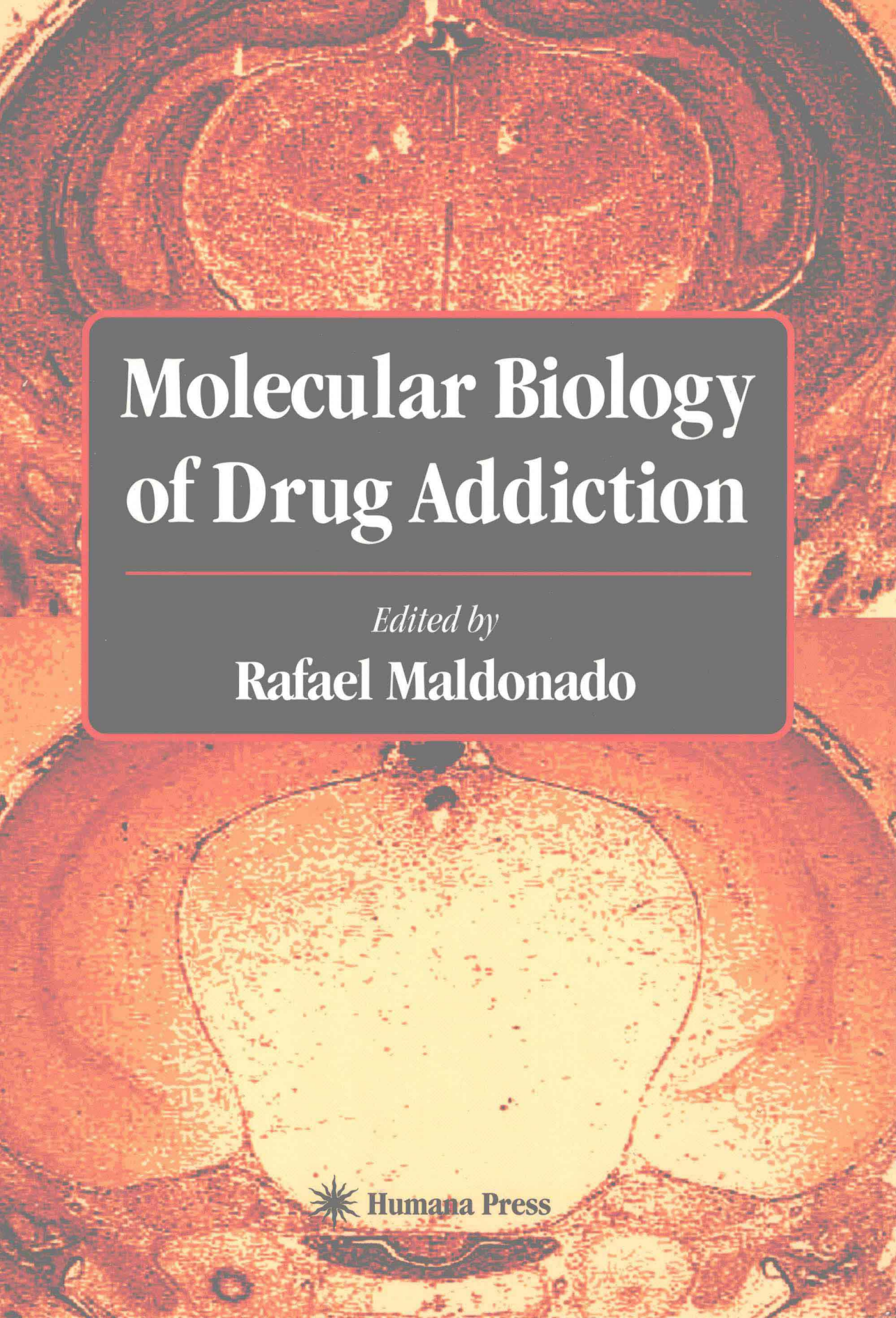

Molecular Biology of Drug Addiction

Edited by

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Preface

The neurobiological mechanisms involved in drug addiction have been investigated for several decades with a variety of pharmacological and biochemical approaches. These studies have associated several neuroanatomical and neurochemical mechanisms with different components of drug-addictive processes, and this has led to the identification of possible targets for new treatment strategies. Progress has been accelerated dramatically in the last few years by novel research tools that selectively remove or enhance the expression of specific genes encoding proteins responsible for the biological responses of these drugs. These new models, most of them obtained from the recent advances in molecular biology's technology, have provided definitive advances in our understanding of the neurobiological mechanisms of drug addiction. Classical behavioral, biochemical, and anatomical techniques have been adapted to take a maximum advantage of these new molecular tools. These recent studies have clarified the different molecular and intracellular mechanisms involved in addictive processes, as well as the interactions among these endogenous neurobiological mechanisms; and they have provided new insights toward identifying other genetic bases of drug addiction.

The main purpose of *Molecular Biology of Drug Addiction* is to offer an extensive survey of the recent advances in molecular biology and complementary techniques used in the study of the neurobiological basis of drug dependence and addiction. Ours is a multidisciplinary review of the most relevant molecular, genetic, and behavioral approaches used in this field. The definitive advances given by the new molecular and behavioral tools now available provide a unique opportunity for such an approach. Each chapter in this book is not simply a review of the research activities of the author's laboratory, but rather provides a critical review of the main advances in the corresponding topic. Sixteen different chapters organized in four parts have been included in the book. The first part is devoted to the advances in the knowledge of the neurobiological mechanisms of opioid addiction provided in the last few years using the new available techniques, and some of the new therapeutic perspectives now opening up in this field. The second part addresses the most recent findings on the molecular, genetic, and neurochemical mechanisms involved in psychostimulant addiction, which have changed some of the basic knowledge of the neurobiological substrate of these processes. The third part of the book is focused on cannabinoid addiction. New molecular tools have also been used recently to elucidate the biological substrate of cannabinoid dependence. The behavioral models now available, which allow evaluation of the different components of cannabinoid dependence, have

