Integration of Directional Mechanosensory Input by Crayfish Interneurons

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SUMMARY AND CONCLUSIONS

1. Interneurons activated by mechanosensory hairs on the crayfish telson respond selectively to directional displacements of the medium; the directions of maximum sensitivity lie 180° apart in approximately the rostrocaudal plane, corresponding to the directional sensitivities of the two populations of primary afferent neurons. We have examined the basis for this selectivity by intracellular recording in the interneurons, correlating subthreshold potentials with activity evoked in identified afferents by bending single hairs or by producing near-field displacements of the medium.

2. Interneurons can usually be caused to discharge by a brief train of impulses in single sensory axons. Unitary EPSPs are associated with arriving afferent spikes in the fourth (sensory) root; each primary interneuron receives convergence from several sensory axons, all sensitive to the same direction of movement. Since each afferent axon is drawn from a pair innervating a single sensory structure, this remarkable specificity of connection is unlikely to depend on an anatomical mode of address.

3. Higher order interneurons receive from directionally sensitive lower order interneurons of the same class, as well as from primary afferents of that class. The responses of such cells may show much more decrement during a train of displacement stimuli than do those of lower order cells. Directionality does not appear to be enhanced.

4. During the "null phase" some interneurons appear to be actively inhibited: bending of single hairs 180° away from the effective direction may produce membrane hyperpolarization and slow spontaneous discharges, and shocks to afferent roots produce mixtures of monosynaptic EPSPs and polysynaptic IPSPs.

INTRODUCTION

Most large interneurons in the abdominal nerve cord of the crayfish respond to input from exoskeletal hair receptors that are distributed over much of the dorsal body surface. Wiersma and Hughes (13) showed that these interneurons differ from one another primarily in the extent of their receptive fields: some receive from many abdominal segments, some from only a single one, and some from a single appendage or region of a segment. More recent studies on the connectivity patterns of these interneurons, based on microelectrode recording of subthreshold excitatory postsynaptic potentials (EPSPs), show that each cell is activated from a single conterminous peripheral region having regular boundaries. Within this region, receptors are likely to connect with the central neuron and therefore to produce unitary EPSPs in it (2, 3, 6). Such receptive fields are normally composed of the innervation zones of several sensory nerve roots; these latter are geographically distinct, having precisely defined and adjoining boundaries in the periphery. Afferents from one of these zones normally show an especially high probability of connection and those from zones flanking it, lower ones, so that the density of input onto an interneuron normally has one geographical peak in the periphery and declines on either side of it. Such systematic variations in input density also occur within the peripheral zone innervated by a single sensory root.

These findings suggest that sensory nerves coming from a given peripheral region carry an anatomical label that determines the connections they will make with central elements during development. Such a view of receptive-field organization requires only that afferent axons maintain, in their central course, a spatial arrangement that accurately reflects the distribution of their endings in the periphery; this view is supported in a general way by a somatotopic representation of the body surface that occurs in the sensory nerves (8). If that representation is conserved in the central ganglia, as it apparently is, a simple form of anatomical address can explain the observed facts of receptive-field organization. It requires that the dendritic pro-
cesses of interneurons branch with greatest density in the region of neuropil containing beams of afferent axons from the center of their receptive fields. The number of connections would thus be highest in this region and decline on either side of it.

The afferent fibers also possess another kind of specificity: they are sensitive to the direction in which the exoskeletal hair is bent (9, 14). Many hairs are dually innervated; one of the afferents responds to headward and the other to tailward bending. In the most carefully studied of the abdominal hair fields, the rostrolateral region of the telson that is innervated by the fourth roots of the sixth ganglion, most of the approximately 70 hairs are dually innervated. Even where two afferent neurons cannot be demonstrated, each single afferent has a preferred direction, and the axis of sensitivity is always nearly parallel to the rostrocaudal axis (14). Near-field water disturbances, such as those evoked by surface waves, thus produce separate population responses in the afferent fibers, one corresponding to forward and the other to tailward displacement.

