The anatomy, physiology, and immunoreactivity of the cells of the enteric nervous system of larval *Manduca sexta*.

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Abstract

This project continues our investigations on the midgut of the larvae of *Manduca* sexta, the tobacco hornworm. In this study, we began to ask the questions of how the nervous system may be controlling the midgut, which will help us to better understand its physiology. Using immunocytochemistry, we looked at the expression of neuromodulators in the midgut and ventral nerve cord of caterpillars of different instars. The antibodies that can be used for immunocytochemistry include: SCPb; serotonin; FMRFamide; and allatotropin. The ultimate goal of this project is to locate and identify putative neurons associated with the midgut that control its function. In addition, we hope to see if there are taste receptor cells that may be located in the Manduca midgut that provide information for the effective control of digestion.

Introduction

• The invertebrate enteric nervous has been a subject of some study, yet not much research has been done on the invertebrate enteric nervous system, described by Bräunig as “terra incognita” (2008).
• The enteric nervous system innervates the digestive and secretory organs, involving complicated networks of ganglia and nerves (Fig. 1, Copenhaver 2007). The midgut is a particularly large organ in insects and contains extensive innervations by the enteric nervous system (Lange and Orchard 1997).
• While more research has been done on the muscular movements of the gut, there is not much known about the sensory cells in the enteric nervous system. Wu et al. (2001) suggests that a chemoreceptor system in the enteric nervous system has a part in regulating appetite, satiety, stomach movement, and digestive hormonal pathways.
• To further define the locations of nerve cells we used immunocytochemistry. Antisera that may recognize the cells of *Manduca sexta* include anti-FMRFamide, Allatostatin 1, serotonin, and small cardioactive peptide B (SCPb) (Lange and Orchard 1997 and Veenstra 2009). This study used serotonin antisera to perform immunocytochemistry on anterior, middle, and posterior sections of the midgut, as well as the nerve cord.

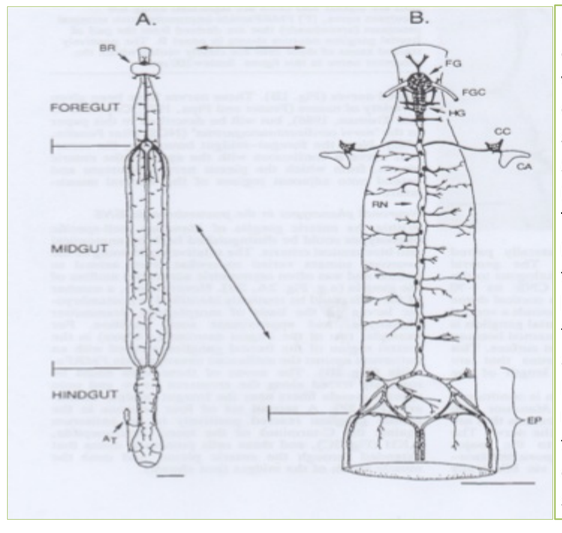

Figure 1. Diagram of the dorsal view of the enteric nervous system of *Manduca sexta* (Copenhaver and Taghert (1991). (A) Overview of the system, highlighting the foregut, midgut, and hindgut. (B) Portion of the midgut showing the ganglia of the enteric nervous system.

