LETTER TO EDITOR

Interactions between earthworm neuroendocrine and immune systems

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To the Editor

We read with interest the recent paper about the importance of studying invertebrate immune-neuroendocrine functions published in the “Vision and Perspectives” section of Inv. Surv. J. (Ottaviani, 2014). The paper reinforces the profoundly important view that the neuroendocrine and innate defense systems of invertebrates and vertebrates can interact in concerted harmony (Cohen and Kinney, 2007). To consolidate the insights propounded in these seminal articles we would like to advocate earthworms as tractable and powerful models for studies on immune-neuroendocrine interactions. Earthworms are relatively easy to culture and manipulate in the laboratory, and deep knowledge of particular aspects of their physiology and molecular genetics is burgeoning rapidly (Stürzenbaum et al., 2009; Liebeke et al., 2014).

Earthworms, as representatives of oligochaete annelids, are metamerically-segmented celomates with a closed circulation and a well-developed nervous system. A segmented celomic cavity communicates with the external environment via dorsal pores, thus the celom ubiquitously contains bacteria, protozoans and fungi, as well as abundant free-floating immunocytes and humoral factors that inhibit microorganism outgrowth (Bilej et al., 2011). In common with other invertebrates, earthworms are devoid of adaptive immunity based on T and B lymphocytes and antibodies; these components are present in jawed vertebrates only. However, the earthworm immune system detects the conserved Pathogen-Associated Molecular Patterns (PAMPs) of microbes by Pathogen Recognition Receptors (PRR), among them the extensively studied Toll-like receptors (Coscia et al., 2011; Skanta et al., 2013). Evidently, earthworms have evolved efficient innate immunity with both cellular and humoral components (Bilej et al., 2011). The immunocytes, according to the term coined out by Ottaviani (2011) for invertebrate cells endowed with attributes of vertebrate macrophages, are represented in earthworms by free-floating amebocytes derived from the lining of the celomic cavity (Parry, 1975). In some lumbricid species amebocytes are accompanied by eleocytes, the latter being detached chloragocytes invested with granules containing species-specific amounts of riboflavin (vitamin B2). Riboflavin is now recognized as a potentiator of immunocompetence and tissue regeneration capacity in earthworms and other organisms (Plytycz and Morgan, 2011; Johnson et al., 2012).

Earthworms stressed by predators or physical/chemical irritants expel celomocyte-containing celomic fluid through the dorsal pores during spasmodic body movements. This ability is commonly exploited for non-invasive retrieval of celomocytes for ex vivo studies and/or for temporal depletion of earthworm celomocytes and celomocyte-derived humoral factors. Depletion of celomocytes is followed by their restoration (Eyambe et al., 1973); amebocyte restoration is faster than eleocyte restoration (Klimek et al., 2011; Santocki et al., 2015).

The earthworm central nervous system (CNS) is a highly differentiated neuroendocrine structure which produces hormones, neurohormones and neurotransmitters (e.g., Takahama et al., 1998; Hartenstein, 2006; Wilhelm et al., 2006; Herbert et al., 2009; Molnar et al., 2015a). The CNS of earthworms is comprised of a ventral nerve cord (VNC) consisting of segmentally repeated ganglia joined longitudinally by connectives and radially by commissures. The first VNC ganglia are fused to form the suboesophageal ganglion which is connected by paired circumpharyngeal connectives to a dorsal cerebral ganglion, often loosely referred to as the ‘brain’. Its anatomical location makes the earthworm brain easy to remove surgically.

Earthworms possess the remarkable ability to regenerate the cerebral ganglion within a few weeks (Lubics et al., 2002; Csoknya et al., 2003; Okrzesik et al., 2013; Molnar et al., 2015b). Our experiments on adult earthworms (Dendrobaena veneta) have shown that amputation of the anterior (brain-containing) segments, or direct surgical brain removal, caused an immediate and pronounced inhibition of reproduction. Reproductive output

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Fig. 1 Reproductive success during 8 consecutive weeks in Dendrobaena veneta either untreated (blue dotted lines), or after surgical brain removal (red solid lines), or surgical brain removal in celomocyte-depleted worms (green dashed lines) measured as: A) numbers of cocoons per worm; B) numbers of hatchlings per worm; C) numbers of hatchlings per cocoon. [This is a modified version of a Figure and Table published in Molnar et al., 2015b]
Collectively, our studies provide empirical support for the notion that the neural and immune systems of earthworms can be demonstrated to be functionally intertwined.

The earthworm experimental model that we have briefly outlined above is unquestionably amenable to detailed examination by a variety of molecular-genetic platforms and by various spatially defining immune-localization microscopic methodologies.

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