PREFACE

In this book the authors present current research in the study of frogs. Frog's neuromuscular junction (NMJ) is a classic and favorite object which have played a leading role in developing understanding of the basic mechanisms of synaptic transmission and secretion of neuromediator. The genetic diversity, neural development and ecological implications of frogs are examined throughout the book. Some of the topics the authors discuss in this compilation include poison storage and maturation in serous cutaneous glands of anurans; a mini review of the suppression of anuran metamorphosis by synthetic chemical compounds; mechanisms of suprathreshold excitation of a frog tectal neuron column by discharge of a single moving edge or darkness detector and their relation to a frog escape reactions; physiological features of blood's system of frogs rana ridibuda pall; and presynaptic voltage-dependent calcium channels at the frog neurmuscular junction.

Chapter 1 – The functional cycle of serous (poison) glands in anuran skin is divided into three phases: biosynthesis, accumulation (or storage), and secretory release. The intermediate phase of poison storage has peculiar traits: it occurs in the syncytial cytoplasm of the gland secretory unit, and may be long-lasting, if encounters with natural predators are infrequent, and serous product release is delayed. Intracytoplasmic secretory deposits have limiting membranes derived from the Golgi apparatus and are classified as vesicles or granules, according to the varying density of their contents. During storage, secretory deposits undergo marked rearrangements in their structures and composition, usually regarded as post-Golgian maturational processes. Serous maturation involves consistent events: vesicle-vesicle and granule-granule merging, as well as interaction between granules and syncytial cytoplasm. Merging processes tend to homogenize serous products by blending various

molecular pools contained in secretory deposits. This maturational activity involves degradation of adhering plasma membranes, pertaining to contiguous vesicles/granules. Secretory granule-syncytium interactions allow exchange of molecules between the two compartments, and entail amplification of the surface of the membranes bounding the granules, in order to enhance molecular flow. Exchange surface increases through two main processes: enlargement of the halo between the secretory product and the limiting membrane of the granule, and/or formation of microvillous-like processes, jutting from the cytoplasm into this translucent compartment. Ultrastructural maturation features reveal marked variations of the aggregation of the poison, ranging from dilution to condensation. As observed in species from several genera, secretory granules may acquire similar ultrastructural traits through condensation, reflecting either maturational programs shared by phylogenetically related taxa or convergent evolution. In some families, condensation causes the secretory granules to have a recurrent substructure that results from a close association of modular subunits (spheroidal or rod-like in shapes) with an average diameter of 90-100 nm. As a whole, ultrastructural findings collected from Old and New World anurans update concepts of serous maturation, which were originally developed by pioneering authors using light microscopy. Key activities in this fundamental phase of poison manufacturing are found in the syncytial cytoplasm that exchanges molecules with the serous product, and the secretory granules that contribute to maturation as active organelles, providing a finely tuned microenvironment.

Chapter 2 – Studies have shown that a large variety of medicines and other chemical pollutants being released into the environment have, in fact, a disruptive or detrimental effect on normal anuran development and health. In this paper the authors survey the literature pertaining to a number of such commonly used synthetic compounds (Cyclosporin A, acetyl-L-carnitine, bisphenol A and related compounds, phthalates and their derivatives, perchlorate and UV filters) in an attempt to better understand the mechanisms underlying such disruption.

Chapter 3 – It is known that a frog retina moving edge and darkness detectors participate in the detection of potentially dangerous objects (cue detection) and may lead to escape reactions. In their studies, the authors had explored features and origins of the response elicited in a frog tectum by burst discharge of a single retina ganglion cell – moving edge or darkness detector.

The experiments were done *in vivo* on frogs *Rana temporaria*. An individual retina ganglion cell (or its retinotectal fiber) was stimulated by current pulses delivered through multichannel stimulating electrode positioned

on the retina. Responses to a discharge of a single retinal ganglion cell were recorded in the tectum by an extracellular carbon-fiber microelectrode positioned in the terminal arborization of the retinotectal fiber in the tectum layer F. Results of the experiments have demonstrated that bursts of spikes of a single retina ganglion cell activate an elementary neuronal network of the tectum (tectum column) containing 6 excitatory neurones: 5 recurrent pear-shaped neurones and 1 efferent pyramidal cell. Two distinct levels of activation of the tectum column by discharging of an individual retina ganglion cell can be distinguished, depending on the strength of discharge. The burst of moderate intensity (2–3 spikes at interspike intervals of 6–10 ms) elicits a lower level of activity of the tectum column, characterized by the persistent excitation of the recurrent pear-shaped neurons due to activation of the persistent L-type Ca^{2+} current in dendrites of those neurons. The lower activity level is reflected in the recordings of the tectal activity by a slow negative wave, generated by the dendritic L-type Ca^{2+} current, and by recurrent excitatory synaptic potentials superimposed on that wave, generated by a persistent firing of the recurrent pear-shaped neurons. The burst of high intensity (4 or more spikes at interspike intervals of 6–10 ms) elicits a higher level of activity of the tectum column, characterized by a suprathreshold excitation of efferent pyramidal neuron of the tectum column due to the NMDA receptor activation. The higher activity level is reflected in the recordings of the tectal activity by a slow negative potential, generated by an activation of the NMDA receptor currents, and by monophasic negative or sometimes biphasic negative-positive spikes superimposed on that potential, generated by a firing of the efferent pyramidal neuron of the tectum column. Activation of the dendritic L-type Ca^{2+} currents is necessary for the activation of the NMDA receptors. That is, the intrinsic recurrent excitation of the tectum column (the lower level of activity) determines transition of the tectum column to the higher level of activity. As the efferent neuron of the tectum column sends the axon to the motor nucleus, its suprathreshold excitation can lead to a frog escape or danger avoidance reactions.