optimized results in this particular field. The last part addresses several molecular, genetic, and behavioral aspects of alcohol and nicotine addiction, which have provided decisive progress in our understanding of these addictive processes.

Molecular Biology of Drug Addiction addresses the main advances in understanding the molecular mechanisms involved in the complex physiological and behavioral processes underlying drug addiction and will, we hope, serve as a useful reference guide for a wide range of neuroscientists. This book also provides basic information of interest for scientists and clinicians interested in the new therapeutic approaches to drug addiction. The different sections of the book are presented by the most relevant scientific personalities for each area. I deeply thank the authors for their effort and expert contribution in the different chapters, and Elyse O'Grady at Humana Press for offering this rewarding opportunity. Finally, I thank Raquel Martín especially for help in manuscript preparation and administrative assistance and Dr. Patricia Robledo and Dr. Olga Valverde for scientific assistance and help in library research.

Rafael Maldonado

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Contents

Part I
Opioid Addiction

1

Molecular Mechanisms of Opioid Dependence by Using Knockout Mice

Brigitte L. Kieffer and Frédéric Simonin

1. Introduction

Opium, extracted from the seed of the poppy *Papaver somniferum*, has been used and abused for several thousand years. This substance is highly efficient to relieve pain or treat dysentery, and also shows strong euphoric and addictive properties. Due to their exceptional therapeutic potential, the active ingredients of opium have been the subject of intense investigations. Morphine, named after Morpheus, the Greek god of dreams, was isolated in 1806 *(1)* and is considered the prototypic opioid compound. This compound retains both analgesic and addictive properties of opium. Despite numerous adverse effects *(2)*, morphine remains the best painkiller in contemporary medicine, and its clinical use is under tight regulation. In 1898 heroin was chemically synthesized by morphine diacetylation, in an attempt to obtain a drug with lower abuse liability. In fact, this morphine derivative showed even higher addictive potential due to its distinct pharmacokinetic properties. Heroin is being illegally abused worldwide and represents a major public health problem. Attempts to dissociate opioid analgesia from opioid addiction have been unsuccessful so far.

