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Invited review

15 years of genetic approaches *in vivo* for addiction research: Opioid receptor and peptide gene knockout in mouse models of drug abuse

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ABSTRACT

The endogenous opioid system is expressed throughout the brain reinforcement circuitry, and plays a major role in reward processing, mood control and the development of addiction. This neuromodulator system is composed of three receptors, mu, delta and kappa, interacting with a family of opioid peptides derived from POMC (β-endorphin), preproenkephalin (pEnk) and preprodynorphin (pDyn) precursors. Knockout mice targeting each gene of the opioid system have been created almost two decades ago. Extending classical pharmacology, these mutant mice represent unique tools to tease apart the specific role of each opioid receptor and peptide *in vivo*, and a powerful approach to understand how the opioid system modulates behavioral effects of drugs of abuse. The present review summarizes these studies, with a focus on major drugs of abuse including morphine/heroin, cannabinoids, psychostimulants, nicotine or alcohol. Genetic data, altogether, set the mu receptor as the primary target for morphine and heroin. In addition, this receptor is essential to mediate rewarding properties of non-opioid drugs of abuse, with a demonstrated implication of β-endorphin for cocaine and nicotine. Delta receptor activity reduces levels of anxiety and depressive-like behaviors, and facilitates morphine-context association. pEnk is involved in these processes and delta/pEnk signaling likely regulates alcohol intake. The kappa receptor mainly interacts with pDyn peptides to limit drug reward, and mediate dysphoric effects of cannabinoids and nicotine. Kappa/dynorphin activity also increases sensitivity to cocaine reward under stressful conditions. The opioid system remains a prime candidate to develop successful therapies in addicted individuals, and understanding opioid-mediated processes at systems level, through emerging genetic and imaging technologies, represents the next challenging goal and a promising avenue in addiction research.

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1. Introduction

Opiates, including morphine, are potent analgesic compounds and represent major therapeutic drugs to treat severe pain. In addition, opiates induce strong euphoria and repeated exposure often leads to dependence and eventually opioid addiction. Milestones in discoveries of the opioid system are shown in Fig. 1. Morphine, the most active component of opium, was isolated in 1805 by Serturner. Opioid receptors were described in 1973, based on opioid binding sites referred as mu, delta and kappa (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). Met- and Leu-enkephalins were characterized in 1975, and altogether three families of endogenous opioid peptides precursors (pre-proenkephalin pEnk, pre-prodynorphin pDyn and proopiomelanocortin POMC) were identified in the late 70's (Goldstein et al., 1979; Guillemin et al., 1976; Hughes et al., 1975; Li and Chung, 1976). Genes encoding opioid peptide precursors were isolated in the early 80's (pEnk (Comb et al., 1982; Gubler et al., 1982; Noda et al., 1982); pDyn (Kakidani et al., 1982); POMC (Nakanishi et al., 1979)). The first opioid receptor gene, encoding delta receptors, isolated by expression cloning in 1992 (Evans et al., 1992; Kieffer et al., 1992), and the two other receptor genes were cloned by homology (Mestek et al., 1995; Simonin et al., 1994, 1995). Opioid receptors belong to the superfamily of G-protein coupled receptors (Kieffer, 1995; Trigo et al., 2010), with coupling to Gi/Go proteins (Law et al., 2000),

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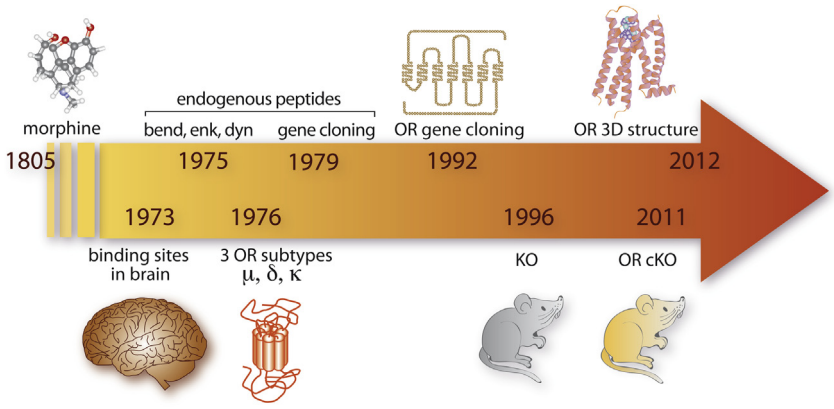

