

REVIEW

The predator and prey behaviors of crabs: from ecology to neural adaptations

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ABSTRACT

Predator avoidance and prey capture are among the most vital of animal behaviors. They require fast reactions controlled by comparatively straightforward neural circuits often containing giant neurons, which facilitates their study with electrophysiological techniques. Naturally occurring avoidance behaviors, in particular, can be easily and reliably evoked in the laboratory, enabling their neurophysiological investigation. Studies in the laboratory alone, however, can lead to a biased interpretation of an animal's behavior in its natural environment. In this Review, we describe current knowledge – acquired through both laboratory and field studies – on the visually guided escape behavior of the crab *Neohelice granulata*. Analyses of the behavioral responses to visual stimuli in the laboratory have revealed the main characteristics of the crab's performance, such as the continuous regulation of the speed and direction of the escape run, or the enduring changes in the strength of escape induced by learning and memory. This work, in combination with neuroanatomical and electrophysiological studies, has allowed the identification of various giant neurons, the activity of which reflects most essential aspects of the crabs' avoidance performance. In addition, behavioral analyses performed in the natural environment reveal a more complex picture: crabs make use of much more information than is usually available in laboratory studies. Moreover, field studies have led to the discovery of a robust visually guided chasing behavior in *Neohelice*. Here, we describe similarities and differences in the results obtained between the field and the laboratory, discuss the sources of any differences and highlight the importance of combining the two approaches.

KEY WORDS: Electrophysiology, Escape, Insects, Looming, Neurobiology, Neurons

Introduction

Escaping from a sudden predator attack or chasing fast-moving prey both require short reaction times. The neural circuits underlying these behaviors therefore have to be straightforward and fast. In fact, as Monk and Pauling (2004) pointed out, 'animals evolved spiking neurons soon after they started eating each other. The first sensory neurons could have been threshold detectors that spiked in response to other animals in their proximity, alerting them to perform precisely timed actions, such as striking or fleeing'. Given their

biological importance, escape behaviors in particular can be easily elicited in the laboratory. This, and the relative simplicity of the underlying circuits operating through giant neurons (see Glossary) to speed up neuronal communication, have made escape responses one of the most traceable behaviors at the neuronal level (Herberholz and Marquart, 2012; Peek and Card, 2016).

In crustaceans, neurophysiological studies on escape behaviors have been carried out mainly in two species, the red swamp crayfish *Procambarus clarkii* and the semiterrestrial crab *Neohelice* (previously *Chasmagnathus*) *granulata*. While studies with crayfish focused on mechanically elicited responses (for a review, see Krasne et al., 2014), investigations with the crab focused on visually elicited responses. A recent review on studies in *Neohelice* (Tomsic, 2016) focused on knowledge of the motion-sensitive giant neurons involved in guiding the crab's escape performance in response to visual stimuli. In the present Review, we compare the neurobiological knowledge acquired from laboratory studies with current understanding of the crab's visual behaviors obtained from studies in the field.

Research on crustaceans as experimental models has contributed to neurobiology topics as diverse as the identification of the major inhibitory neurotransmitter gamma-aminobutyric acid (Kravitz et al., 1962; Edwards et al., 1999a), electrical synaptic transmission (Furshpan and Potter, 1959), principles of neuronal computation (Edwards et al., 1999b), neuromodulatory effects resulting from social experience (Yeh et al., 1996), decision-making processes (Herberholz and Maquart, 2012) and the mechanisms of memory consolidation (Pedreira and Maldonado, 2003; Tomsic and Romano, 2013). These contributions illustrate the amenability of decapod crustaceans for neurophysiological research. Among the advantages offered by these animals are their robustness for experimental manipulations and the presence of central giant neurons from which researchers can make electrophysiological recordings in the intact animal.

Here, we begin by describing the characteristics of crab escape behaviors in the wild and in the laboratory. Then, we outline the visual system of crustaceans and provide a brief description of a variety of motion-sensitive neurons that play a central role in visually guided behaviors (reviewed in Tomsic, 2016). We show that escape behaviors are highly flexible and can be enduringly modified through plastic changes occurring in identified neurons of the optic lobe. We also discuss a newly discovered prey capture behavior of crabs. Finally, through a couple of examples, we highlight the importance of combining field and laboratory experimentation for understanding the neural control of behavior.

Visually elicited avoidance responses of crabs in the natural environment

Neohelice granulata is a robust running grapsid crab, reaching 36 mm across the carapace. Like fiddler crabs, for which the behavioral aspects of predator avoidance have been extensively

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Glossary

Apparent size

The size of an object measured in terms of the angle it subtends on the observer's retina.

Angular velocity of a looming stimulus

The velocity of the image expansion on the observer's retina, measured as the angular velocity of the moving borders.

Giant neuron

A neuron that is comparatively much larger than the other neurons in the tissue. Because neuronal size is one of the factors that increases the velocity of electrical transmission, circuits underlying behaviors that need to be rapidly executed, such as avoidance responses, often include giant neurons. This is particularly observed in invertebrates and lower vertebrates.

Looming stimulus

Two-dimensional representation of a directly approaching object. The dynamic of the image expansion is determined by the size, distance and velocity of the approaching object.

Neuropil

Any area in the nervous system that forms a synaptically dense region composed of mostly dendrites and axons arriving from the somata in the periphery. Retinotopic neuropils are those that hold an orderly representation (a map) of the visual input positions from the retina.

Ommatidia

The single units that comprise the compound eye of arthropods like insects and crustaceans. An ommatidium contains the cornea, a lens and a cluster of 6–9 photoreceptor cells surrounded by support cells and pigment cells. The axon of the photoreceptors projects to the optic neuropils. A compound eye is usually composed of many thousand ommatidia.

Optic ganglia

The optic ganglia of arthropods consist of a number of brain regions that are primarily, although not exclusively, dedicated to the processing of visual information. These include the lamina, the medulla, the lobula and the lobula plate neuropils.

