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REVIEW

The endogenous opioid system in cocaine addiction: what lessons have opioid peptide and receptor knockout mice taught us?

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Cocaine addiction has become a major concern in the UK as Britain tops the European 'league table' for cocaine abuse. Despite its devastating health and socio-economic consequences, no effective pharmacotherapy for treating cocaine addiction is available. Identifying neurochemical changes induced by repeated drug exposure is critical not only for understanding the transition from recreational drug use towards compulsive drug abuse but also for the development of novel targets for the treatment of the disease and especially for relapse prevention. This article focuses on the effects of chronic cocaine exposure and withdrawal on each of the endogenous opioid peptides and receptors in rodent models. In addition, we review the studies that utilized opioid peptide or receptor knockout mice in order to identify and/or clarify the role of different components of the opioid system in cocaine-addictive behaviours and in cocaine-induced alterations of brain neurochemistry. The review of these studies indicates a region-specific activation of the μ-opioid receptor system following chronic cocaine exposure, which may contribute towards the rewarding effect of the drug and possibly towards cocaine craving during withdrawal followed by relapse. Cocaine also causes a region-specific activation of the κ-opioid receptor/dynorphin system, which may antagonize the rewarding effect of the drug, and at the same time, contribute to the stress-inducing properties of the drug and the triggering of relapse. These conclusions have important implications for the development of effective pharmacotherapy for the treatment of cocaine addiction and the prevention of relapse.

Abbreviations

Amy, amygdala; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; Cer, cerebellum; CG, central grey; CgCx, cingulate cortex; CPP, conditioned place preference; CPu, caudate putamen; DA, dopamine; DAT, dopamine transporter; Den, dorsal endopiriform; DG, dentate gyrus; DOPr, δ-opioid receptor; DR, dorsal raphe; End, endorphin; FCx, frontal cortex; FST, forced swim test; Hb, habenula; Hi, hippocampus; HPA, hypothalamic–pituitary–adrenal; Hy, hypothalamus; IC, inferior colliculus; KO, knockout; KOPr, κ-opioid receptor; LH, lateral hypothalamus; MG, medial geniculate; MOPr, μ-opioid receptor; MS, medial septum; NAc, nucleus accumbens; nNOS ir, neuronal nitric oxide synthase immunoreactivity; OB, olfactory bulb; OT, olfactory tubercle; PDYN, preprodynorphin; PENK, preproenkephalin; Pir, piriform; Pit, pituitary; POMC, proopiomelanocortin; Sept, septum; SN, substantia nigra; TCx, temporal cortex; Th, thalamus; Tu, tubercle; VDB, vertical limb of the diagonal band; VMN, ventromedial nucleus of the hypothalamus; VP, ventral pallidus; VTA, ventral tegmental area; WT, wild type

Introduction

Cocaine is a psychostimulant whose use in the European Union has dramatically increased over the last 10 years with Britain topping the European 'league table' for cocaine abuse (European Monitoring Center for Drugs and Drug Addiction, 2011). It is estimated that nearly 1 million people used cocaine last year in England and Wales alone, and the number of cocaine addicts aged 18–24 in treatment has doubled in the last 3 years in the UK (UNODC, 2011). When considering the

physical danger, addictive liability and social harm of all drugs of abuse, cocaine is especially destructive, costing the UK tax payer billions of pounds a year on productivity loss, criminal activity and on social and medical care. Despite this, and although a number of potential targets have been identified (Heidbreder and Hagan, 2005), currently, there is no specific pharmacological therapy with established efficacy for the treatment of cocaine addiction and for the prevention of relapse (Kreek *et al.*, 2002; Somaini *et al.*, 2011).

Cocaine is well known to increase extracellular levels of monoamines by blocking monoamine transporters (Heikkila *et al.*, 1975). Elevation of extracellular dopamine (DA) levels in the mesolimbic dopaminergic system, which is composed primarily of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) has been suggested to play a central role in the reinforcing effect of the psychostimulant, although other brain structures such as the hypothalamus (Hy), bed nucleus of the stria terminalis (BNST), septum (Sept), hippocampus (Hi), caudate putamen (CPu), thalamus (Th), amygdala (Amy) and frontal cortex (FCx) have also been implicated (Koob and Le Moal, 2001; Koob and Kreek, 2007; Le Moal and Koob, 2007). The acute positive reinforcement (i.e. rewarding, euphoric) effect of the drug is thought to involve the activation of the VTA-NAc (reward), BNST and Amy (emotional learning) circuitries (Le Moal and Koob, 2007). The negative withdrawal symptoms that drive drug administration (negative reinforcement) following repeated drug use (dependence state) involve suppression of the aforementioned circuitries and the recruitment of brain stress pathways, namely the Hy and Amy. In terms of relapse, the FCx, involved in impulse control and decision-making, and the basolateral Amy (BLA) have been implicated in drug priming and cue priming reinstatement respectively (Le Moal and Koob, 2007). In addition, other regions such as the Hi (contextual memory) and CPu (habit learning) have been shown to be involved in the transition from recreational drug use to compulsive cocaine abuse, which is characteristic of addiction (Everitt and Robbins, 2005; Le Moal and Koob, 2007). Opioid peptides and receptors are expressed in high density in areas of the reinforcement circuitries and play a key role in their regulation (for extensive review, see Le Merrer *et al.*, 2009).

Three classical opioid receptors, the μ, δ and κ have been identified (see Goldstein and Naidu (1989) and the genes encoding them have been cloned (δ by Evans *et al.*, 1992; Kieffer *et al.*, 1992; Yasuda *et al.*, 1993; μ by Chen *et al.*, 1993; Thompson *et al.*, 1993; Wang *et al.*, 1993; and κ by Meng *et al.*, 1993; Minami *et al.*, 1993; Yasuda *et al.*, 1993; for review, see Corbett *et al.*, 2006; Kieffer 1995) and are referred as μ-opioid (MOP), δ-opioid (DOP) and κ-opioid (KOP) receptors respectively (Borsodi *et al.*, 2011). Endogenous peptides that target opioid receptors have been identified and characterized. Notably, the enkephalins, dynorphins and β-endorphins (ends) are produced by proteolytic cleavage of large protein precursors known as preproenkephalin (PENK), preprodynorphin (PDYN) and proopiomelanocortins (POMC) respectively. Genes encoding these peptide precursors have been cloned (PENK by Gubler *et al.*, 1982; Noda *et al.*, 1982; POMC by Nakanishi *et al.*, 1979; PDYN by Kakidani *et al.*, 1982; Rossier, 1982; see review by Kieffer, 1995) and mice lacking MOP receptor (MOPr) (Matthes *et al.*, 1996; Sora *et al.*, 1997; Tian *et al.*, 1997; Loh *et al.*, 1998; Schuller *et al.*, 1999), DOP receptor (DOPr) (Zhu *et al.*, 1999; Filliol *et al.*, 2000), KOP receptor (KOPr) (Simonin *et al.*, 1998; Ansonoff *et al.*, 2006) or a combination of opioid receptors (MOPr/DOPr, MOPr/KOPr, KOPr/DOPr, MOPr/KOPr/DOPr) (Simonin *et al.*, 2001) as well as mice lacking PDYN (Sharifi *et al.*, 2001; Zimmer *et al.*, 2001), PENK (Konig *et al.*, 1996; Ragnauth *et al.*, 2001) and β-end (Rubinstein *et al.*, 1996) have been generated by homologous recombination by several groups (for review, see Gaveriaux-Ruff and Kieffer, 2002; Kieffer and Gaveriaux-Ruff, 2002).

There is a large body of evidence demonstrating that the endogenous opioid system plays a key role in regulating mood and reward and is central in modulating addictive behaviours. In this context, it is generally accepted that systemic and local region-specific administration of MOPr, and to a lesser extent DOPr, agonists stimulate positive reinforcement, whereas KOPr agonists inhibits positive reinforcement and induces aversion and dysphoria. While there is compelling evidence that the aversive effects of KOPr are mediated via the suppression of DA release in the NAc (Shippenberg and Elmer, 1998; Van Ree *et al.*, 2000), the role of DA in mediating the reinforcing effects of MOPr agonists is less clear. While MOPr and DOPr stimulation induce DA release in the striatum (Di-Chiara and Imperato, 1988; Fusa *et al.*, 2005), at least in animal models, evidence (Robinson and Berridge, 1993; Daglish *et al.*, 2008) suggests that DA may not be critical in mediating the 'high' from opioids in opioid-dependent subjects but the 'drug wanting' instead (Daglish *et al.*, 2008). MOPr and DOPr antagonists have direct aversive-anxiogenic effects and can also suppress the positive reinforcing properties of natural rewards (see review by Colasanti *et al.*, 2011), whereas KOPr antagonists has been shown to facilitate these effects (Van Ree *et al.*, 2000).

Here, we review the evidence demonstrating a dysregulation of different components of the opioid system, which are likely to contribute towards the transition from recreational drug use to compulsive cocaine abuse, which is characteristic of addiction. We focus on the profound effects of chronic cocaine exposure and withdrawal on the opioid receptor (at the mRNA, protein and activity level) and peptide system, primarily in rodent models. This review does not include findings obtained by microarray experiments as this is reviewed elsewhere (Yuferov *et al.*, 2005). Furthermore, we review the studies utilizing opioid peptide and receptor knockout (KO) mice in order to identify and/or clarify the role of different components of the opioid system in cocaine-addictive behaviours and in cocaine-induced alterations of brain neurochemistry. This review will merely focus on the receptor and peptide components of the classical opioid system and does not include endomorphins, nociceptin, nocistatin and opioid receptor-like 1.

