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Original Research Paper

Organization of columnar inputs in the third optic ganglion of a highly visual crab

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ABSTRACT

Motion information provides essential cues for a wide variety of animal behaviors such as mate, prey, or predator detection. In decapod crustaceans and pterygote insects, visual codification of object motion is associated with visual processing in the third optic neuropile, the lobula. In this neuropile, tangential neurons collect motion information from small field columnar neurons and relay it to the midbrain where behavioral responses would be finally shaped. In highly ordered structures, detailed knowledge of the neuroanatomy can give insight into their function. In spite of the relevance of the lobula in processing motion information, studies on the neuroarchitecture of this neuropile are scant. Here, by applying dextran-conjugated dyes in the second optic neuropile (the medulla) of the crab *Neohelice*, we mass stained the columnar neurons that convey visual information into the lobula. We found that the arborizations of these afferent columnar neurons lie at four main lobula depths. A detailed examination of serial optical sections of the lobula revealed that these input strata are composed of different number of substrata and that the strata are thicker in the centre of the neuropile. Finally, by staining the different lobula layers composed of tangential processes we combined the present characterization of lobula input strata with the previous characterization of the neuroarchitecture of the crab's lobula based on reduced-silver preparations. We found that the third lobula input stratum overlaps with the dendrites of lobula giant tangential neurons. This suggests that columnar neurons projecting from the medulla can directly provide visual input to the crab's lobula giant neurons.

1. Introduction

Many crustacean decapods are highly visuals animals that exhibit elaborated visual guided behaviors. Some of these involve visuomotor control, navigation, conspecific and predator recognition, and social communication (Backwell et al., 2000; Christy, 1988a,b; Land and Layne, 1995a,b; Woodbury, 1986). The decapod visual system is composed of a compound eye and three successive optic neuropiles which are contained within the eyestalk: the lamina, the medulla and the lobula (Strausfeld and Nässel, 1980). These neuropiles are organized into retinotopically ordered columnar units intersected by orthogonal tangential strata. Columnar units mainly consist of columnar neurons, while tangential strata comprise lateral processes of columnar neurons, amacrine cells and neurites from tangential cells. Inside of each columnar unit there are different cell types supposed to code parallel channels of visual information (e.g. motion, colour, e-vector polarization). These columnar elements feed different wide field tangential neurons which convey visual information to the midbrain to finally guide specific behaviors (Glantz and McIsaac, 1998; Haag et al., 2010; Paulk et al., 2008; Tomsic et al., 2003).

In decapods and insects, the third optic neuropile, the lobula, is a key neuropile in the processing of motion information (Berón de Astrada and Tomsic, 2002; Glantz and Schroeter, 2006; Horseman et al., 2011; O'Shea and Williams, 1974; Paulk et al., 2008). In decapods, physiological studies in the crab *Neohelice granulata* identified four types of wide field motion sensitive neurons whose dendrites extend tangentially along the lobula and relay motion information to downstream areas of the nervous system (Berón de Astrada and Tomsic, 2002; Medan et al., 2007). These neurons, collectively called lobula giant (LG) neurons respond to focal motion stimuli with strong discharges of action potentials that are superimposed on noisy graded potentials (Berón de Astrada and Tomsic, 2002; Medan et al., 2007). LG neurons present strong responses to the same motion stimuli that evoke animal escape

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behaviors. The response strength of LG neurons tightly correlates with the intensity of the escape across a wide range of experimental conditions. Briefly, LGs activity anticipates the animal escape response by approximately 120 ms; the time courses of the neural responses evoked by different types of visual stimuli reflect the temporal dynamics of the escape; and the neural response also reflects escape behavior modifications that are induced by different types of training and the resultant short- and long-term memory (Oliva et al., 2007; Sztarker and Tomsic, 2008, 2011; Tomsic et al., 2003).

Even though the lobula of decapods has been shown to be a centre of high-level visual integration, there are few studies exploring its neuroarchitecture (Strausfeld and Nässel, 1980; Sztarker et al., 2005). These studies were performed in two groups of animals: crayfishes and crabs. In both groups the lobula is a kidney-shaped neuropile which comprises several tangential strata. According to the position of the arborizations of lobula columnar neurons, the crayfish lobula has been divided in seven main strata (Strausfeld and Nässel, 1980). Three of these strata receive retinotopic inputs from the medulla while the other four strata are defined by the arborizations of columnar neurons that connect the lobula with downstream visual centres. Alternatively the crab's lobula has been classified according to the main orientation of the tangential processes. Based on reduced silver preparations of crab optic ganglia, Sztarker et al. (2005) noted that the pattern of fiber staining in longitudinal sections appears different from that observed in transverse sections. This is because tangential processes extending across columnar neurons run either along the anteroposterior or the lateromedial axis of the neuropile. Thus, the authors defined four layers composed by lateromedial tangential processes (LMT1-4) and five layers composed of anteroposterior tangential processes (APT1-5).

