**Abstract:** Opioid use disorder is classified as a chronic recurrent disease of the central nervous system (CNS) which leads to personality disorders, co-morbidities and premature death. It develops as a result of long-term administration of various abused substances, along with morphine. The pharmacological action of morphine is associated with its stimulation of opioid receptors. Opioid receptors are a group of G protein-coupled receptors and activation of these receptors by ligands induces significant molecular changes inside the cell, such as an inhibition of adenylate cyclase activity, activation of potassium channels and reductions of calcium conductance. Recent data indicate that other signalling pathways also may be involved in morphine activity. Among these are phospholipase C, mitogen-activated kinases (MAP kinases) or β-arrestin. The present review focuses on major mechanisms which currently are considered as essential in morphine activity and dependence and may be important for further studies.

**Keywords:** opioid receptors; adenylate cyclase activity; morphine tolerance and withdrawal signs; mesolimbic system; mitogen-activated kinases (MAP kinases); β-arrestin

1. Introduction

Among opioid drugs, morphine, codeine, fentanyl or buprenorphine are considered the most effective analgesics for application in post-operative and cancer pain. Their chronic administration is associated with a high rate of abuse potential [1]. Moreover, other opioids, including heroin, are used as recreational drugs and have the ability to induce opioid dependence. Substance use disorder is classified as a chronic recurrent disease of the central nervous system (CNS) which leads to personality disorders, co-morbidities and premature death [2,3]. Substance use disorder develops as a result of long-term administration of substances with abuse potential and include physical dependence and/or psychological addiction. Physical dependence is associated with the formation of neuroadaptive changes in the CNS, both at the molecular and cellular level [4–11]. These changes are responsible for the occurrence of characteristic withdrawal signs after cessation of drug-taking. The type and severity of withdrawal signs depend on various factors, such as the type of abused drug, drug doses, time period of drug use, patient’s age, age of the first use of drugs or genetic predispositions [12,13]. Psychological dependence is defined as compulsive drug use to improve the perception of well-being [14]. A typical syndrome of psychological addiction in humans includes intensified drug-seeking behaviour, compromised capacity of will, compulsive drug intake despite awareness of its harmful effects, as well as persistent and recurrent obsession, even after years of abstinence.

Scientific research shows that despite numerous social, psychological and medical projects aimed at reducing the phenomenon of substance abuse, the number of individuals with opioid use disorders...
is steadily increasing worldwide. Nowadays, opioid dependence is considered a global public health crisis. According to the World Health Organization, opioid overdose deaths increased from 69,000 people in 2014 [15] to 118,000 in 2015 [16]. Dramatic increases in maternal opioid use and neonatal abstinence syndrome also has been observed during the last decade. Therefore, in response to the opioid crisis, scientific and government efforts should be focused on several priorities: improving pain management with non-dependent drugs; promoting knowledge of opioid risks; or providing support for cutting-edge research on pain and addiction. The aim of this review is to present current knowledge on mechanisms involved in morphine activity which develop after its acute or chronic administration. The understanding of morphine mechanisms is important for further studies on the activity of the opioidergic system.

2. Morphine and Its Receptors

Morphine and other opioid drugs are able to induce a broad spectrum of pharmacological activity. Occurring in the CNS, they induce strong analgesia, euphoria, sedation, endocrine dysregulation, miosis, antitussive activity or respiratory depression. Additionally, they induce muscle spasms and histamine release in the peripheral nervous system. Observed in clinical practices, there are many opioid drugs that mainly are used as analgesics (Table 1).

| Drug Names | Structural Formula | Indications |
|------------|--------------------|-------------|
| Alfentanil | ![Alfentanil](image) | - anaesthesia in surgery [17] |
| Buprenorphine | ![Buprenorphine](image) | - relieve moderate-to-severe pain [18]  
- substitute treatment for opioid addiction [19]  
- Neonatal Abstinence Syndrome [20] |
| Butorphanol | ![Butorphanol](image) | - treat moderate-to-severe pain [21]  
- relieve acute morphine-induced pruritus [22] |
Table 1. Cont.

| Drug Names | Structural Formula | Indications |
|------------|-------------------|-------------|
| Codeine    | ![Structural Formula](attachment:codeine.png) | - treatment of chronic cough [23]  
- relief of moderate-to-severe pain [24]  
- treat persistent diarrhoea [25] |
| Dextromethorphan | ![Structural Formula](attachment:dextromethorphan.png) | - temporary relief of coughs without phlegm [26] |
| Diphenoxylate | ![Structural Formula](attachment:diphenoxylate.png) | - acute and chronic diarrhoea of various origins [27]  
- reduction in the amount of faecal fluid after ileostomy and colostomy [28] |
| Dihydrocodeine | ![Structural Formula](attachment:dihydrocodeine.png) | - treat moderate-to-severe pain [29]  
- treat dry cough [29]  
- treat diarrhoea [29] |
| Fentanyl   | ![Structural Formula](attachment:fentanyl.png) | - treatment of severe, chronic pain [30]  
- used for surgical anaesthesia [30] |
| Hydrocodone | ![Structural Formula](attachment:hydrocodone.png) | - the management of pain severe enough to require daily, around-the-clock use [31] |
### Table 1. Cont.

| Drug Names | Structural Formula | Indications |
|------------|--------------------|-------------|
| Hydromorphone | ![Hydromorphone](image) | - relieve moderate-to-severe pain [32] |
| Laevodropropizine | ![Laevodropropizine](image) | - treat dry cough [33] |
| Levorphanol | ![Levorphanol](image) | - use in moderate-to-severe pain [34] |
| Loperamide | ![Loperamide](image) | - stop diarrhoea [35] |
| Meptazinol | ![Meptazinol](image) | - relieve moderate-to-severe pain (among others, obstetrics) [36] |
| Methadone | ![Methadone](image) | - treatment of opiate dependence [37] - the treatment chronic, severe pain [38] - treatment of neonatal abstinence syndrome [39] |
| Morphine | ![Morphine](image) | - moderate-to-severe pain relief [40] - used for procedural sedation [40] - sporadically used as an antitussive drug [41] - treatment of Neonatal Abstinence Syndrome [39] |
| Drug Names   | Structural Formula | Indications                                                                 |
|-------------|--------------------|-----------------------------------------------------------------------------|
| Nalbuphine  | ![Nalbuphine](image) | - itching treatment [42]  
- recommended for weak-to-moderately severe pain [42]  
- sedation [43]  
- anaesthesia [44] |
| Nalmefene   | ![Nalmefene](image) | - reduction of alcohol consumption [45]                                       |
| Naloxone    | ![Naloxone](image)  | - Treatment of poisoning, overdose of opioid substances [46]  
- reversal of undesirable effects from opioid used during anaesthesia [47]  
- counteracting the occurrence of opioid-induced constipation [48]  
- substitute treatment of opioid dependence [49] |
| Naltrexone  | ![Naltrexone](image) | - treatment of alcoholism [50]                                                |
| Oxycodone   | ![Oxycodone](image) | - treatment of opioid-induced constipation [48]  
- pain treatment [51] |
Table 1. Cont.

| Drug Names | Structural Formula | Indications |
|------------|--------------------|-------------|
| Pentazocine | ![Structural Formula](image1) | - treatment of moderate-to-severe pain [52]  
- a preeanaesthetic or preoperative medication [52] |
| Pethidine  | ![Structural Formula](image2) | - the treatment of moderate-to-severe pain [53]  
- used as an adjunct to preoperative medications to reduce shivering [53]  
- relief of childbirth pain [54] |
| Remifentanil | ![Structural Formula](image3) | - relief of childbirth pain [54]  
- treatment of moderate-to-severe pain [55]  
- providing anaesthesia and sedation [55] |
| Sulfentanil | ![Structural Formula](image4) | - management of moderate-to-severe pain [56]  
- anaesthesia [56] |
| Tapentadol | ![Structural Formula](image5) | - to help relieve moderate-to-severe pain [57] |
| Tilidine   | ![Structural Formula](image6) | - treatment of pain [58] |
| Tramadol   | ![Structural Formula](image7) | - treat moderate-to-severe chronic pain [59]  
- relief of childbirth pain [59]  
- anaesthesia [60]  
- premature ejaculation [61] |

The pharmacological action of acute doses of morphine is associated with stimulation of the opioid receptors. It interacts predominantly with the μ opioid receptors. Generally, opioid receptors
can be divided into subtypes: $\mu$ ($\mu_1, \mu_2, \mu_3$); $\delta$ ($\delta_1, \delta_2, \delta_3$); and $\kappa$ ($\kappa_1, \kappa_2, \kappa_3$) [13,62]. The novel nociception/orphanin FQ receptor is considered to be a non-opioid branch of the opioid receptor family [13]. Opioid receptors are a group of G protein-coupled receptors [63]. They consist of seven transmembrane domains, three extracellular and three intracellular loops, extracellular amino acid N-terminus and intracellular carboxyl C-terminus. Opioid receptors are located both in the central and peripheral nervous system. The first data on localisation of opioid receptors in the nervous system appeared in 1973 [64]. Nowadays, it is known that opioid receptor subtypes are located in areas involved in: 1) pain transmission, such as the thalamus, rostroventral medulla (RVM), periaqueductal grey area (PAG), pons or in the spinal cord of the dorsal horn; 2) the rewarding system, such as the nucleus accumbens, ventral tegmental area or the cortex; 3) other brain areas, such as the hypothalamus, amygdala, ventral pallidum, globus pallidus, nucleus raphe, hippocampus and olfactory bulb [64–67]. They also occur in peripheral tissues, for example, in the gastrointestinal and in the respiratory tract [67,68].

The localization of opioid receptors in the gut [69] is responsible for regulation of gastrointestinal motility and secretion [70]. Consequently, $\mu$-opioid receptor agonists inhibit gastric emptying, increase pyloric muscle tone, and delay transit through the small and large intestine. All these effects lead to constipation—one of the most impactful adverse effects of morphine and other opioid drugs.

The presence of opioid receptors in the respiratory tract is associated with important clinical indications of morphine [71]. Morphine is used as an antitussive drug, also after surgery within the respiratory system, and in the control of pain due to lung cancer. Conversely, the overdose of morphine induces a high risk of respiratory depression, which is an important limiting factor in morphine therapy [72].

