Preface

Advances in methodologies and experimental models are pivotal to furthering our understanding of central nervous system (CNS) functions in mammals. A most important technology in that regard is "patch-clamp," which was originally developed for monitoring currents through single ion channels in "cell-attached" or "excised patch" configurations. However, current neuroscience studies use patch-clamp primarily for the analysis of membrane potential changes or underlying ion currents in the "whole-cell" mode, with concomitant (defined) dialysis of the cytoplasm, while the "perforated" patch configuration can be applied to retain an intact cellular milieu. Patch-clamp techniques were adapted about two decades ago for studying CNS cells in their natural in situ environment and have since mostly replaced technically more challenging sharp microelectrode recording. Similarly, exciting advances were established for optical approaches using fluorescent dyes, e.g., for monitoring membrane potential, mitochondrial potential, or cytosolic pH. Yet, the most common optical approach is to visualize dynamic changes of the pivotal cytosolic "second-messenger" Ca^{2+}, either in single CNS cells or neural networks that are comprised of neurons and neighboring (micro)glia. Compared to initially used (charge-coupled device) video cameras, optical spatial resolution is notably improved by confocal microscopy while multiphoton imaging allows visualization of cells in deeper CNS layers. Besides, infrared differential interference contrast optics are a convenient low-cost tool for visually targeted patch recording in tissue depths of up to 150 μm. Recently developed genetic tools enable "knock-out" of particular cellular features, such as glutamate receptor subtypes, or allow expression and subsequent imaging of intrinsically fluorescent Ca^{2+}-sensitive or structural proteins in identified CNS cells. "Optogenetics" makes use of genetically inserted, light-activated ion channels that change the activity of specific cells to reveal their functions. Finally, local expression of cell-type-specific proteins is studied using immunohistochemical approaches or molecular tools such as "western blots" or "polymerase chain reaction" for analyzing cytoplasm of individual CNS cells, obtained by extraction after whole-cell recording, or of homogenized nervous tissue.

Until about 30 years ago, most studies on mammalian CNS functions were carried out using in vivo models of diverse mammalian species, mostly cats and rats, despite the fact that various in vitro CNS models were developed already in the 1950s. It was not until the mid-1970s that in vitro conditions were sufficiently well developed to keep these isolated CNS models, including cultures, en bloc ("slab") preparations, and brain slices viable for several hours in solutions that mimicked the composition of cerebrospinal fluid (CSF) (or rather the fluid in the interstitial space within CNS tissues). At the same time, electrical stimulation and single- or multiunit extracellular recording approaches plus sharp microelectrode intracellular recording techniques, such as "single electrode voltage-clamp," were adapted to these models. Until the end of the last millennium, work on acutely isolated brain slices dominated CNS research with emphasis on studying mechanisms of synaptic plasticity associated with long-term potentiation or depression evoked by afferent axon tract stimulation. In these "classical" brain slices, electrical or pharmacological stimulation

was typically needed for evoking neuronal responses, contrary to often pronounced spontaneous activity of the same cells in vivo. It was believed that this limitation is primarily related to the fact that the thickness of brain slices is mostly less than 500 μm for providing sufficient diffusional supply of cells with the energy substrates oxygen and glucose contained in superfused artificial CSF (ACSF). Consequently, neuronal dendrites and axons are partly sectioned, which presumably attenuates network connectivity and thus depresses spontaneous activity. To circumvent this, various laboratories used mainly newborn rodents already since about 30 years ago to develop en bloc models with active neural networks. During the same time period, others succeeded to keep large CNS aspects (up to entire rodent brains) viable by arterial perfusion. During the last decade, procedures for generating active isolated CNS tissues have improved further, e.g., by using an ionic ACSF composition that reflects more closely that of the fluid in the extracellular space of neural networks instead of that in CSF of the subarachnoid space and brain ventricles.

