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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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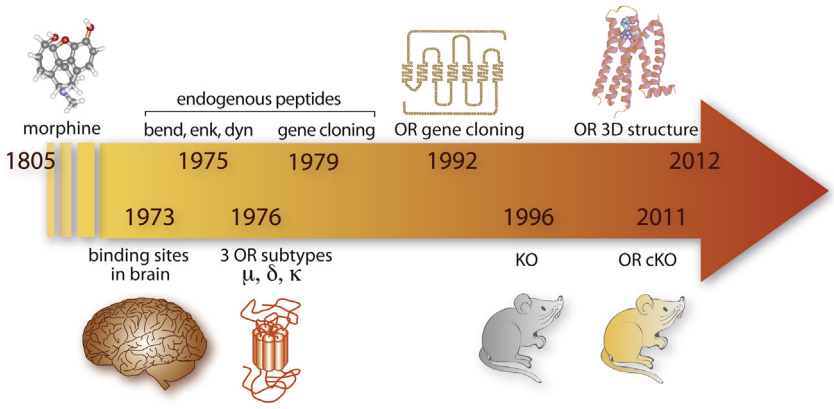

Fig. 1. Milestone discoveries in opioid research. Opium is extracted from poppy seeds (*Papaver somniferum*) and consumed for several thousand years to relieve pain and produce euphoria. Morphine, the most active alkaloid extracted from opium, was the first opioid to be isolated (1805). Opiates act on 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.

Box
Behavioral measures in the mouse.

Behavioral responses examined in mutant mice (Tables 1–6) are briefly explained below.

Conditioned place preference (CPP) or aversion (CPA): pavlovian conditioning based on capacity of the animal to associate the drug effect with the context. If the drug has rewarding effects, mice explore the drug-paired compartment more than the vehicle-paired compartment, and thus show a conditioned place preference (CPP). If the drug is aversive mice avoid the drug-paired box (Conditioned place aversion or CPA). Reinstatement can be measured after a CPP paradigm: drug priming or stress can reinstate preference for the initially drug-paired box after extinction. This test models drug-seeking behavior (Tzschentke, 2007).

Self-administration (SA): operant paradigms model several elements of human drug consumption, and are therefore largely used in rodents. Drug SA in mice (except oral SA), however, is technically difficult, and studies remain scarce. In drug SA models, the animal works to obtain the drug and learns an action/outcome association. Various aspects are investigated: acquisition (under fixed ratio schedule); motivation (under progressive ratio schedule and determination of a breaking point, corresponding to the highest response possible for a single delivery); extinction (response rate after end of drug-delivery); reinstatement (as for CPP). In addition to rewarding effects of the drug, this model enables investigation of motivational aspects of drug intake (Sanchis-Segura and Spanagel, 2006).

Two-bottle choice: In this test, mostly used for measuring alcohol consumption, the animal has access to a water-containing bottle and an alcohol-containing bottle. This access is either continuous (24 h/day) or intermittent (few hours a day or few days a week). The latter closely mimics binge drinking and can be used as a model of relapse by including phases of deprivation (Crabbe et al., 2011).

Locomotor effects and sensitization: Many drugs of abuse increase locomotor activity after acute treatment. Repeated administration of the drug, classically increases this locomotor response, a phenomenon referred to as sensitization that may reflect the transition from voluntary intake to compulsive use (Robinson and Berridge, 2008; Vanderschuren and Pierce, 2010), or vulnerability to drug addiction or drug-induced psychosis in humans (Loweth and Vezina, 2011).

Withdrawal: Chronic drug administration produces physical dependence, which is revealed after cessation of drug exposure. Spontaneous withdrawal is difficult to detect and quantify in animals, therefore physical withdrawal is typically precipitated by treatment with an antagonist, followed by scoring of withdrawal signs. The latter vary with the drug (ptosis, teeth chattering, tremor, paw tremor, wet-dog shakes, sniffing, jumping, diarrhea) and a global score is calculated to measure a general dependence index (Maldonado et al., 1996).

3. Opioid system and opiate drugs

Morphine reward and withdrawal data are shown for the six KO lines in Table 1. Locomotor effects of morphine are presented in Table 6 together with stimulant effects of other drugs of abuse. Genetic studies have definitely established that the mu opioid receptor is required for therapeutic effects as well as unwanted effects of morphine (see Contet et al., 2004). Hence, morphine (Matthes et al., 1996; Nguyen et al., 2012a, 2012b; Sora et al., 2001) and heroin (Contarino et al., 2002) CPPs were abolished in mu KO mice at all the tested doses. Intravenous as well as intra-VTA infusions of the drug observed in wild type animals were also abolished in mutants (Sora et al., 2001; David et al., 2008). In another study, mu KO mice self-administered morphine at levels lower than control mice self-administering saline, perhaps unmasking a kappa/dynorphin-mediated aversive state in these mutants (Becker et al., 2000). Locomotor responses to morphine (Tian et al., 1997; Sora et al., 2001; Chefer et al., 2003; Yoo et al., 2003, 2006; Becker et al., 2000) and heroin administration (Contarino et al., 2002) were eliminated in mu KO animals (see Table 6). Together all the data demonstrate that mu receptors indeed represent the primary *in vivo* molecular target for both most clinically useful (morphine) and most largely abused (heroin) opiates.

The role of delta receptor in reward is debated. Delta KO mice developed a place preference when morphine was paired with the initially non-preferred compartment, but failed to do so when paired to the preferred side of the apparatus (Chefer and Shippenberg, 2009). The authors interpreted this result as a ceiling effect in the biased CPP protocol that was used more than a decrease of rewarding properties of morphine. In another study, using unbiased CPP, delta KO animals did not develop place preference to morphine (Le Merrer et al., 2011). In the same study, mutant mice showed impaired place conditioning to lithium, an aversive stimulus, and showed normal motivation to obtain morphine in a SA paradigm (Le Merrer et al., 2011). Together with a previous study showing intact intra-VTA SA in delta KO mice (David et al., 2008), the data concur to indicate that morphine reward and motivation to obtain the drug are intact in these animals, however drug-context association is impaired. A subsequent study showed that internal or external non-spatial cues (circadian, drug, auditory) predicting drug or food reward restored morphine CPP in delta KO mice, suggesting that only contextual learning is impaired in these mice (Le Merrer et al., 2012). Considering locomotor effects, the stimulant effect of acute morphine was unchanged in delta KO mice (Chefer et al., 2003). However, sensitization or tolerance to this effect, observed upon distinct regimen of chronic morphine administration, was enhanced and reduced respectively (Chefer and Shippenberg, 2009), indicating a role for delta receptors in these adaptive responses to chronic morphine. Otherwise, physical dependence was unchanged in delta KO mice (Nitsche et al., 2002). In conclusion, the delta receptor does not directly mediate morphine reward and likely facilitates contextual learning. Also, as many other systems, this receptor contributes to chronic morphine-induced neuroplasticity. Mechanisms underlying a potential cross talk between delta receptor activity and mu opioid receptor signaling *in vivo* remain unclear (see Pradhan et al., 2011; Stockton and Devi, 2012).