Regulation of opioid receptor and peptide system by chronic cocaine exposure and withdrawal

Opioid receptor regulation by cocaine

Regulation of MOPr. There is large body of evidence indicating a region-specific activation of the MOPr system following

chronic cocaine exposure, which persists for a long time after withdrawal from the drug (Table 1). Several brain regions have been reported to be sites of MOPr regulation by cocaine. The most consistent finding is the increase of MOPr mRNA (Azaryan *et al.*, 1996a,b; 1998; Walters *et al.*, 2005; Leri *et al.*, 2006), binding (Hammer, 1989; Unterwald *et al.*, 1992; 1994; Izenwasser *et al.*, 1996; Azaryan *et al.*, 1996a,b; 1998; Unterwald, 2001) and MOPr activation as measured both by (D-Ala^2, N-MePhe^4, Gly-ol)-enkephalin (DAMGO), stimulated 5′-(γ-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) autoradiography (Schroeder *et al.*, 2003; Bailey *et al.*, 2007b) and by DAMGO inhibition of AC activity (Izenwasser *et al.*, 1996; Schroeder *et al.*, 2003; Bailey *et al.*, 2007b) in the NAc following chronic cocaine exposure. This up-regulation of MOPr did not depend on the administration paradigm and is consistent in animals that have been exposed to continuous cocaine administration (mini-pumps) or repeatedly injected in a 'binge' paradigm or following conditioned place preference (CPP) paradigm (see Table 1). Interestingly, an increase in MOPr mRNA levels (Yuferov *et al.*, 1999) but a decrease in MOPr occupancy (Soderman and Unterwald, 2009) was observed in the NAc following acute cocaine administration in rats, demonstrating dissociation between mRNA and protein levels. However, acute or chronic withdrawal from 14 day 'binge' cocaine administration did not show alterations in MOPr mRNA (Bailey *et al.*, 2005b) or MOPr binding (Bailey *et al.*, 2005a), respectively, in the NAc, demonstrating that these alterations do not persist after the discontinuation of the drug at least in the NAc.

The profile observed for MOPr in the NAc was not reproduced in the CPu. Although no changes in MOPr mRNA and density was observed in the CPu of animals treated with a continuous cocaine administration paradigm (Izenwasser *et al.*, 1996; Azaryan *et al.*, 1996b), or in animals undergoing cocaine CPP (Leri *et al.*, 2006) or after acute withdrawal (Bailey *et al.*, 2005b), an increase in MOPr binding (Unterwald *et al.*, 1992; 1994; 2001; Bailey *et al.*, 2005a) and activity (Schroeder *et al.*, 2003; Bailey *et al.*, 2007b) was observed following chronic 'binge' cocaine administration, which persisted for a long time after the withdrawal of the drug (Bailey *et al.*, 2005a). Surprisingly, no alterations in DAMGO inhibition of adenylate cyclase activity was observed following continuous (Izenwasser *et al.*, 1996) or chronic 'binge' (Unterwald *et al.*, 1993) cocaine administration in the CPu. The lack of association between mRNA and protein receptor level in the CPu suggests that mechanisms other than increased gene expression are responsible for the regulation of MOPr by cocaine in the CPu. Indeed, mechanisms such as decreased degradation of the receptor, increased recycling or/and changes in the levels of the endogenous ligand for the receptors could account for those alterations of MOPr protein and activity. In this regard, although cocaine has been shown to increase β-end in the NAc in rodents (Olive *et al.*, 2001; Roth-Deri *et al.*, 2003; 2006; 2008), β-end deficiency in regulating hypothalamic–pituitary–adrenal (HPA) axis activity has also been suggested based on a study demonstrating hyperresponsivity to removal of glucocorticoid negative feedback in cocaine-abstinent human individuals following the administration of metyrapone (Schluger *et al.*, 1998; 2001). In addition, there is evidence based on rodent studies suggesting a D_2 receptor (Soderman and Unterwald, 2009) and dynorphin (Bailey *et al.*, 2007b)-dependent mechanism in the regulation of MOPr by cocaine.

The FCx and cingulate cortex (CgCx) were also shown to be regions where MOPr expression, binding and activity were profoundly affected by cocaine exposure and withdrawal. MOPr mRNA levels were shown to be increased in the FCx and CgCx following acute cocaine administration (Yuferov *et al.*, 1999), cocaine CPP (Leri *et al.*, 2006) and acute withdrawal from chronic cocaine administration (Bailey *et al.*, 2005b). In addition, although MOPr binding was unchanged in the CgCx following acute cocaine administration (Soderman and Unterwald, 2009), MOPr binding was elevated in the FCx/CgCx following chronic 'binge' (both steady and escalating dose) cocaine administration (Unterwald *et al.*, 1992; 1994; Unterwald, 2001; Bailey *et al.*, 2005a), which persisted for a long time after the withdrawal of the drug (Bailey *et al.*, 2005a). Fourteen-day 'binge' cocaine also increased MOPr activity in the CgCx (Schroeder *et al.*, 2003; Bailey *et al.*, 2007b). Together, these studies clearly indicate an activation of the MOPr system in the FCx, which persists for a long time after the discontinuation of the drug.

Some inconsistencies between mRNA and protein levels and between administration paradigm have been observed in the regulation of MOPr in other brain regions. For instance, while at the mRNA level, there was no modification of MOPr in the Amy following chronic cocaine administration (Zhou *et al.*, 2005), acute withdrawal (Zhou *et al.*, 2005) and CPP (Leri *et al.*, 2006); increased levels of MOPr binding was observed in the BLA following chronic 'binge' administration paradigm (Unterwald *et al.*, 1992; 1994; Unterwald, 2001), which did not persist following 14 day withdrawal period (Bailey *et al.*, 2005a). In contrast, decreased levels of MOPr binding were found following continuous cocaine administration (Hammer, 1989) in the BLA. Again, these inconsistencies probably suggest that mechanisms other than alterations of gene expression contribute towards the regulation of MOPr by cocaine in the Amy and that this depends on the drug administration paradigm.

No alterations of MOPr mRNA levels were observed in the olfactory bulbs (Azaryan *et al.*, 1996b) and no modification of MOPr binding was observed in the olfactory tubercle (OT), ventral pallidus (VP), substantia nigra (SN), VTA, dorsal endopiriform (Den), medial Sept, Hy, Th, vertical limb of the diagonal band, habenula and Hi following chronic cocaine administration (Unterwald *et al.*, 1992; Bailey *et al.*, 2005a) and following long-term withdrawal from chronic cocaine administration (Bailey *et al.*, 2005a). No modification of MOPr mRNA regulation in Hy following CPP (Leri *et al.*, 2006) and withdrawal (Zhou *et al.*, 2005) was observed. MOPr binding was, however, increased in the VP, lateral Hy (LH), medial geniculate, inferior colliculus but decreased in the SN, VTA, dorsal raphe following chronic continuous cocaine administration (Hammer, 1989). These findings indicate a regulatory role for cocaine on the MOPr system in a large number of brain regions, at least at the protein level.

Similar alterations in MOPr were also reported in brains of human cocaine addicts. Increased levels of MOPr availability measured by positron emission tomography were also observed in cocaine abstinent human addicts in the CPu, Th, CgCx, FCx, temporal cortex (Zubieta *et al.*, 1996; Gorelick *et al.*, 2005), demonstrating the translational relevance of this

Table 1

Effect of chronic cocaine exposure and withdrawal on opioid peptide and receptor expression, protein and activity

Opioid system	Administration paradigm	Brain region	mRNA	Protein (binding)	Functional activity	References
MOPr	Acute	FCx, NAc, Amy	↑			(Yuferov *et al.* 1999)
		Hi, Th, Hy	↔			
		NAc		↓		(Soderman and Unterwald, 2009)
		CPu, Tu, CgCx, MCx, VTA		↔		(Soderman and Unterwald, 2009)
	Chronic (repeated)	Amy	↔			(Zhou *et al.*, 2005)
		CgCx, FCx, NAc, CPu, BLA		↑	↑*	(Unterwald *et al.*, 1992; 1994; 2001; Schroeder *et al.*, 2003; Bailey *et al.*, 2005a; 2007b)
		NAc, CPu			↔#	(Unterwald *et al.*, 1993)
		OT, Den, MS, VP, VDB, Hy, Hb, Hi, SN, VTA		↔		(Unterwald *et al.*, 1992; Bailey *et al.*, 2005a)
	Chronic (continuous)	NAc	↑	↑	↑#	(Hammer, 1989; Izenwasser *et al.*, 1996; Azaryan *et al.*, 1996a,b; 1998)
		OB, CPu	↔	↔	↔#	(Izenwasser *et al.*, 1996; Azaryan *et al.*, 1996b)
		VP, LH, MG, IC		↑		(Hammer, 1989)
		SN, VTA, DR, BLA		↓		(Hammer, 1989)
	Acute withdrawal	FCx	↑			(Bailey *et al.*, 2005b)
		CPu, NAc, Amy, Hy	↔			(Bailey *et al.*, 2005b; Zhou *et al.*, 2005)
	Chronic withdrawal	FCx, CgCx, CPu		↑		Bailey *et al.*, 2005a)
		NAc, OT, Den, Sept, VDB, Th, Amy, Hy, Hi,		↔		(Bailey *et al.*, 2005a)
		Limbic Cx, NAc, VDB		↓		(Sharpe *et al.*, 2000)
	CPP	NAc, FCx	↑			(Walters *et al.*, 2005; Leri *et al.*, 2006)
		CPu, Amy, Hy	↔			(Leri *et al.*, 2006)
	Human addicts	CPu, Th, CgCx, FCx, TCx		↑$		(Zubieta *et al.*, 1996; Gorelick *et al.*, 2005; 2008)
		CPu		↓		(Hurd and Herkenham, 1993)
DOPr	Chronic (repeated)	CgCx, OT, NAc, CPu, BLA, SN, VTA		↔	↔*	(Unterwald *et al.*, 1994; Schroeder *et al.*, 2003)
		NAc, CPu			↓#	(Unterwald *et al.*, 1993)
	Chronic (continuous)	NAc, CPu, OB	↔		↔#	(Izenwasser *et al.*, 1996; Azaryan *et al.*, 1996b)
	Acute withdrawal	FCx, Nacb, CPu			↓#	(Perrine *et al.*, 2008)

