Distribution and coexpression of neurochemical markers in the enteric nervous system of embryonic and adult zebrafish

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Introduction

In the last decade, 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 to raise in laboratory conditions. Fertilisation and development occur externally, while the 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) are already known, knowledge concerning the specific characteristics of enteric neurons is still incomplete. We aimed to unravel the neurochemical coding of specific subpopulations of zebrafish enteric neurons.

The zebrafish life cycle

Methods

Using immunological staining methods on isolated intestines from adult and larval (72-96-120 hpf) zebrafish, we demonstrated and quantified the expression of different neurochemical markers in the three functional intestinal segments: the proximal, middle and distal intestine (PI; MI; DI). An antibody against Hu was used as a pan-neuronal marker.

Functional segments

Results

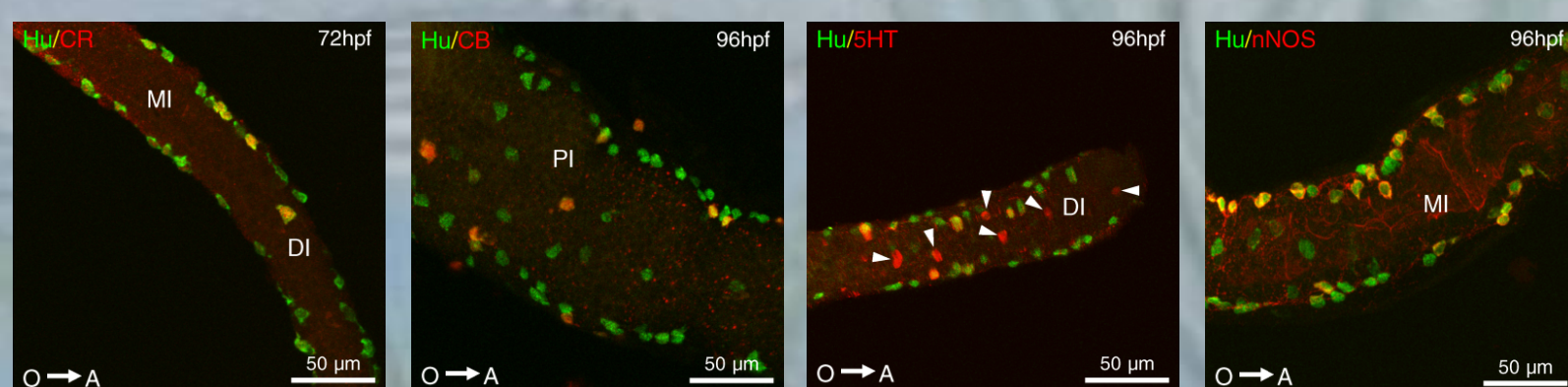### Neurochemical coding in zebrafish embryo's

VIP and PACAP-IR showed a granular appearance and was only detected in enteric nerve fibers from 72 hpf on and in some mucosal cells (*arrowheads*).

