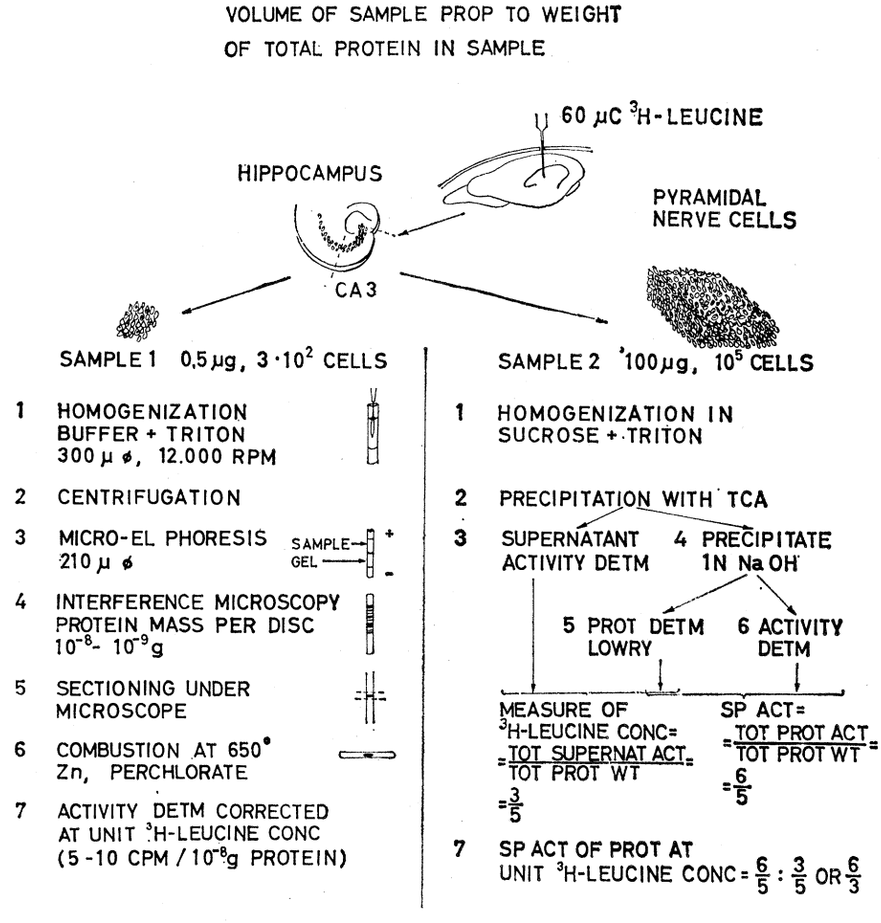


Fig. 1. Pyramidal nerve cell protein separated on 400-$\mu$ polyacrylamide gel. Fractions analyzed (4 and 5) at arrows.

lary. This is then crushed in a special Geiger tube, the gas is released, and the radioactivities are counted. This procedure has an efficiency of 40 percent. From these latter two determinations, the specific activity of the protein is determined. Since this value varies as a result of variations in the local concentration of $^3$H-leucine, it must be corrected. For this correction, the mathematical relation between specific activity and the concentration of $^3$H-leucine must be known. This relation is linear (Fig. 2). Consequently, specific activities of protein fractions from different but anatomically identical brain regions (see below) can be compared by dividing the specific activities by the values of the $^3$H-leucine concentration. The numerical value of this ratio is the slope of the line representing the linear relation between specific activity and $^3$H-leucine concentration, or, formulated in another way: all specific activities are compared at unit $^3$H-leucine concentration. The $^3$H-leucine concentration is obtained as follows. About 100 $\mu$g of pyramidal neurons from the sample is homogenized in 0.5 percent Triton X-100. The solution is precipitated with trichloroacetic acid and washed, and its total radioactivity is determined. It is then dissolved in 1*M* NaOH and the amount of protein in the solution is determined. In the supernatant, the total radioactivity of $^3$H-leucine is determined. The concentration of $^3$H-leucine is obtained as the quotient between the total $^3$H-leucine activity and the amount of protein in the sample. The prerequisite for this procedure is that the amount of protein in the sample can be used as a measure of the sample volume. In identical regions of the two hemispheres or in the brains from different animals, the amount of protein per unit volume varies within narrow limits. Therefore the incorporation of amino acids can be compared only for identical brain regions in the same or in different animals.

