aration is that, unlike those shown in Figs. 1 and 2, there was considerable radioactivity in interstitial and apparently intravascular spaces.

Our observations indicate that, when radioactive substances were selectively introduced into hypoglossal neurons, these substances, or their derivatives, were conveyed down the axons only to the muscle cells of the tongue and that they reached the muscle cells only via these axons—or very nearly so. The labeled molecules apparently crossed the neuromuscular junction into intracellular components of the muscle. We suggest that the proximo-distal conveyance and intercellular transfer of substances from the nerve cell may underlie the so-called trophic and other long-term influence not based on impulses, of peripheral neurons on the metabolism, function, development, differentiation, growth, and regeneration of the structures that they innervate (*10*).

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Fungal Morphogenesis: Ring Formation and Closure by Arthrobotrys dactyloides

Abstract. *The formation and the closure of constricting rings by a nematode-trapping fungus were recorded by means of time-lapse cinephotomicrography. Analysis of the film revealed that hyphal rings resulted from a sequence of morphological events not previously described. Cell inflation and ring constriction were induced by touch, increased temperature, and electrical stimulation. The inflation process was not particularly sensitive to metabolic inhibitors and appears to operate without an expenditure of energy on the part of the cell.*

Fungi frequently display morphological change that can be analyzed biochemically (*1*), and nematode-trapping fungi are typical examples. These microorganisms produce specialized structures that function as traps and enable prey to be captured, killed, and consumed (*2*). The most intricate and remarkable type of trap is the so-called "constricting ring." Constricting rings consist of three curved cells on a short two-celled stalk. They are produced at intervals along a fungal filament and usually grow at right angles to it. When a nematode enters, the cells that comprise the ring inflate rapidly and obliterate the opening. The nematode is trapped by occlusion and is later penetrated by hyphae that originate from the ring cells and spread throughout the carcass, absorbing its content.

We employed time-lapse cinephotomicrography to obtain a record of growth, morphogenesis, and predation by various species of nematode-trapping fungi (*3*). The fungi were grown at 28°C on cornmeal extract agar, in microchambers on slides (*4*). They were photographed at the rate of four frames per minute, using a Reichert Zetopan microscope and a 16-mm Bolex camera that were synchronized and automated for time-lapse work (*5*). Analysis of films depicting morphogenesis in *Arthrobotrys dactyloides* (*6*) revealed that the fungal form is under strict control and that constricting rings are produced by the sequence of events illustrated in Fig. 1.

Hyphal branches destined to differentiate into organelles of capture were highly refractile, robust, and readily identified. As they extended, these specialized branches arched and appeared hook-like (Fig. 1, A and B). Growth and curling continued (Fig. 1C), but in no case was a ring formed by the anticipated, simple union of the tip with its supporting branch. Instead, a bud was consistently formed by the more distal of the two stalk cells (Fig. 1D). It formed in opposition to the advancing tip and the two converged and fused to produce a closed ring (Fig. 1, E–G). After anastomosis, the three cells that comprised the ring increased in size and refractility. They attained an individual width of 5 to 8 μ, a length of 20 to 28 μ, and formed rings 20 to 32 μ in diameter. The biochemical basis for curling of fungal filaments has not been ascertained. It may involve localized differences in the rate of synthesis of cell wall material or increased tension within the structural polymer of the wall because of modification of individual components or linkages. The former condition is responsible for the growth toward light of green plants and some

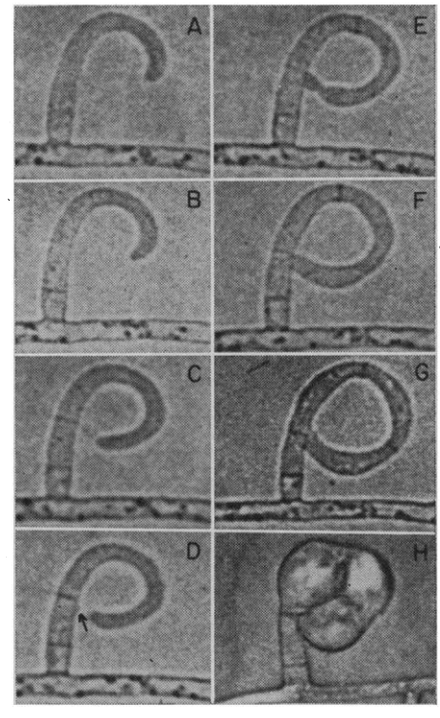

Fig. 1. Ring formation and closure by *Arthrobotrys dactyloides* recorded using time-lapse cinephotomicrography. Rings are initiated as branches that extend to form curved hooks (A–C). The more distal of the supporting cells gives rise to a bud (D) in opposition to the advancing hyphal tip, and the two converge and fuse to produce a closed ring (E–G). Ring cells inflate to triple their normal volume in approximately 0.1 second (H) if induced to do so by touch, increased temperature, or electrical stimulation. An interval of 4 hours was required for this morphological transformation (× 625).

fungi (*7*), and as a model of the latter condition, one can visualize the loop that forms when two points on a length of hose or rope are grasped and twisted in opposite directions (*8*). It is evident from the timing of bud formation by *A. dactyloides* and from the alignment and union of buds with growing tips of the curled hyphae to form traps, that the causes and levels of control responsible for morphogenesis are numerous and complex.