Table 1

Continued

Opioid system	Administration paradigm	Brain region	mRNA	Protein (binding)	Functional activity	References
KOPr	Acute	SN	↔			(Spangler *et al.*, 1997)
	Chronic (repeated)	CPu	↔			(Spangler *et al.*, 1996a; Unterwald *et al.*, 2001)
		NAc, VTA, SN	↓			(Spangler *et al.*, 1996a; 1997; Rosin *et al.*, 1999)
		CgCx, OT, CPu, VTA, Sept		↑		(Unterwald *et al.*, 1994; 2001; Bailey *et al.*, 2007a)
		NAc, BLA, SN		↔		(Unterwald *et al.*, 1994)
		NAc, CPu, CgCx			↔*	(Schroeder *et al.*, 2003)
	Chronic (continuous)	NAc, BLA, End, Sept		↑		(Collins *et al.*, 2002)
	Chronic withdrawal	SN, CPu	↔			(Spangler *et al.*, 1996a)
		NAc, CPu, Septum, BLA		↓		(Turchan *et al.*, 1998; Bailey *et al.*, 2007a)
	Human addicts	NAc, CPu, Amy		↑		(Hurd and Herkenham, 1993; Staley *et al.*, 1997; Mash and Staley, 1999)
PENK	Acute	CPu, Cx	↑			(Daunais and McGinty, 1995; Przewlocka and Lason, 1995)
		Amy	↔			(Turchan *et al.*, 2002)
	Chronic (repeated)	NAc, CPu	↑			(Hurd *et al.*, 1992; Steiner and Gerfen, 1993; Przewlocka and Lason, 1995; Spangler *et al.*, 1996b; 1997; Mathieu-Kia and Besson, 1998; Svensson and Hurd, 1998; Crespo *et al.*, 2001)
		NAc, CPu	↔			(Branch *et al.*, 1992; Hurd *et al.*, 1992; Daunais and McGinty, 1995; Spangler *et al.*, 1996a; Mathieu-Kia and Besson, 1998; Alvarez Fischer *et al.*, 2001; Bailey *et al.*, 2005b; Ziolkowska *et al.*, 2006)
		Pir, Tu	↑			(Crespo *et al.*, 2001)
		FCx, Cx, Amy, Hy, Pit, CG, Cer	↔			(Branch *et al.*, 1992; Daunais and McGinty, 1994; Turchan *et al.*, 2002; Bailey *et al.*, 2005b; Ziolkowska *et al.*, 2006)
	Acute withdrawal	CPu, NAc,	↓			(Przewlocka and Lason, 1995; Crespo *et al.*, 2001)
		Amy	↓			(Crespo *et al.*, 2001)
		CPu, Pir, Tu, NAc	↑			(Crespo *et al.*, 2001)
		FCx, NAc, CPu	↔			(Spangler *et al.*, 1996a,b; Svensson and Hurd, 1998; Arroyo *et al.*, 2000; Bailey *et al.*, 2005b)
	Chronic withdrawal	CPu, Tu, Pir, NAc	↑			(Crespo *et al.*, 2001)
		Amy, VMN	↓			(Crespo *et al.*, 2001)
		CPu, NAc	↔			(Svensson and Hurd, 1998)
	Human addicts	CPu	↓			(Hurd and Herkenham, 1993)

Table 1

Continued

Opioid system	Administration paradigm	Brain region	mRNA	Protein (binding)	Functional activity	References
β-end	Acute	NAc		↑[&]		(Olive *et al.*, 2001)
		Pit		↑[T]		(Moldow and Fischman, 1987)
	Chronic (repeated)	NAc		↑[&]		(Roth-Deri *et al.*, 2003; 2006)
		Hy	↔			(Zhou *et al.*, 2005)
		Pit, plasma		↑[T]		(Forman and Estilow, 1988)
	Acute Withdrawal	Hy	↔			(Forman and Estilow, 1988; Zhou *et al.*, 2005)
	CPP	Hy	↔			(Leri *et al.*, 2006)
PDYN	Human addicts	plasma		↑[T]		(Vescovi *et al.*, 1992)
	Acute	CPu	↑			(Spangler *et al.*, 1997; Zhou *et al.*, 2002)
	Chronic (repeated)	CPu	↑	↑[T]		(Sivam, 1989; Smiley *et al.*, 1990; Hurd *et al.*, 1992; Spangler *et al.*, 1993; 1996a; 1997; Steiner and Gerfen, 1993; Daunais and McGinty, 1995, 1996; Romualdi *et al.*, 1996; Mathieu-Kia and Besson, 1998; Svensson and Hurd, 1998; Werme *et al.*, 2000; Yuferov *et al.*, 2001; Bailey *et al.*, 2005b; Schlussman *et al.*, 2005; Ziolkowska *et al.*, 2006)
		NAc, DG, SN	↑	↑[T]		(Smiley *et al.*, 1990; Hurd *et al.*, 1992; Mathieu-Kia and Besson, 1998; Turchan *et al.*, 2002)
		FCx, Cx, NAc, Hy, Amy, SN	↔			(Spangler *et al.*, 1993; Alvarez Fischer *et al.*, 2001; Yuferov *et al.*, 2001; Turchan *et al.*, 2002; Bailey *et al.*, 2005b; Schlussman *et al.*, 2005; Ziolkowska *et al.*, 2006; Zhou *et al.*, 2008)
	Chronic (continuous)	CPu	↑			(Romualdi *et al.*, 1996)
		Hy	↓			(Romualdi *et al.*, 1996)
		Hi, NAc	↔			(Romualdi *et al.*, 1996)
	Acute withdrawal	CPu, LH, SN	↑	↑[T]		(Sivam, 1989; Smiley *et al.*, 1990; Bailey *et al.*, 2005b; Zhou *et al.*, 2008)
	Chronic withdrawal	CPu, NAc	↓			(Svensson and Hurd, 1998)
		CPu, LH	↔	↔[T]		(Sivam, 1989; Spangler *et al.*, 1996a; Zhou *et al.*, 2008)
	Human addicts	CPu, VP	↑			(Hurd and Herkenham, 1993; Frankel *et al.*, 2008)

*As measured by DAMGO stimulated [^{35}S]GTPγS autoradiography.
[#]As measured by DAMGO inhibition of AC activity.
[&]As measured by microdialysis for β-endorphin release.
[$]As measured by positron emission tomography.
[T]Precursor peptide fragment immunoreactivity.
MCx, motor cortex.

cocaine regulation of the MOPr system in humans as well as the translational reliability of the animal model of chronic 'binge' cocaine administration paradigm not only in terms of mimicking the human pattern of cocaine administration but also in mimicking the neurochemical consequences of chronic cocaine abuse (see also Bailey *et al.*, 2008). It is not clear if the increased availability of MOPr in humans represents a decrease in β-end release in addition to an increase of MOPr in abstinent human individuals. In rodents, although the increase of β-end release in response to acute and chronic cocaine administration has been demonstrated at least in the striatum (Olive *et al.*, 2001; Roth-Deri *et al.*, 2003; 2008), the release of β-end in withdrawn individuals remains to be explored. Increased levels of MOPr availability in frontal and temporal cortical regions was shown to be significantly positively correlated to cocaine craving (Gorelick *et al.*, 2005) and time of relapse to cocaine use (Gorelick *et al.*, 2008) in human cocaine addicts and high MOPr concentration in the PFx, CgCx, NAc and BLA has been shown to be correlated with high impulsivity in humans (Love *et al.*, 2009). Together, these findings clearly demonstrate a region-specific activation of the MOPr system by chronic cocaine exposure at least in the NAc, CPu, FCx, CgCx and Amy, which persists for a long time after the discontinuation of the drug at least in the CPu, FCx and CgCx. Given the role of these regions in decision-making (FCx), impulsiveness (FCx, CgCx, NAc, BLA), emotional learning (Amy) and reinforcement (NAc, CPu, Amy), the previous findings indicate an important role for MOPr in mediating the 'drug wanting' effect of cocaine during cocaine administration and craving of the drug during withdrawal followed by relapse.

Regulation of DOPr. No modification of DOPr mRNA (Azaryan *et al.*, 1996b), receptor binding levels (Unterwald *et al.*, 1994) and DOPr activity as measured by D-penicillamine(2,5)-enkephalin (DPDPE) stimulated [^{35}S]GTPγS autoradiography (Schroeder *et al.*, 2003) was reported in any brain regions of rats treated chronically with cocaine irrespective of treatment paradigm. Surprisingly, chronic 'binge' (Unterwald *et al.*, 1993) but not continuous (Izenwasser *et al.*, 1996) cocaine administration decreased DOPr activity in the NAc and CPu as measured by DPDPE inhibition of adenylate cyclase activity, suggesting that cocaine may result in an uncoupling of the DOPr from the G protein under that administration paradigm. This uncoupling seems to persist during acute cocaine withdrawal, as inhibition of AC by DOPr agonists was attenuated in the FCx, NAc and CPu (Perrine *et al.*, 2008), clearly indicating desensitization of DOPr during acute cocaine withdrawal, which has been shown to be associated with the emergence of emotional withdrawal symptoms (Perrine *et al.*, 2008). In support of this, internalization of DOPr in the NAc has been demonstrated 48 h following a chronic 'binge' cocaine administration paradigm (Ambrose-Lanci *et al.*, 2008). Though limited, these data do not support a regulatory role of cocaine on the DOPr system, at least at the mRNA and protein level. Nonetheless, there is strong evidence to suggest that the regulatory role of cocaine on the DOPr is more likely to be at a signal transduction level.

