dark-adapted retinas have been published elsewhere in conjunction with an historical review[4]. We confirmed the presence of a 500-nm pigment in a few regenerated retinas, but these experiments had not been planned as a biochemical study and our purpose here is only to report qualitative results.

The figure shows that retinas which had been separated from the pigment epithelium showed poor regeneration of pigment (scores in the vicinity of 1). This confirms that bleaching had occurred during the period of light-adaptation and that a bare eyecup will not support regeneration. The retinas which had been next to the pigment epithelium showed good regeneration of pigment (scores of 2–3), regardless of whether the photoreceptors had actually contacted the pigment epithelium or had been separated from it by the thickness of the retina.

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The replacement of peeled retinal tissue upon the pigment epithelium can never be precise at a cellular level, so that even Kühne's original work implied the existence of diffusible factors. Our results with upside down retinas indicate that these factors can traverse the thickness of retina and do not depend on membrane contact between the photoreceptors and the apical microvilli of the pigment epithelium. Investigators have tried since Kühne's time to isolate the substances which diffuse between retina and pigment epithelium during dark-adaptation, but have had limited success[3]. We hope that our observation, which points out that the pigment epithelium can support regeneration across a tangible distance, will help investigators working on the biochemistry of rhodopsin. The ability to obtain regeneration across a significant gap of tissue may allow some new approaches such as microfiltration or the chemical analysis of substances within the gap.

Evidence for a correlation between the latency of an early component of auditory evoked potentials and the brain levels of serotonin in albino rats

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Summary. Changes in brain serotonin levels are correlated with the latency of an early component of auditory evoked potentials (EAEP) in rats. In fact 5-hidroxytryptophan provokes an increase both in serotonin brain synthesis and in the latency of EAEP. On the other hand, PCPA provokes an opposite effect.

Previous findings by histofluorescence methods have revealed serotoninergic structures in some areas of CNS within the acoustic pathways. Serotoninergic neurons have been observed, dorsally and ventrolaterally in respect to the trapezoid body, in the caudal portion of the posterior collicle and the medial geniculate body[1], while serotoninergic nervous endings have been found in the dorsal coclear nucleus, in the inferior olivar complex, in the posterior collicle and in the medial geniculate body[2]. Many authors have hypothesized that cerebral serotonin (5-HT) plays an important role in inhibitory modulation upon the discharge patterns of those nervous structures toward which its fibres run[3,4], thus often giving rise, at a behavioural level, to activities of an inhibitory type[5,6]. We have studied the latency variations of an early component of the cortical acoustic-evoked potentials (EAEP) in the rat according to the levels of brain 5-HT, in order to establish a possible relationship between serotoninergic activity and the central acoustic function.

Materials and methods. Sprague-Dawley adult male rats, weighing 250–300 g, were implanted with 3 chronic stainless-steel electrodes: 1 in the bregma and 1 in the nasion, closely connected with the dura mater, and the 3rd inserted under the periauricular skin[7]. The experiment took place at least 1 week following surgery: 20 clicks at 0.5 Hz, originating from a square pulse of 0.12 msec duration and with an intensity of 100 db (sensation level), were administered by a small speaker placed at 20 cm from the animal in an anechoic room. Brain responses were amplified by an EEG with a flat frequency from 3.2 to 3200 Hz and averaged by a computer with a post-stimuli analysis time of 50 msec. Averaged responses were led into an oscilloscope and recorded with a camera. The experiment started after 30 min adaptation to the new environment conditions, when the exploratory

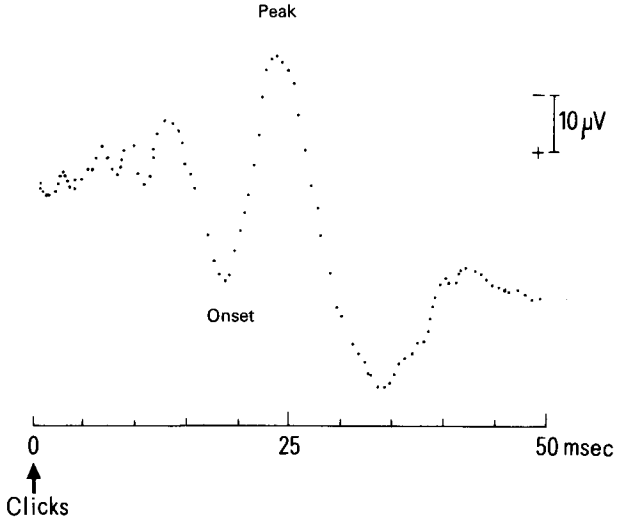

Fig. 1. The averaged response to 20 clicks of 100 db (sensation level) with a post-stimuli analysis time of 50 msec recorded on dura mater at bregma in albino rats is represented.

