# Title page

**Oxytocin and opioid addiction revisited: Old drug, new applications.**

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# Abstract

Opioid addiction has devastating health and socio-economic consequences and current pharmacotherapy is limited and often accompanied by side effects, thus novel treatment is warranted. Traditionally, the neurohypophyseal peptide oxytocin (OT) is known for its effects in mediating reward, social affiliation/bonding and stress, as well as learning and memory. There is now strong evidence that oxytocin is a possible candidate for the treatment of drug addiction and depression-addiction co-morbidities. This review summarizes and critically discusses the preclinical evidence surrounding the consequences of pharmacological manipulation of the oxytocinergic system on opioid addiction-related processes, as well as the effects of opioids on the OT system at different stages of the addiction cycle. The mechanisms underlying the effects of OT in opioid addiction, including oxytocins’ interaction with the monoaminergic, glutamatergic, opioidergic systems and its effect on the amygdala, the hypothalamic-pituitary-adrenal axis and on memory consolidation of traumatic memories are also reviewed. Moreover, we review clinical evidence on the effects of intranasal OT administration on opioid-dependent individuals and discuss the therapeutic potential along with the limitations that OT-based pharmacotherapies manifest. Review of these studies clearly indicates that the oxytocin system is profoundly affected by opioid use and abstinence and points towards the oxytocin system as an important target for developing pharmacotherapies for the treatment of opioid addiction and co-existing affective disorders, and thereby prevention of relapse. Therefore, clinical studies assessing the efficacy of OT-based pharmacotherapies in opioid addiction are warranted.

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Tables of links list key targets and ligands discussed in this article, which are hyperlinked to corresponding entries in http:// [www.guidetopharmacology.org](http://www.guidetopharmacology.org), the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY ([Pawson *et al.*, 2014](#_ENREF_92)) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (a,b[Alexander *et al.*, 2013b](#_ENREF_2); [Alexander *et al.*, 2013c](#_ENREF_3)).

# The oxytocin system

Oxytocin (OT; IUPHAR; ([Alexander *et al.*, 2013a](#_ENREF_1))), a nine amino acid peptide, was discovered by Sir Henry Dale in 1906 ([Dale, 1906](#_ENREF_23)) and was the first peptide hormone to be sequenced and synthesized by Du Vigneaud ([Du Vigneaud *et al.*, 1953](#_ENREF_29); [Tuppy, 1953](#_ENREF_109)) ([du Vigneaud *et al.*, 1954](#_ENREF_28)). OT is synthesised in supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. Besides the well-described peripheral function of OT (uterine contraction, milk ejection), its role as a neurotransmitter/neuromodulator in the brain has recently received increasing attention. OT-producing neurons located in the hypothalamus innervate brain regions associated with stress, reward, mood, fear, emotionality and drug-seeking behaviour, such as the amygdala, septum, nucleus accumbens and the bed nucleus of stria terminalis, where OT receptors are expressed ([see Gimpl *et al.*, 2001](#_ENREF_37)). Upon diffusion, OT binds to nearby OT receptors, but also vasopressin receptors as the latter exhibits >85% homology with OT receptors ([Busnelli *et al.*, 2013](#_ENREF_15)). OT exerts its biological effects by binding to and activating G-protein-coupled receptors ([Kimura *et al.*, 1992](#_ENREF_55)); however, the molecular mechanism(s) of receptor activation and the intracellular signalling events following receptor activation are only partly understood. Activation of the central OT receptors is involved in the modulation of several behaviours including autonomic function, social, sexual, pair-bonding, maternal, anxiety-, depressive- and psychotic-like behaviours (see [Gimpl *et al.*, 2001](#_ENREF_37); [Hashimoto *et al.*, 2012](#_ENREF_40)). Recent advances in the field, both from preclinical and clinical studies, have revealed the potential of OT as an emerging therapeutic target for the treatment of mental disorders characterised by social dysfunction such as autism, social anxiety disorders, depression, borderline personality disorders and schizophrenia ([for extended review see Meyer-Lindenberg *et al.*, 2011](#_ENREF_84))

Based on the key role of oxytocin in social reward and stress regulation, there has been significant interest in the role of oxytocin in addiction. The present review focuses on novel and old, pre-clinical and clinical evidence, suggesting a central involvement of the oxytocinergic system in opioid addiction and addictive-related behaviours and discusses the potential of OT-based pharmacotherapy for the treatment of opioid addiction and relapse prevention. Underlying neurobiological mechanism and limitations of oxytocin use as a pharmacotherapeutic agent are also discussed.

# Oxytocin and the reward system

Increasing interest for the involvement of the oxytocinergic system in drug addiction came from findings suggesting that the brain systems involved in drug reward interact with those involved in natural rewards, such as sexual behaviour and social bonding; early findings from Carmichael et al., ([1987](#_ENREF_16)) outlined the ability of sexual self-stimulation (mediated by the mesolimbic dopaminergic system) to increase plasma OT levels in humans. This findings was recently replicated by de Jong et al., ([2015](#_ENREF_51)), who showed enhanced salivary OT concentrations due to sexual self-stimulation. Pre-clinical findings also showed that partner bonds of prairie voles (a well-characterized monogamous species) are greatly influenced and regulated by the OT neurotransmission in the brain ([Insel *et al.*, 1992](#_ENREF_48); [Insel *et al.*, 1994](#_ENREF_49); [Young, 2003](#_ENREF_123)). Oxytocinergic interactions with the dopaminergic system in the brain were shown to underlie this pair bonding behaviour ([Liu *et al.*, 2003](#_ENREF_79)), indicating possible association between the oxytocinergic and DAergic systems to regulate behaviour. Kovacs et al. ([1990](#_ENREF_68)) showed that both central and peripheral administration of OT acutely increases DA utilisation within the Acb, while chronic systemic administration of OT decreases DA utilisation within the basal forebrain of mice ([Kovacs *et al.*, 1986](#_ENREF_63)).

These interactions of the oxytocinergic with the DAergic systems suggest that oxytocin might have a critical role in the treatment of several DA-related disorders including drug addiction, and its beneficial effects might in fact be mediated by its interactions with several DAergic pathways in the brain.

# Drug addiction cycle

Addiction is often characterised as a cycle of neurochemical and psychological changes that bring about a shift from a impulsive use of a drug to the compulsive use ([see Koob *et al.*, 2008](#_ENREF_58)). Acute administration of drugs of abuse activates the mesolimbic dopaminergic reward pathway in the brain, thus inducing hedonic effects that positively reinforce the user to repeat drug administration. Upon repeated use of the drug, neuroadaptive tolerance to the rewarding effects of the drug is developed and an escalation of the dose is needed in order to achieve the same initial pleasurable effects ([see Koob *et al.*, 2001](#_ENREF_59)). During this period, positive reinforcement associated with acute drug administration is gradually replaced with a negative reinforcement, where the drug is taken to prevent the emergence of a negative withdrawal syndrome upon drug cessation. Acute withdrawal from drug use causes aversive drug-specific physical symptoms that are short-lived and for some drugs of abuse, including opioids, protracted withdrawal could cause long-term emotional impairment (see section below). In fact, drug users who have abstained from drug administration over a long period of time are still vulnerable to relapse to drug-seeking, particularly during re-exposure to the drug itself, to drug-associated environmental cues or following stress ([see Koob *et al.*, 2008](#_ENREF_58)).

