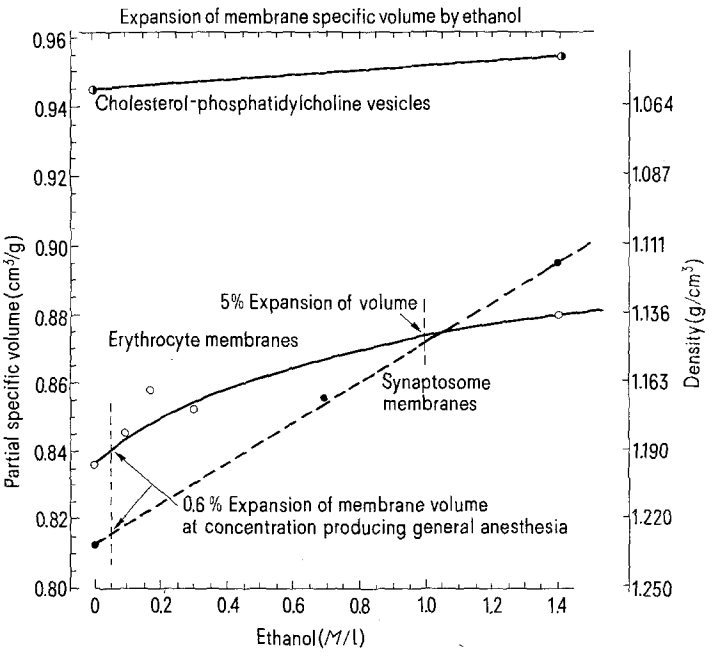

Ethanol expands the specific volume of erythrocyte and brain synaptosome membranes by 0.6% at general anesthetic concentrations (0.05 *M*), and by 5% at local anesthetic concentrations (1 *M*). Liposomes were expanded by only 0.01% and 0.3% at these ethanol concentrations, respect ively. 25 °C.

(control); 0.992487 and 0.993137 for 0.7 *M* ethanol; and 0.987146 and 0.987699 for 1.4 *M* ethanol.

The results for d_m are in the Figure. At concentrations of ethanol which produce general anesthesia[16] (0.05 *M*) the specific volumes (= reciprocal of the specific density) of the membranes increased by 0.5–0.6%. At ethanol concentrations which produce local anesthesia[1] (1 *M*) the specific volume expansions were between 3 and 6%.

The Figure also shows that 0.05 *M* ethanol expanded the specific volume of liposome membranes[17] (lecithin: cholesterol: 1:2 molar ratio) by only 0.01%, in agreement with the membrane-occupying volume of 0.02% by the drug in the membrane phase[1].

Since the specific volume expansion of the biological membranes (0.5%) is many times more than that for lipid membranes (0.01%), this strongly suggests that conformation changes in membrane proteins underly the membrane expansion. Pressure reversal of anesthesia would then involve a return of the protein conformation to its native state, rather than modify the lipids. Although it has been claimed that pressure reverses or antagonizes the anesthetic-induced leakiness or disorder in liposomes[18–20], this is not true antagonism, since pressure by itself changes the leakiness and ordering of lipids[1,18–20] without shifting the dose-response curve[21].

Résumé. Utilisant une nouvelle mesure de densité, de haute précision, on étudia l'effet de l'éthanol sur le poids spécifique des membranes des érythrocytes et des synaptosomes. La concentration de l'éthanol qui produit l'anesthésie générale (0.05 *M*) étendit le volume spécifique des membranes à 0.5–0.6%. Aux concentrations de l'éthanol qui produisent l'anesthésie locale (1 *M*) l'extension du volume spécifique fut de 3 et 6%. Ces résultats supportent la théorie de l'extension des membranes par les anesthétiques et suggèrent que l'extension des constituants protéinés des membranes est plus importante que celle des constituants lipides.

P. SEEMAN[22]

Pharmacology Department, University of Toronto, Toronto M5S 1A8 (Ontario, Canada), 27 December 1973.

