Neuronal subpopulations of the enteric nervous system of larval and adult zebrafish *(Danio rerio)*

L. Uyttebroek(1), M. Dirckx(1), F. Harrisson(1), G. Hubens(1), I.T. Shepherd(3), J.-P. Timmermans(2), L. Van Nassauw(1,2)

(1)Lab. Human Anatomy & Embryology,
(2)Lab. Cell Biology & Histology, University of Antwerp, Belgium;
(3)Dept. Biology, Emory University, Atlanta, USA.

Introduction

In the last decade, the zebrafish emerged as a leading model organism in experimental research, including studies of congenital gastrointestinal diseases. Zebrafish show many advantages over other model organisms. With their small size, zebrafish are easy to keep and raise in laboratory conditions. Fertilization and development occur externally, while embryos are transparent allowing easy observation and manipulation. Within three months after fertilisation, zebrafish are considered adult and are ready to reproduce. While general morphology and development of the enteric nervous system (ENS) of the zebrafish are already known, knowledge concerning the specific characteristics of enteric neurons is still incomplete.

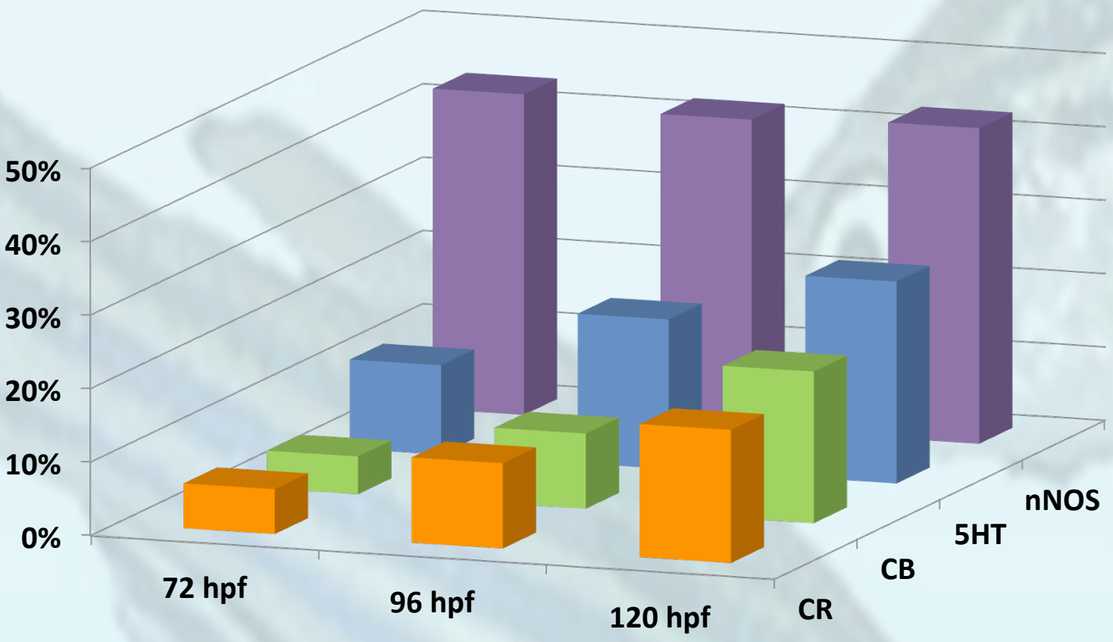
Aim

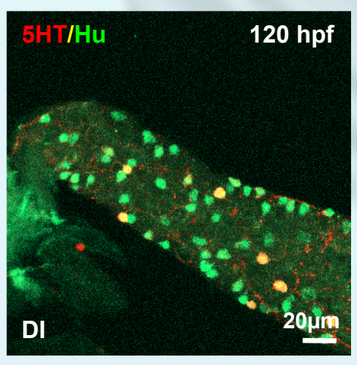
This study aimed to unravel the neurochemical coding of zebrafish enteric neurons, revealing specific subpopulations.

