

Neurochemical characterisation of enteric neurons in the zebrafish (*Danio rerio*) gut

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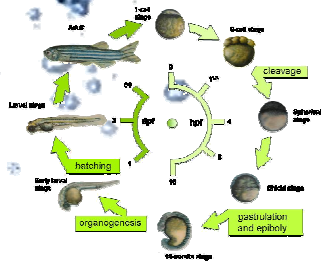
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Introduction

The last decade, the zebrafish emerged as a new leading model system in the study of vertebrate developmental biology. Molecular pathways, organ systems and physiology are well-conserved between the zebrafish and mammals. Optically transparent embryos develop rapidly outside the mother (Fig.1) and are permeable to a wide range of drugs. Within 120 hpf, most major organ systems are present in the larvae. At present, the zebrafish is also used in studies of gastrointestinal congenital diseases. Although the main morphology and development of the zebrafish enteric nervous system are already known, specific knowledge about the functional and morphological characteristics of enteric neurons is still missing. These data are necessary to validate results obtained in genetically modified animals.



The zebrafish life cycle

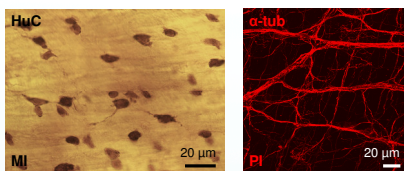
Objective

We aimed to unravel the neurochemical coding of specific subpopulations of zebrafish enteric neurons.

Results

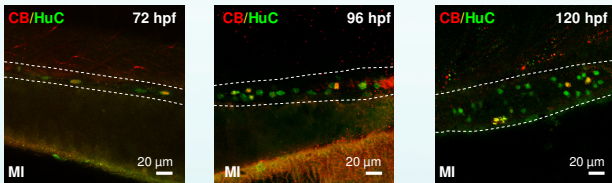
The enteric nervous system in zebrafish...

...consists, in contrast to mammals, of a single plexus of scattered enteric neurons (general markers: HuC/D and acetylated tubulin) in the space between the circular and longitudinal muscle layer.



In embryos ...

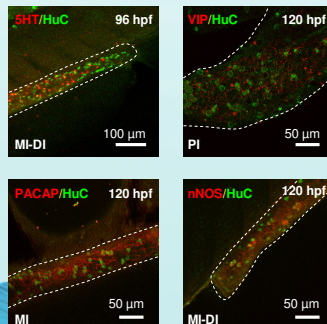
...neuron progenitors appear in the gut at 36 hpf. At 3 dpf neurons are found along the entire gut. Proliferation and differentiation of the enteric nervous system is completed at 5 dpf.



...the inhibitory neuropeptides VIP and PACAP were present from 72 hpf in nerve fibers. The calcium binding proteins (CB and CR) and nNOS were also found around that time in nerve cell bodies. At 96 hpf, some enteric neurons start to express serotonin. Although TH and ChAT were expressed in the central nervous system, they were not detected in the enteric nervous system. 5HT was not only present in neurons, but also in endocrine epithelial cells.

Table : Expression of neurochemical contents in the ENS of zebrafish embryos

		72 hpf	96 hpf	120 hpf
5HT	PI	+	+	+
	MI	+	+	+
	DI	+	+	+
CR	PI	+	+	+
	MI	+	+	+
	DI	+	+	+
CB	PI	+	+	+
	MI	+	+	+
	DI	+	+	+
nNOS	PI	+	+	+
	MI	+	+	+
	DI	+	+	+
PACAP	PI	nf	nf	nf
	MI	nf	nf	nf
	DI	nf	nf	nf
VIP	PI	nf	nf	nf
	MI	nf	nf	nf
	DI	nf	nf	nf



Abbreviations: PI: proximal intestine; MI: middle intestine; DI: distal intestine; 5HT: serotonin; CR: calcitonin; CB: calbindin; nNOS: neuronal nitric oxide synthase; PACAP: pituitary adenylate cyclase-activating polypeptide; VIP: vasoactive intestinal peptide; ChAT: choline acetyl transferase; TH: tyrosine hydroxylase; nf: nerve fibers; hpf: hours post fertilisation; dpf: days post fertilisation; ENS: enteric nervous system

In adults...

...the inhibitory neuropeptides VIP and PACAP, and TH were detected in nerve fibers.

...the other neuronal markers were found in nerve cell bodies.

...the proportion of neurons expressing a neuronal marker was mostly equally distributed along the gut. Only the proportion of 5HT-IR neurons decreased significantly towards the end of the gut.

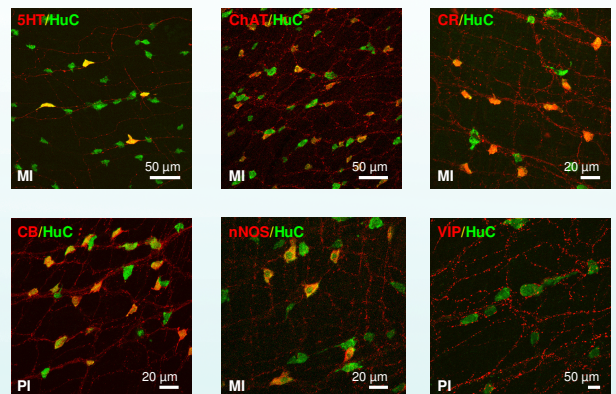
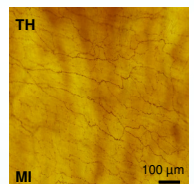
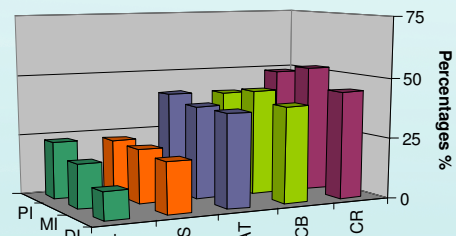


Table : Proportions of neurochemical contents expressed in the ENS of adult zebrafish



	PI	MI	DI
5HT	23,55 *	18,37 *	11,45 *
nNOS	22,8	22,35	21,07
ChAT	41,18	38,35	38,48
CB	40,5	43,59	39,7
CR	48,63	52,27	44,41

*p<0.05

Conclusion

The present results indicate that already at the beginning of enteric neuronal differentiation, several neuronal markers, mainly markers of inhibitory innervation, are expressed in the enteric nervous system. These neuropeptides are suggested to play a significant role in the spontaneous motility activity of the gut observed between hatching (2-3 dpf) and the first feeding (5-6 dpf). The results also support previous data that the enteric nervous system is well-developed before the onset of feeding. In the intestine of the adult zebrafish, the results are indicative of the presence of several populations of enteric neurons and of the existence of regional differences linked to the functions of the three intestinal segments.