# Comorbid drug addiction andmood disorders

Chronic exposure to drugs of abuse, as well as prolonged abstinence from these drugs, is associated with lowered mood, increased anxiety, irritability and social withdrawal and isolation.  It has been estimated that 45% of the drug-dependent population has a comorbid psychiatric disorder, compared with 12% of the non-dependent population ([Farrell *et al.*, 2003](#_ENREF_31)). More specifically, according to epidemiological studies, there is a marked comorbidity (50-60%) between drug addiction and depression ([Guest *et al.*, 2011](#_ENREF_38)), which is a major issue in psychiatry as it is accompanied by more severe symptoms, longer illness duration, higher service utilisation and higher relapse rates ([Alterman *et al.*, 1996](#_ENREF_4); [Brooner *et al.*, 1997](#_ENREF_11); [Kosten *et al.*, 1986](#_ENREF_60)). Therefore, considering that antidepressants ([see Riggs, 2003](#_ENREF_99)) and current addiction pharmacotherapies ([see Kampman *et al.*, 2005](#_ENREF_54)) have limited efficacy and are frequently accompanied by side effects in people suffering from this comorbidity, understanding the neurobiological mechanisms underlying comorbid depression and addiction disorderswill have important therapeutic implications in improving mental health care.

# Impact of opioid addiction on the oxytocinergic system

The impact of opioid addiction on the oxytocinergic system and the effect of OT treatment in modulating the addiction-related behaviours are summarised in tables 1 and 2 respectively.

## Acute opioid administration

Studies showing a clear role of the oxytocinergic system in the acute reinforcing effects of the opiate morphine was among the first evidence pointing towards the involvement of oxytocin in drug addiction. In particular, acute morphine administration was shown to decrease hypothalamic OT release in female rodents ([Clarke *et al.*, 1979](#_ENREF_20); [Haldar *et al.*, 1978](#_ENREF_39)). However, Kovacs et al. ([1987a](#_ENREF_67)) observed increased OT immunoreactivity in extra-hypothalamic regions including hippocampus (Hip), amygdala (Amy) and basal forebrain of male mice, suggesting either differential effects of acute opioid administration on OT neurotransmission in different areas of the brain or a sex-dependent regulation of the oxytocinergic system upon an acute challenge with opioids.

## Chronic opioid administration

Chronic administration of morphine induced a significant decrease in OT immunoreactivity in the Hip, decreased OT mRNA levels within the SON, median eminence and arcuate nucleus of the Hyp (ARC) and reduced brain OT synthesis, and plasma OT levels ([Kovacs *et al.*, 1987a](#_ENREF_67); [Laorden *et al.*, 1998](#_ENREF_74); [You *et al.*, 2000](#_ENREF_121); [Zanos *et al.*, 2014a](#_ENREF_126)). This general down-regulation of the oxytocinergic system following chronic opioid administration, in comparison with the acute stimulatory effects of opioid administration in different brain regions, may be a result of several neuroadaptive changes in the oxytocinergic system caused by chronic exposure to opioids. We showed that this hypo-oxytocinergic tone following chronic administration of opioids is linked with a marked increase in OT receptor binding within the olfactory nuclei and amygdala of mice ([Zanos *et al.*, 2014a](#_ENREF_126)); this effect might comprise a neuroadaptive/compensatory mechanism to counteract the decreased oxytocinergic signalling in the brain. Increased OT receptor binding in the brain following chronic opioid administration might also indicate a hypersensitivity of the oxytocinergic system during this period and should be taken into consideration when choosing the right oxytocin dosing regimen. In fact, while acute administration of low doses of OT lack deleterious side effects in humans ([see MacDonald et al., 2011](#_ENREF_81)), there is uncertainty on the effects of chronic administration of OT at both low and higher doses. Importantly, Peters et al., ([2014](#_ENREF_94)) demonstrated that chronic (15-day) ICV infusion of OT, at a high dose (10 ng/h), induced a paradoxical anxiogenic phenotype in mice. This is particularly important in the case of opioid addiction, where the oxytocin receptor system may be more sensitive based on findings from Zanos et al ([2014a](#_ENREF_126)) showing an upregulation of OT receptors in an animal model of chronic opioid use. There, high doses of OT could be proven deleterious and even worsen the treatment prognosis in this population. Nonetheless, chronic administration of low doses of OT (1 ng/h; 19 days; ICV), prevented psychosocial stress-induced anxiety ([Peters et al., 2014](#_ENREF_94)), indicating a dose-dependent effect of OT. These findings highlight that it may be important for future clinical studies assessing the effects of OT in opioid-dependent individuals, to use low doses of OT, in order to avoid potential undesirable side effects*.*

## Opioid conditioning/self-administration

Interestingly and in contrast with the hypothesis that OT might be a potential target for the treatment of drug addiction, central administration of OT did not prevent the acquisition of morphine-conditioning and it even increased the expression of morphine place preference when it was administered prior to the post-conditioning test in a conditioned-place preference (CPP) study in rats ([Moaddab *et al.*, 2015](#_ENREF_85)). In line with this finding, Van Ree et al., ([1977](#_ENREF_113)) showed that peripherally administered OT slightly facilitated heroin self-administration in rats. These paradoxical effects of OT might be related to the direct actions of OT stimulation on the DAergic system (see Section “Oxytocin and the reward system”). The fact that activation of the central OT receptors directly increases striatal DAergic content ([Georgiou *et al.*, 2015b](#_ENREF_33)) and OT facilitates the effects of DA ([Insel, 2003](#_ENREF_47)), which is directly involved in the reinforcing properties of the drugs of abuse ([Volkow *et al.*, 2006](#_ENREF_116); [Wong *et al.*, 2006](#_ENREF_119)), indicates that OT administration might cause an enhanced morphine-induced conditioned-place preference and/or heroin self-administration via mimicking and thus augmenting the hedonic effects of morphine. Indeed, similar to morphine ([Lintas *et al.*, 2011](#_ENREF_78)), OT administration was shown to increase the firing rate of accumbal neurons ([Moaddab *et al.*, 2015](#_ENREF_85)), indicating that there might be a possible additive effect of morphine and OT on the hyperexcitability of the mesolimbic DAergic neurons, which might have driven the enhanced morphine-induced conditioning. However, the choice of OT dose seems to be critical in determining the beneficial effects of the drug. Indeed, in the morphine CPP study ([Moaddab *et al.*, 2015](#_ENREF_85)), the authors used a dose of 0.2μg (intracerebroventricular; ICV), whereas Ibragimov et al., ([1987b](#_ENREF_46)), using a dose of 0.2ng microinjected into the nucleus accumbens or ventral hippocampus demonstrated that OT was able to abolish heroin self-administration in heroin-dependent rats, an effect that was prevented by OT receptor blockade. These controversies highlight the importance for choosing the correct dose for OT to exert its beneficial effect, especially since OT is known to also bind to the vasopressin receptors at higher doses ([Busnelli *et al.*, 2013](#_ENREF_15)), which would plausibly cause the exact opposite behavioural effects ([Neumann *et al.*, 2012](#_ENREF_88)). In support of this, Peters *et al*., ([2014](#_ENREF_94)) showed dose-dependent differential effects of chronic central administration of OT on anxiety, stress-related behaviours, as well as OT receptor binding in different brain regions..

