SPINAL MOTOR NEURONAL ORGANISATION OF SELECTED LOCAL FRESHWATER FISHES IN RELATION TO MEDIAN FIN INNERVATIONS

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ABSTRACT

Fish fins are appendages which represent the limbs in higher vertebrates. In general, fish moves in the water by body undulation with the assistance of fins. To date, most of the studies focused on the innervations of limbs especially in mammals, such as rats, cats and monkeys. On the contrary, understanding on the innervations of the fish fin is still at its infancy as studies on the relevant field are relatively scarce. Hence, this study aimed to fill the knowledge gap, i.e. to investigate the organisation of spinal motor neurons in relation to the distribution of the median fins. For comparison purposes, four species of juvenile fishes utilised in this study were divided into two groups, namely (i) fish with long and continuous dorsal fin (*Channa micropeltes*, *toman* and *Clarias* sp., *keli*), and (ii) fish with short and non-continuous dorsal fin (*Mystus nemurus*, *baung* and *Pangasius* sp., *patin*). Spinal cord tissue obtained from three different representative segments along the rostro-caudal axis were processed histologically and stained using three neurohistological staining techniques including H&E, Nissl as well as Lillie's Variant of the Weil-Weigert prior to light microscopy level observation. The organisation of motor neurons was correlated with the distribution of fins. Cell column organisation characterised the location of motor neurons into seven cell columns, namely intermediolateral (IL), dorsomedial (DM), ventromedial (VM), central (C), ventrolateral (VL), dorsoventral (DV) and retrodorsolateral (RDL) cell columns. It was observed that median motor neurons (IL, DM and VM cell columns) outnumbered central (C cell column) and lateral motor neurons (VL, DL and RDL cell columns) at all rostral, median, and caudal region of the fish body trunk. Medial motor neurons (IL and DM cell columns) were found in all three segments; thus, were probably innervating the body trunk, for instance to aid in body undulation. On the contrary, DL motor neurons were found only at the dorsal fin area of *Clarias* sp. and *Pangasius* sp.; thus, were

probably innervating the particular fin. To further investigate the innervations of caudal fin muscles through HRP histochemistry, *Clarias* sp. with rounded caudal fin and *Pangasius* sp. with forked caudal fin were selected from each group. The nerve trunks at the caudal fin were transected and soaked in 50% HRP solution. After two days, each fish was sacrificed and its spinal cord was freeze-sectioned using cryostat. The sections were stained using DAB Liquid Enhanced Substrate kit to reveal the motor neurons containing HRP in the cytoplasm which appeared as brownish precipitations. Most of the motor neurons innervating *Clarias* sp. rounded caudal fin were located medially at IL, DM and VM cell columns. In contrast, the motor neurons innervating forked caudal fin of *Pangasius* sp. were located mostly at the ventral areas, namely VM and VL cell columns. The differences of the motor neuronal organisation between the two species could be credited to further muscle differentiation in the caudal fin muscle layers of *Pangasius* sp., which exhibited more complicated organisation than that of the *Clarias* sp. In conclusion, the findings of this study were in agreement with the existing literature that medial motor neurons (IL and DM cell columns) innervate body trunk while more laterally located motor neurons (VM, VL and DL cell columns) innervate distal structure, in this case the fins. Future research can focus, specifically, on the innervations by individual nerve trunk or innervations of individual muscle and/or the research can be conducted at different stages of life cycle of the fish.

