Abstract: Opioids produce strong analgesia but their use is limited by a paradoxical hypersensitivity named opioid-induced hyperalgesia (OIH) that may be associated to analgesic tolerance. In the last decades, a significant number of preclinical studies have investigated the factors that influence OIH development as well as the cellular and molecular mechanisms underlying OIH. Several factors have been shown to influence OIH including the genetic background and gender of experimental animals as well as the opioid regimen. Mu opioid receptor (MOR) variants and interactions of MOR with different proteins were shown important. Furthermore, at the cellular level, both neurons and glia play a major role in OIH development. Several neuronal processes contribute to OIH, like activation of neuroexcitatory mechanisms, long-term potentiation (LTP) and descending pain facilitation. Increased nociception is also mediated by neuroinflammation induced by the activation of microglia and astrocytes. Neurons and glial cells exert synergistic effects, which contribute to OIH. The molecular actors identified include the Toll-like receptor 4 and the anti-opioid systems as well as some other excitatory molecules, receptors, channels, chemokines, pro-inflammatory cytokines or lipids. This review summarizes the intracellular and intercellular pathways involved in OIH and highlights some mechanisms that may be challenged to limit OIH in the future.

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Dear Editor,

Please find enclosed a review article entitled « Opioid-induced hyperalgesia: cellular and molecular mechanisms » by Laurie-Anne Roeckel, Glenn-Marie Le Coz, Claire Gavéraux-Ruff, and Frédéric Simonin that we would like to submit to Neuroscience.

I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience, as well as the other statement that all authors have approved the final article.

We thank you for considering this review for publication in neuroscience.

Frédéric Simonin, PhD

Irkirch, April 11th 2016
Highlights

This review describes:
- The cellular and molecular mechanisms of opioid-induced hyperalgesia (OIH)
- The different factors that influence the development of OIH
- The different molecular targets that are critically involved in OIH
OPIOID-INDUCED HYPERALGESIA: CELLULAR AND MOLECULAR MECHANISMS

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Abbreviations: 5-HT, serotonin; Ab, antibody; β2-AR, β2-adrenergic receptor; β-FNA, β-funaltrexamine; BDNF, brain derived neurotrophic factor; CaMKII, calmodulin kinase 2; CCK, cholecystokinin; CD11b, cluster of differentiation molecule 11b; CNS, central nervous system; COX2, cyclooxygenase 2; CTOP, D-Phe-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH2; Ctrl, controls; CxCR, chemokine receptor; CxCL, chemokine; DAMGO, Tyr-D-Ala-Gly-NMe-Phe-Gly-ol; DOR, delta-opioid receptor; DRG, dorsal root ganglia; EAAT3, excitatory amino acid transporter-3; EPSC, excitatory post synaptic potential; ERK, extracellular signal-regulated kinase; GABA, gamma aminobutyric acid; GFAP, glial fibrillary acidic protein; GLAST, glutamate aspartate transporter; GluN2B, NMDA receptor subunit 2B; GPCR, G protein coupled receptor; HDAC, histone deacetylase; IL-1β, interleukin-1β; IL, interleukin; JNK, c-Jun N-terminal kinase; KCC2, K+/Cl- cotransporter; LPS, lipopolysaccharide; LTP, long-term potentiation; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; MCP-1, monocyte chemoattractant protein 1; MOR, mu-opioid receptor; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; NK-1, neurokinin 1; NMDA, N-methyl-D-aspartic acid; NPFF, neuropeptide FF; NOP, nociceptin; OIH, opioid-induced hyperalgesia; OFQ, orphanin FQ; P2X4R, purinergic receptor P2X; P65, protein 65; PAG, periaqueductal gray matter; PI3K, phosphoinositide 3 kinase; PKC, protein kinase C; PKA, protein kinase A; PLC, phospholipase C; PNS, peripheral nervous system; PTX, pertussis toxin; RVM, rostral ventral medulla; S1P, sphingosine-1-phosphate; SAHA, suberoylanilide hydroxamic acid; SDF-1, stromal derived factor 1; SNL, sciatic nerve ligation; TLR4, toll like receptor 4; TM, transmembrane domain; TNF-α, tumor necrosis factor-α; TrkB, tyrosine kinase B; TRPM8, transient receptor potential member 8; TRPV1, transient receptor potential vanilloid 1;
Abstract

Opioids produce strong analgesia but their use is limited by a paradoxical hypersensitivity named opioid-induced hyperalgesia (OIH) that may be associated to analgesic tolerance. In the last decades, a significant number of preclinical studies have investigated the factors that influence OIH development as well as the cellular and molecular mechanisms underlying OIH. Several factors have been shown to influence OIH including the genetic background and gender of experimental animals as well as the opioid regimen. Mu opioid receptor (MOR) variants and interactions of MOR with different proteins were shown important. Furthermore, at the cellular level, both neurons and glia play a major role in OIH development. Several neuronal processes contribute to OIH, like activation of neuroexcitatory mechanisms, long-term potentiation (LTP) and descending pain facilitation. Increased nociception is also mediated by neuroinflammation induced by the activation of microglia and astrocytes. Neurons and glial cells exert synergistic effects, which contribute to OIH. The molecular actors identified include the Toll-like receptor 4 and the anti-opioid systems as well as some other excitatory molecules, receptors, channels, chemokines, pro-inflammatory cytokines or lipids. This review summarizes the intracellular and intercellular pathways involved in OIH and highlights some mechanisms that may be challenged to limit OIH in the future.

Keywords: Opioid-induced hyperalgesia; morphine; opiates; mu-opioid receptor; neuroinflammation; pain
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INTRODUCTION

Chronic pain is a major health issue in our society that clearly impacts quality of life. 30-40% of the population in United States, and equivalent in Europe, suffer from chronic pain and its total cost has been estimated at 560-635 billion dollars annually (Renfrey et al., 2003, Breivik et al., 2006, Johannes et al., 2010, Breivik et al., 2013). Even if research progresses and new targets appear for treating acute and chronic pain, opioids still represent the gold standard analgesics. However, opioid treatments induce several adverse side effects among which analgesic tolerance and opioid-induced hyperalgesia are of major importance. Analgesic tolerance corresponds to a progressive decrease of analgesia produced by a given dose of opiate upon chronic administration, resulting in the need to increase the opiate dosage in order to maintain the initial analgesic effect. Opioid-induced hyperalgesia (OIH) refers usually to the development of hypersensitivity to painful stimuli observed upon chronic opiates administration. Therefore, the treatment supposed to relief the patient’s pain will, in an opposite way, generate a more exacerbated pain sensation. In clinic, these two phenomena are conflicting since the development of analgesic tolerance will lead to increase opiate dosage, which in turn will induce an enhancement of OIH. In this review we will focus our discussion on OIH, observed in both human and animal models (Angst and Clark, 2006, Silverman, 2009, Crofford, 2010, Chen et al., 2014, Johnson et al., 2014, Hayhurst and Durieux, 2016). OIH is well established in humans in different types of pain such as post-surgical pain (Fletcher and Martinez, 2014), low back pain (Chu et al., 2006), cancer pain (Carullo et al., 2015), musculoskeletal pain (Crofford, 2010), but also in healthy volunteers (Fishbain et al., 2009, Ruscheweyh et al., 2011) and opioid addicts (Compton et al., 2001). Hence, the clinician faces a dilemma to treat or not chronic pain with opioids, known to have a beneficial effect at first, with a risk of noxious consequences.

Nowadays, in clinic, diverse strategies are used in order to reduce OIH. First, the practitioner can change the type of opioid used, i.e. rotate with the different molecules, in order to limit the side effects due to a specific opiate (Mercadante and Arcuri, 2005, Mercadante and Bruera, 2016). This alternative is used in 21 to 44% of patients with cancer pain and considered successful when the pain felt is decreased of at least 33% (Mercadante et al., 2009). Another possibility is to block the glutamatergic system, which is known to be implicated in OIH (for review, see Lee et al., 2011). Ketamine, a classical antagonist of a glutamate receptor, have been shown to be effective in preventing the development of OIH (Ramasubbu and Gupta, 2011), however its chronic administration also produce adverse side effects (Cvrcek, 2008). Finally, usage of non-opioid medication including pregabalin, propofol or COX2 inhibitors can be efficient to prevent OIH (Lee et al., 2011), but clinical data are limited.
Although well characterized in humans, OIH has been less studied than analgesic tolerance in preclinical models. Most opiate molecules commonly used in clinic have been shown to induce OIH, such as morphine, fentanyl and remifentanyl (for review see Angst and Clark, 2006). Moreover, heroin also leads to OIH, as shown in heroin-dependent users before and under methadone or buprenorphine maintenance therapy (Compton et al., 2012). However, the kinetic of action, metabolism and potency of the different opiate molecules can greatly influence the strength and rapidity of OIH development. Morphine, fentanyl and methadone are strong agonists of the mu-opioid receptor, when codeine and oxycodone for example are considered as moderate agonists. In addition, morphine has a low intrinsic activity whereas fentanyl has a high intrinsic activity (Duttaroy and Yoburn, 1995). Therefore, rotation from moderate to strong agonists and from « low » to « high » intrinsic activity agonists may diminish the adverse side effects and increase the potency of the analgesia.

As in humans, diverse opiates can produce OIH in preclinical models, including fentanyl (Celerier et al., 2000), remifentanyl (Aguado et al., 2015), morphine (Mao et al., 1994) and references cited in table 2), and codeine (Johnson et al., 2014). In addition, OIH can also be induced by DAMGO, a synthetic opioid peptide selectively activating mu-opioid receptor (Drdla et al., 2009). Different animal models are available to study OIH. For example, a single fentanyl injection will induce OIH during two to three days (Elhabazi et al., 2012, Laboureyras et al., 2014), repeated morphine administrations will induce long lasting OIH with a return to basal nociceptive threshold around 10 days after the last administration (Richebe et al., 2005, Elhabazi et al., 2014), and after 6 days of daily injections of 1µg of DAMGO in the paw, mice develop hypersensitivity to mechanical stimuli (Rowan et al., 2014a). These models enable to identify the molecular mechanisms underlying OIH as well as to find novel molecules that block its development.