Endogenously, opioid receptors are stimulated by endogenous peptides, such as endomorphins, dynorphins and enkephalins. Endomorphins consist of four aminoacids, including two endogenous ligands (endomorphin-1 and endomorphin-2) that have the highest affinity and selectivity for the $\mu$-opioid receptor in the central and peripheral nervous systems. They are involved in analgesia and reward. Dynorphins (dynorphin A and dynorphin B) exert their effects primarily through the $\kappa$-opioid receptor and have less affinity for the $\mu$-opioid receptor and $\delta$-opioid receptor. Enkephalins (met-enkephalin and leu-enkephalin) produce the effect mainly on $\delta$ receptors, but they also have an affinity for $\mu$ receptors.

3. Molecular Effects of Acute and Chronic Dose of Morphine

A binding of an endogenous (endomorphin molecule) or exogenous (morphine molecule) ligand with an opioid receptor leads to activation of a Go or Gi protein and to subsequent phosphorylation by a family of kinases called the G protein-coupled receptor kinases (GRKs). This induces molecular changes inside the cell, including $\beta$-arrestin binding. G protein is composed of three subunits: $\alpha$, $\beta$ and $\gamma$. Binding of the ligand to the receptor results in opioid receptor activation by GTP binding to the $\alpha$ subunit, while the $\alpha$-GTP complex dissociates from the dimer $\beta\gamma$-subunits. Both complexes, $\alpha$-GTP and dimer $\beta\gamma$, participate in intracellular signal transduction. This leads to an inhibition of adenylylate cyclase activity and a reduction of cyclic adenosine monophosphate (cAMP) levels in the cell [73,74], as well as suppression of the activity of protein kinase A [75,76]. $\alpha$-GTP also activates phospholipase-C (PLC) and mitogen-activated protein (MAP) kinases pathways [77]. PLC hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 increases calcium release from the endoplasmic reticulum that activates calcium-dependent signalling. The activation of potassium channels (G-protein gated inward rectifying potassium channel–GIRK-3) is also observed [78], leading to increased hyperpolarisation of the cell, and, indirectly, to reduced cell excitability [77]. The $\beta\gamma$ dimer directly blocks the calcium channel (P/Q-type, N-type, and L-type channel) and reduces calcium concentration [79] in the cell, leading to suppression of other neurotransmitters. The effect of stimulation of opioid receptors on the activity of potassium and calcium channels was repeatedly confirmed in various brain areas (hippocampus, nucleus locu
coeruleus, the area abdominal caps, etc.), and this mechanism has been considered a key effect for the stimulation of the opioid receptors [80–83].

Chronic exposure to morphine induces the phosphorylation of opioid receptors by GRKs. This phosphorylation prepares opioid receptors for arrestin binding. Arrestin binding blocks further G protein-mediated signalling, thereby, inducing desensitization of opioid receptors [84].

Thus, clinically important pharmacological effects of morphine, induced by a single administration of this substance, are connected with multidirectional, molecular mechanisms which occur within the cell. The molecular effects of morphine are shown graphically in Figure 1.

Figure 1. Molecular mechanisms of morphine action. A binding of ligand with an opioid receptor activates Go or Gi protein. G protein is composed of three subunits: α, β, and γ. The ligand binding results in opioid receptor activation by GTP binding to the α subunit. The α-GTP complex dissociates from the dimer βγ-subunits. Both complexes: α-GTP and dimer βγ, participate in intracellular signal transduction. This leads to an inhibition of adenylate cyclase activity and reduction of cAMP level and protein kinase A inside the cell. The activation of potassium channel and cellular hyperpolarisation is observed. The βγ dimer blocks the calcium channel and reduces calcium concentration inside the cells. The chronic stimulation of opioid receptors by morphine induces the phosphorylation of opioid receptors. sAC–soluble adenylyl cyclase; PKA–protein kinase A; CREB–cAMP response element binding protein; PIP2–phosphatidylinositol biphosphate; PLC–phospholipase C; DAG–diacylglycerol; IP3–inositol triphosphate; MAPK–mitogen-activated protein kinases.

4. Opioid Analgesia

Opioid analgesia is associated strongly with activation of the μ opioid receptors located in CNS. These receptors are localized in subcortical regions of the brain, as previously mentioned, from which the descending pain pathways originate, such as in the thalamus, the PAG and the RVM, as well as in the spinal cord’s dorsal horn [85]. Occurring at the supraspinal level, opioid analgesics stimulate the
μ opioid receptors located on GABAergic interneurons in the RVM, hence, decrease GABA release. Physiologically, GABA, by acting on GABA-A receptors, suppresses the “OFF” cells in the RVM, which subsequently raises the action potential. When the GABA level is reduced, the tonic inhibition of “OFF” cells is relieved (i.e., disinhibition) and the “OFF” cells’ signal suppresses pain perception in the spinal cord (descending pain regulation). Additionally, opioid-induced activation of μ opioid receptors on GABAergic “ON” cells in the RVM inhibits the firing of these cells. Thus, the disinhibition of “OFF” cells and the direct inhibition of “ON” cells produce analgesia, an effect which can be measured using thermal nociception tests [86]. Additionally, the amygdala, a brain area responsible for emotional states, indirectly can modify pain transmission.

Regarding the spinal level, opioid-induced analgesic effects are mediated by the activation of presynaptic μ opioid receptors localized in the dorsal horn of the spinal cord. The triggering of these presynaptic receptors causes membrane hyperpolarization. Such changes in membrane polarization lead to the inhibition of mediators of the pain pathway, such as glutamate, substance P and calcitonin gene-related peptide (CGRP) from nociceptive primary afferent neuron terminals. Consequently, the ascending pain pathway transmission is attenuated.

It should be noted that opioid induced analgesia is a complex process in which μ opioid receptors can be heteromerized with δ or κ opioid receptors. Heterodimeric associations between μ-δ opioid receptors, for example, can be used as a model for the development of novel combination therapies for the treatment of chronic pain and other pathologies [87]. The mechanisms of opioid analgesia are shown graphically in Figure 2.

Figure 2. Mechanisms of morphine analgesia. Regarding the supraspinal level, opioid analgesics stimulate the μ receptors located on GABAergic interneurons in the RVM decreasing GABA release. GABA suppresses the “OFF” cells in the RVM, which subsequently raises the action potential. Additionally, opioid-induced activation of μ opioid receptors on GABAergic “ON” cells in the RVM inhibits the firing of these cells. Observed at the spinal level, opioid-induced analgesia is mediated by the activation of presynaptic μ opioid receptors localized in the dorsal horn of the spinal cord. PAG—periaqueductal gray in midbrain; RVM—rostral ventromedial medulla; GABA—gamma-aminobutyric acid; SP—substance P; CGRP—calcitonin gene-related peptide; NMDA-R—N-methyl-D-aspartate receptor; NK1 R—neurokinin-1 receptor; AMPA-R—α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor.
5. Opioid Rewarding Effects

Generally, the rewarding effect of various addictive substances, including opioids, is associated with stimulation of structures within the mesolimbic system, such as the ventral tegmental area and the nucleus accumbens. This increases the dopamine release in the nucleus accumbens [88] which determines the feeling of pleasure. However, the impulses from other brain structures, such as the ventral striatum, hippocampus, prefrontal cortex or amygdala, also may stimulate the mesolimbic system [8], affecting dopamine levels in the nucleus accumbens. Thus, a dramatic escalation in drug intake, with extended access to drug self-administration, is characterized by a dysregulation of rewarding dopamine pathways in the brain [89–91]. Therefore, the rewarding effect of morphine and other opioids is associated with stimulation of μ opioid receptors localized at the GABAergic terminals of the ventral tegmental area. Such stimulation inhibits GABA release that, in turn, disinhibits dopaminergic neurons and leads to the release of dopamine in the nucleus accumbens that induces feelings of euphoria and promotes the development of drug dependence [13,92,93].

While dopamine plays a crucial role in the rewarding action of morphine, many neurotransmitters and neuromodulators in the CNS affect the dopaminergic system and indirectly modulate various aspects of morphine addiction. These neurotransmitters include glutamate [94,95], serotonin [96], γ-aminobutyric acid (GABA) [97,98], noradrenaline [96,99], adenosine [91], nitric oxide [100], orexin [101], and others. Pharmacological manipulation of these neurotransmitters found in the reward pathway potentially can modify craving for drugs of abuse. The mechanisms of a morphine-induced rewarding effect are shown graphically in Figure 3.

![Figure 3](image_url)

**Figure 3.** Mechanisms of morphine-induced rewarding effect. The rewarding effect of morphine is associated with stimulation of μ opioid receptors localized at the GABAergic terminals in VTA. It inhibits GABA release and disinhibits dopaminergic neurons in NAc. PFC (prefrontal cortex); NAc (nucleus accumbens); HP (hypothalamus); Amy (amygdala); VTA (ventral tegmental area); GABA (gamma–aminobutyric acid); DA (dopamine).

6. Morphine as a Dependent Drug

Chronic morphine abuse leads to physical and psychological dependence [8,12,14,102]. Physical dependence on morphine is manifested by characteristic withdrawal symptoms that can develop after abrupt cessation of drug administration [103,104]. Morphine withdrawal symptoms in people include sneezing, runny nose, cough, abdominal pain, diarrhoea, anorexia, anxiety and other effects [105,106]. Withdrawal effects observed in animals include jumping, paw tremors, teeth chattering, wet dog shakes and diarrhoea [103,105,107,108]. Morphine withdrawal symptoms are evoked in experimental studies either by discontinuation of chronic morphine administration or via the administration of opioid receptor antagonists. Naloxone, the most commonly used opioid receptor antagonist in experimental pharmacology, usually is applied in a dose range from 1 to 6 mg/kg [109–111]. The severity of morphine...
withdrawal symptoms is analyzed on the basis of the number of withdrawal episodes [103,111] (Table 2).