Protocerebral tract

A neural tract composed of many axon bundles that connect the optic ganglia and the lateral protocerebrum (in combination sometimes referred as the lateral brain) with the supraesophageal ganglion (sometimes referred to as the midbrain). In crustaceans, the lateral brain is located near the tip of the eyestalks and, therefore, the protocerebral tract can be very long.

Supraesophageal ganglion

Typically referred to as the brain, this consists of a number of distinct but highly interconnected specialized areas.

investigated in the field, *Neohelice* crabs live in a mud flat environment and form dense populations (Fig. 1A, Fig. 2A). They spend long periods of time out of the water, where they are preyed upon by gulls and other birds (Fig. 1B,D). Similar to fiddler crabs (Hemmi, 2005), *Neohelice* crabs protect themselves from predators by digging individual burrows (Fig. 1A,C) to which they run and where they will hide if they assess a risk to be great enough (Fathala and Maldonado, 2011). Visually elicited escape behavior has been extensively studied in fiddler crabs in the field, by using moving dummies to simulate predator stimuli (e.g. Hemmi, 2005; Smolka et al., 2013). After extensive studies in the laboratory (see below), field studies began to be performed on *Neohelice* using the same approach (Fig. 2A). The two species were found to respond to the dummy predator in a similar manner; both react to an approaching object when its apparent size (see Glossary) is less than 2 deg, such that it is perceived by one or two ommatidia (see Glossary; Hemmi and Pfeil, 2010; Hemmi and Tomsic, 2015). Upon perceiving an

approaching object, the crab's first observable response is to freeze, a strategy that is likely adopted to increase the crab's chances of remaining undetected by the predator, and which also helps to stabilize the image and improve visual information (Hemmi and Tomsic, 2012). However, if the risk to the crab increases because the predator continues to approach, a second strategy consisting of a 'home run' is initiated. This run ends at the burrow entrance from where, if the level of risk continues to increase, the crab will retreat into its burrow (Hemmi, 2005; Hemmi and Pfeil, 2010; Fathala and Maldonado, 2011; Hemmi and Tomsic, 2015). *Neohelice* can also be found wandering in areas without burrows. In the absence of a burrow, the behavioral response to an increasing visual risk is to first freeze, followed by running directly away from the threat and, ultimately, raising the claws and pointing them towards the menace (Fig. 1B).

Visually elicited avoidance responses of crabs in the laboratory

The escape response of crabs can be readily elicited and precisely measured in the laboratory using computer-generated visual stimuli and a simple walking simulator device (Fig. 2Bi). This treadmill-like device consists of a floating Styrofoam ball, which can be freely rotated by the locomoting crab. The path and speed of locomotion are accurately reconstructed over time by recording the rotation of the ball with two optical mice (Oliva et al., 2007). An array of five monitor screens surrounding the animal is used to deliver a wide variety of computer-generated stimuli to different regions of the crab's visual field. The combination of well-controlled visual stimuli and precisely measured responses allows the investigation of different aspects of visually guided behaviors, such as the differential sensitivity across areas of the visual field, the control of the direction and speed of responses, and the processes for decision making based on visual information. These and other characteristics of visually elicited responses have been investigated in the crab *Neohelice*.

Experiments where crabs were challenged with looming stimuli (see Glossary) demonstrated that the escape response is continuously adjusted according to the visual information obtained by the animal. For example, crabs run directly away from a looming stimulus (Fig. 2Bii), but immediately decelerate whenever the object stops approaching (Fig. 2Biii). Moreover, when confronted with looming stimuli representing objects approaching at different velocities (hence with different dynamics of image expansion), crabs regulate their running speed as a function of the velocity of the image expansion. In fact, we found that crabs regulate their speed of escape by computing the angular velocity of looming stimuli (see Glossary; Oliva and Tomsic, 2012) through a set of well-identified brain neurons (see below).

In addition to regulating the speed of escape, crabs also correct their escape direction according to changes in the observed trajectory of the danger. A crab may move away from a threat in two ways: it can keep the same orientation of its body in space but change the course of locomotion; alternatively, it can rotate and visually fixate the predator with the lateral pole of one of the eyes (the part of the eye with maximal optical resolution; Berón de Astrada et al., 2012), and then use its preferred sideways style of running to escape from the danger (Land and Layne, 1995). The sensitivity with which *Neohelice* adjusts its escape direction has been recently analyzed with the walking simulator using translating stimuli (i.e. stimuli moving tangentially to the observer). Under this condition, the crab immediately corrects its running direction following changes in the position of the stimulus of less than 1 deg (Medan et al., 2015). These



Fig. 1. Natural environment of the crab *Neohelice granulata*. (A,C) Crabs inhabit the mudflat environment, forming dense populations. The crab's activity is centered on its burrow (arrow). (B,D) Images of gulls attacking and eating *Neohelice* crabs. Note in B the raised-claw defensive display of the crab. Photos in B and D are courtesy of Lito Montserrat Ferrer.

results demonstrate that while escaping from a visual danger, crabs constantly adjust the speed and direction of the run according to ongoing changes in the flow of visual information.

