sponds to the N-state absorption spectrum only at wavelengths greater than 600 nm (*7*).

At lower light intensities, the LRP responses in these cells show little or no dependence on wavelength of stimulation or of preceding adaptation, except for a small increase in sensitivity in red-adapted cells. Ogden and Stratten (*8*) showed that the LRP action spectrum in such cells, when white-adapted, shows a peak at 535 nm; and we have found the same peak in red- and blue-adapted cells. The absorption spectrum of the N state of the ERP pigment probably has a peak somewhere near 535 nm (Fig. 2). This coincidence of action-spectrum peaks suggests that the same pigment, and in particular its N state, may be responsible for the LRP. However, the increase in sensitivity of the red-adapted cell (which has a reduced proportion of pigment in the N state) apparently also requires some role for the P state.

Clearer evidence for the identity of the ERP and LRP pigments and for the role of the N state in the LRP comes from the observation that strong red illumination (*9*) of a blue-adapted cell (and no other combination) induces an extended depolarization, which can take from seconds up to 30 minutes in the dark to decay back to baseline but can be suppressed by further strong blue illumination (Fig. 1, trace G) (*10*). As with the ERP reversal, the state of color adaptation of the cell—with respect to the presence or absence of a tail in the response to red light—was fully preserved in the dark for at least 60 minutes. Furthermore, the absolute amounts of light necessary to bring about the various LRP and ERP changes are approximately equal. For example, the amount of red light needed to induce a saturated LRP tail in a blue-adapted cell is about that which reverses the ERP response from negative to positive in the same cell (*11*).

Now, according to our hypothesis, red illumination of a blue-adapted cell (the tail-producing combination) activates mainly the N state of the ERP pigment, while all other combinations either activate both states to comparable degrees or activate mainly the P state. The action spectrum for the induction of this tail also follows the N-state absorption spectrum in the narrow region —above 600 nm (Fig. 2)—in which the N-state spectrum is determined.

The action spectrum of the suppression of the tail (Fig. 2), on the other hand, closely follows the absorption spectrum of the P state (that is, the ERP action spectrum of the red-adapted cell).

This suggests that strong activation of the N state of the ERP pigment induces the LRP tail, which can be suppressed or prevented from appearing by strong P-state activation.

Finally, that the tail induction by N-state activation and the tail suppression by P-state activation act on the same membrane process is suggested by bridge measurements. These measurements show decreases in cell resistance roughly in parallel with the courses of the depolarizations during and after the various stimuli, including during the decay or suppression of the tail (*12*).