**Table 2. The experimental procedures in particular phases of addiction.**

| Experimental Procedures for Phases of Addiction |
|-----------------------------------------------|
| Morphine dependence is obtained commonly by chronic administration of increasing doses (from 10 to 50–100 mg/kg) of morphine, twice a day for 5–9 consecutive days. |
| Morphine withdrawal is obtained in morphine dependent animals either by discontinuation of chronic morphine administration or via administration of an opioid receptor antagonist, such as naloxone, at a range of doses from 1 to 6 mg/kg. The severity of morphine withdrawal symptoms is analyzed on the basis of the number of withdrawal episodes, such as jumpings, paw tremors, teeth chattering, wet dog shakes and diarrhea. |
| Morphine tolerance is obtained by repeated administration of the same dose of morphine (10 mg/kg) for several (3–7) consecutive days. Commonly, it is analyzed by comparison of the reaction of animals on noxious stimulus, recorded on the first and last day of morphine administration. Morphine tolerance commonly is measured in behavioural tests, such as the tail immersion test or the hot plate test. |
| Morphine-induced behavioral sensitization is related closely to the environment in which the addictive substance is taken and reflects morphine-seeking behavior in studied animals. It is obtained by administration of a challenge dose of morphine (at range of 1–10 mg/kg) in morphine dependent animals after several days (7–10) of a morphine-free period. It is measured as an increase in locomotor activity of animals, rarely as the enhanced rewarding effect. |

Some authors suggest that the decrease in dopamine concentrations in the mesocorticolimbic system plays a critical role in morphine withdrawal [112–115]. Still, neurotransmitters such as noradrenaline [116], glutamate [117], serotonin [118], orexin [119] and cortisol [120] also may be involved in morphine withdrawal. Furthermore, these changes in neurotransmitters are accompanied by changes in cell signalling pathways such as a significant increase in cAMP level [121] and the deregulation of the MAP kinase pathway (ERK 1/2) [122,123].

Morphine tolerance, the second parameter of physical dependence on this substance, is defined as a need to increase the morphine dose (a rightward shift in the dose–response curve) to achieve the same pharmacological effect [124]. The phenomenon of tolerance develops regarding analgesic, euphoric, sedative, respiratory depressant, and nauseating effects of opioids, but not to their effects on miosis and bowel motility (constipation) [125]. Generally, there are three types of tolerance: pharmacokinetic tolerance; learned tolerance; and pharmacodynamic tolerance. Pharmacokinetic tolerance refers to changes in the distribution or metabolism of the drug, while learned tolerance refers to a reduction in the effects of a drug due to compensatory mechanisms that are learned, such as behaving normally while still intoxicated. The most important form of tolerance relevant to opioids is pharmacodynamic tolerance. This type of tolerance has been related to neuroadaptive changes that take place after long-term exposure to the drug, including changes in receptor density and alteration in receptor coupling to G proteins and signal transduction pathways [126].

During experimental studies, morphine tolerance commonly is analyzed by means of nociception, measured in behavioural tests, like the tail immersion test [103,127] or the hot plate test [128,129] (Table 2). Morphine tolerance may be modified by various neurotransmitters including dopamine [130,131], but also serotonin [132], acetylcholine [133], orexin [134] or endocannabinoids [135] and others.

Behavioral sensitization is a phenomenon involving escalating behavioral responses to repeated exposure to a stimulus such as a drug of abuse like cocaine or opioids, after a drug-free period which can be long-lasting—even to the extent of many years. This effect is related closely to the environment in which the addictive substance is taken. Behavioural sensitization is an important parameter in evaluating the degree of psychological addiction. Animal studies reflect the drug-seeking behaviour in people which often leads to drug use relapse [14]. Experimentally, behavioural sensitization is commonly manifested and measured as an increase in locomotor activity of animals after administration of a challenging dose of the abused substance [136,137]. Furthermore, behavioural sensitization also
may be expressed as the enhanced rewarding effect of the addictive substance. During animal studies, this can be observed in the conditioned place preference test [100,138,139]. Less commonly, sensitization may be observed through the intensification of withdrawal signs after repeated withdrawal periods [91,140] (Table 2).

The development of behavioural sensitization occurs through the neuroadaptive changes observed in glutamatergic and dopaminergic neurotransmission [102,141–143]. Sensitization is associated with an increased dopamine release in the mesolimbic structures [14,144,145]. The main pathways involved in behavioural sensitization are the dopaminergic pathway from the ventral tegmental area and the glutamatergic pathways from the prefrontal cortex, both terminating in the nucleus accumbens.

The expression of morphine sensitization is associated primarily with an increased dopamine release and with alterations in the sensitivity of dopaminergic D1 receptors in the mesolimbic structures, including the striatum, nucleus accumbens, ventral tegmental area, hippocampus and the prefrontal cortex [4–7,91,146,147]. Observed in rats, the pharmacological blockade of D1 receptors impairs the expression of sensitization [148]. Similarly, the administration of D1 and D2 receptor antagonists into the nucleus accumbens also inhibits the development of behavioural sensitization in rats [147]. Moreover, published data show that the increased expression of D1 receptors in the shell of the nucleus accumbens, observed during the morphine sensitization process, is associated with elevated MAP kinase activity (ERK 1/2) and this effect is reduced by the D1 receptor antagonist—SCH 23390 [149]. Alternatively, the development of behavioural sensitization is associated more with the glutamatergic system and with the ventral tegmental area because the antagonists of NMDA and AMPA receptors inhibit the acquisition, but not the expression, of behavioural sensitization [150,151]. Thus, various neuroadaptive changes, including alterations in the density of receptors, the neurotransmitter level or deregulation in cell signalling, can be responsible for the expression and acquisition of behavioural sensitization.

7. Molecular Mechanisms of Morphine Tolerance and Dependence

Considering the cellular level, the major effect of an acute dose of morphine is the decrease in cAMP level and hyperpolarization that is induced by changes in the activity of potassium and calcium channels. However, chronic stimulation of opioid receptors by morphine and other opioid ligands induces adaptive changes within opioid receptors. This leads to a decrease in the acute receptor response. Such changes are essential in controlling the receptor activity as they protect receptors against hyperstimulation, promote signal termination and regulate their expression [86]. Consequently, desensitization, internalization, resensitization or downregulation of opioid receptors is developed. These mechanisms lead to the attenuation of the pharmacological activity of morphine and other opioid drugs, which often is observed after chronic exposure to them.

Regarding chronic morphine exposure, it was previously suggested that the down-regulation of µ receptors was responsible mainly for the reduced morphine activity which was manifested as tolerance. This hypothesis, however, was not confirmed in experiments because chronic exposure to morphine did not produce down-regulation of µ receptors [152,153]. Similarly, internalization of µ receptors was previously considered as a neural mechanism underlying morphine tolerance. However, in vitro studies do not confirm the hypothesis because morphine is able to induce a strong tolerance but its effect on µ receptor internalization is poor [154]. Nowadays, an increasing number of evidences link mechanisms of tolerance with desensitization of µ opioid receptors. It should be underlined that the definitions of cellular tolerance and desensitization are similar and, for many years, these terms have been confused. Both are defined as the reduced capacity to respond to the same drug dose. However, desensitization (expressed as “rapid tolerance” in earlier studies) means a progressive agonist-induced reduction of signal transduction in opioid receptors seen during in vitro models, while tolerance is observed during in vivo models. Desensitization develops directly after opioid exposure and is reversed rapidly in agonist-free circumstances [84]. Rapid desensitization depends on potassium and calcium ion activity, while sustained desensitization is related to enzyme activity (adenylyl cyclase or MAP kinases). Nowadays, the enhanced desensitization of opioid receptors is
considered as being an important mechanism of morphine tolerance, which results from numerous neuroadaptive changes. The desensitization may be caused by increased adenylate cyclase activity and an elevated cAMP level, for example, which, in turn, affects the activity of the cAMP response element-binding protein (CREB) [155]. Additionally, the desensitization involves G protein uncoupling because, in morphine treated animals, the binding of the GTPα complex is reduced in comparison with control animals. Moreover, morphine induced desensitization of μ receptors is associated closely with deregulation of β-arrestin-1 and β-arrestin-2 levels in the cell [156–158]. β-arrestin, a cytosolic protein, is bound to the opioid receptor surface after opioid receptor phosphorylation by a class of serine/threonine kinases (GRKs). Activation of β-arrestin inhibits further cell signalling, which directly produces the receptor desensitization [86,159]. The process of desensitization also is produced by an increase in phosphorylation of MAP kinases. MAP kinases have a large range of potential substrates, including transcription factors controlling gene expression. The role of extracellular signal-regulated kinase 1/2 (ERK1/2) in the effect of chronic morphine administration also was confirmed, although these results are contradictory. Narita et al. [160] and Macey et al. [161] observed that chronic exposure to morphine induced the increase in ERK1/2 phosphorylation, while other authors showed the lack of effect [162–164]. There also are some data showing the role of phospholipase C in morphine-induced desensitization [165]. Phospholipase C increases the level of other secondary neurotransmitters, such as inositol–(1,4,5)–triphosphate (IP3) and 1,2-diacylglycerol (DAG). These lead to the elevation of calcium levels in the cell. The phospholipase C also catalyzes the release of arachidonic acid from cell membranes participating in the formation of inflammation [166]. Phospholipase C plays a significant role in morphine activity. The inhibitors of phospholipase C potentiate the antinociceptive effect of a single dose of morphine and reduce morphine tolerance [167,168].

The molecular effects of morphine tolerance and dependence is demonstrated graphically in Figure 1.