Although OIH is not yet completely understood, different mechanisms have been identified for this adaptive process including sensitization of primary afferent neurons and enhanced release of glutamate by these primary afferents, hyperexcitability of second order neurons to excitatory neurotransmitters, and up-regulation of nociceptive neuromodulators by descending pain controls (See Chu et al., 2008, Lee et al., 2011). More recently, not only neurons but also glial cells have been shown to participate, being activated concomitantly with OIH and involving diverse targets and receptors on both microglia and astrocytes. These mechanisms are summarized in figure 1.

In this review we will focus non-exhaustively on the different factors that can influence OIH as well as on the newest mechanisms discovered over the last decade, in term of cellular and molecular implication.

**FACTORS INFLUENCING OIH**
In relation to mu-opioid receptor

Mu receptor variants

The mu-opioid receptor (MOR) is the main target of opioids used in clinic. MOR activation leads to analgesia, but also to adverse effects such as hyperalgesia. It has been proposed that these two opposite effects are due to specific isoforms of the receptor. MOR is a seven transmembrane domains (7TM) G Protein Coupled Receptor (GPCR) encoded by the OPRM1 gene in human, named Oprm1 in mouse. Since its initial molecular characterization as a 7TM receptor encoded by 4 exons, several variants have been discovered (Law et al., 2013, Pasternak and Pan, 2013, Convertino et al., 2015). In humans, alternative splicing mechanisms result in 7TM, 6TM and 1TM receptor variants. The role of these variants, their relative expression levels as well as distribution in the human nervous system, have not been extensively explored yet. However, 6TM MOR is studied because of its peculiar properties on nociception. The 6TM isoform lacks residues in the N-terminal region corresponding to the extracellular tail and first transmembrane domain. Activation of the 6TM receptor induces excitatory cellular effects by activating Gs (Convertino et al., 2015, Oladosu et al., 2015), while 7TM activation inhibits neurons because of Gi activation. This was shown by the decrease in morphine-induced hyperalgesia obtained after silencing MOR-1K variant in mice (Oladosu et al., 2015). From these results, it was hypothesized that specific MOR isoforms could be involved in OIH while the classical 7TM receptor would be responsible for analgesia. Interestingly, a β2-adrenergic (b2-AR) antagonist reverses hyperalgesia induced either by chronic morphine or by a 6TM-MOR selective agonist in mice (Samoshkin et al., 2015). The authors suggest that the b2-AR antagonist disrupts the 6TM-MOR-b2-AR heterodimer signaling producing OIH. These new findings may be put in relation to previous genetic analyses that identified an haplotype block containing the b2-AR that was associated to OIH (Liang et al., 2006).

Functional interactions of MOR with other GPCRs

In case of tolerance to analgesia, a desensitization of MOR has been observed (Dang and Christie, 2012, Williams et al., 2013). The desensitization corresponds to molecular changes in receptor signaling following repeated exposure to agonists, resulting in a loss of effectiveness or a biased answer to agonist binding (Dang and Christie, 2012, Williams et al., 2013). Two types of desensitization can be distinguished, the homologous desensitization where a receptor type desensitizes itself (i.e. MOR desensitizes MOR), and the heterologous desensitization when other receptors desensitize MOR. Heterologous desensitization has been described between MOR and chemokine receptors above all (Melik Parsadaniantz et al., 2015). For instance, Heinisch and collaborators studied MOR–CxCr4 and MOR-Cx3Cr1 interactions in the periaqueductal gray
matter (PAG), and recorded heterologous desensitization of these receptors by using electrophysiology at the single cell level (Heinisch et al., 2011). Neurons treated with the respective CxCr4 or Cx3Cr1 agonists CxCL12 or Cx3CL1 displayed decreased morphine-induced electrophysiological activity in vitro. In addition, intraspinal CXCL12 administration diminished morphine analgesia, while a CXCR4 antagonist potentiated morphine analgesia, revealing a role for a MOR–CxCr4 crosstalk in the spinal cord in vivo (Rivat et al., 2014). From a molecular point of view, chemokine receptors activate protein kinase C (PKC), which phosphorylates the intracytoplasmic tail of MOR (Williams et al., 2013). Different mechanisms occur for homologous and heterologous MOR phosphorylation, on different serine or threonine residues, that have been reviewed recently (Mann et al., 2015). MOR phosphorylation uncouples MOR from Gi. Then, G protein receptor kinase (GRK) and arrestin are recruited to MOR, leading to MOR internalization and therefore to analgesic tolerance. Chemokine receptor activation can also lead to activation of the ERK pathway that decreases nociceptive thresholds and hence induces hyperalgesia (Melik Parsadanianzt et al., 2015).

MOR heterodimers and OIH
As a member of the GPCR family, MOR can interact with other GPCRs and form heterodimers. High hopes are awarded to the comprehension of opioids heterodimers function. Delta-opioid receptors (DOR) may be involved in OIH, and contrasting results have been obtained on the role of DORs in analgesic tolerance. Indeed, pretreatment with DOR antagonists reverses analgesic tolerance (Beaudry et al., 2015), while tolerance was lost or maintained in DOR knockout mice (Zhu et al., 1999, Scherrer et al., 2009). Heterodimers of MOR and DOR receptors have been characterized, and novel ligands have been developed with mu-agonist and delta-antagonist properties that produce longer analgesia (Harland et al., 2015) or analgesia without development of tolerance (Mosberg et al., 2014). The interaction between MOR and DOR remains a field of investigation and the novel pharmacology of MOR-DOR heterodimers has been reviewed recently (Ong and Cahill, 2014, Fujita et al., 2015). Also, a synergy between MOR and α2-adrenoreceptors was evidenced for analgesia and analgesic tolerance (Milne et al., 2008, Chabot-Dore et al., 2015). MOR heterodimers with other GPCRs have been mainly described in cellular assays. However, some of them have been evidenced ex vivo by either physical association or co-localization in neurons, as recently reviewed (Massotte, 2015). These findings are of great interest because they open a new field of development for analgesic drugs with potency comparable to MOR agonists, but devoid of tolerance and OIH induced by classical opiates. However, the influence of mu heterodimers on OIH and the capacity of these new ligands for OIH attenuation remain mostly unknown.
G protein activation by opiates

Another mechanism underlying OIH consists in a potential shift of MOR coupling from Gi to Gs. Analgesic properties of MOR are initiated by Gi activation in neurons, resulting in neuron hyperpolarization and inhibition of nociception. This classical MOR Gi-mediated pathway seems to be counteracted in OIH by other signaling pathways. Under spinal nerve ligation (SNL)-induced neuropathic condition, MOR coupling to Gs increases in the spinal cord, repeated oxycodone augments SNL-induced MOR-Gs coupling and ultra-low dose naltrexone attenuates this Gs coupling, as well as oxycodone-evoked OIH (Largent-Milnes et al., 2008). The role of Gs has also been evidenced by the use of the Gi protein signalling-disrupter Pertussis toxin (PTX). PTX treatment induces ADP-ribosylation of Gi proteins and therefore prevents their interaction with GPCRs, leading to increased coupling of MOR to Gs protein. Hyperalgesia induced by acute morphine is correlated with an increase of spinal MOR coupling to Gs, and is reversed by ultra-low dose of naloxone (Tsai et al., 2009). In contrast, Gi was found involved rather than Gs in the periaqueductal gray of mice by using an OIH paradigm induced by ultra-low dose of morphine (Bianchi et al., 2009). Further, Gα1-3 and Gαo1 are necessary in this OIH paradigm, as shown by an antisense oligodeoxynucleotide approach (Bianchi et al., 2011). Altogether these data indicate that Gi and Gs involvement in OIH may depend upon the dose and chronicity of morphine, as well as on the nervous system site under study.

Pharmacological blockade of MOR

Because opiates are MOR agonists, and as analgesic tolerance and OIH occur following prolonged treatments with opiates, the prevention of OIH by opioid antagonists has been investigated. Two opioid antagonists are mainly used: naloxone and naltrexone. When either naloxone or naltrexone is administrated under non-pathological conditions, it has no effect on nociception (Celerier et al., 1999, Juni et al., 2006, Corder et al., 2013, Sanna et al., 2014, 2015a). When they are co-administrated with analgesic opiates, they impede analgesia. A pre-treatment with naloxone or with the selective MOR antagonist CTOP prevents low dose opiate OIH (Sanna et al., 2014, 2015a, Sanna et al., 2015b), thereby demonstrating MOR involvement in the development of this acute hyperalgesia. In the other way, naltrexone does not prevent and even aggravates OIH when using 1.6 or 10mg/kg morphine pellets (Juni et al., 2006). Also, naltrexone induces hyperalgesia when given before a morphine analgesic dose (Swartjes et al., 2012). These studies used different protocols for inducing hyperalgesia and for measuring nociception as well as naltrexone vs naloxone (Table 1) that may explain the divergent results.