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References and Notes

1. Similar results were obtained in limited observations on a closely related species, *B. eburneus*.
2. K. T. Brown and M. Murakami, *Nature* **201**, 626 (1964); for a full review see R. A. Cone and W. L. Pak, in *Handbook of Sensory Physiology*, vol. 1, *Principles of Receptor Physiology*, W. R. Loewenstein, Ed. (Springer-Verlag, Berlin, 1971), pp. 345–365.
3. These observations will be presented in detail (P. Hillman, F. A. Dodge, S. Hochstein, B. Knight, B. Minke, in preparation).
4. We have found that *Limulus* ventral photoreceptors exhibit no such color-adaptation dependences.
5. H. J. A. Dartnall, *Brit. Med. Bull.* **9**, 24 (1953).
6. We cannot exclude the possibiity that the two ERP polarities might arise from two different pigments, but at least one of these would in any case have to have two stable states.
7. The pigment process also involves a number of thermally unstable states and thermal and light pathways; these observations will be discussed (B. Minke, S. Hochstein, P. Hillman, in preparation).
8. W. P. Stratten and T. E. Ogden, *J. Gen. Physiol.* **57**, 435 (1971).
9. The intensity is comparable with that of visible sunlight.
10. J. Nolte, J. E. Brown, T. G. Smith [*Science* **162**, 677 (1968)] have reported that, in some cells of the median eye of *Limulus*, ultraviolet light induces an extended depolarizing LRP which can be switched off by exposure to visible light. The repolarization occurs during the visible stimulus, instead of after as in our case. However, the stability of the states involved was not discussed nor was the relation to pigment states determined, since the ERP was not observed. H. M. Brown and W. Wilson have also seen extended depolarizations following red lights in *B. eburneus*. We find no such phenomena in the ventral eye of *Limulus*.
11. Also, these amounts are roughly those needed for each pigment molecule to absorb an average of one photon, if the pigment has an extinction coefficient similar to those of other visual pigments [G. Wald and P. K. Brown, *J. Gen. Physiol.* **37**, 189 (1953)].
12. In particular, suppression of the red-induced voltage tail by blue light goes with a suppression of the red-induced conductance increase and so cannot be the result of an independent process. The inexactness of the correlation between conductance and depolarization is ascribable to the presence in this preparation of a strong stimulus-dependent pump electrogenicity [H. Koike, H. M. Brown, S. Hagiwara, *J. Gen. Physiol.* **57**, 723 (1971)].
13. We thank B. W. Knight and F. A. Dodge for help in preparing the experimenters, H. M. Brown and S. R. Shaw for demonstrating the preparation, R. Werman for criticism of the research and the manuscript, and H. Simhai for technical assistance. Supported in part by the Central Research Fund of Hebrew University.

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Response Decrements in the Cochlear Nucleus of Decerebrate Cats during Repeated Acoustic Stimulation

Abstract. *In the cochlear nucleus of decerebrate, paralyzed cats, multiple-unit responses to an acoustic stimulus showed significant decrements when the stimulus was repeatedly presented once every 5 seconds. These decrements developed in the absence of peripheral receptor adaptation. The responses recovered to the level prior to stimulation when stimulation was withheld for 5 to 10 minutes. Dishabituation by somatic stimulation of the forepaw, however, was less effective than in the intact cat. The continued development of response decrements after strychnine blockade of peripheral olivocochlear influences and central postsynaptic inhibition suggests a mechanism of decreased synaptic effectiveness, which has previously been postulated for neuronal habituation in brainstem and spinal cord.*

The extent to which response decrements develop in the auditory relay nuclei during acoustic habituation has long remained unclear. Particularly in the more peripheral nuclei of the acoustic pathway, for example, the cochlear nucleus, evoked potential studies over the past 10 years have provided both positive (*1*) and negative (*2*) support for such response plasticity. These contradictory data may largely reflect confounding and important variables of acoustic habituation experiments that have only gradually become recognized; for example, the sound-field alterations that accompany movements of the un-

restrained animal, the decrease in sensory input produced by middle ear muscle contractions, and the activating effects of movement and of arousal stimuli on the middle ear muscles. When these variables are controlled, however, by presenting acoustic stimuli through earphones fixed on the head and by blocking ear muscle contractions and body movements with a paralyzing agent, multiple-unit responses from the cochlear nucleus show small but significant decrements during repeated acoustic stimulation (*3*). These response decrements show spontaneous recovery with rest (*3*) and can be dishabituated by a brief electrical shock applied to the skin of the forepaw (*4*). Thus, it seems clear that decrements in the sensory response per se can develop in a specific sensory relay during well-controlled habituation procedures; however, the mechanisms underlying such decrements are obscure. It has been suggested that cochlear nucleus response decrements reflect a buildup of inhibition mediated by recurrent projections from cortex, thalamus, and midbrain reticular formation (*5*) or by inhibitory fibers acting directly on the hair cells of the cochlea (*6*). We have investigated these possible sources of habituation and have shown that significant response decrements continue to develop in the cochlear nucleus after functional deletion of these recurrent forebrain and peripheral inhibitory systems.