8. Epigenetic Mechanisms of Morphine Tolerance and Dependence

Novel data shows that chronic exposure to abused drugs may induce complex epigenetic interactions within a genome thereby regulating patterns of gene expression [169–171]. These epigenetic modifications include changes in DNA methylation [172], histone acetylation and demethylation, alterations in DNA accessibility, and chromatin structure modification. They are inherited despite a lack of effect on DNA structure. First evidence on the role of DNA methylation and histone deacetylation in μ opioid receptor expression was published in 2007 [173]. Observed in P19 mouse embryonal carcinoma cells, hypermethylation of DNA silences μ opioid genes at the transcriptional level and DNA demethylation induces higher μ opioid gene expression. Moreover, μ opioid receptor expression also was increased after pharmacological manipulation, such as administration of a demethylating agent (5′-aza-2′-deoxycytidine) and histone deacetylase inhibitors. It was demonstrated in another study that chromatin modification also participates in μ opioid gene expression [174]. Mashayekhi et al. [175] documented that the alternations in mRNA levels of brain-derivative neurotrophic factor, BDNF, in the ventral tegmental area and the locus coeruleus of rats on the seventh day of morphine abstinence were associated with histone modifications. Another study also confirmed the role of histone methylation in the effects of chronic morphine exposure [176]. Ciccarelli et al. [177] found the augmentation of histone acetylation during naloxone-precipitated morphine withdrawal in the shell of the nucleus accumbens and in the lateral septum. There also are experiments showing that pharmacological inhibition of DNA methylation by 5′-aza-2′-deoxycytidine have an influence on morphine place preference in rats [178,179]. Recent study confirmed the existence of epigenetic changes in numerous brain structures (among others: cerebral cortex, cerebellum, hippocampus, hypothalamus, medulla oblongata, etc.) after acute and chronic exposure to opiates [180].

Although much progress has been made in understanding the mechanisms of opioid activity, little is known about the mechanisms of transcriptional regulation. It seems they are essential regulators
of gene expression. Further studies are necessary to define the precise links between the epigenetic alterations and behavioral effects of morphine and other opioids.

9. Biased Opioid Ligands as a New Class of Opioid Analgesics

Since traditional morphine-like drugs hold many adverse effects (itching, constipation, nausea/vomiting, respiratory depression or abuse liability), recently, the ligand biased at the G protein-coupled receptor has been synthesized. These compounds were perceived to preferentially stimulate certain intracellular pathways over others and produce less side-effects. Thus, such drugs could be safer, more effective and well-tolerated than morphine [181]. Thus far, several G protein-biased μ opioid receptor (GPB–MOR) agonists have been developed [182]. They preferentially activate Gαi protein signalling connected with analgesia over β-arrestin signalling that mediate some undesirable effects. TRV130 (oliceridine), was the first such agonist progressed to clinical trial [183]. Another GPB–MOR ligand is a compound referred to as PZM21, which represents the first example of the structure-based discovery of a biased G protein-coupled receptor ligand. PZM21 showed initial promise in animal studies as a potent analgesic without respiratory depression and morphine-like reinforcing effects [184]. However, although GPB–MOR may produce less respiratory depression and gastrointestinal dysfunction at analgesic doses than currently available opioid analgesics, they retain their abuse liability [185]. Other promising therapeutic candidates for pain and itch relief are G protein-biased agonists for the κ opioid receptor [186]. The example of such a compound is Triazole. It did not alter locomotor activity and did not cause sedation in animal models. These effects appeared to arise from its inability to decrease the dopamine release in mouse striatum, thus not adversely affecting the dopaminergic transmission. Furthermore, this compound did not influence the reward circuit in the brain, thus did not trigger signs of dysphoria and aversion, unlike a typical κ opioid agonist. Generally, the discoveries of new pathways and new ligands at the μ/κ opioid receptor highlight the opportunities for biased ligands as a new class of analgesics providing more efficacious and safer relief from moderate-to-severe pain.

Summing up, morphine, acting on opioid receptors, induces a broad spectrum of pharmacological activity. However, long-term morphine administration generates dysregulation at cellular and molecular levels in the brain, leading to addiction. Despite extensive studies, the effective management of opioid disorders is limited. Therefore, the recognition of mechanisms underlying morphine/opioid dependence seems to be extremely important in searching for new strategies of therapy for morphine/opioid abusers. The present review summarizes the current knowledge on morphine activity and provides a major overview of the mechanisms involved in its acute and chronic exposure.

Author Contributions: Conceptualization, J.L.; Making the table, M.Ł.; Drawing the figures, S.T. and A.M.; Writing—Review and Editing, J.L., M.Ł., S.T., A.M., J.O.-G., J.K.; Supervision J.L.; Edition of Reference M.Ł.

Funding: The review was supported by Statutory Activity of Medical University of Lublin (DS 20/2019).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Nutt, D.J.; King, L.A.; Phillips, L.D. Drug harms in the UK: A multicriteria decision analysis. Lancet 2010, 376, 1558–1565. [CrossRef]
2. Korsgaard, H.O.; Torgersen, S.; Wentzel-Larsen, T.; Ulberg, R. Substance abuse and personality disorder comorbidity in adolescent outpatients: Are girls more severely ill than boys? Child Adolesc. Psychiatry Ment. Health 2016, 10, 8. [CrossRef] [PubMed]
3. Fridell, M.; Bäckström, M.; Hesse, M.; Krantz, P.; Perrin, S.; Nyhlén, A. Prediction of psychiatric comorbidity on premature death in a cohort of patients with substance use disorders: A 42-year follow-up. BMC Psychiatry 2019, 19, 150. [CrossRef] [PubMed]
4. Spanagel, R.; Almeida, O.F.X.; Shippenberg, T.S. Long lasting changes in morphine-induced mesolimbic dopamine release after chronic morphine exposure. Synapse 1993, 14, 243–245. [CrossRef] [PubMed]
5. Spanagel, R.; Shippenberg, T.S. Modulation of morphine induced sensitization by endogenous kappa opioid systems in the rat. *Neurosci. Lett.* 1993, 153, 232–236. [CrossRef]

6. Tjon, G.H.K.; De Vries, T.J.; Ronken, E.; Hogenboom, F.; Wardeh, G.; Mulder, A.H.; Schoffelmeer, A.N. Repeated and chronic morphine administration causes differential long-lasting changes in dopaminergic neurotransmission in rat striatum without changing its δ- and κ-opioid receptor regulation. *Eur. J. Pharmacol.* 1994, 252, 205–212. [CrossRef]

7. Tjon, G.H.K.; Voorn, P.; Vanderschuren, L.J.M.; De Vries, T.J.; Michiels, N.H.L.M.; Jonker, A.J.; Klop, H.; Nestby, P.; Mulder, A.H.; Schoffelmeer, A.N. Delayed occurrence of enhanced striatal preprodynorphin gene expression in behaviorally sensitized rats: Differential long-term effects of intermittent and chronic morphine administration. *Neuroscience* 1997, 76, 167–176. [CrossRef]

8. Koob, G.F.; Ahmed, S.H.; Boutrel, B.; Chen, S.A.; Kenny, P.J.; Markou, A.; O’Dell, L.E.; Parsons, L.H.; Sanna, P.P. Neurobiological mechanisms in the transition from drug use to drug dependence. *Neurosci. Biobehav. Rev.* 2004, 27, 739–749. [CrossRef] [PubMed]

9. Nestler, E.J. Molecular mechanisms of drug addiction. *Neuropsychopharmacology* 2004, 47, 24–32. [CrossRef]

10. Robinson, T.E.; Kolb, B. Structural plasticity associated with exposure to drugs of abuse. *Neuropsychopharmacology* 2004, 47, 33–46. [CrossRef]

11. Motahari, A.A.; Sahraei, H.; Meftahi, G.H. Role of nitric oxide on dopamine release and morphine-dependency. *Basic Clin. Neurosci.* 2016, 7, 283–290. [PubMed]

12. Goodman, A. Neurobiology of addiction. An integrative review. *Biochem. Pharmacol.* 2008, 75, 266–322. [CrossRef] [PubMed]

13. Bodnar, R.J. Endogenous opiates and behavior: 2014. *Peptides* 2016, 75, 18–70. [CrossRef] [PubMed]

14. Robinson, T.E.; Berridge, K.C. The psychology and neurobiology of addiction: An incentive-sensitization view. *Addiction* 2000, 95, 91–117.

15. WHO. Guidelines on Community Management of Opioid Overdose. 2014. Available online: https://www.who.int/substance_abuse/publications/drugs/en/15.06.2019 (accessed on 15 June 2019).

16. WHO. Information Sheet on Opioid Overdose. Available online: https://www.who.int/substance_abuse/information-sheet/en/22 August 2019) (accessed on 22 August 2019).

17. Orfei, P.; Bigetti, E.; Patrizio, A.; Pinto, G. The use of alfentanil for short duration surgery in pediatric anesthesia. *Minerva Anestesiol.* 2000, 66, 123–129. [PubMed]

18. White, L.D.; Hodge, A.; Vlok, R.; Hurtado, G.; Eastern, K.; Melhuish, T.M. Efficacy and adverse effects of buprenorphine in acute pain management: Systematic review and meta-analysis of randomised controlled trials. *Br. J. Anaesth.* 2018, 120, 668–678. [CrossRef] [PubMed]

19. Kumar, R.; Saadabadi, A. *Buprenorphine*; StatPearls Publishing: Treasure Island, FL, USA, 2018.

20. Harricharan, S.; Farah, K. *Buprenorphine Formulations for the Treatment of Opioid Use Disorders: A Review of Comparative Clinical Effectiveness. Cost-Effectiveness and Guidelines*; CADTH Rapid Response Reports; Canadian Agency for Drugs and Technologies in Health: Ottawa, ON, Canada, 2017.

21. Szabova, A.; Sadhasivam, S.; Wang, Y.; Nick, T.G.; Goldschneider, K. Comparison of postoperative analgesia with epidural butorphanol/bupivacaine versus fentanyl/bupivacaine following pediatric urological procedures. *J. Opioid Manag.* 2010, 6, 401–407. [CrossRef]

22. Yokoyama, Y.; Yokoyama, T.; Nagao, Y.; Nakagawa, T.; Magaribuchi, T. Treatment of epidural morphine induced pruritus with butorphanol. *Masui* 2009, 58, 178–182.