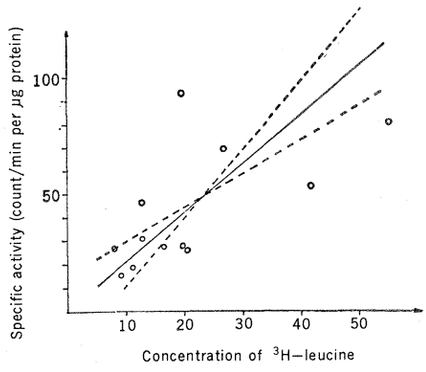


Fig. 2. Linear relation between specific activity of protein and $^3$H-leucine concentration in the CA3 region of hippocampus. Regression lines dotted.

From the protein separated on 400-$\mu$ polyacrylamide gels, the fractions 4 and 5 from the separation front were taken for further analysis. Radiometric determinations were performed on both these fractions which were cut out and combusted together.

Table 1 gives the sum of the amount of protein and activity of fractions 4 and 5. Since there is a linear relationship between the specific activity of $^3$H-protein and the concentration of free $^3$H-amino acid in the region investigated, the uncorrected values of specific activity (Table 1) were divided by the values for the $^3$H-leucine concentration. Such corrected specific activity values can now be compared, provided they are obtained from measurements of identical structures from the two hemispheres of the same brain or from different brains. The corrected specific activity are also shown in Table 1.

The specific activities of the hippocampus nerve cell protein from the animals engaged in motor and sensory activity of the behavioral test are significantly higher than those of the control animals. There is a weak trend ($P = .2$) for the highest protein synthesis to occur in the side of the hippocampus contralateral to the used paw. If the quotient between the corrected specific activity of the learning side and that of the control side for each rat is calculated, thus eliminating the variation between animals, the average value of this quotient is significantly greater than 1 at the 5 percent level ($1.56 \pm 0.29$).

Similar results were obtained when the total amount of unseparated protein of the hippocampal CA3 nerve cells was studied (Table 1). The procedure was identical with that described for correcting for the variation of the $^3$H-leucine concentration.

The incorporation of $^3$H-leucine in both sides of the hippocampal CA3 region from the trained animals is significantly higher ($P < .005$) than that of the untrained rats. There is also a trend to lateralization. To eliminate the variation between animals, we calculated the same quotient between the specific activities of the training side and the control side as determined above. The value is $1.43 \pm 0.22$, which is significantly greater than 1 ($P < .05$).

Thus, as determined by both methods, protein synthesis is significantly higher in the CA3 regions of the hippocampus of the trained rats than it is in the identical structures of the controls. Furthermore, there is a trend for higher protein synthesis to occur in the pyra-

Table 1. Incorporation of $^3$H-leucine in fractionated and unfractionated proteins of the pyramidal nerve cells in hippocampus CA3 from the rat, expressed as specific activities, corrected for the $^3$H-leucine concentration. Numbers in parenthesis, numbers of rats; *n*, number of experiments.

| Hippocampus CA3 | Protein fractions 4 and 5 | | | | | Unseparated proteins |
|---|---|---|---|---|---|---|
| | Protein ($g \times 10^{-8}$) | $^3$H-protein activity (count/min) | Uncorrected specific activity (count/min per $10^{-8}$ g protein) | $^3$H-leucine concentration | Corrected specific activity (count/min per $10^{-8}$ g protein) | Corrected specific activity (count/min per $10^{-8}$ g protein) |
| | | | *Experimental rats* | | | |
| Right side (9) (Contralateral to the used paw) | $2.08 \pm 0.15$ ($n = 7$) | $11.86 \pm 1.53$ ($n = 24$) | $5.70 \pm 0.84$ | $10.73 \pm 1.11$ ($n = 8$) | $0.53 \pm 0.09$ | $0.129 \pm 0.019$ |
| Left side (9) (Contralateral to the preferred paw) | $1.96 \pm .15$ ($n = 7$) | $14.31 \pm 1.46$ ($n = 25$) | $7.30 \pm .93$ | $15.30 \pm 2.32$ ($n = 9$) | $.47 \pm .09$ | $.098 \pm .011$ |
| | | | *Control rats* | | | |
| Average right and left sides (4) | $2.02 \pm 0.15$ ($n = 7$) | $16.40 \pm 1.46$ ($n = 18$) | $8.12 \pm 0.96$ | $30.37 \pm 2.80$ ($n = 8$) | $0.27 \pm 0.04$ | $0.049 \pm 0.005$ |

midal nerve cells contralateral to the used paw.