Regulation of KOPr. As far as the regulation of KOPr by chronic cocaine use is concerned, there are inconsistent findings between KOPr mRNA, receptor binding and KOPr-stimulated [^{35}S]GTPγS studies in the literature. At the mRNA level, chronic 'binge' cocaine decreased KOPr levels in the VTA, NAc, SN (Spangler *et al.*, 1996a; Rosin *et al.*, 1999) but not in the CPu (Spangler *et al.*, 1996a; 1997). No modification of KOPr mRNA was observed following withdrawal (Spangler *et al.*, 1996a). Nonetheless, at the protein level, while no modification of KOPr binding was observed in the NAc, BLA, SN (Unterwald *et al.*, 1994), there was an up-regulation of KOPr binding in the CgCx, OT, CPu, VTA (Unterwald *et al.*, 1994; 2001) and in the Sept (Bailey *et al.*, 2007a) following a chronic 'binge' cocaine administration paradigm. This up-regulation was also observed following chronic continuous cocaine administration in the NAc, BLA, Den and Sept (Collins *et al.*, 2002). Despite this up-regulation in KOPr binding, no increase of KOPr activity was observed in studies of dynorphin 1- to 17-stimulated [^{35}S]GTPγS autoradiography (Schroeder *et al.*, 2003). As in the case of MOPr, this inconsistency between mRNA, protein and [^{35}S]GTPγS data suggests that mechanisms other than alterations of gene expression contribute towards the regulation of KOPr by cocaine. Withdrawal from cocaine administration decreased KOPr binding in the NAc, CPu, BLA and Sept in rodents (Turchan *et al.*, 1998; Bailey *et al.*, 2007a).

These results clearly demonstrate an activation of the KOPr system following chronic cocaine exposure, but in contrast with the MOPr system, it does not persist following withdrawal from the drug. In agreement with these results, an increase in KOPr binding was also observed in the CPu, NAc, Amy of post-mortem brain of people with a history of cocaine abuse (Hurd and Herkenham, 1993; Staley *et al.*, 1997; Mash *et al.*, 2002), demonstrating the translational relevance of this cocaine-induced regulation of KOPr as well as the translational reliability of the animal model of chronic 'binge' cocaine administration paradigm. As KOPr stimulation in the brain produces aversive effects in animals and humans (Zimmer *et al.*, 2001; McLaughlin *et al.*, 2003; Shippenberg *et al.*, 2007), it is likely that this cocaine-induced up-regulation of KOPr in regions associated with reward (CPu, CgCx, VTA) might be part of a protective compensatory neuroadaptive mechanism to counteract the rewarding effect of cocaine and might contribute to the emergence of persistent dysphoria, which is very often reported in humans after the withdrawal of the drug (Gawin, 1991). The functional implications of the alterations of KOPr in brain regions associated with emotional regulation and stress (Amy, Sept) remain to be determined (see KOPr KO section).

Opioid peptide regulation by cocaine

Regulation of PENK. There are several studies that have investigated the effect of chronic cocaine treatment on PENK mRNA, but the results of these studies are inconsistent. For instance, while an increase of PENK mRNA has been reported in the NAc (Branch *et al.*, 1992; Hurd *et al.*, 1992; Przewlocka and Lason, 1995; Mathieu-Kia and Besson, 1998; Crespo *et al.*, 2001) and CPu (Steiner and Gerfen, 1993; Przewlocka and Lason, 1995; Spangler *et al.*, 1996b; 1997; Svensson and Hurd, 1998; Crespo *et al.*, 2001) of rodents following chronic cocaine exposure, a number of other studies have not reported any changes in PENK mRNA in these regions (Hurd *et al.*, 1992; Daunais and McGinty, 1995; Spangler *et al.*,

1996a; Mathieu-Kia and Besson, 1998; Alvarez Fischer *et al.*, 2001; Bailey *et al.*, 2005b; Ziolkowska *et al.*, 2006). In human post-mortem brains, a decrease in PENK mRNA levels with a history of cocaine abuse has been reported in the CPu (Hurd and Herkenham, 1993). It is possible that enkephalins may also affect MOPr regulation, but this is unlikely as a reduction of MOPr levels was observed in the CPu of the same human post-mortem brains where a reduction of enkephalin was observed (Hurd and Herkenham, 1993). In other regions, no changes in PENK mRNA levels were observed in the cortex (Daunais and McGinty, 1994), FCx (Branch *et al.*, 1992; Bailey *et al.*, 2005b), Amy (Turchan *et al.*, 2002; Ziolkowska *et al.*, 2006), Hy, pituitary (Pit), central grey and cerebellum (Branch *et al.*, 1992) following chronic cocaine treatment in rodents. However, cocaine increased PENK levels in the piriform (Pir), tubercle (Tu) in a self-administration paradigm, which persisted following acute and long-term withdrawal (Crespo *et al.*, 2001). Divergent results have also been observed following acute withdrawal from chronic cocaine exposure. While one study reported a decrease of PENK mRNA levels in the CPu and NAc (Przewlocka and Lason, 1995), several others did not demonstrate any changes following acute withdrawal in these regions (Arroyo *et al.*, 2000; Bailey *et al.*, 2005b). A decrease in PENK expression was observed in the Amy following spontaneous withdrawal (Crespo *et al.*, 2001), but no change was observed in the FCx (Bailey *et al.*, 2005b) and in the CPu and NAc (Svensson and Hurd, 1998). Increased levels of PENK mRNA was reported in the CPu, NAc, Tu and Pir and decreased levels were observed in the ventromedial nucleus of the hypothalamus and Amy in animals chronically abstinent from cocaine self-administration (Crespo *et al.*, 2001). These differences could reflect differences in drug administration paradigm or strain/species, and as a result, it is difficult to make clear suggestions about the regulatory role of cocaine on PENK expression other than to say it is commonly susceptible to alterations by cocaine treatment and withdrawal.

Regulation of POMC. More consistent results have been reported for POMC gene expression where no change in mRNA levels were reported following chronic cocaine exposure (Zhou *et al.*, 2005), withdrawal (Zhou *et al.*, 2005) or CPP (Leri *et al.*, 2006) in the Hy, suggesting a lack of POMC regulation by cocaine at least at the level of gene expression in the Hy. However, after acute cocaine administration (Olive *et al.*, 2001) and self-administration (Roth-Deri *et al.*, 2003; 2006) of cocaine, studies have shown increases in β-end release in the NAc, which suggests that β-end might be involved in the initial rewarding properties of cocaine. A cocaine-induced increase in β-end release in the NAc has also been suggested from a study showing a time-dependent decrease in MOPr binding in the NAc following acute cocaine administration in rats (Soderman and Unterwald, 2009). Plasma β-end levels were also shown to be increased following acute cocaine administration (Moldow and Fischman, 1987). Moreover, chronic cocaine administration increased β-end immunoreactivity in plasma, Pit but not in the Hy (Forman and Estilow, 1988), indicating a regulatory role of cocaine on β-end, specifically in the Pit. High levels of plasma β-end have been observed in abstinent human cocaine addicts (Vescovi *et al.*, 1992), supporting the concept of a stimulatory effect of cocaine on β-end release. It is not clear what the β-end release status is in the brain during cocaine withdrawal. β-end deficiency in regulating HPA axis activity has also been suggested based on a study demonstrating hyper-responsivity to removal of glucocorticoid negative feedback in cocaine abstinent human individuals following the administration of metyrapone (Schluger *et al.*, 1998; 2001). More studies need to be carried out in order to clarify the β-end status during withdrawal.

Regulation of PDYN. The most consistent and reliable finding in terms of cocaine-induced regulation of opioid peptide gene expression is undoubtedly the increase of PDYN mRNA levels and immunoreactivity in the CPu following acute, subacute, chronic 'binge' and continuous cocaine treatment (Sivam, 1989; Smiley *et al.*, 1990; Hurd *et al.*, 1992; Spangler *et al.*, 1993; 1996a; 1997; Steiner and Gerfen, 1993; Daunais and McGinty, 1995, 1996; Romualdi *et al.*, 1996; Mathieu-Kia and Besson, 1998; Svensson and Hurd, 1998; Werme *et al.*, 2000; Yuferov *et al.*, 2001; Zhou *et al.*, 2002; Bailey *et al.*, 2005b; Schlussman *et al.*, 2005) as well as following cocaine self-administration (Ziolkowska *et al.*, 2006). This increase has been shown to occur irrespective of treatment protocol. In agreement with these findings, an increase in PDYN was also observed in the CPu and VP of post-mortem brain of people with a history of cocaine abuse (Hurd and Herkenham, 1993; Frankel *et al.*, 2008), demonstrating the translational relevance of this cocaine-induced regulation of PDYN as well as the translational reliability of the animal model of chronic 'binge' cocaine administration paradigm. However, less consistent results have been reported following withdrawal, where a decrease (Svensson and Hurd, 1998), no change (Spangler *et al.*, 1996a) or increase (Sivam, 1989; Smiley *et al.*, 1990; Bailey *et al.*, 2005b) of PDYN mRNA or immunoreactivity has been reported in the CPu. In the NAc, an increase (Smiley *et al.*, 1990; Hurd *et al.*, 1992; Mathieu-Kia and Besson, 1998; Turchan *et al.*, 2002) or no change (Alvarez Fischer *et al.*, 2001; Schlussman *et al.*, 2005; Bailey *et al.*, 2005b; Ziolkowska *et al.*, 2006) in PDYN mRNA or immunoreactivity levels has been reported following chronic 'binge' cocaine administration. This administration paradigm was also shown to increase PDYN mRNA in the dentate gyrus (DG) (Turchan *et al.*, 2002) but not in the Amy (Turchan *et al.*, 2002; Zhou *et al.*, 2008), FCx, Cx (Yuferov *et al.*, 2001) and SN (Spangler *et al.*, 1993). On the contrary, an increase of dynorphin immunoreactivity was observed following chronic 'binge' cocaine treatment in the SN, which persisted in acute withdrawal in rats (Smiley *et al.*, 1990). No change was observed in the Hi (Smiley *et al.*, 1990). In addition, a decrease in PDYN mRNA was observed in the Hy following continuous cocaine administration (Romualdi *et al.*, 1996) but not following chronic 'binge' administration (Yuferov *et al.*, 2001; Zhou *et al.*, 2008), implying that the regulation of PDYN by cocaine in the Hy depends on the paradigm of administration. However, while an increase of PDYN mRNA was observed in the LH following acute withdrawal from chronic 'binge' cocaine, it did not persist into long-term withdrawal (Zhou *et al.*, 2008).

