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Dendritic sprouting and compensatory synaptogenesis in an identified interneuron follow auditory deprivation in a cricket

(neural plasticity/insect hearing/aberrant neural growth/sensory deprivation)

RONALD R. HOY, THOMAS G. NOLEN*, AND GEORGE C. CASADAY†

Section of Neurobiology and Behavior, Seeley G. Mudd Hall, Cornell University, Ithaca, NY 14853

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ABSTRACT **We examined the effect of chronic afferent deprivation on an identified interneuron (Int-1) in the auditory system of the Australian field cricket *Teleogryllus oceanicus*. In normal intact crickets, the auditory afferents from each ear terminate ipsilaterally onto a single Int-1. Each bilaterally paired Int-1 is excited by ultrasound stimulation of its ipsilateral ear but not by the contralateral ear. Unilateral removal of an ear early in postembryonic development deprives the developing Int-1 of ipsilateral auditory innervation. Consequently, the ipsilateral dendrites of the deprived interneuron sprout, grow aberrantly across the ganglionic midline, and terminate specifically in the intact auditory neuropile of the contralateral (unlesioned) side, where they form functional synapses with the contralateral afferents. This unusual compensatory dendritic sprouting restores auditory function to the neuron. Thus, it is demonstrated that the dendritic shape of an identified Int, as well as its synaptic connectivity, is altered as a consequence of chronic sensory deprivation.**

The ability of a neuron to restore its structure and function after lesions have been inflicted on nervous tissue is a fundamental form of neuronal plasticity. Lesions made directly on a neuron by cutting or crushing its axon interrupt a cell's anatomical integrity as well as its physiological functions. However, the neuron may regenerate and thereby restore some measure of structural and functional integrity. A more subtle lesion occurs when a neuron is deprived of its synaptic input even though the neuron is not itself directly damaged. For example, a developing sensory organ can be removed before it makes synapses with target neurons in the central nervous system, effectively deafferenting the latter. Since the morphological targets of afferent terminals are usually the dendrites of the target cell, research has focused on the effect of deafferentation on the dendritic tree of the deprived target neurons. Depending on the species, the target neurons, and the timing of deafferentation, the morphological response of the affected dendrites varies from degeneration to no apparent change, to aberrant sprouting; in general, degeneration is more common. In this paper, we describe the effect of deafferentation in the auditory system of the Australian field cricket *Teleogryllus oceanicus*. We show that an identified auditory interneuron (Int-1) responds to chronic unilateral deafferentation by expanding its dendritic tree into the unlesioned contralateral auditory neuropile and making novel synaptic connections that effectively reafferent the lesioned Int-1, restoring physiological function; some of these results were reported in abstracts (1, 2).

The auditory system of *T. oceanicus* is relatively simple (3, 4). The auditory organ is located on the tibial segment of each foreleg. From there, the ≈70 axons of the receptor cells run within the leg nerve and terminate in the prothoracic ganglion in a localized region of neuropile (auditory neuropile); the auditory terminals are strictly ipsilateral with respect to the ear of origin and do not cross the midline of the prothoracic ganglion. There are thus two separate and symmetrical auditory areas in the prothoracic ganglion, each of which services only its ipsilateral hearing organ. In field crickets, at least six identified auditory Int have been found whose primary dendritic projections are contained within the auditory neuropile (5–7). In *T. oceanicus*, Int-1 is easy to identify as one of these auditory neurons because of its distinctive physiological response to acoustic stimuli and its characteristic morphology (Fig. 1).

METHODS

The crickets used in our experiments were reared locally. The colony was maintained at 30°C on a light/dark cycle of 14:10 and was fed Purina cat chow and water ad libitum.

Operations. Crickets develop postembryonically via a series of free-living nymphs, which undergo 9–10 molts before reaching adulthood (3). Crickets were acoustically deprived on the day of hatching from the egg, or 1 day later, so that only first instar nymphs were operated on in these studies. The ear does not begin to develop until the third nymphal instar (3). The nymphs were immobilized by chilling to 4°C prior to the operation, which consisted of simply amputating the prothoracic leg (foreleg) at the tibial femoral joint. Since the auditory organ in the adult is contained within the tibia, amputation prevents postembryonic development of the auditory organ. In our experiments, only one leg was amputated so that deafferentation was strictly unilateral. Over 90% of operated animals survived to adulthood. The crickets were checked at each larval instar, and regeneration blastema were amputated whenever they appeared.