β-endorphin KO animals compared with wild-type controls spent equal (Niikura et al., 2008) or more (Skoubis et al., 2005) time in the drug-paired compartment, depending on the dose and paradigm used. No modification of morphine CPP could be detected in proenkephalin (pEnk) KO mice (Skoubis et al., 2005), and physical dependence was either decreased (Shoblock and Maidment, 2007) or enhanced in these mice (Nitsche et al., 2002). These results suggest paradoxical negative modulatory roles for the two endogenous peptides in morphine reward (βend) and withdrawal (pEnk), or that compensatory mechanisms have developed in knockout animals.

Morphine CPP was unchanged in mice lacking the kappa opioid receptor (Simonin et al., 1998), as well as dynorphin (Mizoguchi et al., 2010; Zimmer et al., 2001). Prodynorphin KO mice showed unchanged (Mizoguchi et al., 2010; Zimmer et al., 2001) or increased hyperlocomotor activity upon morphine administration (Mizoguchi et al.,

Table 1
Behavioral effects of morphine and heroin in opioid receptor and peptide knockout mice.

Gene KO	Drug of abuse	Behavioral test	Drug of abuse dose, route	Genotype effect	Ref
mu	Morphine	CPP	3 mg/kg, s.c.	Abolished	Matthes et al., 1996
		CPP	10 mg/kg, s.c.	Abolished	Sora et al., 2001
		CPP	10 mg/kg, s.c.	Abolished	Nguyen et al., 2012a
		CPP	10 mg/kg, s.c.	Abolished	Nguyen et al., 2012b
		+ *challenge on d14*	5 mg/kg, s.c.	Abolished	
		SA	2 or 4 mg/0.2 mL, i.c.v. FR1	Lower than saline groups	Becker et al., 2000
		SA	0.1 or 0.3 mg/kg/injection, i.v. FR4	Abolished	Sora et al., 2001
		VTA SA	50 or 100 ng/infusion	Abolished	David et al., 2008
		Withdrawal	20–100 mg/kg, i.p. *(2x/d, 5d)*	Abolished	Matthes et al., 1996
	Heroin	CPP	1 mg/kg, i.p.	Abolished	Contarino et al., 2002
delta	Morphine	CPP preferred side	10 mg/kg, s.c.	Abolished	Chefer and Shippenberg, 2009
		CPP non-preferred side	10 mg/kg, s.c.	Unchanged	
		CPP drug free state	5 mg/kg, s.c.	Abolished	Le Merrer et al., 2011
		CPP under morphine	5 mg/kg, s.c.	Unchanged	
		CPP without cue	10 mg/kg, s.c.	Abolished	Le Merrer et al., 2012
		CPP with cue	5, 10 and 20 mg/kg, s.c.	Unchanged/restored	
		SA	0.25 or 0.5 mg/kg/infusion, i.v. FR1	Unchanged	Le Merrer et al., 2011
			0.25 mg/kg/infusion, i.v. PR	Unchanged	
			0.5 mg/kg/infusion, i.v. PR	Increased	
		VTA SA	50 ng/infusion	Unchanged	David et al., 2008
		Withdrawal	75 mg, pellet *(3d)*	Unchanged	Nitsche et al., 2002
kappa	Morphine	CPP	1 mg/kg, s.c.	Unchanged	Simonin et al., 1998
		Withdrawal	20–100 mg/kg, i.p. *(2x/d, 6d)*	Abolished	Simonin et al., 1998
βend	Morphine	CPP	10 mg/kg, s.c.	Increased	Skoubis et al., 2005
		CPP	5 mg/kg, s.c.	Unchanged	Niikura et al., 2008
pEnk	Morphine	CPP	10 mg/kg, s.c.	Unchanged	Skoubis et al., 2005
		Withdrawal	75 mg, pellet *(3d)*	Increased	Nitsche et al., 2002
		Withdrawal jumping	20 mg/kg, s.c. *(1 injection)*	Decreased	Shoblock and Maidment, 2007
			100 mg/kg, s.c. *(2d)*	Abolished	
pDyn	Morphine	CPP	5 mg/kg, s.c.	Unchanged	Zimmer et al., 2001
		CPP	3.5 mg/kg, s.c.	Unchanged	Mizoguchi et al., 2010
		Withdrawal	20–100 mg/kg, i.p. *(2x/d, 5d)*	Unchanged	Zimmer et al., 2001

Data are shown for each knockout (gene KO) mouse line. Behavioral tests are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO. CPP: Conditioned Place Preference; d: day; SA: self-administration; VTA: ventral tegmental area; FR: fixed ratio; PR: progressive ratio.

2010), suggesting that dynorphin opposes mu receptor signaling for the control of locomotor effects. Several signs of naloxone-induced withdrawal were decreased in morphine-dependent kappa KO mice (Simonin et al., 1998), an effect that could not be observed in pDyn mutants (Zimmer et al., 2001). A tonic role for the kappa/dynorphin system is therefore detected in dependent animals, at receptor level, in agreement with pharmacological studies suggesting protective role of kappa receptor blockade in morphine dependence (Wee and Koob, 2010). Involvement of this anti-reward system (Koob and Le Moal, 2008) is overall better detected in knockout mice under conditions of stress (Bruchas et al., 2010) and in response to non-opioid drugs of abuse (see below).

Table 2
Behavioral effects of cannabinoid in opioid receptor and peptide knockout mice.

Gene KO	Drug of abuse	Behavioral test	Drug of abuse dose, route	Genotype effect	Ref
mu	THC	CPP	1 mg/kg, i.p.	Abolished	Ghozland et al., 2002
		CPA	5 mg/kg, i.p.	Decreased	Ghozland et al., 2002
		Withdrawal	10 mg/kg, s.c. *(5d)*	Unchanged	Lichtman et al., 2001
			30 or 100 mg/kg, s.c. *(5d)*	Decreased	
		Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Unchanged	Ghozland et al., 2002
delta	THC	CPP	1 mg/kg, i.p.	Unchanged	Ghozland et al., 2002
		CPA	5 mg/kg, i.p.	Unchanged	Ghozland et al., 2002
		Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Unchanged	Ghozland et al., 2002
mu delta	THC	CPP	1 mg/kg, i.p.	Decreased	Castane et al., 2003
		Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Decreased	Castane et al., 2003
kappa	THC	CPP	1 mg/kg, i.p.	Unchanged	Ghozland et al., 2002
		CPP without priming	1 mg/kg, i.p.	Present, absent in WT	
		CPA	5 mg/kg, i.p.	Abolished	Ghozland et al., 2002
		Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Unchanged	Ghozland et al., 2002
pEnk	THC	Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Decreased	Valverde et al., 2000
pDyn	THC	CPA	5 mg/kg, i.p.	Abolished	Zimmer et al., 2001
		Withdrawal	20 mg/kg, i.p. *(2x/d, 6d)*	Decreased (trend)	Zimmer et al., 2001
	WIN	SA	6.25 mg/kg/infusion, i.v. FR1	Increased	Mendizabal et al., 2006
			12.5 mg/kg/infusion, i.v. FR1	Abolished	

Data are shown for each knockout (gene KO) mouse line. Behavioral tests are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO. THC: Δ^9-tetrahydrocannabinol; WIN: WIN 55,212-2; CPP: Conditioned Place Preference; CPA: Conditioned Place Aversion; d: day; SA: self-administration; FR: fixed ratio.

