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RESEARCH ARTICLE

Is There Volume Transmission Along Extracellular Fluid Pathways Corresponding to the Acupuncture Meridians?

Weibo Zhang [1], Ze Wang [1], Shuyong Jia [1], Yuying Tian [1], Guangjun Wang [1], Hongyan Li [1], Kjell Fuxe [2,*]

[1] *Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[2] *Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden*
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Abstract
Volume transmission is a new major communication signaling via extracellular fluid (interstitial fluid) pathways. It was proposed by the current authors that such pathways can explain the meridian phenomena and acupuncture effects. To investigate whether meridian-like structures exist in fish body and operate via volume transmission in extracellular fluid pathways, we injected alcian blue (AB) under anesthesia into *Gephyrocharax melanocheir*, which has a translucent body. The migration of AB could be seen directly and was recorded by a digital camera. The fish was then embedded and cut transversely to observe the position of tracks in three dimensions. Eight longitudinal threadlike blue tracks were recognized on the fish. The positions of these threadlike tracks were similar to meridians on the human body. Transverse sections showed that these tracks distributed to different layers of distinct subcutaneous loose connective tissues and intermuscular septa. Lymphatic vessels were sometimes associated with the extracellular blue tracks where the migration of AB occurred. Extracellular fluid pathways were found on fish through their transport of AB. These pathways operating via volume transmission appeared to be similar in positions and functions to the acupuncture meridians in Chinese medicine.

* Corresponding author. Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 17177 Stockholm, Sweden.
E-mail: Kjell.Fuxe@ki.se (K. Fuxe).

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1. Introduction

What are the functions and structures of acupuncture meridians? One of the main functions is to transfer biological signals in order to make possible communication between different parts of peripheral tissues and internal organs to maintain the balance between them according to the theory of traditional Chinese medicine. The common mode of communication is through the nervous system, which operates through nerve impulses releasing neurotransmitters into synapses at a very high speed and with accurate point-to-point communication. Blood running in blood vessels can transfer hormonal signals from the endocrine system, which functions at a much lower speed than synaptic transmission and targets its receptors in tissues as long as the circulation is in operation. The third major mode of communication is called volume transmission, which is a new type of communication, the neurotransmitters and modulators of which after release from cells, especially neurons, diffuse and/or flow as soluble molecules or in extracellular vesicles in the tissues. It uses for transport the volume of the extracellular space of tissues, and perivascular and para-axonal extracellular pathways as well as cerebral spinal fluid pathways [1]. The speed is slow and operates via both short and long distances to reach its receptors. A low hydraulic resistance channel along meridians was found by Zhang et al [2] that may play a role of transferring volume transmission signals in peripheral tissue and can logically explain the meridian phenomena [3,4]. The functions, pathological role, and therapeutic impact of this pathway have been studied as well [5].

In meridian research, most experiments were carried out in mammals, the bodies of which are nontransparent. Few investigators tried fish as an experimental model, which has only a distant relationship to humans. *Gephyrocharax melanocheir* is a fish with a translucent body and was therefore chosen as a new animal model. In our previous experiments, alcian blue (AB) dye was injected into this fish species, and several threadlike tracks were found on the fish body in positions similar to the positions of the meridians on the human body [6]. In the current research, a more penetrating study was carried out by observing the channel position in transverse sections to know the true position of the tracks in three dimensions.

2. Materials and methods

2.1. Animals and reagent preparation

Twenty-nine *G. melanocheir* fish, 3.7–4.3 cm in length and 1.6–2.1 cm in width, were purchased from Beijing Chaolaichun flower market and were used as experimental animals. They were kept in a fish bowl filled with water at a temperature of 24°C and given full access to food and oxygen. AB dye (8 GX; Sigma Co., St Louis, MO, USA) was diluted to 1% in distilled water and filtered through 0.22-μm pore size filter paper. Tricaine powder, a widely used fish anesthetic [7], was diluted to 0.03 g/L in water to keep the fish under anesthesia during the experiment. The experiment was approved by the institutional Animal Care and Use Committee (license number: AE20150118-001) of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences.

2.2. Experimental procedure

The *G. melanocheir* was placed in tricaine solution (0.03 g/L) for anesthesia. When the fish body became inclined, it was taken out and fixed on a piece of sponge in a dish filled with tricaine solution. AB was injected into each fish at a single point, wherein 15 fish were injected at a point near the vertebral column whereas 14 fish were injected close to the dorsal fin. Each fish was injected using an insulin syringe needle (0.1 mm in diameter) connected to a microinjector at a 45° angle to the fish body. Then, 20–25 μL AB was infused at a depth of 1 mm and a rate of 2 μL/min via a microinjection pump (KDS-310-PLUS; KD Scientific, Holliston, MA, USA). During the infusion, the narcosis of the fish was adjusted on the basis of whether the fish wagged its tail through a bottle of tricaine solution, which was connected to a bottle of water via a three-way valve. The needle was pulled out after AB has stopped moving and the residual dye on the surface of the fish body was washed away with water. Then the fish was placed in tricaine solution again

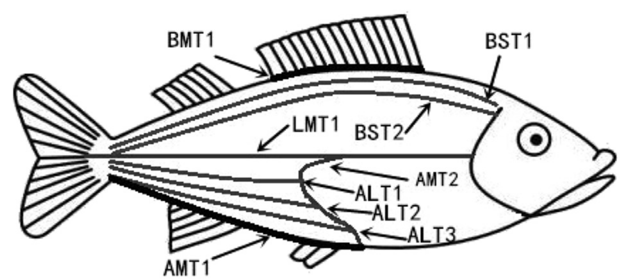

Figure 1 Sketch map of meridian-like tracks. BMT1 is the surface branch of back middle track along the dorsal fin. LMT1 is the superficial part of lateral middle track. BLT1 and BLT2 are the two tracks between LMT1 and BMT1. AMT1 and AMT2 are the two segments of the abdomen middle track. ALT1, AlT2, and ALT3 are three tracks between LMT1 and AMT1. AMT = abdomen middle track; BMT = back middle track; BLT = back lateral track; LMT = lateral middle track.

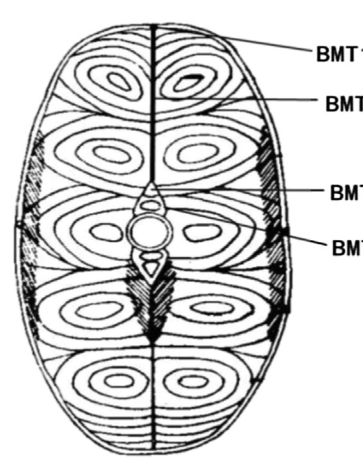

Figure 2 Distributions of BMT1, BMT2, BMT3, and BMT4. BMT = back middle track.