

the histochemical results. The units which were analysed are generally representative of the broad population of 47 motor units previously studied in skunk m. gastrocnemius⁶. Slow (3) and fast (1) contracting units with variable twitch tensions and fatigue characteristics were studied. The only exceptions to the typical nature (for the skunk) of the physiological results were the observations of 'sag' in a slow motor unit and the presence of post-tetanic potentiation in 2 of the slow twitch units. Motor unit 2 (contraction time = 53 msec) showed distinct sag, a property which has been used to separate fast from slow contracting motor units in the cat¹⁴. Another property normally associated with fast contracting units, post-tetanic potentiation¹⁴, was found in slow units 1 and 3.

The histochemical properties of the component fibres of each muscle unit were remarkably uniform. Of the fibres studied in our 4 units only 3 were non-uniform. These all occurred in unit 4. These exceptional fibres were hypertrophied and atypically spheroid in shape, and they were characterized by low staining intensities for all enzymes. These fibres were similar in many respects to the giant fibres previously reported in pig muscle¹⁵. It is our conclusion that the glycogen depletion shown in these fibres is not the result of the stimulation regimen but simply reflects an overall lack of staining.

Our findings are in agreement with the direct correlation of contraction speed with myofibrillar ATPase activity¹⁶ and with similar but indirect correlations found in whole muscles or portions of muscles with different mixtures of histochemical fibre types¹⁷. Further and most importantly our results are compatible with the patterns of physiological and histochemical interrelatedness reported in the rat⁴ and cat⁵ using the same techniques employed in this study.

These results lend strong support to the generalization that histochemical methods can resolve discrete types of muscle fibres which correspond to the muscle unit types generally found in mammalian muscle. The fact that this pattern is found in an animal as functionally and phylogenetically distinct, from the rat and the cat as is the skunk suggests that the observed correlation between histochemical and physiological properties is a trait which may be shared by most mammals.

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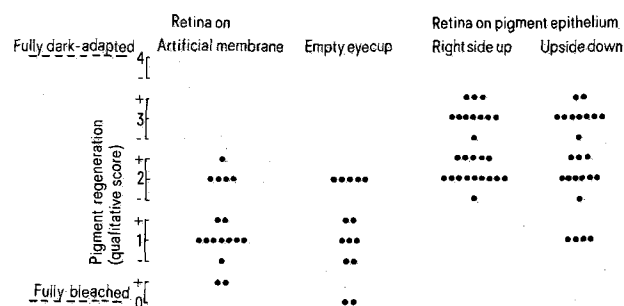
Visual pigment regeneration: Occurrence in frog retina upside down upon the pigment epithelium¹

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Summary. Visual pigment regeneration in the frog requires apposition between the retina and retinal pigment epithelium. However, regeneration occurs nearly as well with the retina upside down (i.e. with the inner limiting membrane facing the pigment epithelium) as right side up.

In 1877, Kühne² found that bleached frog retina would regenerate visual pigment only when it was in apposition to the pigment epithelium. Bridges³ has suggested that the pigment epithelium may be necessary because vitamin A must be esterified during the regeneration cycle. We have had occasion recently to repeat some of Kühne's experiments, and made an additional observation that to our knowledge has not been reported: pigment regeneration occurs nearly as well with the retina upside down upon the pigment epithelium as right side up.



Qualitative assessment of pigment regeneration in the frog retina. The results are from 10 experiments in which placement of the retina right side up and upside down upon the pigment epithelium during dark-adaptation was compared. Each point represents 1 piece of retina from 1 experiment but the number of pieces in each category was not necessarily the same in every experiment.

Material and methods. Under dim red illumination, dark-adapted *Rana pipiens* were decapitated, sections of eyecup were prepared, and the retinas were gently peeled from the pigment epithelium and light-adapted for 15–30 min either as an isolated tissue or after replacement into the eyecups. The retinas were then dark-adapted for 3–5 h in a moist chamber at room temperature, under 1 of 4 conditions: 1. Isolated retina on a moist Nuclepore membrane. 2. Retina in bare eyecup (i.e. no choroid or pigment epithelium). 3. Retina in its normal position (i.e. right side up) upon the pigment epithelium in an eyecup. 4. Retina upside down (i.e. with the inner limiting membrane facing the pigment epithelium) in an eyecup. The retinal fragments averaged 5 mm², and were carefully placed right side up or upside down under direct visual control. They held their position well and became evenly colored after regeneration so that an edge effect could not have accounted for the results.

Results and discussion. The results after dark-adaptation were judged qualitatively, relative to the appearance of freshly peeled, fully dark-adapted retina ('4') or fully bleached retina ('0'). Color photographs of bleached and

1 Acknowledgments. Supported in part by the Medical Research Service of the Veterans Administration and National Eye Institute Grant EY-01678.

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dark-adapted retinas have been published elsewhere in conjunction with an historical review⁴. 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 success³. 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 micro-filtration 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-hydroxytryptophan 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 serotonergic structures in some areas of CNS within the acoustic pathways. Serotonergic 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 body¹, while serotonergic nervous endings have been found in the dorsal cochlear nucleus, in the inferior olivary complex, in the posterior collicle and in the medial geniculate body². 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 serotonergic 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 skin⁷. 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

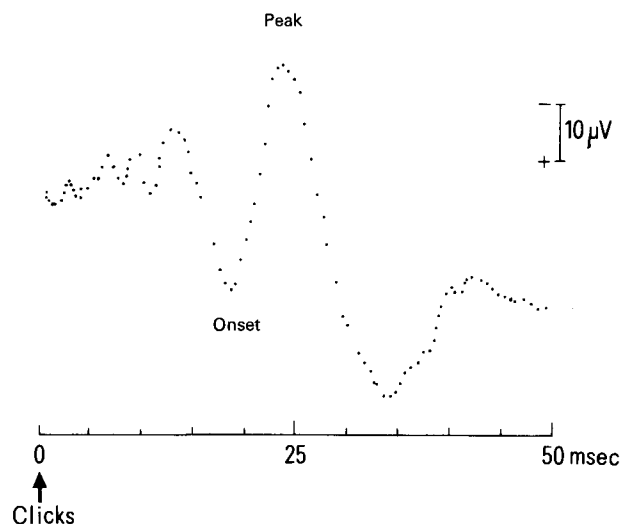


Fig. 1. The averaged response to 20 clicks of 100 db (sensation level) with a post-stimuli analysis time of 50 msec recorded on dura mater at bregma in albino rats is represented.

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