Adult cats were anesthetized with methoxyflurane (Metofane, Pitman-Moore) while venous and tracheal cannulas were inserted and the common carotid arteries were ligated bilaterally. Later, the animals were placed in a stereotaxic instrument and decerebrated. The posterior neocortex was aspirated until the midbrain tectum could be visualized and a midcollicular brainstem transection was then made with a blunt spatula. Blood loss was minimized by avoiding section of the underlying basilar artery and by lightly packing the lesion with Oxycel. Anesthesia was terminated and intravenous Flaxedil infusion was initiated (20 mg initial dose and 20 mg/hour, thereafter) concurrently with artificial respiration; heart rate was continuously monitored. The completeness of brainstem transection was verified after each experiment by gross dissection of the perfused brain.

Multiple-unit recordings from the cochlear nuclei were made through concentric electrodes that consisted of 34-gauge stainless steel wire within 25-gauge stainless steel hypodermic tubing; both components were insulated with Epoxylite except for small bared tip areas. The round window microphonic potential was recorded through a silver ball electrode that was held securely on the round window membrane by cementing the lead wire within a narrow groove drilled in the adjacent bulla. Recordings were amplified by Tektronix 122 preamplifiers and taped on an Ampex FR 1300 FM tape recorder. Histological verification indicated nine electrode placements in the dorsal, two in the anteroventral, and one in the posteroventral subdivisions of the cochlear nucleus.

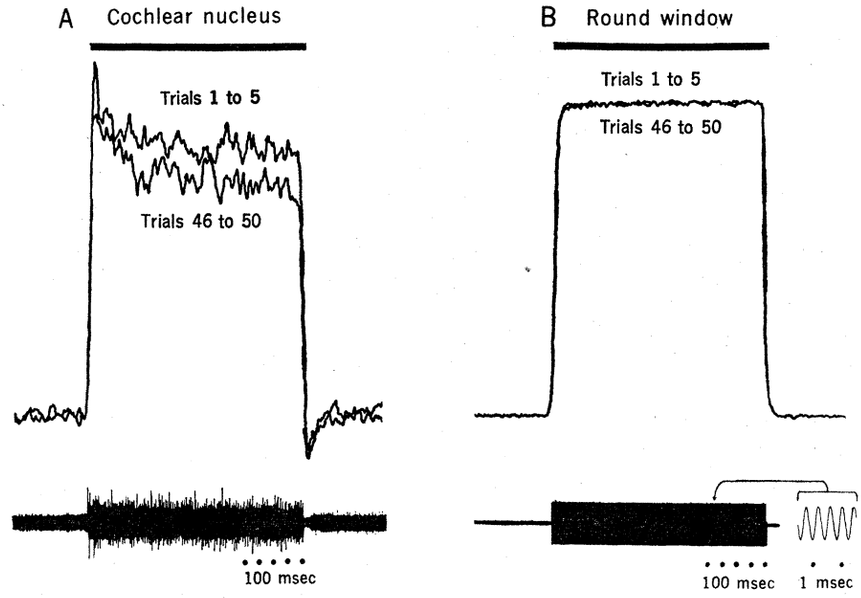

Fig. 1. Responses to repeated tone presentations recorded concurrently from the (A) cochlear nucleus and (B) round window in the decerebrate cat. Integrated multiple-unit activity, computer averaged over five-trial blocks, is shown for habituation trials 1 to 5 and 46 to 50. Activity typical of the cochlear nucleus multiple-unit response and the round window microphonic potential is shown in the lower traces. Duration of the 1.5-second, 2500-hz tone stimulus is indicated by solid bars.

Either a 1.5-second tone (500 to 3000 hz) or white noise, at a sound pressure of 0.6 or 2 dyne/cm^2 referenced to 0.0002 dyne/cm^2, was presented through a miniaturized Tibbetts speaker to the ear ipsilateral to the round window and cochlear nucleus electrodes. A stimulation sequence consisted of 50 stimulations delivered once every 5 seconds.