These data merit only the conclusion that there is a correlation between the increased neuronal protein synthesis and the increased neural activity during the experimental conditions. It seems clear that the electrical activity changes significantly in the hippocampus, at the consolidation of learning. In addition, in his experiments on visual discrimination, Adey (*2*) found spatial as well as temporal changes of the electrical activity within the hippocampus. In early training the phase pattern was reversed to that found in fully trained animals.

The two protein fractions we investigated move near the anode front at electrophoresis. They immediately follow the protein fractions constituting the acidic protein of brain cell, S100 (*13*). This protein is of interest because of its specificity for the central nervous system, its acidic character, and its localization to the nuclei of the neurons. The basic histones of the nucleus are considered to regulate gene activities and the presence of acidic proteins may involve also regulatory effects on the histones.

The results, for example, of Flexner (*6*) in mice, or Agranoff (*14*) in goldfish, and of Albert (*15*) in rats, seem to link protein synthesis to the neural processes responsible for learning and storage of information. Barondes (*16*), on the other hand, has not found experimental evidence for the view that synthesis of a protein is required for memory storage. Our findings, however, are in agreement with those of Flexner and Agranoff. Neither type of finding gives any information about the specificity of proteins.

The trend to lateralization of the highest degree of protein synthesis to the learning side of the hippocampus is interesting but surprising with respect to the wealth of activities reaching and cross-reacting with the limbic area.

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## Response of Ips confusus to Synthetic Sex Pheromones in Nature

Abstract. *The first flight response of bark beetles to synthetic sex pheromones under natural conditions is reported. Two insect species predaceous on this bark beetle also responded. The synthetic compounds delivered in an airstream from a substrate of Carbowax 20M on Chromosorb A appear to elicit the same response as the natural attractant from male-infested bolts of ponderosa pine elicits.*

We report the first flight response of bark beetles to synthetic sex pheromones (*1*) under natural field conditions. These pheromones are three terpene alcohols—compound I: (−)-2-methyl-6-methylene-7-octen-4-ol; compound II: (+)-*cis*-verbenol; compound III: (+)-2-methyl-6-methylene-2,7-octadien-4-ol—which were isolated from frass from male *Ips confusus* (LeConte) (Coleoptera: Scolytidae) (*2*), identified, and synthesized (*3*). These compounds appear to elicit the same response as male-infested bolts of ponderosa pine do; therefore, they are probably the chemical messengers that evoke the concentration flight and subsequent mass attack of *I. confusus*.

Experiments were performed in a gently rolling, treeless brush field to avoid unknown, competing sources of attraction and unpredictable air movements. The site (20 hectares) was located near the south fork of Willow Creek, Madera County, California, in a drainage with a relatively consistent up- and down-valley wind system. Insect traps were placed on 20-m centers in two lines, 400 m apart, perpendicular to the prevailing winds. Each treatment was replicated once in each line, and the trap positions were randomly assigned at the beginning of each experiment. The endemic flying populations were probably emerging from naturally infested, ponderosa pine logging slash within 1 km of the experimental area. All tests were begun when the prevailing winds were moving at 6 to 13 km/hr up the valley toward the infestation. The temperature fluctuated between 20° and 31°C.

The traps consisted of hardware-cloth (mesh of 0.64 to 0.96 cm) cylinders, 30 by 12 cm in diameter, each fastened to the end of a 1.5-m rod driven into the ground. The cylinders were dipped in warm "Stickem Special," so that most of the squares remained open. Air was metered from a portable pressurized tank through a charcoal filter and then through nylon tubing (0.32 cm in outside diameter) to an aluminum tube containing the attractants. We maintained a flow rate of 50 $cm^3$/min with a regulator and a capillary (0.02 cm in inside diameter by 5 cm) in series. The exit port was placed in the center of the trap. One male-infested bolt in each line was the standard source of natural attractant. Each bolt held 20 male beetles confined within preformed entrance tunnels beneath wire screen. The logs were exposed 48 hours after confinement.

The synthetic attractants were confined to an aeration tube consisting of aluminum tubing which had an outside diameter of 1 cm. A solution of 0.5 g of Carbowax 20M in methanol was added to 9.5 g of Chromosorb A, and most of the methanol was evaporated. A solution of 1.5 mg of compound I in pentane was added to 7 ml of the above substrate. The excess pentane was evaporated, and the mixture was poured into a 15-cm length of tubing (section A). A second 15-cm tube (section B) was filled with the same substrate to which a solution of 1.5 mg of compound I, 1 mg of compound II, and 1 mg of compound III in pentane was applied in the same way. Glass-wool plugs were inserted into the ends of the tubes. Then the tubes were joined together with a short piece of rubber tubing. In the