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Authors

Kibaly, Cherkaouia
Xu, Chi
Cahill, Catherine M
et al.

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Non-nociceptive roles of opioids in the CNS: opioids' effects on neurogenesis, learning, memory and affect

Cherkaouia Kibaly[1,*], **Chi Xu**[2], **Catherine M. Cahill**[1], **Christopher J. Evans**[1], **Ping-Yee Law**[1]

[1]Department of Psychiatry and Biobehavioral Sciences, Semel Institute for Neuroscience and Human Behavior, Shirley and Stefan Hatos Center for Neuropharmacology, University of California, Los Angeles, CA, USA.

[2]State Key Laboratory of Natural Medicines, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, China.

Abstract

Mortality due to opioid use has grown to the point where, for the first time in history, opioid-related deaths exceed those caused by car accidents in many states in the United States. Changes in the prescribing of opioids for pain and the illicit use of fentanyl (and derivatives) have contributed to the current epidemic. Less known is the impact of opioids on hippocampal neurogenesis, the functional manipulation of which may improve the deleterious effects of opioid use. We provide new insights into how the dysregulation of neurogenesis by opioids can modify learning and affect, mood and emotions, processes that have been well accepted to motivate addictive behaviours.

The endogenous opioid system consists of approximately 30 different opioid peptides, including β-endorphins, Met[5]enkephalin and Leu[5]-enkephalin, orphanin FQ (also known as nociceptin) and dynorphins. These opioid peptides bind to their cognate G protein-coupled receptors, namely, the μ-opioid peptide receptor (MOP; also known as MOR), δ-opioid peptide receptor (DOP; also known as DOR), κ-opioid peptide receptor (KOP; also known as KOR) and nociceptin opioid peptide receptor (NOP; also known as OPRL1). The endogenous opioids and their receptors are expressed by various cell types and widely distributed throughout the body, including the central and peripheral nervous systems, immune cells, the adrenal medulla and the gonads, with the potential to modulate many

* kcherka@yahoo.fr.

Author contributions

C.K, C.X., C.M.C., C.J.E. and P-Y.L. researched data for the article and made substantial contributions to the discussion of content and to the writing, review and editing of the manuscript before submission.

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different physiological and psychological processes (FIG. 1). From the distribution of the opioid peptides throughout various brain regions, it is clear that the endogenous opioid system has functions beyond the modulation of pain perception. For example, in addition to their actions at the spinal cord and periaqueductal grey matter in modulating pain transmission, enkephalins are located within the amygdala, where they regulate emotional responses. Their presence in the autonomic nuclei of the hypothalamus is important for the regulation of cardiovascular and/or respiratory function, and they exert broad effects on anterior (and posterior) pituitary hormone secretion, including stimulating the release of prolactin, growth hormone and adrenocorticotropic hormone and inhibiting the release of luteinizing hormone, oxytocin and arginine vasopressin.

The hippocampus is a key brain structure crucial in learning and memory, especially in contextual memory. The opioid peptides and their receptors are expressed in all hippocampal regions[1–3], including the dentate gyrus (DG). The action of exogenous opioid drugs in the hippocampus is likely involved in the effects of opioids on learning and memory. Indeed, opioid drugs can impair anterograde and retrograde recall in patients with pain[4]. The other effect of opioids that is clearly linked to learning and memory is the development of addiction. Addiction and relapse have been interpreted as resulting from an abnormally robust memory formed during the opioid-taking experience[5]. Because the hippocampus is also included in the reward circuitry (Fig. 2), the opioid-dependent mnesic effects are also associated with the extensively studied positive (that is, pleasure) and negative (that is, stress, depression and anxiety related to withdrawal) affect and emotion, as well as the incentive salience characterizing the consumption of opiates[6]. The role of opioids in regulating both memory and affect, as well as emotion, is important in view of the current epidemiological context of opioid drug addiction, which is at an all-time high in North America, where opioid overdose is one of the leading causes of death. Indeed, the concept that both affective dysregulation and learning are the driving forces behind addictive behaviours, where the disruption of consolidating the contextual memory associated with drug experiences and rewards may have therapeutic potential to prevent relapse[6–8], has gained attention in the past decade[5].