Fig. 1. Milestone discoveries in opioid research. Opium is extracted from poppy seeds (*Papaver somniferum*) and consumed for several thousand years to relieve pain and produce euphoria. Morphine, the most active alkaloid extracted from opium, was the first opioid to be isolated (1805). Opiates act on the nervous system, where they specifically activate receptors (1973), which are normally stimulated by a family of endogenous neurotransmitters, β-endorphin, enkephalins and dynorphins (1975). Several opioid receptors subtypes were further described based on receptor pharmacology (1976). Gene cloning occurred in early 80's for peptide precursors (1979) and early 90's for opioid receptors (1992). Opioid receptors genes (*Oprm1, Oprd1* and *Oprk* encoding mu-, delta- and kappa-opioid receptor; *pomc*, *pEnk* and *pDyn* encoding peptide precursors) were targeted in mice by homologous recombination, and mice lacking the mu receptor and enkephalins were available first (1996). Recently, refinement of *in vivo* targeted mutagenesis techniques led to the first conditional knockout mouse for the opioid system, with a delta receptor deletion restricted to primary afferent nociceptive neurons (2011). The 3D crystal structure of all three receptors was elucidated very recently (2012). OR: opioid receptor, KO: knockout mouse, cKO: conditional knockout mouse. Detailed references are in the text.

and their structure was solved at high-resolution by X-ray crystallography (Granier et al., 2012; Manglik et al., 2012; Wu et al., 2012). The opioid system is broadly expressed in the nervous system, particularly within the neurocircuitry of addiction (Koob and Volkow, 2010). Both peptides and receptors are present in areas associated with reward, motivation, learning and stress (Le Merrer et al., 2009; Mansour et al., 1995), and therefore play a key role in many aspects of addictive behaviors (see Lutz and Kieffer, 2013).

All the known drugs of abuse activate reinforcing brain circuitries (Koob and Volkow, 2010). These drugs, however, recruit distinct molecular targets in the brain and show notable differences in their pharmacological actions, which has led researchers and physicians to classify them into distinct groups. Opiates, acting directly at opioid receptors, produce sedative effects in addition to euphoria, and are therefore known as narcotics. In contrast, psychostimulants that include cocaine, amphetamine and methamphetamine, provide immediate euphoria with a feeling of intellectual and physical power, and indifference to pain and fatigue, mainly via direct stimulation of dopaminergic transmission. Nicotine, a major component of tobacco, is also considered a mild stimulant and α-nicotinic receptors constitute their molecular target. Relaxing and euphoric sensations searched by marijuana users arise from the stimulation of CB1 receptors by cannabinoids, including the most active component delta9-tetrahydrocannabinol (THC). Finally, the most widely abused licit drug is alcohol, targeting several receptors and ion channels in the brain and representing a major health problem (Hyman, 2008). It is now well established that the endogenous opioid system plays an important role in acute and chronic effects of all these drugs. The exact nature of opioid receptor or peptide involved has been clarified over the years, largely owing to genetic approaches, and this large set of data is overviewed here.

Drug abuse is a major threat to public health (Compton et al., 2007; Gustavsson et al., 2011). For 40 years, NIDA has supported extensive research towards understanding molecular bases of drug abuse (Everitt et al., 2008; Nestler, 2005; Pierce and Wolf, 2013), and developing innovative strategies for treatment (Heilig et al., 2011; Kalivas and Volkow, 2011; Koob et al., 2009; Pierce et al., 2012; Volkow and Skolnick, 2012). We are extremely grateful to NIDA for long-standing support to our efforts in developing genetic mouse models for opioid research. Knockout (KO) mice for the opioid system, developed by others and us, have been extensively studied and broadly shared within our research community. In this review, we have gathered data from these KO mice that have accumulated in the past fifteen years (for previous reviews see Contet et al., 2004; Kieffer and Gaveriaux-Ruff, 2002), and enabled identification or clarification of the specific role of each component of the opioid system in drug reward and addiction. Note that the opioid system plays a central role in pain processing, but this particular aspect will not be reviewed here (see recent reviews in Bodnar, 2012; Gaveriaux-Ruff and Kieffer, 2011; Woolf, 2011).

We will first summarize behavioral responses of null mutant mice to opiates, then overview reports investigating the effects of other drugs of abuse, including cannabinoids, psychostimulants (cocaine, MDMA, amphetamine), nicotine and alcohol in these mice, and finally conclude on the respective roles of opioid peptides and receptors, and perspectives of opioid research in the area of drug abuse. Whereas data from receptor KO mice have unambiguously clarified receptor roles *in vivo*, data from peptide KO mice are by essence more complex (low receptor selectivity) and the latter mutants still deserve further investigations.

2. Behavioral measures in the mouse

At present, behavioral paradigms to model distinct aspects of addiction (for a review see Everitt et al., 2008; Koob et al., 2009) in rodents remain limited, particularly for mice (see Box). Several well-described behavioral models in rats have nevertheless been successfully adapted to mice, and largely applied to mutant animals. Among these, voluntary/operant testing (two-bottle choice, TBC and self-administration, SA) addresses some aspects of binge intoxication and/or excessive consumption, and conditioned place preference (CPP) examines drug reward. Withdrawal and the negative effect of drug abstinence can be revealed by conditioned place aversion (CPA) and drug-induced physical withdrawal, and preoccupation/anticipation can be tested by drug-, cue- or stress-induced reinstatement of CPP. Finally locomotor activation by drugs of abuse, and sensitization to this effect upon repeated treatment, are also typical responses studied in rodents although no human correlate exists for this behavior. Data from all these tests are summarized in Tables 1–6, and main findings are summarized below.