lateral geniculate body from the optic radiations by means of a semimicroelectrode (tip diameter, about 20 μm). This electrode recorded simultaneously from many units, the amplitude of the impulses being practically uniform. The activity was then fed into a band-pass filter with cutoffs at 6 and 600 hertz. The filter output was sent to an averaging computer. The averaged responses to an alternating grating looked approximately sinusoidal. The amplitude of the responses obtained from a kitten for gratings of various spatial frequencies and orientations are reported in Fig. 2. The frequency of the grating to which the kitten had been exposed was 0.45 cycle/deg. All three curves, for the vertical, horizontal, and oblique gratings, show a dip in the region of this frequency. This experiment has been repeated in six kittens.

When recordings of the response to gratings located in the central part of the visual field were made with the same technique from the optic tract, we did not find any significant reduction of the response for the spatial frequency of the early exposure. This experiment however, may not be conclusive, since the recording electrode might select populations of fibers unaffected by exposure.

In initial experiments, a reduction in sensitivity for the spatial frequency of exposure was found also at a single unit level, both in some geniculate cells and in some complex neurons of the striate cortex. In our experiments the cortical cells did not show any tuning in favor of the vertical orientation, the orientation of the periodic grating to which the kittens were exposed (*7*).

It is known from psychophysical experiments in man that inspection of a grating of high contrast strongly increases the contrast threshold for a grating of the same spatial frequency (*8*). We demonstrated that the simple cortical neurons of the adult cat respond similarly (*9*). If a grating of high contrast drifts across the receptive field of a cortical neuron, its response to a grating of low contrast is dramatically reduced for 25 to 30 seconds. The reduction is limited to a narrow band of spatial frequencies around that of the high contrast grating to which the neuron receptive field was exposed. This is exactly the same phenomenon reported here for kittens, except that the neural modification brought about by early exposure is probably permanent (*10*). In kittens the modification of neural responses occurs primarily at the geniculate level, where the receptive fields are concentrically organized. It is not surprising, therefore, that the phenomenon was independent of orientation.

Our experiments seem to contradict the generally accepted concept that exposure to a given pattern during the critical period increases the sensitivity of the visual system for that pattern. We have found an instance in which this sensitivity is decreased.

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Cyclic Guanosine Monophosphate: Elevation in Degenerating Photoreceptor Cells of the C3H Mouse Retina

Abstract. *As a result of an early deficiency in cyclic nucleotide phosphodiesterase activity, guanosine 3′,5′-monophosphate accumulates in retinal photoreceptor cells before they begin to degenerate. It is suggested that degeneration of the photoreceptor cells is related to an imbalance in their metabolism or function which is caused by the elevated levels of cyclic guanosine monophosphate.*

The neural retina of the mouse is organized in layers that contain morphologically and functionally distinct classes of neurons (*1*). The outer layers contain the light-sensitive photoreceptor cells with their characteristic rod outer segments, and the innermost layers contain the ganglion cells and their axons, which converge to form the optic nerve. The bipolar or inner nuclear layer, which contains several neuronal cell types, is sandwiched in between these layers.

An autosomal recessive mutation (*rd*) of C3H/HeJ mice (*2*) results in the degeneration of all the photoreceptor cells of this retina during the second and third postnatal week (*1*). The inner bipolar and ganglion layers survive the disease and retain their layered organization. Biochemical studies have shown that the C3H retina is abnormal before the first signs of degeneration can be observed in the photoreceptor cells at the eighth postnatal day (*3*).

During the development of the normal retina, a receptor-specific phosphodiesterase (PDE), which hydrolyzes cyclic nucleotides to 5′-mononucleotides, increases in activity as the photoreceptor cells differentiate and mature. In the developing C3H retina, this enzyme activity is never observed. The receptor-specific PDE has been studied in homogenates of the normal retina (*3*) and in isolated rod outer segments of photoreceptor cells (*4*), where it exhibits a low affinity for cyclic nucleotides [high Michaelis constant (K_m) for PDE]. But the role of this high K_m-PDE in the metabolism or function of normal photoreceptor cells, as well as the identity of its substrate in vivo, has remained speculative.