The visual system and motion detection of crabs

Crabs have two compound eyes located at the tip of movable eyestalks (Fig. 3A,B). The ommatidia are spherically distributed, except for a narrow band of cuticle on the medial side of the eyestalk. This band, however, does not impose a blind spot, because at its borders the ommatidia are oriented in a way that covers the medial visual space. Therefore, crabs possess monocular views of 360 deg (Zeil and Al-Mutari, 1996; Smolka and Hemmi, 2009). The eye of *Neohelice* is composed of about 9000 ommatidia, and presents a rim of maximal optical resolution around the eye's equator and in the lateral part of the eye (Berón de Astrada et al., 2012). The visual nervous systems of decapod crustaceans and insects are thought to be homologous (Sinakevitch et al., 2003; Sztarker et al., 2005, 2009; Ma et al., 2012; Sombke and Harzsch, 2015), containing the retina and a series of nested retinotopic neuropils (see Glossary) that, from periphery to center, are the lamina, the medulla and the lobula complex, which includes the lobula and the lobula plate (Fig. 3C,D). These neuropils are organized in vertical columns that correspond to the ommatidial array, so that each column brings information from a particular part of the visual field (Sztarker et al., 2005; Berón de Astrada et al., 2013). At the level of the medulla, each column in *Neohelice* has been estimated to contain about 50 different classes of transmedullary neurons (Sztarker and Tomsic, 2014). Visual information is processed while flowing centripetally through the columnar neurons, and it is collected by relatively few extensive tangential neurons whose receptive fields encompass large portions of visual space (Fig. 3E). Some of these neurons project their axon through the protocerebral tract (see Glossary) towards the supraesophageal and the contralateral optic ganglia (see Glossary).

Decades ago Wiersma and collaborators, recording extracellularly from fibers in the protocerebral tract of a variety of decapod species, described several classes of motion-sensitive elements (reviewed in Glantz and Miller, 2002; Glantz, 2014). More

recently, intracellular recording followed by neuronal staining revealed that those motion-sensitive fibers correspond to the axons of lobula giant (LG) neurons (Berón de Astrada and Tomsic, 2002). So far, four different classes of motion-sensitive LG neurons have been described in *Neohelice* (Fig. 3E); these different neuron classes have significant similarities as well as important differences (Sztarker et al., 2005; Medan et al., 2007; for a recent review, see Tomsic, 2016). Certain aspects of these four classes of LG neurons are discussed below.

LG neurons and the escape behavior

Two classes of LG neurons, named monostratified lobula giant 1 and 2 (MLG1 and MLG2, respectively), are thought to be central elements involved in the continuous regulation of the run speed and direction when the crab escapes from a visual danger. Both neuron types respond to looming stimuli by increasing their firing rate according to the dynamics of the image expansion (Fig. 3F), and in a manner that closely corresponds with running speed (Oliva et al., 2007). The MLG1 neurons form an ensemble of 16 elements distributed over the lateromedial lobula axis (the axis that maps the 360 deg azimuthal positions of visual space; Berón de Astrada et al., 2011). There are more MLG1 units dedicated to cover the lateral visual field, i.e. the area of maximal optical resolution, which is used by the animal to fixate moving objects. Morphological as well as physiological measurements show that the receptive fields of neighboring MLG1 elements overlap extensively, suggesting that the information on object position is encoded as a population vector (Medan et al., 2015). Thus, with its elements having receptive fields oriented toward different azimuthal positions, the MLG1 system is perfectly suited to encode and convey information on the positions of objects, which is required to escape directly away from a visual threat.

Interestingly, the vertical receptive field center of the MLG1 elements is at the level of the eye's equator, coinciding with the horizontal rim of maximal optical resolution observed in the ommatidial array. Because crabs align the eye's equator with the horizon (Zeil, 1990), the acute visual rim and the vertical receptive field center of the MLG1 neurons are specialized for the