Table 1: Effect of opioid receptor antagonists on OIH
<table>
<thead>
<tr>
<th>Kind of treatment</th>
<th>Dose</th>
<th>Administration</th>
<th>Effect</th>
<th>Opioid antagonist</th>
<th>Nociception test</th>
<th>Result on OIH</th>
<th>Reference</th>
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<tbody>
<tr>
<td>morphine</td>
<td>1µg/kg</td>
<td>Subcutaneous injection</td>
<td>Acute</td>
<td>CTOP, 0.001µg</td>
<td>Latency before paw licking on hot plate 52.5°C</td>
<td>Prevents OIH</td>
<td>(Sanna et al., 2014, 2015a, Sanna et al., 2015b)</td>
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<td>ultra low dose</td>
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<td>Naloxone, 1mg/kg</td>
<td>Latency before paw licking on hot plate 52.5°C</td>
<td>Prevents OIH</td>
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<td>Morphine</td>
<td>1.6mg/kg</td>
<td>Osmotic pump</td>
<td>Chronic</td>
<td>Naltrexone, 30mg</td>
<td>Latency before tail withdrawal in tail immersion 47.3°C</td>
<td>Provokes</td>
<td>(Juni et al., 2006, Juni et al., 2008, Juni et al., 2010)</td>
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<td>low dose</td>
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<td>24h before</td>
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MOR has been shown mandatory for morphine analgesia (Gaveriaux-Ruff, 2013, Pasternak and Pan, 2013) but its role in OIH is still unclear as shown above with the contrasting effects of opioid antagonists on OIH. In addition, some studies show no involvement of opioid receptors in OIH. For example, naloxone had no effect on hyperalgesia induced by remifentanyl infusion in healthy human volunteers (Chu et al., 2011). Toll like receptor-4 (TLR4) has been proposed as a mediator of OIH (Hutchinson et al., 2010, Hutchinson et al., 2011, Grace et al., 2015). Therefore, the findings on naltrexone suggest that the opioid antagonists could reverse morphine hyperalgesia by antagonizing TLR4. The neuroinflammatory aspects of OIH including studies on TLR4 are summarized in part 4 of this review.

**Other factors influencing OIH**

OIH is a phenomenon observed in humans and modeled in experimental animals. In both clinical and preclinical studies, pain perception depends on the gender (Hampton et al., 2015), and on genetic profiles (Denk et al., 2014). As summarized in the following paragraphs, OIH is also under the influence of gender and genetic background, and depends of the dose of opioid used.

**Gender**
Most of the teams working on pain or OIH have used males rather than females (Mogil, 2012). However, a few teams have related different profiles of OIH between males and females, although a sex-dependent factor for OIH is not clearly established yet. In their paper, Holtman et al., (Holtman and Wala, 2005, 2007) have found increased morphine induced-hyperalgesia in females as compared to males, in agreement with higher analgesic tolerance in females (Hopkins et al., 2004). In comparison, no sex difference was observed for OIH in C57BL/6J mice under morphine infusion (40mg/kg/d or 1.6mg/kg/d; (Juni et al., 2010). But interestingly, in a previous paper, the same authors noticed a stronger hyperalgesia in CD-1 females as compared to males, for morphine infusion at 1.6mg/kg/d but not at 40mg/kg/d (Juni et al., 2008). These results suggest a potential interaction for the influences of gender, genetic background and opioid dose (Bodnar and Kest, 2010).

Genetic background

The influence of the genetic background on OIH has been clearly demonstrated in rodent models. Liang and colleagues have compared 23 strains of inbred mice in a chronic morphine protocol (40mg/kg/d for 4 days), and have found strain differences for OIH (Liang et al., 2006, Liang et al., 2014b). As an example, C57BL/6J mice display a great hyperalgesia whereas 129/S mice present no or a weak OIH under a same morphine protocol (Liang et al., 2006, Liang et al., 2014b, Oladosu et al., 2015). An influence of the strain in the development of OIH has also been observed in rats (Laboureyras et al., 2014).

Opiate Regimen

It has been shown that the dose of opiate administered impacts both analgesia and hyperalgesia. In this context, a morphine dose under 1mg/kg can be considered as a low dose in rodents. Low as well as ultra-low doses induce acute hyperalgesia (Bianchi et al., 2011, Milne et al., 2013, Alizadeh et al., 2014, Sanna et al., 2015a, Sanna et al., 2015b) while a treatment over 1mg/kg is rather analgesic (Sanna et al., 2014). The same applies to buprenorphine for which doses over 20 µg/kg elicits analgesia in rats, whereas a dose of 0.1 µg/kg induces hyperalgesia (Wala and Holtman, 2011). Similarly to OIH elicited by chronic high opioid doses, hyperalgesia induced by ultra-low dose of opioids could also be affected by the gender (Holtman and Wala, 2005, 2007). Interestingly, repeated treatment with low doses of morphine produces tolerance to hyperalgesia (Holtman and Wala, 2005, Wala et al., 2011, Milne et al., 2013) while a chronic treatment with analgesic doses of morphine is classically known to cause hyperalgesia (Lee et al., 2011). It is noteworthy that low-dose of opiates also led to OIH and higher post-operative morphine consumption in chronic pain patients with osteoarthritis scheduled for orthopedic surgery (Hina et al., 2015).
Classically, in rodent models, opioid systemic administration is performed through intraperitoneal, subcutaneous and intramuscular injections (Jin et al., 2015). Intravenous administrations are mainly used to obtain fast answers. Moreover, the role of precise central areas can be explored by injections into the area themselves. Opiates produce analgesia, analgesic tolerance and OIH via the different routes, intraperitoneal (Tumati et al., 2012), subcutaneous (Bianchi et al., 2011), intrathecal (Milne et al., 2013) and many other papers), and intradermally (Araldi et al., 2015).

MOR is known to be present in the central and peripheral nervous systems (CNS, PNS). The analgesic effects of MOR activation on either CNS or PNS have been explored, and the analysis of mice with a conditional MOR deletion in peripheral Nav1.8+ sensory neurons has revealed the involvement of these receptors in opiate-induced analgesia (Weibel et al., 2013, Stein, 2016). In the future, the investigation of particular opioid receptor populations in OIH may be determined by using genetic approaches and/or injection of opioids in specific areas.

Opiate metabolites
The different morphine metabolites display differential properties. The main metabolites obtained following in vivo morphine administration are morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). In human, morphine is converted into 44-55% of M3G and 9-10% of M6G (Andersen et al., 2003, Frolich et al., 2011), and numerous metabolites have been identified in blood of patients treated with morphine (Laux-Biehlmann et al., 2013). M6G is estimated to be 2-5 times more analgesic than morphine (Coller et al., 2009, Lotsch, 2009), whereas M3G is considered as hyperalgesic and devoid of any analgesic property (Komatsu et al., 2009, Due et al., 2012, Swartjes et al., 2012). Codeine can also produce hyperalgesia, and it is known that about 10% of this opiate is metabolized into morphine (Johnson et al., 2014). Interestingly, patients have differential reactions to morphine related to their genetic polymorphism (Lotsch and Geisslinger, 2010). For example, patients A118G homozygotes show less analgesia to morphine (De Gregori et al., 2012). Altogether, these findings suggest that a case-by-case analysis and understanding of OIH in patients may allow solving this major health problem.

MECHANISMS AND TARGETS DESCRIBED FOR OIH AT THE NEURONAL LEVEL

At the neuronal level, several possible mechanisms for opioid-induced hyperalgesia have been studied including (i) sensitization of primary afferent neurons (ii) sensitization of second order neurons to excitatory neurotransmitters and (iii) adaptation of pain descending control leading to up-regulation of nociceptive neuromodulators and enhanced release of glutamate by primary afferents (See Chu et al., 2008). In the last few years, several novel neuronal targets have been
involved in these phenomena. This section will particularly focus on the most recent studies describing neuronal mechanisms and targets associated with development of OIH. They are summarized in figure 2.

**Long-term potentiation**

Long-term potentiation (LTP) is a sensitization of homosynapses leading to an enhanced strength of the synapse and its signal transduction. This will lead to a hypersensitivity of the system and so possibly to hyperalgesia in models of persistent pain. LTP has been shown to occur at synapses between C fibers and neurons from the superficial layers of the spinal cord dorsal horn (Liu and Sandkuhler, 1997), being a prime site of the nociceptive processing. This phenomenon can also occur at the level of heterosynapses inducing a spread sensitization to the neighbouring synapses leading to secondary hyperalgesia (Scanziani et al., 1996). The link between LTP and hyperalgesia induced by persistent pain has been revealed by drugs able to block LTP that can also prevent hyperalgesia, such as ketamine (Klein et al., 2007, Zhou et al., 2010) or minocycline (Zhong et al., 2010). More recently, LTP has also been shown to be involved in OIH. Indeed, the withdrawal following remifentanl infusion has been shown to lead to a potentiation of the synapse between nociceptive C fibers and neurons in superficial dorsal horn of the spinal cord (Drdla et al., 2009). In another study, the withdrawal from the three opiates morphine, fentanyl and remifentanl induced an increase in C fibers field potential (Drdla-Schutting et al., 2012). Other arguments are in favour of a role of LTP in OIH: the three opioid receptors are present on primary afferents C fibers and in neurons of the superficial spinal dorsal horn (Besse et al., 1990) and LTP and OIH share common signalling pathways. For example, the neurotrophin BDNF that is released by glial cells and play a role in the development of OIH (Ferrini et al., 2013) has been shown to be involved in the maintenance of LTP (Zhou et al., 2010). Moreover, secondary messengers such as CaMKII, PKC, PKA and PLC are part of both OIH and LTP hypersensitivity signalling, and their inhibition can prevent LTP (Chen and Huang, 1991, Yang et al., 2004, Drdla et al., 2009). Also of major importance, LTP and OIH require NMDA receptors (Mayer et al., 1999, Sandkuhler, 2009) and can be prevented by NMDA receptor antagonists including ketamine (Benrath et al., 2005, Haugan et al., 2008), and MK801 (Frankiewicz et al., 1996, Li et al., 2001). Finally, glial cells and cytokines are mediators of LTP and OIH. In the spinal cord, IL-1β and TNF-α can enhance the frequency and/or amplitude of spontaneous EPSCs leading to LTP (Zhang et al., 2011). Cytokines have differential effects in naïve and neuropathic animals with respectively either no effect or the induction of LTP (Zhong et al., 2010). IL-1β and TNF-α will act mostly through the activation of glial cells (although some neurons also express their receptors) to induce the release of other mediators that will trigger LTP and hyperalgesia (Gruber-Schoffnegger et al., 2013).
Taken together, these data argue in favor of a link between LTP and OIH, LTP being a mechanism participating to the setup and maintenance of long lasting OIH.