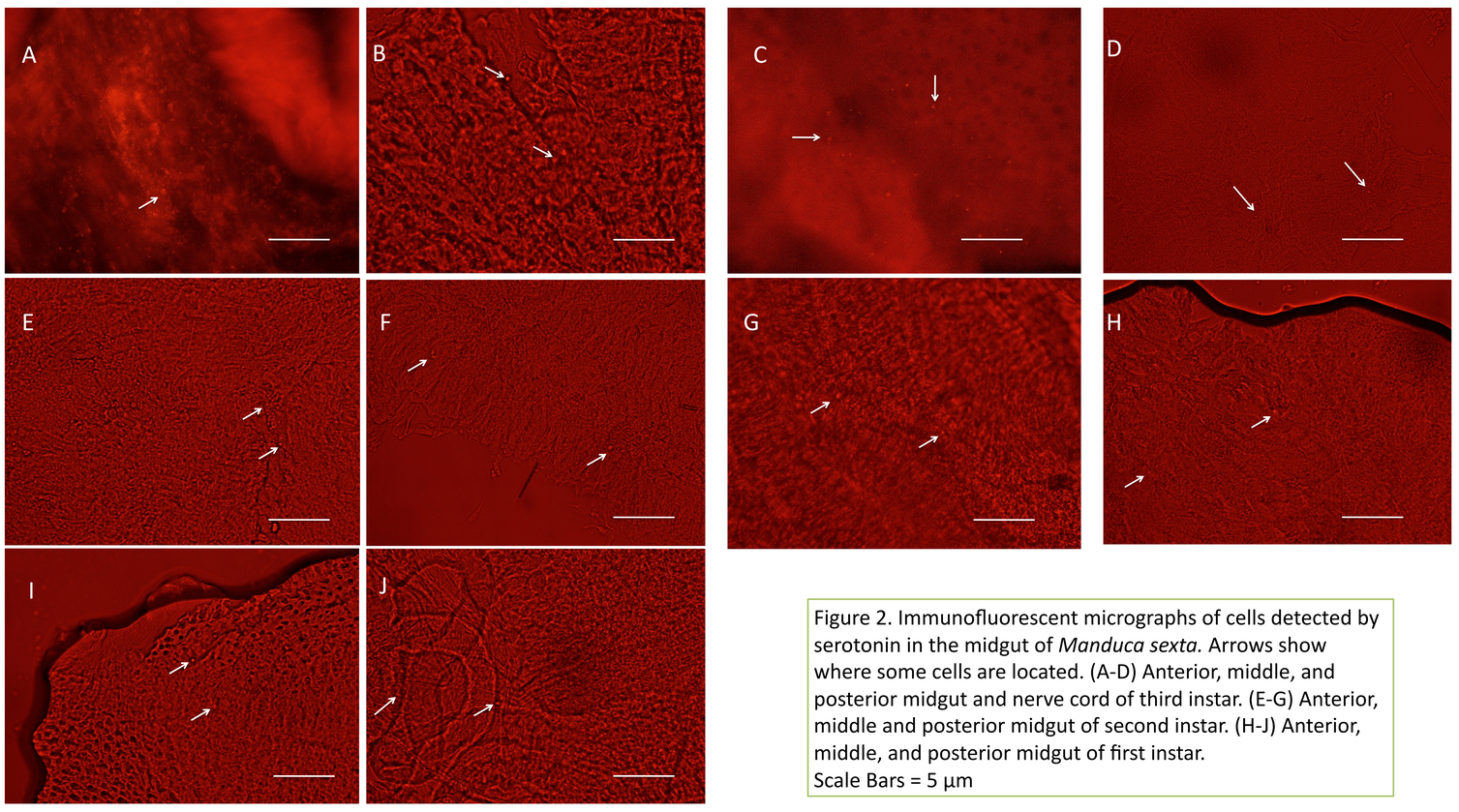

Figure 2. Immunofluorescent micrographs of cells detected by serotonin in the midgut of *Manduca sexta.* Arrows show where some cells are located. (A-D) Anterior, middle, and posterior midgut and nerve cord of third instar. (E-G) Anterior, middle and posterior midgut of second instar. (H-J) Anterior, middle, and posterior midgut of first instar.
Scale Bars = 5 µm

Methods

Sectioning
• Fixed midgut sections from *Manduca sexta* were sectioned at 20 um using a cryostat and placed on gel-coated slides. The slides were dried overnight on a slide warmer.
Immunocytochemistry
• In the immunocytochemistry process, the slides were rinsed with PBT and incubated in PBT with 10% normal goat serum (NGS). The PBT with normal goat serum was replaced with the primary antibody with 10% NGS in PBT, serotonin (1:1000), FMRFamide (1:1000), allatostatin 1 (1:1000), or SCPb (1:250). The tissue was washed with PBT and PBT with 10% normal goat serum, and then replaced with the secondary goat anti-rabbit antibody (IgG) in 10% FBS in PBT (1:500). After washing a final time in PBT, alkaline phosphatase reaction mixture was applied to slides in the dark until the color developed. Slides were washed with PBS with 10mM EDTA for a minimum of 5 minutes and then dehydrated through in an ethanol series. The slides were mounted and viewed under a microscope.
•In a second immunoctyochemistry procedure, whole tissue was incubated with the serotonin primary antibody and secondary antibody, anti-rabbit Trit C with 10% NGS (1:400) and viewed under an epifluorescent microscope.

Results

• The first immunocytochemistry procedure resulted in nonspecific staining by all the antibodies.
• The second immunocytochemistry procedure revealed serotonin binding in specific areas of the *Manduca* midgut, as shown in Figure 2.

Discussion

• The results from the first immunocytochemistry procedure suggest that there was nonspecific binding by the different antibodies used. Therefore, the procedure was simplified to looking at whole mounts of first, second, and third instars.
• Finding where specific antibodies bind in the midgut may reveal where certain taste receptor cells are located. This study will continue using serotonin, as well as other antibodies, such as to SCPb, FMRFamide, and allatostatin 1.

Acknowledgements

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