## Opioid Tolerance

OT was also shown to modulate short- and long-term opioid tolerance. In particular, both peripheral and central OT administration dose-dependently attenuated the development of analgesic morphine and heroin tolerance in rodents, and a single OT injection also blocked the expression of heroin tolerance following repeated heroin administration ([Kovacs *et al.*, 1985c](#_ENREF_64); [Kovacs *et al.*, 1984](#_ENREF_66); [Kovacs *et al.*, 1998](#_ENREF_70); [Kovacs *et al.*, 1987c](#_ENREF_71)). These results strongly suggest that OT can rapidly modulate both the early development, as well as the expression of an already developed opioid tolerance. Interestingly, OT treatment reduced heroin self-administration in heroin-tolerant ([Kovacs *et al.*, 1985b](#_ENREF_62)), but not in non-tolerant rats ([Kovacs *et al.*, 1998](#_ENREF_70)), although it inhibited the development of tolerance to morphine-induced hyper-locomotion in mice (Kovacs and Telegdy, 1987), which might indicate differential effect of OT on the adaptive *versus* acute opioid tolerance processes. Importantly, the lateral septum was shown to mediate the inhibitory effect of OT on heroin self-administration since direct microinjection of OT within this brain region abolished heroin self-administration in heroin-tolerant rats ([Ibragimov *et al.*, 1987a](#_ENREF_45)). While the effect of OT in reducing opioid tolerance may be desirable in increasing the therapeutic efficacy of opioid replacement therapy, it may also be dangerous as it could lead to opioid toxicity in patients receiving opioid replacement therapy, especially in cases of accidental opioid overdose. In addition, if tolerance to the respiratory depressant effects of opioids is also reduced by OT, this effect could lead to a higher risk of opioid overdose, which has been also suggested to be the case with the ethanol effect on tolerance to the respiratory depressant effects of opioids ([Hill *et al.*, 2016](#_ENREF_43)).

## Opioid Withdrawal/relapse

The first evidence for a role of OT on the regulation of opioid withdrawal came from Kovacs *et al*., ([1985d](#_ENREF_65)) who showed that peripheral OT administration decreases naloxone-precipitated morphine withdrawal symptoms in rodents. To unravel the mechanism underlying the inhibitory effect of OT on opioid withdrawal symptoms, later studies investigated the effect of pharmacologically-induced opioid withdrawal on OT neurotransmission. In particular, Bicknell *et al*., ([1988](#_ENREF_8)), demonstrated that naloxone-precipitated morphine withdrawal, a protocol which is widely used to precipitate acute physical opioid withdrawal symptoms, increases plasma OT levels, as well as the firing rate of SON OT neurons in chronically morphine-treated lactating rats. Additionally, naloxone administration also produced a large increase in OT levels within the CSF of opioid-dependent rats ([Coombes *et al.*, 1991](#_ENREF_21)). Finally, naloxone-precipitated morphine withdrawal increased *Fos* protein expression within the SON ([Johnstone *et al.*, 2000](#_ENREF_50); [Murphy *et al.*, 1997](#_ENREF_86)) and OT mRNA levels within the median eminence (ME) and PVN ([Laorden *et al.*, 1998](#_ENREF_74)), which may illustrate an increase in the biosynthesis of OT. These findings are somewhat unexpected since OT treatment prevents naloxone-precipitated withdrawal symptoms at a time point where OT neurotransmission is already enhanced. Taken together, these results might indicate a possible OT receptor-independent mechanism of action of exogenously administered OT on the regulation of opioid withdrawal physical symptoms. Indeed, it has been demonstrated that OT might exert an OT receptor-independent action at GABAA receptor δ subunit to regulated addiction-related processes ([Bowen *et al.*, 2015](#_ENREF_10)).

In contrast to the pharmacologically-induced morphine withdrawal findings, recent studies have demonstrated a differential regulation of the oxytocinergic system following non-precipitated, long-term spontaneous withdrawal from opioid treatment in mice. Zanos *et al*. ([2014a](#_ENREF_126)) found decreased hypothalamic OT levels and increased OT receptor binding in the olfactory nuclei, piriform cortex, septum and amygdala following spontaneous (non-precipitated) withdrawal from chronic escalating-dose morphine administration in mice. These neuroadaptive alterations of the oxytocinergic system were concomitant with the emergence of emotional deficits including depressive-, anxiety-like behaviors and social impairment at least in an animal model setting. We also demonstrated that carbetocin, an oxytocin analogue, was able to prevent morphine withdrawal-induced emotional impairment, as well as stress- and priming-induced reinstatement of morphine conditioned preference in mice ([Georgiou *et al.*, 2015b](#_ENREF_33); [Zanos *et al.*, 2014a](#_ENREF_126)). These findings demonstrate the ability of OT treatment to reduce the physical and emotional symptoms of opioid abstinence, suggesting a promising pharmacotherapy for comorbid mood and substance abuse disorders, as well asrelapse prevention; thus, warranting a clinical investigation in opioid abusers and abstinent individuals undergoing detoxification.

## Mechanisms underlying the effect of OT in opioid addiction

The mechanism/s by which OT exerts its effect on drug-related behaviours are complex and not fully understood. Here, we outline some of the main suggested mechanisms based on pre-clinical and clinical evidence.

