J. exp. Biol. **152**, 305–312 (1990)
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INHIBITORY CONNECTIONS UNDERLYING THE DIRECTIONAL SENSITIVITY OF THE EQUILIBRIUM SYSTEM IN THE CRAYFISH *PROCAMBARUS CLARKII*

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Accepted 6 April 1990

Summary

1. Neuronal mechanisms underlying the directional sensitivity of the crayfish equilibrium system were studied in the brain by intracellular recording combined with mechanical statocyst hair deflection.

2. Five primary afferents were successfully characterized. Three of them showed a decrease in response to inward hair deflection. The remaining two showed the opposite directional response.

3. Directional sensitivity was found in six interneurones. Two of them were excited during inward hair deflection while the other four were excited during outward deflection. Both groups exhibited active inhibition during hair deflections in the opposite direction.

4. This 'null-phase inhibition' appeared to arise from the convergence of the two classes of afferents onto an interneurone with the opposite sign.

5. Three identified descending statocyst interneurones, S3, S6 and S7, were found to receive excitatory input from one statocyst and inhibitory input from the other.

6. The results thus indicated that the directional sensitivity of the crayfish equilibrium system was achieved by selective excitatory connections between the interneurone and the directionally arranged receptor and sharpened by inhibitory mechanisms.

Introduction

The neuronal mechanisms underlying the directional sensitivity of the equilibrium system of the crayfish *Procambarus clarkii* have been extensively studied (Takahata and Hisada, 1979, 1982*a*,*b*). Equilibrium responses are primarily controlled by a pair of specialized gravity-sensing organs, statocysts, that are located in the basal segments of the antennules. Sensory hairs are aligned in a crescent shape on the floor of the statocyst. The principles of operation of the statocyst were first described in the crayfish *Astacus fluviatilis* (Stein, 1975). In this species, all the hairs are polarized structurally towards the centre of the crescent.

Key words: crayfish, directional sensitivity, equilibrium system.

Any tilt of the animal was thought to result in a specific pattern of deflection of the hairs distributed along the sensory crescent. Eye movements in response to selective stimulation of the statocyst hairs were observed, and showed indirectly that the relative magnitude of the excitation from a hair depended on the degree of coincidence between its polarization direction and the direction of shear, being maximal if both directions corresponded. In *Procambarus clarkii*, it was shown by extracellular recording from the statocyst nerve that the sensory neurones attached to the hairs had the same directional sensitivity, with maximal excitatory responses to hair deflection towards the centre of the crescent and maximal suppression for the opposite direction. Because of the crescent arrangement of the planes of polarization, the direction of hair deflection that elicited an excitatory response in the receptor was observed to vary with the location of the hair. Thus, it was suggested that a body tilt around a particular body axis would produce a regional distribution of excitation and suppression of receptors around the crescent (Takahata and Hisada, 1979). In each circumoesophageal connective of the crayfish, several statocyst interneurones have been found that receive input only from receptors located in a specific region of the sensory crescent and which show the same directional sensitivity (Takahata and Hisada, 1982*a*,*b*; Nakagawa and Hisada, 1989). These studies have suggested that the directional tuning of these statocyst interneurones is based on a selective connection onto them from a crescent-like distribution of polarized sensory hairs.

Selective connections to directionally arranged receptors have been shown to account for directionality of interneurones in other systems (e.g. giant interneurones of the cockroach, Westin *et al.* 1977; Walthall and Hartman, 1981). Different mechanisms of directional sensitivity have also been reported in other systems. In the crayfish telson, many hairs are dually innervated, with one afferent responding to headward bending and the other to tailward bending (Wiese, 1976). Some interneurones receive not only excitatory input from receptors with the same directional sensitivity but also indirect inhibitory input from ones with the opposite sensitivity. This 'null-phase inhibition' (Wiese *et al.* 1976) sharpens the system's directional sensitivity. Central inhibitory interactions have also been reported to sharpen directional sensitivity in other vertebrate and invertebrate sensory systems (Precht, 1974; Edwards and Palka, 1974; Palka and Olberg, 1977; Matsumoto and Murphey, 1977; Levine and Murphey, 1980; Reichert *et al.* 1983). In this paper we show, on the basis of intracellular recording in the brain, that the directional sensitivity of the crayfish equilibrium system is attained by selective excitatory connections and is enhanced by such inhibitory mechanisms.