Functional (behavioural) significance of the two levels of activity of the tectum column is considered. The lower level may implement the mechanism of a short-term memory about potentially dangerous stimulus. The higher level, then, implements a mechanism of danger avoidance reaction leading to a crouch or jump.

Chapter 4 – Physiological features of blood's system of frogs *Rana ridibunda Pall* is seasonal cyclicity of hematopoietic activity and maturation of cells in peripheral blood channel. In the current study is investigated

histological picture of hematopoiesis centers and morphology and age structure of circulating erythrocytes in to the blood channel according to the electrophoretic mobility of the cells with into account changes of the seasonal activity of hematopoiesis.

Hematopoiesis of frogs occurs in to the bone marrow, spleen, and liver, wall of the bowel and also in peripheral blood. In the blood system of frogs is some redistribution of function due to the different centers of hematopoiesis and heterogeneity of cells populations. According obtained data the base type of hematopoiesis for *Rana ridibunda Pall* is splenic-medullary. The role of born marrow is dominant; it is found all the sprouts of hematopoiesis as in the period of activation and also at the damping of hematopoiesis. The main place of localization of erythropoiesis in the organism of *Rana ridibunda Pall* is bone marrow, spleen and liver. Lymphopoiesis is concentrated mainly in the spleen. Progenitor cells of granulocytopoiesis are found in small amounts in to the bone marrow and spleen. It is revealed differences in the proliferative activity of erythroid cells in the different of seasonal periods. Proliferative activity of progenitor cells of erythropoiesis is decreased in a bone marrow in the period of attenuation of hematopoiesis and it is increased in to the liver in spring-summer period.

In the peripheral of blood of frogs was observed the polymorphism of cells form and their maturation. In the period of attenuation of hematopoiesis the number of progenitor of erythro- and granulocytopoiesis was decreased. The forms of lympho- and thrombocytopoiesis in the peripheral channel were not identified. In the blood channel of frogs was observed the erythrocytes without nucleus, cells forms with cytoplasmic processes and nucleus on the stage of amitosis. In the period of physiological anabiosis the numbers of old and worn-out functionally forms of erythrocytes in the peripheral blood was decreased as compared with periods of activity and attenuation of hematopoiesis. While during spring-summer activation of hematopoiesis the percentage of old and worn-out forms were increased. Analyzing the seasonal dynamics of indicators of blood are marked more stable indicators of male compared with female. Sec differences of hematological parameters are more expressed in the time of the outbreak of hematopoiesis.

The current research has important practical meaning and it is aimed on the study of mechanisms of adaptation to seasonal changes of environment. By seasonal observations is studied of histology pictures of liver as additional center of hematopoiesis in the spring-summer period and analyzed quantitative changes and proliferative potential of progenitor cells to the preparing of

anabiosis and established of dynamic of proliferative activity of hematopoietic cells in the bone marrow, spleen and liver.

Chapter 5 – Calcium ions entering the nerve ending through voltage-dependent calcium channels (VDCC) during action potential generation initiate neurotransmitter release and thereby play an important role in the synaptic transmission of excitation. It is commonly accepted that, in contrast to central nervous system, where neurosecretion is mediated via the opening of various types of voltage-dependent calcium channels, only one type of calcium channels (N or P/Q) is involved in exocytosis into the peripheral nervous system of adult vertebrate animals. In frog synapses, these are N-type channels ($Ca_V2.2$), which are blocked by ω-conotoxin GVIA. However, there is data indicating possible participation of other types of presynaptic calcium channels in the regulation of exocytosis in peripheral synapses of cold-blooded animals. The authors' investigations have shown the existence of different types of VDCCs in the frog neuromuscular junction and indicated their possible role in the modulation of both the amount of neurotransmitter quanta released in response to a nerve impulse and the time course of their secretion. Using immunohistochemistry, the authors detected the expression of α_{1A}, α_{1B}, α_{1E}, α_{1G}, α_{1H}, α_{1I}, α_{1D}, α_{1F} subunits of P/Q-, N-, R-, T-, and L-type VDCCs. Using fluorescent and electrophysiological techniques the authors have demonstrated that specific Ca^{2+}-channel blockers reduced the action potential-induced calcium transient and decreased spontaneous and evoked neurotransmitter release. Blockage of high-threshold Ca^{2+}-channels resulted in synchronization of the release kinetics. The authors' data indicate the functional expression of voltage-dependent calcium channels of N-, P/Q-, L-, R- and T-types participating in the modulation of the quantal acetylcholine release from frog motor nerve endings.