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[21] Appendix: Equation 1 is derived as follows. $d_{ms} = (m_b + m_{mw} + m_m)/(V_b + V_{mw} + V_m)$, where m_b, m_{mw} and m_m are the masses (in g per test-tube) of buffer medium, membrane water, and dry membrane, respectively, and where V_b, V_{mw} and V_m are the corresponding volumes (in ml per tube). Dividing all 6 terms by V_s (= volume of suspension in tube), and substituting W for m_m/V_s, m_m/d_m for V_m, and $d_b V$ for m_b, one obtains $d_{ms} = [(d_b V_b/V_s) + (m_{mw}/V_s) + W]/[(V_b/V_s) + (V_{mw}/V_s) + W/d_m]$. Further substituting f for V_b/V_s, and h for m_{mw}/V_s, and rearranging, one arrives at equation 1.

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Time Dependence of a Neurochemical Correlate of a Learning Task: a Non-Disruptive Approach to Memory Consolidation

Learning and memory constitute the acquisition and retention of information in time. Most techniques, such as electro-convulsive shock[1], anaesthetics[2] and inhibitors of protein and RNA synthesis[3,4] used to establish the time course of memory consolidation are 'disruptive' in that they depend on the extinction of memory; furthermore, the results based on these techniques are conflicting. It is felt that the time course of a neurochemical correlate

of a continuous learning task may assist the sequential analysis of memory consolidation without disrupting the memory trace.

SHASHOUA[5] has demonstrated that fish have the ability to learn to swim in the normal position following the attachment of corks to their ventral surfaces. This learning task includes a memory component that is inhibited by puromycin[5], cyclohexamide and actinomycin[6]. Shashoua successfully used the ratio of uridine to cytidine in newly synthesized brain RNA as an index of base composition changes in the fish on completing the learning task[5]. He subsequently showed that changes in the uridine/cytidine (U/C) ratio were independent of stress, exercise or intense electrical activity[7].