Anatomy. All anatomical studies were done in adult crickets. The anatomy of Int-1 was revealed by two different staining techniques. First, Int-1 was stained by means of retrograde uptake of cobalt chloride into the cell from its cut axon in the neck connective. The cobalt was precipitated as its sulfide salt, and the neuron was visualized in whole mount by conventional methods (8). In some preparations, the cobalt-stained ganglion was embedded in paraffin and cut at 30 μm so that the cell could be silver-stained by the Timm's intensification procedure (9). The backfill method has advantages because Int-1 takes up cobalt reliably, and therefore a large number of animals could be processed. Second, in a smaller number of preparations, the cell was stained by intracellular injection of the fluorescent dye Lucifer Yellow with glass micropipette electrodes (10). The advantage of this method is that it permitted us to record the neuron's responses to acoustic stimuli as well as stain it with certainty. Thus,

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Abbreviation: Int, interneuron(s).

*Present address: Department of Psychology, Yale University, New Haven, CT.
†Present address: Digital Equipment Corporation, Maynard, MA.

unambiguous identification of Int-1 was made on the basis of its characteristic physiology and anatomy, which have been extensively studied. Both intracellular and backfill staining techniques served to reinforce separate conclusions drawn from each.

Physiology. All recordings were made in adults, because only adults can hear. Intracellular and extracellular recordings were made from Int-1 by conventional recording techniques. Extracellular recordings were made by applying suction electrodes to the cervical connectives, thus permitting us to monitor activity in the axon of Int-1 as it ascends in the connective (11). Intracellular recordings were made in the prothoracic ganglion in the region of the auditory neuropile, thus permitting us to monitor activity from Int-1 dendrites (12). The auditory stimulus consisted of electronically generated sound pulses (rise–fall times, 5 msec; duration, 30–500 msec). The carrier frequency of the stimulus tones was produced by a Hewlett-Packard 200 CD oscillator. The tones were attenuated (H-P 350D), amplified (Crown 150 amplifier), and delivered through a piezoelectric tweeter (Motorola). A second stimulus system permitted two tones to be delivered simultaneously in order to test for two-tone suppression (11). The speakers were placed level with the cricket, 90° to the left and right with respect to the animal's longitudinal body axis. Peak sound pressure levels were measured at the recording site (B&K 2209 meter). All sound-pressure levels are expressed in decibels (dB) relative to 20 μPa.

RESULTS

Chronic Deprivation Alters Dendritic Shape. The anatomy of Int-1 has been described (5–7, 11, 12), but it will be outlined here to clarify the present study. There are only two Int-1s in the prothoracic ganglion, one in each half-ganglion, each neuron the mirror-image of the other, and each with a