By considering the KOPr and PDYN data together, we can conclude that the PDYN/KOPr system is under profound regulatory control by cocaine and is activated following

chronic cocaine exposure. As discussed earlier, it is likely that this cocaine-induced activation of the KOPr/PDYN system in regions associated with reward (CPu, NAc, VTA, CgCx) might be part of a protective compensatory neuroadaptive mechanism to counteract the positive reinforcement effect of cocaine. In addition and considering the role of PDYN/KOPr in inducing dysphoria, it is likely that the alterations observed in brain regions associated with stress (Sept, Amy, Hy) may contribute to the emergence of persistent dysphoria and in triggering relapse in response to stress, which is very common in human cocaine addicts after the withdrawal of the drug (Gawin, 1991). Recent studies carried out with the use of selective pharmacological tools and in KO mice (discussed in the following section) have shed further light into the role of PDYN/KOPr system in cocaine addiction.

Cocaine responses in opioid receptors and peptide KO mice

Opioid receptor KO mice

MOPr KO mice. A great body of literature over the last 50 years has provided clear evidence with the use of pharmacological tools that the MOPr plays an important role in mediating the positive reinforcing effects of natural rewards as well as of opiate and non-opiate drugs of abuse including cocaine (for extensive review on this topic, see Shippenberg and Elmer, 1998; van Ree *et al.*, 2000; Le Merrer *et al.*, 2009). Briefly, administration of selective MOPr antagonists attenuates the development of cocaine CPP (Schroeder *et al.*, 2007; Soderman and Unterwald, 2008) and reduces cocaine self-administration (Ward *et al.*, 2003) and reinstatement (Tang *et al.*, 2005) of cocaine-seeking behaviour in rats, clearly suggesting that the MOPr system is involved in the rewarding as well as the relapse potential of the psychostimulant.

The use of MOPr KO mice clearly demonstrated that the MOPr is the primary molecular target for morphine as morphine CPP (Matthes *et al.*, 1996; Sora *et al.*, 2001; Mizoguchi *et al.*, 2003) and self-administration (Becker *et al.*, 2000) were completely abolished in KO animals. Although MOPr KO studies demonstrated a clear role of MOPr in mediating the positive reinforcing effects of some non-opioid drugs of abuse such as nicotine (Berrendero *et al.*, 2002), delta9-tetrahydrocannabinol (Ghozland *et al.*, 2002) and alcohol (Roberts *et al.*, 2000; Becker *et al.*, 2002), the same was not true for cocaine reinforcement. Cocaine CPP was shown to be unchanged (Contarino *et al.*, 2002), increased (Becker *et al.*, 2002) or decreased (Hall *et al.*, 2004) in MOPr KO (Table 2), which gives a unclear picture of the role of MOPr in cocaine reinforcement and is certainly not in agreement with the pharmacological manipulations described earlier. The discrepancy of results between studies could be due to differences in genetic background of mice strain used, differences in gender of animals and/or differences in experimental protocol used (dose of cocaine, number of conditional sessions, etc.). While the study conducted by Becker *et al.* (2002) used mixed 129/Ola × C57BL male mice (F2 generation), Hall *et al.* (2004) used congenic C57 mice (F10 generation) of mixed sexes. Indeed, strains of mice differ considerably in their behavioural and neurochemical effects of drugs of abuse (e.g. Cunningham *et al.*, 1992; Orsini *et al.*, 2005; Glatt *et al.*, 2009; Bailey *et al.*, 2010), and genetic background of mouse strain has been shown repeatedly to influence the phenotypic and neurochemical consequences of gene KO (Hummel *et al.*, 2004; Yoo *et al.*, 2010). This really points towards the importance of using KO mice of homogeneous genetic background by backcrossing over several generations in order to dilute the effect of background strain. In addition, differences in cocaine rewarding effects have been shown between male and female mice (Anker and Carroll, 2011), which might also explain the discrepancies observed. This points towards the importance of using same-sex KO mice in behavioural experiments to minimize the gender effect on phenotypic changes in KO mice. Finally, in most studies, only one or two doses of cocaine was tested for cocaine CPP experiments, which could mask dose–response shift in sensitivity of these mice to the rewarding effect of cocaine. This demonstrates the need to conduct CPP experiments in KO mice with a range of different doses of cocaine. In contrast to cocaine CPP and in agreement with pharmacological studies, cocaine self-administration was reduced in male congenic C57BL MOPr KO in a dose–response manner (Mathon *et al.*, 2005), suggesting a role for MOPr in the operant and reinforcement effect of cocaine.

In addition to CPP and self-administration, other behavioural tests are very commonly used in order to address other aspects of cocaine-addictive behaviours such as behavioural sensitization. Behavioural sensitization is a phenomenon whereby repeated exposure to psychostimulant drug elicits progressive enhancement of behavioural responses, which persists after withdrawal from the drug (Robinson and Becker, 1986) and is thought to reflect neuroadaptive/neuroplastic alterations that mediate drug-seeking behaviour (Kalivas and Stewart, 1991; Kalivas *et al.*, 1993; Pierce and Kalivas, 1997). As with CPP, there are discrepancies among studies investigating the locomotor stimulating and sensitizing effects of cocaine in mice lacking MOPr. A decrease (Yoo *et al.*, 2003; Chefer *et al.*, 2004) or no change (Becker *et al.*, 2002; Contarino *et al.*, 2002; Hall *et al.*, 2004; Lesscher *et al.*, 2005) of cocaine-induced locomotor activity and/or locomotor sensitization was observed in MOPr KO mice. As for CPP, the discrepancy in locomotor and sensitization effects of cocaine in MOPr KO mice are likely to be due to gender differences as well as to genetic background. Mixed sexes of mice were used in the Contarino *et al.* (2002), Hall *et al.* (2004), Yoo *et al.* (2003; 2006) studies, whereas only male mice were used in the Chefer *et al.* (2004), Lesscher *et al.* (2005) and Hummel *et al.* (2004) studies. Hummel *et al.* (2004) specifically carried out cocaine-induced locomotor and sensitization studies in MOPr KO mice of different genetic backgrounds under the same experimental conditions in order to address the influence of genetic background in phenotypic changes in mice lacking MOPr. An increase in the acute locomotor and sensitization effect of cocaine was observed in congenic C57BL/6J MOPr KO mice (>N10 backcrossing to C57BL/6J) versus wild type (WT); a decrease was observed in the mixed 129S6 × C57BL/6J MOPr KO, whereas no changes were observed in isogenic 129S6 MOPr KO (Hummel *et al.*, 2004), clearly displaying that genetic background does influence phenotypic changes in mice lacking MOPr.

Table 2

Behavioural and neurochemical effects of cocaine in opioid receptor and peptide knockout mice

Gene	Gender and background	Dose	Behavioural effects	Biochemical effects	Reference
MOPr	Male	5 or 10 mg·kg^{-1}, i.p.	↓ CPP (5 mg·kg^{-1})(5 mg·kg^{-1})		(Becker *et al.*, 2002)
	129/Ola × C57BL/6 Crossed to CB6F1	Two or four parings	↑ CPP (10 mg·kg^{-1})		
	F2 used for the study				
		20 or 40 mg·kg^{-1}, i.p.	↔ Locomotion		
	Male, female	10 mg·kg^{-1}, i.p.	↔ CPP		(Contarino *et al.*, 2002)
	129Sv × C57BL/6 F2 used for the study	Three parings			
		30 mg·kg^{-1}, i.p.	↔ Locomotion		
	Male, female	2 × 5 or 10 mg·kg^{-1}, s.c.	↓ CPP (10 mg·kg^{-1})		(Hall *et al.*, 2004)
	129SvEv × C57BL/6J	Two parings			
	>F10 used for the study				
		5, 10,20 mg·kg^{-1}, s.c.	↔ Locomotion		
		20 mg·kg^{-1}, s.c., 5 days	↔ Sensitization		
	Male, Female	15 mg·kg^{-1}, i.p. (injection for 6 days, 6 days withdrawal then challenge with initial dose)	↓ Locomotion	↓ nNOS-ir in DG	(Yoo *et al.*, 2003; 2006)
	129/Ola × C57BL/6		↓ Sensitization	↔ nNOS-ir in Cx, CPu	
	Male	10 or 20 mg·kg^{-1}, i.p.	↓ Locomotion (20 mg·kg^{-1})	↔ DA in NAc	(Chefer *et al.*, 2004)
	129/Sv × C57BL/6				
	>N10 backcrossed to C57BL/6				
	F15 used for the study				
	Male	0.4, 0.8, 1.6 μg·kg^{-1}·1.6 μL^{-1}, i.v.	↓ Self-administration (1.6 μg·kg^{-1}·1.6 μL^{-1})		(Mathon *et al.*, 2005)
	129Sv × C57BL/6				
	N6 or N7 backcrossed to C57BL6/Jico				
	Male	3, 10, 20 or 30 mg·kg^{-1}, i.p.	↔ Locomotion		(Lesscher *et al.*, 2005)
	129Sv × C57BL/6				
	N6 or N7 backcrossed to C57BL6/Jico				
		10 mg·kg^{-1}, i.p., (Injection for 11 days, 72 h withdrawal and then challenge with 20 mg·kg^{-1})	↔ Sensitization		
	Male	15 mg·kg^{-1}, i.p. (injection for 10 days, 7 days withdrawal then challenge with initial dose)	↔ Locomotion		(Hummel *et al.*, 2004)
	129S6 × C57BL/6J		↓ Sensitization		
	Male	Same as previous	↑ Locomotion		
	129S6 × C57BL/6J		↑ Sensitization		
	≥ N10 backcrossed to C57BL/6J				
	Male	Same as previous	↓ Locomotion		
	129S6 MOPr KO × F10 C57BL/6J		↓ Sensitization		
	F1 used for the study				
	Male	Same as previous	↔ Locomotion		
	129S6 × C57BL/6J crossed to 129S6		↔ Sensitization		