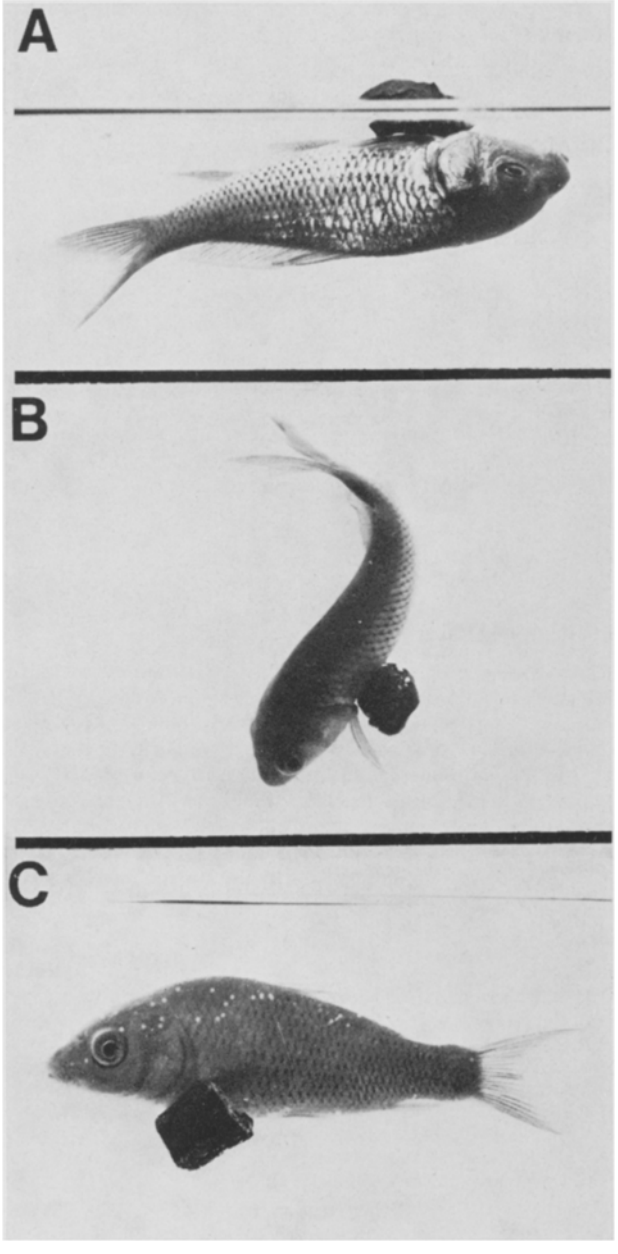

Fig. 1. Stages in the ability of the fish to swim in the normal position following float attachment. A) 30–60 min after float attachment (upside-down fish). B) 1–2 h (intermittent diving). C) 4 h (swimming in the normal position).

We have further examined the time course of changes in the U/C ratio of newly synthesized brain RNA during, on completion of, and following the same learning task in carp. SHASHOUA only applied this analysis after completion of the learning task.

Materials and methods. The continuous learning task depended upon the ability of a fish, *Cyprinus carpio* L., to learn to swim in the normal position after the attachment of a float to its ventral surface. Polystyrene floats (1.5 ml/20 g fish) were sutured 5 mm caudal to the lateral fins. By 4 h all fish had learned to compensate for the float and to swim in the normal position (Figure 1).

In all experiments 10 μCi of the pyrimidine precursor orotic acid-5-H^3 (26.4 Ci/m*M*), dissolved in 10 μl of sterile normal saline, was injected into the subarachnoid space through the anterior cranium. Injections were always given 4 h before the fish were killed. Each measurement was on 4 pooled fish brains; at each time-period studied, 5 experimental (with floats) and 5 control (sham-operated) measurements were made. Controls were handled to the same extent as the experimentals and were sham-operated at the same time that the floats were attached to the experimental group.

At the end of each time period assayed, the brains were immediately removed from the fish and washed with 0 °C *Tris* buffer, pH 7.6. Cytoplasmic RNA was extracted by a cold phenol technique[8]. The RNA was purified and freed from adsorbed nucleotides and then hydrolyzed in KOH. The bases were separated by TLC on cellulose plates (Machery Nagel cellulose 300) using a solvent system of propan-2-ol, HCl and water (60:8:27). Cytidine and uridine were eluted and counted in Instagel (Packard).

Results. In the first experiment, changes in the U/C ratio during and after the learning task were measured at intervals of 2, 4, 6, 8, 16 and 24 h after operation. At 2 and 8 h of float attachment, the experimental U/C values differ significantly from the controls, Figure 2 ($P < 0.01$ in both cases). Thus the changes in the ratio are not uniform during learning but are maximal at 2 h of learning and about 4 h after completion of the task. The change observed 4 h after completion of the task (8 h of float attachment) might be related to the consolidation of long term memory, since this time interval is of the same order of magnitude seen in disruptive studies of long term memory[9].

That this change is not due to continued float attachment is shown by another set of experiments in which the floats were removed at the time when the experimental fish had learned to swim in the normal position. All the fish were injected then and killed 4 h later. In this series, 5 experimental (20 fish) and 5 control (20 fish) measurements were made. The U/C ratios were 1.74 ± 0.39 (S.E.M.) and 0.56 ± 0.02 (S.E.M.) respectively and are significantly different ($P < 0.02$).

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Discussion. The U/C ratio changes at 2 and 8 h might be related to a high proportion of hybridizable RNA molecules in the brain with short half-lives (2.5–4 h)[10]; such molecules may facilitate adaptations to changes in physiological conditions by an alteration in protein synthesis[11]. Changes in ribosomal and tRNA[12] could also contribute to any alterations in RNA species. The early changes observed during learning are difficult to interpret in terms of information storage and may well represent non-specific changes associated with the onset of a challenging learning task such as arousal.