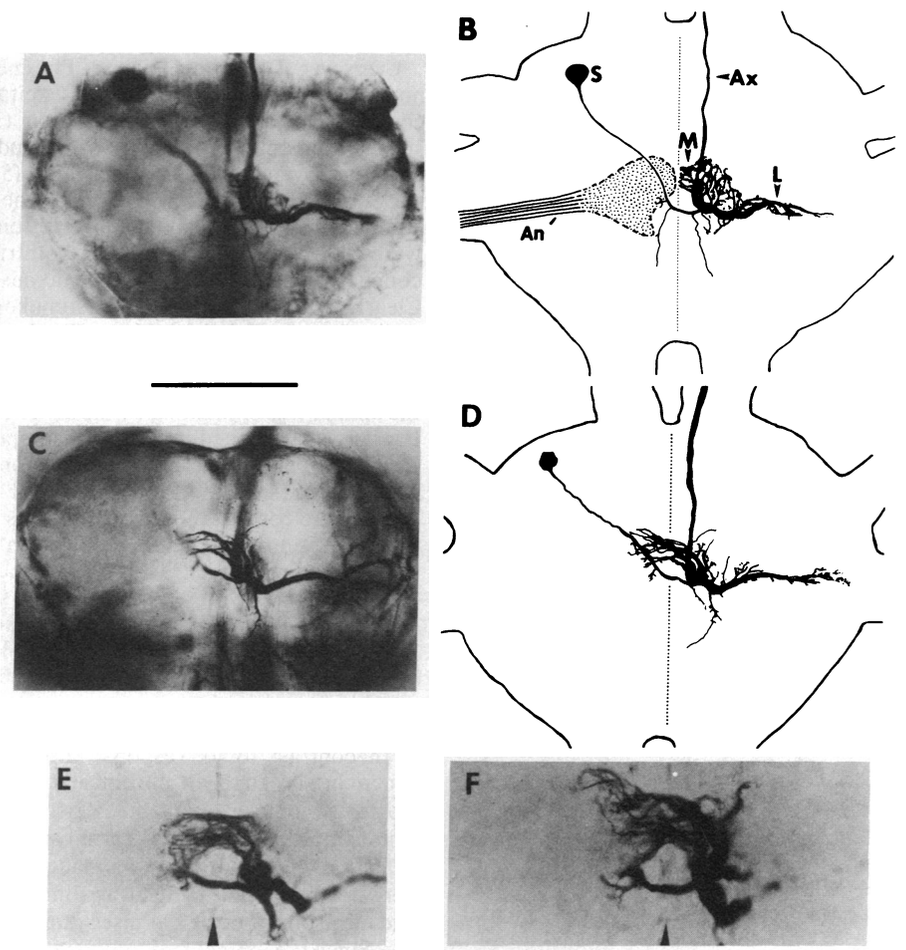

FIG. 1. Anatomy of dendritic sprouting in deafferented Int-1s. (*A*) A normal Int-1 is shown in the photograph of a prothoracic ganglion prepared in whole mount. The neuron had been impaled with an intracellular microelectrode and injected with the dye Lucifer Yellow. A camera lucida drawing of the same neuron is shown in *B* and the relevant portions of the cell are labeled: S, cell body (soma) of the neuron; M, medial dendrites, which are the postsynaptic targets for the afferent terminals of the auditory nerve fibers; the medial dendrites ramify throughout the terminal field of the auditory afferents (the auditory neuropile). The lateral dendrites (L) lie along the tract of fibers formed in part by the incoming auditory afferent axons; they were not studied. The axon (Ax) of Int-1 ascends to the brain by way of the cervical connectives (where the neuron's activity may be recorded by means of extracellular suction electrodes). The contralateral auditory nerve (An) is drawn to show the extent of the auditory afferent terminal regions, where synapses are made with Int (stippled area). Note that Int-1 medial dendrites would be entirely contained within the terminal projection of the ipsilateral ear. (*C*) The effect of chronic deafferentation can be seen in this photograph of a whole mount preparation. Int-1 was stained by retrograde uptake of cobalt chloride in the cervical connective. (*D*) The preparation in *C* was redrawn as a camera lucida preparation for clarity. Notice that the medial dendrites have crossed the midline of the ganglion and terminate in the auditory neuropile on the contralateral side. (*E* and *F*) Int-1s from two additional deafferented animals. Each had been stained by injection from an intracellular microelectrode filled with Lucifer Yellow. These ganglia were then sectioned in the horizontal plane to permit a more detailed examination of the dendrites, but at the cost of viewing only part of the cell in a given section. Arrowheads mark the ganglion midline and clearly show that the medial dendrites cross over. Histological examination confirmed that they had sprouted into the contralateral auditory neuropile of the contralateral half of the ganglion. (Bar = 300 μm for *A–D*; 200 μm for *E* and *F*).

characteristic L-shaped morphology (Fig. 1 *A* and *B*). The lateral arm gives rise to medial and lateral sets of dendritic arborizations. The vertical arm forms the axon, which ascends in the neck connective and terminates in the brain (1). The medial dendritic arborization overlaps the terminal field of the primary auditory fibers from the ipsilateral ear, forming a discrete bean-shaped projection (Figs. 1 and 2). Each auditory organ gives rise to a strictly ipsilateral terminal field (auditory neuropile; see refs. 4 and 13); auditory afferents do not cross the midline of the prothoracic ganglion (4).