Table 2

Continued

Gene	Gender and background	Dose	Behavioural effects	Biochemical effects	Reference
DOPr	Male 129/Sv × C57BL/6 >N10 backcrossed to C57BL/6 F15 used for the study	10 or 20 mg·kg^{-1}, i.p.	↑ Locomotion (10 mg·kg^{-1})	↓ DA in Nac (20 mg·kg^{-1})	(Chefer *et al.*, 2004)
KOPr	Male 129S6 × C57BL/6J	5, 10 or 15 mg·kg^{-1}, i.p.	↑ Locomotion	↑ DA in Nac (10 mg·kg^{-1}) ↓ c-Fos, jun B mRNA	(Chefer *et al.*, 2005)
	Male 129S6 × C57BL/6J N10 backcrossed to C57BL/6J	15 mg·kg^{-1}, i.p., 8 days (injection for 5 days, 3 days withdrawal then challenge with initial dose)	↓ Sensitization ↑ Sensitized state		
	Gender not specified 129S6 × C57BL/6J >N10 backcrossed to C57BL/6	15 mg·kg^{-1}, s.c. 2 days pairing	↔ CPP (unstressed) ↓ CPP (FST stress)		(McLaughlin *et al.*, 2006a)
	Male 129S6 × C57BL/6J backcrossed to C57BL/6	15 mg·kg^{-1}, s.c. four pairings	↓ CPP reinstatement (Foot shock & FTS)		(Redila and Chavkin, 2008)
PENK	–	–	–	–	–
β-end	Male C57BL/6J × 129/SV >N10 backcrossed to C57BL/6J	15, 30,60 mg·kg^{-1} i.p.	↓ Locomotion		(Marquez *et al.*, 2008)
		15, 30, 60 mg·kg^{-1} i.p. 2 pairings	↓ CPP		
PDYN	Male 129/SV × C57BL/6, N10 backcrossed to C57BL/6 F15 used for the study	10 and 20 mg·kg^{-1}, i.p.	↓ Locomotion (20 mg·kg^{-1})	↓ DA levels in NAc	(Chefer and Shippenberg, 2006)
	Male 129/SV × C57BL/6J background ≥N10 backcrossed to C57BL/6J	3 × 15 mg·kg^{-1}/day, i.p. 14 days	↔ Locomotion ↑ Sensitization ↔ Stereotypy	↓ D2 binding in CPu ↔ D1, DAT binding ↓ MOP activity increase* ↓ Corticosterone levels	(Bailey *et al.*, 2007b)
	Gender not specified 129/SvEvTac × C57BL/6 >N10 backcrossed to C57BL/6J	15 mg·kg^{-1}, s.c. two pairings	↔ CPP (unstressed) ↓ CPP (FST stress)		(McLaughlin *et al.*, 2006b)
	Male 129/SvEvTac × C57BL/6 backcrossed to C57BL/6J	15 mg·kg^{-1}, s.c. four pairings	↓ CPP reinstatement (foot shock and FTS)		(Redila and Chavkin, 2008)

*As measured by DAMGO stimulated [^{35}S]GTPγS autoradiography.

Studies using pharmacological tools have demonstrated that MOPr contributes towards the elevation of DA release in the NAc response to cocaine (Shippenberg and Elmer, 1998; Van Ree *et al.*, 2000). In contrast with these studies, no significant genotype effect was detected in either basal or acute cocaine-induced NAc DA levels in MOPr KO (Chefer *et al.*, 2004). This discrepancy may account for compensatory changes taking place in other CNS systems in KO mice, which could influence DA release in the NAc.

The effect of chronic cocaine administration on neuronal nitric oxide synthase (nNOS) expression in the Hi of WT and MOPr KO was investigated in order to assess if cocaine induction of nNOS is mediated by MOPr. Chronic cocaine administration induced up-regulation of the expression of nNOS in the DG of the Hi in WT mice but that was attenuated in MOPr KO clearly suggesting an involvement of nNOS pathway in the mechanism of action of cocaine (Yoo *et al.*, 2006).

DOPr KO mice. The use of pharmacological manipulation has consistently demonstrated that the DOPr is involved, although to a lesser extent than the MOPr, in mediating the positive reinforcing effects of opioid and non-opioid drugs of abuse including cocaine (for review, see Le Merrer *et al.*, 2009). Selective DOPr antagonists decrease systemic cocaine self-administration when microinjected in the NAc but increase cocaine self-administration when injected in the VTA and had no effect when injected into the Amy, indicating that the DOPr can modulate cocaine reinforcement depending on the brain site (Ward *et al.*, 2003).

Studies on DOPr KO mice demonstrated an anxiogenic and depressive phenotype in these animals (Filliol *et al.*, 2000), and DOPr KO exhibited an increase in ethanol self-administration (Roberts *et al.*, 2001), clearly suggesting a role for DOPr on emotional regulation and drug reinforcement. The locomotor activity of DOPr KO mice maintained on a pure C57BL/6J background in response to acute cocaine administration was investigated. Acute cocaine-induced locomotor activity was significantly increased in DOPr KO, clearly suggesting a modulatory role for DOPr in the acute psychomotor effect of cocaine (Chefer *et al.*, 2004). Further behavioural tests such as cocaine CPP, sensitization and self-administration need to be carried out to demonstrate the role of DOPr in cocaine addiction behaviours.

Studies conducted with pharmacological tools have suggested that cocaine-induced release of DA in the NAc is partly mediated by a DOPr dependent mechanism (Shippenberg and Chefer, 2003), which is likely to contribute towards the positive reinforcing effect of cocaine. In agreement with these studies, Chefer *et al.* (2004) showed a diminished release of DA in the NAc of DOPr KO in response to acute cocaine, clearly suggesting a role for the DOPr in mediating the release of DA in response to cocaine in the NAc. The mechanism underlying the role of DOPr in mediating the cocaine-induced DA release is not clear. However, there is evidence supporting an involvement of glutamatergic and free radical dependent mechanisms (Billet *et al.*, 2004; Fusa *et al.*, 2005). A mechanism also involving MOPr–DOPr heteromeric interactions may also be plausible.

KOPr KO mice. It has been well established that activation of KOPr produces motivational and neurochemical effects that oppose those of MOPr (Shippenberg *et al.*, 1998; Van Ree *et al.*, 2000). This was confirmed in KOPr KO mice, which did not experience CPP aversion in response to a KOPr agonist, in contrast to WT mice, which did (Simonin *et al.*, 1998). In terms of neurochemistry, KOPr agonists have been repeatedly shown to decrease DA release in the NAc, whereas activation of MOPr increases it (Di-Chiara and Imperato, 1988). This was also confirmed in the KOPr KO mice, which were found to have higher basal levels of DA in the NAc compared with WT in a freely moving microdialysis study (Chefer *et al.*, 2005), indicating a tonic inhibitory role of KOPr on DA release. This result was replicated both in KOPr KO mice maintained on a pure C57BL/6J or a mixed 129S6 × C57BL/6J background (Chefer *et al.*, 2005), indicating that that KOPr-mediated regulation of DA release is not influenced by background strain of mouse.

It is also broadly accepted that activation of the KOPr system has anti-addictive properties by antagonizing the acute reinforcing/rewarding effect of cocaine (see Wee and Koob, 2010). Selective KOPr agonists were shown to consistently block cocaine-induced CPP in rodents (Crawford *et al.*, 1995; Mori *et al.*, 2002; Zhang *et al.*, 2004), to decrease cocaine self-administration in rats (Glick *et al.*, 1995; Schenk *et al.*, 1999) and monkeys (Mello and Negus, 1998), to attenuate cocaine-induced behavioural sensitization and to decrease cocaine-induced DA release in the NAc (Heidbreder *et al.*, 1993; 1995; 1998; Heidbreder and Shippenberg, 1994; Shippenberg *et al.*, 1996; Zhang *et al.*, 2004; 2005), clearly suggesting an important role for the KOPr system in modulating the rewarding effect of cocaine by opposing cocaine-induced DA release.

