CHAPTER 5: CONCLUSION

Returning to the objectives posed in the beginning of this study (Chapter 1), the following points are corresponded to each of the objective.

1) To describe the gross morphology and general histology of the spinal cord of a locally available frog species, i.e. *Fejervarya limnocharis*.

   The spinal cord of *Fejervarya limnocharis* revealed a typical organisation at the gross morphological level. This cylindrical rostral portion of the central nervous system, in general, displayed gradual reduction in diameter as it descends caudally with two distinct widening or enlargements. They were localised at the cervical and lumbar regions, which most possibly correlated to nerve innervation to the fore- and hindlimbs of the frog, respectively.

   Based on results from neurohistological stainings, i.e. H&E, Thionin and Lillie's variant modification of Weil-Weigert method, a transverse section demonstrated a core grey matter, enclosed by an outer layer of white matter, arranged in the shape of a butterfly. The general architecture of the frog spinal cord varied at different segmental levels. The cervical spinal segment had the biggest diameter owing to the enlargement. They contained the most portion of white matter, which composed of collected ascending and descending pathways, and also high density of large motor neurons. Similarly at lumbar level, spinal sections also displayed enlargement and presence of motor neuron pool. However, the lumbar segment had relatively less white matter than cervical cord since most efferent nerves making up the descending pathway had exited rostrally and afferent nerves started to form the ascending pathway. The segment in between these two enlargements was of the thoracic region.

It had smaller diameter and contained less grey matter. The sacral region, which was located caudal to the lumbar level, exhibited the smallest diameter with the least grey and white matter compositions.

2) To provide cytoarchitectonic information on the spinal cord organisation of *Fejervarya limnocharis* using basic neurohistological staining and immunohistochemistry.

In agreement with Ebbeson (1976), Sasaki (1977), Mensah and Thompson (1978) as well as Adli and coworkers (1988), the central grey matter of the Nissl-stained frog spinal cord could not be strictly addressed based on the widely known Rexed's laminar organisation. Nonetheless, the frog spinal grey cytoarchitectonic subdivision in accordance with the Ebbeson's approach was fairly comparable to the laminar arrangement in a more flexible manner. It was revealed that a homolog region or field was likely to represent one or more laminae, i.e. dorsal field was equivalent to lamina I to IV, lateral field was corresponded to lamina V to VI, ventrolateral field, ventromedial field, lateral motor and medial motor fields were represented by lamina VII, lamina VIII and lamina IX, respectively while central field coincided with lamina X.

On cytoarchitectonic level, the Nissl- and Golgi-stained frog spinal grey matter was morphologically heterogeneous with neuronal cells differing in shape and size. The identifiable neuronal somas were primarily categorized based on four major shapes with highest average population proportion in the respective order: spindle, triangular, polygonal and tear. However at the level of analysis, there was no specific pattern of cell arrangement to accurately define the investigated fields.

Nonetheless, the frog spinal cord tissue architecture showed a strong resemblance to those of higher vertebrates (mammals, reptiles and birds) on a general basis. However, a certain degree of differences in the cytoarchitecture between frog and other amniotes was often traced back to the nature of its spinal grey that exhibited poor differentiation in neuronal cell organisation**.** Thus, neurobiological research in amphibians and other non-mammalian vertebrates should be continuously explored following the unresolved issues in the cytoarchitectonic organisation of the frog spinal cord. For example, work involving localization of NeuN (or Neuronal Nuclei), a neuron-specific nuclear protein in the spinal cord to further substantiate the neuronal characterization and distribution.

3) To delineate the distribution of selected nociception-related neurotransmitters (enkephalin, substance P and serotonin) in the frog spinal cord via immunohistochemical techniques

From the immunohistochemical analysis, the immunoreactivities of the nociceptive-related neurotransmitters; ENK, SP and 5HT were identified in the frog spinal cord, particularly in areas within the dorsal and lateral field of grey matter. ENK-like immunoreactive cell bodies were demonstrated in the mediolateral band of grey matter while none of such for SP and 5HT at all segmental regions. The ENK- and SP-like immunoreactive fibres and varicosities were found richest along the mediolateral band that was located at the base of the dorsal field to the lateral field. On the contrary, those of 5HT were concentrated at the dorsal field. While the distribution pattern of 5HT was generally consistent to that reported in other amniotes, those of ENK and SP were almost exclusive to frogs (Lorez and Kemali, 1981; Adli et al., 1988; Partata et al., 2002). The differences in the neurotransmitter labeling patterns noted between frogs and other animal species may be accounted for the different cytoarchitectonic organisation of the spinal cord. The locations where these three neurotransmitters were present suggest their potential roles in the integration of nociceptive transmission. Their distributions in frog spinal cord, especially in the dorsal horn, the site of afferent nerve terminals (Adli et al., 1988), reflected the possible involvement in spinal nociceptive effects of SP and antinociceptive action of serotonin and enkephalin, as similarly observed in mammals. Their widespread occurrence of positive immunoreactivities therefore, allowing ENK, SP and 5HT to be considered as useful candidates for physiological studies of their functions or actions in the frog spinal cord.

On the last note, it should be stressed that this current study only allowed correlation of spinal cord information between frogs and other animal species to be made. Due to the limitation and constraints of techniques used, future supplementary scientific advances are required to address the remaining obscure issues of the frog spinal cord, including the detailed aspects of spinal cord cytoarchitecture and hodology, as well as the other nociceptive-related neurochemical pathways and the mechanisms involved. It is also of interest to determine the occurrence and distribution of neurotransmitters within the spinal cord in different animal species before extrapolation on such data to be made from one species to another. However, the current findings support the idea that basic pattern in the spinal cord are present throughout the vertebrates, thus reinforced the potentials of using non-mammalian neural system as model for neurobiological studies.