M. Berón de Astrada · J. Sztarker · D. Tomsic

Visual interneurons of the crab *Chasmagnathus* studied by intracellular recordings in vivo

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Abstract Comparative physiology of visual systems has become an important field of investigation. However, despite the fact that Crustacea represents a major phylogenetic group, research on the physiology of vision of these animals is scant and almost limited to the crayfish. We developed a preparation to study in vivo the visual nervous system of a semiterrestrial crab through intracellular recordings. The response to a pulse of light was investigated in 206 interneurons from 38 animals. Seventy-eight of these neurons could be classified by functional criteria as sustaining cells, dimming cells, nonspiking hyperpolarizing cells and nonspiking depolarizing cells. Quantitative description is provided for the first two groups and qualitative description is given for the last two. The remaining neurons presented a broad range of different types of phasic responses to light. Although semiterrestrial crabs are behaviorally more reactive to visual stimuli than the crayfish, the general physiological properties of identified lamina and medullary neurons of *Chasmagnathus* resemble those of the crayfish. The results described here represent the first attempt to study the visual system of crabs by intracellular recordings and constitute the beginning of a project aimed to investigate the neuronal functions underlying behavioral responses elicited by visual stimuli.

Key words Visual interneurons · Crustacea · In vivo intracellular recording

Abbreviations DN dimming neuron · EPSP excitatory postsynaptic potential · IPSP inhibitory postsynaptic potential · SN sustaining neuron

Introduction

The degree of evolutive convergence of elements of the visual systems among phylogenetically distant species as diverse as insects and mammals is receiving great attention (Osorio and Bacon 1994; Somlyo and Walz 1995; Rind and Simmons 1999). It has been found that the sensitivity to a moving bar of specific neurons in the lobula of insects is remarkably similar to that of neurons of the visual cortex of mammals, suggesting that evolutionary convergence in visual processing is not limited to early pathways (O’Carroll 1993). Insects, like mammals, seem to possess mechanisms for extracting spatial features from visual scenes (Srinivasan et al. 1993; Giurfa et al. 1996). However, in spite of the general interest in the comparative physiology of vision (Weckstrom and Laughlin 1995), investigation of visual processing is mainly performed in vertebrates and insects. In Crustacea, with the exception of Wiersma’s pioneering work on visual processing in several different species (e.g., Waterman et al. 1964; Wiersma 1966; Wiersma and Yamaguchi 1967a, 1967b; York and Wiersma 1975; Wiersma et al. 1982), the research is scarce and has been limited almost exclusively to the crayfish (e.g., Sandeman et al. 1988, 1995; Glantz 1994, 1996).

The visual system of higher decapods usually consists of five optic ganglia contained in the eyestalk, which are connected to the supraesophageal ganglion by the protocerebral tract (Sandeman et al. 1992). Recording extracellularly from the optic nerve of different species, Wiersma and coworkers identified and studied three main groups of visual neurons: sustaining (SNs), dimming (DNs) and moving-sensitive neurons (MSNs). Unfortunately, extracellular recordings provide limited information other than changes in the spike activity of individual neurons. Besides, no information can be obtained extracellularly from nonspiking interneurons. To investigate the biophysical and synaptic events taking place in individual neurons, intracellular recordings are required. Information from intracellular recordings has

M. Berón de Astrada · J. Sztarker · D. Tomsic (✉)
Laboratorio de Neurobiología de la Memoria,
Dep. Biología, Universidad de Buenos Aires,
Pabellón 2 Ciudad Universitaria (1428),
Buenos Aires, Argentina
E-mail: tomsic@bg.fcen.uba.ar
Fax: +54-1-145-763321
been attained for SNs and DN s (Kirk 1982; Kirk et al. 1982, 1983) as well as for nonspiking interneurons like amacrine, lamina monopolar and tangential cells in the crayfish only (Waldrop and Glantz 1985; Wang-Bennett and Glantz 1987a, 1987b; Pfeiffer and Glantz 1989; Pfeiffer-Linn and Glantz 1991). Nevertheless, from even rough observations on the behavioral reactions of different decapods, there can be no doubt that visual cues play quite different roles in their reactions (Wiersma, 1966; Wiersma and Yamaguchi 1967a). This difference may explain why, contrasting with the absence of physiological studies, behavioral studies on vision have been far more extensively performed in crabs than in crayfish (e.g., Horridge 1966a, 1966b; Sandeman 1978a, 1987b; Barnes and Na lbach 1993; Tomsic et al. 1993, 1996, 1998a, 1988b; Land and Layne 1995; Blanke et al. 1997; Layne 1998). In addition, crabs have conquered a wide variety of ecological niches for which they possess particular adaptations of their visual system (Zeil et al. 1986; Nalbach and Nalbach 1987), and constitute a highly radiative group that provides rich material for the study of ecological adaptations (Osorio and Bacon 1994).

