those leaning into +/+ pigment epithelium were relatively normal suggests that the site of aberrant interaction between the defective pigment epithelial cell and the photoreceptor is at or by way of the tip of the rod outer segment, the site of contact between the two cell types.

Survival of some photoreceptor cells under mutant pigment epithelium may be mediated by adjacent, normal pigment epithelial cells. This is consistent with the finding that outer segments underlying patches of mutant pigment epithelium are more normal at the periphery of the patch than those under the center of the patch. If amelioration of the disease proves to be mediated by a diffusible substance, it may be possible in future work to isolate the substance from normal pigment epithelial cells and provide it exogenously to the mutant retina or pigment epithelium or both, and thereby retard or alleviate the degenerative disorder.

This study and others (*14, 16*) demonstrate the usefulness of experimental chimeras in determining whether particular neurological mutations are acting intrinsically, within the most obviously affected cell type, or extrinsically, within either an adjacent or distant interacting cell (for example, by a circulating factor). The rat chimeras are particularly informative, for we can conclude not only that the *rdy* gene is acting extrinsically to the photoreceptor cell, but also that the pigment epithelial cell is the actual site of the gene action (*17*). As far as we are aware, this is the first neurological mutation in mammals in which a primary site of mutant gene action can be assigned to a particular cell type.

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7. CDF is the abbreviation for inbred cesarian-derived Fischer rats obtained from Charles River Breeding Laboratories. The rats are albinos (*c/c*) and are +/+ at the *p* and *rdy* loci; therefore, the CDF × RCS F_1 hybrids are +/*c*, +/*p*, and +/*rdy*.
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Lysergic Acid Diethylamide– and Mescaline-Induced Attenuation of the Effect of Punishment in the Rat

Abstract. *At a dose as low as 1 microgram per kilogram of body weight, lysergic acid diethylamide (LSD) significantly decreased the suppressive effect of electric shock on licking behavior of the rat. Attenuation of punishment was also obtained with mescaline, but neither dimethyltryptamine nor Δ^9-tetrahydrocannabinol was active in this test. Cyproheptadine and α-propyldopacetamide, drugs that interfere with the function of neurons that contain serotonin, have a behavioral effect similar to that of LSD and mescaline, which suggests that the attenuation of punishment produced by these hallucinogens may result from decreased activity of such neurons.*

Pharmacological interference with the function of neurons that contain serotonin (5-hydroxytryptamine) restores behavior suppressed by electric shock. Such attenuation of the effect of punishment occurs after the administration of antagonists of serotonin receptors (*1, 2*). A similar effect is obtained after depletion of serotonin by *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase (*3*). Benzodiazepines and barbiturates also diminish the effect of punishment on responding (*4*). Among their many neuropharmacological effects, these "antianxiety" drugs decrease serotonin turnover (*5*). This effect on serotonin metabolism is also produced by several drugs with hallucinogenic properties, including lysergic acid diethylamide (LSD) (*6*), mescaline (*7*), dimethyltryptamine (DMT) (*8*), and Δ^9-tetrahydrocannabinol (THC) (*9*). Therefore, I tested these hallucinogens in a procedure sensitive to benzodiazepines (*10*). I found that LSD and mescaline significantly reduce the effect of punishment.

The procedure was identical to that used by Vogel *et al.* (*10*). The apparatus was a clear Plexiglas box (38 by 38 cm) with a black Plexiglas compartment (10 by 10.5 cm) attached to one wall and an opening 5 by 7.5 cm between the two. The entire apparatus had a stainless steel grid floor. A metal water tube extended 1.5 cm into the small box 4 cm above the grid. A drinkometer circuit (Grason-Stadler, model E4690A) was connected between the drinking tube and the grid floor. An unscrambled shock (0.75 ma) was administered by switching the connection to the tube and grid from the drinkometer to a shock generator (Aim BioSciences, model 507).

