sponse to the honeyeater reflects fear of a frightening stimulus comparable to that of a real predator, we conducted another experiment. Six blackbirds that had been habituated and then conditioned to the honeyeater were presented the stuffed little owl between 1 day and several days after the last honeyeater test. Responses to the two stuffed birds did not differ significantly (Wilcoxon matched-pairs signed-ranks two-tailed test). Hence, at least under the conditions of testing, cultural transmission of honeyeater recognition leads to a response resembling that to a genuine predator. Whether transmission can lead to still higher response levels when associated with more dangerous predators than little owls needs investigation.

Cultural transmission embodies a type of nongenetic transfer of information that probably protects the observer in a dangerous situation in one or more of the ways mentioned. The transfer would be even more effective if there were no appreciable loss of information when passed on in chainlike fashion. The length of such a chain would depend on several constraints, notably the decrement of transmission due both to the information lost in each single learning act and to forgetting. We tested this hypothesis by making the observer bird in one trial the teacher in the next trial and so on. From the second trial on, the honeyeater was presented at the end of the hallway in a way such that it could be seen by both the new teacher and the observer. There was no discernible decrement of information transfer through a total of six presentations, involving six observers that were subsequently teachers. Response strength in all six cases was less during conditioning than in the subsequent test ($P = .05$, Wilcoxon matched-pairs signed-ranks one-tailed test).

Our demonstration of cultural transmission of enemy recognition has probably revealed only one function of mobbing, namely, enhanced fitness of the observer. The benefits accruing to the teacher remain to be investigated. Since blackbird dispersal is viscous (*24*), kinship effects or other more immediate benefits seem possible. Although we have established one function of mobbing, we are far from understanding which selection pressures have molded the adaptedness of this behavior (*23*).

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Light Stimulates Tyrosine Hydroxylase Activity and Dopamine Synthesis in Retinal Amacrine Neurons

Abstract. *Retinal dopamine-containing amacrine neurons are rapidly activated by light, as shown by an increase in the rate of dopamine formation in vivo and a concomitant increase in the activity of tyrosine hydroxylase, measured in vitro with a subsaturating concentration of pteridine cofactor. Activation of tyrosine hydroxylase also occurs when isolated eyes from rats killed in the dark are exposed to a strobe light. Studies of amacrine neurons should provide basic data about the biochemical processing of visual information, as well as the physiological presynaptic regulatory mechanisms of dopamine-containing neurons.*

In the retina, the neurotransmitter dopamine is found in some amacrine neurons (*1*) that play an important role in local circuit processing of visual information. We found that in amacrine cells of the rat, the activity of tyrosine hydroxylase (E.C. 1.14.16.2), the rate-limiting enzyme for dopamine formation, is modulated by environmental lighting. The enzyme is rapidly activated when either the whole animal, or the isolated eye of dark-adapted rats, is exposed to light. When the light is removed, enzyme activity decreases. Activation is apparently the result of a decrease in the Michaelis constant of tyrosine hydroxylase for its pteridine cofactor. Concomitant with enzyme activation is a fourfold increase in the rate of formation of dopamine by amacrine neurons. To our knowledge, the retinal dopamine-containing amacrine neurons are the only dopamine-containing neurons that can be experimentally activated and inactivated by a physiological stimulus. The retinal amacrine neuronal system should provide valuable data about the biochemical processing of visual information, the molecular mechanisms of tyrosine hydroxylase activation, and the regulation of dopamine synthesis.

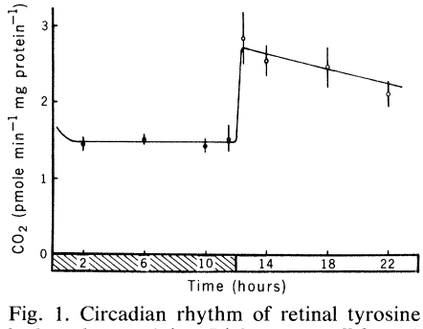

Fig. 1. Circadian rhythm of retinal tyrosine hydroxylase activity. Lights were off from 0 to 12 hours. Solid circles represent samples taken in the dark and open circles represent samples taken in the light. Data are expressed as mean ± standard error for five duplicate determinations.

Male Sprague-Dawley rats (Zivic-Miller, 190 to 210 g) were exposed to 12 hours of light per day for 3 days before

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Table 1. Light-induced activation of retinal tyrosine hydroxylase during the dark phase of the light-dark cycle. All rats were killed during the third hour of darkness. The rats in group 1 were killed and their retinas dissected under red light. The rats in group 2 were exposed to 15 minutes of room light prior to being killed. Tyrosine hydroxylase activity was measured as picomoles of CO_2 produced per minute per milligram of protein.