Consolidation of long term memory by an alteration of synaptic transmission, changes in glial activity or post-synaptic receptor modification might involve the synthesis of new or increased levels of exiting proteins. This would require an increase in transcription and translation and in possible changes in cytoplasmic RNA species and would help to explain changes in the U/C ratio observed on completion of the learning task. Having shown that a neurochemical correlate of learning is time dependent, a non-disruptive approach together with the measurement of the exact half-lives, locations, and specificity of any such neurochemical correlates may prove profitable to a sequential analysis of memory.

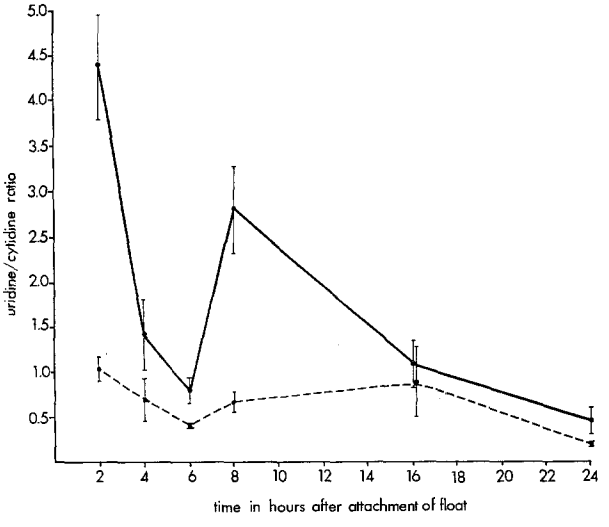

Fig. 2. Time course of an index of base composition (uridine/cytidine ratio) during (0–4 h) and after (4–24 h) the learning task. In each case the label was injected 4h before measurement. Solid line, experimentals; interrupted line, controls. For each point, $n = 5$ (where $n = 1$, 4 brains are pooled); values are given as means $\pm$ S.E.M.

Zusammenfassung. Veränderungen am neurochemischen Korrelat des Erinnerungs- und Lernvermögens im Fisch erwiesen sich als zeitabhängig.

C. J. WOOLF, G. H. WILLIES, H. R. HEPBURN and C. ROSENDORFF[13]

Department of Physiology, University of the Witwatersrand Medical School, Hospital Street, Johannesburg (South Africa), 19 November 1973.

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Phosphorus Metabolism in Grape Buds During Floral Initiation

The importance of P nutrition in grape production has been emphasized by many workers[1–3]. It has been reported that application of P fertilizers at the time of floral initiation and differentiation enhanced the number of fertile buds[4] with accelerated accumulation of P[5,6]. It has also been observed that there was a tremendous upsurge in the nucleic acid P during floral initiation and differentiation[1]. The present study was undertaken with a view to investigating the incorporation of soil applied P into various phosphorus compounds in the buds of vigorously growing Anab-e-Shahi (AS) and less vigorous Bangalore Blue (BB) Pachadraksha (PD) and Kali Sahebi (KS) grape cultivars, during floral initiation.

Radioactive phosphorus in the form of 'carrier free' *ortho*-phosphoric acid at 0.5 mCi/plant was injected into the holes in the soil, made around the plants, 5 days before normal time of floral initiation[5]. Bud samples were collected from the current shoots at the time of floral initiation from all cultivars and the phosphorylated compounds were fractioned[7] after removing the dry scales and tomentose hairs. 1 ml in each of the fractions was dried in cupped planchets under infrared lamp and assayed for ^{32}P activity with the aid of Philips GM counter. The mean activity of ^{32}P of all fractions of P are given in the Table.

The results indicated that the incorporation of ^{32}P in the buds was more in vigorously growing AS than in less vigorous cultivars. The high rate of incorporation of ^{32}P in the buds of vigorously growing varieties might be due to the presence of a greater number of large-sized conducting vessels[8] which in turn could have efficiently transported the applied P into the buds.

Among the various fractions of P, the nucleic acid had more activity to an extent of 68 to 79% of total activity with a predominant amount in DNA fraction in AS and KS. This conspicuous increase in the nucleic acid fraction supports findings in vitis[9,10] *Chenopodium album*[11] and

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