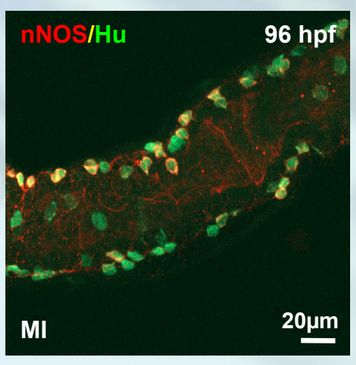
Results

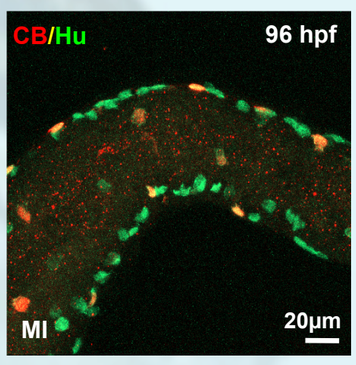
Neurochemical coding of zebrafish embryos

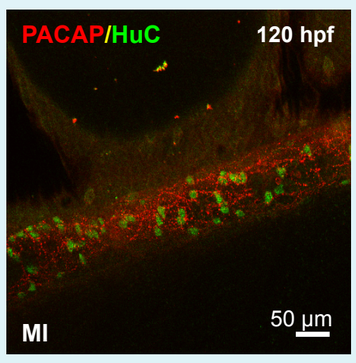
All markers are present from 72 hpf on. The number of immunoreactive cells increases between the different time points measured. High levels of nNOS are found over the entire length of the gut at 72 hpf. Those levels stay high during development. Nerve fibres showing VIP- and PACAP–IR are present from 72 hpf on.

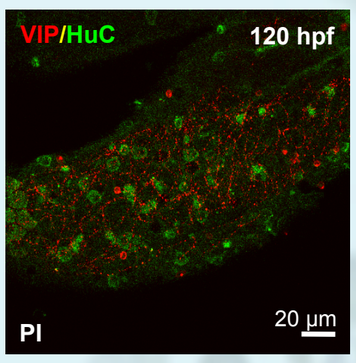


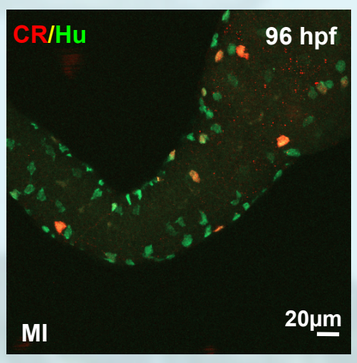






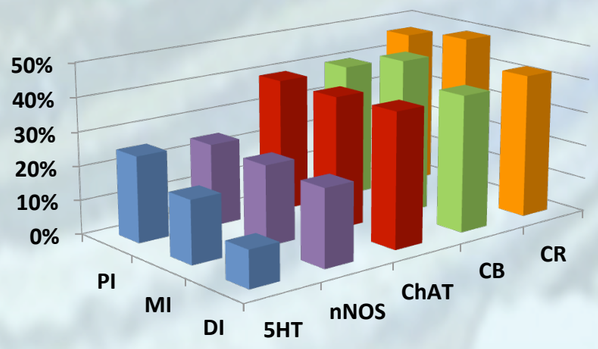






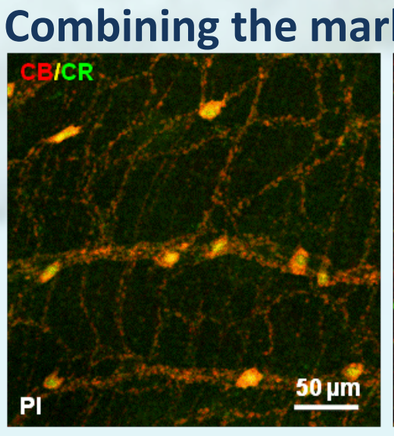
Neurochemical coding of adult zebrafish

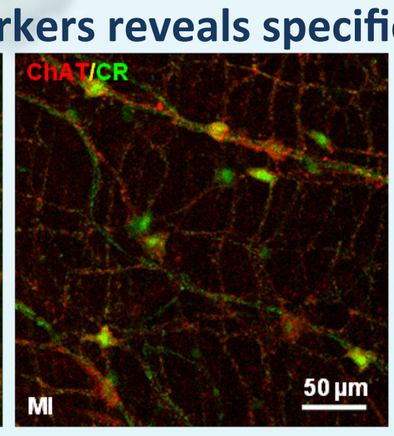
Quantification of different neuromodulators against the pan-neuronal marker HuC reveals following percentages of neuron-populations.