Unilateral deprivation has a striking effect on the morphology of Int-1. The medial dendrites sprout and grow extensively across the ganglionic midline to form a rich contralateral arborization uncharacteristic of intact normal animals. Examination of cobalt- or Lucifer-stained and sectioned ganglia reveals that these aberrant contralateral dendrites are confined specifically to the contralateral auditory neuropile (Figs. 1 and 2). In control animals, the auditory projections of contralateral (intact) auditory neuropile and the contralateral Int-1 were examined. Examination of six animals revealed that the auditory fibers on the intact side remained ipsilateral, although in one case two auditory afferent fibers strayed slightly across the midline; in all cases the contralateral Int-1 appeared normal—in particular, the medial dendrites maintained an ipsilateral projection.

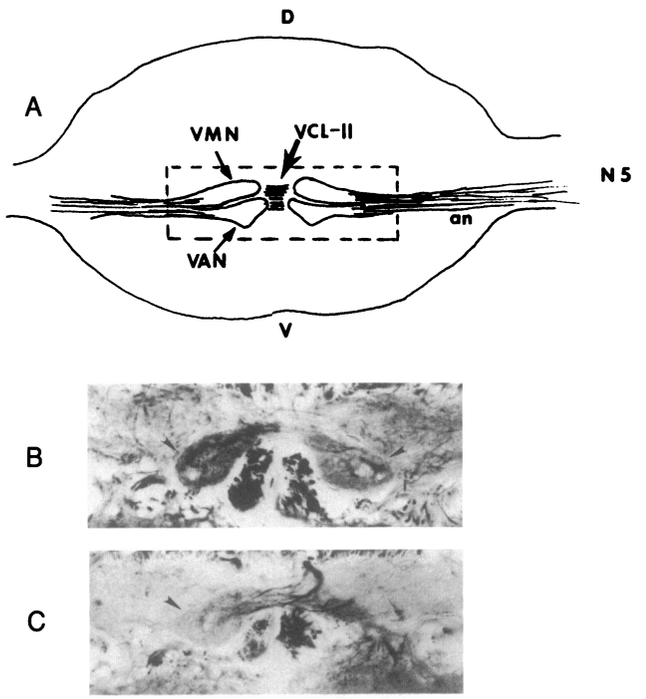

FIG. 2. Medial dendritic fields of Int-1 before and after chronic auditory deprivation. Transverse sections through the prothoracic ganglion at the level of the auditory neuropile (*A*). Schematic in *A* shows salient features: N5 (leg nerve), an (auditory tract), VAN (ventral auditory neuropile, within which the medial dendrites of Int-1 arborize), VMN (ventral medial neuropile), and VCL-II (a commissure) are landmarks for anatomical reference. D, dorsal; V, ventral. Dashed lines demarcate the neuropile region shown in *B* and *C*. (*B*) Cobalt-filled medial dendritic arborizations from both left and right Int-1s, made from an intact animal (control). Each bean-shaped arborization (arrowheads) is derived from its ipsilateral Int-1 and branches profusely within each VAN. The two darkly staining structures that lie below each dendritic projection, one on each side of the ganglionic midline, are the ventral median tracts. These longitudinal tracts were also stained with cobalt and are shown in *C*. (*C*) Cobalt-filled medial dendritic arborization from the right Int-1 in an animal that had been chronically deafferented on the right side. The dendritic arborization crosses the ganglionic midline and terminates within the left VAN (arrowhead). (Bar = 200 μm.)

