any stimulation of growth of the root tissue.

Shoot dry weights became greater in the mycorrhizal than in the nonmycorrhizal plants after differences in plant height were evident. Thus, the results indicate that low resistances to water transport are associated with increased shoot growth in VA mycorrhizal plants. Nevertheless, the increased growth appeared slightly before differences in resistance were detectable (compare A and B, Fig. 1). Unless our experiments were incapable of measuring small initial changes in resistance, it seems probable that growth stimulation is not caused by the changes in resistance which we observed. On the other hand, if small differences in resistance were present from the onset of growth stimulation, but were unobserved, then the higher tissue water potentials which occurred in mycorrhizal plants may have contributed to the increase in growth (*12*).

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Cholinergic Sensitivity: Normal Variability as a Function of Stimulus Background

Abstract. *The sensitivity of the normally innervated iris sphincter to its neurotransmitter, acetylcholine, and to related agents varies inversely with the preexisting physiological stimulus background, that is, the environmental light intensity. This normal variability suggests the existence of a negative feedback mechanism whereby sensitivity of the effector cell is modulated by a product of neuronal activity.*

Supersensitivity develops after the denervation of structures which are normally under the control of either voluntary or autonomic innervation (*1*). Development of subsensitivity to cholinomimetic drugs, as a result of topical or systemic long-term treatment with an inhibitor of cholinesterase (*2*, *3*), has also been demonstrated in a variety of tissues, and evidence has been presented that supersensitivity and subsensitivity represent opposing expressions of the same basic phenomenon (*4*). All of the methods used to induce such alterations in the sensitivity of effector organs involved drastic interference with normal physiological function.

We have recently demonstrated (*4*) that alterations in the sensitivity of the iris to pilocarpine can also be induced by alteration in the normal physiological stimulus (environmental light) for the neuronal outflow to this organ. Both eyes of the cats used in these previous experiments were, however, sympathetically decentralized to minimize the mydriatic effects of emotional state; and one eye of each cat had been parasympathetically denervated to show that the effects of environmental lighting are mediated by parasympathetic innervation.

Our experiments demonstrate that stimulus deprivation results in supersensitivity and overstimulation leads to subsensitivity of the iris to cholinomimetics in the absence of any surgical or pharmacological interference with normal innervation. Furthermore, these experiments show that sensitivity of the iris to its normal neurotransmitter, acetylcholine (ACh), is also affected by the same physiological means. These findings imply that normal cholinergic sensitivity is a dynamic rather than a static entity. Maintenance of target organ sensitivity within a normal range can best be explained, at present, on the basis of an inverse relationship between the local concentration of the neurotransmitter and the concentration of cholinergic receptors at the membrane of the effector cell (*4*).

Four cats (2.5 to 4.5 kg) were selected without regard to sex on the basis of ease of handling. The external portion of the nictitating membranes was removed under Nembutal anesthesia to eliminate interference with measurement of pupillary size and with the topical application of drugs. The cats were kept for 1 week under each of the lighting conditions shown in Fig. 1. At the end of each conditioning period a dose-response curve to pilocarpine hydrochloride (ophthalmic solution, Alcon Laboratories) was obtained. The cats were put in the dark for 30 to 45 minutes before the first measurement and remained there

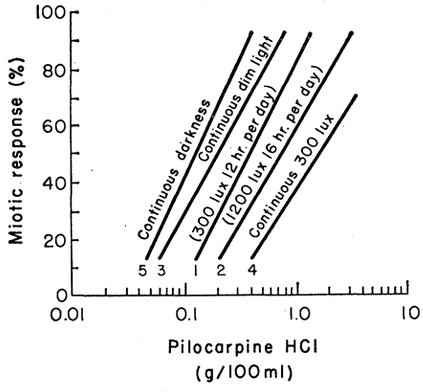

Fig. 1. The effects of preexisting stimulus background on the sensitivity of cat irises to topically applied pilocarpine. Four cats were maintained for 7 days under each of the lighting conditions indicated on the graph in the sequence shown by the number next to each line. The regression lines were obtained by the method of least-squares on all response values between 10 and 90 percent of the initial pupillary diameter. All measurements were made in complete darkness. Each regression line is based on 16 to 24 points obtained on eight eyes and covers at least a ninefold range of drug concentrations. The results show that the sensitivity of the iris to this cholinomimetic is dependent on preexisting stimulus background; 1 lux = 1 lumen/m^2.

throughout the 3 to 4 hours required for completion of the study (*5*). None of the preconditioning environments had a measurable effect on the initial pupil size. The drug was applied to the corneal surface at a constant volume of 25 μl at 45-minute intervals, the concentration being increased in threefold steps until the maximum miotic effect was evident. Pupillary (horizontal) diameter was measured with an infrared image converter (*4, 5*) before the application of any drug and at the end of each 45-minute period.

The results (Fig. 1) demonstrate that the sensitivity of the cat iris is dependent on the preexisting intensity of environmental lighting. Stimulus deprivation leads to a supersensitivity, whereas overstimulation depresses the normal sensitivity of the iris. All the dose-response regression lines are approximately parallel, and the extent of alteration in sensitivity is proportional to the magnitude of change in stimulus background.