**Channels**

NMDA – glutamatergic system

OIH share common mechanisms with chronic pain, and one of them is the involvement of the glutamatergic system and particularly of the NMDA glutamate receptors. NMDA receptors are located presynaptically on the central terminals of primary afferent neurons as well as postsynaptically on spinal dorsal horn neurons (Antal et al., 2008). Several studies show that the administration of a NMDA receptor antagonist diminishes or prevents OIH (Larcher et al., 1998, Celerier et al., 2001, Li et al., 2001). With electrophysiology experiments, it has been demonstrated that a chronic opioid treatment increases presynaptic NMDA receptor activity in spinal dorsal horn as well as decreases the postsynaptic NMDA receptor activity (Zhao et al., 2012b). The hypothesis of Zhao and colleagues is that chronic opioid administration can induce a translocation of PKC to the plasma membrane leading to the activation of presynaptic NMDA receptor by the removal of the Mg2+ blocker from the receptor. This results in promoting NMDA receptor trafficking to the plasma membrane to increase its activity, ending up with a higher release of glutamate (Zhao et al., 2012b). Correlated with the observation of a decrease of the glutamate transporters GLAST and GLT1 after a sustained morphine administration (Mao et al., 2002), this will induce an enhancement of glutamate level in the synaptic cleft, and hence an overstimulation of the synapse that could lead to hyperalgesia.

A new study from Gong and collaborators underlines the importance of the peripheral nervous system in OIH and more precisely of the DRG through the upregulation of the glutamate transporter EAAT3 and NMDA receptor subunit GluN2B (Gong et al., 2016). They found that sustained morphine administration increases the excitability of small diameter DRG neurons but not of large neurons. Mu-opioid receptors are expressed by small diameter neurons of the DRG (Scherrer et al., 2009, Wang et al., 2010) and when activated, can lead to an increase in intracellular calcium signalling and release of excitatory amino acids, resulting in an overexcitability of the fibers (Gong et al., 2016).

**KCC2 and Cl\(^{-}\) homeostasis**

Disruption of Cl\(^{-}\) homeostasis at the level of the dorsal horn spinal cord has been shown to be critical for the development of hyperalgesia in different persistent pain syndromes including inflammatory and neuropathic pain (Zeilhofer et al., 2012). Trans-synaptic reduction in the expression of the potassium chloride exporter KCC2 is considered a key mechanism that leads to a
shift in neuronal anion gradient associated with the development of hyperalgesia in neuropathic pain (Coull et al., 2003, Coull et al., 2005). Moreover, modulation of KCC2 expression results from the release of brain-derived neurotrophic factor (BDNF) by activated microglia and further TrkB receptor activation in lamina 1 neurons (Coull et al., 2005). This mechanism has recently been shown to underlie hyperalgesia induced by opiates but not analgesic tolerance (Ferrini et al., 2013), see below for details.

TRPV1

The capsaicin receptor TRPV1 is a non selective cation channel activated by heat and chemical ligands that plays a role in transduction of noxious chemical and thermal stimuli (Caterina et al., 2000). This channel is present at both central and peripheral terminals of primary sensory neurons (for review see Julius, 2013). Several pieces of evidence indicate that TRPV1 is involved in the development of OIH. Chronic use of opiates can repeatedly stimulate TRPV1-expressing primary afferents and increase the nociceptive input leading to the sensitization of spinal dorsal horn neurons and hyperalgesia (Zhou et al., 2010). Different TRPV1 antagonists (e.g. AMG0347, SB366791) are able to suppress tolerance and OIH (Chen et al., 2008, Vardanyan et al., 2009, Zhou et al., 2010), TRPV1 KO mice did not develop thermal and tactile hypersensitivity after chronic morphine administration (Vardanyan et al., 2009) and TRPV1 mRNA is increased in DRG and spinal cord of rats after chronic morphine treatment. Moreover, the TRPV1 channel can be found in association with β-arrestin2, which permits its regulation through its desensitization and internalization (Por et al., 2012). Recently, MOR agonists have been shown to sequester β-arrestin2 to MOR therefore attenuating the TRPV1/β-arrestin2 interaction to amplify TRPV1 activity in peripheral sensory neurons and contribute to symptoms of OIH (Rowan et al., 2014b). The same mechanism was observed with DOR and the DOR-selective agonist SNC80 resulting in sensitization of TRPV1 and behavioural signs of OIH (Rowan et al., 2014a).

TRPM8

The menthol receptor TRPM8 is another member of the transient receptor potential channel (TRP) superfamily that has been shown to be responsible for mild cold sensation in mammals (Bautista et al., 2007). Like TRPV1, TRPM8 is expressed in primary nociceptors but in a distinct neuronal population in naïve animals, indicating that the detection of hot and cold noxious stimuli may be operated by different cells (Basbaum et al., 2009). In a recent study, sustained stimulation of MOR has been shown to suppress TRPM8 activity in DRG neurons by promoting its internalization (Shapovalov et al., 2013). This phenomenon has been proposed to form the basis of cold analgesia induced by opiates. Moreover, both morphine cold analgesia and hyperalgesia were suppressed in
TRPM8 deficient mice suggesting that functional interaction between mu-opioid receptor and TRPM8 are critical for both phenomena.

5HT3 and descending pain facilitation

Descending serotoninergic neurons from the rostral ventromedial medulla have clearly been involved in the facilitation of nociceptive signaling in different models of persistent pain (Donovan-Rodriguez et al., 2006, Svensson et al., 2006, Dogrul et al., 2009) as well as in the development of hyperalgesia and analgesic tolerance induced by opiates (Vanderah et al., 2001). The 5HT-3 receptor is the only ligand-gated cation channel with excitatory function in the 5-HT receptor family. It is expressed both in spinal dorsal horn and primary afferent neurons and has been identified as an important player in the modulation of pain hypersensitivity by descending serotoninergic neurons (Lopez-Garcia, 2006). Recently, pharmacological blockade of 5-HT3 receptor with ondansetron by systemic or intrathecal administrations was shown to significantly prevent and reverse OIH and tolerance indicating that adaptions to chronic opiate treatments are also controlled by descending serotoninergic processsing from the rostral ventromedial medulla (Vera-Portocarrero et al., 2007, Bannister et al., 2011, Liang et al., 2011). This hypothesis was further supported by experiments showing that, ablating NK-1 receptor expressing cells from lamina I of the dorsal horn completely blocks the development of hyperalgesia induced by chronic morphine administration. Indeed these cells have been proposed to act as a critical component in an ascending pathway, which result in activation of descending facilitation from the brainstem (Suzuki et al., 2002). Very recently, Guo and colleagues have shown that selective activation of neuronal 5-HT3 receptor leads to hypereactivity of microglia and astrocytes, which result in spinal sensitization and pain hypersensitivity (Guo et al., 2014).

EphrinB receptors

Ephrin receptors form the largest known subfamily of receptor tyrosine kinases. They are activated by membrane bound ligands called ephrins. EphrinB receptors have largely been shown to regulate the development of glutamatergic synapses and their plasticity in adult nervous system by interaction with NMDA receptors (Dalva et al., 2000, Henderson et al., 2001, Takasu et al., 2002). More recently, EphrinB receptor blockers have been shown to inhibit the induction and maintenance of hyperalgesia and allodynia induced by nerve injury as well as hyperexcitability of nociceptive small DRG neurons, sensitization of dorsal horn neurons and LTP of synapses between primary nociceptors (C fibers) and spinal neurons. A recent study further extends these observations by showing that an antagonist of ephrinB receptor inhibits remifentanyl-induced hyperalgesia in rats (Xia et al., 2014). Moreover, similarly to what was observed in a model of nerve-injury,
ephrinB1 and its receptor were found up-regulated in spinal cord dorsal horn of rats treated with remifentanil.

**mTOR**
mTOR is a Serine/Threonine kinase forming two different complexes: mTORC1 with the protein raptor, is sensitive to the antibiotic rapamycin and is involved in protein translation through the regulation of phosphorylation/activation of downstream effectors such as p70S6K and 4E-BP1 (for review see Hay and Sonenberg, 2004, Lutz et al., 2015). mTORC2, with the protein rictor, is rapamycin insensitive and can regulate the phosphorylation of PKB/Akt and PKC proteins (Hay and Sonenberg, 2004). mTOR, p70S6K and 4E-BP1 mRNAs and proteins are present in DRG and spinal dorsal horn neurons but not in astrocytes or microglia (Xu et al., 2010). A recent study from Xu and colleagues focused on morphine-induced hyperalgesia and demonstrated that MOR activation induces the phosphorylation and thus activation of mTOR and its downstream effectors (Xu et al., 2014). Moreover, MOR, mTOR, PI3K/Akt and NMDA receptor subunit NR1 were shown to colocalize in neurons of the spinal dorsal horn and pharmacological inhibition of mTOR action decreased morphine tolerance and hyperalgesia (Xu et al., 2014). Altogether, these data are in favour of a role of mTOR activation in the spinal cord in morphine-induced hyperalgesia mechanism through the increase of protein translation in the spinal dorsal horn (Xu et al., 2014, Lutz et al., 2015, Xu et al., 2015).

