

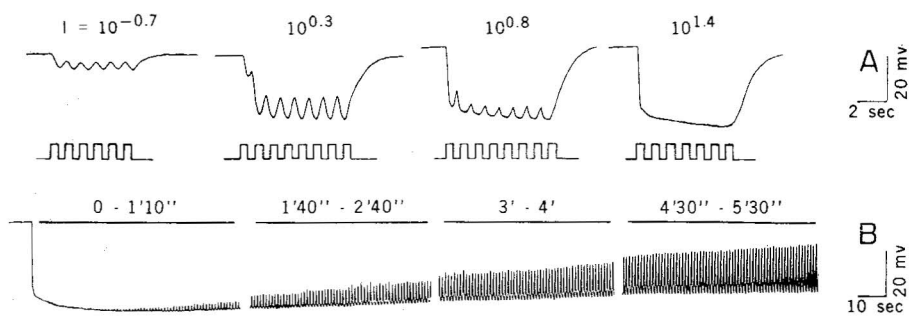


Fig. 2. Intracellular recording of horizontal cells (S-potentials) using glass micropipettes filled with 2M KCl. Signals were led through a Bak ELSA-4 negative-capacitance amplifier and written out on a Brush Mark 220 recorder. (A) Responses to short bursts of intermittent light of graded intensity, I . Although the more intense stimuli produced a deeper hyperpolarization of the unit, flicker resolution is progressively diminished until, at higher intensities, the membrane potential saturates. (B) Prolonged exposure of the brightest stimulus in the series ($10^{1.4}$ quanta $\mu\text{m}^{-2} \text{sec}^{-1}$), recorded at a paper speed five times slower than in the upper tracings. Note that only minimal flicker following is seen during the first minute. However, as the cell slowly adapts to the light, the membrane repolarizes and flicker responses become larger.

utes, the membrane slowly repolarizes. During this time, small flicker responses are detected, which continue to grow and finally reach maximal amplitude in about 6 minutes. More intense stimuli give rise to higher CFF values, but require longer waiting times for flicker recovery—for example, up to 20 minutes when $I = 10^4$ quanta $\mu\text{m}^{-2} \text{sec}^{-1}$.

The phenomenon we report here is closely related to the results of Dowling and Ripps (2), who recorded increment thresholds from the skate retina which, over a response range of 7 log units, followed the Weber-Fechner relation ($\Delta I/I = \text{constant}$) without saturating. Their studies showed that ΔI is a time-dependent variable which, at high background luminances, required waiting periods of 20 to 30 minutes before stable values could be attained. In a later study (3), they suggested that this unusual property may reflect an underlying mechanism which exists to prevent saturation of skate photoreceptors. We believe that the same mechanism is responsible for the shifting of the skate photoreceptor to an operating characteristic which enhances its temporal resolution. This very slow process is in contrast to the rapid discrimination achieved by cones in mixed retinas when presented with high rates of intermittent stimulation.

The experimental data reported here seem to establish by conventional tests that all the responses are due to a single photoreceptor type—a rod. However, double branched curves for isolated receptor potentials, intracellular S-potentials, and ERG b-waves could also be explained by summation between receptor types. The spectral analysis shows that if this is the case all receptor types have the same photopigments. Final resolution of this point would seem to require intracellular recordings from individual skate photore-

ceptors. Nonetheless, the anatomical and physiological evidence makes it seem likely that there are no cones in the skate retina.

If skate rods have a dual response, is this property to be found in all rods, or is it unique to the skate rod? Only intracellular recordings of rods in other species (8) will satisfactorily answer this question. In any case, one requirement for efficient temporal resolution would be that the decay of the receptor potential change from the slow form normally typical of rods to the brisk off-response normally characteristic of cones (9). Such a property of skate rods, if it exists, is a subtle one, since no dramatic changes were noted in preliminary electron microscopic observations (10).

Whatever the mechanism, the end result is that the rods are able to resolve high rates of intermittent stimuli and increments against backgrounds far more in-

tense than those required to produce short-term rod saturation (11). We suggest that this is possible because skate rods possess the means to alter the basic nature of their response to light and behave in a fashion very similar to that classically ascribed to cones.

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Lysergic Acid Diethylamide: Effect on Histone Acetylation in Rabbit Brain

Abstract. *Lysergic acid diethylamide increased acetylation of histones in rabbit cerebral hemispheres and midbrain 30 minutes after intravenous administration of the drug at doses of 10 and 100 micrograms per kilogram of body weight. Evidence for the stimulation of acetylation in individual histone bands was obtained after separation by electrophoresis on polyacrylamide gels.*

Little is known of the possible effects of psychotropic drugs on gene activity in the brain, particularly when the drug dosage is comparable to levels utilized by man. As covalent modification of chromosomal proteins may be associated with regulation of gene activity (1), we analyzed the effect of *d*-lysergic acid diethylamide (LSD) on the acetylation of histones in various re-

gions of the rabbit brain. It has been shown previously that brain nuclei contain histone acetylase and that the activity of this enzyme increases with neural maturation (2). We now report the effect of LSD at dosages comparable to concentrations that cause distortion of sensory perception in humans (3).

LSD was administered intravenously to

Table 1. Effect of LSD on acetylation of brain histones. LSD (9) in 100 μ l of saline was injected into the ear vein of male New Zealand white rabbits (1 kg). Control animals received saline alone. After 30 minutes, 1 mc of sodium [3 H]acetate (3.7 c/mmole) was injected through cannulas into each lateral ventricle of the brain. After 15 minutes the brain was removed, and histones were purified from isolated nuclei (10). Each value is the mean $\pm$ the standard error of the mean of three experiments.