The presence of opioid sites of action in the hippo-campus also explains the opioid modulation of neurogenesis, which is a phenomenon that occurs in two neurogenic brain regions, one of them being the DG[9–16]. Adult neurogenesis refers to the generation of new neurons in adult brain[17] (FIC. 3a), and it has been widely recognized to influence cognitive processes[18] such as affect, mood, emotion[19–21], learning and memory[22,23]. Activity of the new neurons selectively integrated into existing circuitry can be influenced by animal experiences[24] and may modify the hippocampal synaptic plasticity involved in learning and memory (that is, long-term potentiation (LTP))[25]. Thus, neurogenesis presents itself as a common mechanism through which opioids can alter affect, mood, emotion, learning and memory. Targeting neurogenesis in adults will provide new perspectives for the development of antiaddiction therapies. The other non-nociceptive opioid effects, such as respiratory depression, have not been correlated to learning and memory, affect and emotion, or neurogenesis to our knowledge. This Review highlights how opioid drugs can modulate adult neurogenesis in order to change learning and affect (that is, anxiety and depression). The term ‘opioids’ is often used instead of ‘opiates’ because of the difficulty in

distinguishing between the direct effect of opiates on biological processes and the opiate-dysregulated endogenous opioid system, which in turn can regulate the same processes.

Opioid effects on neurogenesis

Many reports describe the opioid-receptor-mediated regulation of neurogenesis[17] in both the embryonic and adult brain, along with in vitro studies using neural stem and progenitor cells (NSPCs) extracted from the brains of mice and rats. The results suggest the universal modulation by opioids of both embryonic and adult neurogenesis in a variety of brain sites. The difference between exogenous and endogenous opioids is noteworthy, because the modulatory effects of endogenous opioids are considered physiological, while the addictive effects of exogenous opioids, which are the primary focus of this Review, represent the pathological status.

Neural progenitor proliferation.

Although the majority of NSPCs in the CNS undergo differentiation and finally lose their capacity to divide, actively dividing cells in both embryonic and adult brains have nonetheless been observed. As an indispensable process in the maintenance of the stem pool and the generation of functional differentiated cells[17,26,27], the proliferation of NSPCs is a crucial step in the regulation of neurogenesis (FIG. 3a). Thus far, the regulation of NSPC proliferation is con-sistently observed in response to many factors, including MOP-targeting opioid drugs[17].

The antiproliferative effects of opioids on embryonic NSPC proliferation were shown in the dorsal telencephalon of the E15.5 embryonic mouse. Acute in utero morphine treatment resulted in a prolonged C2/M phase in both radial glial and basal progenitor cells, suggesting a role of morphine in inhibiting cell cycle progression and embryonic neurogenesis in the developing cortex[28]. More recently, it was found that acute morphine exposure inhibited the proliferation of NSPCs separated from cortices of E14 mice, which was reversed by the addition of naloxone, a non-selective antagonist of opioid receptors[29]. Similar results were discovered in NSPCs isolated from embryonic rat telencephalon, as chronic morphine treatment inhibited the proliferation rate of NSPCs in a concentration-dependent manner, and opioid receptor involvement was confirmed by a naloxone blockade.

The modulating effect of the opioid system on adult NSPC proliferation has been widely shown. This effect was first discovered by the labelling of 5-bromo-2’-deoxyuridine (BrdU)-positive cells in the DG of adult rats, which indicated an antiproliferative effect of both morphine and heroin[9]. Similarly, the cell cycle modulation of NSPCs in the adult mouse subgranular zone (SGZ) by morphine was further supported by using two endogenous cell cycle markers, proliferating cell nuclear antigen (PCNA) and phosphorylated histone H3 (pHisH3), along with BrdU labelling[10]. The impaired NSPC proliferation in adult animals was shown to be reversible, and drug withdrawal resulted in a rebound increase in proliferating cells, which was rescued to the normal level after 2 weeks of withdrawal[11].

There are two noteworthy aspects of adult NSPC proliferation inhibition by morphine. First, the administration paradigm determines the effect of modulating NSPC proliferation. For

example, pellet implantation, which produces a high and stable morphine concentration in the blood, resulted in a significant decrease in NSPC proliferation, whereas intermittent, single daily morphine injections had no significant effect[13]. Second, morphine modifies adult NSPC proliferation in a stagespecific manner[14]. For example, chronic morphine inhibited the proliferation of type 2b and type 3 NSPCs rather than cells of other stages[30]. The results above sup-port the antiproliferative effect of MOP-targeting opioid receptor agonists in both embryonic and adult NSPCs. In human fetal brain-derived NSPCs, a robust expression of KOP was observed and, in contrast to morphine, κ-agonists such as U50,488 and dynorphin 1–17 stimulated the proliferation and migration of these cells[31]. The effect of endogenous dynorphin remains unclear. The data point to differential effects of targeting different opioid receptor types.