CR-, CB-, 5HT- and nNOS-IR were present from 72 hpf on in neuronal cell bodies. More than 40% of the enteric neurons are nNOS-positive, their proportion remaining constant over time. The proportions of CR, CB and 5HT neurons increase over the course of time. In the intestinal wall, there are Hu-negative 5HT-positive cells (*arrowheads*), presumptive 5HT-producing enterochromaffin cells.
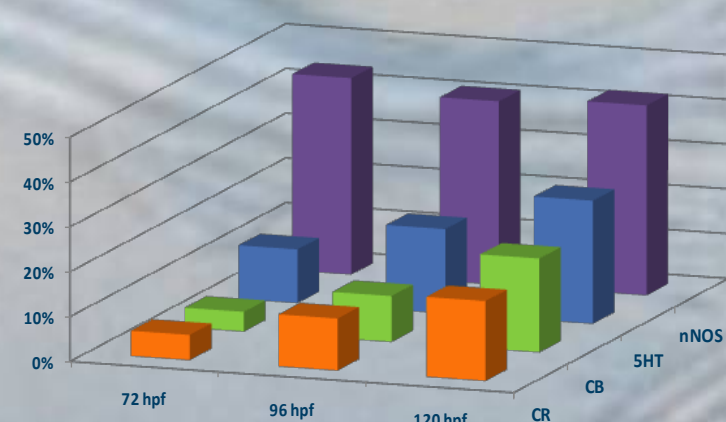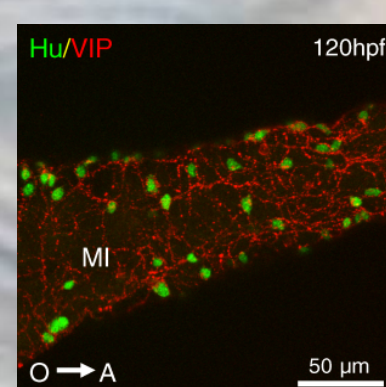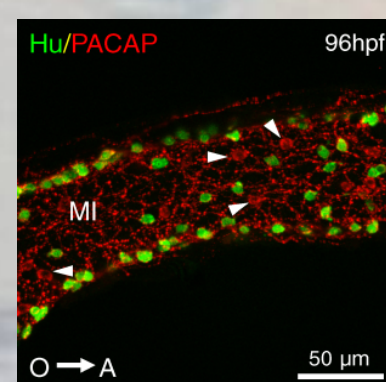
|	72 hpf	96 hpf	120 hpf
CR	6%	12%	18%
CB	4%	10%	21%
5HT	12%	19%	27%
nNOS	44%	41%	42%

Expression of neurochemical contents in the ENS of zebrafish embryos

Neurochemical coding in adult zebrafish

In the adult zebrafish intestine, VIP and PACAP displayed a similar staining pattern as in the embryo, although treatment with colchicine reveals VIP and PACAP-IR in the neuronal cell bodies.

IR for ChAT, CR, CB, 5HT and nNOS was predominantly found in neuronal cell bodies. The proportion of nNOS, ChAT, CB and CR show no regional differences, while the percentage 5HT-positive neurons decreases significantly along the length of the intestine. Quantification and combination of these neuronal markers revealed five different subpopulations.
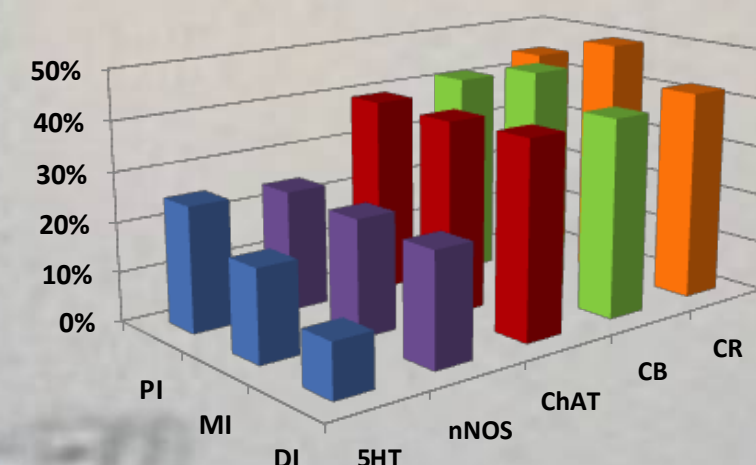
Proportions of neurochemical contents expressed in the ENS of adult zebrafish

Colocalization with...	Neurons expressing...				
	CB	CR	ChAT	nNOS	5HT
CB	---	100%	±93%	100%	0%
CR	100%	---	±94%	100%	0%
ChAT	±86%	±85%	---	±11%	0%
nNOS	±37%	±39%	±7%	---	0%
5HT	0%	0%	0%	0%	---