Similar responses have been reported briefly in interneurons activated by afferents from mechanoreceptors on the uropods and telson (15). These observations suggest that the division of this sensory system into submodalities detecting rostral and caudal displacement is preserved in the central nervous system. Such an organization would require that labels for direction as well as for peripheral location be used in specifying central connections between sensory axons and interneurons during development.

We have examined these patterns of connection by stimulating single, identified peripheral sensilla and recording intracellularly from central interneurons to examine the effects. The results show that directional selectivity is preserved in most primary interneurons by an absolute specificity of connection with afferents having a given directional sign. Single afferents may connect with many interneurons having the same directional sensitivity; conversely, a given interneuron connects with many afferents responding to the same direction of hair displacement. Some higher order elements display a more complex input organization in which inhibitory interaction between lower order interneurons with different directional sensitivity seems to be important.

**METHODS**

Crayfish (*Procambarus clarkii*, both sexes) were prepared as described previously (14). The abdominal nerve cord was isolated, leaving the fourth roots intact and connected to the uropods and telson, and pinned out in a Silgard-lined preparation dish. The isolation procedure preserves natural input to central interneurons from hair receptors located in the rostral half of the telson. The connectives between the fifth and sixth ganglia and the ventral surface of the sixth ganglion were desheathed.

Glass suction electrodes of varying tip diameters were used to record the discharge of interneurons in the connective or to monitor activity in fourth-root afferents en passant. Interneurons were isolated by fine dissection, leaving their connections in the sixth ganglion intact, and drawn into the tips of the suction electrodes for recording. Root recordings were made by attaching a suction electrode of approximately the same diameter as the root to the latter; sometimes these same electrodes were used to deliver electrical stimuli to afferents. We used 3 M KCl-filled glass microelectrodes for intracellular recording; these were pulled with Fiberglas wisps to aid in filling, and those selected for use had resistances between 20 and 50 MΩ. Signals were amplified by a high-impedance cathode follower with negative-capacity feedback (WPI). Such microelectrodes were advanced through the ventral surface of the sixth ganglion until units were impaled that could be driven by mechanical agitation of the fourth-root receptive field, and also responded with at least subthreshold activity to near-field water disturbances. The analysis presented in this paper is based upon 34 units that met these criteria.

Natural stimuli were delivered in a variety of ways; most of these are described in the previous paper (14). Single hairs were moved backward and forward along the rostrocaudal axis by a fine wire loop slipped around the hair; the wire was mounted on a small speaker coil carried on a micromanipulator. To activate the entire field of hairs with synchronous near-field water movement a plastic sphere or cylinder was moved up and down by a speaker coil through the interface between air and the van Harreveld’s solution (12) used to bathe the preparation. Driving voltages for the speaker coils in both cases were derived from the power-amplified output of a variable-frequency sine-wave generator.

**RESULTS**

**Classes of interneurons**

We classified interneurons as sensitive to water displacements and established their directional sensitivity by moving a plastic sphere or
horizontal cylinder up and down sinusoidally through the meniscus of the preparation chamber (see METHODS). This produced alternating rostral and caudal displacements of the medium at the stimulus frequency, and allowed ready characterization of responding units recorded by suction electrodes from filaments or divisions of the 5-6 connective. Figure 1 shows samples of such responses from a pair of interneurons having opposite directional sensitivity that were isolated by dissection from the same region of the 5-6 connective. Typically, serial recording from bundles dissected in a single connective revealed 6–10 units that responded with multiple spikes during either the rostral or caudal phase of displacement. We assume, without direct evidence, that units with similar directional sensitivity and other properties differ from one another in the regional distribution of their sensitivity along the sixth-segment appendages.

Because the experiments were necessarily done under conditions in which reflection of surface waves occurred, precise measurements of phase and of following frequency were not made. Maps of the directional sensitivity of the interneurons, made by delivering identical sinusoidal stimuli at various compass points from a preparation of the isolated tail, revealed a distribution much like that for primary afferent fibers: responses 10° away from directly rostral or caudal were significantly reduced. Responses sometimes followed stimulus repetition rates as high as 20 Hz, but interneurons showed considerable variation in temporal characteristics.

