1 General Introduction

1.1 Background and History

The adaptation to terrestrial life by amphibians involved the development of limbs and the central nervous system modifications subserving tetrapod locomotion. Although some amphibians are limbless (Gymnophonia), and other possess rudimentary extremities (e.g. Amphiuma), anurans and most urodeles have developed fairly large extremities. The extensive muscle mass and surface area of the limbs have been underwritten by enlargements of the spinal cord at those segmental levels concerned with afferent and efferent innervation. The spinal cord anurans manifests, on the whole, a somewhat higher degree of differentiation than that of urodeles and the intumescentia cervicalis and the intumescentia lumbalis are conspicuous features in the frog.

Because of its relative shortness (11 segments), “typical vertebrate” organization, and accessibility, the frog spinal cord has become a model system for the study of a variety of spinal mechanisms. Although more than eighty years ago, SALA (1892) published the first comprehensive anatomical observations on the frog spinal cord based on Golgi preparations, most of our information has been obtained in the last ten years. Except for a rare thorough study, such as CRUCE (1974a, b), the available literature contains little in depth analysis of the frog spinal cord, most studies being superficial examinations of one component at a time. While there is available today a considerable amount of data on many features of cord organization, many important aspects have received little attention. The existing information about spinal afferents, for example, is complete as far as the light microscopic level of analysis is concerned, but almost nothing is known about efferent projections to the brain or the source of such fibers. Golgi studies are limited to less than a handful and these are not systematic in terms of characterization of cell types at various spinal levels. Although the data is decidedly fragmentary, an attempt is made in this Chapter to tie together the available pieces and, by adding new material from our own laboratory, provide an overview of the ranid spinal cord.

To this end, the data on spinal afferents, and cytoarchitecture, has been used to subdivide the grey matter into architectonic fields similar to those described by REXED (1964) in the cat. A Golgi study was undertaken to elucidate that aspect of the Chapter. A study on the ascending spinal projections was also completed (EBBESSON, 1969) and included.

This Chapter deals primarily with data from the genus Rana; *Rana catesbeiana* and *Rana pipiens*. As far as we can tell, these species have nearly identical spinal cords. Except for variation in cord length among tailless amphibians, there appears to be no substantive interspecific variation in basic spinal organization. It is important, however, that generalizations not be made from one or two species and it must be emphasized that the choice of experimental species remains an important variable when comparing the details of ranid cord organization.

1.2 Plan of the Chapter

The basic organization of the ranid spinal cord will be discussed first. This will include examining gross morphology with an analysis of normal sections through various levels of the cord stained with the Nissl and Golgi methods. Cell groups will be described along with dendritic fields of cells within these various spinal cord nuclei, emphasizing their possible relationships to afferent projections. Spinal afferents will be discussed in the Section 2 and the ascending projections will be covered in the third. Sections 4 and 5 are brief reviews of some recent histochemical and neurophysiological studies respectively.

1.3 Material and Methods

While this Chapter is to a large degree a review of the literature, we have included data from two unpublished experiments. One is a Golgi study of the spinal cord in adult specimens of *R. catesbeiana* and the other is a study of the ascending spinal projections in the same species. For the Golgi-Cox impregnations we followed the technique of RAMON-MOLINER (1970), as well as a modification which was suggested to us by CLAIRAMBault.
A total of 19 adult frogs (R. catesbeiana) were used in the study on ascending spinal projections. Spinal cord hemisections were made at levels between the calamus and the IVth spinal nerves. A 10% solution of Tricaine methanesulfonate (Sandoz MS-222) administered intramuscularly was used for anesthesia. Lesions were made with a Van Graef cataract knife under direct observation.

The frogs were kept in a group tank where the water temperature ranged from 24° to 26° C and air temperature from 21° to 23° C. The animals were killed 10 to 28 days after the lesion with an overdose of anesthesia and perfused through the heart with normal saline followed by 10% formalin in saline. The brain and spinal cord were immediately exposed and stored in situ with the dissected cranium and vertebral column in 10% formalin for 1–2 months before processing.

The entire brain and spinal cord were embedded in egg yolk (Ebbesson, 1967) before transverse sections were made on the freezing microtome at 33 µm. Every 6th section was prepared according to a Uranyl nitrate modification of the Nauta method (Method 6 in Ebbesson, 1970b). Other adjacent sections were stained with a modification of the Fink-Heimer method (Method 7 in Ebbesson, 1970b). A third set of adjacent sections were stained for cell bodies with cresylecht violet (Fernstrom, 1958).

The patterns of fiber and terminal degeneration were recorded on projected drawings of the selected silver sections, and are illustrated in Figures 20–31. In the drawings, fibers of passage are indicated by large dots or broken lines and loci of terminal degeneration by small dots. The corresponding adjacent Nissl sections were photographed with Kodalith Ortho high contrast film (Ebbesson and Rubinson, 1971). Reversed enlargements were made to match the drawings and are shown on the left of Figures 20–31.

2 The Basic Organization

2.1 Gross Features

The ranid spinal cord is connected with each of the eleven pairs of spinal nerves by means of a small ventral and a large dorsal root (Figs. 1 and 2). The spinal ganglia are found in the dorsal roots to which their cells give origin. Cervical and lumbar enlargements occur in the cord in relation with the large roots of the nerves that supply the upper and lower limbs. The lower end of the cord is reduced to a slender cone and is relatively long in anurans and contains a rudimentary central canal and nondescript neuroectodermal cells, mostly of glial type. This represents the reduced remnant of the cord’s caudal portion which, before metamorphosis, innervated the musculature of the tail at larval stages.