This contribution to the *Neuromethods* series exemplifies the application of a majority of the above-mentioned and other technologies to mostly active in vitro preparations from basically different CNS regions with a diversity of functions. Specifically, Chapter 1 by *Trapp and Ballanyi* deals with neurons in rodent brainstem slices that control vagal outflow. It outlines how "tonic" activity of these cells is modulated by metabolic processes and how underlying mechanisms are studied with single channel and whole-cell patch-clamp techniques, gas- and ion-sensitive microelectrodes, optical photomultiplier-based techniques, and diverse molecular approaches. Further emphasis is on how methods for slice generation and storage plus superfusate composition affect properties of these vagal neurons and neurons in general. Chapter 2 by *Ruangkittisakul et al.* delves deeper into the latter topic by pointing out the particular importance of superfusate K^+, Ca^{2+}, and glucose, and also of physical dimensions of newborn rodent en bloc and slice preparations for spontaneous activity of respiratory neurons in the lower brainstem. Further, they exemplify that whole-cell and suction electrode recording (for neuronal population activity) combined with multiphoton/confocal Ca^{2+} imaging is used for investigating contributions of neurons *versus* glia to respiratory rhythm. Chapter 3 by *Moore et al.* summarizes techniques for studying (spontaneous) activity in neurons of human fetal cortex slices, focusing on how slices of this almost gel-like tissue can be generated and how developing electrical properties such as immature Na^+ action potentials can be discriminated from imperfect whole-cell recording conditions in these delicate cells. Chapter 4 by *Fish et al.* deals with histological characterization of physiologically determined fast spiking interneurons in slices of the dorsolateral prefrontal cortex of monkeys. It outlines particularly how high-resolution confocal imaging is combined with sophisticated optical analyses for elucidating structure-function relationships for these cells. Chapter 5 by *Nakamura et al.* describes neural networks in the suprachiasmatic nucleus of the hypothalamus that continue to generate circadian rhythm in acute slices or slice cultures. It shows how these circuits depend on experimental conditions, such as time of day for their generation, and how they are analyzed with patch-clamp plus multiunit activity recording, molecular approaches, and Ca^{2+} or genetic bioluminescence imaging. Chapter 6 by *Stachniak et al.* deals with other hypothalamic networks that regulate plasma osmolarity in the body and whose responses to osmotic stimuli are studied with patch-clamp and immunohistochemical approaches in conventional and thick hypothalamic slices. In Chapter 7 by *McKay et al.*, patch-clamp recording and histological analysis are used to show that repetitive synaptic input establishes in vivo–like activity in rat cerebellar slice neurons and that biophysical neuron properties change during postnatal development. The latter findings are important for comparing in vivo data from adult animals with in vitro

findings that are often obtained in preparations from juvenile animals. Chapter 8 by *Sanchez-Vives* shows, using primarily whole-cell and multiunit activity recording, that patterns of spontaneous rhythmic activities in slices of adult cerebral cortex depend on animal species, superfusate composition, and temperature. Chapter 9 by *Broicher and Speckmann* reports how spontaneous and evoked neuronal activities in acute cortical slices from patients who needed surgical removal of brain tissue are analyzed by combining electrophysiological approaches with voltage imaging. Chapter 10 by *Luhmann and Kilb* outlines how cellular properties and network activity are analyzed in intact in vitro preparations of neonatal rodent cerebral cortex. Chapter 11 by *Kantor et al.* deals with the use of suction electrode recording and Ca^{2+} plus morphological multiphoton/confocal imaging for studying spontaneous network oscillations in hippocampal, neocortical, and *locus ceruleus* slices from newborn rats and piglets. Chapter 12 by *De Curtis et al.* describes methods for arterial perfusion of isolated guinea pig brains that retain functional and interacting neural networks. Examples are given for spontaneous and electrically evoked activities that are analyzed under normal conditions and upon evoked seizures or ischemia with extra- plus intracellular electrophysiological approaches, ion-sensitive microelectrodes, and voltage plus Ca^{2+} imaging. Chapter 13 by *Day and Wilson* describes a juvenile rat model for independent dual perfusion of carotid bodies and lower brainstem for analysis of contribution to respiratory rhythm of peripheral and central chemoreceptors, respectively. Chapter 14 by *Biggs et al.* outlines how to generate organotypic spinal cord slices for investigating with electrophysiologic and Ca^{2+} imaging approaches mechanisms of pain-related central sensitization. Chapter 15 by Mandadi et al. reviews slice and en bloc cord preparations for studying locomotor networks with electrophysiologic and Ca^{2+} imaging approaches.

Due to space limitation, other established or recently developed isolated CNS preparations and their applications could not be dealt with, such as tonically active *substantia nigra* networks, isolated optic nerves or *corpus callosum* slices for studying axon-glia interactions, or (organotypic) brain slices with intact connectivity of distinct regions, e.g., between the thalamus and the cortex. However, the in vitro approaches and methodologies described here are most likely applicable to further improve the latter models and to develop corresponding models of not yet intensively studied structures such as *nucleus ruber*, *superior colliculus*, or basal ganglia.

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