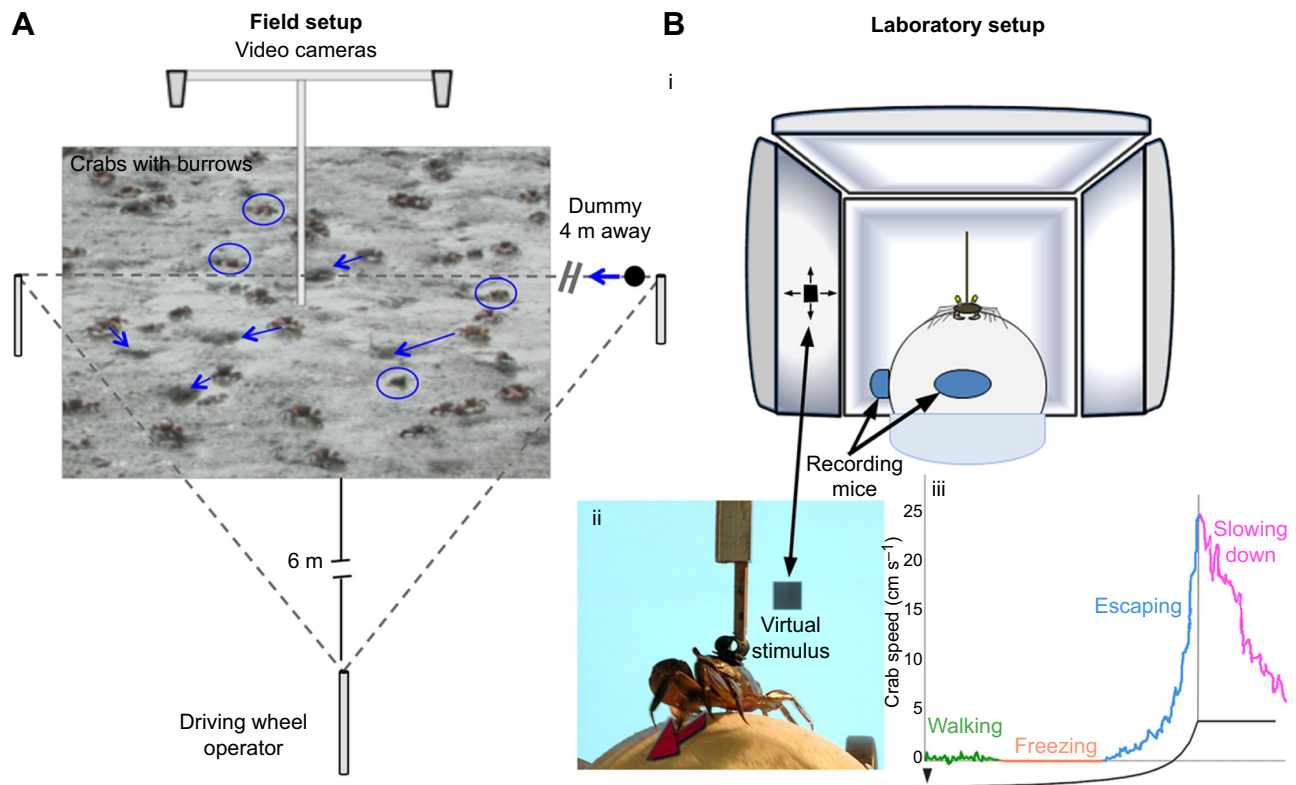


Fig. 2. Field and laboratory setups for analyzing visually elicited behaviors. (A) Experiments in the field are performed with the method developed by Hemmi (2005). Briefly, it consists of recording the response of crabs to a dummy predator attached to a fishing line that moves overhead (note, the camera field of view of the crabs and burrows is not to scale). The line with the dummy is pulled by a driving wheel from the operator position. In this context, crabs invariably escape towards their burrows (blue arrows), and those that are already at the burrow entrance (blue circles) may descend into it. (B) Experiments in the laboratory are mostly performed using computer-generated visual stimuli, which can be displayed simultaneously or separately on five flat-screen monitors located 20 cm above and around the animal. The locomotor activity is studied using a walking simulator device consisting of a water-supported styrofoam ball that can be freely rotated by the animal. The rotations of the ball are recorded by two optical mice. The crab is held in position by a weightless rod attached to its carapace (further details in Oliva et al., 2007). (Bii) Picture of a crab running on a ball in the direction indicated by the red arrow, to escape from a 2D representation of an approaching object (looming stimulus). (Biii) The graph shows the running speed of a single crab during its response to a simulated object approaching at constant velocity. The curved line represents the angular size of the looming stimulus, which expanded from 4 to 60 deg in 5 s, with the arrowhead representing the start of expansion. The colored trace represents the animal's performance. Initially, the crab was walking (green), then froze for a short time (orange). This was followed by running away with a speed that matched the velocity of stimulus expansion (blue). At the end of the expansion, the crab immediately began to decelerate (magenta).

perception of events that take place a few degrees above and below the horizon. In addition, MLG1 neurons are sensitive to horizontally rather than to vertically moving objects (Medan et al., 2015). These optical and neuronal features are clear adaptations to the vertically compressed mudflat world of the crab, where most object motion corresponds to the movements of neighboring crabs along the horizontal plane (Fig. 1A).

The firing rate of MLG1 neurons follows the dynamic of expansion of looming stimuli only to the extent that the image expansion remains below 35 deg, i.e. during the early stage of escaping (Oliva and Tomsic, 2014). In contrast, the apparently unique MLG2 neuron, with its receptive field that encompasses the whole 360 deg, was found to respond to stimuli approaching from anywhere around the crab, and to encode looming information for images expanding beyond 35 deg. Using a wide variety of stimulus dynamics, we found that the MLG2 neuron faithfully encodes the angular velocity of looming stimuli, and thus conveys the information that is used by the animal to continuously adjust its running speed (Oliva and Tomsic, 2016).

As discussed above, a crab's response to visual threats is more complex than performing a single escape run. When a crab faces an approaching object, its first strategy is to freeze, but if the object

continues to approach, the crab will run away. Furthermore, if the predator approaches too fast or it is too close, the crab may raise its claws towards the threatening stimulus (Scarano and Tomsic, 2014). The decision to implement any of these defensive strategies depends on the risk assessment made by the animal on the basis of the visual information on the stimulus (but see below). In addition to the MLG1 and MLG2 neurons, bistratified lobula giant type 1 and 2 neurons (BLG1 and BLG2, respectively) have been partially investigated in an attempt to understand the neural basis of the crab's choice of strategy when responding to visual threats. BLG1 neurons appear to have some sensitivity to stimulus elevation, which makes these elements potentially capable of encoding the distance to an object (Hemmi and Zeil, 2003) or of categorizing a visual object by its elevation (Layne et al., 1997). In contrast to the three neuronal classes already described, the BLG2 neuron responds to a looming stimulus at the very beginning of its expansion (Fig. 3F), when the freezing response occurs (Fig. 2Biii), and the activity of this neuron stops with further approach of the stimulus, when the MLG1 and MLG2 neurons start to fire (Fig. 3F) and the crab begins to run away (Fig. 2Biii) (Oliva, 2010).

Therefore, motion-sensitive LG neurons are thought to play an important role in the implementation of defensive responses. The