Responses of interneurons to input from single receptors

Dually innervated hairs that produced prominent impulses in the fourth root on each direction of bending were selected for stimulation; in general, these belonged to the S and L groups (6) and were among the lowest threshold receptors for near-field water disturbances (14). In the first experiments, multiunit recordings of interneurons in the connective between fifth and sixth ganglia were made with large suction electrodes. Placements on the ventrolateral margin of the connective, approximately centered on area 85 (13), yielded responses from interneurons with large axons, each with spikes of characteristic amplitude and each showing directionally selective responses. Examples of such responses are seen in Fig. 3, 6, and 8B, where records from large suction electrodes monitoring the activity of interneurons in the 5-6 connective are included. In response to movement of single hairs, these units normally gave 1–3 spikes for each movement direction at frequencies below 5 Hz; above this value occasional failures were observed, but the responses that remained held a fixed relationship to stimulus phase. Placements in other areas occasionally also revealed responding units, and some of these showed repetitive discharges following single deflections of the hair. These results demonstrated that a brief train of impulses in a single afferent axon is adequate to produce discharge in some interneurons.

Connections of directionally sensitive afferents with primary interneurons

Calabrese (2) has demonstrated that interneurons responding to mechanoreceptive hairs of the kind stimulated in these experiments may be classified as primary or higher order by the latency and duration of their excitatory postsynaptic potentials. Primary interneurons display prominent unitary EPSPs associated with arriving afferent impulses; compound EPSPs resulting from synchronous afferent volleys have short latency (≤ 2 ms), last no longer than 40 ms, and produce no more than 2 or 3 impulses even at high stimulus intensities. We recorded intracellularly from such neurons to examine the selectivity of the connections they received from directionally sensitive afferents. In some of the experiments a single hair was stimulated or the entire receptive field was activated directionally, and several interneurons were penetrated in sequence; in others, several dually innervated hairs were stimulated successively in order to study their effects on a single postsynaptic cell.

In the most extended experiment of the first type, stable penetrations of 11 interneurons sensitive to hair movement were made in a pe-
period of about 3 h, and in 9 of these cells unitary EPSPs were produced in response to one direction of deflection but not the other. Three of these interneurons responded to headward bending, and the rest to tailward bending. In other preparations, tailward-sensitive interneurons were encountered much more often than headward-sensitive ones: of 34 directionally sensitive interneurons recorded intracellularly in our experiments, only 5 were responsive to the headward direction of bending. Since this predominance of tailward-sensitive elements is not observed in series of units recorded extracellularly from the connective, we suppose that some factor influencing the frequency of microelectrode penetration is responsible for it.

Where single hairs were stimulated, the unitary EPSPs recorded in the interneuron clearly corresponded to afferent impulses initiated by bending the hair in one direction but not the other (Fig. 2). By the criteria given above (cf. ref 16), these connections are monosynaptic; the effects recorded in higher order interneurons are distinctively different (see below).

The convergence of directionally similar afferent pathways onto the same interneuron is illustrated by the records in Figs. 3 and 4. Figure 3 compares the responses of two elements to sinusoidal movements of the medium in the rostral-caudal plane. Several low-threshold hairs are bent synchronously in the same direction by such stimuli; all of the unitary EPSPs in each interneuron resulted from the same direction of movement—tailward in the case of the lower record, headward in the case of the upper record. Figure 4 shows responses of the same interneuron to stimulation of two adjacent hairs in the S row (6). In each case, only activation of the headward afferent produced unitary EPSPs in the interneuron.

One identified interneuron failed to display such selectivity. In Fig. 5, quasi-intracellular records from interneuron A (17) show that unitary EPSPs are produced by both phases of displacement of the entire hair field.

Responses of higher order interneurons

Many interneurons in the abdominal connectives are driven mainly by other interneurons, although they may also receive input directly from primary afferent fibers. These interneurons also display longer latency (≥3 ms) compound EPSPs, a long duration that produce, at high stimulus intensities, repetitive impulse trains containing 10 or more impulses (2).