We have considered adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) as possible substrates for the high K_m-PDE reaction in vivo. Quantitative histochemical and biochemical studies have revealed that (i) cyclic AMP is concentrated in the inner layers of the retina where it is associated with neuronal function and (ii) the content of cyclic AMP and the activity of adenylate cyclase are both low in normal photoreceptor cells (*5*). That the metabolism of cyclic AMP is minimal in photoreceptor cells is also suggested by investigations of the immature retina of C3H mice, where cyclic AMP concentrations do not increase in the photoreceptor cells, even though the cells are deficient in cyclic nucleotide PDE activity (*5*). These findings cast serious doubt as to

whether cyclic AMP is available to serve as substrate for the PDE enzyme of photoreceptor cells in vivo.

In contrast, the high concentrations of guanosine nucleotides in the retina (*6*) and the greater affinity of the high K_m-PDE enzyme for cyclic GMP than for the other cyclic nucleotides (*4*) encouraged us to investigate the distribution of cyclic GMP in the normal retina and its possible involvement in photoreceptor degeneration in the *rd* retina.

The amount of cyclic GMP was measured in developing retinas of DBA/1J (normal) and C3H/HeJ (*rd*) mice by Murad's binding protein assay (*7*) with some modifications (Fig. 1). Cyclic GMP in the normal retina increases postnatally, nearly doubling between day 6 and day 12 (Fig. 1A). In the developing *rd* retina, the content of cyclic GMP is normal for 6 days and then it increases, reaching a peak value at days 14 to 16. Subsequently, cyclic GMP decreases, and by 22 days stabilizes at a value less than that of the normal retina. When the amount of cyclic GMP per milligram of protein is considered, the pattern of change in cyclic GMP during development is similar to that per retina; but, in the phase of declining cyclic GMP concentrations, the curves for the normal and *rd* retinas do not intercept (Fig. 1B). High concentrations of cyclic GMP in the *rd* retina between postnatal days 6 and 19 appear to result from the balance of two events, one producing an increase in the cyclic GMP content and the other causing a decrease. The rise in cyclic GMP content of the *rd* retina at day 6 occurs at the time when the deficiency in activity of a high K_m-PDE can first be observed (*3*); its accumulation in the *rd* photoreceptor cells could be accounted for by their inability to hydrolyze cyclic GMP. The decline in cyclic GMP content after day 16 corresponds to the period in which the photoreceptor population is depleted (*2*); the death and degeneration of the photoreceptor cells would disperse the accumulated cyclic GMP in these cells and thereby reduce the content of cyclic GMP in the *rd* retina.

We performed quantitative histochemical studies to establish the level of cyclic GMP in the photoreceptor and inner layers of the normal and *rd* mouse retina. About 60 percent of the cyclic GMP in the normal retina of mature and immature mice is found in the photoreceptor layer (Table 1). At day 15, when the number of photoreceptor cells in the *rd* retina is already reduced, the amount of cyclic GMP per milligram of protein in the inner layers of the *rd* retina is comparable to that of a 15-day-old normal animal. However, the diseased photoreceptor layer of this retina contained four- to fivefold more cyclic GMP per milligram of protein than the photoreceptor layer of the normal. These

Table 1. Concentrations of cyclic GMP in microdissected layers of freeze-dried retinas. Eyes frozen in situ were sectioned at −20°C and lyophilized; samples (200 to 400 μg) of either the complete retina, the photoreceptor layer, or the bipolar and ganglion cell layers were prepared from unstained sections of normal and *rd* eyes. The layers were divided by cutting in the plane of the outer synaptic layer. The samples were extracted and assayed for cyclic GMP (see Fig. 1). Values represent means of duplicate assays on individual samples that were microdissected by separate dissectors; eyes from at least two litters of mice were used.

Samples	Cyclic GMP (pmole per mg protein)		
	DBA adult	DBA day 15	C3H day 15
Photoreceptor layer	660	625	2002
	675	615	2765
			2755
Bipolar and ganglion cell layer	419	414	407
	442	425	425
Complete retina	489	501	912
	577	535	766
			831
			819