Group	Condition	N	Tyrosine hydroxylase activity
1	Dark	4	2.0 ± 0.2
2	Light (15 minutes)	5	4.9 ± 0.6*

*$P < .005$.

experimentation. The light intensity inside each cage was about 400 lux. Rats were decapitated and retinas dissected and frozen within 5 minutes. Rats killed during the dark phase were processed as above under a red photographic safety light (Kodak Wratten filter No. 1). Tyrosine hydroxylase activity was measured with 0.4 m*M* 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) by the decarboxylase-coupled assay of Waymire *et al.* (*2*) as modified by Zivkovic *et al.* (*3*). We used an incubation time of 20 minutes. Dopamine and norepinephrine were determined by gas chromatography–mass spectrometry (*4*), with deuterated-dopamine and deuterated-epinephrine being used as internal standards. The rate of dopamine synthesis was estimated from the depletion of retinal dopamine after α-methyl-*p*-tyrosine administration (*5*). Protein was assayed by the method of Lowry *et al.* (*6*).

Retinal tyrosine hydroxylase activity, when measured in the presence of a subsaturating concentration of $DMPH_4$, showed a circadian rhythm (Fig. 1). Tyrosine hydroxylase activity was low and constant during darkness and increased rapidly after the lights went on. After the lights went off, enzyme activity decreased. Increased tyrosine hydroxylase activity in light-exposed rats was also observed in assays in which we used a subsaturating concentration of tetrahydrobiopterin, the presumed natural cofactor of tyrosine hydroxylase.

A group of rats adapted to the normal 12-hour light-dark cycle were exposed to 15 minutes of light during the third hour of darkness (Table 1). Again, exposure of the animals to light increased tyrosine hydroxylase activity. This result demonstrates that the observed increase in activity is an effect of the light, and is not dependent on an internal biological rhythm. In another study, rats were killed during the dark phase and their eyes removed under red light. The isolated eyes were exposed for 60 seconds to high-intensity strobe light (2 Hz) and the retinas were removed under white light in 30 seconds. This brief exposure to light significantly increased tyrosine hydroxylase activity (Table 2), supporting the notion that the increase of enzyme activity is intrinsic to the eye. Kinetic analysis indicated that 15 minutes of exposure to light significantly decreased the apparent Michaelis constant of retinal tyrosine hydroxylase for $DMPH_4$, but had no effect on the apparent maximum velocity (V_{max}) (*7*). Similar activation of striatal tyrosine hydroxylase has been reported to occur after neuroleptic administration (*3*) or electrical stimulation (*8*).

The steady-state concentration of retinal dopamine was not significantly different in the light and the dark (Fig. 2). The rate of decline of dopamine after α-methyl-*p*-tyrosine administration, however, was significantly faster in the light than in the dark and, consequently, the calculated rate of synthesis was four times higher in the light. A more rapid rate of decline of the catecholamine after α-methyl-*p*-tyrosine treatment is associated with increased neuronal firing rates (*9*). Norepinephrine was not detected in the retina. Our limit of sensitivity was about 0.5 pmole.

Our experiments demonstrate that retinal tyrosine hydroxylase activity increases concomitantly with an increase of dopamine synthesis as a direct effect of light acting on the retina. Presumably,

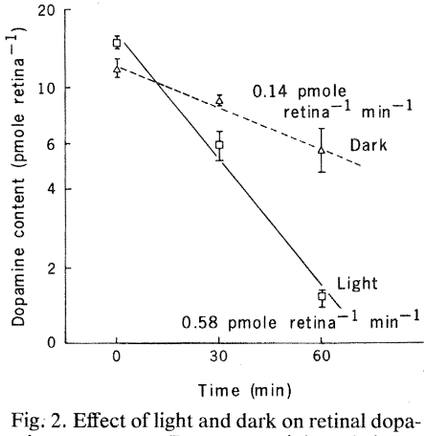

Fig. 2. Effect of light and dark on retinal dopamine turnover. Rats were injected intraperitoneally with α-methyl-*p*-tyrosine methyl ester (300 mg/kg) at 2:00 hours (dark, triangles) and 14:00 hours (light, squares) and killed 0, 30, or 60 minutes after injection. The *y*-axis indicates the dopamine content and the *x*-axis is the time after injection. The calculated synthesis rate is given at the end of each dopamine-depletion line. Data points represent mean ± standard error for five samples.

Table 2. Light-induced activation of retinal tyrosine hydroxylase in isolated eyes. All rats were killed during the third hour of darkness and the eyes were removed under red light. The isolated eyes were exposed to 60 seconds of strobe light (Grass Photo-Stimulator, Model PS1, 2 Hz intensity 8). Tyrosine hydroxylase activity was measured as picomoles of CO_2 produced per minute per milligram of protein.

Group	Condition	N	Tyrosine hydroxylase activity
1	Dark	10	2.0 ± 0.2
2	Strobe	6	3.1 ± 0.4*

*$P < .005$.

this effect is mediated through synaptic interactions among photoreceptors and other retinal neurons. These findings are consistent with reports that exposure to light synaptically depolarizes amacrine neurons (*10*), increases the release of dopamine from the perfused eye (*11*), and increases the rate of dopa accumulation after decarboxylase inhibition (*12*). The rapid response of the enzyme to light suggests that the moment-to-moment regulation of dopamine synthesis in response to changes in neuronal activity occurs through alterations in the molecular properties of tyrosine hydroxylase, possibly by an allosteric mechanism.

In conclusion, the retina appears to be a useful system for studying the physiological presynaptic regulatory mechanisms of dopamine-containing neurons.

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