Such interneurons exhibited the same kind of connection specificity as did the primary interneurons. Of 12 units that could clearly be classified as higher order according to these criteria, all received direct or indirect excitatory input from afferents having the same directional sign. Figure 6 illustrates some of the ways in which such units differed from primary interneurons. Repetitive stimulation produced a more dramatic decline in the response: the first few stimulus cycles resulted in an EPSP barrage (A), but as the stimulus was continued, components of this depolarization dropped out, leaving a single unitary EPSP (beginning of record B). The latter clearly cannot be attributed to activity in a single afferent axon, since no corresponding impulse can be seen in the fourth-root record. Later, in B, this EPSP failed in all-or-none fashion without visible changes in the fourth-root record. This EPSP must therefore have been produced by single impulses in a directionally sensitive interneuron of lower order. After its failure, very weak input associated with primary afferents having the same directional sensitivity remained.

Role of inhibition

In addition to receiving connections from directionally sensitive afferents and (in some cases) from lower order interneurons, some interneurons also appear to be inhibited during the null phase of hair movements. In Fig. 7, compound EPSPs evoked by stimulation of the ipsilateral fourth root were recorded intracellularly in order to test their efficacy during both phases of movement of a single hair. The pene-

![FIG. 2. Responses of a sixth ganglion interneuron (intracellular record, bottom trace) to sinusoidal displacement of a single L row hair. The stimulus monitor (middle trace) indicates tailward movement of the hair by downward deflection. Top trace, extracellular record from the fourth root. Each discharge of the tailward-sensitive afferent (large spikes) produces a unitary EPSP in the interneuron; other unitary EPSPs occur occasionally, even during the opposing movement, but these are due to the unmonitored spontaneous discharges of hair receptors having afferent axons in different roots. Stimulus period, 550 ms; voltage calibration, 10 mV.](image-url)
FIG. 3. Responses of two different interneurons, recorded sequentially in the same preparation, to sinusoidal displacements of the medium (bottom traces). Tailward displacements are upward. Top trace: monitor of afferent activity in the intact fourth root; gain increased in record B. Second trace: monitor of activity in the ipsilateral 5-6 connective; third trace: intracellular recording from sixth ganglion interneurons. Depolarizations composed of distinct unitary EPSPs are seen during headward deflection in A and during tailward deflection in B; these are lacking in the null phase of displacement, and there is an indication of hyperpolarization in B. Calibrations: 10 mV for A, 20 mV for B. Stimulus frequency, 2 Hz.

FIG. 4. Responses of the same interneuron to sinusoidal movements of two adjacent sensory hairs. Traces arranged as in Fig. 3; records are from the interneuron in Fig. 3A. A: displacement of hair S5; B, of hair S4. Headward displacements are upward. Stimulus frequency, 5 Hz; voltage calibration, 10 mV.
FIG. 5. Responses of interneuron A (17) to sinusoidal displacement of the medium (bottom trace). Recordings of unitary EPSPs and spikes are made by a suction electrode fitted around the cut end of the dissected axon (cf. ref 2). Top trace: monitor of afferent activity in the fourth root; middle trace: interneuron record. Note that unitary EPSPs and spikes appear during both phases of displacement in this cell. Stimulus period, 300 ms.

The treated cell was sensitive to headward movements of the hair. When such EPSPs were evoked during the tailward phase of hair movement, their amplitude was reduced and their time course markedly shortened, suggesting an inhibitory conductance increase in the post-synaptic cell.

The proposal that some interneurons receive inhibition in one movement phase and excitation in the other is consistent with the mixed character of the input to many abdominal interneurons (3, 7, 16). Figure 8A illustrates the kind of complex EPSP/IPSP sequences that can occur in higher order interneurons when the ipsilateral fourth root is stimulated electrically. An early EPSP component, having a latency consistent with monosynaptic connections from primary afferent neurons, is followed by a deep hyperpolarization. The latency of this IPSP is greater than 3 ms and it, therefore, must be

FIG. 6. Responses of a higher order interneuron to single-hair displacement. Top trace: monitor of interneuronal activity in ipsilateral 5-6 connective. Second trace: afferent monitor, fourth root. Third trace: intracellular record from sixth ganglion interneuron. Bottom trace: stimulus monitor; headward deflection is upward. Thirteen cycles are deleted between the end of A and the beginning of B. Note that different interneurons identifiable in the top trace are associated with headward and tailward deflection of this single hair; but none of these is accountable for the intracellularly recorded EPSPs. Stimulus period, 170 ms; voltage calibration, 20 mV.
polysynaptic; in this cell it developed at a lower threshold than the EPSP, but this situation is not usual. At high stimulus intensities, as shown in Fig. 8A, the IPSP leads into a sustained depolarization beginning at about 40 ms; several impulses may be discharged from this plateau. The late depolarization is clearly associated with synaptic excitation: responses to high-intensity contralateral root stimulation exhibit only the IPSP and also lack the delayed depolarization.