We conclude that unilateral auditory deprivation induces the medial dendrites of the affected Int-1 to sprout and grow into the contralateral auditory neuropile. These conclusions are supported by several lines of anatomical investigation. Over 50 unilaterally deafferented animals were stained by the cobalt-backfill technique and examined in whole mount, as in Fig. 1; in every case the medial dendrites of the affected Int-1 grew across the ganglionic midline into the contralateral auditory neuropile. Examination of >100 normal (unlesioned) ganglia revealed Int-1s with ipsilateral projections of their medial dendrites. Twelve ganglia from unilateral auditory-deprived crickets were sectioned in the horizontal (Fig. 1) or transverse (Fig. 2) planes, and they confirmed that the reactive crossover dendrites had terminated in the contralateral auditory neuropile. Additional confirmation came from five experiments in which the deafferented Int-1 had been recorded from and stained intracellularly with Lucifer Yellow-filled microelectrodes. Again, in all five cases the deafferented Int-1 exhibited aberrant crossover dendrites that projected into the intact contralateral auditory neuropile (Fig. 1 *C–F*).

Crossover Dendrites Restore Physiological Function. In normal unlesioned animals, the excitatory input to Int-1 comes solely from the ipsilateral ear (1). Previous studies suggest that Int-1 is a second-order auditory neuron that is directly driven by auditory afferents (5–7, 11, 12). Fig. 3*A* shows the normal response of Int-1 when an intact cricket is stimulated with ultrasound (30-kHz tones). The recordings were made simultaneously from both right and left cervical connectives, which contain the axons of the two Int-1s. Clearly, since the sound pulses stimulate both ears, bilateral excitation occurs in both Int-1s. To show that each Int-1 was driven only by its ipsilateral ear, the right foreleg was amputated (removing the ear); the right Int-1 fell silent when the animal was stimulated by ultrasound (Fig. 3*B*). The intact left leg (ear) retained its acoustic competence and drove the ipsilateral Int-1. Thus, acute deafferentation abolished the excitatory response of the ipsilateral Int-1 to sound and, clearly, the contralateral intact ear was incapable of exciting it. Another normal characteristic of Int-1 in *T. oceanicus* is two-tone suppression (11). Int-1 responds to a suprathreshold ultrasonic tone with a tonic burst of spikes [Figs. 3 and 4 (Normal)]. If a second tone, in this case 5 kHz, is presented simultaneously with the excitatory 30-kHz tone, the neuron responds less vigorously than to the 30 kHz tone alone; the combination tone has a suppressive effect on Int-1 [Fig. 4 (Normal)].

In contrast to its response after acute deafferentation, when Int-1 had been chronically deafferented throughout postembryonic development, it became excitable by acoustic stimulation of the contralateral ear. This is shown in Fig. 4 (Deaff.). The recording was made from a chronically deafferented Int-1. The animal was stimulated with a suprathreshold ultrasonic tone of 200-msec duration and 30-kHz frequency. Since the animal had only one foreleg (hence one ear), it is clear that stimulating the contralateral ear excited the chronically deafferented Int-1. We can be certain that this neuron was indeed Int-1 for several reasons. First, the intracellular recording made from the neuron revealed two response properties characteristic of Int-1: (*i*) the neuron was tonically excited by ultrasound stimulation, and (*ii*) the neuron showed two-tone suppression, as described above. Second, when the same cell was injected with Lucifer Yellow at the conclusion of the physiological tests, it was revealed to have the L-shaped morphology of Int-1. In addition, this deafferented Int-1 had dendrites crossing over into the contralateral auditory neuropile, providing the anatomical basis for excitation arising from the contralateral ear. Moreover, amputation of the contralateral ear abolished the cell's