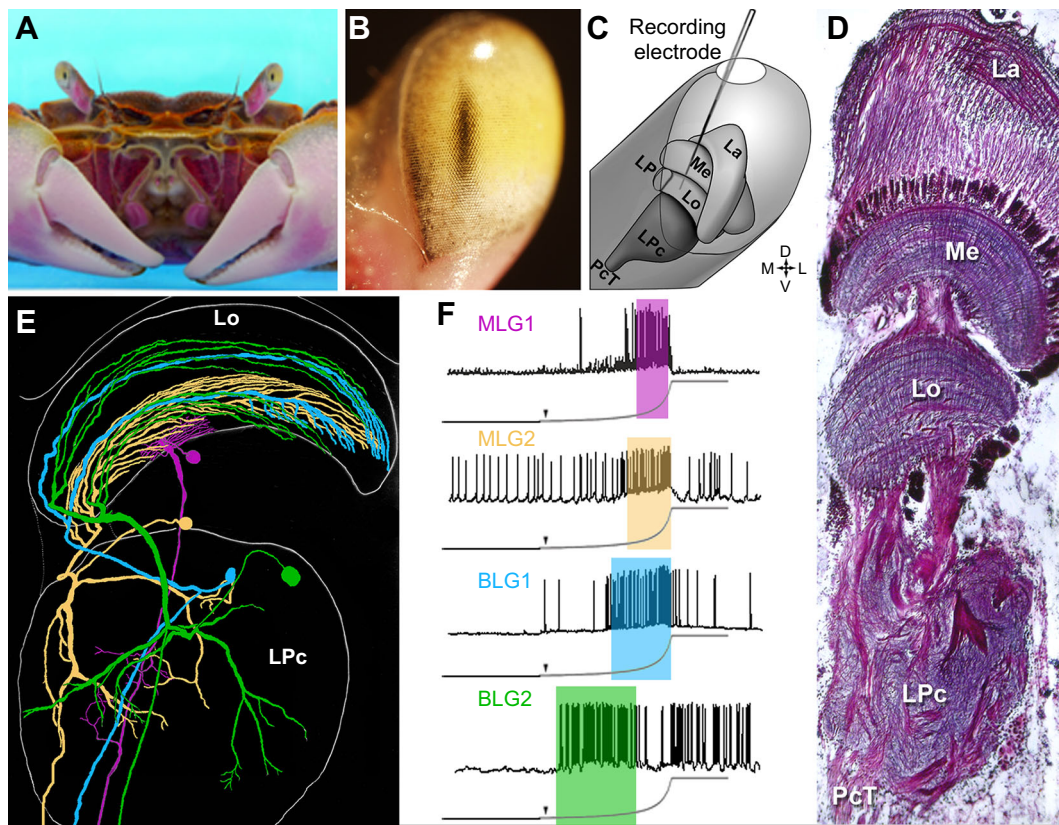


Fig. 3. The visual system and motion detector neurons of the crab. (A) The two compound eyes of crabs are mounted on movable eyestalks. (B) *Neohelice* has about 9000 ommatidia spherically distributed around the tip of the eyestalk. (C) Within the eyestalk is a series of neuropils that are easily accessible for *in vivo* intracellular recording. From periphery to center, the retinotopic neuropils are the lamina (La), the medulla (Me), the lobula (Lo) and the lobula plate (LP). The optic lobe also includes a number of non-retinotopic neuropils that comprise the lateral protocerebrum (LPc). The optic lobe is connected to the supraesophageal ganglion through the protocerebral tract (PcT). D, dorsal; V, ventral; M, medial; L, lateral. (D) Bodian staining of the optic lobe showing the retinotopic columnar arrangement of the La, the Me and the Lo (the LP is not seen in this view). (E) Schematic representation, based on morphological reconstructions from separate intracellular staining experiments, of the four different classes of identified lobula giant (LG) neurons depicted with different colors: magenta, MLG1; yellow, MLG2; blue, BLG1; green, BLG2. (F) Response of the four different LG neurons (with colors corresponding to those in E) to a looming stimulus with a dynamic of expansion represented by the curved line below each trace (4 to 60 deg in 5 s). Colored rectangles denote the period of maximum response of each neuron. Compare these responses with the crab's behavioral performance to an identical looming stimulus (as shown in Fig. 2Biii), and notice the correspondence between the temporal course of the freezing and the response of the BLG2 neuron at the beginning of the stimulus expansion, and then between the accelerated run and the increase in the rate of firing in the other LG neurons during the late phase of the stimulus expansion.

feature detection differences found among the distinctive giant neurons of the lobula, the anatomical proximity of these neurons (Fig. 3E) and the matching of their particular responses with different aspects of the defensive response to visual stimuli suggest that the microcircuit of the LG neurons can operate as a decision-making node that coordinates visual behavioral strategies (Tomsic, 2016).

Recently, we have discovered the existence of another class of giant neurons arborizing in the lobula, lobula plate and lateral protocerebrum. Contrasting with the previously described LG neurons, these new neurons show a remarkable directional preference for objects moving in the horizontal plane. Such a property makes these neurons candidates to contribute to the directional control of visually guided behaviors. We are currently performing experiments to further identify and characterize these neurons (F. Scarano, J. Sztarker and D.T., unpublished results).

Long-term changes of the escape response and their underlying neural basis

Although escaping from visual stimuli is an innate behavior, it can be heavily modified by a variety of factors. Investigations in *Neohelice* have shown that the escape response can be modulated in a transient or long-lasting manner by factors as diverse as the time of

day (circadian rhythm; Pereyra et al., 1996), the time of year (seasonal period; Sztarker and Tomsic, 2008), the ecological risk of predation (predator abundance; Magani et al., 2016) or learning and memory (Tomsic et al., 2009). These behavioral adaptations were found to be reflected by response changes occurring in the LG neurons. Below, we briefly discuss two examples of long-lasting changes, the first relating to memories induced by repeated presentation of a threatening stimulus and the second relating to differences in predation risk between populations.

Learning and memory

Changes in the crab's escape response following repeated presentation of a visual danger stimulus (VDS: an object moving overhead) have been extensively investigated in *Neohelice* using behavioral, pharmacological, electrophysiological and molecular approaches. Depending on the number and frequency of VDS presentations, different types of behavioral changes involving distinct physiological and cellular mechanisms can be induced (reviewed in Tomsic and Romano, 2013; Tomsic and Maldonado, 2014). Performing 15 training trials each separated by 2 s induces a rapid and deep reduction in the crabs' response; however, the normal response is recovered in 15 min. In contrast, if the same

number of trials is performed but separated by 3 min, one can induce a slower reduction in escape response that will last for several days (e.g. Pedreira et al., 1998). Yet, if the visual layout of the testing session differs from the one in which animals were trained, for example by changing a plain for a textured environment or vice versa, their escape response fully recovers (Tomsic et al., 1998). Therefore, this enduring behavioral modification is stimulus and context specific, and thus has been called ‘context-signal memory’ (CSM). The temporal course of escape reduction during training, as well as the duration of the memory formed with different stimulation frequencies, was found to be accounted for by parallel changes occurring in the LG neurons (Tomsic et al., 2003). Moreover, the activity of LG neurons has been shown to reflect important attributes of visual memory, such as stimulus generalization and stimulus recognition (Sztarker and Tomsic, 2011). These neurons, however, are not capable of accounting for the context component of the CSM (Sztarker and Tomsic, 2011), which has been recently found to involve neurons in the hemiellipsoid body (Maza et al., 2016). Interestingly, the hemiellipsoid body of crustaceans is thought to be homologous to the insect mushroom body, a brain center involved in complex memory (Wolff and Strausfeld, 2016).

