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Functional Properties of Individual Neuronal Branches Isolated in Situ by Laser Photoinactivation

Abstract. *The functional properties of an isolated dendritic branch of an identified sensory interneuron in the cricket were studied. The branch responded to wind stimuli directed at the animal and displayed a distinct directional sensitivity to those stimuli. A technique was used that allows a neuron to be specifically lesioned in a semi-intact preparation during intracellular recording. Lesioning was achieved by dye-sensitized photoinactivation with a laser epi-illumination stereomicroscope.*

The function of a neuron depends on the structure of its dendritic and axonal branches in two important respects. First, the location of a neuron's branches in the nervous system determines the subset of other cells with which it may interact. Second, the fine structure of the branches determines how synaptic inputs and endogenously generated currents are integrated and transformed into "meaningful" output to other cells. The relations between neuronal structure and function have been studied extensively by experimental and theoretical neurobiologists over the past three decades. However, there has been no way to test directly how the structure of a cell affects its integrative properties, since such a test would require changing the structure of the cell in situ during physiological recording.

We report here a technique for ablating specific regions of individual neurons during intracellular recording. Using the technique, which involves dye-sensitized photodynamic inactivation, we isolated a single dendritic branch of an identified sensory interneuron and determined the response properties of that branch to sensory stimuli.

When a neuron is filled with a fluorescent dye and illuminated with intense light of the appropriate wavelength, the cell becomes functionally inactivated (*1*). Neither the illumination nor the dye alone harms the tissue; it is the interaction between the two that results in photooxidative damage (*2*). Studies have shown that the axon of a single neuron can be selectively inactivated by filling the entire cell with dye but irradiating only the nerve containing the axon (*1, 3*). The illuminated section of the axon is killed and the rest of the cell remains intact. For the present study this technique was substantially refined so that single dendrites of dye-filled neurons could be inactivated during physiological recording. To allow the placement of microelectrodes into neurons in a semi-intact preparation, it was necessary to use a dissecting stereomicroscope. In order to project a light beam small enough to illuminate single dendritic branches, we developed a laser epi-illuminator attachment for the microscope. With dichroic mirrors and filters the

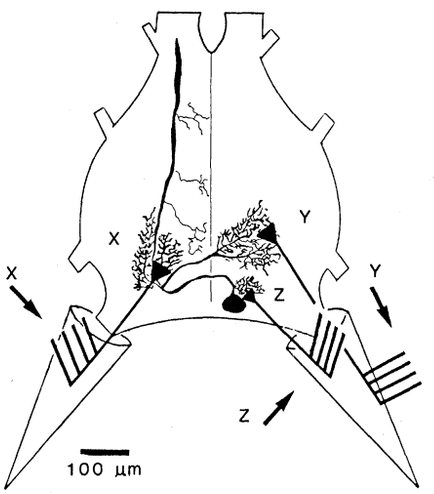

Fig. 1. Interneuron 10-3 and its presumed excitatory inputs (camera lucida drawing of a whole-mount preparation of this neuron stained with cobalt and intensified with silver). Each of the three dendritic regions has been labeled with a letter (X, Y, or Z). The three classes of afferents presumed to provide excitatory input to 10-3 are shown on the cerci (represented as cones). Afferents that overlap with dendrite X respond best to wind stimuli directed at the lateral face of the left cercus. Wind directed at the medial face of the right cercus activates afferents that overlap with dendrite Z and wind directed at the right front of the animal activates afferents that overlap with dendrite Y.

beam of a helium-cadmium laser was directed down through the objective of the microscope, producing a beam diameter of 30 μm at the tissue. All these components were attached to a movable optical bench so that the apparatus could be translated in three dimensions with respect to the preparation without disturbing the intracellular microelectrodes (*4*).

The neuron we studied is a primary sensory interneuron located in the terminal abdominal ganglion of the cricket *Acheta domesticus*. This cell, 10-3, is one of a class of wind-sensitive mechanosensory interneurons (*5*) involved in the escape response of the animal (*6*). The amplitude of this cell's response depends on the direction of the wind stimulus with respect to the animal's body. This "directional sensitivity" is thought to be determined by the cell's dendritic structure (*7*).

The primary afferent input to neuron 10-3 is monosynaptic excitation (*7, 8*) from sensory neurons associated with filiform hairs on the cerci (*9*), two antenna-like sensory appendages located at the rear of the cricket's abdomen. These sensory neurons are activated by mechanical displacement of the associated hair in response to air currents (*10*). Each sensory neuron displays a strong directional selectivity: it fires a barrage of action potentials when the hair is deflected in a particular direction and is silent when the hair is deflected in the opposite direction (*11*). Sensory afferent neurons have been divided into several classes according to their directional selectivity, and each class terminates in a discrete region of the terminal ganglion (*7, 8*).