### Interactions with the monoaminergic system

*Dopamine*: The most characterised link between the oxytocinergic and the DAergic systems stems from the fact that OT-mediated social affiliative behaviours are also linked to key alterations in the DAergic reward system ([Skuse *et al.*, 2009](#_ENREF_105); [Young *et al.*, 2004](#_ENREF_124)). OT is known to modulate DA turnover and OT receptors have been shown to functionally interact with the DA D2 receptor in the nucleus accumbens ([Romero-Fernandez *et al.*, 2012](#_ENREF_100)). Thus, it is perhaps not surprising that the DAergic system is involved in the mechanism(s) underlying the effect of OT in modulating addictive-related behaviours. For instance, Qi et al., ([2008](#_ENREF_97)) demonstrated that OT administration prevents methamphetamine-induced hyperlocomotion via decreasing methamphetamine-associated reduction on DA turnover in the mesolimbic system of the brain. In addition, intra-prefrontal cortex administration of OT prevented amphetamine-induced impaired pair bond formation via blocking amphetamine-induced increases in DA levels in the Acb ([Young *et al.*, 2014](#_ENREF_122)). With regards to opioid addiction, Georgiou *et al*., ([2015b](#_ENREF_33)) showed that administration of the oxytocin analogue carbetocin increases DA turnover in the striatum of mice, which was associated with the ability of the drug to prevent both priming- and stress-induced reinstatement to opioid conditioned-place preference.

*Noradrenaline*: Some preliminary evidence for an interaction between the oxytocinergic and noradrenergic systems exists. OT administration enhances [noradrenaline](http://topics.sciencedirect.com/topics/page/Norepinephrine) release in the [SON nucleus](http://topics.sciencedirect.com/topics/page/Supraoptic_nucleus) of the hypothalamus, which then activates [hypothalamic](http://topics.sciencedirect.com/topics/page/Hypothalamus) OT neurons ([Onaka *et al.*, 2003](#_ENREF_90)). Importantly, we have recently shown that the prevention of morphine primed-reinstatement of opioid seeking behaviour following administration of OT is directly associated with the ability of OT to suppress striatal noradrenaline turnover in mice ([Georgiou *et al.*, 2015b](#_ENREF_33)), thus suggesting the presence of a noradrenergic mechanism underlying the beneficial effect of OT in opioid relapse prevention. Nonetheless, this was not the case with stress-induced reinstatement indicating differential regulation of OT-noradrenaline interactions in mediating diverse reinstatement triggers.

*5-hydroxytryptamine (serotonin):* Preliminary data suggest possible interactions between the oxytocinergic and serotonergic systems, which might be implicated in the modulation of several neuropsychiatric disorders. For example, [serotonergic](http://topics.sciencedirect.com/topics/page/Serotonergic) terminals originating from the dorsal and median [raphe nuclei](http://topics.sciencedirect.com/topics/page/Raphe_nuclei) were shown to project to the PVN magnocellular neurons ([Larsen *et al.*, 1996](#_ENREF_75); [Sawchenko *et al.*, 1983](#_ENREF_103)), where they possibly regulate OT release via an interaction with the serotonin receptors ([Ho *et al.*, 2007](#_ENREF_44); [Jorgensen *et al.*, 2003](#_ENREF_52)). In addition, administration of  a serotonergic [agonist](http://topics.sciencedirect.com/topics/page/Agonist) to healthy individuals increased plasma OT levels ([Lee *et al.*, 2003](#_ENREF_76)). Although the involvement of the serotonergic system in the mechanisms underlying the effects of OT on opioid addiction-related behaviours has not been investigated to date, it is important to pursue research aiming to understand whether OT-based pharmacotherapies, via interacting with the serotonergic system, are effective in treating opioid addiction-mood disorder comorbidities, considering the evidence that serotonin reuptake inhibitor antidepressant drugs (i.e., citalopram and fluvoxamine), may exert their antidepressant effects partly via interacting with the oxytocinergic system ([de Jong *et al.*, 2007](#_ENREF_25); [Swaab *et al.*, 2000](#_ENREF_107); [Uvnas-Moberg *et al.*, 1999](#_ENREF_112)).

### Interactions with the glutamatergic system

While the role of glutamatergic neurotransmission in opioid addiction has not yet been identified, there is evidence to suggest a key involvement of glutamate in the pharmacological effects of OT in modulating addictive-related behaviours. In particular, Qi et al., ([2009](#_ENREF_96)) showed that OT treatment abolished stress-induced, but not methamphetamine-priming increases in glutamate levels in the medial prefrontal cortex of mice undergoing reinstatement of methamphetamine conditioned-place preference. Interestingly, this effect was associated with the ability of OT to prevent stress-induced, but not drug-primed reinstatement to methamphetamine place preference.

### Role of the amygdala

Intranasal OT has been shown to decrease anxiety via reducing amygdala reactivity in response to threat ([Baumgartner *et al.*, 2008](#_ENREF_6); [Domes *et al.*, 2007](#_ENREF_27); [Kirsch *et al.*, 2005](#_ENREF_56); [Labuschagne *et al.*, 2010](#_ENREF_72)). Since there is high comorbidity between drug addiction and anxiety disorders, it is plausible that OT may act within this precise brain network to induce its restorative effect on recovering emotional impairment in drug-dependent individuals. Indeed, we have shown that chronic administration of morphine ([Zanos *et al.*, 2014a](#_ENREF_126)), cocaine ([Georgiou *et al.*, 2015a](#_ENREF_32); [Georgiou *et al.*, 2016b](#_ENREF_35)), methamphetamine ([Georgiou *et al.*, 2016a](#_ENREF_34); [Zanos *et al.*, 2014b](#_ENREF_127)) and nicotine ([Zanos *et al.*, 2015](#_ENREF_125)) induces an upregulation of the OT receptor binding in the amygdala of mice, indicating a possible common neuroadaptation of the oxytocinergic system in response to different classes of drugs of abuse. Although the exact mechanisms underlying these neuroadaptations need further investigation, it is likely that these changes of the amygdalar OT receptor system are involved in the modulation of drug/emotional impairment comorbidity. Given the anxiolytic, antidepressant and social-enhancing effects of OT administration in humans when administered via an intranasal spray ([Baumgartner *et al.*, 2008](#_ENREF_6); [Di Simplicio *et al.*, 2009](#_ENREF_26); [Kirsch *et al.*, 2005](#_ENREF_56)), or in animal models when administered centrally or peripherally ([Dabrowska *et al.*, 2011](#_ENREF_22); [Windle *et al.*, 2004](#_ENREF_117)), this dysregulation (upregulation) of the OT receptor system in the amygdala may constitute a common neurobiological mechanism to plausibly counteract the negative emotional state induced by chronic drug administration. Therefore, the use of an OT-based pharmacotherapy to preferably jump-start the amygdala to attenuate emotional distress, including anxiety, and activate stress-coping mechanisms could be an important area for research and further our understanding of the role of the amygdalar oxytocinergic system in controlling substance use.