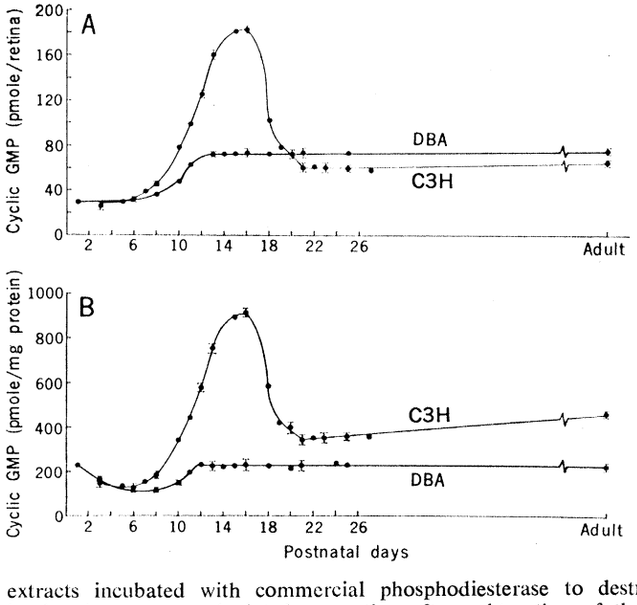

Fig. 1. Cyclic GMP concentrations during development in freshly dissected retinas of normal and *rd* mice. Two retinas from each age or strain of mice were homogenized in 0.1*N* HCl and extracted at 100°C for 2 minutes (*9*). The extract was cooled, centrifuged, and adjusted to *p*H 4.0 by the addition of 1*M* sodium acetate. For each sample, a duplicate series of four tubes was set up in an ice bath. The tubes were marked 20, 10, 5, and 0, respectively, representing the number of picomoles per 10 μl of unlabeled cyclic GMP added as internal standards. The components of the reaction mixture were then added as follows: 10 μl of the appropriate concentration of standard cyclic GMP, 10 μl of retinal extract, 10 μl of ^{3}H-labeled cyclic GMP, and 30 μl of cyclic GMP binding protein (JLB Laboratories, Los Angeles). If standard cyclic GMP was omitted, it was replaced by 10 μl of 50 m*M* sodium acetate buffer, *p*H 4.0. The tubes were placed in a mixture of ice and water for 2 hours, the contents were diluted with 1 ml of 20 m*M* KH_2PO_4, *p*H 6.0, and filtered through cellulose acetate filters. The discs were washed with phosphate buffer, placed in counting vials, and shaken with Aquasol until dissolved; the radioactivity was then counted in a Nuclear-Chicago scintillation counter. The reciprocals of the values for the radioactivity retained with the filters were calculated and plotted against the amount of standard cyclic GMP added to the samples (*10*). This gives a linear curve for tissue extracts and for the tissue extracts incubated with commercial phosphodiesterase to destroy endogenous cyclic GMP (blanks). (A) Amounts of cyclic GMP in the tissue were calculated per retina after subtraction of the appropriate blank. Similar results were obtained when retinal extracts were applied to anion-exchange columns of AG1-X2, Cl^-, 200–400 mesh resin (Bio-Rad) and cyclic GMP was eluted (*11*) and assayed by the binding protein method, or when retinal extracts were analyzed with the radioimmunoassay for cyclic GMP (*12*). (B) Showing the data expressed as per milligram of protein is informative since cells are maturing in the normal retina and degenerating in the *rd* retina during development (*13*). The protein content in each homogenate was determined by the method of Lowry *et al.* (*14*). The amount of cyclic GMP in the *rd* retina after 20 days remains above normal; this is probably related to adaptive changes in cyclic GMP metabolism of the surviving cells of the *rd* retina.

observations establish that the high levels of cyclic GMP in the immature *rd* retina are caused by the accumulation of cyclic GMP in the photoreceptor layer.

Our findings indicate that normal photoreceptor cells hydrolyze cyclic GMP by means of their specific high K_m-PDE enzyme and those of the *rd* retina accumulate cyclic GMP due to their deficiency in high K_m-PDE activity. The imbalance in cyclic GMP metabolism of *rd* photoreceptors occurs before the onset of their degeneration and, most probably, causes disturbances in the metabolism or function of these cells. It is premature to speculate on the specific action of cyclic GMP in these cells but, generally, cyclic nucleotides act to regulate cellular function (*8*). This suggests that elevated cyclic GMP will affect the homeostatic balance of the *rd* photoreceptor cells and, if this effect is significant, it could produce changes that result in the degeneration of those cells.