Percentages of colocalization between the different markers tested
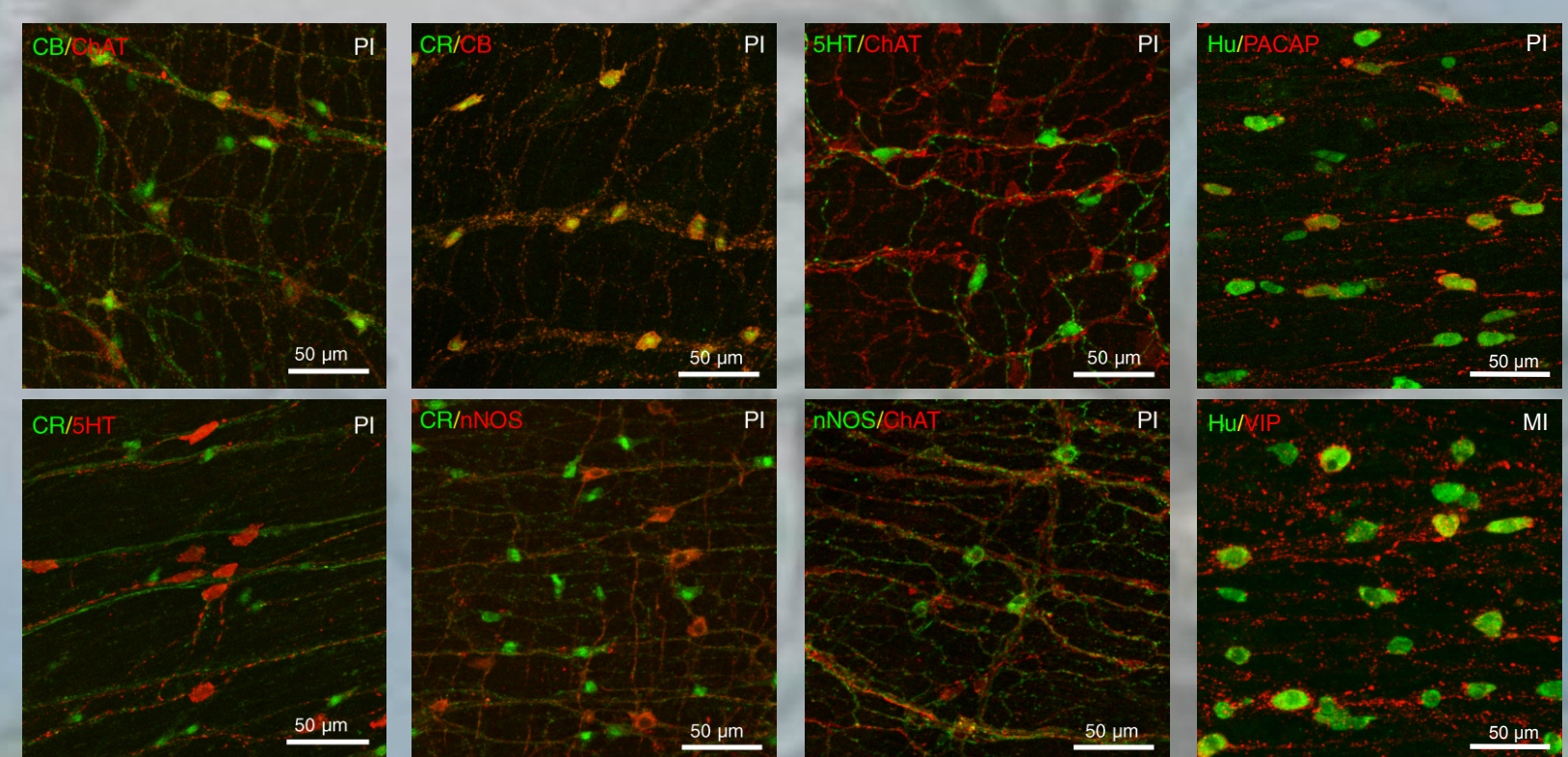
Conclusion

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 the adult zebrafish, these results reveal the presence of different populations of enteric neurons :

Population	Proportion	Presumptive class
CB/CR/ChAT/nNOS	± 3%	Interneurons
CB/CR/nNOS	± 15%	Inhibitory motorneurons
CB/CR/ChAT	± 35%	Intrinsic sensory neurons or ascending interneurons
ChAT	± 5%	-
5HT	± 18%	Ascending excitatory motorneurons or secretomotor neurons