Table 3
Behavioral effects of psychostimulant in opioid receptor and peptide knockout mice.

Gene KO	Drug of abuse	Behavioral test	Drug of abuse dose, route	Genotype effect	Ref
mu	Cocaine	CPP	5–10 mg/kg i.p.	Rightward shift	Becker et al., 2002
		CPP	10 mg/kg, i.p.	Unchanged	Contarino et al., 2002
		CPP	5 mg/kg, s.c.	Unchanged	Hall et al., 2004
			10 mg/kg, s.c.	Decreased	
		CPP	30 mg/kg, i.p.	Unchanged	Nguyen et al., 2012a
		SA	0.4, 0.8 or 1.6 μg/inf, i.v. FR1	Decreased	Mathon et al., 2005
	MDMA	CPP	10 mg/kg, i.p.	Unchanged	Robledo et al., 2004
	Amphetamine	CPP	1 mg/kg, i.p.	Unchanged	Marquez et al., 2007
kappa	Cocaine	CPP ± forced swim stress	15 mg/kg, s.c.	Unchanged/no effect of stress	McLaughlin et al., 2006a
		CPP	15 mg/kg, s.c.	Unchanged	Redila and Chavkin, 2008
		Stress-induced reinstatement		Abolished	
		Cocaine prime test	15 mg/kg, s.c.	Unchanged	
βend	Cocaine	CPP	30–60 mg/kg i.p.	Rightward shift	Marquez et al., 2008
		CPP	30 mg/kg, i.p.	Abolished	Nguyen et al., 2012a
pDyn	Cocaine	CPP ± forced swim stress	15 mg/kg, s.c.	Unchanged/no effect of stress	McLaughlin et al., 2003
		CPP + social defeat stress	15 mg/kg, s.c.	Decreased	McLaughlin et al., 2006b
		CPP	15 mg/kg, s.c.	Unchanged	Redila and Chavkin, 2008
		Stress-induced reinstatement		Abolished	
		Cocaine prime test	15 mg/kg, s.c.	Decreased	

Data are shown for each knockout (gene KO) mouse line. Behavioral tests are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO. CPP: Conditioned Place Preference; d: day; SA: self-administration; FR: fixed ratio.

4. Opioid system and cannabinoids

Both pharmacological studies and genetic approaches provide considerable evidence suggesting that cannabinoid and opioid systems interact bi-directionally to regulate both neurochemical effects of drug and behavioral responses (Trigo et al., 2010; Vigano et al., 2005). Although mechanisms underlying functional interactions remain unclear, receptors from the two systems show overlapping distribution in various brain structures, and potential heterodimer formation between CB1 and mu opioid receptors has been suggested from *in vitro* studies (Maldonado et al., 2011; Solinas et al., 2008). Data summarizing cannabinoid effects in KO mice for the opioid system are shown in Table 2.

THC-induced CPP was unchanged in delta or kappa KO mice (Ghozland et al., 2002), but was abolished in mu KO mutants (Ghozland et al., 2002) and the double mu–delta KO line (Castane et al., 2003), suggesting that mu receptors mediate rewarding properties of THC. Interestingly conditioned place aversion (CPA), typically observed at a high dose of THC in wild-type mice, was abolished in both pDyn (Zimmer et al., 2001) and kappa KO mice (Ghozland et al., 2002). The latter observations indicate that the kappa/dynorphin system mediates aversive effects of THC, another facet of cannabinoid effects. This was further supported by facilitated self-administration of WIN, a cannabinoid agonist, in pDyn KO mice (Mendizabal et al., 2006). It has long been established that mu and kappa receptors oppositely regulate hedonic homeostasis (Spanagel et al., 1992) and it is therefore possible that the same opposing activities of the two opioid receptors mediate the well-known dual euphoric/aversive effects of cannabinoids. Notably, the delta receptor does not seem involved in all these THC effects, at least from knockout mice analysis (Ghozland et al., 2002).

THC withdrawal upon chronic THC treatment was reduced in pEnk KO mice (Valverde et al., 2000) and double mu–delta KO mice (Castane et al., 2003). Reduced THC withdrawal was also detected in mu KO animals, at high doses of THC (Lichtman et al., 2001). Single mutants for pDyn (Zimmer et al., 2001), mu, delta or kappa

Table 4
Behavioral effects of nicotine in opioid receptor and peptide knockout mice.

Gene KO	Behavioral test	Drug of abuse dose, route	Genotype effect	Ref
mu	CPP	0.5 or 0.7 mg/kg, s.c.	Abolished	Berrendero et al., 2002
	CPP	1 mg/kg, i.p.	Abolished	Walters et al., 2005
		2 mg/kg, i.p.	Unchanged	
	Withdrawal	10 mg/kg/d, minipump *(6d)*	Decreased	Berrendero et al., 2002
delta	CPP	0.17 mg/kg, s.c.	Abolished	Berrendero et al., 2012
	SA	15 μg/kg/infusion 10d, i.v. FR1	Unchanged	Berrendero et al., 2012
		30 μg/kg/infusion 10d, i.v. FR1	Decreased	
		30 μg/kg/infusion, i.v. PR	Decreased	
	Withdrawal	8.77 mg/kg/d, minipump *(6d)*	Unchanged	Berrendero et al., 2012
βend	CPP	0.5 mg/kg, s.c.	Abolished	Trigo et al., 2009
	Withdrawal	10 mg/kg/d, minipump *(6d)*	Unchanged	Trigo et al., 2009
pEnk	CPP	0.5 mg/kg, s.c.	Abolished	Berrendero et al., 2005
	Withdrawal	25 mg/kg/d, minipump *(6d)*	Decreased	Berrendero et al., 2005
pDyn	CPP	0.5 mg/kg, s.c.	Unchanged	Galeote et al., 2009
	SA	5.2–85.5 mg/kg/infusion, i.v. FR1	Leftward shift	Galeote et al., 2009
		5.2, 10.6, 21.3 or 85.5 mg/kg/infusion, i.v. PR	Unchanged	
		42.7 mg/kg/infusion, i.v. PR	Decreased	
	Withdrawal	25 mg/kg/d, minipump *(6d)*	Unchanged	Galeote et al., 2009

Data are shown for each knockout (gene KO) mouse line. Behavioral tests are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO. CPP: Conditioned Place Preference; d: day; SA: self-administration; FR: fixed ratio; PR: progressive ratio.

Table 5
Alcohol behavioral effects in opioid receptor and peptide knockout mice.