The cochlear nucleus multiple-unit data and round window microphonic potentials were analyzed by integrating the activity through a resistance-capacitance integration system (*7*) and by averaging successive responses in five-trial blocks with the use of a computer of average transients (Mnemotron). In some cases, the area of the integrated responses was also planimetrically measured, and these data were used for regression and variance analyses to determine the significance of the progressive response decrements.

Among the 43 acoustic stimulation series carried out on decerebrate, paralyzed cats, 84 percent showed progressive cochlear nucleus response decrements when plots of averages for successive responses in five-trial blocks were superimposed (Fig. 1A). The amount of response reduction during 50 stimulations ranged from 10 to 45 percent and, in general, the decrements developed most rapidly within the first ten trials. In order to establish the significance of this effect, 28 habituation series were statistically analyzed. A linear regression analysis of the acoustic responses over successive trials indicated that significant decrements developed ($P < .05$) in 23 of the 28 series (82 percent); a typical example of such response decrement is plotted in Fig. 2.

After a rest period, the response decrements showed spontaneous recovery (Fig. 2). In most cases, recovery to the level prior to stimulation required a 10-minute rest period, which indicated a decremental effect of relatively long duration, that is, more persistent than

classical neural refractory periods or cochlear nucleus afterpotentials (*8*). Whereas partial or complete recovery occurred in all cases, dishabituation was not easily induced by somatic stimulation (a 0.5-second train of 18-hz, 1-msec-duration pulses to the dorsum of the forepaw), the one stimulus modality tried thus far. In intact cats this stimulus was adequate to dishabituate the cochlear nucleus response decrements that developed during identical stimulation procedures (*4*); however, it produced only weak and transient response reversals in the decerebrate cat. This difference would seem to reflect the inability of the forepaw stimulus to influence the cochlear nucleus in the decerebrate preparation. It has been shown that electrical stimulation of the paw produces a strong response in the inferior colliculus (*9*), a site from which recurrent fibers project to the cochlear nucleus (*10*). Functional deletion of the inferior colliculus in these decerebrate preparations may thus have removed the pathway through which, in the intact animal, the dishabituating effects of somatic stimulation were exerted.

The above data indicated that cochlear nucleus response decrements could develop independently of any recurrent inhibition from cortical, thalamic, or midbrain levels. The peripherally directed influence of the olivocochlear system, known to modify cochlear nucleus responsiveness by inhibition of the cochlear hair cells (*6*), was next examined. In order to measure possible changes in receptor responses, we recorded the round window microphonic potential (a reflection of hair cell activation) concurrently with recordings from the cochlear nucleus in six animals. During acoustic stimulation significant response decrements developed in the cochlear nucleus that were not paralleled by significant alterations in the cochlear microphonic (Figs. 1 and 2). The recording procedure was appropriately sensitive as indicated by the fact that the microphonic potential decreased in amplitude when the body temperature of the animal was cooled several degrees. Thus, the development of cochlear nucleus response decrements in the absence of changes in the microphonic potential indicated that receptor adaptation was not responsible for the phenomenon.

In an additional series of studies, the possibility of a buildup of postsynaptic inhibition at either the peripheral receptor level or centrally was tested by strychnine administration. Strychnine has been shown to block the inhibition of hair cell activity mediated by the olivocochlear bundle (*11*), as well as centrally mediated postsynaptic inhibition (*12*). Approximately 15 minutes after intravenous injection of strychnine sulfate (0.2 mg/kg) the cochlear nucleus response to acoustic stimulation had become enhanced, reflecting the effects of the inhibitory blockade. In eight subsequent habituation sequences, however, clear decrements in the cochlear nucleus response to repeated tones developed that were statistically significant ($P < .05$). These decrements were reversible and spontaneous recovery occurred over a 10-minute period of rest. Thus, the response attenuation was not dependent on either peripheral or central buildup of strychnine-sensitive postsynaptic inhibition.