ABSTRAK

Sirip ikan merupakan appendaj yang mewakili anggota badan dalam vertebrata tinggi. Secara amnya, ikan bergerak dalam air melalui pergerakan badan dengan bantuan sirip. Pada masa kini, kebanyakan kajian memfokus kepada pensarafan anggota badan terutamanya mamalia, seperti tikus, kucing dan monyet. Sebaliknya, pemahaman tentang pensarafan sirip ikan adalah masih di peringkat awal oleh kerana kajian dalam bidang yang berkaitan adalah masih kurang. Oleh itu, kajian ini ingin mengisi ruang maklumat berkaitan, iaitu untuk mengkaji/menyiasat hubung kait organisasi neuron motor korda spina dengan kedudukan sirip tengah. Untuk tujuan perbandingan, empat spesies ikan juvenil yang digunakan dalam kajian ini telah dibahagikan kepada dua kumpulan, iaitu (i) ikan dengan sirip dorsal yang panjang dan bersambung (*Channa micropeltes*, toman, dan *Clarias* sp., keli), serta (ii) ikan dengan sirip dorsal yang pendek dan tidak bersambung (*Mystus nemurus*, baung, dan *Pangasius* sp., patin). Tisu korda spina diambil dari tiga wakil segmen yang berlainan sepanjang paksi rostro-kaudal dan diproses secara histology dan diwarnakan menggunakan tiga jenis kaedah pewarnaan neurohistologi, iaitu *H&E, Nissl* dan *Lillie's Variant of the Weil-Weigert* sebelum penelitian tahap mikroskopik cahaya. Organisasi neuron motor dikaitkan dengan kedudukan sirip-sirip ikan. Organisasi kolum sel mencirikan lokasi neuron-neuron motor kepada tujuh kolum sel yang merangkumi kolum sel *intermediolateral* (IL), *dorsomedial* (DM), *ventromedial* (VM), *central* (C), *ventrolateral* (VL), *dorsoventral* (DV) dan *retrodorsolateral* (RDL). Didapati bahawa neuron motor median (kolum sel IL, DM and VM) melebihi neuron motor tengah (kolum sel C) dan lateral (kolum sel VL, DL and RDL) di ketiga-tiga bahagian rostral, tengah dan kaudal tubuh badan ikan. Neuron motor median (kolum sel IL dan DM) ditemui di ketiga-tiga segmen tersebut; dengam itu berkemungkinan mengawal tubuh badan, contohnya membantu

pergerakan undulasi badan ikan. Sebaliknya, neuron motor DL hanya didapati di kawasan sirip dorsal *Clarias* sp. dan *Pangasius* sp,; maka berkemungkinan mensarafi sirip ikan tersebut. Untuk mengkaji pensarafan otot sirip kaudal secara lebih mendalam dmelalui *HRP histochemistry*, *Clarias* sp. dengan sirip kaudal berbentuk bulat dan *Pangasius* sp. dengan sirip kaudal yang bercabang telah dipilih dari setiap kumpulan. Cabang utama saraf yang terdapat di sirip kaudal ikan telah dipotong dan direndam di dalam larutan *HRP* 50%. Selepas dua hari, setiap ikan tersebut dikorbankan dan korda spinanya telah dikerat nipis secara beku mengunakan kriostat. Keratan nipis tersebut diwarnakan dengan menggunakan *DAB Liquid Enhanced Substrate kit* untuk menyerlahkan neuron motor yang mengandungi *HRP* di dalam sitoplasma sebagai pemendakan berwarna perang. Kebanyakan neuron motor yang mensarafi sirip kaudal bulat *Clarias* sp. terletak di kawasan median pada kolum sel IL, DM dan VM. Sebaliknya, neuron motor yang mensarafi sirip kaudal bercabang *Pangasius* sp. kebanyakannya didapati di kawasan ventral, iaitu kolum sel VM dan VL. Perbezaan organisasi neuron motor antara kedua-dua spesies berkemungkinan disebabkan oleh proses pembezaan lanjut lapisan otot sirip kaudal *Pangasius* sp. yang mempamerkan organisasi lebih kompleks berbanding dengan yang terdapat pada *Clarias* sp. Secara kesimpulannya, penemuan kajian ini adalah sama dengan bahan-bahan literasi yang sedia ada, bahawa neuron motor median (kolum sel IL dan DM) mensarafi tubuh badan manakala neuron motor yang terletak secara lebih lateral (kolum sel VM, VL dan DL) mensarafi struktur distal, iaitu sirip ikan dalam kes ini. Kajian masa depan boleh memfokus, khususnya, kepada pensarafan oleh cabang utama saraf individu atau pensarafan otot individu dan/atau kajian boleh dilaksanakan pada peringkat yang berlainan dalam kitaran hidup ikan.

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Kwong Soke Chee

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LIST OF SYMBOLS AND ABBREVIATIONS

c	caudal
C	central
CC	central canal
d	dorsal
DAB	3,3'-diaminobenzidine
DL	dorsolateral
DM	dorsomedial
H_2O_2	hydrogen peroxide
HRP	horseradish peroxidase
IL	intermediolateral
l	left
m	midline
MS222	tricaine methanesulfonate
NBF	neutral buffered formalin
r	rostral
RDL	retrodorsolateral
TBS	Tris-buffered saline
v	ventral
VL	ventrolateral
VM	ventromedial
µ	micro