23. Chung, K.F.; Pavord, I.D. Prevalence, pathogenesis, and causes of chronic cough. *Lancet* 2008, 371, 1364–1374. [CrossRef]

24. Peechakara, B.V.; Gupta, M. *Codeine*; StatPearls Publishing: Treasure Island, FL, USA, 2018.

25. Palmer, K.R.; Corbett, C.L.; Holdsworth, C.D. Double-blind cross-over study comparing loperamide; codeine and diphenoxylate in the treatment of chronic diarrhea. *Gastroenterology* 1980, 79, 1272–1275. [CrossRef]

26. Koskela, H.; Naaranlahti, T. Drug therapy for cough. *Duodecim* 2016, 132, 455–460. [PubMed]

27. Leung, F.W.; Rao, S.S. Approach to fecal incontinence and constipation in older hospitalized patients. *Hosp. Pract. (1995)* 2011, 39, 97–104. [CrossRef] [PubMed]

28. Schiller, L.R. Treatment of Fecal Incontinence. *Curr. Treat. Options Gastroenterol.* 2003, 6, 319–327. [CrossRef] [PubMed]

29. Leppert, W. Dihydrocodeine as an opioid analgesic for the treatment of moderate to severe chronic pain. *Curr. Drug Metab.* 2010, 11, 494–506. [CrossRef] [PubMed]
30. Schug, S.A.; Ting, S. Fentanyl Formulations in the Management of Pain: An Update. *Drugs* 2017, 77, 747–763. [CrossRef] [PubMed]

31. Dhillon, S. Hydrocodone Bitartrate ER (Hysingla® ER): A Review in Chronic Pain. *Clin. Drug Investig.* 2016, 36, 969–980. [CrossRef]

32. Davis, M.P.; McPherson, M.L.; Mehta, Z.; Behm, B.; Fernandez, C. What Parenteral Opioids to Use in Face of Shortages of Morphine, Hydromorphone, and Fentanyl. *Am. J. Hosp. Palliat. Med.* 2018, 35, 1118–1122. [CrossRef]

33. Laso

34. Gudin, J.; Fudin, J.; Nalamachu, S. Levorphanol use: Past, present and future. *Clin. Drug Investig.* 2017, 37, 411–426. [CrossRef] [PubMed]

35. Johnson, K.; Gerada, C.; Greenough, A. Treatment of neonatal abstinence syndrome. *West. J. Med.* 2003, 179, 196–201. [CrossRef] [PubMed]

36. Singer, J.; Jank, A.; Amara, S.; Stepan, P.D.; Kaisers, U.; Hoehne, C. Efficacy and Duration of Parenteral Pethidine or Meptazinol and Regional Analgesia for Pain Relief during Delivery. A Comparative Observational Study. *Geburtshilfe Frauenheilkd.* 2016, 76, 964–971. [CrossRef]

37. Johnson, K.; Gerada, C.; Greenough, A. Treatment of neonatal abstinence syndrome. *Arch. Dis. Child. Fetal Neonatal Ed.* 2003, 88, F2–F5. [CrossRef] [PubMed]

38. Anderson, I.B.; Kearney, T.E. Medicine Cabinet Use of methadone. *West. J. Med.* 2000, 172, 43–46. [CrossRef] [PubMed]

39. Siu, A.; Robinson, C.A. Neonatal Abstinence Syndrome: Essentials for the Practitioner. *J. Pediatr. Pharmacol. Ther.* 2014, 19, 147–155.

40. Murphy, P.B.; Barrett, M.J. Morphine; StatPearls Publishing: Treasure Island, FL, USA, 2018.

41. Bolser, D.C. Pharmacologic Management of Cough. *Otolaryngol. Clin. N. Am.* 2010, 43, 147–155. [CrossRef] [PubMed]

42. Logash, M.; Pokotylo, P.; Zboina, B.; Stepień, R.B. Nalbuphine: Some aspects of the research and applications. *Med. Stud.* 2017, 33, 146–154. [CrossRef]

43. Zeng, Z.; Lu, J.; Shu, C.; Chen, Y.; Guo, T.; Wu, Q.; Yao, S.; Yina, P. A Comparison of Nalbuphine with Morphine for Analgesic Effects and Safety: Meta-Analysis of Randomized Controlled Trials. *Sci. Rep.* 2015, 5, 10927. [CrossRef] [PubMed]

44. Kubica-Cielińska, A.; Zielińska, M. The use of nalbuphine in paediatric anaesthesia. *Anaesthesiol. Intensive Ther.* 2015, 47, 252–256. [CrossRef] [PubMed]

45. Laux, G. Update Psychopharmacotherapy. *Med. Monatsschr. Pharm.* 2017, 40, 4–14.

46. Behar, E.; Bagnulo, R.; Coffin, P.O. Acceptability and Feasibility of Naloxone Prescribing in Primary Care Settings: A Systematic Review. *Prev. Med.* 2018, 114, 79–87. [CrossRef]

47. Jordan, M.R.; Morrisonpone, D. *Naloxone*; StatPearls Publishing: Treasure Island, FL, USA, 2018.

48. Liu, M.; Wittbrodt, E. Low-dose oral naloxone reverses opioid-induced constipation and analgesia. *J. Pain Symptom. Manag.* 2002, 23, 48–53. [CrossRef] [PubMed]

49. Sokol, R.; LaVertu, A.E.; Morrill, D.; Albanese, C.; Schuman-Olivier, Z. Group-based treatment of opioid use disorder with buprenorphine: A systematic review. *J. Subst. Abuse Treat.* 2018, 84, 78–87. [CrossRef] [PubMed]

50. Gastfriend, D.R. A pharmaceutical industry perspective on the economics of treatments for alcohol and opioid use disorders. *Ann. N. Y. Acad. Sci.* 2014, 1327, 112–130. [CrossRef] [PubMed]

51. Gkekges, I.D.; Minis, E.E.; Iavazzo, C. Oxycodone/naloxone in postoperative pain management of surgical patients. *J. Opioid Manag.* 2018, 14, 52–60. [CrossRef] [PubMed]

52. Wang, N.; Wang, L.; Gao, Y.; Zhou, H.; Wang. J. Analgesic Effect of Preoperative Pentazocine for Laparoscopic Cholecystectomy. *Carcinus* 2016, 8, e948. [CrossRef] [PubMed]

53. Yasaee, R.; Saadabadi, A. *Meperidine*; StatPearls Publishing: Treasure Island, FL, USA, 2018.

54. Leong, W.L.; Sng, B.L.; Sia, A.T. A comparison between remifentanil and meperidine for labor analgesia: A systematic review. *Anesth. Analg.* 2011, 113, 818–825. [CrossRef] [PubMed]

55. Bushuven, S.; Kreuer, S.; Kranke, P. Remifentanil Up2date—Part 1. *Anaesthesiol. Intensivmed. Notfallmed. Schmerzther.* 2017, 52, 543–553.
56. Mandel, J.E. Considerations for the use of short-acting opioids in general anesthesia. *J. Clin. Anesth.* **2014**, *26*, SI-57. [CrossRef]

57. Zajaczkowska, R.; Przewlocka, B.; Kocot-Kepsm, M.; Mika, J.; Leppert, W.; Wordliczek, J. Tapentadol—A representative of a new class of MOR-NRI analgesics. *Pharmacol. Rep.* **2018**, *70*, 812–820. [CrossRef]

58. Wittert, G.; Hope, P.; Pyle, D. Tissue distribution of opioid receptors gene expression in the rat. *Brain Res.* **2013**, *155*, 161–168. [CrossRef] [PubMed]

59. Bravo, L.; Mico, J.A.; Berrocoso, E. Discovery and development of tramadol for the treatment of pain. *Expert Opin. Drug Discov.* **2017**, *12*, 1281–1291. [CrossRef] [PubMed]

60. Khan, A.H.; Rasaily, D. Tramadol Use in Premature Ejaculation: Daily versus Sporadic Treatment. *Indian J. Psychol. Med.* **2013**, *35*, 256–259. [CrossRef] [PubMed]

61. Waldhoer, M.; Bartlett, S.E.; Whistler, J.L. Opioid receptors. *Annu. Rev. Biochem.* **2004**, *73*, 953–990. [CrossRef]

62. Dhawan, B.N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P.B.; Portoghese, P.S.; Hamon, M. International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* **1996**, *48*, 567–592.

63. Pert, C.B.; Snyder, S.H. Opiate receptor: Demonstration in nervous tissue. *Science* **1973**, *179*, 1011–1014. [CrossRef] [PubMed]

64. Gray, A.C.; Coupar, I.M.; White, P.J. Comparison of opioid receptor distributions in the rat central nervous system. *Life Sci.* **2006**, *79*, 674–685. [CrossRef] [PubMed]

65. Wood, J.D.; Galligan, J.J. Function of opioids in the enteric nervous system. *Neurogastroenterol. Motil.* **2004**, *16*, 17–28. [CrossRef] [PubMed]

66. Zajaczkowska, R.; Przewłocka, B.; Kocot-Kępka, M.; Mika, J.; Leppert, W.; Wordliczek, J. Tapentadol—A representative of a new class of MOR-NRI analgesics. *Pharmacol. Rep.* **2018**, *70*, 812–820. [CrossRef] [PubMed]

67. Law, P.Y.; Wong, Y.H.; Loh, H.H. Molecular mechanisms and regulation of opioid receptor signaling. *Curr. Opin. Neurobiol.* **1998**, *8*, 351–356. [CrossRef]
81. Zamponi, G.W.; Snutch, T.P. Modulating modulation: Crosstalk between regulatory pathways of presynaptic calcium channels. *Mol. Interv.* 2002, 2, 476–478. [CrossRef] [PubMed]

82. Torrecilla, M.; Marker, C.L.; Cintora, S.C.; Stoffel, M.; Williams, J.T.; Wickman, K. G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. *J. Neurosci.* 2002, 22, 4328–4334. [CrossRef] [PubMed]

83. Torrecilla, M.; Quillinan, N.; Williams, J.T.; Wickman, K. Pre- and postsynaptic regulation of locus coeruleus neurons after chronic morphine treatment: A study of GIRK-knockout mice. *Eur. J. Neurosci.* 2008, 28, 618–624. [CrossRef]

84. Allouche, S.; Noble, F.; Marie, N. Opioid receptor desensitization: Mechanisms and its link to tolerance. *Front. Pharmacol.* 2014, 5, 280. [CrossRef] [PubMed]

85. Fields, H. State-dependent opioid control of pain. *Nat. Rev. Neurosci.* 2004, 5, 565–575. [CrossRef]

86. Raehal, K.M.; Bohn, L.M. β-arrestins: Regulatory role and therapeutic potential in opioid and cannabinoid receptor-mediated analgesia. *Handb. Exp. Pharmacol.* 2014, 219, 427–443.