Gene KO	Behavioral test	Drug of abuse dose, route	Genotype effect	Ref
mu	TBC limited access	10%	Unchanged	van Rijn and Whistler, 2009
	TBC	10%	Decreased	Becker et al., 2002
	TBC	2–32%	Decreased (female)	Hall et al., 2001
	TBC	10%	Decreased	Roberts et al., 2000
	Oral SA	5–10%	Abolished	
	Oral SA following TBC		Abolished	
	CPP	2 or 4 g/kg	Unchanged	Becker et al., 2002
	CPP	2 g/kg	Abolished (female)	Hall et al., 2001
	Withdrawal	liquid diet 0.8–5%	Earlier signs	Ghozland et al., 2005
delta	TBC limited access	10%	Increased	van Rijn and Whistler, 2009; van Rijn et al., 2010
	Oral SA	5–10%	Increased	Roberts et al., 2001
	TBC following SA	10%	Increased	
kappa	TBC limited access	10%	Decreased	van Rijn and Whistler, 2009
	TBC	3–12%	Decreased	Kovacs et al., 2005
βend	TBC	7%	Increased	Grisel et al., 1999
	TBC +/− mild foot shock	8%	Decreased/no effect of stress	Racz et al., 2008
	SA	75 mg/kg; 2h session/9d; i.v. FR3	Acquisition in KO but not WT	Grahame et al., 1998
	Oral SA	3–6%	Unchanged	Hayward et al., 2004
	Withdrawal	forced drinking 16%	Unchanged	Racz et al., 2008
pEnk	TBC	2–10%	Unchanged	Koenig and Olive, 2002
	TBC	8%	Unchanged	Racz et al., 2008
	TBC + foot shock		Decreased (male)	
	Oral SA	3–6%	Unchanged	Hayward et al., 2004
	CPP	2 g/kg, i.p.	Unchanged	Koenig and Olive, 2002
	Withdrawal	forced drinking 16%	Unchanged	Racz et al., 2008
pDyn	TBC	2–8%	Increased	Femenia and Manzanares, 2012
	TBC	3–12%	Decreased (female)	Blednov et al., 2006
	TBC	8%	Increased	Racz et al., 2012
	TBC + foot shock		Prolonged/WT	
	TBC	4–10%	Unchanged	Sperling et al., 2010
	CPP	2 g/kg, i.p.	Increased	Femenia and Manzanares, 2012
	CPP drug free state	2 g/kg, i.p.	Unchanged (female)	Nguyen et al., 2012c
	CPP priming	2 g/kg, i.p. challenge 1 g/kg	Increased (female)	
	CPP	2 g/kg, i.p.	Unchanged	Blednov et al., 2006
	Conditioned taste aversion	2.5 g/kg, i.p.	Unchanged	
	Withdrawal	4 g/kg, p.o.	Increased	Femenia and Manzanares, 2012
		4 g/kg, i.p.	Unchanged	Blednov et al., 2006

Data are shown for each knockout (gene KO) mouse line. Behavioral tests are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO; CPP: Conditioned Place Preference; d: day; SA: self-administration; TBC: two-bottle choice; FR: fixed ratio.

receptors (Ghozland et al., 2002) otherwise showed normal THC withdrawal. The data together suggest that an endogenous enkephalinergic tone, acting jointly at mu and delta receptors, contributes to the development of physical dependence to THC.

5. Opioid system and psychostimulants

Multiple studies have pointed out a role for opioid receptors and their endogenous ligands in psychostimulant − particularly cocaine-addiction (for a recent review, see Yoo et al., 2012, and Table 3). Cocaine self-administration was dose-dependently reduced in mu KO mice (Mathon et al., 2005), and cocaine CPP was maintained (Contarino et al., 2002; Hall et al., 2004; Nguyen et al., 2012a) or decreased (Hall et al., 2004) depending on dose and experimental conditions (number of pairings, number and duration of conditioning sessions). These data indicate that mu receptors mediate, at least in part, cocaine reward. A rightward shift of the CPP dose–response curve was observed in both mu (Becker et al., 2002) and β-endorphin (Marquez et al., 2007) KO mice, suggesting decreased cocaine sensitivity in the two lines and a possible implication of mu/βend signaling in cocaine reinforcement. Place preference studies were also conducted in mu KO for amphetamine (Marquez et al., 2007) and MDMA (Robledo et al., 2004) but no phenotype could be detected.

The rewarding properties of cocaine were examined using CPP in mice lacking either kappa receptors or preprodynorphin. Preference for the drug-paired compartment was maintained in both animal models (McLaughlin et al., 2006a, 2003; Redila and Chavkin, 2008). In presence of stress, cocaine CPP is typically increased in wild type mice but remained unchanged in kappa and pDyn KO mice (forced-swim stress in McLaughlin et al. (2006a); McLaughlin et al. (2003); social defeat stress in McLaughlin et al. (2006b), indicating that the kappa/dynorphin system contributes to the stress-mediated response. Within this line, stress-induced reinstatement of extinguished cocaine CPP was decreased in pDyn KO, although this was not observed in kappa KO mice (Redila and Chavkin, 2008).

Another well-known effect of psychostimulants is drug-induced hyperlocomotion (Table 6). In some reports, the locomotor response to cocaine was reduced in mu KO mice (Chefer et al., 2004; Yoo et al., 2006, 2003) as well as in βend KO mice (Marquez et al., 2008), while in many other mu KO studies, this cocaine effect was unchanged (Becker et al., 2002; Chefer et al., 2004; Contarino et al., 2002; Hall et al., 2004; Lesscher et al., 2005). Furthermore, sensitization to locomotor effects of cocaine was reduced (Yoo et al., 2006, 2003), maintained (Lesscher et al., 2005), or enhanced (Hummel et al., 2004), depending on the mouse genetic background (Hummel et al., 2004) and the pattern of drug exposure (administration regimen and timing of injections) (Allouche et al., 2013; Puig et al., 2012). In mu KO mice also, methamphetamine-induced locomotion, was decreased at one dose, maintained in lower and higher doses, and no behavioral

Table 6
Drugs of abuse locomotor effects in opioid receptor and peptide knockout mice.