NSPC differentiation and maturation.

Differentiation is the process that occurs when proliferating NSPCs give rise to offspring with different lineages and phenotypes. Regarding neurogenesis, neuronal differentiation of NSPCs and the following maturation of neuroblasts are crucial steps in determining the rate of newborn neuron generation[32]. The initiation of differentiation and line-age determination occurs at a stage as early as that of type 2 cells that feature limited self-renewal and transient amplification[33], which is therefore a crucial stage in the study of neurogenesis modulated by opioids.

It was reported that enkephalin and U69,593, agonists of MOP and KOP, respectively, promoted the differentiation of embryonic stem cells (ESCs) to neuronal-oriented precursors via the activation of extracellular-signal-regulated kinase (ERK)[34]. However, both opioid agonists inhibited NSPC-derived neurogenesis and astrogenesis via their corresponding receptors by means of p38-mediated and ERK-mediated pathways, respectively[35]. This report was supported by a recent in vitro study, as neuronal differentiation of primary embryonic NSPCs was inhibited by morphine sulfate at both early and late stages of cellular differentiation, implicating the role of opioids in fetal brain development[36]. Findings from studies using neural ESCs support the role of the third type of opioid receptor, DOP, in embryonic neurogenesis and neuroprotection, as its selective agonist SNC80 could promote neural differentiation through the activation of Trk-dependent tyrosine kinase, along with the association of PI3K, PKC, CaMKII and MEK[37]. Moreover, buprenorphine, a partial agonist of MOP and antagonist of DOP and KOP, was found to decrease the pro-liferation and differentiation of cultured rat embryonic NSPCs by inhibiting brain-derived neurotrophic factor (BDNF) expression[38]. The effects of opioids on NSPC differentiation may rely on the administration paradigm, the targeted opioid receptors and the origins of embryonic NSPCs.

The role of opioids in the differentiation of adult NSPCs and the maturation of new neurons and glia has been clearly demonstrated by recent studies. The phenotypes of DG granule cells were shown to be substantially altered by repeated morphine administration, and a marked rebound was detected after 1 week of withdrawal, suggesting that morphine inhibits the neuronal differentiation of DG granule cells[11]. Moreover, it was found that chronic morphine administration pre-vented neuronal maturation by increasing the percent-age of type 2b NSPCs while decreasing that of type 3 cells[31]. By contrast, in vitro experiments

showed that MOP and DOP antagonists were capable of promoting neuronal differentiation while inhibiting glial differentiation, supporting the involvement of opioid agonists in adult NSPC differentiation[39]. In the sub ventricular zone-olfactory bulb (SVZ-OB) system, however, it was found that naloxone inhibited neuronal differentiation induced by paced mating in female rats, indicating a role of endogenous opioids in facilitating, rather than inhibiting, the maturation of adult-born neurons[40]. In a recent study in mice, KOP agonists were found to promote oligodendrocyte differentiation and myelination, with therapeutic implications for multiple sclerosis[41]. Thus, it is clear that the endogenous opioid system, which modulates neural growth and development, is mimicked by exogenous opioids that disrupt neurogenesis by inhibiting the maturation of neurons[42].

Our recent research further elucidated the effects of opioids on the differentiation and maturation of adult NSPCs. Although morphine and fentanyl are both agonists of MOP, only morphine can modulate NSPC differentiation by inducing astrocyte-preferential differentiation. This ability of morphine to control mechanisms of cell fate determination is attributed to its regulation of the miR-181a-Prox1-Notch1 pathway, which is a result of different signalling mechanisms of the two agonists leading to MAPK activation[15] (FIG. 3b). Furthermore, the effect of morphine is due to its ability in activating the PKCε-TRBP-Dicer pathway, which modulates the processing of microRNA maturation[43]. Fentanyl, however, activates ERK via a β-arrestin-dependent pathway, thus resulting in decreased expression of miR-190, which targets and inhibits neurogenic differentiation 1 (NeuroD1), a transcriptional factor implicated in neuronal differentiation and maturation[44] (FIG. 3b). NeuroD1 also participates in opioid mediated neurogenesis, as its overexpression rescues the morphine-induced loss of late-stage progenitors and immature neurons[16]. These studies reveal the complexity of mechanisms that control NSPC differentiation and exemplify the mechanism of biased agonism in the regulation of adult neurogenesis by opioids.

