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Serotonin expression in the optic lobes of cavernicolous crustaceans during the light–dark transition phase: Role of the lamina ganglionaris

Dalila Benzid [a], Laetitia De Jong [a], Christophe Lejeusne [b], Pierre Chevaldonné [b], Xavier Moreau [a,*]

[a] *ER "Biodiversité and Environnement", Case 18, Université de Provence, 13331 Marseille cedex 3, France*
[b] *UMR CNRS 6540 "DIMAR (Diversité, Evolution et Ecologie Fonctionnelle Marine)", Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France*

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Abstract

The expression pattern of serotonin neurotransmitter (5-HT) was investigated in the optic lobes of the marine cave mysid *Hemimysis margalefi* (Crustacea, Mysidacea) during the dark to light and light to dark transitions by fluorescence immunohistochemistry. The intensity of the expression was evaluated according to the number of immunoreactive elements. Experiments were carried out under laboratory controlled conditions of illumination. The results show that some structures as the X-organ and medulla terminalis are always immunoreactive during light or dark periods. Other ones as the rhabdom of the retinular cells (rh), the distal pigment cells (dpc), the internal medulla (im), the external medulla (em), and the sinus gland (sg) are immunoreactive during light exposure. In these latter structures, immunoreactivity is quickly detected after illumination (30 min) while, back to darkness, it takes longer to disappear (1 h for rh and dpc, 2 h for im and more for em and sg). The 5-HT expression pattern in the lamina ganglionaris is different. During the dark to light transition, immunoreactive cells are already detected after 30 min of light exposure; after 1 h of exposure, the lamina ganglionaris expresses many immunoreactive cells, then, they are less numerous after 2 h; finally, the detection limit is reached after 3 h of exposure. During the light to dark transition, 5-HT is expressed in the lamina ganglionaris only after 1 h of darkness. The number of 5-HT neurones was increased after 2 h of darkness but no reactivity is detected after one night. To go further, same experiments were carried out in an other mysid species, *Leptomysis lingvura* that lives at same depth but in a shallow lagoon. In contrast to *H. margalefi*, *L. lingvura* is not subject to nycthemeral migrations. A different 5-HT expression pattern in the lamina ganglionaris has been observed: immunolabelling was detected as soon as 30 min, whatever the considered transition phase, dark to light or light to dark. These results are discussed considering the behaviour of each species. We suggest that the expression pattern of 5-HT in the lamina ganglionaris can play the role of a photoreception signal integrator during the light–dark transition phases and that cavernicolous mysid crustaceans can be considered as a good model to study the mechanisms of determination and regulation of circadian migrations of zooplankton.

Keywords: *Lamina ganglionaris*; Light–dark transitions; Mysidacea; Serotonin; Zooplankton

* Corresponding author. Tel.: +33 4 91 10 64 12; fax: +33 4 91 10 62 65.
E-mail address: xavier.moreau@up.univ-mrs.fr (X. Moreau).

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1. Introduction

Mysid crustaceans are important elements of the marine food webs. They live in various habitats and have been recorded from shallow to deep waters in the world oceans and at all latitudes and longitudes; moreover, some live in marine caves where they can account for a significant part of the community biomass. In the open sea, many planktonic species accomplish vertical and circadian migrations that are homologous with the nycthemeral migrations of some cave mysids (Passelaigue and Bourdillon, 1986). The distribution and the circadian migrations of cavernicolous *Hemimysis* species have been previously studied (Chevaldonné and Lejeusne, 2003; Coma et al., 1997; Macquart-Moulin and Passelaigue, 1982; Passelaigue and Bourdillon, 1986). However, the physiological mechanisms involved in the control of these migrations are unknown.

Crustaceans display a variety of circadian rhythms that are controlled by periodic functions of the nervous system (Fanjul-Moles and Prieto-Sagredo, 2003). In the Decapoda crustaceans, although some of these rhythms are well documented, the regulation processes are not completely understood. It has been suggested that some tissues of the optic lobe, such as the lamina ganglionaris (Aréchiga and Rodríguez-Sosa, 1998), the retina (Aréchiga and Rodríguez-Sosa, 1997; Escamilla-Chimal et al., 1998), and the X-organ-sinus gland complex (Sáenz et al., 1997) may play a role in this regulation.

In decapods, serotonin (5-hydroxytryptamine, 5-HT) is involved in the regulation of several behaviours and biological functions by acting either as a classical neurotransmitter, a neuromodulator or a neurohormone (Beltz, 1999). Indeed, 5-HT plays a key role in feeding and reproductive behaviours and also aggressive, postural and social behaviours (Edwards et al., 1999; Harris-Warrick and Kravitz, 1984; Kravitz, 1988; Livingstone et al., 1980; Weiger, 1997; Yeh et al., 1997). In the decapod optic lobes, 5-HT has been located in all neuropiles. Although 5-HT has been proposed to modulate the release of some hormones from neurosecretory cells (Sáenz et al., 1997) such as the crustacean hyperglycaemic hormone (CHH), the red pigment dispersing hormone (RPDH) and the neurodepressing hormone (NDH) (Crow and Bridge, 1985; García and Aréchiga, 1998; Kravitz, 1988), 5-HT plays a major role in the response to light or obscurity. Indeed, the 5-HT content in the entire eyestalk is higher at night (Fingerman and Fingerman, 1977) and 5-HT has been shown to enhance the sensitivity to light in crayfish retinal photoreceptors (Aréchiga et al., 1990). However, little is known about the role of this biogenic amine in Crustacea other than Decapoda. In Mysidacea, 5-HT neurons have been evidenced and mapped in the brain and optic lobes only recently (Moreau et al., 2002).

As light intensity is thought to be the major factor influencing the migrations of crustaceans, the purpose of this study was to investigate the effects of various conditions of illumination, especially during the light–dark and dark–light transition phases, on the expression pattern of 5-HT in the optic lobes of two mysid species easily kept alive in laboratory conditions. These investigations were conducted on a Mediterranean endemic marine cave species that exhibit nycthemeral migrations, *Hemimysis margalefi* Alcaraz, Riera and Gili, 1986, and on *Leptomysis lingvura* G.O. Sars, 1866, a shallow lagoonal species living at comparable depth and not subject to circadian migrations.

2. Materials and methods

2.1. Experimental designs

During winter 2003–2004, biological material was collected off the French Mediterranean coast: the specimens of *H. margalefi* were obtained from the Figuier cave (15 m depth) near Marseille and *L. lingvura* from the Brusc lagoon near Toulon. Experiments were carried out under artificial lighting (cool-white fluorescent light at 150 μmol m^{-2} s^{-1}). Adult specimens of *H. margalefi* were randomly sampled under low red light intensity.

For both species, eight groups of 10 randomly chosen specimens were maintained one night (12 h) in the dark at 21 °C in natural seawater. For each species, one group was fixed in the dark after the 12 h in darkness. Four groups were illuminated with cool-white fluorescent light and respectively fixed after 30 min, 1 h, 2 h or 3 h of illumination. Three groups were illuminated with cool-white fluorescent light for 3 h and then, placed back in the dark and, respectively, fixed in darkness after 30 min, 1 h or 2 h. The period of light exposure was not longer than 3 h in order to preserve the functional integrity of the visual system of the cave mysids.

For each condition, the specimens were fixed in saline phosphate buffer (PBS, 0.1 M pH 7.2) containing 4% paraformaldehyde (PFA) and used for immunoassays. Fixed samples were stored at 4 °C until immunohistochemical assay.