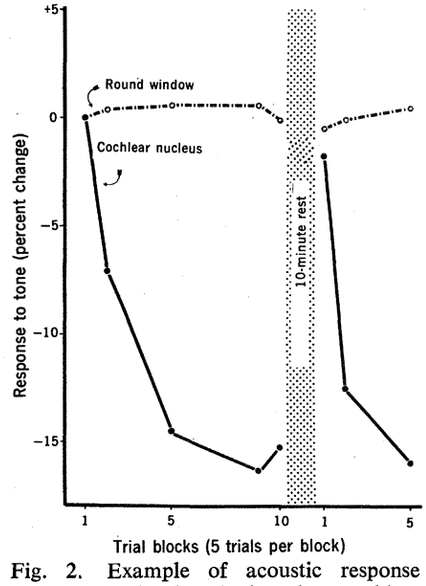

Fig. 2. Example of acoustic response decrement developed in the cochlear nucleus of the decerebrate cat (solid line). The concurrently recorded microphonic potential of the round window remains essentially constant (dashed line). Each point represents the average integrated response area for a five-trial block. After a 10-minute rest period, spontaneous recovery of the cochlear nucleus response is almost complete; with subsequent resumption of tone presentations, habituation again develops.

Our experiments support and extend earlier observations of cochlear nucleus unit response decrements in intact cats during repeated acoustic stimulation (*3*). These decrements, previously shown to be independent of sound-field alterations and ear muscle contractions, have now been shown to develop (i) in the absence of cortex, thalamus, and midbrain, (ii) without parallel changes at the peripheral receptor level, and (iii) after strychnine blockade of postsynaptic inhibition.

The stimulus parameters utilized in the present study, that is, interstimulus interval, number, duration, and intensity, are similar to those generally utilized in electrophysiological studies of vertebrate neuron habituation, although other studies have often used shorter interstimulus intervals, for example, 1-second intervals (*13*). The decremental process described above for cochlear nucleus neurons is clearly not due to effector fatigue; sensory adaptation, monitored by round window recordings, did not develop during repeated stimulation; infringement of successive stimuli on the neuronal refractory period (*8*) could not have occurred with the 5-second interstimulus intervals utilized. The time necessary for recovery after neuronal response decrement, from one or a few minutes for partial recovery to 10 minutes for complete recovery in our study, again are similar to those noted for recovery of habituated vertebrate neurons (*13*). Whereas in the decerebrate preparation, electrical stimulation of the paw did not easily produce dishabituation, this stimulus did reverse similar cochlear nucleus response decrements in the intact cat (*4*). This suggests an inability of the somatic stimulus to influence the cochlear nucleus of the decerebrate cats because of the functional deletion of the projection pathway, rather than an inability of cochlear neurons to be dishabituated.

Response decrements to repeated stimulation that show recovery over time when the stimulus is withheld are generally considered to exemplify habituation (*13, 14*), particularly when factors of peripheral fatigue and neural refractoriness can be ruled out (*15*). Whereas habituation suggests neither a single explanation nor mechanism, it serves as a convenient umbrella under which to group phenomena sharing certain characteristics of response plasticity. We suggest that the decremental phenomena of the decerebrate cochlear nucleus comply with the generally accepted criteria for neuronal habituation.

As the inferior colliculus showed no ongoing activity nor acoustic responsiveness in the decerebrate preparations, its contribution to descending modulation can be ruled out. Below the level of the inferior colliculus, four projection pathways containing recurrent fibers to the

cochlear nucleus have been described; for example, the olivocochlear bundle, the intermediate acoustic stria, the dorsal acoustic stria, and the trapezoid body (*10*). The efferent fibers of both the olivocochlear bundle and intermediate acoustic stria originate in the superior olivary complex and terminate in the ventral cochlear nucleus (*10*), whereas the recurrent fibers of the dorsal acoustic stria and trapezoid body originate in the nuclei of the lateral lemniscus and terminate in the dorsal cochlear nucleus (*10*). The role of these brainstem systems in the development of cochlear nucleus response decrements is as yet not known, but, conceivably, could be an important one.