Materials and methods

Adult crayfish, *Procambarus clarkii* Girard, of either sex, measuring 8.5–10.5 cm in length, were used. The preparations were essentially the same as those in a previous report (Nakagawa and Hisada, 1989). However, to facilitate intracellular microelectrode access to the brain, the animal was placed head-down

in a chamber filled with crayfish saline (van Harreveld, 1936). The antennules were kept level in order to deliver direct mechanical stimulation to the statocyst hairs. Small groups of sensory hairs in the lateral region of the crescent were deflected towards or away from the centre by a miniature hook probe (Nakagawa and Hisada, 1989). To determine the effectiveness of the stimulation, we recorded the extracellular spike activity of both the optic nerve motor bundle and the circumoesophageal connective on the side contralateral to the stimulated statocyst. Glass microelectrodes filled with a 3% solution of Lucifer Yellow were inserted into the deutocerebrum on both sides. After the directional responses of the statocyst-related neurones had been examined intracellularly, the dye was injected into them ionophoretically.

Results and Discussion

Here, we will identify two possible additional mechanisms that contribute to the directional sensitivity of the crayfish equilibrium system. The first is the bi-directional sensitivity of some interneurones arising from excitation of some afferents in response to deflection of statocyst hairs away from the centre of the crescent (Fig. 1). The neurones were judged to be afferents on the basis of the following observations. (1) Their action potentials arose directly from the baseline. No underlying postsynaptic potentials were visible. (2) No cell bodies were stained in the brain. (3) The neurones appeared to enter the brain nearly at the entrance of the statocyst nerve. So far, we have obtained successful intracellular recordings from five primary afferents. Three of these showed a decrease in response to hair deflection towards the crescent centre and an increase in response to a deflection in the opposite direction. The remaining two showed the opposite directional responses, like those reported in a previous study (Takahata and Hisada, 1979). Each of these two groups contained afferents which projected ipsilaterally or bilaterally (Fig. 1). The structural mechanisms underlying this novel directionality of the afferents are not clear. Two possibilities exist. (1) As in the telson, the statocyst sensory hairs are dually innervated, with one of the afferents responding to inward, and the other to outward, deflection. (2) Some statocyst sensory hairs are functionally polarized away from the centre of the crescent and are innervated by afferents which respond to an outward deflection. In *Astacus* each hair was shown to possess three sensory neurones in an ultrastructural study (Schöne and Steinbrecht, 1968). Patton (1969) reported that two or three qualitatively different receptors were driven simultaneously by movements of the same hair in *Homarus americanus*. Two of these responded to lateral deflection of the hair from its rest position, while the third was excited when the hair was returned to rest from a lateral deflection. This would tend to support the first possibility. In contrast, in *Nephrops*, several rows of statolith hairs were found in the lateral regions of the crescent with different polarizations (Newland and Neil, 1987). Also, sensory hairs oriented in opposite directions have been found in vertebrate vestibular sensory areas (Flock, 1964; Spoendlin, 1964). These