Brain region	Histone acetylation per milligram of protein (10 ³ count/min)		
	Control	LSD dose	
		10 μ g/kg	100 μ g/kg
Cerebral hemispheres	62.9 $\pm$ 1.1	80.7 $\pm$ 3.9	112.9 $\pm$ 7.7
Midbrain	27.4 $\pm$ 3.7	52.0 $\pm$ 7.2	59.8 $\pm$ 5.4
Cerebellum	8.6 $\pm$ 0.5	7.9 $\pm$ 0.1	8.0 $\pm$ 1.5

young rabbits. The drug accumulates rapidly within the brain (4). After 30 minutes, the histone acetylation was assayed by the introduction of [3 H]acetate into the brain for a short period. As shown in Table 1 a moderate dosage of LSD (10 μ g per kilogram of body weight) stimulated the incorporation of [3 H]acetate into histone by 28 percent in the cerebral hemispheres and by 90 percent in the midbrain (expressed over saline control values). The increase was 80 percent and 118 percent, respectively, at the higher drug dosage (100 μ g/kg). No change was detectable in the cerebellum at either LSD concentration. This result could not be attributed to an absence of LSD in the cerebellum as studies with [3 H]LSD have demonstrated a wide distribution of the drug in this region of the brain (4).

The following observations indicated that the data given in Table 1 reflect a stimulation of histone acetylation rather than a change in histone synthesis. The intracerebral injection of puromycin did not significantly reduce [3 H]acetate incorporation into cerebral hemisphere histone in either control or LSD-treated animals

(Table 2), and more than 80 percent of the radioactivity in the labeled histone was released by acid hydrolysis (5). Similar results were obtained for the midbrain and cerebellum.

In order to verify that specific histones undergo increased acetylation, histones were fractionated by electrophoresis on 6.25M urea-polyacrylamide gels (6). The acetylation profile of histones isolated from cerebral hemisphere nuclei of LSD-treated and saline control animals is shown in Fig. 1. The greatest stimulation was shown by histones $f_{2a2} + f_{2b}$ (161 percent) followed by f_3 (128 percent) and then f_{2a1} (71 percent). A similar pattern of acetylation was evident for histones from midbrain nuclei (Fig. 2); however, the percentage increases were greater, that is, $f_{2a2} + f_{2b}$ (215 percent), f_3 (151 percent), and f_{2a1} (85 percent). Acetylation of histone f_1 was not detectable in either brain region.

The isotope was administered through cannulas that had been inserted stereotactically into the lateral ventricles of the cerebral hemispheres on the day preceding the administration of the drug (7). This

Table 2. Effect of puromycin on acetylation of cerebral hemisphere (counts per minute per milligram of protein) histones. Experimental procedures were as given in Table 1, except that puromycin at 100 μ g per ventricle was included with the isotope injection.

Treatment	Acetylation (10 ³ count/min)
Control	62.1
Control + puromycin	59.5
LSD (100 μ g/kg)	111.6
LSD + puromycin	106.3

procedure permitted a reproducible delivery of the isotope into the ventricles for distribution through the brain by the cerebral spinal fluid. An autoradiographic analysis indicated that periventricular regions were primarily labeled during the isotope labeling period.

In summary, LSD stimulated the acetylation of specific histones in rabbit cerebral hemispheres and midbrain 30 minutes after the intravenous administration of the drug. We have also observed a stimulation of RNA synthesis in isolated brain nuclei 2½ hours after administration of LSD in vivo (8).

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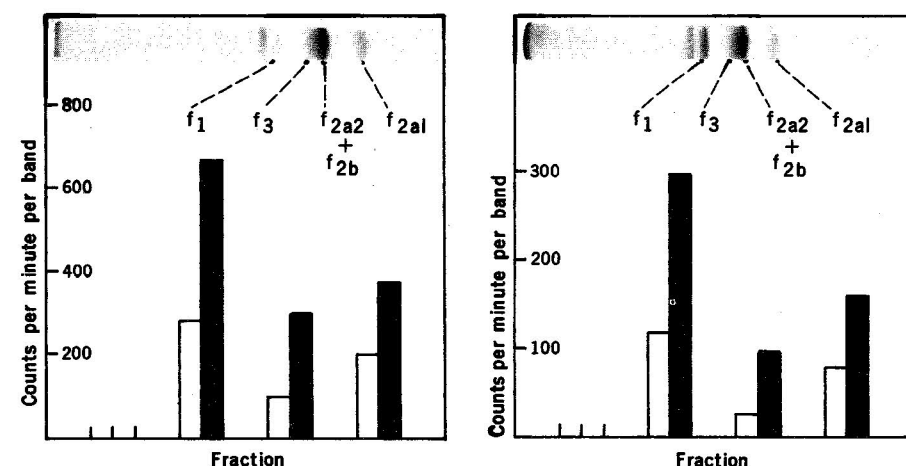


Fig. 1 (left). Acetylation profile of cerebral hemisphere histones after LSD. LSD (100 μ g/kg) and sodium [3 H]acetate were administered to young rabbits as outlined in Table 1. Histones purified from isolated nuclei were subjected to electrophoresis on 6.25M urea-15 percent polyacrylamide gels (6, 10, 11). Equal amounts of histone (50 μ g) were used for each treatment. LSD treatment, closed bars; saline control, open bars. Fig. 2 (right). Acetylation profile of midbrain histones after LSD. Procedure was similar to that for data in Fig. 1. LSD treatment, closed bars; saline control, open bars.