Gene KO	Drug of abuse	Locomotor stimulation	Drug of abuse dose, route	Genotype effect	Ref
mu	Morphine	Locomotion	2.3 mg/kg, i.p.	Abolished	Tian et al., 1997
		Locomotion	5 or 10 mg/kg, s.c.	Decreased/saline	Becker et al., 2000
		Locomotion	10 mg/kg, s.c.	Abolished	Sora et al., 2001
		Locomotion	10 or 20 mg/kg, s.c.	Abolished	Chefer et al., 2003
		Locomotor sensitization *(6d inj)*	10 mg/kg, s.c. *d1*	Abolished locomotion	Yoo et al., 2003, 2006
		+ *challenge on day 12*	10 mg/kg, s.c. *d12*	Abolished	
	Heroin	Locomotion	3 mg/kg, i.p.	Abolished	Contarino et al., 2002
	THC	Locomotor tolerance *(2x/d, 5d)*	20 mg/kg, i.p.	Unchanged	Ghozland et al., 2002
	Cocaine	Locomotion	20 or 40 mg/kg, i.p.	Unchanged	Becker et al., 2002
		Locomotion	30 mg/kg, i.p.	Unchanged	Contarino et al., 2002
		Locomotion	15 mg/kg, i.p.	Abolished	Yoo et al., 2003, 2006
		Locomotion	10 mg/kg, i.p.	Unchanged	Chefer et al., 2004
			20 mg/kg, i.p.	Decreased	
		Locomotion	20 mg/kg, s.c.	Unchanged	Hall et al., 2004
		Locomotion	3, 10, 20, or 30 mg/kg i.p.	Unchanged	Lesscher et al., 2005
		Locomotor sensitization *(6d inj)*	15 mg/kg, i.p.		Yoo et al., 2003, 2006
		+ *challenge on day 12*	15 mg/kg, i.p.	Decreased	
		Locomotor sensitization *(10d inj)*	15 mg/kg, i.p.		Hummel et al., 2004
		+ *challenge on day 17*	15 mg/kg, i.p.	Decreased in 129S6xC57BL/6J Increased in C57BL/6J	
		Locomotor sensitization *(5d inj)*	20 mg/kg, s.c.	Unchanged	Hall et al., 2004
		Locomotor sensitization *(11d inj)*	20 mg/kg, i.p.		Lesscher et al., 2005
		+ *challenge on day 14*	10 mg/kg, i.p.	Unchanged	
	Methamphetamine	Locomotion	1.25 mg/kg, i.p.	Unchanged	Shen et al., 2010
			2.5 mg/kg, i.p.	Decreased	
			10 mg/kg, i.p.	Unchanged	
		Locomotor sensitization *(7d inj)*	0.62 mg/kg, i.p.	Abolished	
	Nicotine	Locomotion	0.7, 1 or 3 mg/kg, s.c.	Unchanged	Berrendero et al., 2002
		Locomotor sensitization *(2x/d, 7d)*	0.05 mg/kg, s.c. *d1*	No effect (WT and KO)	Yoo et al., 2004
			0.05 mg/kg, s.c. *d7*	Abolished	
		+ *challenge on day 11*	0.05 mg/kg, s.c.	Abolished	
		Locomotor sensitization *(2x/d, 7d)*	0.05 mg/kg, s.c. *d1*	No effect (WT and KO)	Yoo et al., 2005
			0.05 mg/kg, s.c. *d7*	Abolished	
	Alcohol	Locomotion	0.75, 1.25 or 1.75 g/kg, i.p.	Abolished	Ghozland et al., 2005
		Locomotion	0.5 or 1.2 g/kg, i.p.	Decreased (trend)	Hall et al., 2001
delta	Morphine	Locomotion	10 or 20 mg/kg, s.c.	Unchanged	Chefer et al., 2003
		Locomotor sensitization *(5d inj)*	20 mg/kg, s.c.	Unchanged, faster	Chefer and Shippenberg, 2009
		Challenge on day +7	5 mg/kg, s.c.	Increased	
		Challenge on day +33	5 mg/kg, s.c.	Unchanged	
		Locomotor tolerance *(3d)*	25 mg pellet, s.c.	Decreased	
	THC	Locomotor tolerance *(2x/d, 5d)*	20 mg/kg, i.p.	Unchanged	Ghozland et al., 2002
	Cocaine	Locomotion	10 mg/kg, i.p.	Increased	Chefer et al., 2004
			20 mg/kg, i.p.	Unchanged	
	Nicotine	Locomotion	0.35, 1.05 or 2.10 mg/kg, s.c.	Unchanged	Berrendero et al., 2012
mu delta	THC	Locomotion	20 mg/kg, i.p.	Unchanged	Castane et al., 2003
	Cocaine	Locomotion	5 or 15 mg/kg, i.p.	Unchanged	Chefer et al., 2005
			10 mg/kg, i.p.	Increased	
		Locomotor sensitization *(5d inj)*	15 mg/kg, i.p. *d1*	Increased locomotion	
		+ *challenge on day 8*	15 mg/kg, i.p. *d8*	Abolished	
kappa	THC	Locomotor tolerance *(2x/d, 5d)*	20 mg/kg, i.p.	Decreased	Ghozland et al., 2002
	Cocaine	Locomotion	5 or 15 mg/kg, i.p.	Unchanged	Chefer et al., 2005
			10 mg/kg, i.p.	Increased	
		Locomotor sensitization *(5d inj)*	15 mg/kg, i.p. *d1*	Increased locomotion	
		+ *challenge on day 8*	15 mg/kg, i.p. *d8*	Abolished	
βend	Cocaine	Locomotion	15, 30, or 60 mg/kg, i.p.	Decreased	Marquez et al., 2008
	Nicotine	Locomotion (horizontal)	1 or 3 mg/kg, s.c.	Unchanged	Trigo et al., 2009
		Locomotion (vertical)	1 mg/kg, s.c.	Increased	
			3 mg/kg, s.c.	Unchanged	
	Alcohol	Locomotor sensitization *(12d inj)*	2 g/kg, i.p.		Sharpe and Low, 2009
		+ *challenge on day 13 or 14*	1.2 g/kg, i.p.	Unchanged	
pEnk	THC	Locomotion	20 mg/kg, i.p.	Unchanged	Valverde et al., 2000
	Nicotine	Locomotion	1, 3 or 6 mg/kg, s.c.	Unchanged	Berrendero et al., 2005

Table 6 (*continued*)

Gene KO	Drug of abuse	Locomotor stimulation	Drug of abuse dose, route	Genotype effect	Ref
pDyn	Morphine	Locomotion	5 mg/kg, s.c.	Unchanged	Zimmer et al., 2001
		Locomotion	4.2 mg/kg, s.c.	Increased	Mizoguchi et al., 2010
			5 mg/kg, s.c.	Unchanged	
	THC	Locomotion	20 mg/kg, i.p.	Unchanged	Zimmer et al., 2001
	Cocaine	Locomotion	10 or 15 mg/kg, i.p.	Decreased	Chefer and Shippenberg, 2006
		Locomotor sensitization *(14d inj)*	15 mg/kg, i.p. *d1*	Unchanged	Bailey et al., 2007
			15 mg/kg, i.p. *d3, 7 and 14*	Increased	
	Nicotine	Locomotion	1, 3 or 6 mg/kg, s.c.	Unchanged	Galeote et al., 2009
	Alcohol	Locomotion	2 g/kg, i.p.	Unchanged	Nguyen et al., 2012c

Data are shown for each knockout (gene KO) mouse line. Measures of locomotor stimulation and sensitization are detailed in the Box. Unchanged: no genotype effect; increased: KO shows higher response compared to wild-type (WT); decreased: KO shows lower response compared to WT; abolished: no response in KO; d: day; inj: injection.

sensitization was found (Shen et al., 2010), therefore altogether, evidence exists that mu receptor activity contributes to locomotor effects of cocaine, and the adaptive response to repeated exposure to the drug.

Cocaine-induced locomotion was also investigated in delta KO mice, showing an increased response to cocaine in these mutant animals (Chefer et al., 2004). Locomotion stimulation upon cocaine administration was maintained or increased (Chefer et al., 2005) in kappa KO animals depending on the dose, and maintained (Bailey et al., 2007) or decreased (Chefer and Shippenberg, 2006) in pDyn KO mice, indicating contrasting effects of the kappa/dynorphin system in this response. Similarly, locomotor sensitization was abolished in kappa KO mice (Chefer et al., 2005), and increased in pDyn KO animals (Bailey et al., 2007), suggesting a dissociation of kappa receptors and dynorphins in the locomotor stimulant effect of cocaine.