Population-specific predation risks

Neohelice crabs are restricted to living in estuaries and bays, which can be separated by several hundred kilometers, and their larvae are unlikely to travel long distances through the ocean. Therefore, populations in different regions are thought to be genetically isolated from one another. Along its wide, but discontinuous, geographic distribution, the species experiences different tidal regimes (with water depths ranging from a few centimeters to 9 m), water salinities (from near 0 to 60 ppt), environmental structures (mud flats versus vegetated areas) and predation risks (areas where aerial predators are abundant or scarce) (Luppi et al., 2012). Consequently, *Neohelice granulata* has been proposed as an excellent model for studying intra- and inter-population variability (reviewed in Spivak, 2010). In a recent study, we showed that crabs inhabiting an area with a high risk of bird predation respond more strongly to VDS (but not to other visual and non-visual stimuli) than crabs that inhabit an area of low predation risk. Remarkably, the behavioral differences were reflected by a difference in the response of the LG neurons. Neurons from animals of the population with the stronger escape response responded with a larger number of spikes to VDS than neurons from animals of the less reactive population. The difference in the VDS-elicited spikes occurred without detectable differences in the input signals and the resting properties of the LG neurons of the two populations. Therefore, the difference in the number of elicited spikes must arise from intrinsic differences in the input–output transfer function between the LGs of the two populations. These results represent an exceptional example of the effect of an ecological pressure observed at the level of individual identified neurons (Magani et al., 2016).

The predator behavior of *Neohelice*

Neohelice prey on smaller individuals of the same species and on small fiddler crabs of the species *Uca uruguayensis* (Fig. 4A; Daleo et al., 2003). Unpublished results (D.T.), obtained by using the same method as that described above to investigate the escape response in the field (Fig. 2A), revealed that *Neohelice* display conspicuous chasing responses. A small dummy moved at ground level elicits a strong pursuing response in almost every crab that is less than 40 cm away. The pursuit usually ends with the capture of the dummy prey

(Fig. 4B). In contrast, the same dummy moved 10 cm above the ground elicits an escape behavior (Fig. 4C). Therefore, crabs seem to apply a rule of thumb to categorize certain moving objects as prey or predators, causing the crabs to run after or away from the stimulus, respectively. This rule seems to be: if the visual object is small and moves at ground level, it is a prey, whereas if it is large or moves overhead, it is a predator (Fig. 4D). Using the methodology developed to perform *in vivo* intracellular recording (Berón de Astrada and Tomsic, 2002), in combination with computer-generated visual stimuli (Medan et al., 2007), we are currently searching for neurons that may be involved in the behavioral responses to small moving objects. Fig. 4E shows one such neuron, which is characterized by having a very wide receptive field and sensitivity for small objects moving at the level of the horizon. These neurons of the crab resemble the well-known small target movement detector (STMD) neurons from the lobula of dragonflies, which are involved in the prey capture behavior of this animal (e.g. Nordström, 2012).

Lessons from field and laboratory studies

Ideally, behavior should be investigated within the complexity of the animal’s natural environment. However, investigations of the neurobiology subserving behavior are feasible only under laboratory conditions. To bridge the gap, the behavior elicited in the laboratory must reflect the essential features of the behavior occurring in the natural environment. In addition, when searching for neural elements underlying a particular behavior using computer-generated stimuli, it is first necessary to validate the effectiveness of such stimuli for eliciting that behavior. It is worth remembering, however, that the stimuli, the contextual situation and the measuring conditions used to investigate behavioral responses in the laboratory differ from those that an animal experiences in its natural environment. Such differences may affect the animal’s behavioral performance, as we discuss below.

Differences in the thresholds for eliciting anti-predator strategies

The main anti-predator strategies of freezing, escaping and defending with the claws as shown by crabs in the field are also present in the laboratory. The probability of eliciting one or another of these strategies depends on the stimulus features and, therefore, it can be manipulated in the laboratory. For instance, in the laboratory, a looming stimulus that increases its apparent size by 1.5 deg brings about a freezing response; if it grows above 7 deg, it triggers the escape run, whereas if it expands fast enough or becomes too big, it induces a claw-raising display (Oliva, 2010; Oliva and Tomsic, 2012). The thresholds for triggering these behavioral components are typically much lower in the field (Fig. 5A) than in the laboratory. For instance, in the field, crabs with burrows have been found to run away from an approaching object when its angular size is as small as 1–2 deg. The discrepancy has been interpreted in terms of the contextual differences. In the field, the presence of the nearby burrow likely promotes an earlier decision to run away towards the safety of the burrow, whereas in the laboratory, the lack of a reachable shelter would favor freezing as the most suitable strategy, until this proves perilous because the object continues to approach, in which case the animal finally decides to run away (Hemmi and Tomsic, 2015).