**Hyperalgesic priming**
Hyperalgesic priming is a mechanism that was first identified in an inflammatory pain model in which the short lasting hyperalgesia induced by carrageenan in rats was shown to induce a phenomenon of latent pain sensitization in primary nociceptors that resulted in a long lasting increased response to inflammatory mediators including prostaglandin E2, 5-hydroxytryptamine or adenosine A2 receptor agonist (Aley et al., 2000). Latent pain sensitization was further prevented by the administration of a PKC epsilon inhibitor. In a recent work, Araldi and collaborators showed that intradermal injection of DAMGO induces mechanical hyperalgesia as well as a marked prolongation of the hyperalgesia induced by further administration of prostaglandin E2 (Araldi et al., 2015). They further showed that these two phenomena are attenuated by a PKA inhibitor and by antisense oligonucleotides against PLC-β3. However, this effect was reversible, indicating that both PKA and PLC-β3 are involved in the expression, but not the induction or maintenance of DAMGO-induced hyperalgesia and hyperalgesic priming. Moreover, Goi protein does not seem to be involved, as pertussis toxin did not affect the hyperalgesia produced by DAMGO while the G-protein β/γ both DAMGO-induced
and hyperalgesic priming. Altogether, these data clearly highlight a critical role of primary nociceptors in the development of OIH and that repeated opioid exposure can also induced hyperalgesic priming like other nociceptive stimuli although with some differences in the mechanisms (Araldi et al., 2015).

NEUROINFLAMMATORY MECHANISMS IN OIH

Besides neuronal regulations, neuroinflammatory cells have been identified as key actors in these opioid-induced phenomena, and their role in OIH has been reviewed recently (Grace et al., 2015). These cells include oligodendrocytes, astrocytes, microglia, perivascular macrophages, endothelial cells and infiltrating immune cells (Grace et al., 2015, Thomas et al., 2015), although most studies were carried on astrocytes and microglia. The neuroimmune mechanisms in OIH appear to constitute one facet of the glial cell contribution to chronic pain. Since the initial discussion on the tetrapartite synapse for central pain sensitization (De Leo et al., 2006), several reviews on the role of neuroimmune activation in chronic pain have been published (Stein and Machelska, 2011, Calvo et al., 2012, von Hehn et al., 2012, Ji et al., 2013, Mika et al., 2013, Grace et al., 2014, Ji et al., 2014, Austin et al., 2015, Old et al., 2015, Ren and Dubner, 2015, Yaksh et al., 2015).

Neuroimmune mechanisms for OIH have been first demonstrated by using inhibitors of glial cell activity. These inhibitors include the glial cell blockers pentoxyfylline, propentofylone, fluorocitrate and minocycline, as well as proinflammatory cytokines antagonists (see Hutchinson et al., 2011). It is noteworthy that while many reports have described the importance of neuroinflammation in analgesic tolerance, only a dozen have focused on immune mechanisms for OIH. The different studies, which report that blockade of immune activation diminishes OIH are summarized in table 2. Most articles show that inhibition of inflammation attenuates hyperalgesia induced by repeated morphine doses. Interestingly, the only one paper in which animals where chronically treated with remifentanyl showed that minocycline did not prevent the development of OIH (Aguado et al., 2015). Differences in drugs (remifentanyl versus other opiates) and administration protocols (intravenous versus other route of administration) may contribute to this divergent result. In any case, this raises the notion that both opioid type and administration schedule may have a significant impact on analgesic tolerance and OIH.

It is important to note that among papers describing a link between neuroinflammation and OIH, most of them used male rats or mice, and only two have involved female rats (Wilson et al., 2011, Due et al., 2012). However, gender has recently been reported to have a major influence on the immune mechanisms mediating pain (Sorge et al., 2015, Mapplebeck et al., 2016). In male mice, peripheral nerve injury induces an increase in P2X4 receptor expression and BDNF release by
spinal microglia that will activate TrkB receptor on neurons, leading to neuronal disinhibition and hyperalgesia. In contrast, female mice microglia do not show these responses, and the suppression of microglia activation reversed hypersensitivity in males but not in females. It is suggested that, in females, peripheral nerve injury rather elicits T lymphocytes activation resulting in hyperalgesia (Sorge et al., 2015, Mapplebeck et al., 2016). Similarly, in male rats the microglial inhibitor minocycline increases morphine analgesia and lowers morphine-induced glial activation whereas it has no effect on morphine analgesia in female rats and tends to increase some glia activation markers (Posillico et al., 2015). As microglia appears to be more involved in pain and OIH in males as compared to females, upcoming studies focusing on neuroimmune mechanisms involved in the development of OIH should therefore consider analyzing both males and females. Moreover, species, strain and opioid category will also have to be regarded in studies on neuroimmune mechanisms for OIH (see Table 2). Finally, as mentioned above for females, peripheral immune cells may also regulate OIH mechanisms. Indeed, in damaged tissues they release pronociceptive mediators and trigger analgesia by releasing endogenous opioids, therefore playing a dual role in pain control (Machelska, 2011, Sacerdote et al., 2012, Ninkovic and Roy, 2013, Basso et al., 2014)

Astrocytes and microglia contribute to OIH

At first, several papers showed that morphine exposure triggers astrocyte activation. Acute morphine exposure leads to GFAP, IL-1β and matrix metalloprotease-9 upregulation in DRG satellite glial cells, as shown by co-staining with GFAP. Furthermore, 5-7 day of chronic morphine is required for spinal astrocyte activation and IL-1β upregulation in astrocytes (Berta et al., 2012, Berta et al., 2013). On the other hand, LPS-induced Ca2+ response in astrocytes is attenuated by the mu-agonist endomorphin in vitro (Block et al., 2013). A specific action on neurons and astrocytes vs microglia was shown following intrathecal chronic morphine in rats, as reflected by augmented levels of NF-kB-phospho-p-65 in both spinal neurons and astrocytes but not microglia (Bai et al., 2014). In this study, the TLR4 antagonist LPS-RS prevents p-65 phosphorylation as well as tolerance and OIH. A selective activation of astrocytes was also found in response to an ultra low morphine dose inducing OIH (Sanna et al., 2015a). Morphine at 1 µg/kg produces hyperalgesia and the activation of spinal astrocytes but not microglia. Astrocytic levels of p-JNK are increased by morphine while JNK and NMDA antagonists prevented the development of OIH. Similarly, buprenorphine at ultralow dose (0.1 µg/kg) produces hyperalgesia and activates spinal astrocytes while microglia did not show enhanced reactivity (Gerhold et al., 2015). Moreover, spinal administration of 5-HT2 receptors antagonists prevents descending facilitating pathways,
Table 2: Neuroimmune inhibitors decrease OIH

<table>
<thead>
<tr>
<th>Drug name and dose</th>
<th>Chronic opioid name and dose</th>
<th>Species Gender</th>
<th>OIH</th>
<th>Tolerance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1ra 100 µg i.t.</td>
<td>Morphine 10 µg i.t.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Johnston et al., 2004)</td>
</tr>
<tr>
<td>Anti-Cx3cr1 Antibody 10 µg i.t.</td>
<td>Morphine 10 µg i.t.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Johnston et al., 2004)</td>
</tr>
<tr>
<td>IL-10 by adenovirus 5 µg i.t.</td>
<td>Morphine 10 µg i.t.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Johnston et al., 2004)</td>
</tr>
<tr>
<td>AMD3100 Cxcr4 antagonist 10 mg/kg</td>
<td>Morphine 10 mg/kg</td>
<td>Rats Females</td>
<td>Reduced</td>
<td>Not tested</td>
<td>(Wilson et al., 2011)</td>
</tr>
<tr>
<td>Minocycline 30-100 mg/kg i.p.</td>
<td>Remifentanil 240 µg /kg/h i.v.</td>
<td>Rats Males</td>
<td>No effect</td>
<td>No effect</td>
<td>(Aguado et al., 2015)</td>
</tr>
<tr>
<td>Mac-1 Ab-saporin 20-36 µg i.t.</td>
<td>Morphine 10 mg/kg s.c.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>No effect</td>
<td>(Ferrini et al., 2013)</td>
</tr>
<tr>
<td>Bdnf-cKO in microglia</td>
<td>Morphine 10-40 mg/kg s.c.</td>
<td>Mice Males</td>
<td>Reduced</td>
<td>No effect</td>
<td>(Ferrini et al., 2013)</td>
</tr>
<tr>
<td>IL-1ra/sTNFR/ anti-IL6 Ab 100 µg /30 µg / 0.08 µg i.t.</td>
<td>Morphine 10 mg/kg s.c.</td>
<td>Rats Males*</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Raghavendra et al., 2002)</td>
</tr>
<tr>
<td>Propentofyline 1-10 µg i.t.</td>
<td>Morphine 10 mg/kg s.c.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Raghavendra et al., 2004)</td>
</tr>
<tr>
<td>Pentoxifylline 50 mg/kg i.p.</td>
<td>Morphine 10-40 mg/kg s.c.</td>
<td>Mice Males#</td>
<td>Reduced</td>
<td>Not tested</td>
<td>(Liang et al., 2008)</td>
</tr>
<tr>
<td>IL-1ra 100 mg/kg i.p.</td>
<td>Morphine 20 mg/kg Codeine 21 mg/kg</td>
<td>Mice Males</td>
<td>Reduced</td>
<td>Not tested</td>
<td>(Johnson et al., 2014)</td>
</tr>
<tr>
<td>LPS-RS 20 µg i.t.</td>
<td>Morphine 10 µg i.t.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>Reduced</td>
<td>(Bai et al., 2014)</td>
</tr>
<tr>
<td>IL-1ra 100 µg i.t.</td>
<td>M3G 0.75 µg i.t.</td>
<td>Rats Males</td>
<td>Reduced</td>
<td>No analgesia</td>
<td>(Lewis et al., 2010)</td>
</tr>
<tr>
<td>Compound 15 Tlr4 inhibitor</td>
<td>M3G 10 mg/kg i.p.</td>
<td>Rats Females</td>
<td>Reduced No analgesia</td>
<td>(Due et al., 2012)</td>
<td></td>
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</tbody>
</table>

All studies were performed on naïve animals except in * Raghavendra et al. 2002 where rats underwent spinal nerve transection, and in # Liang et al. 2008 where OIH was followed by hind paw incision.
diminishes astrocytes activation and reverses buprenorphine-induced hyperalgesia. This indicates that ultralow-dose of buprenorphine induces OIH through a 5-HT mediated mechanism in astrocytes (Gerhold et al., 2015).