Opioids have been classified as narcotic drugs (from the Greek word for stupor), due to their pharmacological profile very distinct from that of other drugs of abuse, such as pyschostimulants (cocaine, amphetamine), cannabinoids, nicotine, or alcohol *(3)*. As for other substances of abuse, though, opioid addiction typically develops in four stages *(4)*: (a) the initiation phase, in which drug exposure produces positive subjective effects (euphoria); (b) the maintenance phase, in which drug-taking becomes compulsive, indicating that dependence has developed; (c) withdrawal, which develops when drug levels decrease in the body and is recurrently experienced by drug abusers; and (d) craving—or the intense desire to use the drug—and relapse, which are most critical from a therapeutic standpoint. Not every individual exposed to opioids will develop addiction, depending on social, contextual, or perhaps genetic factors *(5)*. However, opioids are considered strongly addictive, and it has been proposed that incremental—perhaps irreversible—neuroadaptations profoundly modify the central nervous system (CNS) following repeated opioid exposure, and contribute to the establishment of opioid dependence *(6)*.

Opioid addiction is a complex phenomenon. Opioid drugs act by activating opioid receptors distributed throughout the CNS and stimulate a number of pathways, among

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which the so-called reward pathways located in the limbic system *(7)* are particularly relevant to the addictive process. Repeated opioid stimulation will modify and dysregulate opioid receptor activity and, consequently, interfere with a tightly regulated endogenous opioid system *(8)*, which is critically involved in the control of natural rewards and motivation *(7,9)*, as well as responses to stress *(10)* and pain *(11)*. The endogenous opioid system itself interacts with other neurotransmitter systems, and long-term exposure to exogenous opioids may ultimately remodel associated neuronal networks within brain circuits *(12)* and activate antiopioid systems that counteract opioid effects *(13–15)*, thereby modifying hedonic homeostasis *(16)*.

Recent research aims at clarifying the molecular mechanisms of neuroadaptations to chronic opioids. Cellular models have highlighted regulatory processes, which occur at the level of opioid receptors and their associated signaling proteins, and are believed to contribute to the development of opioid tolerance and withdrawal. Receptor uncoupling from second messenger systems, receptor downregulation, and adenylyl cyclase upregulation were largely shown in neuroblastoma cells expressing opioid receptors endogenously *(17)*. Agonist-induced receptor phosphorylation, desensitization, internalisation, and trafficking were demonstrated more recently using recombinant opioid receptors (e.g., *18–24*). These studies, however, addressed a limited aspect of opioid adaptations, and the link between early agonist-induced events and integrated behavioral responses remains to be established. In vivo, biochemical studies have confirmed upregulation of the cAMP pathway in several brain areas, shown modifications of tyrosine hydroxylase, glutamate receptor subunits, or cytoskeleton protein levels, and proposed a role for growth and transcription factors in the establishment of opioid addiction *(6,25)*.

Gene manipulation in rodents provides a unique mean to correlate molecular events with complex behavior, and is now used to study substance abuse. Possible approaches include (a) targeted gene inactivation using homologous recombination in embryonic stem cells (knockouts), (b) gene overexpression by egg microinjection (transgenics), (c) gene overexpression by viral-mediated gene transfer in adult mice, and (d) gene downregulation by antisense oligonucleotides (26). In this chapter we will focus on gene knockout models, in an attempt to analyze what these unique genetic tools have taught us about opioid addiction. Recently, a number of null mutant mice have been subjected to chronic morphine treatments and their responses found to differ from their wild-type controls (*see* Tables 1–3). These observations have highlighted a role for a number of known genes in behavioral responses to opioids, and allow us to establish a connection between the activity of these genes and molecular neuroadaptations subsequent to chronic opioid treatments in vivo.