### Hypothalamic-pituitary-adrenal (HPA) axis activity

There is evidence to support a regulatory role of the HPA axis activity on the anxiolytic and antidepressant effects of OT. Specifically, i.c.v administration of OT decreased stress-induced corticosterone release in rats ([Windle *et al.*, 1997](#_ENREF_118)). Moreover, intra-PVN administration of an OT receptor [antagonist](http://topics.sciencedirect.com/topics/page/Receptor_antagonists) increased basal [ACTH](http://topics.sciencedirect.com/topics/page/Corticosterone) levels, while it reduced ACTH release in response to a forced-swim stress in male rats ([Neumann *et al.*, 2000a](#_ENREF_87); [Neumann *et al.*, 2000b](#_ENREF_89)). These findings indicate a possible tonic inhibition of the HPA axis activity by OT and an enhancing action under stress conditions. With regards to opioid addiction, we have shown that the effect of carbetocin in preventing stress- ([Zanos *et al.*, 2014a](#_ENREF_126)) and priming-induced ([Georgiou *et al.*, 2015b](#_ENREF_33)) reinstatement of morphine-seeking does not depend on changes in the HPA axis activity, since we did not observe any effects of carbetocin on plasma corticosterone levels following either priming- or stress- induced reinstatement in mice. However, the effects of OT or OT-based drug administration on the central corticotropin-releasing factor (CRF) system cannot be precluded since there is evidence for a direct regulation of the CRF neurotransmission by OT ([Bulbul *et al.*, 2011](#_ENREF_13); [Jurek *et al.*, 2015](#_ENREF_53); [Pati *et al.*, 2015](#_ENREF_91)).

### Extinction of traumatic memories

Although not tested in the context of drug addiction, there is compelling evidence to suggest that OT facilitates extinction of memories associated with fear. For instance, ICV administration of OT prior to fear conditioning does not appear to have any effect on fear learning; however later fear extinction is facilitated by OT, while OT receptor antagonists administration impairs extinction learning and retrieval ([Singewald *et al.*, 2015](#_ENREF_104)). Therefore, it is conceivable that OT may be able to alleviate the affective emotional consequences of drug addiction and prevent relapse by interfering with the consolidation of fear memories, making these memories weaker and more susceptible to extinction ([Singewald *et al.*, 2015](#_ENREF_104)). This hypothesis warrants further exploration.

### Interactions with the endogenous opioid system

Opioid peptide regulation of the OT system has been suggested to at least partly underlie the effects of opioid drugs on the OT system. In fact, opioid peptide neuronal fibers and terminals are located in close proximity with OT neurons within the Hyp ([Bicknell *et al.*, 1988](#_ENREF_8)). Moreover, μ-opioid receptors (μ receptors) are highly expressed in the Hyp, and particularly within the SON and PVN nuclei, where oxytocinergic neurons project from ([Atweh *et al.*, 1983](#_ENREF_5)). These studies indicate possible interactions between the opioid and oxytocinergic systems. Indeed, it was recently demonstrated with the use of receptor autoradiographic binding in μ receptor knockout mice, the presence of brain region-specific interactions between the μ receptor and OT receptor systems ([Georgiou *et al.*, 2015b](#_ENREF_33); [Gigliucci *et al.*, 2014](#_ENREF_36)), which may be involved in the effects OT on the modulation of opioid-associated behaviours discussed in this review. Furthermore, a remarkable decrease in OT gene expression was observed in the Acb of mice lacking the μ receptor gene ([Becker *et al.*, 2014](#_ENREF_7)), further supporting a close interaction between the opioidergic and oxytocinergic system.

## OT as a potential pharmacotherapy for opioid addiction: From bench to bedside

### Clinical studies

There is currently a limited number of clinical trials investigating the efficacy of OT in the treatment of drug addiction. With regards to opioid addiction, there have been only two clinical studies to-date that assessed the effects of intranasal OT in opioid-dependent patients ([Stauffer *et al.*, 2016](#_ENREF_106); [Woolley *et al.*, 2016](#_ENREF_120)). The main outcome of both studies demonstrates a safe and good tolerability profile of OT administration in opioid-dependent individuals, even after repeated administration for two weeks. In a randomized, double-blind, placebo-controlled, crossover study, Wooley *et al*., ([2016](#_ENREF_120)) reported that intranasal OT administration (40 IU) did not improve cue-induced craving in opioid-dependent subjects receiving opioid replacement therapy. In contrast, in a placebo-controlled trial of individuals undergoing methadone replacement treatment for opioid and co-occurring cocaine use disorder, placebo-treated patients reported an increase for heroin craving, while individuals who received intranasal OT treatment (40 IU; two times daily x two weeks) did not exhibit increased craving response ([Stauffer *et al.*, 2016](#_ENREF_106)), providing some promise for the treatment of this population. No evidence of a reduction of opioid tolerance following OT administration was observed in these trials. This is especially important considering the findings of OT-induced opioid tolerance observed in animals, which could have potentially led to fatal overdose

### Therapeutic potential of OT in opioid addiction treatment and addiction-emotional impairment comorbidities

In light of the literature reviewed here, OT has unambiguously a key role in mediating several opioid addiction related behavioural and neurochemical processes and can be considered a promising target for the treatment of opioid dependence and emotional impairment comorbidity. One important factor that distinguishes OT from currently available medications is that it does not show abuse or addiction potential. In fact, the doses used in the pre-clinical studies, which revealed that OT induces conditioned-place preference, are way higher than the doses used in the clinical trials ([Liberzon *et al.*, 1997](#_ENREF_77)). Evidence also suggests that patients treated with OT could not discriminate between placebo or the actual drug ([MacDonald *et al.*, 2011](#_ENREF_81)), further supporting the lack of rewarding properties of OT at least at that doses ranging from 18-40 IU. However, future studies should assess the possibility of any rewarding effects following chronic administration of OT in humans.

Another unique property of OT that is particularly important for the treatment of opioid addiction and/or comorbid mood disorders is related to its prosocial effects ([Churchland *et al.*, 2012](#_ENREF_19)). Prolonged use of drugs of abuse results in disintegration of the social lives of drug addicts and may lead to social isolation and poor decision-making in their social domain at the expense of compulsive pre-occupation with the drug and its related cues ([Dawe *et al.*, 2009](#_ENREF_24); [Volkow *et al.*, 2011](#_ENREF_115)). Impaired social behaviours have been linked with the propensity of addicts to relapse after long-term abstinence ([Tokar *et al.*, 1975](#_ENREF_108)). Therefore, considering the therapeutic effects of social support programs (e.g. Alcoholics Anonymous, Narcotics Anonymous) and the benefits of social rehabilitation and social reintegration in keeping addicts abstinent from the drug ([Koerner, 2010](#_ENREF_57); [McGregor *et al.*, 2012](#_ENREF_83)), the current findings for the pro-social effects of OT may implicate its use as an adjunct to Cognitive Behavioural Therapy as a novel effective “psycho-biological therapy” for the prevention of relapse to drug-seeking. In support of this, OT and social support have been shown to interact and exert a stress-buffering effect following a psychosocial stress challenge in humans ([Heinrichs *et al.*, 2003](#_ENREF_41)) Moreover, there is clinical evidence for a beneficial role of OT in the treatment of other disorders characterised by social cognitive impairment including autistic spectrum disorders and schizophrenia ([Carter, 2007](#_ENREF_17); [Heinrichs *et al.*, 2007](#_ENREF_42)).

