Preface

This book is on *N*-methyl-D-aspartate receptors (NMDARs), and more precisely on the methods that can be used to study NMDARs at various levels. NMDARs are cation channels that are gated by the major excitatory neurotransmitter glutamate. NMDAR-mediated signaling is involved in normal development, plasticity, learning, memory, and high cognitive functions. NMDARs play an important role in temporal integration of neuronal network activity and long-term alterations in synaptic structure and function. There is long known association of NMDAR dysfunctions with various neurodevelopmental disorders and excitotoxicity. Recent human genetic studies revealed the existence of multiple alterations in NMDARs subunits genes in numerous brain diseases, such as intellectual disability, autism spectrum disorders, or epilepsy, hence nominating NMDARs as promising targets for pharmacological treatment.

This book puts together the methods and techniques that are in use in leading laboratories to ensure a better understanding of the NMDAR structure-function relations, principles, and rules by which NMDARs operate in brain processing under normal and pathological conditions. Written by world-renowned experts, developers, and experienced users, this book provides thorough and detailed coverage of readily reproducible laboratory protocols that will be of interest to scientists, clinicians, or industry professionals working in this field.

Indeed, the book covers many aspects of NMDARs biology and the different ways this can be addressed. In a very comprehensive and up-to-date introductory Chapter 1, Hansen, Yi, Perszyk, Menniti, and Traynelis summarize everything you always wanted to know about NMDARs—and were not afraid to ask. They review the relationship between NMDARs structure and function, the diversity and significance of NMDAR subtypes in the CNS, as well as principles and rules by which NMDARs operate in the CNS under normal and pathological conditions. NMDARs subunits have different temporal and spatial expression patterns in the brain and NMDAR subtypes also vary according to the cell types and subcellular localization; accordingly, in Chapter 2 Pallesi-Pocachard describes the way transcriptional expression of the different genes encoding those various subunits can be quantified using reverse-transcription PCR. Whereas defects in several NMDARs subunits have been consistently reported in different types of brain disorders, notably GluN2A in epileptic encephalopathies of the epilepsy-aphasia spectrum, unexpected findings point to the high rate of somatic mutations of this GluN2A subunit in melanoma. The way such somatic mutations can be found in melanoma samples is described here by Prickett, Gartner, and Samuels in Chapter 3. NMDARs functioning can also be disturbed by nongenetic factors. One such factor is the presence of autoantibodies directed against NMDARs. How the existence of NMDARs autoantibodies can be searched and studied in the context of encephalitis is reported here by Gastaldi, Waters, and Vincent in Chapter 4. NMDARs structure-function relations and genetic variations can be studied by reconstituting them in cells transfected with the appropriate constructs for the expression of the corresponding subunits. This can be done in non-neuronal cells not expressing any native NMDARs as described by Bruneau and Szepetowski in Chapter 5, notably using magnetofection, allowing to study in isolation subunit-specific properties of NMDARs and their mutants in a physiological context. On the other hand transfection of NMDARs in primary cultured

neuronal cells expressing NMDARs endogenously, as shown in Chapter 6 by Marwick and Hardingham, allows to experimentally vary the NMDAR composition in the neurons, in order to investigate NMDAR trafficking and regulation in a physiological context, as well as synapse formation, synaptic activity, and cell survival. In Chapter 7 Yi, Traynelis, and Hansen describe the method to selectively express in heterologous expression systems recombinant triheteromeric GluN1/2A/2B receptors without interfering co-expression of diheteromeric GluN1/2A and GluN1/2B receptors; this enables quantitative evaluation of functional and pharmacological properties of triheteromeric GluN1/2A/2B receptors, which are presumably the most abundant native NMDARs in the adult cortex and hippocampus. This chapter is followed by logically linked chapters describing the methods for studying NMDARs function using electrophysiological and imaging techniques. In Chapter 8 Rozov and Jappy describe a method for electrophysiological functional analysis of recombinant channels expressed in host cells using a fast agonist application system. This method allows adequate assessment of ion permeation, kinetics, drug sensitivity, and other electrophysiological properties of the NMDAR-mediated currents using a wide range of the receptor activation protocols. Perrio, Nicole, and Buisson in Chapter 9 describe the methods for preparation of the fluorescent GluN2B specific probe, evaluation of GluN2B binding and inhibition potency of the probe by calcium imaging, and the labeling and visualization of GluN2B by confocal GluN2B imaging in living cortical neurons. This approach provides important insights into receptor dynamics and trafficking as well as into biophysical microenvironment of the binding site. In Chapter 10 Tian and Ye provide a protocol for the incorporation of two UV-sensitive crosslinking unnatural amino acids into NMDARs using the genetic code expansion technique to engineer light-sensitive NMDARs for imaging studies. Reduced recombinant systems provide a unique opportunity to study the biophysical properties of NMDAR channels with known subunit compositions. For a detailed molecular, morphological, and cellular analysis of NMDARs and the consequences of their dysfunction, they have to be studied in their natural environment in a particular cell type and/or in synapses and neuronal networks in animal models. Hence in Chapter 11 Sprengel, Eltokhi, and Single describe in detail the different methodological steps for successful gene targeting and generation of conditional NMDAR mutant mouse lines in which the hypomorphic Grin allele can be activated at specific time points and in specific cell types allowing functional analysis of the mutated NMDARs in living animals *in vivo* and in brain slices *in vitro*. In Chapter 12 Pons-Bennaceur and Lozovaya describe the methods to study electrophysiological properties of NMDAR-mediated component of spontaneous or evoked excitatory postsynaptic currents by extracellular stimulation or by stimulating synaptically connected neurons in brain slices. This approach allows pinpointing the basic functional properties of NMDARs that are specific to identified brain regions, neurons, and synapses in wild type or genetically manipulated mice. NMDARs are not only present in neuronal cells but also found in a wide variety of non-neuronal cells, within the brain or even in peripheral tissues. In Chapter 13 Kirchhoff describes in detail the methodology on how to study the physiology of NMDARs in astrocytes. This includes electrophysiological characterization of NMDAR-mediated currents in brain slices, analysis of the NMDAR kinetics in acutely isolated, single astrocytes, and visualization of NMDARs localization by Ca^{2+} imaging using confocal laser-scanning microscopy. In Chapter 14 Peineau, Degos, Verney, and Gressens describe the complementary steps to demonstrate the presence and functions of endogenous NMDARs in microglia using a wide range of biological tools with a dedicated strategy. Makhro, Kaestner, and Bogdanova in Chapter 15 present the methodological approaches for detection of the erythroid NMDARs activity in red blood cell

fractions of low, medium, and high density, or in single cells. In Chapter 16 Khazipov describes a procedure using NMDARs single-channel activity recorded in neurons in cell-attached mode as a tool for noninvasive measurements of the neuronal membrane potential in living cells. Finally, in Chapter 17 Keller, Bouteiller, and Berger introduce a computational approach as a complementary tool to explore NMDARs function based on the knowledge provided by various experimental results. They describe a general computational method aiming at developing kinetic Markov chain-based models of NMDARs subtypes that can be used to make predictions on NMDARs properties and on their role in synaptic function under various physiological and pharmacological conditions.

With this book, we expect that the readers will find different and complementary ways NMDARs can be studied nowadays. The different chapters do not only reflect the diversity of the corresponding approaches, the importance of NMDARs, and their complexity, but also the progresses that have been and are still being made at various and intertwined levels.

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