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activities ceased and the 1st EEG signs of drowsiness appeared. In order to change brain 5-HT synthesis, the following drugs were used: 5-hydroxytryptophan (5-HTP) was given at a dose of 50 mg/kg b.wt; L-tryptophan (L-Try) at the dose of 100 mg/kg b.wt; p-chlorophenylalanine(PCPA), a rather selective tryptophan hydroxylase inhibitor[8], at a dose of 100 mg/kg daily for 3 days. For all treatments, the route of administration was i.p. at a volume of 2 ml/100 g b.wt. A group of 4 rats (A) were treated 1st with saline, to establish if the experimental handling of the animals might influence the EAEP's latency, then with 5-HTP and finally with L-Try, at intervals of 48 h according to a latin square design. A trial of 2 consecutive EAEP was recorded at 30 and 60 min after each treatment. A 2nd group of 4 animals (B) was treated with PCPA and 2 consecutive recordings were obtained 24 h after the last drug administration. 15 min after the last click, the animals received 5-HTP at a dose of 50 mg/kg, and a trial of 2 consecutive auditory-evoked potentials was recorded 30 and 60 min after this treatment. After each treatment, groups of 4 animals were stimulated, and then killed at different time intervals. Controls animals were only acoustically stimulated. The brain was immediately taken, to measure cerebral 5-HT according to the method of Curzon and Green[9]. Statistical evalutation was made by Student's t-test.

Results. At first, 2 check records were obtained before each drug administration. As seen in figure 1, the sequence of auditory-evoked potentials showed a negative wave (EAEP) at the onset, and the peak mean latencies were very similar to those described by Kern et al. in guinea-pigs[10]. Results of drug treatment on the latency of EAEP are recorded in figures 2 and 3. The administration of 5-HTP to group A caused latency increase of the onset(os) and the peak(p) of EAEP's at 30(os = + 6.8%; p = + 7.8%) or 60 min(os = + 6.8%; p = + 12.3%) after its administration. The increases of 5-HT were, respectively, 67.3% at 30 min, and 70.7% at 60 min. L-Try administration did not cause in these animals any appreciable variation in the EAEP's latency. After this treatment, the increase of 5-HT were 17.8% and 13.2% at 30 and 60 min respectively. Saline did not modify either EAEP's latency or 5-HT levels. After PCPA administration to the animals of group B, we observed a shortening of EAEP's peak latency (p = − 4.7%). Such decrease was blocked and reversed by a subsequent administration of 5-HTP, the EAEP's latency being greater than that of the untreated animals after this treatment. This increase reached a maximum after 30 min (os = + 8.9%; p = + 7.8%), being still higher after

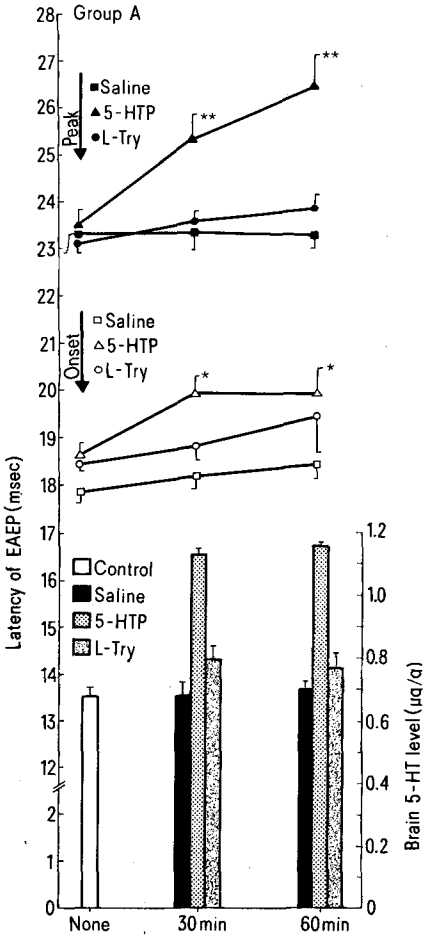

Fig. 2. The graphs represent the mean latency trend of EAEP's onset and peak; vertical bars are SEM; asterisks indicate statistically significant differences from untreated animals (*p < 0.05, **p < 0.005). The columns represent the brain 5-HT levels express in µg/g; vertical bars are SEM. For the doses used, the time intervals and other details, see the text.