### Limitations

One concern for studies looking at effects of exogenously administered OT is that it has a very short plasma (3-5 min) and central (30 min) half-life ([Ludwig *et al.*, 2006](#_ENREF_80); [Uvnas-Moberg, 1998](#_ENREF_111)). However, intranasal administration of OT has been shown to induce more prolonged release of at least 80 min ([Burri *et al.*, 2008](#_ENREF_14)) and has extended biological (endocrine and sexual) activity, even after a single dose in humans ([Uvanas-Moberg *et al.*, 2005](#_ENREF_110)). Moreover, intranasally administered OT has been shown to cross the BBB and to exert central effects ([Born *et al.*, 2002](#_ENREF_9); [Chang *et al.*, 2012](#_ENREF_18); [Pedersen *et al.*, 2013](#_ENREF_93)). Nonetheless, the development of smaller non-peptide OT agonists with high specificity for central OT receptors is undoubtedly desirable.

Although the outcome from multitude of clinical trials using intranasal oxytocin treatment points towards a safe profile of the drug ([MacDonald *et al.*, 2011](#_ENREF_81)), there are some unanswered questions related to its safety following chronic use in drug-dependent individuals. In fact, high doses of intravenous OT have been associated with cardiovascular side-effects including hypotension and myocardial ischemia ([Dyer *et al.*, 2011](#_ENREF_30)) or electrolyte imbalances due to its structural similarity to arginine vasopressin and its effects in the kidneys ([Rasmussen *et al.*, 2004](#_ENREF_98)). Importantly, OT administration at high doses could also activate the vasopressin V1A receptors in the brain, which may actually lead to the opposite behavioural responses ([Neumann *et al.*, 2012](#_ENREF_88)). Concerns also include the safety of OT administration in females at different reproductive phases due to the peripheral effects of OT (i.e. milk ejection, labour induction), as well as the regulation of OT by the gonadal hormones ([McCarthy, 1995](#_ENREF_82); [Zhang *et al.*, 1991](#_ENREF_128)).

Moreover, caution needs to be applied when choosing the dose of OT for chronic intranasal administration. In fact, Peters *et al*., ([2014](#_ENREF_94)) showed that chronic ICV infusion of OT (15 days) at a high dose (10 ng/h), induces an anxiogenic phenotype; however, low doses of OT(1 ng/h for 19 days) prevented psychological stress-induced hyper-anxiety in rats. These findings highlight the need for a deeper understanding of chronic treatment and dose-dependent effects of OT before we consider OT for long-term therapeutic use for the treatment of psychiatric conditions such as addiction.

# Concluding remarks

Pre-clinical and clinical evidence clearly indicates OT’s potential as an effective next-generation treatment (possibly as an ad-hoc medication) for opioid addiction and comorbid mood disorders, as well as prevention of relapse. Therefore, there is a need for future clinical studies to directly assess the effect of OT-based pharmacotherapies in the different stages of opioid addiction and to determine doses that would avoid any detrimental side effects.

# Author contributions

All authors wrote/critically reviewed parts of the manuscript.

# Conflict of Interest

Authors declare no conflict of interest.