87. Gomes, I.; Gupta, A.; Filipovska, J.; Szeto, H.H.; Pintar, J.E.; Devi, L.A. A role for heterodimerization of μ and δ opiate receptors in enhancing morphine analgesia. *Proc. Natl. Acad. Sci. USA* 2004, 101, 5135–5139. [CrossRef]

88. Di Chiara, G. Role of dopamine in the behavioural actions of nicotine related to addiction. *Eur. J. Pharmacol.* 2000, 393, 295–314. [CrossRef]

89. Anderson, S.M.; Pierce, R.C. Cocaine-induced alterations in dopamine receptor signaling: Implications for reinforcement and reinstatement. *Pharmacol. Ther.* 2005, 106, 389–403. [CrossRef] [PubMed]

90. Briand, L.A.; Flagel, S.B.; Garcia-Fuster, M.J.; Watson, S.J.; Akil, H.; Sarter, M.; Robinson, T.E. Persistent alterations in cognitive function and prefrontal dopamine D2 receptors following extended, but not limited, access to self-administered cocaine. *Neuropsychopharmacology* 2008, 33, 2969–2980. [CrossRef] [PubMed]

91. Listos, J.; Baranowska-Bosiacka, I.; Wąsik, A.; Talarek, S.; Tarnowski, M.; Listos, P.; Lupina, M.; Antkiewicz-Michaluk, L.; Gutowska, I.; Tkacz, M.; et al. The adenosinergic system is involved in sensitization to morphine withdrawal signs in rats-neurochemical and molecular basis in dopaminergic system. *Psychopharmacology* 2016, 233, 2383–2397. [CrossRef] [PubMed]

92. Wise, R.A.; Rompre, P.P. Brain dopamine and reward. *Annu. Rev. Psychol.* 1989, 40, 191–225. [CrossRef] [PubMed]

93. Johnson, S.W.; North, R.A. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* 1992, 12, 4. [CrossRef]

94. Sepehrizadeh, Z.; Bahrololoumi Shapourabadi, M.; Ahmadi, S.; Hashemi Bozchlou, S.; Zarrindast, M.R.; Sahebgharani, M. Decreased AMPA GluR2, but not GluR3, mRNA expression in rat amygdala and dorsal hippocampus following morphine-induced behavioural sensitization. *Clin. Exp. Pharmacol. Physiol.* 2008, 35, 1321–1330. [CrossRef] [PubMed]

95. Farahmandfar, M.; Karimian, S.M.; Zarrindast, M.R.; Kadivar, M.; Afrouzi, H.; Naghdhi, N. Morphine sensitization increases the extracellular level of glutamate in CA1 of rat hippocampus via μ-opioid receptor. *Neurosci. Lett.* 2011, 494, 130–134. [CrossRef]

96. Reith, M.E.; Li, M.Y.; Yan, Q.S. Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration. *Psychopharmacology* 1997, 134, 309–317. [CrossRef]

97. Carr, D.B.; Sesack, S.R. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse* 2000, 38, 114–123. [CrossRef]

98. Frankowska, M.; Wydra, K.; Faron-Grecka, A.; Zaniewska, M.; Kuśmider, M.; Dziedzicka-Wasylewska, M.; Filip, M. Alterations in gamma-aminobutyric acid(B) receptor binding in the rat brain after reinstatement of cocaine-seeking behavior. *Pharmacol. Rep.* 2008, 60, 834–843.

99. Zaniewska, M.; Filip, M.; Przegalinski, E. The Involvement of Norepinephrine in Behaviors Related to Psychostimulant Addiction. *Curr. Neuropharmacol.* 2015, 13, 407–418. [CrossRef] [PubMed]

100. Sahraei, H.; Zarei, F.; Eidi, A.; Oryan, S.; Shams, J.; Khoshbaten, A.; Zarrindast, M.R. The role of nitric oxide within the nucleus accumbens on the acquisition and expression of morphine-induced place preference in morphine sensitized rats. *Eur. J. Pharmacol.* 2007, 556, 99–106. [CrossRef]
101. Alijanpour, S.; Tirgar, F.; Zarrindast, M.R. Role of dorsal hippocampal orexin-1 receptors in memory restoration induced by morphine sensitization phenomenon. *Neuroscience* 2016, 312, 215–226. [CrossRef] [PubMed]

102. Robinson, T.E.; Berridge, K.C. The incentive sensitization theory of addiction: Some current issues. *Philos. Trans. R. Soc. B* 2008, 363, 3137–3146. [CrossRef]

103. Listos, J.; Baranowska-Bosiacka, I.; Talarek, S.; Listos, P.; Orzelska, J.; Fidecka, S.; Gutowska, I.; Kolas, A.; Rybicka, M.; Chlubek, D. The effect of perinatal lead exposure on dopamine receptor D2 expression in morphine dependent rats. *Toxicology* 2013, 310, 73–83. [CrossRef] [PubMed]

104. Diana, M.; Pistis, M.; Muntoni, A.; Gessa, G. Profound decrease of mesolimbic dopaminergic neuronal mRNA levels in the lateral hypothalamus and striatum are enhanced by morphine withdrawal. *J. Endocrinol.* 2006, 191, 3137–3146. [CrossRef] [PubMed]

105. Harris, A.C.; Gewirtz, J.C. Acute opioid dependence: Characterizing the early adaptations underlying drug withdrawal. *Psychopharmacology* 2005, 178, 353–366. [CrossRef] [PubMed]

106. Evans, C.J.; Cahill, C.M. Neurobiology of opioid dependence in creating addiction vulnerability. *F1000Research* 2016, 5. [CrossRef]

107. Schulteis, G.; Markou, A.; Gold, L.H.; Stinus, L.; Koob, G.F. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. *J. Pharmacol. Exp. Ther.* 1994, 271, 1391–1398. [PubMed]

108. Zhang, Z.; Schulteis, G. Withdrawal from acute morphine dependence is accompanied by increased anxiety-like behavior in the elevated plus maze. *Pharmacol. Biochem. Behav.* 2008, 89, 392–403. [CrossRef]

109. Done, C.; Silverstone, P.; Sharp, T. Effect of naloxone-precipitated morphine withdrawal on noradrenaline release in rat hippocampus in vivo. *Eur. J. Pharmacol.* 1992, 215, 333–336. [CrossRef]

110. Diaz, S.L.; Kemmling, A.; Balero, G.N. Baclofen reestablishes striatal and cortical dopamine concentrations during naloxone-precipitated withdrawal. *Neurochem. Int.* 2003, 42, 293–298. [CrossRef]

111. Hooshmandi, M.; Hosseinzamiri, N.; Janahmadi, M.; Khakpai, F.; Rohampour, K.; Doostmohammadi, M. Antagonism of orexin type-1 receptors (OX1Rs) attenuates naloxone-precipitated morphine withdrawal syndrome in rat dorsal hippocampus. *Pharmacol. Biochem. Behav.* 2017, 158, 39–48. [CrossRef] [PubMed]

112. Koob, G.F.; Stinus, L.; Le Moal, M.; Bloom, F.E. Opponent process theory of motivation: Neurobiological evidence from studies of opiate dependence. *Neurosci. Biobehav. Rev.* 1989, 13, 135–140. [CrossRef]

113. Acquas, E.; Di Chiara, G. Depression of mesolimbic dopamine transmission and sensitization to morphine during opiate abstinence. *J. Neurochem.* 1992, 58, 1620–1625. [CrossRef] [PubMed]

114. Diana, M.; Pistis, M.; Muntoni, A.; Gessa, G. Profound decrease of mesolimbic dopaminergic neuronal activity in morphine withdrawn rats. *J. Pharmacol. Exp. Ther.* 1995, 272, 781–785. [PubMed]

115. Elman, I.; Borsook, D.; Volkow, N.D. Pain and suicidality: Insights from reward and addiction neuroscience. *Prog. Neurobiol.* 2013, 109, 1–27. [CrossRef]

116. Fox, M.E.; Rodeberg, N.T.; Wightman, R.M. Reciprocal catecholamine changes during opiate exposure and withdrawal. *Neuropsychopharmacology* 2017, 42, 671–681. [CrossRef]

117. Sepulveda, M.J.; Hernandez, L.; Rada, P.; Tucci, S.; Contreras, E. Effect of precipitated withdrawal on extracellular glutamate and aspartate in the nucleus accumbens of chronically morphine-treated rats: An in vivo microdialysis study. *Pharmacol. Biochem. Behav.* 1998, 60, 255–262. [CrossRef]

118. Zhang, G.; Wu, X.; Zhang, Y.M.; Liu, H.; Jiang, Q.; Pang, G.; Tao, X.; Dong, L.; Stackman, R.W., Jr. Activation of serotonin 5-HT(2C) receptor suppresses behavioral sensitization and naloxone-precipitated withdrawal symptoms in morphine-dependent mice. *Neuropsychopharmacology* 2016, 101, 246–254. [CrossRef]

119. Zhou, Y.; Bendor, J.; Hofmann, L.; Randesi, M.; Ho, A.; Kreek, M.J. Mu opioid receptor and orexin/hypocretin mRNA levels in the lateral hypothalamus and striatum are enhanced by morphine withdrawal. *J. Endocrinol.* 2006, 191, 137–145. [CrossRef] [PubMed]

120. Matinfar, M.; Esfahani, M.M.; Aslany, N.; Davoodi, S.H.; Parsaee, P.; Zarei, G.; Reisi, P. Effect of repeated morphine withdrawal on spatial learning, memory and serum cortisol level in mice. *Adv. Biomed. Res.* 2013, 2, 80. [PubMed]

121. Meye, F.J.; van Zessen, R.; Smidt, M.P.; Adan, R.A.; Ramakers, G.M. Morphine withdrawal enhances constitutive µ-opioid receptor activity in the ventral tegmental area. *J. Neurosci.* 2012, 32, 16120–16128. [CrossRef] [PubMed]
Vanderschuren, L.J.; Pierce, R.C. Sensitization processes in drug addiction. In Mol. Interv. 2003, 142.