The findings discussed above indicate several critical signalling cascades that are responsible for the alterations of neurogenesis induced by opioids. These cascades include MAPKs and other signalling cascades of transcriptional factors, such as the Pax6-Ngn2-Tbr2-NeuroD1-Tbr1 and Prox1-Notch1 signalling pathways[17]. More recently, it was discovered that the regulator of G protein signalling 4 (RGS4) could regulate STAT5B-directed responses, which in turn modulate opioid-induced neurite outgrowth and neuronal differentiation[45]. These signalling cascades, along with biased agonism (the ligand-dependent functional selectivity of a receptor for certain pathways), represent common mechanisms underlying opioid effects on neurogenesis.

Adult neurogenesis in humans and rodents.

The role of adult neurogenesis disruption by long-term opioids has mainly been studied using addiction-related behaviours such as reward, opioid-associated contextual memory and mood changes[46,47]. Manipulations that increase adult hippocampal neurogenesis, either by environ-mental enrichment, chronic antidepressant treatment or exercise, are correlated with reduced drug taking and relapse. Conversely, manipulations that decrease neurogenesis, such as stress and schizophrenia, are associated with increased drug taking and relapse[47]. Most of these behavioural data have been shown in rodents. However, the importance could be

disputed because of the current controversy regarding whether the adult human brain, like the rodent brain, continues to generate new neurons. Indeed, a study published in March 2018 by Sorrells et al.[48] concluded that neurogenesis in the hippocampus decreases throughout childhood and stops in the adult (after adolescence). Research on neurogenesis-regulated processes with animals would be useless for medical advances if the adult human brain could not make new neurons. However, a more recent report from April 2018 by Boldrini et al.[49] showed the contrary, namely, that neurogenesis is preserved in 79-year-old healthy adult humans. None of the patients presented psychiatric or chronic illness or drug and/or alcohol history, which, like their lifestyle, can influence the birth of new cells. The known history of the analysed post-mortem brains in the more recent study and the more accurate methodology tend to add more validity to the persistence of neurogenesis throughout human ageing. Moreover, over the past two decades, birth dating studies in different brain areas using BrdU[50] or 14C (REF.[51]) have supported the existence of adult human neurogenesis[52]. Despite the controversy, there is still the possibility that activities such as exercise and/or the administration of neurogenic molecules could reactivate an endogenous program of neurogenesis and generate new neurons in the human brain, as it has been demonstrated in rodents[53,54] and monkeys[55]. Therefore, strategies that target neurogenesis to rescue opioid-dependent learning and memory impairments should continue to be explored.

Opioid system, learning and memory

The strong association of environmental, and possibly interoceptive, cues with various hedonic aspects of drug experience is considered maladaptive. Indeed, this pathological opioid-associated memory undoubtedly renders abstinent patients more vulnerable to relapse when exposed to opioid-associated cues and/or context.

Opioid-associated memory.

The reinforcing effects of opioids and the underlying motivational circuit charac-terize opioid-associated learning. The opioid hedonic effect increases the motivation for further consumption by reinforcing the memories of the association between the opioid's feeling of relief (negative reinforcement) and/or pleasure (positive reinforcement), the drug taking and the exteroceptive and interoceptive cues[56]. These opioid-associated memories are also reinforced by a negative affect following dissipation of the opioid hedonic effect and precipitation of physical and emotional withdrawal, which motivate the individual to carry out compulsive drug seeking[6]. The rewarding and/or reinforcing effects of opioids influence memory processes, from encoding and storage to retrieval (Supplementary Box 1), with the opioid reward circuitry sharing the same major neural substrates as the memory system[6] (FIG. 2).