After 1 week of conditioning in continuous room light the maximum miotic response to pilocarpine was depressed; thus, line 4 of Fig. 1 is incomplete. After conditioning in continuous bright illumination (1200 lumen/m^2), of greater intensity than normal room light, the irises of two of the cats became insensitive to pilocarpine. The maximum response obtainable on all four eyes of the other two cats was also depressed to less than half the normal maximum response. A significant regression line could not be obtained; thus, results on this conditioning are not indicated in Fig. 1. This extreme subsensitivity was, however, still completely reversible when the cats were again maintained under normal room light cycle (12 hours at 300 lumen/m^2; 12 hours in dark).

The sensitivity of the iris to ACh could not be studied in vivo because topical application of this hydrolyzable agent has no miotic effect on the cat eye. The variability of sensitivity to ACh was, therefore, tested in vitro on enucleated rat eyes. Hooded male rats (Long-Evans, 250 to 300 g) were divided randomly into two groups. One group was kept in the dark and the other in continuous room light for 5 to 7 days. At the end of this period, the rats were killed by crushing of the cervical cord (rats conditioned in darkness were killed in the dark). The eyes were enucleated, and the corneas were removed to facilitate the access of drugs to the irises. Four or five pairs of eyes representing both groups were put in specially designed trays and immersed in Ringer solution kept at 36°C; a mixture of 5 percent CO_2 and 95 percent O_2 was bubbled into the solution. After ½ hour of incubation, the pupillary diameters were measured through a stereomicroscope with an ocular micrometer (*6*). At this time all eyes were fully dilated and the pupillary sizes in the two groups were not significantly different. Small volumes of acetylcholine iodide or pilocarpine hydrochloride solution were then added to the bathing fluid, the drug concentration being increased threefold at 5-minute intervals.

The ACh dose-response curve obtained for the irises of rats which were kept in continuous room light was shifted to the right compared to that for the irises of rats kept in complete darkness (Fig. 2). The extent of variability in the sensitivity to ACh in vitro is less than that to pilocarpine (Fig. 2). A full miotic response to pilocarpine could not be induced in those eyes exposed to continuous room light. The differences between ACh and pilocarpine in the extent of changes in sensitivity suggest that these two agonists may act on two different populations of receptors. Preliminary experiments show that the dose-response curves for the choline esters methacholine and carbamylcholine closely resemble in shape and relative displacement those of ACh.

Our experiments demonstrate that the sensitivity of the iris sphincter to both its neurotransmitter and to cholinomimetics can be altered by purely physiological means. In these experiments a period of at least 5 days of conditioning was arbitrarily selected. Preliminary experiments indicate, however, that in rats subsensitivity develops within hours of exposure to room light after 5 days of stimulus deprivation. It thus appears likely that the iris sphincter, as well as other similarly innervated target organs, undergoes day-to-day and possibly hour-to-hour variations in sensitivity to neuronal input. These variations may not have an important physiological significance, but they do demonstrate that sensitivity of the normal target organ is a dynamic rather than a static entity. It follows that a mechanism must exist to maintain this sensitivity within normal physiological range.

Although the nature of this control mechanism is not understood, there is increasing evidence that the primary factor in the control of target-organ

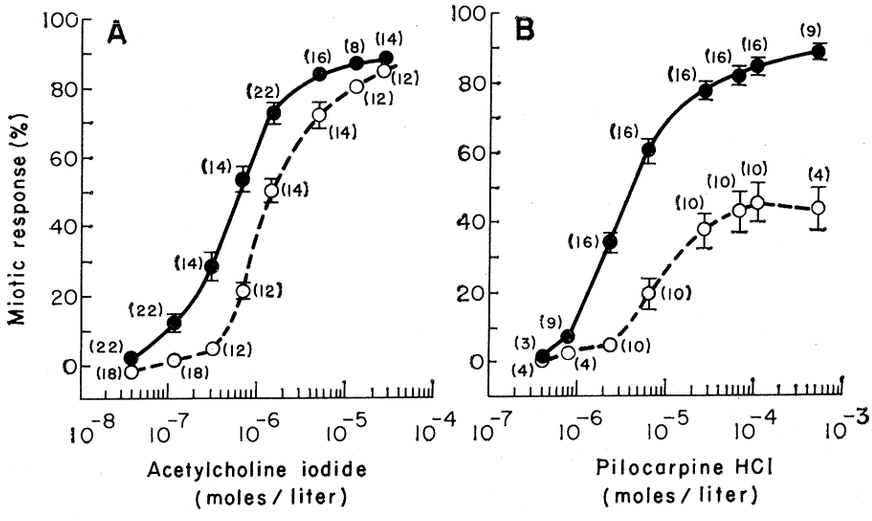

Fig. 2. The effects of stimulus deprivation (●) and overstimulation (○) on the sensitivity of the enucleated rat eye to the miotic effects of acetylcholine and pilocarpine. Dose-response curves were determined in vitro after the rats were kept for 5 to 7 days in complete darkness (stimulus deprivation) or continuous room light (overstimulation). The points represent the mean response values obtained on the number of eyes shown in parentheses; limits indicate one standard error of the mean. Dose-response curves to both ACh and pilocarpine were shifted to the left in animals kept in complete darkness compared to those kept in continuous room light. The maximum miotic response to the alkaloid cholinomimetic, pilocarpine, was suppressed by overstimulation, whereas the maximum response to ACh remained unchanged.