6. Opioid system and nicotine

Among psychostimulants, nicotine is the primary component of tobacco that maintains smoking habits. The drug acts as a nicotinic acetylcholine receptor agonist to produce relaxation and enhanced cognitive performance, and is strongly addictive. Pharmacological and genetic studies have provided evidence for a critical role for the opioid system in nicotine addiction (for recent reviews, see Berrendero et al., 2010; Drews and Zimmer, 2010; Hadjiconstantinou and Neff, 2011; Tuesta et al., 2011), and knockout studies addressing nicotine reward and withdrawal are summarized in Table 4.

Rewarding properties of nicotine were altered in pEnk, βend, mu and delta KO mice, as shown by decreased nicotine CPP in these mutant mice (Berrendero et al., 2002, 2005, 2012; Trigo et al., 2009; Walters et al., 2005). In agreement, enhanced extracellular dopamine induced by nicotine in the nucleus accumbens was attenuated in mice lacking pEnk (Berrendero et al., 2005) and delta receptors (Berrendero et al., 2012). Also, the acquisition of nicotine SA was decreased in delta KO mice (Berrendero et al., 2012), further substantiating the notion that delta/pEnk receptor signaling contributes to reinforcing properties of nicotine. In contrast, self-administration of a low nicotine dose was increased in pDyn KO mice (Galeote et al., 2009) suggesting that, as for THC, dynorphin may contribute to aversive effects of nicotine. It would be interesting to pursue similar experiments in kappa KO mice to confirm this hypothesis.

mu/pEnk signaling seems involved in nicotine dependence. Withdrawal signs of chronically nicotine-treated pEnk (Berrendero et al., 2005) and mu KO (Berrendero et al., 2002) mice were attenuated, while no difference with wild-type controls was observed for pDyn (Galeote et al., 2009), delta (Berrendero et al., 2012) and βend (Trigo et al., 2009) KO mice. Finally, the mu receptor also contributes to nicotine-induced locomotor sensitization (Yoo et al., 2005, 2004), see Table 6.

7. Opioid system and alcohol

Alcohol produces euphoria, among many other effects, and acts on several molecular targets in the brain. A recent analysis of 37 KO mouse lines has provided evidence that alcohol consumption is controlled by multiple physiological systems (Blednov et al., 2012). Among these, endogenous opioids represent an important neurobiological component of alcohol intake and dependence (Gianoulakis, 2009; Koob et al., 2003). Extensive research has implicated endogenous opioid peptide release in alcohol consumption, and naltrexone, a general opioid antagonist, showed some efficacy in the treatment of alcoholism (Koob et al., 2009). Knockout mice have provided key insights into opioid mechanisms underlying alcohol-related behaviors (see Table 5). Mice lacking mu opioid receptors did not self-administer alcohol under several conditions, including oral self-administration and the two-bottle choice, and did not display conditioned place preference to alcohol (Becker et al., 2002; Hall et al., 2001; Roberts et al., 2000), demonstrating that mu receptors are essential to consumption and motivation for alcohol. mu receptor also plays a role in alcohol withdrawal as the absence of mu receptor accelerated the progression of physical signs of withdrawal (Ghozland et al., 2005). Finally, no locomotor stimulation was observed following alcohol administration in mu KO mice (Ghozland et al., 2005 and Table 6), and altogether data show a prominent role of mu receptors in many aspects of alcohol effects.

Opposing mu receptor mutants, delta KO mice showed increased alcohol consumption in TBC (Roberts et al., 2001; van Rijn et al., 2010; van Rijn and Whistler, 2009) and oral SA combined with TBC (Roberts et al., 2001) paradigms and their innate anxiety returned to wild-type levels after alcohol SA (Roberts et al., 2001). Given the important role of delta in reducing emotional responses (Filliol et al., 2000), increased alcohol intake in these mutants may reflect a self-medication approach to alleviate high levels of anxiety (for a recent review, see Chu Sin Chung and Kieffer, 2013). Interestingly, pEnk KO animals showed intact rewarding effect of alcohol and a normal pattern of alcohol consumption (Koenig and Olive, 2002), however alcohol drinking was modified in pEnk KO under stressful conditions. The latter observation supports a role for delta/pEnk signaling in regulating emotional responses that may impact on alcohol consumption. β-endorphin may also be involved since alcohol intake was reduced (Racz et al., 2008), unchanged (Hayward et al., 2004) or increased (Grahame et al., 1998; Grisel et al., 1999) in βend KO mice.

Paradoxically, mice lacking the kappa receptor showed reduced preference and alcohol consumption in TBC paradigms (Kovacs et al., 2005; van Rijn and Whistler, 2009), which contrast with increased reinforcing effects of other drugs of abuse in these mice. Using similar

TBC testing, pDyn KO mice showed increased voluntary consumption (Femenia and Manzanares, 2012; Racz et al., 2012) suggesting that the kappa receptor and dynorphins regulate alcohol intake via distinct mechanisms. Alcohol CPP was unchanged (Blednov et al., 2006; Nguyen et al., 2012c; Sperling et al., 2010) or increased (Femenia and Manzanares, 2012) in mice lacking pDyn. The latter observation is in agreement with the TBC data and the reported aversive-like activity of dynorphin peptides. pDyn KO mice otherwise showed normal increase in stress-induced alcohol preference (Racz et al., 2012; Sperling et al., 2010), but developed stronger withdrawal signs after chronic alcohol (Femenia and Manzanares, 2012). As mu receptors therefore, pDyn influences several aspects of responses to alcohol, and future studies will examine whether kappa/pDyn signaling indeed operates in alcohol abuse.

8. Discussion and concluding remarks

Knockout studies have highlighted very distinct roles for each component of the opioid system in drug reward and dependence: the mu receptor is a convergent molecular target mediating rewarding properties of all drugs of abuse, the kappa receptor opposes mu receptor signaling in the control of hedonic homeostasis, and also mediates aversive effects of cannabinoids and nicotine, and the delta receptor most likely modulates drug consumption indirectly, by improving emotional states or facilitating drug-context association (see Lutz and Kieffer, 2012, 2013). Confronting data from receptor KO and peptide KO mice is a difficult task, since ideally behavioral responses of the six knockout lines should be examined in parallel, using the same experimental setting. This was performed with the three receptor lines for some responses, but was never achieved for the six lines together. Also studies from constitutive gene deletions have sometimes yielded results which are discordant with behavioral pharmacology, often attributed to compensatory mechanisms that may develop in genetically modified animals (Kieffer and Gaveriaux-Ruff, 2002; Portugal and Gould, 2008). Altogether however, data analysis across the literature allows identification of potential endogenous receptor/peptide systems operating in drug reinforcement processes, and reveals differing mechanisms across the distinct classes of drugs of abuse (Figs. 2 and 3).

8.1. Role of mu signaling in drug reward

mu receptor is essential for rewarding effects of opiates as well as non-opiate drugs (cannabinoids, psychostimulants and alcohol). Both pEnk and βend (Roth-Deri et al., 2008) are involved in rewarding effects of non-opioid drugs of abuse, with a demonstrated implication of βend for cocaine and alcohol, whereas nicotine or cannabinoid reward has been little explored so far for the two peptides.