On the other hand, the acoustic nerve projects a terminal branch of each fiber to both dorsal and ventral cochlear nuclei and, in both nuclei, internuncial cells with intrinsic fiber connections relay the incoming stimulus through local polysynaptic circuits, a situation known to favor the development of response plasticity. In the isolated spinal cord, for example, response decrements of dorsal horn sensory neurons develop during repeated cutaneous stimulation; such habituation is readily observed in the absence of recurrent projections from higher levels and is a function of cells with polysynaptic, rather than monosynaptic, contacts with the primary afferent fibers (*16*). Thus, on the basis of current data, it would seem equally probable that intrinsic, rather than recurrent, circuits of the cochlear nuclei mediate the response decrements.

The continued decrement of the acoustic responses after strychnine blockade of postsynaptic inhibition is again similar to observations made during repeated somatosensory stimulation in the isolated spinal cord (*17*). Moreover, the low frequency of stimulation (once every 5 seconds) during which response decrements developed, and the time required for recovery (5 to 10 minutes), involve durations too long to reflect pre- or postsynaptic inhibition (*12*). Therefore, whereas a buildup of inhibition has been suggested as the source of cochlear nucleus response habituation (*5*), the unit response decrements described above seem best attributed to a progressive reduction of excitation either within intrinsic circuits or recurrent pathways to the cochlear nucleus. This conclusion is consonant with the synaptic depression hypothesis of habituation (*13, 17, 18*), which attributes decrements of neuronal response to decreased effectiveness of synaptic transmission through mechanisms such as transmitter depletion or diminished subsynaptic sensitivity.