This spatial mapping of wind direction by afferent terminals must have important functional consequences for an interneuron with dendrites located in these regions. Interneuron 10-3 (Fig. 1) has three dendritic branches, each located in a different neuropil region containing afferents from sensory cells responsive to a different wind direction. Consequently, each branch should exhibit a directional sensitivity to wind stimuli that is different from that of the whole cell. The overall directional sensitivity of the cell should represent a summation of the directional sensitivities of all three dendritic branches.

To test this hypothesis directly, it was necessary to compare the directional sensitivity of an individual dendrite with that of the entire cell in the same preparation. This was done by isolating a single dendrite from the rest of the neuron and testing its response to wind stimuli from different directions. The soma was first impaled with a microelectrode and filled iontophoretically with 6-carboxyfluorescein (*12*). The directional sensitivity of the neuron was then characterized by recording the responses of the cell to calibrated wind puffs delivered at 11 positions around the animal's body (*13*). Sample records obtained at two different stimulus orientations are shown in Fig. 2B. In each record the response of the cell consisted of a depolarization and a burst of spikes that was maintained throughout the duration of the wind stimulus. Each response was measured and plotted in polar coordinates according to wind stimulus orientation (Fig. 2C) (*14*).

The interneuron was maximally sensitive to wind from the left side of the animal's body. To relate each response to selective activation of a different set of afferents, the movements of the hairs on the cerci were observed at each stimulus orientation. After observing which of the classes of hairs were deflected, we used the anatomical maps of Bacon and Murphey (*7*) and Walthall (*8*) to predict which of the dendrites would be activated by the stimulus. This is indicated by the letters around the perimeter of the graph (Fig. 2C).

The heavy outline in Fig. 2C represents the directional sensitivity of the cell with inputs onto all three dendrites. In order to characterize the directional sensitivity of dendrite Z alone, the laser was aimed at the cell at the position indicated by the arrow in Fig. 2A. Immediately after a 3-second illumination there was an initial decrease in input resistance, followed by a steady increase (as measured at the cell body). Within 15 minutes input resistance had stabilized at approximately twice the initial value (Fig. 2D). Such an increase is a crucial control in all such experiments, demonstrating that there is no residual current shunt caused by the lesion. A change in morphology was observed as a result of the photoinactivation: the section of dendrite receiving direct illumination was bleached and the dendrite constricted at points about 20 μm on either side of the targeted area.

After the selective photoinactivation, 10-3's directional sensitivity was measured a second time. The result, plotted as the dashed outline in Fig. 2C, represents the directional sensitivity of dendrite Z alone. The directional sensitivity was shifted toward the left rear of the animal relative to the control. The maximum response was obtained at position 1, which corresponds to wind directed at the medial face of the right cercus. According to the anatomical map this is the optimum stimulus orientation for afferents that overlap with dendrite Z (*6*). The

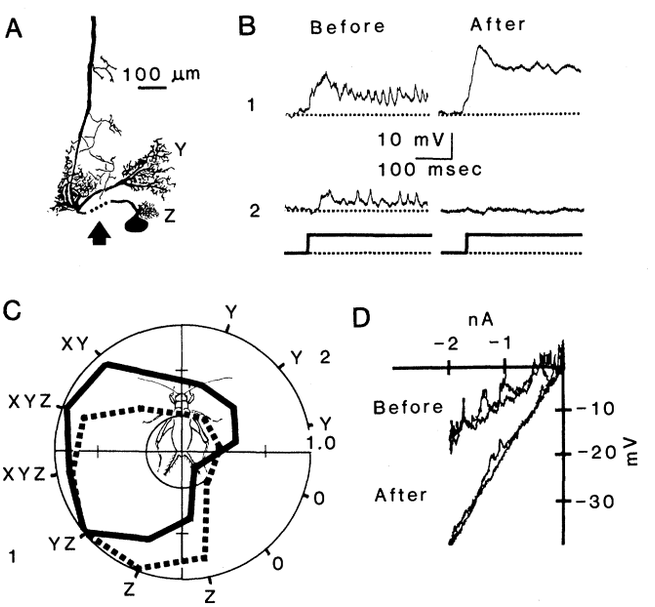

Fig. 2. Isolation of dendrite Z by selective photoinactivation. (A) The site of the selective lesion (arrow) on interneuron 10-3. (B) Intracellular recordings from the cell body of 10-3, showing the responses of the cell to wind from two different directions before and after the lesion. The action potentials appear as small (5- to 10-m*V*) spikes in these records because of their attenuation between the spike-initiating zone and the passive cell body. Individual postsynaptic potentials are not resolvable at this level, and merge to form the observed depolarization. The step functions below the responses indicate the onset of the wind stimulus. Wind from position 1 activates afferents that synapse with dendrites Y and Z. Wind from position 2 activates inputs that synapse with dendrite Y alone. (C) Directional sensitivity of interneuron 10-3 before (heavy outline) and after (dashed outline) the selective lesion. Both outlines were scaled to their own maximum values. The letters around the perimeter of the graph indicate which of the three dendrites should be activated by a wind stimulus from that direction. After photoinactivation the directional sensitivity was shifted toward inputs specific for dendrite Z. Responses to inputs specific for dendrite Y were eliminated. (D) Current voltage relation measured before and after photoinactivation. The slope of the curve increases after the selective lesion, indicating an increase in input resistance.

response was larger after the irradiation. This increase in response amplitude is due to the increase in input resistance, as discussed above. Conversely, the response at wind position 2 was eliminated. According to the anatomical observations wind from position 2 should activate only dendrite Y. Note that no action potentials were visible in the responses recorded after the laser ablation. This indicates that the spike-initiating zone in 10-3 was located beyond the targeted region of the neurite (*15*). All the data shown in Fig. 2 were obtained from a single experiment. The experiment was repeated four times with similar results.