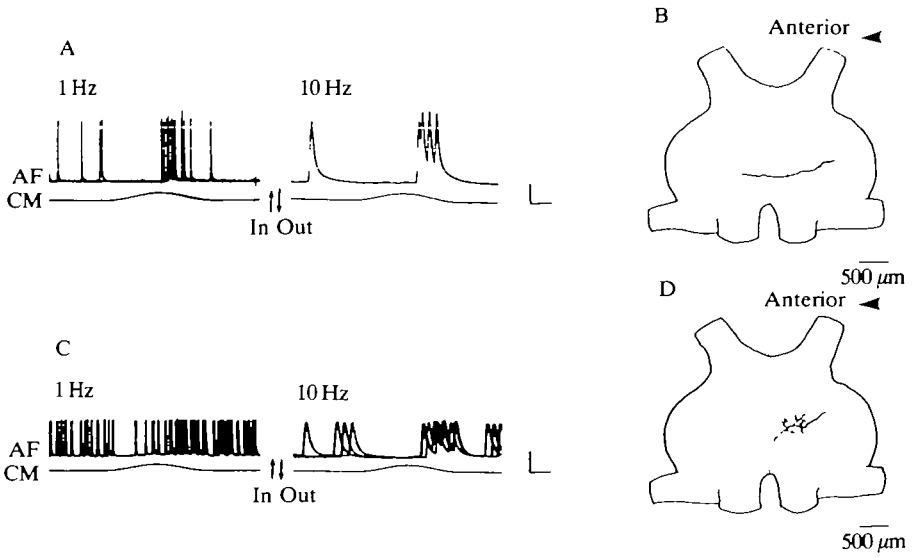

Fig. 1. Responses and structures of two statocyst afferents with the opposite directional sensitivity to that previously reported. (A) Responses of a bilateral type afferent to right statocyst sensory hair deflection. The left and right panels show the responses to stimulation at 1 and 10 Hz, respectively. The upper trace is the intracellular recording from the afferent (AF). The lower trace shows the current applied to the stimulating probe (CM). An upward excursion indicates an inward movement of the sensory hairs. The afferent shows an inhibitory response to a deflection towards the crescent centre and an excitatory response to a deflection in the opposite direction. (B) Morphology of the afferent from which the records shown in A were taken. (C) Responses of a unilateral type afferent to hair deflection. The experimental conditions and the presentation of data are the same as in A. This afferent also shows inhibition and excitation in response to inward and outward hair deflection, respectively. (D) Morphology of the afferent from which the records shown in C were taken. In A and C, calibrations, 10 mV, 0.2 s (left panels); 10 mV, 20 ms (right panels). B and D are viewed dorsally. The arrowheads indicate the side of statocyst stimulation. The anterior direction is towards the top.

observations provide precedents for the second possibility. A re-examination of the morphology of statocysts, including the inner hair row (Takahata and Hisada, 1979), in more detail is necessary to correlate this opposite directionality with statocyst morphology.

We have found six interneurones that are directionally sensitive and show opposite directional responses depending on the direction of hair deflection. Except for one projecting interneurone, all of them are local interneurones. Two of them were excited during inward deflection and actively inhibited by incoming IPSPs during outward deflection. The remaining four were actively inhibited during inward deflection and excited during outward deflection. Fig. 2 shows the morphology and typical responses of these interneurones. It should be noted that IPSPs or EPSPs could clearly be observed in response to outward deflection of the

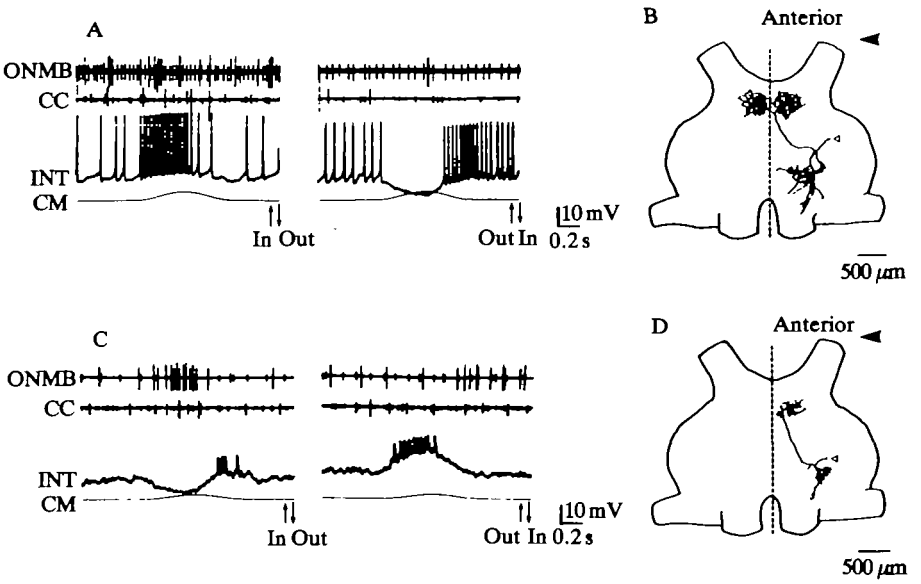

Fig. 2. Responses and structures of statocyst-related interneurones which show opposite directional responses depending on the direction of hair deflection. (A) Responses of a local interneurone in the right deutocerebrum to right statocyst sensory hair deflection. In both panels, from the top, the first trace is from the left optic nerve motor bundle (ONMB), the second is from the left circumoesophageal connective (CC), the third is an intracellular recording from the local interneurones (INT), and the lowest trace indicates the current applied to the stimulating probe (CM). In the left panel, an upward excursion indicates an inward movement of the sensory hairs, whereas, in the right panel, an upward excursion indicates outward movement of the sensory hairs. The interneurone is excited during inward deflection and actively inhibited during outward deflection. (B) Morphology of the local interneurone from which the records shown in A were taken. (C) The experimental conditions and presentation of data are the same as in A. The interneurone is actively inhibited during inward deflection and excited during outward deflection. (D) Morphology of the local interneurone from which the records shown in C were taken. B and D are viewed dorsally. The arrowheads indicate the side of statocyst stimulation. The open triangles show the position of the soma. Anterior is towards the top.

hairs. These potential changes cannot be attributed to the abolition of spontaneous afferent discharge. These findings suggest that: (1) statocyst sensory afferents are functionally endowed with two opposite directional sensitivities; (2) a given interneurone can be postsynaptic to both classes of afferents, but it is connected with opposite signs, with one afferent being excitatory and the other inhibitory; and (3) this convergence of the afferents of the two classes sharpens the directional sensitivity of the interneurone by the null-phase inhibition mechanism (Wiese *et al.* 1976) during hair deflection in the non-preferred direction.