Secondly, microglia have been shown activated under chronic opioid OIH conditions. Chronic morphine and codeine treatments lead to augmented CD11b but not GFAP expression in trigeminal ganglions and spinal cord (Johnson et al., 2014). As well, microglia were shown in vitro to release proinflammatory cytokines and nitric oxide upon morphine or DAMGO stimulation (Merighi et al., 2013). Ferrini and colleagues have also identified a specific role of microglia in OIH (Ferrini et al., 2013). They showed that both microglia depletion by spinal anti-Mac-1-saporin treatment and the conditional inactivation of BDNF gene in microglia prevent chronic morphine OIH while sparing tolerance. They demonstrated that the microglia to neuron signaling cascade involves P2X4R upregulation in microglia that release BDNF (see below for detailed mechanisms). Interestingly, these findings have identified a pathway that contributes to OIH without affecting analgesic tolerance, dissociating the two phenomena (Ferrini et al., 2013). The reverse has been observed in patients with low back pain that had analgesic tolerance without OIH (Chu et al., 2012, Richebe et al., 2012). To conclude, these different studies indicate that a number of microglial actors and mechanisms participate in the development OIH.

Finally, concurrent activation of astrocytes and microglia has also been observed under chronic morphine conditions. In particular, chronic morphine injections in rats activate both spinal microglia and astrocytes, together with TNF-α release (Tumati et al., 2012). In neuropathic animals also, both spinal astrocytes and microglia are activated by chronic morphine. Furthermore, glial activation and morphine analgesia are reduced following a chronic co-treatment with botulinum toxin, suggesting a potential clinical application (Vacca et al., 2013). Coincident activation of microglia and astrocytes also occurred in the ventrolateral periaqueductal gray matter (vIPAG) following repeated morphine, suggesting an additional site for glia-mediated OIH in addition to spinal cord (Eidson and Murphy, 2013a). Also, persistent peripheral inflammation attenuates chronic morphine-induced activation of astrocytes and microglia in the vIPAG together with lowered analgesic tolerance, correlating the two phenomena (Eidson and Murphy, 2013b). Accordingly, similar results were found on the role of peripheral inflammation on analgesic tolerance (Zollner et al., 2008). Lin and co-workers published a recent method for quantifying the different parameters of microglia and astrocyte activation found in OIH (Lin et al., 2015b) that will help in future studies focused on glia activation in OIH. Interestingly, whereas morphine triggers OIH and astrocytes-microglia activation, some novel endomorphin derivatives induce less tolerance together with a lack of OIH or glia activation (Zadina et al., 2015). This indicates that mu opioid
agonists that possess good analgesic properties without glia-mediated adverse effects can be developed.

**Molecular actors of OIH in neuroinflammatory cells**

In the recent years, several novel glial targets have been associated with development of OIH. This section will focus on the studies describing these newly identified actors that are summarized in figure 3 for microglial cells and figure 4 for astrocytes.

**Toll like receptor 4 (TLR4)**

Among neuroimmune mechanisms, TLR4 activation has been shown to contribute to opioid-induced tolerance and hyperalgesia. Blockade of TLR4 activation potentiates and prolongs acute opioid analgesia (Hutchinson et al., 2010), suggesting a role in analgesic tolerance. Also, the TLR4 antagonist LPS-RS prevents OIH and tolerance as well as p-65 phosphorylation in the spinal cord (Bai et al., 2014). Furthermore, morphine and other opiates were reported to bind directly to - and activate TLR4 by molecular modeling and in vitro cellular assays (Hutchinson et al., 2010). In contrast, morphine, fentanyl, naltrexone and β-FNA lowered TLR4 activation in a similar functional cell assay (Stevens et al., 2013). The notion that morphine elicits analgesic tolerance and hyperalgesia through TLR4 activation raised much interest and debate (Skolnick et al., 2014, Watkins et al., 2014, Grace et al., 2015). To our knowledge until now, four studies have investigated TLR4 implication in OIH by genetic approaches, through in vivo experiments with TLR4 deficient mice (Due et al., 2012, Ferrini et al., 2013, Johnson et al., 2014, Mattioli et al., 2014). Main features from these papers are recapped in Table 3. Two of these studies found no OIH in TLR4 mutants while the two others reported that OIH was preserved. In addition, two papers found intact analgesic tolerance in TLR4 deficient mice (Fukagawa et al., 2013, Mattioli et al., 2014) while tolerance was not investigated in the other OIH studies. These conflicting findings on the implication or lack of implication of TLR4 in OIH may be explained by disparities in experimental conditions including mouse strains, sanitary status that may activate TLR4 to different extents, morphine regimen as well as OIH evaluation. Besides, TLR9 was found involved in in vitro microglia responses to morphine, in a MOR-dependent manner (He et al., 2011). Also, TLR2, TLR5, TLR8 and microRNA-124 expression was regulated by morphine in microglia culture experiments (Qiu et al., 2015). Future analyses will be required to understand more deeply the implication of TLRs in opioid-induced analgesic tolerance and OIH.

**Table 3 : Opioid Induced Hyperalgesia studies on TLR4 deficient mice**
<table>
<thead>
<tr>
<th>Mouse lines</th>
<th>Opiate treatments</th>
<th>Sensitivity assays</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 Ctrls B10ScNJ mutants</td>
<td>Morphine-3 glucuronide 25 mg/kg</td>
<td>Von Frey filaments Hot plate</td>
<td>OIH in Ctrls No OIH in mutants</td>
<td>(Due et al., 2012)</td>
</tr>
<tr>
<td>Balb/c Ctrls TLR4-KO mutants</td>
<td>Morphine 20mg/kg Codeine 21mg/kg 2x/day/4 days</td>
<td>Von Frey filaments</td>
<td>OIH in Ctrls No OIH in mutants</td>
<td>(Johnson et al., 2014)</td>
</tr>
<tr>
<td>C3H/HeOuJ Ctrl C3H/HeJ mutants B10ScSNJ Ctrls B10ScNJ mutants</td>
<td>Morphine 10-40mg/kg 2x/day/7 days</td>
<td>Von Frey filaments</td>
<td>OIH in Ctrls OIH in mutants</td>
<td>(Mattioli et al., 2014)</td>
</tr>
<tr>
<td>C3H/HeOuJ Ctrl C3H/HeJ mutants</td>
<td>Morphine 10-40mg/kg 2x/day/7 days</td>
<td>Von Frey filaments</td>
<td>OIH in Ctrls OIH in mutants</td>
<td>(Ferrini et al., 2013)</td>
</tr>
</tbody>
</table>

Cytokines, chemokines and chemokine receptors

In addition to the TLRs, several cytokines, chemokines and chemokine receptors were identified as important for OIH or tolerance (Melik Parsadaniantz et al., 2015). Neutralizing antibodies to MCP-1 or CX3CR1 administrated intrathecally reduce analgesic tolerance or OIH (Johnston et al., 2004, Zhao et al., 2012a). Activation of CXCR4 receptor by the chemokine CXCL12 counteracts acute morphine analgesia in naïve rats (Rivat et al., 2014). Along the same lines, chronic morphine upregulates SDF1/CXCL12 and CXCR4 levels whereas CXCR4 blockade reversed OIH (Wilson et al., 2011). More broadly, the SDF1/CXCL12 - CXCR4 system is considered as a general modulator of chronic pain (Luo et al., 2016). Interestingly, a study on cancer patients has reported increased CXCL1 levels in the cerebrospinal fluid of the opioid-tolerant group (Lin et al., 2015a). In the same paper, morphine analgesia in rats is decreased by CXCL1 infusion and improved by CXCL1-neutralizing antibody or CXCR2 antagonist administration (Lin et al., 2015a). These studies pointed to MCP-1/CCR2, CX3CL1/CX3CR1, SDF1/CXCR4 and CXCL1/CXCR2 as major chemokine systems involved in OIH. In addition, chemokines such as CCL3, CCL2, CCL5 and CXCL8 can alter the function of the mu-opioid receptor by desensitizing it through the activation of their
respective GPCR receptors (Zhang et al., 2004). On the cytokine side, the IL-1 receptor antagonist IL-1ra (Johnston et al., 2004, Johnson et al., 2014), a combination of IL-1, TNF-α and IL-6 blockers (Lewis et al., 2010) as well as the anti-inflammatory cytokine IL-10 (Johnston et al., 2004) prevent OIH (Table 2).

In connection with these results, TNF-α and IL-1β contribute to high frequency stimulation-induced hyperalgesia and synaptic LTP potentiation by activating their receptors on glial cells in the spinal cord (Gruber-Schoffnegger et al., 2013).

**ATP, P2X4 receptor and BDNF**

Following the initial demonstrations that morphine promotes microglia migration via MOR and P2X4 receptor dependent signalling (Takayama and Ueda, 2005, Horvath and DeLeo, 2009), inhibition of P2X4 receptors activity on microglia was shown to prevent morphine analgesic tolerance (Horvath et al., 2010). Recently, genetic tools in mice have been used to confirm those findings and show that OIH implicates two mechanisms, a MOR-dependent upregulation of P2X4 receptors on microglia, and a MOR-independent gating of BDNF release (Ferrini et al., 2013). Spinal BDNF activates TrkB on neurons and downregulates the potassium-chloride co-transporter KCC2, leading to the disinhibition of GABAergic neurons and hyperalgesia (see Grace et al., 2015, Trang et al., 2015) for reviews). Interestingly, BDNF signaling from microglia has been previously shown important in learning and memory (Parkhurst et al., 2013), indicating a common mechanism for memory and persistent pain.