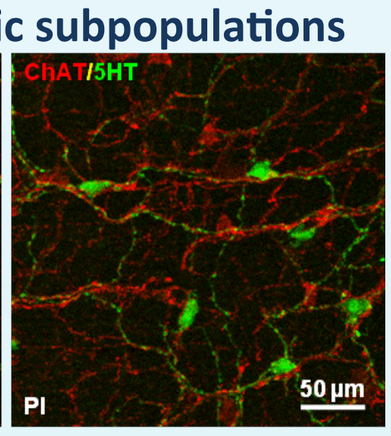


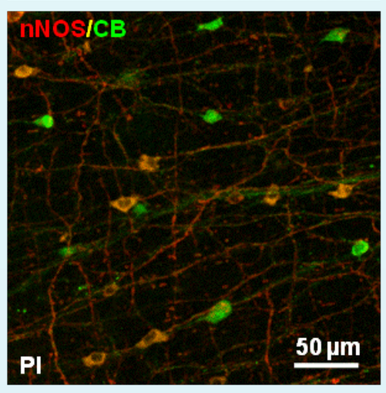
	PI	MI	DI	MEAN
5HT	25% ± 8	18% ± 4	11% ± 3	**18%**
nNOS	24% ± 2	24% ± 3	23% ± 3	**23%**
ChAT	39% ± 6	39% ± 6	40% ± 7	**39%**
CB	41% ± 7	46% ± 5	40% ± 6	**43%**
CR	44% ± 4	49% ± 5	42% ± 9	**45%**

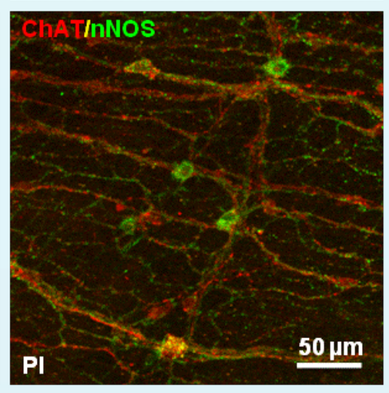
Combining the markers reveals specific subpopulations

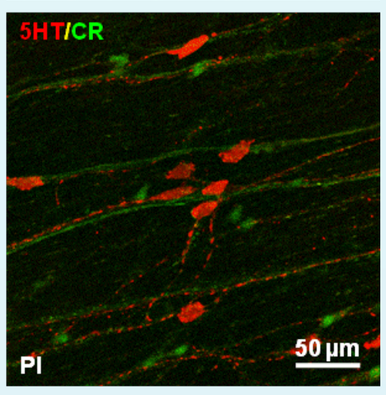












CB	CR	ChAT	nNOS	5HT
	100%	90-95%	100%	0%
100%		90-95%	100%	0%
70-80%	70-80%		15-25%	0%
45-50%	45-50%	10-20%		0%
0%	0%	0%	0%	

Conclusion

The results indicate that at the beginning of enteric neuronal differentiation, several neuronal markers are expressed. They are suggested to play a role in the spontaneous motility activity of the gut between hatching (2-3 dpf) and first feeding (5-6 dpf). These data support previous reports that the ENS is well-developed before the onset of feeding. nNOS has been suggested to play a role in the development of the ENS. In the intestine of adult zebrafish, some subpopulations are identified. The amount of 5HT-IR neurons is, in comparison to mammals, much more abundant.

SUBPOPULATIONS:

- **ChAT/CB/CR/nNOS**
- **CB/CR/nNOS**
- **CB/CR/ChAT**
- **ChAT/nNOS**
- **5HT**

List of abbreviations: CB: calbindin; CR: calretinin; nNOS: neuronal nitric oxide synthase; DI: distal intestine; dpf: days post fertilisation; ENS: enteric nervous system; hpf: hours post fertilisation; MI: mid intestine; PI: proximal intestine; PACAP: pituitary adenylate cyclase-activating peptide; VIP: vasoactive intestinal peptide

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