8.2. Role of kappa signaling in drug aversion

The important role of kappa/dynorphin in dysphoric effects of drugs of abuse has been reviewed recently (Shippenberg et al., 2007; Wee and Koob, 2010). The set of data summarized here supports the notion that kappa receptors mainly interact with pDyn-derived peptides to limit drug reward and mediate dysphoric aspects for some drugs (cannabinoids, nicotine). Moreover, and only under stressful conditions, kappa/dynorphin activity increases sensitivity to cocaine reward. The kappa/dynorphin partnership regulating alcohol intake, however, requires further studies.

8.3. Role of delta signaling in drug reward

Data indicate that delta receptor activity reduces levels of anxiety and depressive-like behaviors, and that enkephalin is involved in this process (Chu Sin Chung and Kieffer, 2013; Lutz and Kieffer, 2012; Pradhan et al., 2011), and it is likely that delta/pEnk signaling also regulates alcohol intake through similar mechanisms.

8.4. Clinical perspectives

Many pharmacotherapies to treat addiction have been developed in the past decades, but have often shown modest efficacy or acted on sub-populations of patients (Potenza et al., 2011; Volkow and Skolnick, 2012). Clinical studies also showed reduced relapse rate in patients receiving behavioral therapy (alcohol), and in general individual differences, including genetic vulnerability, need be considered (Heilig et al., 2011). The question of whether novel opioid compounds could lead to more efficient treatments is under intense investigations. Naltrexone, a general opioid antagonist, was the first opioid medication with FDA approval to reduce the level or frequency of drug intake (Pettinati and Rabinowitz, 2006). Methadone treatment, targeting mu receptors, was a pioneering substitution approach to treat heroin addiction, and a recent report describing eight compounds effective in the treatment of alcohol (acamprosate, naltrexone), opioid (buprenorphine, methadone, naloxone) and nicotine (nicotine, varenicline, bupropion) addiction, shows that mu receptors remain a prime target in most successful treatments for addiction (Pierce et al., 2012). Delta agonists may be efficient to limit disruption of emotional responses in addicted

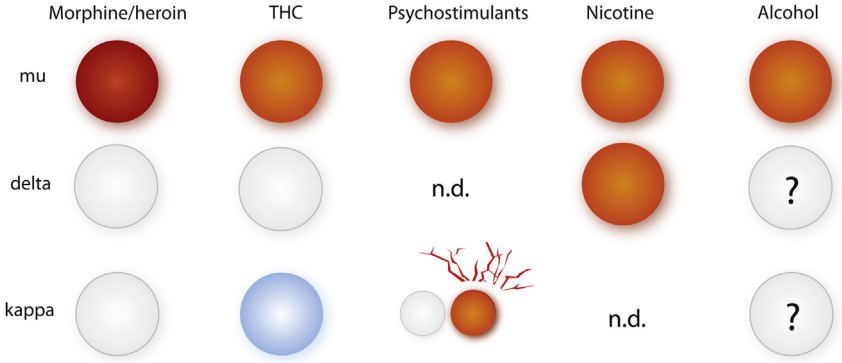

Fig. 2. Involvement of opioid receptors in drug reward. The scheme summarizes data from receptor KO mice and highlights the role of each receptor in drug reward. The mu opioid receptor mediates rewarding properties of both opioid and non-opioid drugs of abuse. With the exception of nicotine, the delta receptor does not seem involved in drug reward. The kappa receptor mediates dysphoric effects of THC and favors cocaine reward after stress (red lines). The role of delta and kappa receptor in alcohol intake is under investigation (see text). Circles indicate euphoria (red/orange), no effect (white) or dysphoria (blue); n.d: not determined in receptor KO mice.