Differences in the direction of escape

There is also a conspicuous difference in the direction of running under field and laboratory conditions. Escape behaviors in the field were recorded from crabs that possessed a burrow; hence, regardless

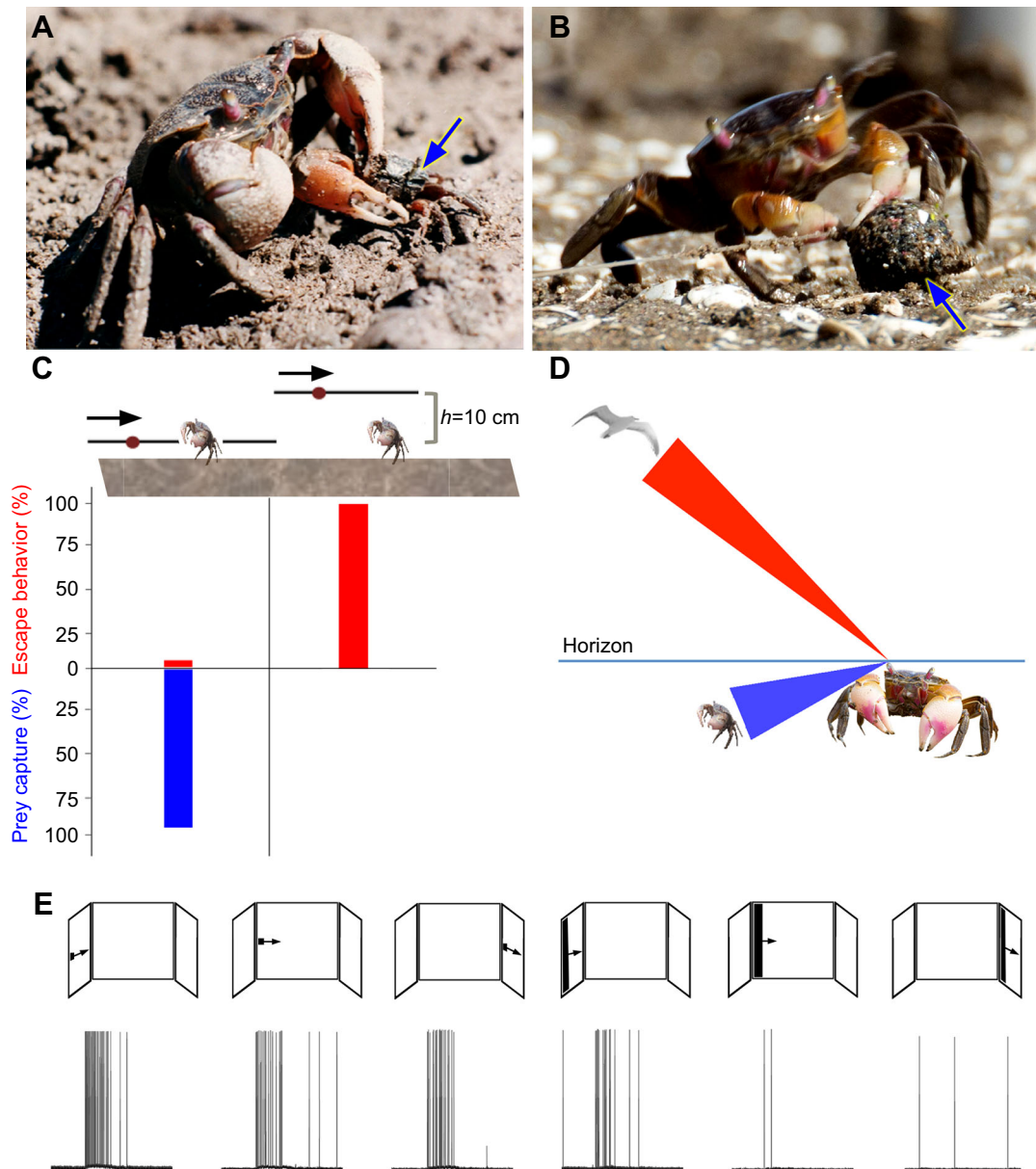


Fig. 4. The prey and predator behavior of *Neohelice granulata*. (A) *Neohelice* crabs prey on smaller individuals of the same species and on fiddler crabs (*Uca uruguayensis*, arrow), as this picture shows. (B) A robust chasing behavior can be reliably elicited in *Neohelice* by using a small dummy prey (arrow) moved at ground level, which the crab pursues, captures and furiously attempts to tear apart with its claws. (C) The same dummy moving either at ground level or overhead (at a height of 10 cm) exclusively triggers the prey capture behavior (18 out of 19 individuals) or the escape behavior (16 out of 16) of this crab, respectively. (D) The crab clearly categorizes a moving object as a prey item or a predator, and this seems to be based mainly on the object elevation (see 'The predator behavior of *Neohelice*'). (E) A small target detector neuron that might be involved in the prey capture behavior. This wide-field neuron responds to small rather than to large objects moving horizontally at the level of the crab. The scheme above each trace indicates the type of stimulus and the screen where it was presented. Photo in A is courtesy of Pablo Ribeiro.

of the direction of the stimulus approach, the direction of escape was always towards the burrow (even if this meant that a crab had to run towards the approaching predator; Fig. 5B,D). In contrast, on the laboratory treadmill there was no possible shelter available. Therefore, crabs in the laboratory invariably run directly away from the looming stimulus (Fig. 5C,E).

Concluding remarks

In *Neohelice*, the analyses of behavioral responses to different visual stimuli in the laboratory, including the evaluation of visual learning and memory capabilities, has guided the identification of LG

neurons as central players underlying those behaviors. The activity of these neurons can be stably recorded intracellularly in the living and almost intact animal, a feature that has allowed their morphological description together with the physiological characterization of their responses to the stimulus eliciting the behavior. The knowledge gained from these studies, in combination with our associated neuroanatomical studies of the visual nervous system of the crab (Sztarker et al., 2005, 2009; Berón de Astrada et al., 2011; Sztarker and Tomsic, 2014), has contributed to strengthening the idea that the optic neuropils of crustaceans and insects are homologous.