In agreement with the aforementioned studies, the acute effect of cocaine on locomotor behaviour and striatal DA release was enhanced in KOPr KO mice relative to WT maintained on a mixed 129S6 × C57BL/6J background (Chefer *et al.*, 2005). In addition, in contrast to WT mice, repeated administration of cocaine did not induce locomotor sensitization in KOPr KO mice (Chefer *et al.*, 2005). This might signify the development of a chronically sensitized state in the absence of the KOPr. The reduction of cocaine-induced immediate early gene expression observed in KOPr KO mice (Chefer *et al.*, 2005) provides additional evidence of a 'cocaine-sensitized' phenotype for KOPr KO mice.

Although it was initially accepted that the activation of the KOPr system has anti-addictive properties, there has been recent emerging evidence showing that it can also trigger cocaine reinstatement via a stress-dependent mechanism (see review by Wee and Koob, 2010). While KOPr antagonists inhibited stress-induced, but not cocaine-primed, reinstatement of cocaine self-administration in rats (Beardsley *et al.*, 2005) and stress-induced cocaine CPP in mice (Carey *et al.*, 2007; Redila and Chavkin, 2008), KOPr agonists induced reinstatement of extinguished cocaine-associated place preference (Redila and Chavkin, 2008), clearly suggesting that stress can induce reinforcing effects of cocaine via a KOPr-mediated mechanism. This suggestion was further reinforced by recent studies carried out in KOPr KO mice. While no differences in cocaine CPP was observed between WT and KOPr KO mice in the absence of stress, repeated swim stress potentiated the rewarding effect of cocaine in WT but not in KOPr KO mice, further demonstrating that KOPr activation

induced by stress may be both necessary and sufficient for potentiating the reinforcing actions of cocaine (McLaughlin *et al.*, 2006a). In support of this, foot shock stress and forced swim-induced reinstatement of cocaine CPP was absent in mice lacking the KOPr gene (Redila and Chavkin, 2008).

PENK KO mice. Endogenous PENK has been postulated to mediate the positive reinforcing effects of several drugs of abuse (Gianoulakis, 1993; Berrendero *et al.*, 2005; Marinelli *et al.*, 2005; Shoblock and Maidment, 2007). No alterations of basal levels of DA was observed in the NAc of PENK KO mice versus WT (Berrendero *et al.*, 2005), indicating a lack of tonic regulation of DA release by PENK, at least at a basal state. There are no studies to our knowledge investigating the effect of cocaine on PENK KO mice.

β-end KO mice. Endogenous end has been suggested to modulate positive reinforcement and pleasurable effects of a range of drugs of abuse including cocaine (Olive *et al.*, 2001), alcohol (Gianoulakis, 1993; Jarjour *et al.*, 2009) and nicotine (Roth-Deri *et al.*, 2008). Cocaine has been shown to increase β-end in the NAc (Olive *et al.*, 2001), raising the possibility that this neuropeptide may be important in rewarding effects of the drug. Locomotor activity and CPP effects of cocaine were both reduced in mice lacking β-end in a dose-dependent manner (Marquez *et al.*, 2008), further demonstrating that β-end plays a modulatory role in the motor stimulating and rewarding effects of acute cocaine. Further studies are warranted for the investigation of the operant and chronic effect of cocaine in these animals.

PDYN KO mice. Activation of the endogenous dynorphin system has been proposed to oppose positive reinforcement/reward by means of KOPr activation, resulting in tonic inhibition of DA release and signalling (Zhang *et al.*, 2004; Chefer *et al.*, 2005; Shippenberg *et al.*, 2007). Dynorphin A (1–17) administered directly in the CPu of mice was shown to decrease basal DA release in a dose-dependent manner by more than 60% (Zhang *et al.*, 2004). Dynorphins have also been implicated in behavioural responses to stress. i.c.v. administration of dynorphin A (1–17) was shown to potentiate the immobility response to stress, which was blocked by selective KOPr antagonists (McLaughlin *et al.*, 2003). In addition, when exposed to an inescapable physical or psychological stressor, rodents demonstrate stress-induced analgesia that is blocked by KOPr selective antagonists (Takahashi *et al.*, 1990; McLaughlin *et al.*, 2003; 2006a; Aldrich and McLaughlin, 2009). This effect was also blocked in PDYN KO mice, suggesting that KOPr activation by dynorphin contributes to behavioural responses to stress (McLaughlin *et al.*, 2003).

In terms of responses to cocaine effects, administration of dynorphin 1–17 directly in the CPu was shown to decrease cocaine-induced CPP to attenuate the locomotor effect of the drug and to block the elevation of striatal DA levels in response to cocaine in mice (Zhang *et al.*, 2004). These data, together with the fact that cocaine induces PDYN expression in the striatum (as discussed earlier), demonstrates a critical role of dynorphin in suppressing DA release and, as a result, in opposing the rewarding and reinforcing effect of cocaine. As a consequence, dynorphin could be characterized as an 'anti-addictive' peptide. In agreement with this hypothesis, we showed an enhancement of locomotor sensitization following chronic 'binge' cocaine administration in mice lacking PDYN gene (Bailey *et al.*, 2007b). In terms of DA release, Chefer and Shippenberg (2006) showed that the deletion of dynorphin in mice, decreased basal DA levels and attenuated the cocaine-induced elevation of DA in the NAc. This paradoxical phenomenon might be due to compensatory up-regulation of KOPr observed in PDYN KO mice (Clarke *et al.*, 2003), which could be activated by endogenous met-enkephalin and/or β-end. We also showed that the deletion of dynorphin induced a decrease of striatal D2 receptors in chronic 'binge' cocaine-treated mice but not in WT animals (Bailey *et al.*, 2007b), further demonstrating the protective role of dynorphin in opposing D2 receptor down-regulation, which is thought to be associated with increased vulnerability to the reinforcing effect of the drug (Nader and Czoty, 2005).

In complete agreement with data obtained from KOPr KO mice, use of Ppdyn KO confirmed the role of endogenous dynorphin in stress-inducing effects of cocaine and in triggering cocaine reinstatement via a stress-like mechanism. Genetic deletion of PDYN abolished stress-induced reinstatement of cocaine CPP in mice (Redila and Chavkin, 2008). Moreover, while no differences in cocaine CPP was observed between WT and PDYN KO mice in the absence of stress, repeated swim or foot shock stress potentiated the rewarding effect of cocaine in WT but not in PDYN KO (McLaughlin *et al.*, 2003), further demonstrating that activation of the dynorphin system induced by stress may be both necessary, and sufficient, for the potentiation of the reinforcing actions of cocaine. The mechanism underpinning this effect is not clear, but it might possibly involve the HPA axis and/or extra-hypothalamic mechanisms (e.g. Amy). We demonstrated that while chronic 'binge' cocaine elevated corticosterone levels in WT mice, this effect was blunted in PDYN KO (Bailey *et al.*, 2007b), clearly suggesting a PDYN mechanism in the HPA axis stress-activating effect of cocaine. In support of this finding, Wittmann *et al.* (2009) showed an attenuation of stress-induced corticosterone levels in PDYN KO mice.

Conclusions/therapeutic implications

Together, the opioid peptide/receptor regulation and gene KO studies have clearly demonstrated the induction of profound neuroadaptive changes in the opioid system, which may contribute towards the progressive transition from impulsive drug taking to compulsive cocaine abuse (Figure 1).

- Cocaine causes a region-specific activation of the MOPr system, which we postulate to contribute towards the positive reinforcement/hedonic effect of cocaine (partly via increasing DA release in the NAc) and towards cocaine craving during withdrawal followed by relapse. Cocaine causes a region-specific activation of the MOPr system, which we postulate to contribute towards the positive reinforcement/hedonic/drug wanting effect of cocaine (partly via increasing DA release in the NAc) and towards cocaine craving during withdrawal followed by relapse.