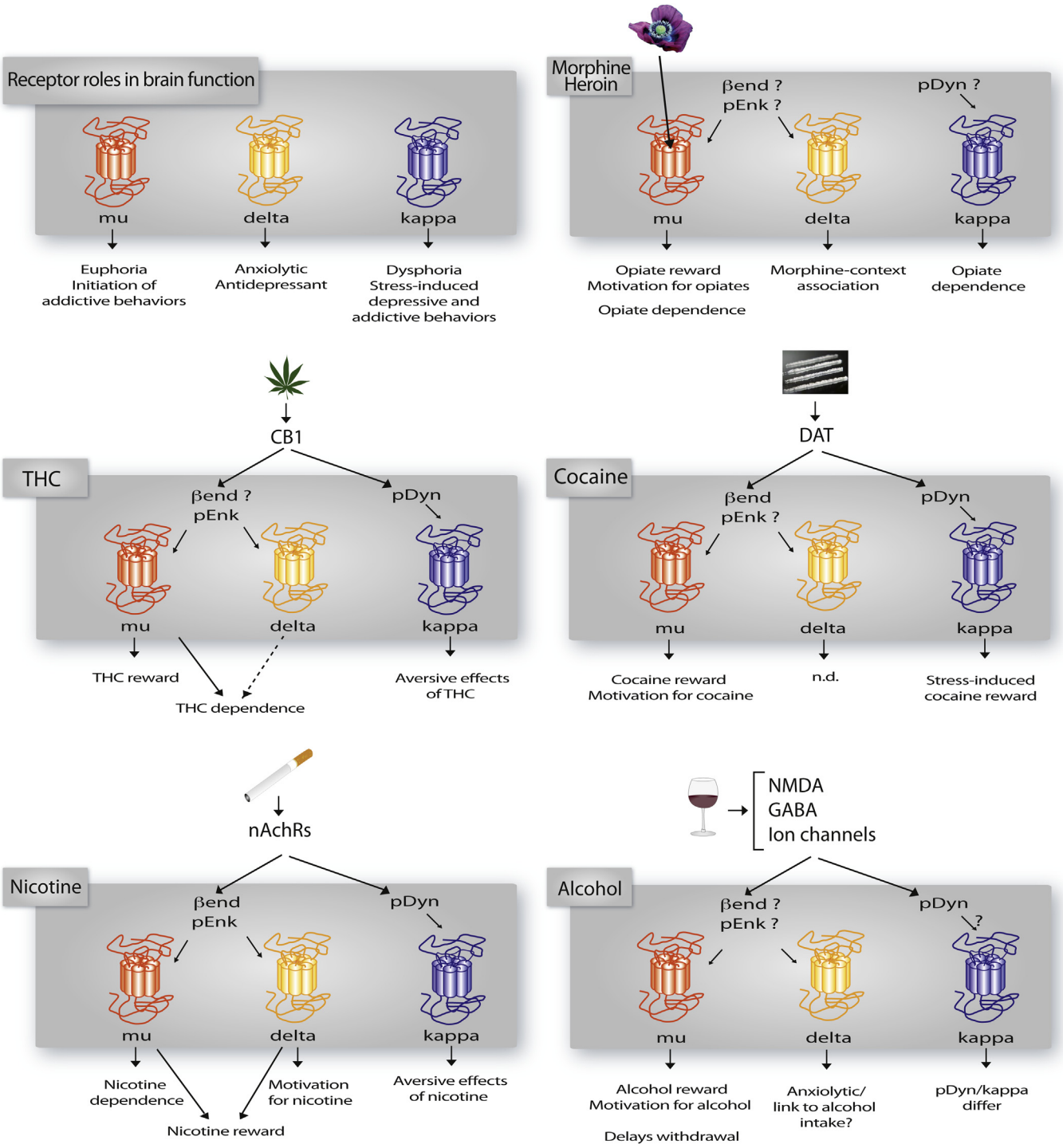

Fig. 3. Distinct roles of opioid receptors and peptides in addiction-related effects of drugs of abuse. The upper left scheme summarizes known roles of opioid receptors in brain functions related to hedonic homeostasis and mood (from Lutz and Kieffer, 2012). In the five other panels, we propose mechanisms implicating opioid receptors and/or peptides in addiction liability of each class of drugs of abuse, as inferred from both receptor and peptide knockout mouse data reviewed here. "Reward" and "drug-context association" refer to CPP data, "aversive effects" to CPA data, "motivation for the drug" to SA experiments, and "dependence" to scores of physical withdrawal under antagonist treatment. Data from locomotor studies are not included (see summary in Table 6). Opiates: peptide KO mice show paradoxical (β-end/reward, pEnk/withdrawal) or no (pDyn/withdrawal) phenotype. THC: β-end KO mice not tested; cocaine: pEnk KO mice not tested; nicotine: β-end KO mice tested for reward but not withdrawal; alcohol: β-end KO mice show contrasting phenotypes and pEnk show a phenotype under stress. Altogether, data from peptide KO mice, combined with those from receptor KO mice, concur to substantiate involvement of a kappa/dynorphin system in dysphoric states associated to drugs of abuse, although this may not apply to alcohol. Data also suggest a role for mu/β-end signaling in cocaine and nicotine reward, and implication of delta/pEnk signaling to regulate alcohol intake.

individuals (Lutz and Kieffer, 2012). Delta drugs have been developed to treat chronic pain and depression, and are currently being tested in the clinic, but their use in indications related to drug abuse has not been considered, as yet (Gaveriaux-Ruff and Kieffer, 2011). Preclinical research has definitely established that kappa receptor activity plays a role in addiction-related behaviors, with a prodepressant-like activity (see review Lutz and Kieffer, 2013). Kappa antagonists are therefore promising candidates for pharmacotherapies in stress- and

addiction-related disorders, and may attenuate compulsive drug intake (Wee and Koob, 2010) or specific symptoms of depressive disorders, depending on the administration time point (Knoll and Carlezon, 2010). Finally, considering the growing evidence of co-morbidity between addiction and depression, possible improvement of addiction therapies may arise from the combination of substitution treatments (mu agonists such as methadone, or partial agonists such as buprenorphine) with kappa antagonists or delta agonists, for treating patients with comorbid conditions (Lutz and Kieffer, 2013).

Further development of delta and kappa opioid drugs will join the growing body of studies addressing other targets, such as gamma-aminobutyric acid receptors and voltage-gated ion channels. These drugs will likely complete other non-pharmacological therapies, including transcranial magnetic stimulation or behavioral, cognitive therapies and group therapies considered very effective in long-term treatments (Addolorato et al., 2012; Volkow and Skolnick, 2012).

9. Future directions – addressing the neural circuit by genetic approaches

9.1. Conditional knockout

Conventional knockout approaches have proved valuable to tease apart respective contributions of opioid receptor and peptides in several aspects of drug abuse. Further important developments in addiction research involve investigation of molecular mechanisms operating at the level of neuronal circuits underlying the distinct aspects of addiction (Koob and Volkow, 2010). Therefore, genetic approaches targeted at specific brain sites or neuronal populations are required (Fowler and Kenny, 2012; Gaveriaux-Ruff and Kieffer, 2007; Heldt and Ressler, 2009), among which conditional gene knockout using the Cre/loxP system has received great attention (Nagy, 2000). In the addiction field, several studies using this technology have provided invaluable insights into circuit mechanisms of drug reward. Site-specific deletion of α4-containing nAChR (McGranahan et al., 2011) as well as NMDA receptor NR1 subunit (Wang et al., 2010) has revealed involvement of NMDA receptors expressed in dopaminergic neurons in nicotine reward. Mice lacking CREB specifically in the cerebral cortex were tested for cocaine self-administration and showed a role for CREB in mediating cocaine reinforcement in this brain structure (McPherson et al., 2010). A comprehensive analysis of behavioral and autonomic effects of THC in several conditional lines has revealed implication of the CB1 receptor expressed at the level of forebrain glutamatergic neurons (CB1CamKIIa-Cre mice), cortical glutamatergic neurons (CB1NEX-Cre mice) and dopaminergic neurons (CB1Drd1a-Cre mice), but not GABAergic neurons (CB1Dlx5/6-Cre mice) (Monory et al., 2007). Also a conditional knockout approach using Pet1-Cre mice, targeting the transcription factor *Lmx1b* in developing serotonergic neurons of the hindbrain, showed that central serotonergic neurons modulate supraspinal pain but are not involved in morphine reward (Zhao et al., 2007). So far, only one conditional line has been reported for opioid receptors and peptides, demonstrating a key role of delta receptors expressed in primary nociceptive neurons in delta analgesia and the control of chronic pain (Gaveriaux-Ruff et al., 2011). It is expected that conditional lines for the opioid system, targeting the neurocircuitry of addiction, will be instrumental to understand circuit mechanisms underlying opioid-mediated drug effects and plasticity.

9.2. Optogenetics and brain imaging

More recently, a novel area of investigation has emerged with the development of optogenetic approaches to manipulate specific neuronal populations in live animals (Fowler and Kenny, 2012). For example, light-mediated phasic activation of dopaminergic neurons in the VTA produced a place preference in a CPP paradigm (Tsai et al., 2009) and the specific light-activation of cholinergic neurons from nucleus accumbens reduced cocaine reward (Witten et al., 2010). The specific manipulation of mu, delta or kappa receptor expressing neurons will be of great interest towards understanding neuronal connectivity and plasticity while addiction develops. Within this line, non-invasive neuroimaging and functional connectivity techniques, now developed in small rodents, offer promises in translational medicine (Dalley et al., 2009; Jasinska et al., 2013), and neuroimaging of opioid receptor and peptide genetic mutants may provide invaluable information towards understanding the human disease.

9.3. New animal models

Behavioral testing in mice is limited, however new models have been developed to better characterize several stages of the addiction cycle, or protracted abstinence and relapse (for example: Goeldner et al., 2011; for reviews see O'Brien and Gardner, 2005; Spanagel, 2003) Animal research is expanding in this direction for brain disorders in general (Ahmed, 2010; Berton et al., 2012; Nestler and Hyman, 2010). Also, automated multidimensional systems now enable recording behavior of mice living in social groups to characterize novelty-seeking trait, anxiety, impulsivity, compulsivity and motivation, and such systems can be successfully applied to study behavioral adaptations to drugs of abuse (Radwanska and Kaczmarek, 2011). Also, drosophila or zebra fish are model organisms that allow rapid genetic screens and are being developed in the context of drug abuse (Kaun et al., 2012; Klee et al., 2012; Stewart et al., 2011).

Ultimately, the combination of emerging technologies at molecular, circuit and behavioral levels holds enormous potential to discover novel mechanisms operating at integrated level. The opioid system remains a prime candidate to develop successful therapies in addicted individuals, and understanding opioid-mediated processes at systems levels represents a challenging goal in addiction research.

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