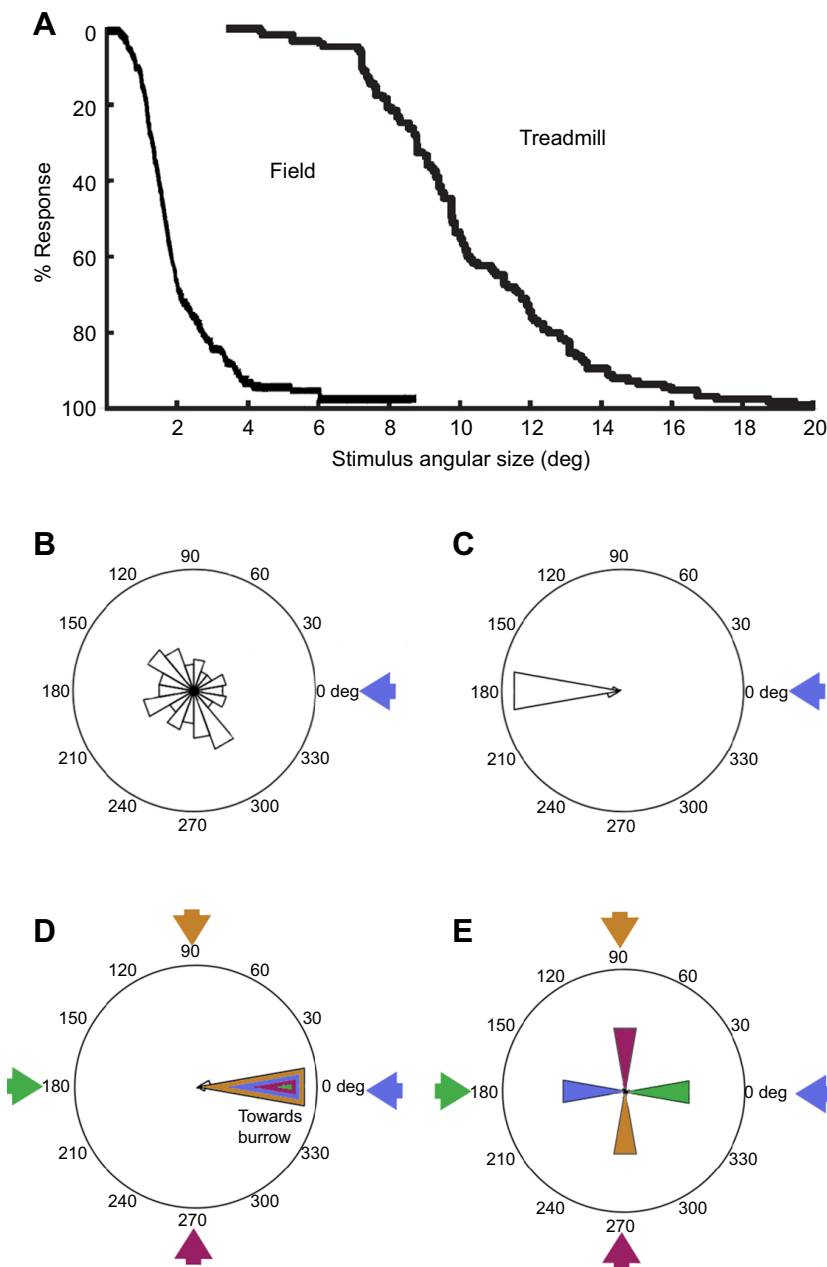


Fig. 5. The escape response in the field and in the laboratory. (A) The graph shows the percentage of crabs that initiate escape as a function of apparent stimulus size. In the field, crabs initiate their escape towards their burrows at much smaller stimulus angular sizes than in the laboratory. The earlier response in the field (i.e. when the stimulus is at a greater distance), is likely due to the possibility of reaching an available refuge. (B–E) Polar plots showing the probability of animals running in different directions in field (B,D) and laboratory (C,E) experiments. (B) In the field, the escape direction is independent of the trajectory of the approaching stimulus (blue arrow), whereas (C) in the laboratory, the escape direction is determined by the stimulus trajectory. (D) In the field, the direction of escape is entirely dependent on the direction of the animal's burrow. (E) The lack of an available refuge in the laboratory setup compels animals to always run directly away from the stimulus. In D and E, the color of the escape direction matches the color of the corresponding stimulus.

The investigation of visual motion processing in the arthropod optic neuropils has been mainly, although not exclusively, connected with flow field analysis and course control (e.g. Borst, 2014; Ullrich et al., 2015). To fulfill their biological role, these tasks require responses to be sustained during continuous or repeated visual stimulation. This has led to the idea that plasticity serving visual memory necessarily occurs at levels of processing deeper than the optic neuropils. However, our studies have revealed a strong parallel between behavioral changes in performance and changes in the responses of the LG neurons, across a variety of stimuli and circumstances. Moreover, the LG neurons have been shown to share some remarkable properties, such as the ability to integrate visual information with mechanosensory information from the animal's legs (Berón de Astrada and Tomsic, 2002; Medan et al., 2007), the integration of binocular information (Sztarker and Tomsic, 2004) and the capacity to hold long-term visual memory traces (Tomsic et al., 2003; Sztarker and Tomsic, 2011). Therefore,

our results clearly show that the lobula is not simply a visual-processing neuropil: it also possesses features that are often ascribed to higher neural centers.

Investigations of the crab's behavior under laboratory conditions have shown the essential features of the behavior elicited by predators in the natural environment, but they have also revealed significant differences, the most important of which can be explained by the lack of the burrow in the laboratory. The field studies have allowed us to make sense of these differences, but they have also led us to new discoveries, such as the fascinating pursuit behavior displayed by the crab. Remarkably, field experiments have shown that crabs will reliably escape from or chase after exactly the same moving object, depending on whether the object is moving overhead or at ground level, respectively. In that context, crabs clearly use a simple rule to categorize an object as a prey item or a predator. This system offers an attractive possibility for investigating the neurobiology of a decision-making process

leading to opposite responses, i.e. to run after or away from a moving object. Furthermore, investigations of the chasing behavior and of the STMD neurons of the crab will allow comparisons with the STMD neurons of dragonflies, which might provide further evidence to support the homology between the optic neuropils of insects and crustaceans.

Competing interests

The authors declare no competing or financial interests.

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