After 48 hours of water deprivation, each rat (200 g, male, albino, Sprague-Dawley) was placed in the apparatus 30 minutes after the drug or saline was injected. It was allowed to find the drinking tube and to lick 20 times, after which shocks were administered for each tube contact during a 2-second period. The cycle of 20 licks without shock followed by 2 seconds of vulnerability to shock was repeated until 3 minutes after the first shock was delivered. During this 3-minute session, the total number of shocks was recorded automatically. Each daily experiment included one group treated with saline and four groups treated with a drug (there were eight rats in each group). Drugs were either dissolved in saline or suspended in distilled water with one drop of Tween 80, and they were ad-

Table 1. Neuropharmacological effects of hallucinogenic drugs on serotonin with respect to their effect on punished behavior.

Drug	Serotonin			Net synaptic action
	Turnover	Uptake	Release	
Restores punished behavior				
LSD	Lowers	Weak blocker	Decreases	Antagonistic
Mescaline	Lowers	Weak blocker		Antagonistic
Does not restore punished behavior				
DMT	Lowers	Potent blocker	Increases	Agonistic
THC	Lowers	Potent blocker		Agonistic

ministered intraperitoneally in a volume equivalent to 1 ml per kilogram of body weight.

In control animals, licking behavior punished with electric shock is suppressed; administration of LSD decreased such suppression. A significant increase in the number of shocks accepted was observed at doses of 1 and 3 μg/kg (Fig. 1). The highest dose tested, 10 μg/kg, was less effective in diminishing the effect of punishment.

Three other drugs with hallucinogenic properties were also tested for their influence on the effect of punishment on behavior. Mescaline, at doses of 10 and 30 mg/kg, was found to produce a significant increase in the number of shocks accepted (Fig. 1). Over a wide dose range, neither DMT nor THC had any effect. At the highest doses of these two drugs, licking actually decreased; this effect was accompanied by gross disturbances of behavior.

All four of these drugs influence neurons that contain serotonin. Their neuropharmacological effects, however, are exceedingly complex. In order to relate the neuropharmacological effects of these hallucinogens to their behavioral effects, several nonhallucinogenic compounds that share some of their actions on serotonin were also studied (Fig. 2).

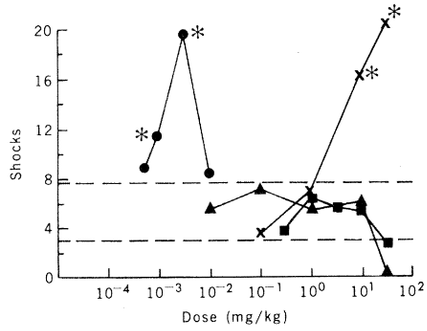

Fig. 1. Dose-response curves for the effect of LSD (●), mescaline (x), THC (▲), and DMT (■) on the mean number of shocks during the 3-minute test session. Each point represents data from at least eight rats. Those significantly beyond the range of saline-treated rats (parallel dashed lines) are indicated with an asterisk (Mann-Whitney U test).

Cyproheptadine, an antagonist of serotonin receptors, and α-propyldopacetamide (α-PDAC), an inhibitor of tryptophan hydroxylase, were capable of attenuating the effect of punishment at doses of 3.0 and 500 mg/kg, respectively. Chlorimipramine, a relatively selective blocker of serotonin uptake, was ineffective at the doses tested.

In pigeons and rats, two antagonists of serotonin receptors, methysergide and bromo-LSD, increase the rate of responses that are punished (*1*). Both drugs are derivatives of lysergic acid. Cinanserin and cyproheptadine, antagonists of serotonin that are not lysergic acid derivatives, also attenuate the effect of punishment (Fig. 2) (*2*). These results suggest that the effect of LSD may be related to a decrease in the activation of serotonin receptors, a consequence of its ability to decrease firing of neurons of the raphe nuclei (*11*) and to decrease serotonin turnover (*6*), rather than to any special feature of the lysergic acid molecule itself.