**Tachykinin NK1 receptor**

In the spinal cord, the tachykinin NK1 receptor has been shown to be involved in glia activation during OIH. Morphine withdrawal-induced glial cell activation and OIH are attenuated by the tachykinin NK1 receptor antagonist L-732,138, indicating a role for the tachykinin-NK1 receptor system in OIH. In addition, an opioid agonist-NK1 antagonist bivalent compound did not activate spinal glial cells and prevented OIH. This indicates that substance P-triggered glia activation and OIH with sustained morphine can be prevented by targeting opioid and NK1 receptors concomitantly (Tumati et al., 2012).

**Sphingolipid ceramides**

In glial cells, the sphingolipid ceramide system has been described as an actor in OIH. The sphingosine-1-phosphate (S1P) and its GPCR are expressed in the spinal cord and DRG, structures well known to be involved in nociceptive processing. Also, there is an overlapping distribution of S1P receptor and mu-opioid receptor in different CNS regions like the striatum, amygdala or spinal cord (Salvemini et al., 2013). OIH is correlated to sphingolipid ceramide upregulation in rat spinal astrocytes and microglia under chronic morphine infusion (Muscoli et al., 2010). Also the
metabolite S1P, TNF-α, IL-1β and IL-6 was upregulated. Likewise, intrathecal administration of ceramide inhibitors prevented OIH, concurrently with normalized spinal inflammatory cytokines. This may refer to a previous study where the neuraminidase inhibitor oseltamivir was shown to block ganglioside-regulated morphine analgesic tolerance and low dose-morphine hyperalgesia in mice (Crain and Shen, 2004). In addition to OIH, the sphingolipid pathway is considered as a therapeutic target for chronic pain management (Salvemini et al., 2013). Another argument for the implication of the ceramide pathway in OIH is that the activation of TLR4 (see above) leads to an enhanced production of ceramides by glial cells, suggesting that this receptor could be involved in OIH development by triggering the ceramide pathway.

Superoxides and peroxinitrites
Spinal peroxynitrite has been involved in OIH. Indeed, the superoxide-sparing peroxynitrite decomposition catalyst SRI-110 prevents morphine-induced hyperalgesia and analgesic tolerance. SRI-110 lowers morphine-induced upregulation of TNF-α, IL-1β and IL-6 while it increases IL-4 and IL-10 anti-inflammatory cytokines (Little et al., 2013). Therefore, restoring spinal mitochondrial function by removal of superoxides and peroxynitrites may increase the levels of anti-inflammatory mediators and ensuing pain relief.

OTHER SYSTEMS INVOLVED IN OIH
Several molecular targets have been described to play a role in the development of OIH in the past decades. However, for most of these targets the mechanism of action remains poorly understood. This section aims to summarize the current knowledge on these targets.

Anti-opioid peptides
Several neuropeptides have been shown to display pro-nociceptive activity that opposes antinociceptive action of opiates. They have been proposed to be part of a homeostatic equilibrium in which exogenous administration of opiates triggers the release of these peptides that in turn counteract the analgesic action of opiates thus leading to the development of hyperalgesia and analgesic tolerance. The most studied peptides include cholecystokinin, neuropeptide FF (NPFF) and OFQ/Nociceptin as well as the opioid peptide dynorphin. The anti-opioid action of these peptides and their receptors has already been described in several reviews (Rothman, 1992, McNally, 1999, Ossipov et al., 2003, Mouledous et al., 2010, Toll et al., 2016).

CCK has long been proposed to act as an endogenous anti-opioid (Faris et al., 1983). Several studies have shown that morphine triggers the release of CCK in the spinal cord (de Araujo Lucas et al., 1998, Gustafsson et al., 2001) and CCK receptor antagonists have been shown to prevent the
development of antinociceptive tolerance to morphine (Dourish et al., 1990, Hoffmann and Wiesenfeld-Hallin, 1994). Moreover, pharmacological blockade of CCK2 receptors in the rostral ventral medulla (RVM) have been shown to block the development of hyperalgesia induced by continuous systemic morphine indicating that endogenous CCK activity in RVM may decrease spinal analgesic effect of opiates by activating descending pain facilitatory mechanism to exacerbate spinal nociceptive sensitivity (Xie et al., 2005).

Both neuropeptide FF and OFQ/nociceptin induce hyperalgesia and reverse morphine analgesia when administered intracerebroventrically, but display antinociceptive effect when administered intrathecally suggesting that these peptides could display both pro and antinociceptive properties (Simonin, 2006, Toll et al., 2016). However, systemic administration of NPFF receptor antagonist RF9 have been shown to completely prevent the development of hyperalgesia and analgesic tolerance induced by chronic opiates administration indicating that NPFF and its receptors represent a bona fide anti-opioid system (Simonin et al., 2006, Elhabazi et al., 2012). Although the consequence of systemic pharmacological blockade of OFQ/nociceptin receptor on the development OIH has not been investigated so far, systemic administration of a selective antagonist (J-113397) as well as the genetic blockade of NOP receptor or OFQ/nociceptin precursor genes produced a significant increase of mouse nociceptive behaviour in inflammatory pain models. These data indicate that for this system the spinal antinociceptive action prevails over supraspinal pronociceptive effects (Depner et al., 2003, Rizzi et al., 2006).

Kappa-opioid receptor and its endogenous ligand dynorphin have also been shown to counteract mu-opioid receptor action in different brain regions (Pan, 1998). However, systemic administration of kappa-opioid receptor selective agonists produce analgesic effect indicating that, similarly to OFQ/nociceptin system, the antinociceptive action of kappa-opioid receptor and dynorphin prevails over anti-analgesic actions of this system (Simonin et al., 1998). Recently, the kappa receptor system has been shown implicated in pain aversion (Cahill et al., 2014). Therefore, this system may also contribute to aversive mechanisms underlying hyperalgesia induced by chronic opiates.

**GPCRs and intracellular pathways**

In addition to the so-called anti-opioid peptides and their receptors several other GPCRs as well as intracellular targets have been involved in the development of OIH.

A genetic analysis in mice pointed to β2 adrenergic receptor as an important player in the development of OIH, which was further confirmed by pharmacological and genetic blockade of this receptor (Liang et al., 2006). This study concluded that genetic variants of β2 adrenergic receptor
gene could explain the differences observed between different strains of mice in the development of OIH.

Selective antagonist of melanocortin 4 receptor (HS104) has also been shown to prevent the development of analgesic tolerance induced by chronic morphine infusion in rats as well as hyperalgesia that was observed after drug withdrawal (Kalange et al., 2007). Moreover, pharmacological blockade of this receptor during the induction of morphine tolerance was further shown to reduce the activation of astrocytes as well as the expression of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and to upregulate the anti-inflammatory cytokine IL-10 in the rat spinal cord (Niu et al., 2012).

At the intracellular level, protein kinase Cγ was shown to play an important role in the development of OIH. Indeed, PKCγ deficient mice displayed an enhancement of fentanyl acute analgesia as well as a complete absence of secondary hyperalgesia (Celerier et al., 2004). This effect could be due to the fact that Ca2+-sensitive protein kinases C, particularly the γ isoform catalyzes the NMDA receptor phosphorylation upon stimulation of opioid receptors leading to sensitization to painful stimuli (Celerier et al., 2004). Moreover, thermal hypernociception induced by morphine in mice was further shown to be inhibited by the PLC inhibitor U73122 and the PKC blocker, calphostin C as well as by antisens phosphodiester oligonucleotides directed against PLCβ3 and PKCγ suggesting that PLCβ3/PKCγ/NMDA pathway is stimulated upon morphine administration and play an opposing role in morphine analgesia (Galeotti et al., 2006). More recently, hyperalgesia induced by ultra-low dose of morphine was shown to be dependent on ERK1/2-c-JUN signalling pathway at different levels of the central nervous system including the spinal cord (Sanna et al., 2014, 2015a, Sanna et al., 2015b). In this latter region JNK was shown to be activated specifically in astrocytes suggesting that activation of neuronal MOR could lead to the activation of ERK1/2-c-JUN pathway in astrocytes, thus contributing to hyperalgesia induced by ultra-low doses of morphine.

**EPIGENETIC AS A NOVEL MECHANISM FOR OIH**

Epigenetic adaptations reflect the impact of the environment on DNA leading to a modulation of gene transcription. Two main mechanisms are involved: the cytosine methylation of the DNA leading to a repression of gene transcription and the histone acetylation, increasing the access for gene transcription. Histone deacetylases (HDAC) inhibitors are able to diminish pain sensitivity, indicating that epigenetic mechanisms are involved in chronic pain (Denk et al., 2014, Descalzi et al., 2015).
The first evidence for a link between OIH and epigenetic is that opiates users have an increase of methylation on the MOR promoter gene that will influence its transcription (Doehring et al., 2011), suggesting that opiates may inhibit MOR gene demethylation (Doehring et al., 2013). Another study showed that modifications of the degree of histone acetylation induced by morphine in the spinal cord participate in the regulation of morphine tolerance, dependence and OIH (Liang et al., 2013). Indeed, the concomitant treatment with chronic morphine and the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) enhances OIH in naïve mice (Liang et al., 2014a) and induce changes in BDNF and prodynorphin gene histones acetylation that will modify their expression (Liang et al., 2013, Liang et al., 2014a). On the another hand, the HDAC inhibitors trichostatin A and valproic acid have been shown to reverse neuropathy-induced decrease in morphine analgesia in mice (Uchida et al., 2015). Neuropathic damage decreases MOR expression in DRG, and the HDAC inhibitors restore morphine analgesia by upregulating MOR mRNA expression to non-injury levels. Both studies were performed on young adult C57BL/6 male mice, but Liang and collaborators used an escalating morphine treatment of pain-naïve mice whereas the study by Uchida and colleagues was performed with nerve-injured mice acutely treated with morphine. BDNF is also found upregulated following a repeated morphine treatment inducing OIH, with a downregulation of BDNF exon IV methylation level. In addition, the DNA methylation inhibitor 5-aza-2’-deoxycytidine upregulates BDNF expression and augments OIH, implicating an epigenetic control of BDNF in OIH (Chao et al., 2016). Altogether this shows a contribution of epigenetics in OIH that will be influenced by the animal pain state and opioid regimen.