2. The Behavioral Models

The manifestations of opioid addiction and dependence can be evaluated in mice using a large panel of behavioral models *(27)*. The reinforcing properties of opioids are currently investigated using conditioned place preference (CPP) or self-administration (SA) procedures. The development of tolerance is observed at the level of opioid analgesia. Typically, tail withdrawal latencies are measured in response to thermal or mechanical pain (tail flick, tail immersion, tail pinch, or hot plate). Latencies are prolonged following acute treatment (analgesia) and gradually return to control values under

Table 1
Effects of Morphine in Knockout Mice of the Opioid System[a]

Gene knockout	Acute morphine	Tolerance to analgesia	Morphine reward	Morphine withdrawal	Reference
MOR	Analgesia abolished		CPP abolished	Somatic and vegetative signs absent	*28*
	Hyperlocomotion abolished				*29*
			SA below saline		*30*
DOR	Analgesia unchanged	Abolished (TF)			*31*
				Somatic signs unchanged	Pintar J., personal communication
KOR	Analgesia unchanged		CPP unchanged	Somatic signs reduced	*32*
PreproENK		Abolished (TF)			*31*
				Somatic signs unchanged	Pintar J., personal communication

[a]CPP, conditioned place preference; SA, self-administration; TF, tail-flick.

Table 2
Effects of Morphine in Knockout Mice for Neuropeptides and Receptors[a]

Gene knockout	Acute morphine	Tolerance to analgesia	Sensitization to hyperlocomotion	Morphine reward	Morphine withdrawal	Reference
CB1	Analgesia unchanged	Unchanged (HP, TI)		SA abolished	Somatic signs reduced	*33*
				DA increase in Nuc Acc abolished		*34*
	Hyperlocomotion unchanged		Abolished	CPP abolished	Withdrawal CPA unchanged	*35*
				SA abolished		*36*
					Somatic signs reduced	*37*
D2R	Analgesia unchanged; hyperlocomotion unchanged			CPP abolished	Somatic signs unchanged	*38*
				CPP maintained in naive but absent during withdrawal	Somatic signs unchanged; withdrawal CPA abolished	*39*
DAT	Analgesia unchanged; hyperlocomotion abolished			CPP enhanced	Some somatic signs reduced (but not jump)	*40*

NK1	Analgesia unchanged; hyperlocomotion abolished			CPP abolished	Jump abolished; other somatic signs unchanged; withdrawal CPA reduced	*41*
GluR-A and GluR-A(R/R)[b]	Analgesia unchanged; hyperlocomotion unchanged	Abolished in GluR-A; unchanged in GluR-A(R/R) (TF)	Context-independent sensitization abolished		Somatic signs reduced in GluR-A, unchanged in GluR-A(R/R))	*42*
OFQ/N	Analgesia unchanged	Unchanged (TI)			Jump increased	*43*
ORL-1	Analgesia unchanged	Reduced (TP)				*44*
	Analgesia unchanged	Reduced (TP and TF)			Jump reduced; other somatic signs abolished	*45*
αCGRP	Analgesia reduced; SIA abolished	Unchanged (TF)		Heroin SA unchanged	Somatic signs reduced	*46*
IL6	Analgesia reduced; SIA abolished	Faster (HP)				*47*

[a]CPA, conditioned place aversion; CPP, conditioned place preference; DA, dopamine; HP, hot plate; Nuc Acc, nucleus accumbens; SA, self-administration; SIA, stress-induced analgesia; TF, tail flick; TI, tail immersion; TP, tail pinch.

[b]In GluR-A(R/R) mutant mice the Q582 residue of the GluR-A subunit is replaced by an arginine residue, which reduces the calcium permeability and channel conductance of receptors containing this subunit.