Opioid-associated learning necessitates primarily MOP activation, as demonstrated by the abolishment of morphine[57·58] and heroin[59] conditioned place preference (CPP) in MOP−/− mice and by the reduction of morphine self-administration in these mutant mice[60] (BOX 1; Supplementary Table 1). DOP mediates drug-context learning, as indicated by impaired morphine CPP in DOP−/− animals[61]. However, DOP is involved in the contextual encoding

but not the motivational aspect of a drug-associated experience because non-spatial cues predicting drug reward (for example, sound) restored morphine CPP in DOP−/− mice[62]. KOP activation does not appear to be directly involved in opioid-associated memory, as suggested by unchanged morphine CPP in KOP−/− mice[63] and dynorphin knockout mice[64]. Regarding endogenous opioids, it remains unclear how they control maladaptive opioid-associative learning and memory, although dysregulation of a β-endorphin basal inhibitory tone may be involved in contextual-associated learning, as suggested by increased morphine CPP in β-endorphin−/− mice[61,65,66] (BOX 2).

During chronic morphine exposure, prepro enkephalin upregulation in the hippocampus[67] or other endogenous opioids may interfere with hippocampal neurogenesis functioning, which potentially under-lies opioid-associated memory[16,46] (FIG. 4). Endogenous opioids decrease maturing neuron survival, as observed in MOP−/− mice[12]. Opiates such as morphine reduce the proliferation of progenitors and stimulate gliogenesis at the expense of neuronal differentiation[15]. To draw the mechanisms by which the modulation of neurogenesis could underlie the development of strong but maladaptive opioid-associated memories, we must consider neurogenesis modulation in correlation with every step of an opioid-associated memory task and during withdrawal. Indeed, neurogenesis modulation affects drug-associated learning processes in a time-dependent manner[53,68]. In one of the rare correlative studies, morphine was shown to decrease NeuroD1 activity, possibly via inhibition of CaMKIIα, the day of the CPP test (day 15 — one day after the conditioning session)[69]. During that day, the mice did not receive a morphine injection. Thus, it is unclear whether the NeuroD1 decrease in activity is related to morphine context acquisition during training or to the beginning of abstinence or extinction (that is, the new association ‘context-no morphine’). Furthermore, an absence of reducing effect of fentanyl on NeuroD1 activity[69] reflects a biased agonism that adds more complexity to the role of neurogenesis in opioid-associated learning and/or memory.

Chronic morphine decreases dendritic spine stability in rodent primary hippocampal neuronal cultures (a downstream event modulated by NeuroD1)[70–74] and also modulates hippocampal-dependent LTP[71]. LTP, a substrate of learning and memory, is influenced by neurogenesis[5,25]. There is confusion in interpreting the role of LTP in opioid-associated memory because there are few studies that correlate the time course of the LTP modulation by the opioids with each process of associative learning[75,76]. However, one of the few reports demonstrated that acute morphine infusion (1 hour) in lateral perforant path (LPP)-granule cell synapses of the DG attenuated LTP induction, while chronic morphine infusion for 72 hours augmented LTP[75]. Similarly, hippocampal slices from morphine-dependent rats maintained in artificial cerebrospinal fluid (ACSF) with morphine (to prevent spontaneous withdrawal) did not express CA1 LTP. By contrast, hip-pocampal slices from morphine-dependent rats maintained in ACSF with naloxone (precipitated withdrawal) or ACSF alone (spontaneous withdrawal) displayed LTP[76]. Therefore, the morphine-mediated enhanced LTP may not be involved in so-called opioid-cue association learning but may be the result of the dissipation of the morphine’s effects during withdrawal. This hypothesis of increased LTP reflecting morphine tolerance is supported by the fact that, unlike acute morphine, endogenous opioid blockade by naloxone, CTAP (MOP-targeting) or nor-BNI (KOP-

targeting) increases mossy fibre tetanus-induced LTP in wild-type rodents[77]. Moreover, morphine decreases glutamatergic transmission in primary hippocampal neurons[78].

Non-opioid-associated memory.

