CHAPTER 5

5.0 GENERAL DISCUSSION

The four fishes studied represented rostrally and caudally oriented cerebellum. There were some similar features seen across all four and also within specific orientation; both from gross morphological and neurohistological aspects. For example, cerebellum of all four fishes remains a dorsally located non-convoluted hindbrain structure. The cerebellum is attached to the dorsal part of the brainstem. Their swimming pattern also showed not much difference between each other. Thus, the morphological features of cerebellum looked at in this study did not reflect the swimming pattern of fish, although the cerebellum is known as a movement's coordinator. Neurohistologically, all four fishes cerebellar tissue displayed peripherally located cerebellar cortex with intensely stained granular layer making up the innermost area and the white matter contained fibers of afferent and efferent pathways. From the results obtained in Chapter 2, 3 and 4, the findings apparently showed that all four fish species also portrayed specific features either gross morphologically and/or neurohistologically.

Most of the established histological studies were done on fishes with caudally oriented cerebellum such as goldfish (Ikenaga et al., 2002; Ikenaga et al., 2005; Hans et al., 2006), zebrafish (Bae et al., 2009), medaka (Yuji et al., 2010), mormyrid (Meek et al., 1986; Campbell et al., 2006; Campbell et al., 2007). Prior to this study, no published literature was found regarding the histology of rostrally directed cerebellum, although Bass (1982) reported that the cerebellum among teleost species have various form and size (Hans et al., 2006). Moreover, none of them ever mentioned the orientation of the cerebellum studied. Thus, it is advisable to state the orientation of cerebellum being studied in future works so that readers would not be misunderstood with the assertion that cerebellum only has one orientation.

All four fishes exhibited corpus cerebelli, valvula cerebelli and eminentia granularis. This is in agreement with other studies which stated that the fish cerebellum has similar three subdivisions (Pouwels, 1978; Glickstein and Jan, 1998; Ikenaga et al., 2006; Campbell et al., 2007; Gunther, 2008; Yuji et al., 2010). These three subdivisions determined the morphology and function of that cerebellum (Hans, 2006). However, the valvula cerebelli could only be observed histologically. It protruded from the corpus cerebelli and located beneath the optic tectum, as was reported by Sarnat and Netsky (1981). In contrast, human cerebellum is composed of the cerebellar hemispheres and a midline structure called vermis, which is known as corpus in fish cerebellum. The vermis is considered homologous to corpus cerebellum (Ikenaga et al., 2006). Corpus cerebellum was identified as the largest structure in fish cerebellum as seen in this study, while vermis is the smallest part in human cerebellum. However, there is a dispute regarding the equivalent of corpus cerebelli in human. This is because according to Pansky and Allen (1980), anterior and posterior lobes of human cerebellum comprise the corpus cerebelli, on the contrary to the statement by Ikenaga et al. (2006).

There are conflicts amongst researchers regarding the name given to one of the subdivisions. The structure referred to as eminentia granularis in this study has also been referred to caudal lobe (Campbell et al., 2007; Yuji et al., 2010), auricles (Glickstein and Jan, 1998), vestibulolateral lobe and flocculus in tetrapods (Ikenaga et al., 2006). One of the works on caudal lobe of mormyrid, an electroreceptive teleost, was by Campbell and co-researchers (2007). Their findings showed that the eminentia granularis is different from caudal lobe as the eminentia granularis gives rise to the parallel fibers of the posterior caudal lobe. In addition, the histological feature of the posterior caudal lobe is irregular compared to that of the corpus and valvula, and there were less apparent organization of the cerebellar elements. According to Hans et al. (2006), the eminentia granularis forms visible bulges at the caudal lateral part of the cerebellum. As normally seen in many teleost species, a molecular layer is continuous with that of the cristae cerebellaris. The finding in this current study is in agreement with the report. Nevertheless, the eminentia granularis gross morphology in caudally oriented cerebellum of *jelawat* and *tilapia* is hardly observed compared to the one in rostrally oriented cerebellum of *keli* and *baung*, which is more visible and easy to differentiate. The existence of the eminentia granularis was affirmed by histology.

The cerebellum of mormyrid fish exhibits unusual gross morphology and histology. Other than its huge size, its valvula cerebellum was easily observed compared to other fishes because it has ‘pushed’ the tectum aside to cover the entire surface of the brain (Glickstein and Jan, 1998). Larsell (1967), quoted by Hans et al. (2006), said that the most rostral and posterior portions of the cerebellum were formed by the valvula and vestibulolateral lobe, respectively. However, this fact is not applicable for rostrally directed cerebellum in this study because the valvula only comprised of the ventral portion of the cerebellum, as it extended anteriorly from the corpus cerebelli. Thus, in cerebellum work, it is important to state the orientation of the cerebellum being studied to avoid misleading readers.