It appears that this dendrite does have a distinct directional sensitivity. Thus the responses of each of the three dendrites may summate to produce the overall directional sensitivity of the interneuron. By performing similar experiments in which the laser beam was aimed at different parts of the dendritic tree of neuron 10-3, we have greatly refined our knowledge about the relation between neuronal form and function in this system. The response properties of this sensory interneuron can now be interpreted in terms of synaptic interactions at single dendritic branches.

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14. The cell's response was quantified by measuring the area under the waveform for the first 100 msec during the wind puff. The maximum response value for each curve was set at 1.0 and all responses were scaled with respect to that maximum. Each value is plotted in polar coordinates according to stimulus orientation with respect to the animal's body.
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Selective Extraction of Small and Large Molecules from the Cerebrospinal Fluid by Purkinje Neurons

Abstract. *Cerebellar Purkinje neurons accumulated propidium iodide, granular blue, and horseradish peroxidase conjugated to wheat germ agglutinin but not unconjugated horseradish peroxidase, bisbenzimide, or Evans blue when these compounds were infused into the lateral cerebral ventricles of awake, unrestrained rats. Accumulation of propidium iodide by Purkinje neurons of the vermis was associated with a reproducible behavioral abnormality characterized by truncal tremor, ataxia, and nystagmus. Both the accumulation of propidium iodide in Purkinje cells and the behavioral abnormality were prevented by prior intracerebroventricular administration of ouabain or colchicine, drugs that block neuronal transport processes. The ability of cerebellar Purkinje neurons to extract small and large molecules from the cerebrospinal fluid has important implications for their physiology and pathology.*

Proteins injected into the lateral cerebral ventricles can enter brain parenchyma along the perivascular spaces of blood vessels (*1*) and can then be pinocytosed by neurons and glia (*2*). In a recent study certain plant lectins were accumulated from the cerebrospinal fluid (CSF) by Purkinje neurons of neonatal mice, while no uptake was observed in mature mice (*3*). However, the Purkinje neurons in the adult human brain have been found to contain immunologically recognizable light-chain proteins that are also present in the CSF (*4*). The possibility that Purkinje neurons can extract and accumulate molecules from the CSF could have important physiological and pathological consequences, since these neurons have large dendritic arrays in close proximity to the CSF. In this study we examined the accumulation by rat brain Purkinje cells of two proteins, horseradish peroxidase (HRP) and HRP conjugated to wheat germ agglutinin (HRP-WGA), and of four fluorescent dyes, propidium iodide (PI), granular blue, bisbenzimide, and Evans blue (*5*) after their intracerebroventricular injection. Since PI can enter the CSF after intravenous administration (*6*), we also examined the accumulation of PI by Purkinje cells after its intravenous administration.

Adult male Sprague-Dawley rats (300 to 350 g) were anesthetized and implanted with a 23-gauge metal cannula in the lateral cerebral ventricle. Five to 14 days later the animals received (without anesthesia) a unilateral intraventricular injection of 10 μl of a test substance. The compounds injected were HRP (10 percent; $n = 3$), HRP-WGA (1 percent; $n = 3$), PI (0.1 to 0.5 percent; $n = 7$), granular blue (1 percent; $n = 2$), bisbenzimide (5 percent; $n = 2$), and Evans blue (2 percent; $n = 3$). Behavioral responses were observed during the injection and postinjection periods. At various periods after the injections each rat was anesthetized with pentobarbital (60 mg/kg) and perfused with a fixative (*7*) for 20 minutes. The fixative was then washed out by an additional perfusion with 0.1*M* sodium phosphate buffer containing sucrose (15 percent, weight to volume). Brain sections were cut on a freezing microtome. Sections from rats that had received injections of fluorescent dyes were mounted and examined under a Leitz fluorescent microscope. Sections from rats that had received HRP or HRP-WGA were treated with 3,3′,5,5′-tetramethylbenzidine before mounting (*8*).

No behavioral abnormalities were noted during or after intraventricular infusion of HRP, HRP-WGA, bisbenzimide, or Evans blue. There was a brief burst of grooming behavior during the infusion of granular blue, but the rats behaved normally during the subsequent 4 hours. PI caused a reproducible behavioral abnormality, beginning at the end of the infusion period, which was characterized by an oscillating rotatory tremor of the head and body and prominent rotatory nystagmus. No tremor was present in the limbs. By 10 minutes after onset the rats