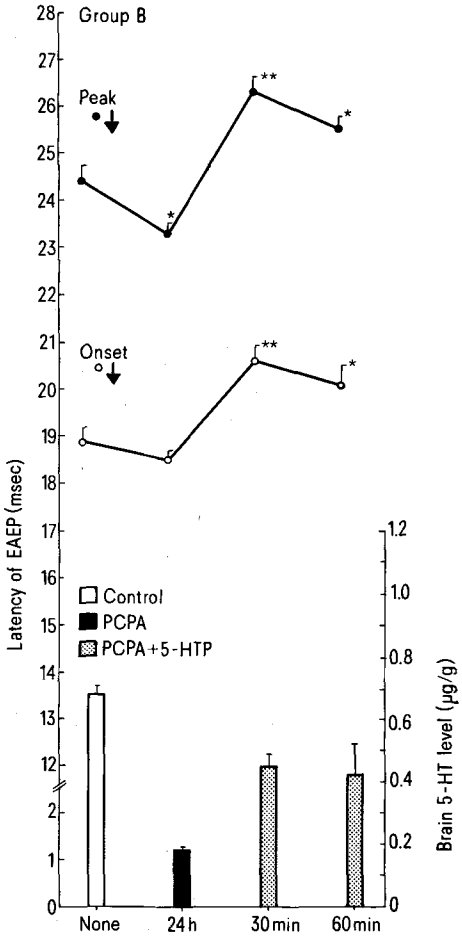

Fig. 3. The graphs represent the mean latency trend of EAEP's onset and peak; vertical bars are SEM; asterisks indicate statistically significant differences from untreated animals (*p < 0.05, **p < 0.005). The columns represent the brain 5-HT levels express in µg/g; vertical bars are SEM. For the doses used, the time intervals and other details, see the text.

60 min (os = + 6.1%; p = + 4.3%). After PCPA administration, 5-HT levels decreased 27.4%. After a subsequent administration of 5-HTP, brain levels of 5-HT reached values of 66% and 62% at 30 and 60 min, respectively, as compared with the controls.

Discussion. Our data strongly support a direct relationship between the levels of brain 5-HT and EAEP's latency. The administration of 5-HTP, which causes a strong increase of the levels of brain 5-HT, is able to raise the EAEP's latency (onset and peak), both in experiment A and in experiment B. The only difference into the effect of 5-HTP treatment in experiment B seems to be a shorter lasting effect on latency. The fact that the 5-HT content after PCPA treatment cannot be restored to normal level by the following 5-HTP administration, may be responsible for the short duration of the effect. On the other hand, PCPA, which causes a strong decrease of cerebral 5-HT, is able to reduce the EAEP's peak latency. However, the increase of EAEP's latency, caused by the administration of 5-HTP, was higher, in absolute values, as compared with the decrease of EAEP's latency caused by the administration of PCPA. The evoked cortical responses are an objective parameter of the function of these nervous pathways. The lag between the stimuli and the responses are in direct relationship with the induction or the inhibition of the nervous pathways afferent to the interested system. Our results show mainly the inhibitor effect upon the central acoustic pathways by increasing the synthesis of brain 5-HT. The lack of a strong corresponding decrease of EAEP's latency by diminishing the levels of this monoamine may probably be due to the known properties of the CNS, as the conduction velocity of a stimulus does not increase beyond a limit, due to the biophysical properties of the nerve fibres. Such inhibitory action can be exerted through the activation of serotoninergic structures, whose presence has been observed along the nervous acoustic pathways. Finally, it can be hypothesized that feedback mechanisms, through the 5-HT synthesis, regulate the stimuli adaptation in the animals[11].

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Lipofuscin accumulation in squirrel monkey spinal cord consequent to protein malnutrition during gestation[1]

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Summary. The formation of lipofuscin pigment in the anterior horn cells of the cervical spinal cord has shown in the fetuses and neonates under the extrinsic influence of maternal protein deprivation during the gestation period in the squirrel monkeys.

Although the healthy squirrel monkeys of the genus *Saimiri*, in their adult life, are characterized by the presence of lipofuscin pigment in the various areas of the nervous system, its appearance as early as the fetal or neonatal period has not been observed or described by earlier workers. Lipofuscin pigment, generally referred to as the 'aging pigment', appears in small amounts in the neurons of the adult animals including the humans and increases quantitatively as the organism ages[2,3]. Such an accumulation of lipofuscin pigment in the aging nerve cells could lead to significant changes in normal cellular physiology, which have a direct bearing on important neurophysiological functions[4]. In normal healthy animals, lipofuscin pigment is not observed until after the age of 3 months in rhesus monkeys[5] and 2 or 2.5 years in hogs and dogs[6,7].

We report that typical lipofuscin pigment, histochemically identical to the aging or 'wear and tear' pigment[8], accumulates in the anterior horn cells of the spinal cord of the squirrel monkeys born to mothers given protein deficient diets during most of the gestation period. The day of conception was determined by physical palpation of the uterus on a fixed day every week and cytologic examination of the vaginal smears. Before conception, the squirrel monkeys weighed 620–710 g. Beginning at day 35 of conception, the critical period of rapid brain growth, 59 squirrel monkeys were maintained in 2 groups picked up at random from the colony on ad libitum high protein (25% calories from casein as a protein source) and low protein (8% calories from proteins) regimens with 40% calories from a fat source and supplemented with vitamins and minerals. The higher level of calories from fat appears to improve the taste of food and helps increase the total caloric intake[9,10]. Fetuses from 2 animals in each group were removed by cesarian section at 115 days and 140 days of gestation. Together with the neonates these constituted the 28 animals we investigated. Whereas the healthy neonates weighed about 115 g, the malnourished neonates weighed around 80 g. The caloric intake of the mothers in high and low protein group ranged from 80 to 120 calories per animal per day. No statistically significant difference in the total caloric intake was observed in the 2 groups, except during the first 30 days in the low protein group. The average intake

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