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Presynaptic Inhibition: Primary Afferent Depolarization in Crayfish Neurons

Abstract. *Inhibition of transmission between tactile sensory neurons and interneurons in the crayfish was investigated by intracellular recording in the presynaptic processes. Inhibition is correlated with a depolarization of the presynaptic process, as in the mammalian spinal cord; the depolarization is accompanied by a conductance increase, and is mediated by interneurons that can be excited by a variety of routes.*

Presynaptic inhibition is a process of wide occurrence, in which synaptic efficacy is reduced by preventing the release of transmitter from presynaptic endings, instead of by increasing the conductance of the postsynaptic membrane. In the mammalian spinal cord it is correlated with a depolarization of primary afferent terminals (*1*). The mechanism by which transmitter release is reduced is unknown, however, because intracellular records from the presynaptic axons must be made at a great distance from the terminals, or cannot be made at all (*2*). We here report that primary afferent depolarization (PAD) can be recorded from crustacean sensory neurons at sites close to the region in which they release excitatory transmitter. We have

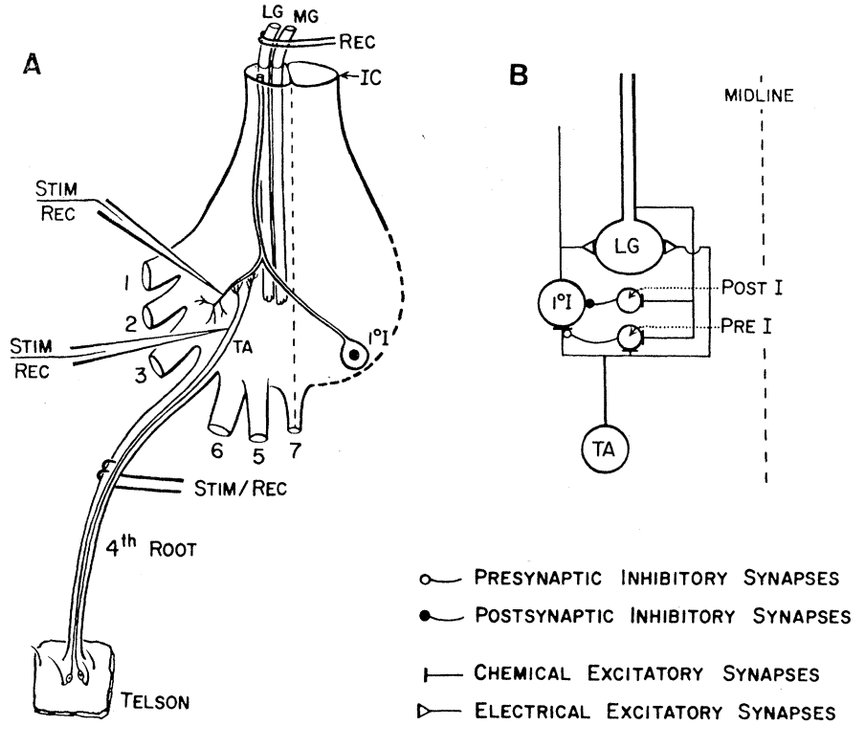

Fig. 1. Preparation and circuit diagram. (A) Composite diagram of the sixth abdominal ganglion preparation (ventral view), displaying the main neural elements and their known anatomical relations. The fourth ganglionic roots, which innervate tactile hairs on parts of the telson and sixth abdominal segment, were left intact. Suction electrodes were used for both stimulation and monitoring of the root activity. The lateral giant fiber was recorded as shown, and stimulated in a more anterior interganglionic connective with a suction electrode. Intracellular electrodes were inserted through the ventral surface of the desheathed ganglion to penetrate either fourth root tactile afferents or first-order tactile interneurons. (B) Simplified circuit diagram indicating known and postulated interactions between the elements discussed in the text. This diagram is based on the work of Zucker (*6*) and Krasne and Bryan (*5*) as well as the experiments discussed here. Connections between the lateral giant and the inhibitory interneurons may be polysynaptic. Abbreviations: *IC*, interganglionic connectives; *LG*, lateral giant fiber; *MG*, medial giant fiber; *POST I*, postsynaptic inhibitory interneuron; *PRE I*, presynaptic inhibitory interneuron; *1°I*, first order tactile interneuron; and *TA*, telson hair tactile afferent. In (A) and (B) only one hemiganglion is shown; all elements are bilaterally symmetrical.