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References and Notes

1. R. Hernandez-Peon and H. Scherrer, *Fed. Proc.* **14**, 71 (1955); J. T. Marsh, D. A. McCarthy, G. Sheatz, R. Galambos, *Electroencephalog. Clin. Neurophysiol.* **13**, 224 (1961); W. R. Webster, C. W. Dunlop, L. A. Simons, L. M. Aitkin, *Science* **148**, 654 (1965); L. A. Simons, C. W. Dunlop, W. R. Webster, L. M. Aitkin, *Electroencephalog. Clin. Neurophysiol.* **20**, 485 (1966).
2. F. G. Worden and J. T. Marsh, *Electroencephalog. Clin. Neurophysiol.* **15**, 866 (1963); J. T. Marsh and F. G. Worden, *ibid.* **17**, 685 (1964); W. O. Wickelgren, *J. Neurophysiol.* **30**, 1424 (1968).
3. S. B. Holstein, J. S. Buchwald, J. A. Schwafel, *Brain Res.* **16**, 133 (1969); M. Kitzes and J. Buchwald, *Exp. Neurol.* **25**, 85 (1969).
4. J. S. Buchwald and G. L. Humphrey, in *Progress in Physiological Psychology*, E. Stellar and J. Sprague, Eds. (Academic Press, New York, in press), vol. 5.
5. R. Hernandez-Peon, *Electroencephalog. Clin. Neurolphysiol.* **13** (Suppl.), 101 (1960). J. E. Desmedt, in *Neurological Mechanisms of the Auditory and Vestibular System*, G. L. Rasmussen and W. F. Windle, Eds. (Thomas, Springfield, Ill., 9160), p. 152.
6. R. Galambos, *J. Neurophysiol.* **19**, 424 (1956). J. E. Desmedt, *J. Acoust. Soc. Am.* **34**, 1478 (1962). J. Fex, *Acta Physiol. Scand.* **55** (Suppl.), 189 (1962).
7. J. S. Buchwald, S. B. Holstein, D. S. Weber, in *Methods in Physiological Psychology—Bioelectric Recordings*, R. Thompson and M. Patterson, Eds. (Academic Press, New York, in press).
8. H. S. Gasser, *Ohio J. Sci.* **41**, 145 (1941); G. L. Gerstein, R. A. Butler, S. D. Erulkar, *J. Neurophysiol.* **31**, 526 (1968).
9. J. S. Buchwald, S. B. Holstein, J. Schwafel, *Anat. Rec.* **163**, 160 (1969); S. B. Holstein, G. L. Humphrey, J. S. Buchwald, *Physiologist* **13**, 224 (1970)
10. G. L. Rasmussen, in *Neural Mechanisms of the Auditory and Vestibular Systems*, G. L. Rasmussen and W. F. Windle, Eds. (Thomas, Springfield, Ill., 1960), p. 105.
11. J. E. Desmedt and P. Monaco, *Proc. Int. Pharm. Meet. 1st 8*, 183 (1962).
12. J. C. Eccles, *The Physiology of Synapses* (Springer-Verlag, New York, 1964), pp. 190–192, 234–236.
13. J. P. Segundo and C. C. Bell, in *Short-Term Changes in Neural Activity and Behavior*, G. Horn and R. Hinde, Eds. (Cambridge Univ. Press, Cambridge, 1970), pp. 77–94; E. R. Kandel and W. A. Spencer, *Physiol. Rev.* **48**, 63 (1968).
14. J. Bruner and J. Kehoe, in *Short-Term Changes in Neural Activity and Behavior*, G. Horn and R. Hinde, Eds. (Cambridge Univ. Press, Cambridge, 1970), pp. 323–359.
15. R. A. Hinde, in *ibid.*, pp. 3–40.
16. B. G. Wicklegren, *J. Neurophysiol.* **30**, 1424 (1967); P. D. Wall, *J. Physiol. London* **188**, 403 (1967); P. M. Groves, R. DeMarco, R. F. Thompson, *Brain Res.* **14**, 521 (1969).
17. W. A. Spencer, R. F. Thompson, D. R. Nielson, Jr., *J. Neurophysiol.* **29**, 253 (1966).
18. G. Horn, *Nature* **215**, 707 (1967).
19. Supported by PHS grants NB 05437 and MH 06415. Computing assistance was obtained from the Health Sciences Computing Facility, University of California, Los Angeles, sponsored by NIH Special Research Resources grant RR-3.
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Silk Moth Eclosion: Hormonal Triggering of a Centrally Programmed Pattern of Behavior

Abstract. *The emergence of the adult cecropia silk moth from the pupal skin involves a stereotyped series of abdominal movements—the pre-eclosion behavior. This behavior, triggered by a neurosecretory hormone, consists of three phases that are characterized by the relative frequency and pattern of movements. Electrical recordings from a nerve cord with severed peripheral nerves demonstrate that the pre-eclosion behavior is prepatterned in the abdominal ganglia. In response to the hormone, the entire 1.25-hour behavioral program can be activated and "read off" in the absence of sensory feedback.*

In the giant silk moths, the control of adult emergence involves two separable neural components that are linked together by a neurosecretory hormone—the eclosion hormone (*1*, *2*). The first component is centered in the brain and involves an extraoptic photoreceptor, a biological clock, and a neuroendocrine output. The second component involves the neural circuitry that gives rise to the various behavior patterns in the adult emergence sequence and is independent of the brain. Of special interest is the first part of the emergence sequence—the pre-eclosion behavior. This behavior primarily involves abdominal movements and is performed by isolated abdomens after injection of homogenates containing the eclosion hormone (*2*). We report here that the pre-eclosion behavior arises from a centrally generated program of behavior that is activated and "read off" in response to a hormonal signal.

In the cecropia moth (*Hyalophora cecropia*), the pre-eclosion behavior is complex and consists of three distinct phases (Fig. 1) (*1*, *2*):

1) The behavior is initiated with a hyperactive period that lasts approximately 0.5 hour and involves abdominal rotations and various ventral "twitches."