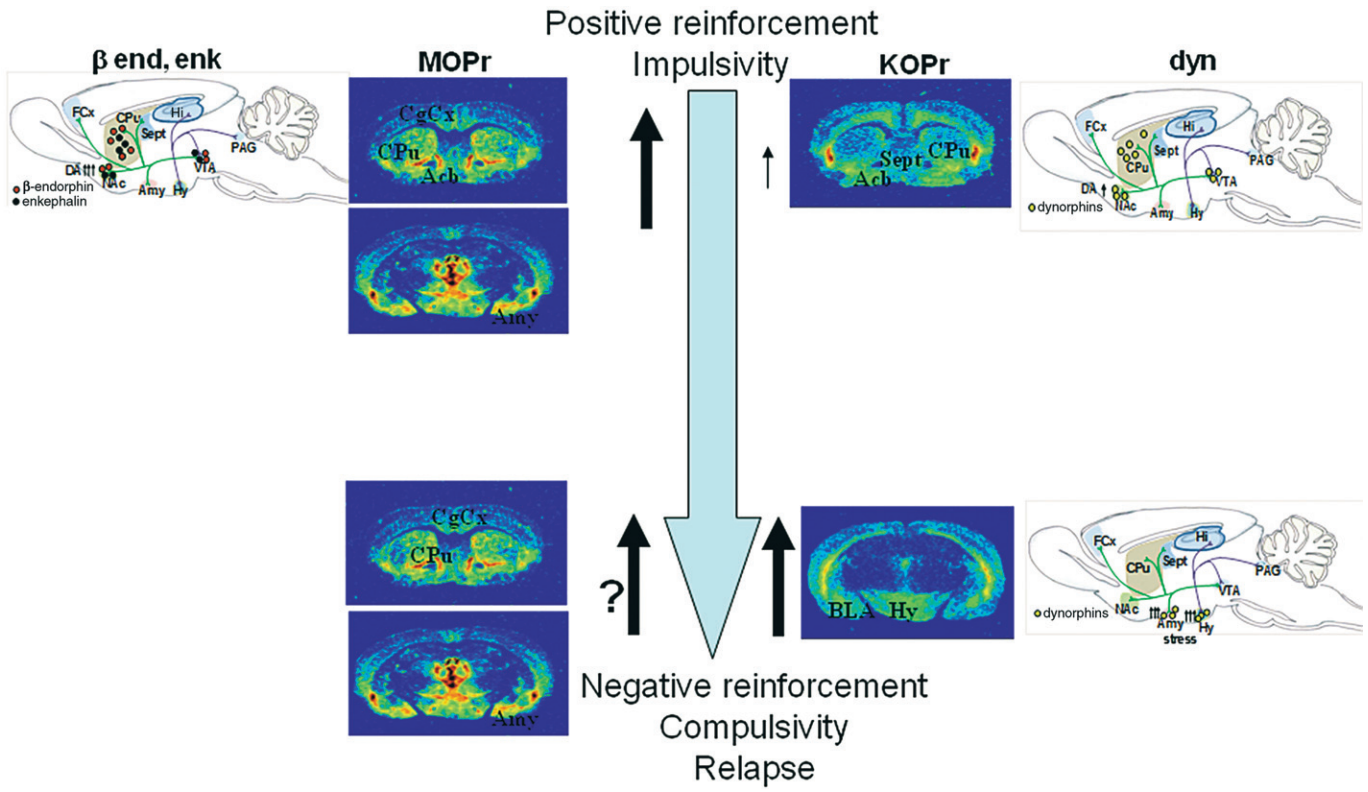

Figure 1

Endogenous opioid neurocircuitries hypothesized to be recruited during the transition from positive to negative reinforcement in cocaine addiction (founded on figures from Le Moal and Koob, 2007; Wee and Koob, 2010). Top left: illustrates an increase in MOPr activity in regions of the brain involved in reward (NAc, CPu, Amy) and impusivity/decision-making (FCx, CgCx), which drives the positive reinforcement effect of cocaine. It also illustrates the involvement of β-endorphin (β end) and enkephalin (enk) in the reinforcement effect of cocaine via their interaction with the mesolimbic dopaminergic system. Top right: illustrates an activation of the dyn/KOPr system in regions of brain regions involved in reward. This activation opposes the positive reinforcement effect of cocaine via its interaction with the mesolimbic dopaminergic system. Bottom left: illustrates an increase in MOPr activity in regions of the brain involved in drug priming (FCx, CgCx) and cue priming reinstatement (Amy). It is not clear what effect this may have in terms of driving negative reinforcement and compulsive drug taking. Bottom right: illustrates an activation of the dyn/KOPr system in brain regions involved in stress responses (Hy, Amy), which will drive negative reinforcement and compulsive cocaine taking and trigger relapse. The images are computer-enhanced autoradiograms of coronal mouse brain sections labelled with MOPr and KOPr selective ligand [^{3}H]DAMGO and [^{3}H]CI977 respectively (for detailed autoradiography methods, see Kitchen *et al.*, 1997). The sections shown are from the level of the caudate (Bregma 0.86 mm) and from the thalamus (Bregma –1.94 mm). The arrows indicate an increase of positive or negative reinforcement and the size of the arrow represents the magnitude of the effect. The mesolimbic dopaminergic system (green line), which projects from the VTA to various parts of the brain, is illustrated. dyn, dynorphin; enk, enkephalin; PAG, periaquetactal grey.

More specifically, we postulate that cocaine would activate MOPr, leading to opioid-induced DA release. There is evidence to suggest that dopaminergic (Azaryan *et al.*, 1996a; Ambrose *et al.*, 2004) and non-dopaminergic (Bailey *et al.*, 2007b) mechanisms may mediate this MOPr activation.

- Cocaine also causes a region-specific activation of the KOPr/PDYN system, which we postulate to antagonize the positive reinforcement effect of cocaine, via opposing DA release in the NAc, but also to contribute to the stress-inducing properties of the drug and the triggering of relapse.

These conclusions have important implications for the development of effective pharmacotherapy for the treatment of cocaine addiction and the prevention of relapse. Indeed, one could suggest that MOPr antagonists and KOPr agonists would be more effective as an acute intervention to suppress cocaine reward and craving than for a long-term treatment (Aldrich and McLaughlin, 2009). Indeed, the non-selective opioid receptor antagonist naltrexone has been tested in clinical trials as a possible treatment of cocaine cravings with mixed results (Heidbreder and Hagan, 2005; Modesto-Lowe *et al.*, 1997), possibly due to the anxiety provoking effect of naltrexone (see review of Colasanti *et al.*, 2011). In terms of KOPr agonism, although centrally acting KOPr agonists are known to induce dysphoria and sedation in humans (Pfeiffer *et al.*, 1986; Mello and Negus, 2000), and for that reason the therapeutic development has been limited (Barber and Gottschlich, 1997; DeHaven-Hudkins and Dolle, 2004), they were shown to decrease symptoms of mania in bipolar disorder patients with no adverse effects (Cohen and Murphy, 2008). As a result, KOPr agonists might be of benefit for the treatment of acute cocaine cravings. On the other hand, the KO data suggest that KOPr antagonists would be better suited

as a therapeutic to prevent stress-induced relapse in cocaine-dependent individuals and as consequence would help cocaine-abstinent individuals to maintain their abstinence state. The mixed opioid receptor ligand buprenorphine, which has mixed MOPr agonist/KOPr antagonist activity, was used in combination with naltrexone in heroin and cocaine addicts in a clinical study (Gerra *et al.*, 2006). The outcomes of the study demonstrated enhanced compliance and drug abstinence from heroin and cocaine compared with naltrexone alone in dependent individuals (Gerra *et al.*, 2006), further demonstrating the therapeutic potential of selective KOPr antagonists or a combination of MOPr/KOPr antagonists in cocaine addiction.

Problems, limitations and future directions

Despite its significant contribution towards understanding the mechanisms underlying the effects of cocaine, KO mouse technology does not come without its problems and limitations. Firstly, classic opioid receptor/peptide KO have the opioid receptor or peptide globally deleted from the animal, that is, in all brain regions, spinal cord, peripheral and immune sites. As a result, it is not possible to pinpoint the role of the precise anatomical brain sites or circuitry where the specific receptor or peptide is localized in modulating certain behavioural effects associated with cocaine use such as sensitization, stereotypy, impusivity, habit formation, emotional memory formation, anxiety, positive and negative reinforcement, reinstatement, CPP, etc. For instance, the role of MOPr in the FCx is postulated to be involved in impulsivity (Love *et al.*, 2009), whereas the role of MOPr in the NAc is critical in reward processing (Van Ree *et al.*, 2000). MOPrs in both these regions are up-regulated following chronic cocaine administration; however, the use of conventional KO mice is unable to pinpoint the functional/behavioural consequence of each of these region's specific up-regulations. The development of conditional KO mice that will enable spatial control over the gene deletion will surely allow more fine-tuning in our understanding of the role of receptors and peptides in specific regions involved in addictive biology. Transgenic Cre technology will permit the deletion of opioid peptide or receptor in specific neuronal populations (e.g. GABAergic or aminergic neuron) and, in combination with neuroanatomical imaging approaches, will provide further knowledge of the molecular and cellular mechanisms by which different components of the opioid system would influence addictive properties of cocaine *in vivo* (Le Merrer *et al.*, 2009).

Furthermore, the genetic background of mouse strain has also been repeatedly shown to influence the phenotypic consequences of gene KO mice as evidenced, for example, by the discrepancy of results between cocaine sensitization, locomotor and CPP studies in MOPr KO mice discussed in this review. This really points towards the importance of using KO mice of homogeneous genetic background by backcrossing over >10 generations.

Furthermore, there is a need to take advantage of opioid transgenic technology to investigate the role of specific components of this system in later stages of cocaine addiction (reinstatement, extinction, habit formation, etc) as well as in the adverse effect of the drug (seizures, psychosis, neurotoxicity, cardiotoxicity, cognitive impairment). Indeed, opioid receptor/peptide KO technology was used predominantly to investigate the role of the endogenous opioid system in modulating the locomotor, behaviour sensitization and drug conditioning effects of cocaine, and a limited number of studies have investigated the influence of those genes in the development of cocaine dependence, extinction, reinstatement and consolidation, which are characteristics of later stages of drug addiction. In addition, although cocaine consumption is well known to be associated with serious cardiovascular complications that may lead to death (Vasica and Tennant, 2002; Afonso *et al.*, 2007), the mechanism underlying its cardiotoxicity is not well understood (Fan *et al.*, 2009). Despite the unique benefit that KO technology can provide to investigate these mechanisms, there has been a very limited use of these animals in this specific field.

Although opioid KO mice have been used for the investigation of genetic components of addiction and there is a large body of literature demonstrating the contribution of 'environment' at different stages of the drug addiction cycle, there is very limited literature on the interaction of these two factors, which are generally accepted to play a crucial role in drug addiction. Transgenic mice provide a unique model to study the influence of environment on genes and vice versa in an addiction setting. The further understanding of those interactions is crucial not only to provide new knowledge on the neuropathology of the disease and on risk factors that may influence drug addiction vulnerability but also for the developing of more effective pharmacotherapy for the treatment of this disease.

A final point that has to be made here is that based on recent genome-wide association studies (GWAS) of addiction in humans and multitude of genetic studies carried out in mice, the concordance between human and animal studies have been disappointing. However, this is not the case for MOPr and PDYN where human polymorphisms of these genes have been repeatedly reported to affect the vulnerability of cocaine addiction (for review, see Kreek *et al.*, 2005; Yuferov *et al.*, 2010). Nonetheless, there is a need for closer interaction between human and animal geneticists so that information about candidate genes underlying drug addiction detected by GWAS in human populations is used to inform development of novel transgenic animal models that will better model the genetics of human addiction.

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Conflict of interest

Authors declare they have no conflict of interest.

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