There have also been some studies that differentiate the corpus cerebelli into lobes, for example, the one done by Meek et al. (1986). *Gnathonemus petersi*, a weakly electric fish exhibits a highly developed cerebellum that is divided into four lobes, numbered C_1 to $C_{4.}$ The basic cellular organization of teleost cerebellum was found to be similar with that in mammals. The teleost cerebellums also consist of three layers, namely outermost molecular layer, Purkinje layer and innermost granular layer, even including the fibres. This is in agreement with the findings in mammals (Ottoson, 1983).

There are questions about the relationship between the sizes of brain and ‘behavioral complexity’. Although human in general possess more complex behaviors than the elephants and whales, yet the brains of elephants and whales are 4 to 5 times the size of a human being’s brain. However, the animal’s brain size in proportion to its body size must still be taken into account because an animal with a larger body surface will definitely need larger sensory areas in its brain (Dubuc, 2002) and sensory input will definitely affect behavior.

Actually the differences in the evolutionary development of specific parts of the brain have greater effect on behavior than brain size does. For example, the cerebellum which plays important role in coordinating movements is reported to have a constant percentage value when its weight is compared to the brain’s total weight in all mammals (Dubuc, 2002). The fishes that have large corpus probably exhibits the well-coordinated truncal movements in swimming, chiefly in changing direction and compensating for shifting water currents (Sarnat and Netsky, 1981). This is corroborated by the *jelawat* studied which had the highest mass of cerebellum and displayed the forked caudal fin. These criteria lead to the assertion which was reflected physiologically that *jelawat* was the fastest swimmer compared to the other three fishes studied.

Apparently, there are various different factors that influence the brain size; from neuron number, the size of the dendritic fields, the complexity of the circuitry linking them, and the processing arrangements required for a big amount of sensory input. Mantas have enlarged cerebellum and they demonstrate very maneuverable swimming behavior for their size. It is possible to say that the enlarged cerebellum might be due to the requirement to process information from the proprioceptors regulating their flapping flights. As for the *Mustelus,* it is an active free-swimming shark and has a much more developed cerebellum than the sluggish bottom-dwelling *Scyliohinus,* while *Isurus* which has the most elaborately-folded cerebellum, is a very active short fin mako shark (Bone and Moore, 2008).

As has been traditionally reported in mammal cerebellum (Eccles, 1967) regarding the parallel fibers, mossy fibers and climbing fibers in the cerebellum; these fibers are also present in fish cerebellum (Ikenaga et al., 2002). Thus, the finding in this study about the presence of bundle fibers in the core portion of cerebellum that could be observed clearly in longitudinal section was corroborated by the earlier findings. The somatotopic projection of the various afferent fiber systems upon the cerebellar cortex and the distribution being limited to certain regions of the cerebellar cortex (Sarnat and Netsky, 1981) could not be ascertained in this study.

In all the four fishes studied, it was easy to discern the cerebellar characteristics of *tilapia* from the other three fishes. *Tilapia* with caudally directed cerebellum was the only fish that performed carangiform locomotion. In contrast, *jelawat* with the same orientation of cerebellum, as well as *keli* and *baung* with the rostrally directed cerebellum, performed subcarangiform mode of swimming. Moreover, both *keli* and *baung* displayed the same body shape, which was torpedo-shaped. However, this shape was not displayed by fishes with caudally directed cerebellum, where *tilapia'*s compressed body had wide vertical height compared to *jelawat*. *Tilapia* also had no barbels as seen in other three fishes. From neurohistological aspect, the peculiar neurons that could not be identified as Purkinje, Golgi or eurydendroid cells (see Chapter 4, section 4.2.4.3, figure 4.45) were only observed in *tilapia's* cerebellar tissue. In addition, a 'dome-like' pattern of the densely stained innermost area in transverse section of *tilapia's* cerebellar tissue was observed, in contrast to the other three fishes where the ‘butterfly/bird-like’ with expanding wing pattern was observed. The reasons behind all of these differences in *tilapia* are inscrutable. It is worthwhile for this matter to be further studied.

The locomotion of the fish by means of the undulation of the trunk in propulsion through water seems similar with the rhythmic movements of the trunk in many reptiles. However, due to the additional factors such as greater external forces like shifting water currents, vertical movements and depth control, swimming requires more coordination (Sarnat and Netsky, 1981). In this study, the differences in morphological and histological characteristics of the cerebellum between these four fishes could not be fully correlated to the swimming pattern and body form of the fishes. It is probable that the variation in specific neuronal circuitry activities receiving different stimulations from other parts of the brain for each of the fish was a major factor in determining the feature of cerebellar gross morphology and histology. This speculation however needs to be ascertained by further studies from neuroanatomical and neurophysiological aspects with more representatives from each cerebellar orientation.

Very few researchers were keen on dealing with non-mammalian neural tissues due to factors such as the non-availability of species; they are mostly found in tropical countries. Besides, the intricacy of the processing of non-mammalian neural tissue through histological and histochemical procedures is also one of the factors. There are no standard protocols in delivering satisfactory results that could be exactly replicated across different labs. In addition, less reward is reaped from the labor put into neural studies involving these animals due to the lack of neurohistological details (of the neural tissue) which could be used as reference.