Ring closure is induced by touch and by increased temperature. It is extremely fast, requiring less than 0.1 second, and, on inflation, the cell volume more than triples (Fig. 1H). Under natural conditions, rings constrict when nematodes move into their openings. In the laboratory, micromanipulation with a fine needle can be substituted for prey, but application of water at 50°C, as described by Muller (*9*), is the simplest means of inducing ring constriction. We were successful in triggering closure of rings electrically, but activity was localized to the area immediately surrounding the cathodic microelectrode. The mechanism of cell inflation has not been adequately explained. Touch and temperature somehow initiate an irreversible change in wall structure, decreasing wall pressure and increasing permeability of the cell to water. Plasmolytic estimates of the osmotic potential of ring cells before and after inflation indicate that the concentration of solute in stimulated cells must triple (*9*), but the timing and rate of this process and the nature and origin of the osmotically active material are not known as yet.

In order to determine whether inflation is metabolically linked and requires an expenditure of energy on the part of the cell, *A. dactyloides* was grown on the surface of cornmeal extract agar in petri dishes and treated at *p*H 6.0 with solutions of iodoacetate, mercuric chloride, sodium azide, and sodium cyanide at concentrations ranging from 10^{-1} to $10^{-4}M$. After being exposed to the inhibitors for 15 minutes, ring closure was induced electrically. Cells that had been treated with iodoacetate, mercuric chloride, and sodium cyanide at 10^{-1} and $10^{-2}M$ failed to inflate. However, more dilute solutions of these compounds were inactive, and the system was not influenced adversely by $10^{-2}M$ or less of sodium azide. The fact that inflation was resistant to catalytic quantities of various metabolic inhibitors indicates that it is a passive rather than an active process and supports the suggestion that physicochemical phenomena, including changes in osmotic potential, and not energy requiring biochemical reactions, are operative in ring closure.

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Opposite Synaptic Actions Mediated by Different Branches of an Identifiable Interneuron in Aplysia

Abstract. *Among the identifiable cells in the abdominal ganglion of* Aplysia californica *are five that generate bursting rhythms endogenous to the cells. In the four bursting cells of the left upper quadrant the rhythm is modulated by a unitary inhibitory postsynaptic potential; in the bursting cell of the right lower quadrant the rhythm is modulated by a unitary excitatory postsynaptic potential. Both the excitatory and inhibitory postsynaptic potentials are mediated by separate branches of a single interneuron. The pharmacological properties of the double action interneuron as well as those of the follower cells suggest that a single transmitter (acetylcholine) is involved in both the excitatory and the inhibitory action of the interneuron.*

Certain chemical transmitter substances can produce different actions at different synapses. For example, acetylcholine (ACh) mediates excitation at the vertebrate neuromuscular junction and inhibition at the sinoauricular node of the vertebrate heart (*1*). In the marine mollusc *Aplysia* ACh appears to be, in the same ganglion, the excitatory synaptic transmitter on certain cells (D cells) and the inhibitory synaptic transmitter on other (H cells) (*2*). These findings indicate that the nature of synaptic transmission is determined not only by the chemical structure of the transmitter substance but also by the properties of the postsynaptic cell (*2, 3*). It is therefore theoretically possible for a single interneuron to produce opposite synaptic actions with the same transmitter via different branches on different follower cells. One cell would then serve as an inhibitory interneuron for some cells and as an excitatory interneuron for others. Although such interneurons have been postulated (*2*) and their presence inferred from indirect data (*4*), no such interneuron has yet been described.

In the course of studying direct and common connections among identifiable cells in the abdominal ganglion of *Aplysia californica*, we have encountered a cell that mediates inhibition to some cells in the ipsilateral hemiganglion and excitation to some cells in the contralateral hemiganglion. We now report some properties of this interneuron and of some of the cells to which it is synaptically connected (*5*).

Within the abdominal ganglion of *Aplysia californica* there are 30 cells that can be identified on the basis of a number of physiological and morphological criteria (*6*). Figure 1A illustrates the position of the 19 identifiable cells on the dorsal surface. The five cells whose connections we will describe are stippled on the drawing. These cells appear to form a distinct functional group since they are the only identifiable cells to show a regular bursting rhythm and since they all send their efferent axon into the pericardial branch of the genital nerve (*6*).

There is good evidence that in all five neurons the bursting rhythm is