This suggestion is supported by the behavioral effect of α-PDAC, which decreases serotonin turnover by inhibiting tryptophan hydroxylase. Chlorimipramine also shares some of the neuropharmacological actions of LSD, but it differs in certain respects, which may account for its lack of influence on punished behavior. Like LSD (and α-PDAC), chlorimipramine decreases serotonin turnover (*12*). Also, like LSD, it decreases the activity of neurons of the raphe nuclei (*13*). However, unlike LSD which is relatively weak (*14*), it blocks the uptake of serotonin and, at some doses, actually increases the release of serotonin, an effect opposite that of LSD (*15*). In terms of synaptic activity, therefore, chlorimipramine would potentiate serotonin (that is, exhibit an agonistic action) rather than decrease its activity as would LSD (exhibit an antagonistic action) (Table 1).

Similar reasoning can explain the behavioral effects obtained with the other hallucinogenic drugs tested. Mescaline, which had a similar effect on punished behavior, shares with LSD the ability to decrease the firing rate of raphe neurons (*16*) and the turnover of serotonin (*7*); it too only weakly blocks serotonin uptake (*14*). On the other hand, DMT and THC failed to restore punished behavior. These two drugs share some of the neuropharmacological effects of LSD but have certain actions in common with chlorimipramine. Turnover of serotonin is decreased by DMT (*8*); however, it is a potent inhibitor of serotonin uptake (*17*). There is also evidence that DMT may cause serotonin to be released from presynaptic terminals (*18*). In a dose of 20 mg/kg (intraperitoneal injection), THC (like LSD) decreased serotonin turnover (*9*). Lower doses (for example, 5 mg/kg), although still behaviorally active, had no effect on serotonin (*19*). Furthermore, THC is also a potent inhibitor [IC_{50} (inhibitory concentration) = $1 \times 10^{-7}M$] of serotonin uptake (*20*). Consequently, it is not surprising that DMT and THC do not attenuate the effects of punishment. Although both decrease serotonin turnover, each has an agonistic action at serotonin receptors that would be expected to counter any decrease in the function of serotonin-containing neurons that it might produce (Table 1).

In the main, the behavioral effects of LSD consist of decreases in ongoing behavior. This occurs only at doses that are two or more log units higher than those effective in my study. A few reports indicate that in low doses (10 to 50 μg/kg) LSD can facilitate responding (*21*). In those studies, as in this one, when the dose of LSD was increased, behavior no longer increased. Such a dose-dependent effect of LSD is reflected in its electrophysiological action. Low intravenous doses (10 to 20 μg) decrease the firing rate of raphe neurons (*11*) (presumably as a consequence of a direct effect

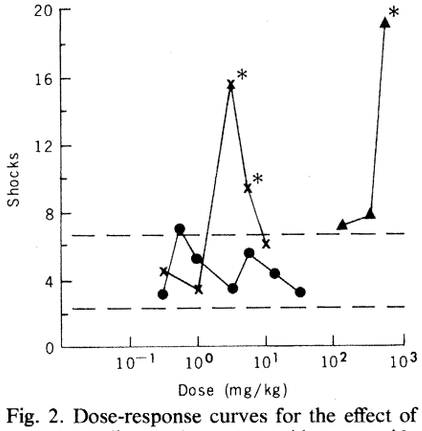

Fig. 2. Dose-response curves for the effect of cyproheptadine (x), α-propyldopacetamide (▲), and chlorimipramine (●) on the mean number of shocks during the 3-minute test session. The analysis is described in the legend to Fig. 1.

on these neurons) and increase the neuronal activity in brain areas innervated by raphe neurons (*22*). Higher doses of LSD decrease activity in these areas (*23*). These findings and the results of my study suggest that the decrease in raphe neuronal activity and the consequent release of inhibition in areas innervated by raphe neurons is responsible for the increase in punished behavior produced by LSD. This interpretation supports the evidence (*1–3*) for the role of neurons that contain serotonin in mediating the suppression of behavior by aversive stimuli.