Units showing responses resembling those of Fig. 8A are encountered regularly in microelectrode penetrations made in the sixth abdominal ganglion. Figure 8B demonstrates that in at least some of the cells, the property of receiving mixed inhibition and excitation from ipsilateral fourth-root afferents is associated with directional sensitivity.

The only decisive proof that an interneuron receives null-phase inhibition requires direct evidence that IPSPs or summed hyperpolarization occur in the appropriate stimulus phase or that spontaneous firing is reduced then. Unfortunately, experiments of this kind involving natural water waves are difficult to interpret. Some afferents show spontaneous discharge when their hairs are in the resting position; displacement away from that position can suppress the discharge, and the results of such a displacement would therefore resemble active inhibition. The position of a single hair in a loop can be adjusted so that this difficulty is avoided, and since some lower order interneurons can be activated by trains of impulses in a single afferent neuron, we attempted to demonstrate null phase inhibition by such stimulation. Figure 9 shows that, during response to sinusoidal rostral and caudal movements of a hair, the membrane potential dips below its resting level during the null phase. This effect cannot be accounted for by the abolition of spontaneously occurring EPSPs, since "silent" periods of the order of a stimulus cycle length do not produce hyperpolarization.

In Fig. 10, the effect of maintained bending of a single hair in the null direction is examined. The headward-sensitive interneuron maintained
a fairly regular spontaneous activity in the absence of stimulation; bending of a single S-row hair in the caudal direction produced a train of afferent spikes (arrow) and a transient inhibition of the spontaneous discharge.

DISCUSSION

Directional selectivity and specific connections

Primary afferents in the telson region all appear to have the same set of directional preferences, i.e., directly rostral and directly caudal, and the angular sensitivity of interneurons does not appear to differ significantly from that of afferents. Neither is there evidence for subpopulations of interneurons sensitive to displacement vectors other than directly rostral and directly caudal. In some other systems, "sharpening" of afferent directional sensitivity is attained as a consequence of central inhibitory interactions; this mechanism appears to be used in the central giant interneurons of some insects (5). There is no suggestion that central inhibitory interactions play such a role in the system studied here.

A second way to achieve precise directional tuning in central interneurons is to have a regular peripheral distribution of various preferred bending directions of the sensilla, accompanied by selective connections between afferents from a local area and particular interneurons. Very specific connectivity based on peripheral location of afferents is known in arthropods (1) and appears to be the mechanism used, for example, in generating input to the very direction-specific interneurons found in the locust wind-detection system (4).

But the crayfish mechanoreceptors are not separated into many direction classes; afferents connected to the same hair respond with fairly broad directional tuning to stimuli originating from directions 180° apart. Furthermore, regional cues do not appear useful for generating directional specificity in interneurons; instead, the specificity emerges...
through a connection pattern in which certain interneurons accept only afferents having one directional sign, and reject those from the very same sensillae having the opposite sign. The only form of connection specificity previously known in this system (or, in fact, in other arthropod ganglia) is based on the anatomical ordering of afferent fibers as they enter the ganglion, which reflects the topography of their peripheral origins. The existence of a different principle for the formation of specific connections raises questions about the nature of the labels employed in "addressing" the directional afferents. Are these labels essentially anatomical, like those apparently used in elaborating regional receptive fields, or is some other form of labeling used, e.g., chemical? To date we have not found any evidence for anatomical segregation of afferents having different directional sensitivity (8), and it therefore seems likely that another kind of address is used in generating the proper connections.