Wolf, M.E. LTP may trigger addiction. In Goodman and Gilman’s The Pharmacological Basis of Therapeutics; Hardman, J.G., Limbird, L.E., Eds.; McGraw-Hill: New York, NY, USA, 2001; pp. 569–619.

Mitra, S.; Sinat, R.S. Perioperative management of acute pain in the opioid-dependent patient. Anesthesiology 2004, 101, 212–227. [CrossRef]

Cecchi, M.; Capriles, N.; Watson, S.J.; Akil, H. Differential responses to morphine-induced analgesia in the tail-flick test. Behav. Brain Res. 2008, 194, 146–151. [CrossRef]

Célérier, E.; Yazdi, M.T.; Castañé, A.; Ghozland, S.; Nyberg, F.; Maldonado, R. Effects of nandrolone on acute morphine responses, tolerance and dependence in mice. Eur. J. Pharmacol. 2003, 465, 69–81. [CrossRef]

Joharchi, K.; Jorjani, M. The role of nitric oxide in diabetes-induced changes of morphine tolerance in rats. Eur. J. Pharmacol. 2007, 570, 66–71. [CrossRef]

Zarrindast, M.R.; Dinkoub, Z.; Homayoun, H.; Bakhtiarian, A.; Khavandgar, S. Dopamine receptor mechanism(s) and morphine tolerance in mice. J. Psychopharmacol. 2002, 16, 261–266. [CrossRef] [PubMed]

Ozdemir, E.; Bagívan, I.; Gursoy, S. Role of D1/D2 dopamine receptors antagonist perphenazine in morphine analgesia and tolerance in rats. Bosn. J. Basic Med. Sci. 2013, 13, 119–125. [CrossRef] [PubMed]

Singh, V.P.; Jain, N.K.; Kulkarni, S.K. Fluoxetine suppresses morphine tolerance and dependence: Modulation of NO-cGMP/DA/serotonergic pathways. Methods Find. Exp. Clin. Pharmacol. 2003, 25, 273–280. [CrossRef] [PubMed]

Gawel, K.; Gubala-Bruzda, E.; Dziedzic, M.; Jenda-Wojtanowska, M.; Marszalek-Grabska, M.; Silberring, J.; Kotlinska, J.H. Cholinergic activation affects the acute and chronic antinoceptive effects of morphine. Physiol. Behav. 2017, 169, 22–32. [CrossRef] [PubMed]

Abdollahi, H.; Ghaemi-Jandabi, M.; Azizi, H.; Semnanian, S. The role of orexin type-1 receptors in the development of morphine tolerance in locus coeruleus neurons: An electrophysiological perspective. Brain Res. 2016, 1646, 91–97. [CrossRef] [PubMed]

Wilson-Poe, A.R.; Lau, B.K.; Vaughan, C.W. Repeated morphine treatment alters cannabinoid modulation of GABAergic synaptic transmission within the rat periaqueductal grey. Br. J. Pharmacol. 2015, 172, 681–690. [CrossRef] [PubMed]

Liu, X.S.; Hou, Y.; Yan, T.L.; Guo, Y.Y.; Han, W.; Guan, F.L.; Chen, T.; Li, T. Dopamine D3 receptor-regulated NR2B subunits of N-methyl-d-aspartate receptors in the nucleus accumbens involves in morphine-induced locomotor activity. CNS Neurosci. Ther. 2014, 20, 823–829. [CrossRef] [PubMed]

Guegan, T.; Cebrà, J.P.; Maldonado, R.; Martin, M. Morphine-induced locomotor sensitization produces structural plasticity in the mesocorticolimbic system dependent on CB1-R activity. Addict. Biol. 2016, 21, 1113–1126. [CrossRef] [PubMed]

Shippenberg, T.S.; Heidbreder, C. Sensitization to the conditioned rewarding effects of cocaine: Pharmacological and temporal characteristics. J. Pharmacol. Exp. Ther. 1995, 273, 808–815.

Manzanedo, C.; Aguilar, M.A.; Rodríguez-Arias, M.; Miñarro, J. Sensitization to the rewarding effects of morphine depends on dopamine. J. Neuroreport. 2005, 16, 201–205. [CrossRef]

Rothwell, P.E.; Gewirtz, J.C.; Thomas, M.J. Episodic withdrawal promotes psychomotor sensitization to morphine. Neuropsychopharmacology 2010, 35, 2579–2589. [CrossRef] [PubMed]

Vanderschuren, L.J.; Kalivas, P.W. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. Psychopharmacology 2000, 151, 99–120. [CrossRef] [PubMed]

Wolf, M.E. LTP may trigger addiction. Mol. Interv. 2003, 3, 248–252. [CrossRef] [PubMed]

Vanderschuren, L.J.; Pierce, R.C. Sensitization processes in drug addiction. In Behavioral Neuroscience of Drug Addiction 2010; Springer: Berlin/Heidelberg, Germany, 2010; pp. 179–195.
144. Le Moal, M.; Simon, H. Mesocorticolimbic dopaminergic network: Functional and regulatory roles. *Physiol. Rev.* 1991, 71, 155–234. [CrossRef] [PubMed]

145. Robinson, T.E.; Berridge, K.C. The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res. Rev.* 1993, 18, 247–291. [CrossRef]

146. Kalivas, P.W.; Duffy, P. Sensitization to repeated morphine injection in the rat: Possible involvement of A10 dopamine neurons. *J. Pharmacol. Exp. Ther.* 1987, 241, 204–212.

147. Reisi, Z.; Bani-Ardalan, M.; Zarepour, L.; Haghparast, A. Involvement of D1/D2 dopamine receptors within the nucleus accumbens and ventral tegmental area in the development of sensitization to antinociceptive effect of morphine. *Pharmacol. Biochem. Behav.* 2014, 118, 16–21. [CrossRef]

148. Jeziorski, M.; White, F.J. Dopamine receptor antagonists prevent expression, but not development, of morphine sensitization. *Eur. J. Pharmacol.* 1995, 275, 235–244. [CrossRef]

149. Borgkvist, A.; Valjent, E.; Santini, E.; Hervé, D.; Girault, J.A.; Fisone, G. Delayed, context- and dopamine D1 receptor-dependent activation of ERK in morphine-sensitized mice. *Neuropharmacology* 2008, 55, 230–237. [CrossRef]

150. Jeziorski, M.; White, F.J.; Wolf, M.E. MK-801 prevents the development of behavioral sensitization during repeated morphine administration. *Synapse* 1994, 16, 137–147. [CrossRef]

151. Carlezon, W.A., Jr.; Rasmussen, K.; Nestler, E.J. AMPA antagonist LY293558 blocks the development, without blocking the expression, of behavioral sensitization to morphine. *Synapse* 1999, 31, 256–262. [CrossRef]

152. Stafford, K.; Gomes, A.B.; Shen, J.; Yoburn, B.C. Mu-Opioid receptor downregulation contributes to opioid tolerance in vivo. *Biochem. Behav.* 2001, 69, 233–237. [CrossRef]

153. Dang, V.C.; Christie, M.J. Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. *Br. J. Pharmacol.* 2012, 165, 1704–1716. [CrossRef] [PubMed]

154. Anselmi, L.; Jaramillo, I.; Palacios, M.; Huynh, J.; Sternini, C. Ligand-induced beta-arrestin-2 determines morphine tolerance but not dependence. *Nature* 2000, 408, 720–723. [CrossRef] [PubMed]

155. Nestler, E.J. Reflections on: “A general role for adaptations in G-Proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function”. *Brain Res.* 2016, 1645, 71–74. [CrossRef] [PubMed]

156. Bohn, L.M.; Gainetdinov, R.R.; Lin, F.T.; Lefkowitz, R.J.; Caron, M.G. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. *Nature* 2000, 408, 720–723. [CrossRef] [PubMed]

157. Bohn, L.M.; Lefkowitz, R.J.; Gainetdinov, R.R.; Peppel, K.; Caron, M.G.; Lin, F.T. Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science* 1999, 286, 2495–2498. [CrossRef] [PubMed]

158. Fan, X.L.; Zhang, J.S.; Zhang, X.Q.; Yue, W.; Ma, L. Differential regulation of beta-arrestin 1 and beta-arrestin 2 gene expression in rat brain by morphine. *Neuroscience* 2003, 117, 383–389. [CrossRef]

159. Al-Hasani, R.; Bruchas, M.R. Molecular mechanisms of opioid receptor-dependent signaling and behavior. *Anesthesiology* 2011, 115, 1363–1381. [CrossRef]

160. Narita, M.; Ioka, M.; Suzuki, M.; Narita, M.; Suzuki, T. Effect of repeated administration of morphine on the activity of extracellular signal regulated kinase in the mouse brain. *Neurosci. Lett.* 2002, 324, 97–100. [CrossRef]

161. Macey, T.A.; Bobeck, E.N.; Hegarty, D.M.; Aicher, S.A.; Ingram, S.L.; Morgan, M.M. Extracellular signal-regulated kinase 1/2 activation counteracts morphine tolerance in the periaqueductal gray of the rat. *J. Pharmacol. Exp. Ther.* 2009, 331, 412–418. [CrossRef] [PubMed]

162. Ferrer-Alcón, M.; García-Fuster, M.J.; La Harpe, R.; García-Sevilla, J.A. Long-term regulation of signaling components of adenylyl cyclase and mitogen-activated protein kinase in the pre-frontal cortex of human opiate addicts. *J. Neurochem.* 2004, 90, 220–230. [CrossRef] [PubMed]

163. Muller, D.L.; Unterwald, E.M. In vivo regulation of extracellular signal-regulated protein kinase (ERK) and protein kinase B (Akt) phosphorylation by acute and chronic morphine. *J. Pharmacol. Exp. Ther.* 2004, 310, 774–782. [CrossRef] [PubMed]