In humans, opioid drug administration affects daily task performances requiring forms of memory that use reinforcers (that is, motivators) different from opioids[4,79,80] (Box 1; Supplementary Box 1 and Supplementary Table 2). In rodents, acute and chronic morphine and/or heroin impairs hippocampus-dependent spatial acquisition[81,82] and retention and retrieval when injected after acquisition[83]. The same retention deficits were observed after β-endorphin, endomorphin 1 and endomorphin 2 administration[84,85]. By contrast, the opioid antagonist, either naloxone or naltrexone, facilitates spatial acquisition[86] and retention[87]. These results suggest that there is an endogenous opioid basal tone that inhibits learning, similar to that observed in β-endorphin−/− mice tested for morphine-associated memory[65]. The naltrexone-mediated or naloxone-mediated improved learning might result from enhanced stress generated from the endogenous basal tone removal[88]. The role of the endogenous opioid tone inhibition in enhancing spatial learning is questioned by the behavioural discrepancies between the genetic backgrounds and mutations of knockout animals. Studies using MOP−/− mice with a 100% C57BL/6 background and MOP exon 1 excised exhibited intact acquisition and first retrieval[86,89–91]. By contrast, MOP−/− mice with a hybrid genetic background (that is, 50% 129SVJ and 50% C57BL/6 or 129/Ola-C57BL/6) or MOP exons 2and 3 deleted displayed impaired spatial learning[57,92,93]. These discrepancies between the different genetic backgrounds need to be clarified.

The aforementioned opioid-induced mnesic deficits in tasks using reinforcers other than opioids may involve neuronal plasticity and neurogenesis modulation. Less well described is the opioid-antagonist-mediated learning facilitation in 100% C57BL/6 mice, which could be of interest for the development of promnesic treatments. One of the few proposed mechanisms suggests that chronic naltrexone increases hippo-campal glutamatergic neurotransmission to facilitate learning[77,86]. However, studies using MOP−/− mice with a 129SVJ background showed, by contrast, impaired learning associated with attenuated LTP in the CA3 (REF[92]) and the DG[94]. The lower level of neurogenesis in the 129SVJ strain of mice compared with the 100% C57BL/6 mice should be taken into account when considering the behavioural and electrophysiological differences[95]. Again, the contradicting results linked to the differences between genetic backgrounds must be resolved. The other hypothesis relies on the fact that naltrexone increases the number of new neurons[39,96]. The inhibition of the MOP endogenous tone may stimulate neurogenesis to positively influence long-term memory or even prophylactically delay or improve neuronal dysfunction in neurodegenerative disorders. No studies have assessed the effects of an opioid antagonist on neurogenesis in relation to both memory and neurodegeneration (for example, such as observed in dementia), but it is noteworthy that depot naltrexone treatment of opioid use disorder is having unanticipated success in reducing opioid craving[97].

Treatment of maladaptive memory.

Current treatments such as methadone replacement therapy or naltrexone maintenance treatment do not address the issue of addiction in opioid addicts, who must follow these

therapies throughout their lives to maintain permanent abstinence[98]. One of the strategies adopted by researchers to prevent relapse in patients recovering from opioid dependence is to pharmacologically enhance cue-exposure therapy (CET). During CET, subjects are exposed to repeated extinction trials that result in cues and context previously associated with drug taking becoming associated with the lack of drug[98–100]. Drug candidates for the development of anti-relapse treatments combined with CET act through direct modulation of the neurotransmission systems. A partial coagonist of the glutamatergic NMDA receptor, D-cycloserine, and an uncompetitive NMDA antagonist, memantine, are among these candidates and have the advantage of having been clinically tested[101,102]. D-Cycloserine facilitates the extinction of morphine-withdrawal-associated place aversion in morphine-dependent rats[102] and has already achieved positive results in CET for people with fear and anxiety disorders[102]. Memantine was clinically effective in reducing physical withdrawal signs in patients with heroin dependence[101] and attenuates the maintenance of morphine self-administration in mice[103].

Another approach focuses on the phenomenon of reconsolidation occurring in parallel to learning the new ‘cue-no drug’ association during extinction and CET[100,104]. Reconsolidation is the process in which the formerly acquired cue-drug memory becomes labile when reactivated or retrieved during extinction and subsequently restabilized into long-term storage through new protein synthesis after being subjected to reminders[100,104]. Treatments that interfere with reconsolidation would lessen the cue-drug association, its drug-seeking triggering effect and the probability of relapse. A few attempts to disrupt reconsolidation in opioid addiction were made by inhibiting ERK, protein synthesis with rapamycin, muscarinic acetylcholine receptors with scopolamine, NMDA receptors with ketamine and β-adrenergic receptors with propranolol[99,105]. All abolished the previously learned morphine CPP when administered after re-exposure to a drug-paired environment. Other potential new treatments that interrupt reconsolidation, such as blocking PKA activity with the inhibitor Rp-cAMPs, were effective in attenuating cue-induced reinstatement in cocaine self-administration[99] but need to be tested in opioid addiction.