Finally, epigenetic could represent a new track to create personalized treatments in order to limit the development of opiates side effects including OIH. Profiles of DNA methylation or acetylation are specific from an individual and can be obtained with a genetic diagnosis. Hence, knowing these personal characteristics could help to treat patients individually e.g. with HDAC inhibitors in addition to other drugs. However, epigenetic modifications are not specific from genes involved in pain pathways and OIH, therefore side effects could be also induced (for review, see Doehring et al., 2011).

**CONCLUSION**

In this review, we present the numerous mechanisms and actors that have been recently identified in OIH. Interestingly, some of them are similar to those involved in chronic pain. In neuropathic or inflammatory pain models, a tetrasynapse composed of the central terminal of primary afferent neurons, projection neurons, astrocytes and microglia has been demonstrated in the spinal cord dorsal horn (Grace et al., 2014). In non-pathological conditions, the glutamatergic signaling is sought to transmit nociceptive message to supraspinal areas. This phenomenon is characterized by a
short duration because of compensations and regulations by environmental cells. GABAergic inhibitory neurons and endogenous opioid systems are thus activated and decrease neuroexcitatory responses and thereby alleviate pain. Additionally, glutamate transporters present on neurons or astrocytes (EEAC or GLT-1 respectively), recapture glutamate in the synapse cleft and so contribute to the decrease of neuroexcitation induced by the stimulus, and participate to pain relief.

Both chronic pain and OIH show a disruption of these regulatory processes. In case of OIH, the recapture of glutamate by neurons or astrocytes is reduced, increasing synaptic concentrations of glutamate and thus neuroexcitation by elevating intracellular Ca2+ concentrations. Other actors will take part to OIH. Activated KCC2 channels block inhibitory GABA-R on neurons. Furthermore, LTP induced by high Ca2+ intracellular concentrations, and reactive oxygen species are involved in the maintenance of neurons hypersensitivity. Consequently, activated neurons produce chemokines, which in turn stimulate microglial cells. Activated microglia will then produce molecules responsible of neuroinflammation: chemokines, proinflammatory cytokines. Astrocytes are also activated in this context and feed this neuroinflammatory environment. The chronological order of tetrasynapse cells activation is currently under debate, but the fact that glial cells and neurons play a role in the development of OIH appears well established. Specific MOR variants, with different number of TM domains, have been shown to induce hyperalgesia under acute exposure to opiates. This suggests a change of the signaling pathways engaged in case of hyperalgesia compared to those involved in analgesia. In addition, other receptors could be involved in OIH including other GPCRs or TLR4.

The environment also appears to have a great impact on OIH development. According to the gender, age, genetic background, immune conditions, pain and opiate treatment history as well as the doses of opiates administered, and maybe to the age or the immune conditions, OIH establishment and development and maintenance may differ. All these OIH influencing factors identified in preclinical studies make these studies close to the clinical conditions of OIH. Indeed, each patient is a unique case. The opiate treatment is initiated by different painful situations and each patient has his own health history with, or without opiate pre-exposure. Each patient has also his own genetic profile and life environment. This patient is a man or a woman, young or aged, and all these factors will influence the establishment of OIH, its intensity persistence and. Preclinical studies give various hints on pathways involved in OIH, each one in specific conditions. Preclinical studies show that OIH implicate complex mechanisms, reflects somehow the clinical reality, and provide hints for the development of strategies to avoid OIH.

Finally, tolerance to analgesia may be described as a consequence of OIH. Because OIH lowers the basal nociceptive level, analgesia will be masked by OIH, leading to analgesic tolerance. From a mechanistic point of view, this phenomenon may contribute to tolerance beside or in
addition to the decrease of functional MOR induced by chronic exposure to opiates, contributing to the loss of analgesic effects of opiates (Williams et al., 2013). In the future, the respective contribution of OIH and other mechanisms in the development of analgesic tolerance will need further investigation.

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**LEGENDS TO FIGURES**

**Figure 1. Tetrasynapse activation contributes to OIH.**
Morphine activates neurons, astrocytes and microglia by inhibiting glutamate recapture and inducing production of pro-inflammatory molecules. In response to its activation, each cell will produce more excitatory substances such as pro-inflammatory chemokines, cytokines, ATP or NO contributing to the establishment and the maintenance of OIH.

ERK, Extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; NFkB, nuclear factor kappa B; NO, nitric oxide; LTP, long-term potentiation.

**Figure 2. Neuronal pathways involved in OIH**
Activation of neuronal MOR by its agonists (ie morphine) induces regulation of intracellular mechanisms involved in OIH. Morphine activates mTOR signalling taking part in neuroexcitation and OIH. Morphine can also block the glutamate transporter EEAC1 involved in glutamate recapture. Synaptic concentrations of glutamate are thus increased. Glutamate will play an important role, through its receptor NMDA and the LTP to provoke OIH. Other factors are involved such as TrkB, the BDNF receptor, that will diminish KCC2 action, modulating the Cl- homeostasis, leading to a switch in the role of GABA from inhibitory to stimulatory and thus producing OIH. Activated glial cells and neurons by morphine will contribute to OIH by producing cytokines such as IL1β or TNF-α involved in LTP. Altogether, these mechanisms participate to the establishment and persistence of OIH.

BDNF, brain derived neurotrophic factor; Cx3Cl1, chemokine Cx3Cl1; EAAC1, glutamate transporter EAAC1; GABA-R, Gamma Aminobutyric Acid receptor; GRK, G protein coupled receptor kinase; IL1β, Interleukin beta; IP3, inositol triphosphate; KCC2, K+/Cl- cotransporter 2; LTP, long-term potentiation; MOR, mu-opioid receptor; mTOR, mammalian target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; NK1, neurokinin 1 receptor; NMDA-R, N-methyl-D-aspartic acid receptor; PKC, protein kinase C; SP, substance P; TNFα, Tumor Necrosis Factor alpha; TrkB, Tyrosine Kinase B; TRPV1, Transient Receptor Potential vanilloid 1.

**Figure 3. Microglia activation contributes to OIH.**
Morphine binds to its specific receptor MOR or to TLR4 and will activate molecular intracellular cascades. This will trigger the activation of microglial cells. In addition, diverse pro-inflammatory molecules such as chemokines, cytokines and ATP also participate in reinforcing this activation and OIH.

BDNF, brain derived neurotrophic factor; Cx3C11, chemokine Cx3C11; Cx3Cr1, CX3C chemokine receptor 1; CCL, chemokines with CC motif; CXCL, chemokines with CXC motif; ERK, Extracellular signal-regulated kinase; IL, interleukin; MOR, mu-opioid receptor; NFkB, nuclear factor kappa B; NO, nitric oxide; P2X4-R, purinergic receptor P2X4; S1P, sphingosin-1-phosphate; TLR4, toll like receptor 4; TNFα, tumor necrosis factor alpha; R, receptor.

**Figure 4. Astrocytes are actors of OIH.**

Concomitant actions of opiates and cytokines induce intracellular cascades leading to production and release of cytokines that confer an activated state to astrocytes. In addition, morphine is able to block the glutamate transporter (GLT-1) resulting in an increase of glutamate in the synaptic cleft, which will then reverberate on neuronal excitation.

GLT1, glutamate transporter 1; IL-1β, Interleukin-1β; IL1R, interleukin 1 receptor; IL-6, Interleukin 6; JNK, junk kinase; MOR, mu-opioid receptor; NFkB, nuclear factor kappa B; NO, Nitric Oxide; P2Y-R, purinergic receptor P2Y; P65, protein 65; PKC, Protein Kinase C; TNFα, tumor necrosis factor alpha; TNFαR, tumor necrosis factor alpha receptor.
Pro-inflammatory cytokines, Chemokines, ATP, NO, Glutamate

NFkB, P65, JNK, ERK, p38

Astrocyte, microglia, presynaptic neuron, postsynaptic neuron

Glu-transporters, Glu-receptors

OIH

Peripheral sensitisation

Glu-transporters, Glu-receptors

activated microglia

mTOR, Ca2+, NO, LTP

Central sensitisation

Morphine

Figure 1 Roeckel et al.
Figure 2 Roeckel et al.

Activated microglia, neurons, astrocytes, endothelial cells

Proinflammatory cytokines, NO, ATP

Microglial activation

Figure
Click here to download Figure: figures 2 Roeckel et al_color_online.pdf
Proinflammatory cytokines, NO, ATP

Activated microglia, neurons, astrocytes, endothelial cells

Figure 3 Roeckel et al.
Proinflammatory cytokines, NO, ATP

Activated microglia, neurons, astrocytes, endothelial cells

Morphine

MOR

GLT-1

PKC

Ca²⁺

P2Y-R

ATP

NFκB

p65

pJNK

IL-1R

TNFα-R

Morphine

astrocytes

Glutamate

IL-1β

TNFα

IL-6

ATP