164. Asensio, V.J.; Miralles, A.; García-Sevilla, J.A. Stimulation of mitogen-activated protein kinase kinases (MEK1/2) by mu-, delta- and kappa-opioid receptor agonists in the rat brain: Regulation by chronic morphine and opioid withdrawal. *Eur. J. Pharmacol.* 2006, 539, 49–56. [CrossRef] [PubMed]
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
177. Ciccarelli, A.; Calza, A.; Santoru, F.; Grasso, F.; Concas, A.; Sasso
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
175. Mashayekhi, F.J.; Rasti, M.; Rahvar, M.; Mokarram, P.; Namavar, M.R.; Owji, A.A. Expression levels of the
180. Barrow, T.M.; Byun, H.M.; Li, X.; Smart, C.; Wang, Y.X.; Zhang, Y.; Baccarelli, A.A.; Guo, L. The e
179. Zhang, J.J.; Han, J.; Sui, N. Okadaic acid blocks the e
172. Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002, 16, 6–21. [CrossRef] [PubMed]
173. Hwang, C.K.; Song, K.Y.; Kim, C.S.; Choi, H.S.; Guo, X.H.; Law, P.Y.; Wei, L.N.; Loh, H.H. Evidence of
174. Hwang, C.K.; Kim, C.S.; Kim, D.K.; Law, P.Y.; Wei, L.N.; Loh, H.H. Up-regulation of the mu-opioid receptor
176. Sun, H.; Maze, I.; Dietz, D.M.; Scobie, K.N.; Kennedy, P.J.; Damez-Werno, D.; Neve, R.L.; Zachariou, V.;
178. Liu, P.; Zhang, J.; Li, M.; Sui, N. Distinctive Roles of 5-aza-2
170. Novikova, S.I.; He, F.; Bai, J.; Cutrufello, N.J.; Lidow, M.S.; Undieh, A.S. Maternal cocaine administration in
167. Smith, F.L.; Lohmann, A.B.; Dewey, W.L. Involvement of phospholipid signal transduction pathways in
166. Nabemoto, M.; Mashimo, M.; Someya, A.; Nakamura, H.; Hirabayashi, T.; Fujino, H.; Kaneko, M.; Okuma, Y;
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
177. Ciccarelli, A.; Calza, A.; Santoru, F.; Grasso, F.; Concas, A.; Sasso
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
175. Mashayekhi, F.J.; Rasti, M.; Rahvar, M.; Mokarram, P.; Namavar, M.R.; Owji, A.A. Expression levels of the
180. Barrow, T.M.; Byun, H.M.; Li, X.; Smart, C.; Wang, Y.X.; Zhang, Y.; Baccarelli, A.A.; Guo, L. The e
179. Zhang, J.J.; Han, J.; Sui, N. Okadaic acid blocks the e
172. Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002, 16, 6–21. [CrossRef] [PubMed]
173. Hwang, C.K.; Song, K.Y.; Kim, C.S.; Choi, H.S.; Guo, X.H.; Law, P.Y.; Wei, L.N.; Loh, H.H. Evidence of
174. Hwang, C.K.; Kim, C.S.; Kim, D.K.; Law, P.Y.; Wei, L.N.; Loh, H.H. Up-regulation of the mu-opioid receptor
176. Sun, H.; Maze, I.; Dietz, D.M.; Scobie, K.N.; Kennedy, P.J.; Damez-Werno, D.; Neve, R.L.; Zachariou, V.;
178. Liu, P.; Zhang, J.; Li, M.; Sui, N. Distinctive Roles of 5-aza-2
170. Novikova, S.I.; He, F.; Bai, J.; Cutrufello, N.J.; Lidow, M.S.; Undieh, A.S. Maternal cocaine administration in
167. Smith, F.L.; Lohmann, A.B.; Dewey, W.L. Involvement of phospholipid signal transduction pathways in
166. Nabemoto, M.; Mashimo, M.; Someya, A.; Nakamura, H.; Hirabayashi, T.; Fujino, H.; Kaneko, M.; Okuma, Y;
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
177. Ciccarelli, A.; Calza, A.; Santoru, F.; Grasso, F.; Concas, A.; Sasso
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
175. Mashayekhi, F.J.; Rasti, M.; Rahvar, M.; Mokarram, P.; Namavar, M.R.; Owji, A.A. Expression levels of the
180. Barrow, T.M.; Byun, H.M.; Li, X.; Smart, C.; Wang, Y.X.; Zhang, Y.; Baccarelli, A.A.; Guo, L. The e
179. Zhang, J.J.; Han, J.; Sui, N. Okadaic acid blocks the e
172. Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002, 16, 6–21. [CrossRef] [PubMed]
173. Hwang, C.K.; Song, K.Y.; Kim, C.S.; Choi, H.S.; Guo, X.H.; Law, P.Y.; Wei, L.N.; Loh, H.H. Evidence of
174. Hwang, C.K.; Kim, C.S.; Kim, D.K.; Law, P.Y.; Wei, L.N.; Loh, H.H. Up-regulation of the mu-opioid receptor
176. Sun, H.; Maze, I.; Dietz, D.M.; Scobie, K.N.; Kennedy, P.J.; Damez-Werno, D.; Neve, R.L.; Zachariou, V.;
178. Liu, P.; Zhang, J.; Li, M.; Sui, N. Distinctive Roles of 5-aza-2
170. Novikova, S.I.; He, F.; Bai, J.; Cutrufello, N.J.; Lidow, M.S.; Undieh, A.S. Maternal cocaine administration in
167. Smith, F.L.; Lohmann, A.B.; Dewey, W.L. Involvement of phospholipid signal transduction pathways in
166. Nabemoto, M.; Mashimo, M.; Someya, A.; Nakamura, H.; Hirabayashi, T.; Fujino, H.; Kaneko, M.; Okuma, Y;
165. Bianchi, E.; Lehmann, D.; Vivoli, E.; Norcini, M.; Ghelardini, C. Involvement of PLC-beta3 in the e
177. Ciccarelli, A.; Calza, A.; Santoru, F.; Grasso, F.; Concas, A.; Sasso-Pognetto, M.; Giustetto, M. Morphine
175. Mashayekhi, F.J.; Rasti, M.; Rahvar, M.; Mokarram, P.; Namavar, M.R.; Ovji, A.A. Expression levels of the
BDNF gene and histone modifications around its promoters in the ventral tegmental area and locus ceruleus of rats during forced abstinence from morphine. Neurochem. Res. 2012, 37, 1517–1523. [CrossRef] [PubMed]
176. Sun, H.; Maze, I.; Dietz, D.M.; Scobie, K.N.; Kennedy, P.J.; Damez-Werno, D.; Neve, R.L.; Zachariou, V; Shen, L.; Nestler, E.J. Morphine epigenomically regulates behavior through alterations in histone H3 lysine 9 dimethylation in the nucleus accumbens. J. Neurosci. 2012, 32, 17454–17464. [CrossRef] [PubMed]
177. Ciccarelli, A.; Calza, A.; Santoro, F.; Grasso, F.; Concas, A.; Sassò-Pognetto, M.; Giustetto, M. Morphine withdrawal produces ERK-dependent and ERK-independent epigenetic marks in neurons of the nucleus accumbens and lateral septum. Neuropharmacology 2013, 70, 168–179. [CrossRef]
178. Liu, P.; Zhang, J.; Li, M.; Sui, N. Distinctive Roles of 5-aza-2’-deoxycytidine in Anterior Agranular Insular and Basolateral Amygdala in Reconsolidation of Aversive Memory Associated with Morphine in Rats. Front. Behav. Neurosci. 2016, 10, 50. [CrossRef]
179. Zhang, J.J.; Han, J.; Sui, N. Okadaic acid blocks the effects of 5-aza-2’-deoxycytidine on consolidation, acquisition and retrieval of morphine-induced place preference in rats. Neuropharmacology 2014, 86, 282–293. [CrossRef]
180. Barrow, T.M.; Byun, H.M.; Li, X.; Smart, C.; Wang, Y.X.; Zhang, Y.; Baccarelli, A.A.; Guo, L. The effect of morphine upon DNA methylation in ten regions of the rat brain. Epigenetics 2017, 12, 1038–1047. [CrossRef]
181. Chen, X.T.; Pitis, P.; Liu, G.; Yuan, C.; Gotchev, D.; Cowan, C.L.; Rominger, D.H.; Koblish, M.; Dewire, S.M.; Crombie, A.L; et al. Structure-activity relationships and discoveries of a G protein biased μ opioid receptor ligand, [3-methoxyxthiophen-2-yl]methyl][2-[[(9R)-9-(pyridin-2-yl)-6-oxaspiro-[4.5]de can-9-yl]ethyl]amine (TRV130), for the treatment of acute severe pain. J. Med. Chem. 2013, 56, 8019–8031. [CrossRef] [PubMed]
182. Cheng, J.X.; Cheng, T.; Li, W.H.; Liu, G.X.; Zhu, W.L.; Tang, Y. Computational insights into the G-protein-biased activation and inactivation mechanisms of the μ opioid receptor. Acta Pharmacol. Sin. 2018, 39, 154–164. [CrossRef] [PubMed]
183. Madariaga-Mazón, A.; Marmolejo-Valencia, A.F.; Li, Y.; Toll, L.; Houghten, R.A.; Martinez-Mayorga, K. Mu-Opioid receptor biased ligands: A safer and painless discovery of analgesics? Drug Discov. Today 2017, 22, 1719–1729. [CrossRef] [PubMed]

184. Manglik, A.; Lin, H.; Aryal, D.K.; McCorvy, J.D.; Dengler, D.; Corder, G.; Levit, A.; Kling, R.C.; Bernat, V.; Hübner, H.; et al. Structure-based discovery of opioid analgesics with reduced side effects. Nature 2016, 537, 185–190. [CrossRef] [PubMed]

185. Negus, S.S.; Freeman, K.B. Abuse Potential of Biased Mu Opioid Receptor Agonists. Trends Pharmacol. Sci. 2018, 39, 916–919. [CrossRef]

186. Ranjan, R.; Pandey, S.; Shukla, A.K. Biased Opioid Receptor Ligands: Gain without Pain. Trends Endocrinol. Metab. 2017, 28, 247–249. [CrossRef] [PubMed]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).