**Number of elements**

We cannot tell exactly how many directionally sensitive interneurons represent the sixth-segment appendages in the central nervous system. The number is certainly small, probably 20 or less; but it includes some of the most prominent elements in the abdominal connectives. In samples of units obtained by extracellular recording from filaments dissected in the 5-6 connective, headward-sensitive elements are about as abundant as tailward-sensitive ones. We tentatively conclude that the paucity of headward units in our intracellular sample must be attributed to some feature that makes microelectrode penetration of such cells inherently more difficult. Relatively small size seems the likeliest explanation. If this is right, the interneurons preserve a size relationship already found among the primary afferents, where tailward-sensitive axons are usually larger than headward-sensitive ones (14).

**Synaptic organization**

The convergence of primary afferents onto first-order interneurons must have a ratio between 20 and 100. There are about 70 mechanoreceptive hairs in the fourth-root innervation field in an adult crayfish; of these, 20 or less are of the low-threshold type normally excited by near-field water disturbances. In our experiments, interneurons excited by an afferent from one of these hairs tended to receive connections from most of the others as well: central neurons also receive input from afferents traveling in other roots, and the breadth of such receptive fields is one of the defining characteristics of the interneuron.

Not all input connections are of equal efficacy. The strongest appear to be barely below the strength necessary to discharge the postsynaptic neuron with single impulses. But the experiments reported here show that trains of several impulses in a single afferent are adequate to discharge a number of the more sensitive interneurons.

For nearly all the interneurons in our sample, there was clear evidence of directionally selective connections. The exception was interneuron A, a high-threshold, phasic element that discharges "naturally" only when the caudal appendages are touched or the interface between the air and the bathing medium is broken near the animal. This cell is one of the identified interneurons in the lateral giant escape circuit (17). It, and perhaps other primary interneurons as well, participate in rapid withdrawal from intense mechanical stimuli. There seems little reason for it to preserve any sort of directional information.

**Inhibition and higher order interactions**

No primary inhibition is found in the directionally selective interneurons, consistent with the conclusion (3) that afferent neurons in this system are not capable of mediating it. Several lines of evidence indicate that, in some interneurons, inhibition mediated by polysynaptic pathways occurs during the null phase of hair movement. Spontaneous activity in such interneurons can be slowed by appropriate movements of single hairs, and test EPSPs evoked in the null phase of hair movements are diminished; in neurons showing such responses, IPSPs (sometimes mixed with EPSPs) can be evoked by shocking the appropriate afferent roots.

Until more is known of the behavioral significance of near-field water disturbances to crayfish, little can be said of the importance of the higher order neurons. Neither the limits of directional localization nor the extent of frequency sensitivity have been measured. There are indications that the presence of inhibitory connections from interneurons sensitive to hair movements in the null direction may be associated, in those elements that do not exhibit response decrement, with an enhanced ability to follow higher frequencies. Such connections could thus serve to stabilize the phase of interneuronal responses. There is another, still untested possibility: since laterally placed sources of near-field disturbance produced mixed responses from headward- and tailward-sensitive
afferents, a mechanism for enhancing the contrast between them might be used in the localization of sources.

One function of the interneuronal hierarchy seems clear. At least some higher order interneurons show much more dramatic response decrements than the elements receiving direct afferent connections. These cells signal the start of a series of oscillatory water movements, but they stop responding quite quickly when the same frequency is maintained. Analogous elements are found in other central systems for processing mechanoreceptive input. For example, Roeder (11) has shown that trains of acoustical pulses to the hearing organs of noctuid moths excite some interneurons on each repeated cycle, but some are more liable. Still others fire only to mark the beginning of a train. Similarly, in interneurons responding to directionally sensitive wind receptors in locusts, some higher order central elements show especially rapid adaptation (4). Our results suggest that such properties could be the simple outcomes of a hierarchical ordering of the interneurons.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Deborah Cowley and Jody McVittie, and thank Drs. Peter Getting and Jeffrey Wine for helpful discussions. This work was supported by a grant from the National Institutes of Health (NS-02944) to D. Kennedy, and by a fellowship from the Deutsche Forschungsgemeinschaft (Wi 363/4) to K. Wiese.

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