For more than a decade we have been studying a visual learning process in the semiterrestrial crab Chasmagnathus granulatus. Briefly, upon a sudden presentation of a visually dangerous stimulus, i.e., an object moving overhead, the crab displays an escape response. However, following repetitive presentation of the stimulus the response decline and remains reduced for several days (Lozada et al. 1990; Pedreira et al. 1995; Tomsic et al. 1993, 1996, 1998a). This memory process has been investigated with behavioral, pharmacological and molecular approaches (for reviews see Maldonado et al. 1997; Tomsic et al. 1998b). The present paper is the beginning of a project aimed to investigate the neural processes underlying the responses of Chasmagnathus to visual stimuli with electrophysiological techniques. In this paper we introduce a new preparation developed to perform stable intracellular recordings in an intact and awaken living animal, and present the first description of the general physiological properties of visual interneurons of a crab.

**Materials and methods**

**Animals**

Animals were adult male *C. granulatus* crabs 2.7–2.9 cm across the carapace, collected from water less than 1 m deep in the *rias* (narrow coastal inlets) of San Clemente del Tuyu, Argentina, and transported to the laboratory, where they were lodged in plastic tanks (35 cm × 48 cm × 27 cm) filled to 0.5 cm depth with water. Water used in tanks and other containers during experiments was prepared with lw-Marine (Winex-Germany), salinity 1.0–1.4%, pH 7.4–7.6. The holding room was maintained on a 12 h light-dark cycle (light on 0700–1900 hours). Animals were fed rabbit pellets (Nutrients, Argentina) every 3 days and after feeding the water was changed. Temperature of both holding and experimental rooms as well as the alley between them was maintained within a range of 20–24°C. Experiments were carried out mostly at daytime and within the first two weeks after the animal’s arrival.

In most crustaceans, the optic lobes contain the retina and five neuropils, classically called lamina, external medulla, internal medulla, terminal medulla, and hemiellipsoid body. The first three neuropils lying behind the retina (lamina, external medulla and internal medulla) are considered by Strausfeld and Nässel (1981) to be clearly homologous with the lamina, medulla and lobula of the insects, and they suggest that these terms be used as well for the crustacean neuropils. They use the term “lateral protocerebrum” to refer to the terminal medulla and hemiellipsoid body complex. The older terminology is, however, firmly entrenched in the literature, and to avoid confusion we have adhered to it in referring to the five neuropils (Sandeman et al. 1988).

**Physiological preparation**

A new preparation was developed to perform stable intracellular recordings in vivo. Briefly, the crab was firmly held by its body sides using an adjustable clamp, allowing free movements of the walking legs but reducing movements of the chelae (Fig. 1). The eyestalks were cemented to the carapace at an angle of 70°–80° from the horizontal line, which corresponds to their normal seeing position. The ommatidia are not equally distributed around the equator of the eye and, in particular, the area looking towards the medial part of the animal does not possess facets. Instead, this side is covered by a projection of the eyestalk cuticle, that ends in a rather spherical peninsula covering less than one third of the dorsal surface of the eye (Fig. 1). A tangential cut performed with a sharp scalpel allowed to remove this small piece of thin cuticle (about 500 μm in diameter) located at the tip of the eyestalk, without causing damage to the ommatidial area. Once prepared in this way,