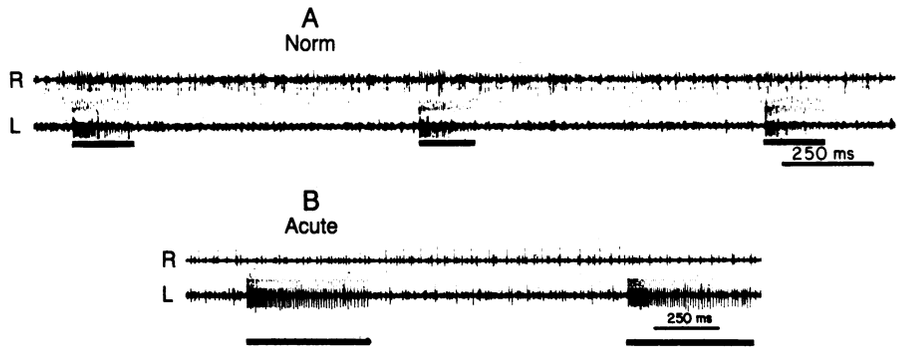

FIG. 3. Physiology of acute unilateral deafferentation. (*A*) Norm, the normal response of both Int-1s (R, right; L, left). Both responded to excitatory 30-kHz tones (80 dB suprathreshold). Solid bars below the spike traces indicate stimulus periods. Stimulation by 30 kHz excited both Int-1s. (*B*) Acute, the right foreleg (and ear) had been amputated just prior to the recording. The sound stimulus (solid bars) no longer excited the right Int-1, but clearly continued to stimulate the left Int-1 (same stimulus parameters in both *A* and *B*). This demonstrates that ultrasound stimulation of Int-1 is mediated by the ipsilateral ear, and that stimulation of the contralateral ear is not sufficient. The recordings were made extracellularly in the neck connectives.

responsiveness to sound; thus, the contralateral ear was the sole source of auditory input into the neuron.

Thus, the physiological data demonstrate that the deprived Int-1 is reafferented, presumably via the crossover dendrites, and that auditory function is restored with a high degree of specificity, restoring even two-tone suppression, a complex response not found in the other six auditory interneurons.

DISCUSSION

Our results show that the dendrites of an identified auditory interneuron react to unilateral auditory deprivation by sprouting, growing uncharacteristically into the intact contralateral auditory neuropile, and forming synapses there. This is an unusual compensatory growth response by dendrites. Comparable studies show that both deprivation and deafferentation more commonly have negative effects on

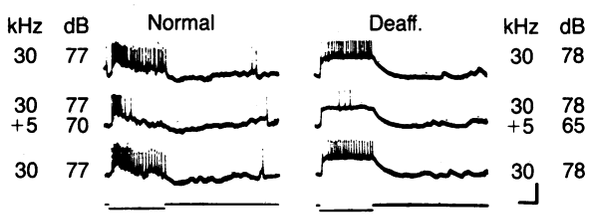

FIG. 4. Physiology of chronic unilateral auditory deprivation. For comparison, the responses of an Int-1 from an intact animal (Normal) are presented alongside those from an Int-1 that had been unilaterally deafferented by amputation of the ipsilateral ear since hatching from the egg (Deaff.). The normal Int-1 responds to a 30-kHz tone played at 77 dB suprathreshold with tonic excitation. The chronically deafferented Int-1 also responds to a 30-kHz tone played at 78 dB suprathreshold. In a normal animal, Int-1 responds to a combination tone (5 kHz and 30 kHz) to a lesser extent than to 30 kHz alone. This is two-tone suppression (see text). The deafferented Int-1 also shows two-tone suppression. In both cases, the suppressive effect of the combination tone is not long-lasting, as shown by the bottom panels, which are presentations of the 30-kHz tone alone, given immediately after presentation of the combination tone. The recordings were made intracellularly from the dendritic region of the neuron, hence the appearance of spikes rising off of the excitatory postsynaptic potential. The neuron from which the Deaff. recording was made was stained with Lucifer Yellow and its aberrant medial dendrites are shown in Fig. 1*F*. Note that the responses recorded in the Deaff. Int-1 are mediated by the intact contralateral ear (see text). Broken bar at the bottom of the records is the auditory stimulus period. Calibrations are as follows: Normal, ordinate = 29 mV, time = 105 msec; Deaff., ordinate = 32 mV, time = 105 msec.

dendritic growth; reports of dendritic sprouting are rare, and functional reconnection is even rarer, as will be seen below.

In our study, the crickets were lesioned within a day after hatching from eggs, and weeks before any auditory afferents would have grown into the central nervous system (3). Clearly, the survival of the deprived Int is not dependent on innervation by ipsilateral auditory afferents; however, since its crossover dendrites synapse with contralateral auditory afferents, these afferents might "rescue" the neuron. This is not likely because we have found that bilaterally deprived crickets possess clearly recognizable Int-1s (unpublished results).

