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Cardiac Neuroanatomy and Chronotropic Modulation of the Adult Giant Danio Heart

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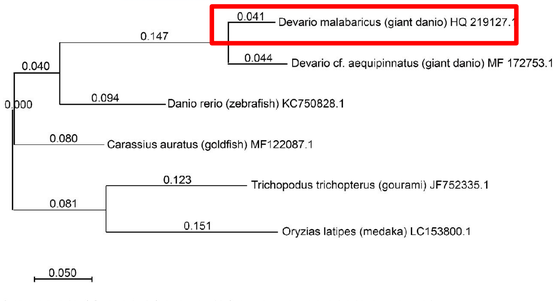
Abstract

Studies in non-mammalian models species have contributed significantly to our understanding of the biology and the nature of innervation in the heart. The giant danio (*D. malabaricus*) is a teleost fish species closely related to zebrafish, that is also capable of heart regeneration. We previously described the development and maturation of the giant danio (GD) heart. However, little is known about its innervation. We hypothesized that the pattern of innervation in the GD heart is anatomically and physiologically complex, and that the heart is responsive to physiological modulation similar to that seen in cyprinid fish and mammals. Using various neuronal markers and electron microcopy, we described the presence, distribution, and nature of nerves in the GD heart. Our study shows first that fine intrinsic cardiac nerve fibers are present throughout the heart chambers. Second, nerve soma and ganglia are highly concentrated at nerve plexuses located near the sinoatrial (SA) and atrioventricular (AV) junctions. However, the volume density of axonal processes located over the ventral aorta_ is highest over the corpus of the bulbus arteriosus. Third, using an *ex vivo* GD heart preparation, we found that the GD heart responded to both adrenergic and cholinergic agonists, in a manner that mirrors mammalian and teleost hearts. Taken together, our studies show that the GD heart displays complex patterns of innervation, and conserved cardiac physiological responses, and strongly suggest that the GD could be used as a viable model for investigating cardiac biology.

Methods



Animal: Giant Danio (Devario malabaricus)
Tissue Processing: Heart fixed in 4% PFA, Permeabilized in 0.5% Triton X in PBS. Cryoprotection: 30% sucrose, 10 µm
Imaging: Nikon AR Confocal,, Zeiss LightSheet,, Zeiss SEM, Zeiss Apotome, BioTwin TEM, Indus Doppler.

Results

1. Giant Danio Cardiac Development

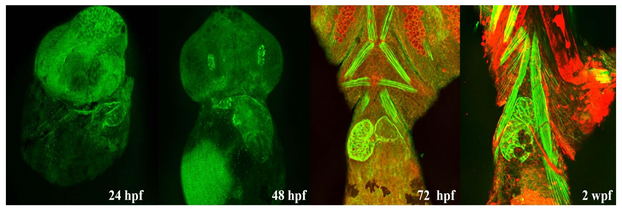


Figure 1. Heart development in the giant danio. MYH1 staining of embryonic and early larval heart.

2. Measuring chronotropic and inotropic response in the GD heart

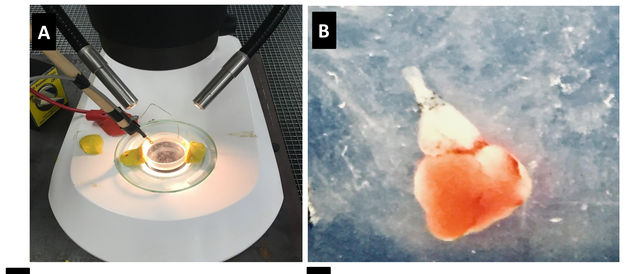


Figure 2. Set up for EKG recordings of Ex vivo giant danio heart (A, B). EKG tracing of control and isoproterenol-treated heart (C), and heart rate measurement with various pharmacological agents (D). (E), Doppler set up for non-invasive measurements of flow velocity through the AV and the bulboventricular valves