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# Tables

Table 1: Effects of oxytocin on opioid- -induced addictive related behaviours.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Addictive substance** | **Administration paradigm** | **Animal model** | **Oxytocin administration paradigm** | **Effect of oxytocin** | **Reference** |
| **Morphine** | **Morphine tolerance:**  37.5 mg morphine.HCl pellet, s.c./48 hours | Male CFLP mice (25±5g) housed in groups | OT: 50 μg and 100 μg/ animal, s.c. (2 hours prior to morphine pellet implantation) | ↓development of tolerance | ([Kovacs *et al.*, 1985d](#_ENREF_65)) |
| **Morphine tolerance:**  30 mg/kg morphine.HCl, s.c. and 5 hours later 5 mg/kg morphine.HCl, s.c. | Male albino inbred mice (25±5g) housed in groups | OT: 1 μg i.c.v. or intra-CPu (1 hour prior the tolerance-inducing dose of morphine) | ↔ development of tolerance | ([Sarnyai *et al.*, 1988](#_ENREF_102)) |
| **Morphine tolerance:**  30 mg/kg morphine.HCl, s.c. and 5 hours later 5 mg/kg morphine.HCl, s.c. | Male albino inbred mice (25±5g) housed in groups | OT: 1 μg microinjection into: posterior olfactory nucleus, CeA, ventral hipoccampus (1 hour prior the tolerance-inducing dose of morphine) | ↓ development of tolerance | ([Sarnyai *et al.*, 1988](#_ENREF_102)) |
| **Morphine tolerance:**  60 mg/kg morphine.HCl, s.c. and 5 hours later 1 mg/kg morphine.HCl, s.c. | Male CFLP mice (25±5g) housed in groups | OT: 0.002 mg/kg, s.c. (1 hour prior the tolerance-inducing dose of morphine) | ↓ development of tolerance | ([Kovacs *et al.*, 1987b](#_ENREF_69)) |
| **Morphine tolerance:**  100 mg/kg morphine.HCl, s.c. and 5 hours later 1 mg/kg morphine. HCl, s.c. | Male CFLP mice  (25±5 g) housed in groups | OT: 0.002 mg/kg, s.c. (1 hour prior the tolerance-inducing dose of morphine) | ↓ development of tolerance | ([Kovacs *et al.*, 1987b](#_ENREF_69)) |
| **Morphine tolerance:**  37.5 mg morphine.HCl pellet, s.c./48 hours  then, morphine.HCl (5 mg/kg, s.c.) | Male CFLP mice, housed in groups | OT: 50 μg, s.c. (24 hours prior to pellet implantation) | ↓ development of tolerance | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Morphine tolerance:**  37.5 mg/ morphine.HCl pellet, s.c./48 hours  then, morphine.HCl (5 mg/kg, s.c.) | Male CFLP mice, housed in groups | OT: 0.005 or 0.5 μg/1μl, i.c.v. (24 hours prior to pellet implantation) | ↓ development of tolerance | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Morphine tolerance:**  37.5 mg morphine.HCl pellet, s.c./48 hours  then, morphine.HCl (5 mg/kg, s.c.) | Male CFLP mice, housed in groups | OT: 0.5 ng/1μl, into the dorsal Hip or the Acb (24 hours prior to pellet implantation) | ↓ development of tolerance | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Morphine tolerance:**  37.5 mg morphine.HCl pellet, s.c./48 hours  then, morphine.HCl (5 mg/kg, s.c.) | Male CFLP mice, housed in groups | OT: 0.5 ng/1μl, into the CPu, VTA or external cortical surface (24 hours prior to pellet implantation) | ↔ development of tolerance | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Morphine-induced conditioned-place preference:**  Acquisition: 3 conditioning days (morphine. HCl, 5 mg/kg/day, s.c.) | Male Wistar rats (250-300 g), housed in groups | OT: 0.2 μg, i.c.v., 5 min prior to each conditioning session (both prior to morphine and prior to saline injections) | ↔ acquisition of morphine CPP | ([Moaddab *et al.*, 2015](#_ENREF_85)) |
| **Morphine-induced conditioned-place preference:**  Expression: 3 conditioning days (morphine. HCl, 5 mg/kg/day, s.c.) | Male Wistar rats (250-300 g), housed in groups | OT: 0.2 μg, i.c.v., 5 min prior to the post-conditioning session | ↑ expression of morphine CPP | ([Moaddab *et al.*, 2015](#_ENREF_85)) |
| **Physical signs following precipitated withdrawal:**  Day 1: 2 x 20 mg/kg, i.p.  Days 2-4: 2 x 40 mg/kg i.p. (morning and afternoon injections)  Naloxone: 4 mg/kg, i.p. (1 hour after the morning morphine injection daily) | Female Wistar rats (130-150 g) housed individually | OT: 1.0 μg/animal, s.c. (1 hour prior to each morphine injection daily) | ↑ physical dependence (decreased body weight) | ([van Ree *et al.*, 1976](#_ENREF_114)) |
| **Naloxone-precipitated withdrawal:**  37.5 mg morphine.HCl pellet, s.c./74 hours  Naloxone (74 hours after pellet implantation): 1 mg/kg, i.p. | Male CFLP mice, housed in groups | OT: 50 μg, s.c., (24 hours prior to pellet implantation) | ↑ latency of naloxone-precipitated withdrawal | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Naloxone-precipitated withdrawal:**  37.5 mg morphine.HCl pellet, s.c./74 hours  Naloxone (74 hours after pellet implantation): 1 mg/kg, i.p. | Male CFLP mice, housed in groups | OT: 0.005 or 0.5 μg/1μl, i.c.v. (24 hours prior to pellet implantation) | ↑ latency of naloxone-precipitated withdrawal | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Naloxone-precipitated withdrawal:**  37.5mg morphine.HCl pellet, s.c./74 hours  Naloxone (74 hours after pellet implantation): 1mg/kg, i.p. | Male CFLP mice, housed in groups | OT: 0.5 ng/1μl, into the dorsal Hip or the mesolimbic Acb (24 hours prior to pellet implantation) | ↑ latency of naloxone-precipitated withdrawal | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Naloxone-precipitated withdrawal:**  37.5 mg morphine.HCl pellet, s.c./74 hours  Naloxone (74 hours after pellet implantation): 1 mg/kg, i.p. | Male CFLP mice, housed in groups | OT: 0.5 ng/1μl, into the CPu, VTA or external cortical surface (24 hours prior to pellet implantation) | ↔ latency of naloxone-precipitated withdrawal | ([Kovacs *et al.*, 1984](#_ENREF_66)) |
| **Naloxone-precipitated withdrawal:**  37.5mg morphine.HCl pellet, s.c./72 hours  Naloxone (72 hours after pellet implantation): 1 mg/kg, i.p. | Male CFLP mice  (25±5 g) housed in groups | OT: 50 μg and 100 μg/ animal, s.c. (2 hours prior to morphine pellet implantation) | ↓ withdrawal symptoms | ([Kovacs *et al.*, 1985d](#_ENREF_65)) |
| **Spontaneous withdrawal:**  20-100 mg/kg/day, for 7 days, i.p. morphine sulfate  Withdrawal: 7 days in home cage without injections | Male C57BL/6J mice (20-25 g), housed individually | Carbetocin: 6.4 mg/kg, i.p. (15 min prior to FST, EPM/ 5 min prior to 3-CB test/ | ↓ withdrawal-induced depressive-, anxiety-like behaviours, sociability deficits | ([Zanos *et al.*, 2014a](#_ENREF_126)) |
| **Stress-induced reinstatement morphine CPP:**  10 mg/kg, s.c., for 4 conditioning days (morphine sulfate in the afternoon)  Stress: forced-swim stress (6-min total) | Male C57BL/6J mice (20-25 g), housed individually | Carbetocin: 6.4 mg/kg, i.p. (5 min prior to swim stressor for reinstatement) | ↓ stress-induced reinstatement of morphine conditioned place preference | ([Zanos *et al.*, 2014a](#_ENREF_126)) |
| **Priming-induced reinstatement of morphine CPP:**  10 mg/kg, s.c., for 4 conditioning days (morphine sulfate in the afternoon)  Priming: 2 mg/kg, i.p. (morphine sulfate) | Male C57BL/6J mice (20-25 g), housed individually | Carbetocin: 6.4 mg/kg, i.p. (5 min prior to morphine priming injection) | ↓ morphine-primed induced reinstatement of morphine conditioned place preference | ([Georgiou *et al.*, 2015b](#_ENREF_33)) |
| **Heroin** | **Self-administration:**  5 days of fixed ratio schedule of reinformcement: 0.25 ml of heroin solution (0.125 mg/ml/infusion), i.v.; experimenter delivered two initial heroin injections by pressing the lever | Male Wistar rats (200-230 g) housed individually | OT: 1.0 μg/animal, s.c. (1 hour prior to experimentation daily) | ↑ self-administration (slightly) | ([Van Ree *et al.*, 1977](#_ENREF_113)) |
| **Self administration:**  0.05 mg/kg s.c., 2 x daily/4 days + 0.4 mg/kg, s.c., 2 x daily/3 days  Followed by  7 days of progressive ratio schedule of reinformcement: 0.25 ml of heroin solution (0.125 mg/ml/infusion), i.v. | Male Wistar rats (200-220 g) housed individually | OT: 1.0 μg/animal, s.c. (1 hour prior to self-administration session on the day 7) | ↓ self-administration | ([Kovacs *et al.*, 1985a](#_ENREF_61)) |
|  | **Development of heroin tolerance (escalating-dose):**  2 x daily i.p. injections (100, 200, 400, 800, 800, 800 μg/kg)  **Self-administration:**  On day 7 of heroin injections, rats were placed in self-administration chambers; fixed-ratio reinforcement: 50 μl of heroin solution (0.4 g/l) – flow rate 5 μl/sec; schedule terminated upon stable level of responding for 3 consecutive days (usually 7-8 days) | Male Sprague-Dawley rats (250 ± 30 g) housed individually | OT: intra-accumbal or injections directly into the ventral Hip; 2 ng; treatment block of saline/OT/saline/OT 1.0 μg/animal, s.c. (1 hour prior to self-administration session daily) | ↓ self-administration | ([Ibragimov *et al.*, 1987b](#_ENREF_46)) |

A detailed summary on the effects of oxytocin or oxytocin-based drugs administration on the behavioural effects of opioids in rodents. ↑ increase; ↓ decrease; ↔ no effect.