We interpret our experiments as evidence that deprivation causes dendritic sprouting. In developmental studies of the orthopteran nervous system, neurons sometimes produce extra branches (usually axons) that are later retracted before reaching adulthood (14, 15). We have traced Int-1 from the adult back to the seventh instar in unlesioned normal specimens, and at each nymphal stage they possess the typical L-shaped morphology with medial dendrites restricted to the ipsilateral hemiganglion (unpublished observations). Thus, while we cannot rule out supernumerary dendritic branching earlier in development, we do not believe that the reactive crossover dendrites of deprived Int-1s are developmental vestiges, particularly because we can reproduce the results reported here by deafferenting adults (unpublished results). Moreover, recent experiments show that a deafferented Int-1 can later be reafferented by regeneration of the auditory nerve—remarkably, Int-1 possessed both aberrant crossover and ipsilateral dendrites; the reactive dendrites had not retracted and were functional (16).

Comparable studies in vertebrates and invertebrates indicate that the reaction of Int to deafferentation is variable, but that dendritic sprouting followed by functional reconnection is rare. In the vertebrate auditory system, deprivation or deafferentation has degenerative effects on postsynaptic relay neurons (17–20).

The example most directly comparable to the present study is the cercal nerve–giant Int system of cockroaches, locusts, and crickets. Deafferentation involves amputation of a cercus (a posterior sensory appendage that bears sound- and wind-sensitive hairs) either upon hatching or in later nymphal instars. The result of deafferentation can be assessed by its effect on the dendritic morphology of any or all of the seven postsynaptic giant neurons. In the cricket, deafferentation has a slightly negative growth effect on the dendrites of postsynaptic Int (21, 22). In the cockroach, cercal deafferentation has apparently no measurable effect on giant Int dendrites (23, 24). It is interesting that the only case in which

cercal removal was found to cause dendritic sprouting in giant Int occurs in grasshopper embryos, when cercal amputation was done before the Int was innervated by cercal afferents (25), just as we had done in Int-1. However, the stage at which afferents are removed does not explain sprouting in Int-1, because we can demonstrate that true deafferentation (removal of already existing afferents in nymphal instars or even in the adult) also causes medial dendrites to sprout, cross the midline, and become functionally innervated by the contralateral auditory afferents (unpublished observations). Another interesting comparison is the finding of Shankland *et al.* (25) that the dendritic sprouting in one part of a dendritic arbor appeared to be at the expense of outgrowth elsewhere on the neuron, suggesting that the amount of dendritic membrane may be limited (by intrinsic factors). While we have not made a quantitative analysis of dendritic branching in Int-1, it is our impression that whenever deprivation induces sprouting of the medial dendrites, there appears to be a concomitant decrease in the complexity and number of lateral dendrites.

Presumably, this sort of neuronal plasticity has behavioral consequences. Cockroaches (24, 26) and crickets (27) have been shown to make compensations for behavioral deficits produced by auditory deafferentation, although it may take weeks to occur. Dendritic sprouting by Int is not likely the explanation in cockroaches (24), but it could explain behavioral compensation in crickets, although this is yet to be demonstrated.

In conclusion, our findings in Int-1 are unusual because chronic sensory deprivation was shown to alter the shape of the dendritic arborization of an identified neuron, as well as the pattern of its synaptic connectivity. This contrasts sharply with the more commonly degenerative response of neurons to chronic sensory deprivation or deafferentation.

Notes Added in Proof. (*i*) It has been brought to our attention that a study on the developing retina of the rat has demonstrated dendritic sprouting in retinal ganglion cells and that these data provide evidence of dendritic competition for afferents (28). (*ii*) Recently, a thorough study of the neuroanatomy of the tracts of the prothoracic ganglion of the cricket, *Gryllus campestris*, has been published (29). The neuroanatomy of *G. campestris* and *T. oceanicus* appear so similar that we suggest that the commissure that we call VCL–II in Fig. 2 should be reidentified as the anterior ring tract, in accordance with the designations of Wohlers and Huber (29). (*iii*) K. Schildberger, D. Wohlers, and F. Huber (personal communication) have confirmed our findings of dendritic sprouting in a neuron homologous to our Int-1 in the cricket *Gryllus bimaculatus* and, in addition, find sprouting in two other neurons.

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