3. Innervation of the Adult Heart

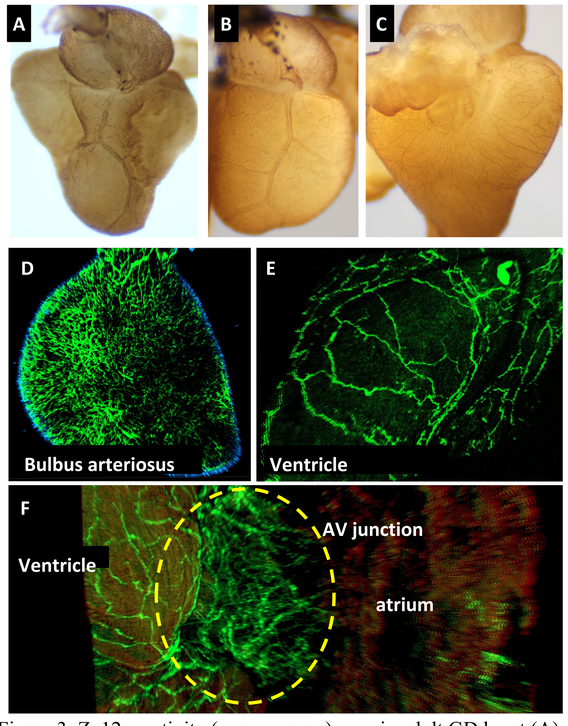


Figure 3. Zn12 reactivity (nerves-green) seen in adult GD heart (A) ventral, (B) side, (C) dorsal, and on the bulbus (D), ventricle (E) and the AV junction (F).

4. Innervation pre-vascularization

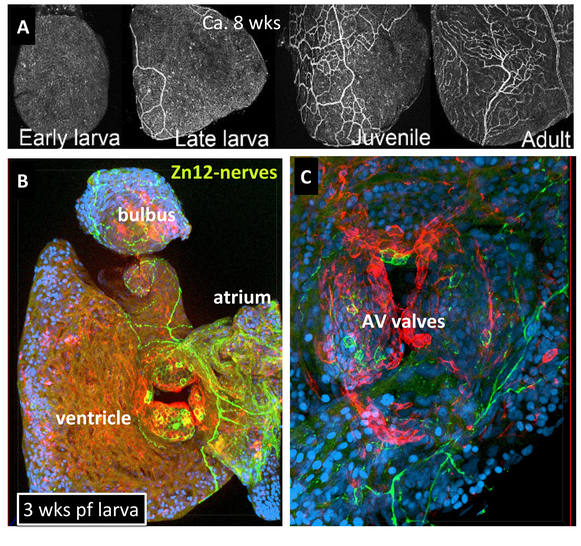
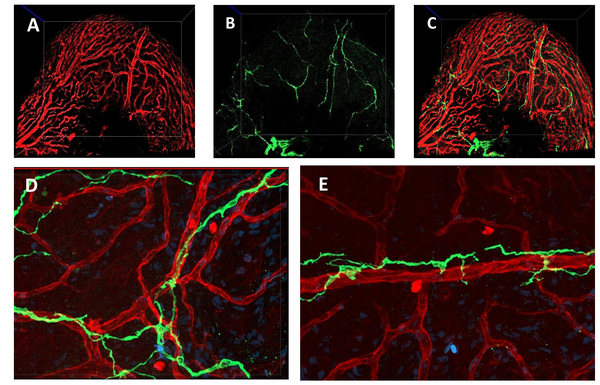


Figure 4. (A) timing of coronary vascularization with BS lectin. (B) Zn12 stained nerves in 3 weeks pf larvae. (C) reactivity (nerves-green) and valve (BS lectin-red) at the atrioventricular junction (3wpf).

Results

5. Neurovascular Patterning



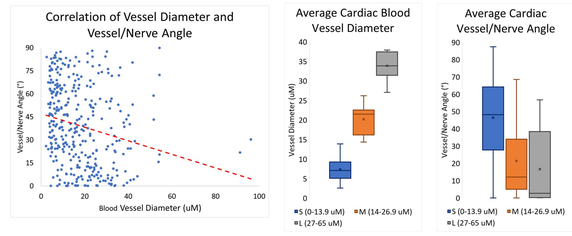


Figure 5. (A) Ventricular coronary vessels stained with BS lectin-red. (B) Cardiac nerves Zn12-green, and (C) overlay of nerves and vessels in a adult GD heart. (D,E) Neurovascular pattern in juvenile GD heart with nerves (green) running alongside large vessels (red). Neurovascular spatial correlation between vessels and nerves in hearts.

Conclusion

- Our findings suggest that the Giant Danio is a viable non-mammalian model for adult heart structure and function studies.
- Our findings suggest that in the fish heart the innervation precedes coronary vascularization.
- We have observed a close spatial correlation between large vessels and nerves.
- We hypothesize that the established innervation pattern in the early larval stage may provide guidance cues to the emerging coronary vasculature.

Acknowledgements

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