The punishment-attenuating effect of LSD occurred at extremely low doses. Since this behavioral effect is a common feature of most clinically effective antianxiety drugs (*4*), in low (possibly nonhallucinogenic) doses, LSD and mescaline may also prove effective in treating anxiety.

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Cholera Toxin Induces Pineal Enzymes in Culture

Abstract. *Addition of choleragen to rat pineal organ cultures caused a long-lasting stimulation of adenylate cyclase activity, and this was followed by increases in serotonin* N*-acetyltransferase and cyclic adenosine monophosphate phosphodiesterase activities. These effects of choleragen were not blocked by the β-adrenoceptor antagonist propranolol, but the increases in cyclic adenosine monophosphate phosphodiesterase and serotonin* N*-acetyltransferase activities could be prevented by the protein synthesis inhibitor cycloheximide. The results indicate that cholera toxin can mimic the induction of pineal enzymes that normally follows β-adrenoceptor activation and suggest that increased cyclic adenosine monophosphate is a necessary and sufficient signal for such changes in enzyme activity.*

The pineal hormone melatonin (5-methoxy-*N*-acetyltryptamine) (*1*) is synthesized from 5-hydroxytryptamine by the enzymes serotonin *N*-acetyltransferase (SNAT) (*2*) and hydroxyindole *O*-methyltransferase (*3*). The activities of these enzymes are regulated by the sympathetic innervation to the pineal, and they can be induced by addition of norepinephrine or other β-adrenoceptor agonists to pineals maintained in culture (*4*). The norepinephrine-stimulated increase in the activity of SNAT appears to be the key regulatory step in the formation of melatonin (*4*), and this response seems to involve a norepinephrine-stimulated adenylate cyclase and increased intracellular cyclic adenosine monophosphate (cyclic AMP) (*5*). In agreement with this hypothesis, high concentrations of the cyclic AMP analog dibutyryl cyclic AMP, or the phosphodiesterase inhibitor theophylline (*4*), can induce SNAT in cultured pineals. However, it has otherwise not been possible to separate the effects of adenylate cyclase activation from the effects of β-receptor stimulation. In the present experiments, we describe the use of cholera toxin (choleragen) as an alternative and novel tool for investigating the regulation of enzyme activities in the rat pineal organ. The only known mechanism of action of choleragen is an irreversible activation of adenylate cyclase in intact cells (*6*) following its binding to a cell-surface receptor, the ganglioside GM_1 (*7*). We find that choleragen activates adenylate cyclase and increases SNAT activity in pineal cultures. We also show that choleragen causes an increase in cyclic AMP phosphodiesterase (E.C. 3.1.4.17) in the pineal and that this results in a decrease in cellular cyclic AMP levels toward normal values, although adenylate cyclase remains maximally activated.

Pineal bodies were rapidly removed after decapitation of male Sprague-Dawley rats (150 to 200 g) and placed in a defined culture medium (see legend to Fig. 1). The pineals were maintained in culture for 12 hours to allow degeneration of adrenergic nerve endings and to stabilize enzyme levels and β-adrenoceptor sensitivity. They were then transferred to Krebs-Ringer solution (16 pineals in 2 ml) either with or without 50 μg of choleragen per milliliter and incubated at 37°C for 15 minutes, after which they were returned to culture for further periods of up to 24 hours. Choleragen had no effect if added directly to the culture medium, presumably because the high ganglioside content of the culture medium effectively neutralized the toxin. The pineals were removed from culture at various times, and enzyme activities or cyclic AMP content were determined (see legend to Fig. 1).

Adenylate cyclase activity was increased by more than threefold 1 hour after exposure to choleragen and showed no significant decline in activity for up to 24 hours after such exposure (Fig. 1A). The magnitude of this response and the irreversible activation are typical of the response to choleragen of adenylate cyclases in whole cells of many other types (*7*). The SNAT activity also increased after exposure to choleragen. This effect was significant after 4 hours and maximal after 6 hours; SNAT activity remained significantly elevated at 24 hours (Fig. 1B). Serotonin *N*-acetyltransferase in-