The second mechanism is that several identified descending statocyst interneurones connect with opposite signs to the statocysts of the two sides. In previous

papers, we have shown that these interneurones receive their major input from only one of the statocysts (Takahata and Hisada, 1982*a*,*b*; Nakagawa and Hisada, 1989). In this study, however, some descending statocyst interneurones were found to receive excitatory input from one statocyst and inhibitory input from the other, which we once thought to be the non-input statocyst. This is evident in the response of interneurone S7. We have demonstrated previously that this interneurone receives excitatory input from the statocyst ipsilateral to its axon (Nakagawa and Hisada, 1989). However, since the background discharge of this interneurone is very low, an inhibitory input, even if it existed, would not have been detected by measuring changes in the spike frequency. However, intracellular recordings from neurites in the brain clearly demonstrate inhibition of the neurone. Fig. 3 shows the response of S7 to inward deflection of the statocyst hairs on the side contralateral to the axon. The spike discharge was suppressed during the stimulation. The inhibition became clear when the background discharge frequency was increased (about 20 spikes s^{-1}) by intracellular injection of current. Interneurones S6 and S3 also showed a similar inhibition in response to inward deflection of the hairs of the contralateral and ipsilateral statocyst, respectively.

Spatial information has been shown to be transmitted in two different modes; by the pattern of excitation of the maculae and by the overall amount of excitation (Schöne, 1975). With respect to the former, the crayfish equilibrium receptor system was thought to be of relatively simple construction. This was based largely on the crescent-like distribution of inward-preferring polarization of sensory hairs and selective excitatory connections between receptors in specific regions and particular interneurones (Takahata and Hisada, 1979, 1982*a*,*b*). However, in this

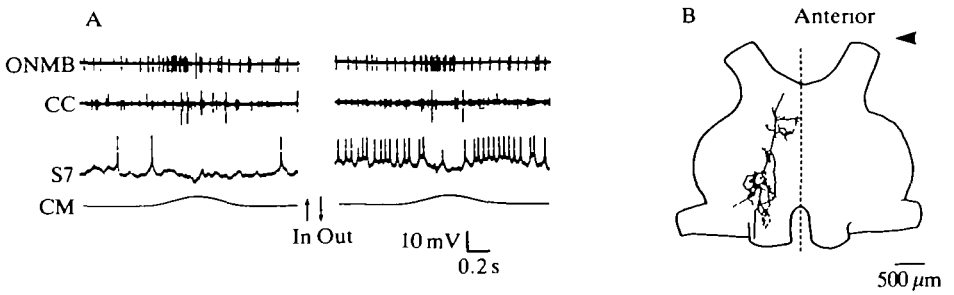

Fig. 3. Responses and structure of the identified descending statocyst interneurone S7. (A) Responses of S7 to contralateral statocyst sensory hair deflection at 1 Hz. In both panels, from the top, the first trace is from the left optic nerve motor bundle (ONMB), the second trace is from the left circumoesophageal connective (CC), the third trace is an intracellular recording from S7 in the left deutocerebrum (S7). The bottom trace indicates current applied to the stimulating probe (CM), with an upward excursion indicating inward movement of the sensory hairs. In the right panel, the spike discharge is increased by +1 nA intracellular current injection (about 20 spikes s^{-1}). The spike discharge of S7 is suppressed during inward hair deflection. (B) Morphology of S7 from which the records shown in A were taken. B is viewed dorsally. The arrowhead indicates the side of statocyst stimulation. Anterior is towards the top.

study, we have shown that additional mechanisms are involved in the equilibrium system. The first mechanism is the convergence of the two classes of afferents onto the interneurones that form the null-phase inhibitory connections. The second is the central inhibition of some identified descending statocyst interneurones derived from the differential connections from the statocysts of the two sides. Thus, several mechanisms which have been reported in other sensory systems also exist in the crayfish and serve to sharpen the directional sensitivity of the equilibrium system.

We are grateful to Dr H. Robinson for reading the manuscript and for constructive suggestions. This work was supported by Grants-in-aid from the Japanese Ministry of Education, Science and Culture to HN (no. 01790321) and to MH (nos 61480016 and 01480021).

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