sensitivity is the local concentration of the neurotransmitter (*2, 4*). We have shown recently that a variety of manipulations which modify the sensitivity of the iris sphincter have only one apparent common feature—their effect on the release or destruction of ACh. Furthermore, even in the absence of nerve terminals, ACh is able to depress the sensitivity of the iris sphincter to cholinomimetics (*4*). These and other experiments led to the conclusion that the sensitivity of the target organ is inversely related to the long-term local concentration of the neurotransmitter (*7*). Our results are consistent with this concept, since overstimulation can be expected to lead to a local accumulation of the neurotransmitter, whereas stimulus deprivation can be expected to lower the concentration of ACh at the site of the target organ.

The available evidence suggests that changes in the sensitivity of smooth muscle, as well as other innervated target organs, result from changes in the concentration of cholinergic receptors on the effector cell membrane (*4*). If receptors, like other membrane components, undergo a continuous turnover, changes in concentration result from alterations in either the rate of synthesis or the rate of inactivation of receptor material. Thus, sensitivity of the target organ could be controlled by a negative feedback of ACh (or a product of the interaction of ACh and the receptor) on the rate-limiting step in receptor synthesis. Such a mechanism would be consistent with the observation (*8*) that at least in some systems the synthesis of new protein (or proteins) is required for the achievement of supersensitivity after denervation. The work of Warren and Glick (*9*) indicates, however, that in mammalian cells the rate of synthesis of membrane components is relatively constant, whereas turnover is a variable. Thus, the possibility that changes in cholinergic sensitivity result from changes in the rate of receptor turnover must be considered. A relationship between the frequency of activation of the receptor and the duration of its functional life provides a simple mechanism to account for alterations in receptor turnover rate and concomitant changes in cholinergic sensitivity. Detailed studies on the rates of development of sensitivity changes and on the specificity of these changes should help to distinguish between these possibilities and to elucidate other aspects of maintenance of normal cholinergic sensitivity. The reversible physiological alteration in target organ sensitivity, combined with the in vitro technique of dose-response assays, as used in the present experiments, provides a uniquely suitable system for such studies.

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Butterfly-Plant Coevolution: Has Passiflora adenopoda Won the Selectional Race with Heliconiine Butterflies?

Abstract. *Hooklike trichomes of* Passiflora adenopoda *provide a specific, effectively absolute defense against heliconiine butterfly larvae, a major class of* Passiflora *herbivores. It is suggested that since mechanical defenses are usually more selective in their action against herbivores, they usually are evolved by a plant only after it has accumulated a series of chemical defenses.*

It has long been assumed that many structural features of plant surfaces function to give the plant resistance to the attack of herbivores (*1*). Indeed, the effectiveness of the large thorns of *Opuntia* in discouraging herbivores seems self-evident. However, very little is known about the possible defensive roles of many small plant hairs or trichomes. In fact, a recent review on the biology of plant hairs practically ignores this subject (*2*). Evidence is presented here that at least one such structure, a trichome of *Passiflora adenopoda* D.C. (Passifloraceae), acts as a specific and highly efficient deterrent against heliconiine butterfly larvae, a major class of *Passiflora* herbivores in tropical America (*3*). To my knowledge, this represents the best documented example of the role and mode of action of a cuticular structure in preventing herbivore attack.

All observations on *Passiflora adenopoda* were carried out in an insectary at Stanford. The vine was grown from

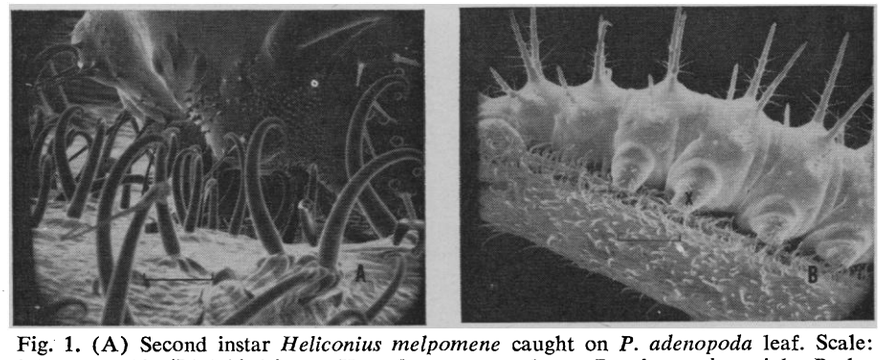

Fig. 1. (A) Second instar *Heliconius melpomene* caught on *P. adenopoda* leaf. Scale: 0.1 mm: ×80. (B) Third instar *H. melpomene* caught on *P. adenopoda* petiole. Proleg marked x is enlarged in Fig. 2. Scale: 1 mm; × 8.5.