Recently, we have developed a preparation for studying the population calcium response of columnar elements of crab's optic ganglia (Berón de Astrada et al., 2013). By applying dextran-conjugated calcium indicators in the medulla we stained the medulla columnar neurons that project into the lobula and studied the physiology of lobula columnar elements. However, a detailed morphological characterization of the lobula input strata of crabs has been lacking. Thus, in the present study we employed mass stainings to morphologically characterize the lobula input strata and to allocate these strata in relation to the layers composed of tangential processes (Sztarker et al., 2005). We first show that mass staining with dextran-conjugated fluorescent indicators effectively allows us to identify the principal input elements from the two best known optic neuropiles of decapoda, the lamina and the medulla. Then, we characterize the input strata of the less explored lobula neuropile. We found that in the crab, there are four lobula input strata; that these strata are composed of different number of substrata; and that the thickness of the strata encompasses the shape of the lobula. Finally, by staining the lobula layers composed by lateromedial tangential processes (LMT layers), and studying the autofluorescence profile of the neuropile, we allocate the lobula input strata in relation to the layers composed of tangential processes.

2. Material and methods

2.1. Animals

Animals were adult male *Neohelice granulata* (=*Chasmagnathus granulatus*) crabs measuring 27–30 mm between the lateral carapace spines (Fig. 1A). They were collected from water less than 1 m deep in the rias (narrow coastal inlets) of San Clemente del Tuyú, Argentina, and transported to the laboratory where they were lodged in plastic tanks (35 × 48 × 27 cm) filled to 0.5 cm depth with artificial seawater (salinity 1.0–1.4%, pH 7.4–7.6; Pedreira et al., 1998). The holding room was maintained at 20–24 °C on a 12 h light: dark cycle.

2.2. Dye staining

Mass staining with dextran coupled to fluorescent molecules was performed using dextran-Alexa Fluor 488 or dextran-Texas Red (3000 MW, Molecular Probes). Dextran-conjugated fluorescent indicators are commonly applied to neural pathways where the fluorescent dextrans are taken up by neurons and transported in an anterograde and retrograde direction (Grienberger and Konnerth, 2012; Utting et al., 2000). Thus, to stain different neuronal pathways of the crab's visual system a fine glass micropipette coated with crystals of dextran-conjugated dye was gently inserted into the retina or the optic neuropiles (Fig. 1B; Gelperin and Flores, 1997).

To apply the dye into the retina, the crab was held in an adjustable clamp and placed on ice under a dissecting microscope. The glass micropipette coated with dextrans was inserted through the cornea into the retina. The probe was gently rotated and then removed after 5–10 s, leaving a spot of dye behind. To apply the dextran into the optic neuropiles, the left eyestalk was fixed in horizontal position. A small piece of cuticle was removed and the optic neuropiles were exposed in order to apply the dextran in the neuropile of interest. After depositing the dye, the piece of cuticle removed from the eyestalk was put back in place and cemented with calcium hydroxide.

Twenty four hours after the dye application, animals were cold anaesthetized and the optic lobe was dissected and fixed overnight (4% paraformaldehyde in phosphate buffer 0.1 M, pH 7.2). After five 20-min washes with phosphate buffer, the tissue was dehydrated in ethanol series and cleared in methyl salicylate. In some preparations, the staining was performed with the two fluorescent conjugated dyes but we observed no difference in the neuronal elements stained.

2.3. Images

The optic lobes were imaged as whole mounts in a slide which has a side to side perforation that enables to image the tissue from a posterior and an anterior view. At one side, a coverslip was glued with transparent varnish. The optic lobe was placed in the resultant chamber. The preparation was covered with another coverslip and scanned with a confocal microscope (Olympus, Fluoview FV 1000 or FV 300-Lasers Ar488 nm; HeNe green 543 nm). Optical slices were acquired at 1–5 μm intervals. The illustrations presented here are *z* projections of the three-dimensional stacks adjusted for brightness and contrast.

2.4. Coordinates of the optic lobe and planes of optical sectioning

Neohelice has mobile eyestalks normally oriented at an angle of 50° from the horizontal plane (Fig. 1A and B). Such orientation complicates the definition of a reference system. Thus, for the sake of simplicity, we refer to a coordinate system in which the eyestalk is oriented at 90° from the horizontal plane (Berón de Astrada et al., 2011). Transverse sections were obtained by performing the optical sectioning along the long axis of the eyestalk and along the lateromedial axis of the animal. Longitudinal sections were obtained by performing sections along the long axis of the eyestalk and along the anteroposterior axis of the animal.