*Abbreviations*: Acb, nucleus accumbens; CPP, conditioned place preference; CPu, caudate-putamen; HCl, hydrogen chloride; Hip, hippocampus; s.c., subcutaneous; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.v., intravenous; OT, oxytocin; VTA, ventral tegmental area.

Table 2: Effects of opioids on the oxytocinergic system.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Addiction phase** | **Administration paradigm** | **Animal Model** | **Effect on Oxytocin** | **Reference** |
| **Morphine** | **Acute** | 4μg, i.v. | Lactating Wistar rats | ↓ hypothalamic OT release | ([Clarke *et al.*, 1979](#_ENREF_20)) |
| 10 mg/kg, s.c. | Swiss Webster mice between the 11th and 22nd day of lactation | ↓ OT release during suckling | (Haldar and Sawyer et al. 1978) |
| 5 mg/kg, s.c. | Male CFLP mice (25±5 g) housed in groups | ↑ OT immunoreactivity in Hip, Amy and basal forebrain | (Kovacs et al. 1987a) |
| 5 mg/kg, i.v. | Virgin Sprague-Dawley female rats (~270 g) | ↓ spontaneous activity of SON OT neurons  ↓ plasma OT levels | ([Pumford *et al.*, 1991](#_ENREF_95)) |
| 0.1-1.5 μg/μl, i.c.v. | Virgin Sprague-Dawley female rats (~300 g) | ↓ spontaneous activity of SON OT neurons | ([Pumford *et al.*, 1991](#_ENREF_95)) |
| **Chronic** | Morphine pellet (37.5 mg morphine.HCl), s.c. | Male CFLP mice (25±5 g), housed in groups | ↔ OT immunoreactivity in Amy and basal forebrain | (Kovacs et al. 1987a) |
| Osmotic mini-pump (75 mg), s.c., 1 on day 0, 2 on day 2 and 3 on day 4. On day 8 morphine.HCl (30 mg/kg.i.p.) | Male Sprague-Dawley rats (230-270 g) housed in groups | ↓ OT immunoreactivity in the Hip, SON, ME and ARC  ↓ OT peptide levels in SON and ME  ↔ OT peptide levels in PVN | ([Laorden *et al.*, 1997](#_ENREF_73); [Laorden *et al.*, 1998](#_ENREF_74)) |
| Osmotic mini-pump, s.c., 10μg/h/40hrs then 20μg/h/40hrs and then 50 μg/h/40hrs | Lactating, primiparous Sprague-Dawley female rats (2-4 days post-partum) | ↔ plasma OT levels | ([Bicknell *et al.*, 1988](#_ENREF_8)) |
| Osmotic mini-pump, s.c., 10μg/h/40hrs then 20μg/h/40hrs and then 50 μg/h/40hrs | Virgin Sprague-Dawley female rats (~270 g) | ↔ firing rate of active non-phasic OT neurons | ([Pumford *et al.*, 1991](#_ENREF_95)) |
| 20-100 mg/kg/day, for 7 days, i.p. morphine sulfate | Male C57BL/6J mice (20-25 g), housed individually | ↑ OT receptor levels in the olfactory nuclei, PirCx and Amy  ↓ hypothalamic OT levels | ([Zanos *et al.*, 2014a](#_ENREF_126)) |
| **Naloxone -Precipitated withdrawal** | Morphine: osmotic mini-pump, s.c., 10μg/h/40hrs then 20μg/h/40hrs and then 50μg/h/40hrs  Naloxone (day 5; following morphine administration):  5 mg/kg, i.v. | Lactating, primiparous Sprague-Dawley female rats | ↑ plasma OT levels;  ↑ firing rate of OT neurons (SON) | (Bicknell *et al*., 1988) |
| Morphine sulfate (20-40 mg/kg) x 5 days, i.c.v (1 μl/hr)  Naloxone (day 5; after i.c.v. morphine):  5mg/kg, i.v. | Virgin Sprague-Dawley female rats (243-287 g) housed individually | ↑ plasma OT levels;  ↑ OT levels in CSF | ([Coombes *et al.*, 1991](#_ENREF_21)) |
| Osmotic mini-pump (75 mg), s.c., 1 on day 0, 2 on day 2, 3 on day 4.  Naloxone.HCl 1 mg/kg, s.c. (on day 7) | Male Sprague-Dawley rats (200-210 g) housed in groups | ↑ OT mRNA levels in the ME and PVN | ([Laorden *et al.*, 1998](#_ENREF_74)) |
| Morphine: osmotic mini-pump, s.c., 10μg/h/40hrs, then 20μg/h/40hrs, then 50μg/h/40hrs  Naloxone (day 5; after last morphine infusion):  5 mg/kg, i.v. | Virgin Sprague-Dawley female rats (~250 g) housed individually | ↑ plasma OT levels  ↑ OT peptide levels in the mediolateral septum  ↔ OT levels in the dorsal Hip  ↔ OT levels in the nucleus of tractus solitarius | ([Russell *et al.*, 1992](#_ENREF_101)) |
| Morphine: osmotic mini-pump, s.c., 10μg/h/40hrs, then 20μg/h/40hrs, then 50μg/h/40hrs  Naloxone (day 5 after last morphine infusion):  5 mg/kg, i.v. | Virgin Sprague-Dawley female rats housed individually | ↑ OT SON neuron post-spike excitability in morphine-dependent rats and to a lesser extend in morphine-naïve rats | ([Brown *et al.*, 2005](#_ENREF_12)) |
| **Spontaneous withdrawal** | 20-100 mg/kg/day, for 7 days, i.p. morphine sulfate  Withdrawal: 7 days in home cage without injections | Male C57BL/6J mice (20-25 g), housed individually | ↑ OT receptor levels in the olfactory nuclei, MS, VDB, LS, PirCx and Amy | ([Zanos *et al.*, 2014a](#_ENREF_126)) |

A detailed summary of the effects of opioid drugs on the oxytocinergic system. ↑ increase; ↓ decrease; ↔ no effect.

*Abbreviations*: Amy, amygdala; ARC, arcuate nucleus; CSF, cerebrospinal fluid; HCl, hydrogen chloride; Hip, hippocampus; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.v., intravenous; LS, lateral septum; ME, median eminence; MS, medial septum; SON; supraoptic nucleus of the hypothalamus; OT, oxytocin; PirCx, piriform cortex; PVN, paraventricular nucleus of the hypothalamus; s.c., subcutaneous; VDB, ventral limb of the diagonal band of Broca.