An emerging anti-relapse strategy corresponds to the time-dependent manipulation of adult hippocampal neurogenesis via a pharmacological agent. Adult hippocampal neurogenesis has a time-dependent differential effect on drug-associated memory[68]. Our recent data published in 2017 regarding a novel synthetic compound, KHS101, which is known to specifically accelerate neuronal differentiation[54], showed that KHS101 injected before morphine CPP training prolonged the CPP extinction whereas when administered after morphine-induced CPP training, KHS101 shortened extinction[53]. The increase in neurogenesis that occurs after learning a cue-drug association or that occurs during a period of abstinence weakens the retention of the cue-drug association and the propensity of relapse. Molecules such as KHS101 or surgical interventions that stimulate neurogenesis such as NSPC transplantation[106] may have therapeutic potential in drug or opioid addiction. These strategies based on modulating neurogenesis have therapeutic potential, not only because they can weaken drug-associated memory and behaviour but also because of the involvement of neurogenesis in regulating emotional disorders such as depression and anxiety.

Anxiety and depression

Opioids are superior analgesics for treating moderate-to-severe pain because of their ability to diminish not only the sensory experience (pain intensity) but also the emotional, affective dimension (that is, how much it bothers the individual) pain. Thus, opioid analgesics have the ability to allow one to dissociate from their pain such that they no longer care about it, in addition to decreasing pain intensity. This ability of opioids to modulate affect transcends to mood disorders, and MOP agonists, at least acutely, have been recognized for decades to have antidepressant effects[107]. MOP agonists, with the exception of tianeptine, are not generally prescribed for depression[108]. Buprenorphine (a partial MOP agonist with KOP antagonist proper-ties) is effective in alleviating symptoms of treatmentresistant depression[109], and the KOP antagonism may contribute to its success in treating the negative affect experienced during addiction cycles. Chronic pain is often comorbid with mood disorders, which can range from 30–100% depending on the painaetiology. This co-occurrence of psychopathology in patients with chronic pain negatively influences their pain intensity, pain-related disability and the effectiveness of treatment (BOX 3). Additionally, long-term opioid use can precipitate depressive episodes[110]. We recently proposed that learning and memory processes are engaged in creating the association between opioid use and negative reinforcement (relief of negative affect, including during withdrawal) and that this memory contributes to drug relapse following prolonged periods of abstinence, because the learned association between opioid use and negative reinforcement may be generalizable to different stressors[7]. We surmise that in patients with chronic pain, the salient value associated with initial opioid use is pain relief, but it transitions to the alleviation of stress or depressed mood causative, or not, to the occurrence of pain. We proposed that subsequent stressors will trigger memories of the learned associations between opioid use and drug relief of dysphoric states, which could trigger drug relapse7. Many elegant studies have demonstrated that adult hippocampal neurogenesis contributes to the effectiveness of antidepressants[18,111], and it is not unreasonable that the antidepressant actions of opioids and the learned association between opioid use and negative reinforcement may engage adult neurogenesis. Although most studies suggest that MOP agonists negatively affect or disrupt hippocampal neurogenesis[9–11,15–17,28,29,31,35,36,43,46,112], the opposite may occur during cycles of withdrawal or via KOP, as KOP antagonists have antidepressant actions[113]. Furthermore, KOP is expressed on neural stem cells and NSPCs in the hippocampus, suggesting that KOP may modulate neurogenesis[114].

The link between neuropsychiatric disorders and adult DG neurogenesis has been widely recognized[115]. Stress and anxiety phenotypes are associated with a transient reduction in neurogenesis[116]. Humans with major depressive disorder have smaller hippocampi and fewer mature granule cells in the DG, which may be a result of reduced neurogenesis[117]. Supporting this association is the observation that the ablation of neurogenesis gives rise to anxiety and depression-like behaviour in rodents[118]. Moreover, recent studies revealed the requirement of new DG neurons for antidepressant efficacy[119,120]. More direct evidence supporting the association between adult neurogenesis and mood disorders was obtained by manipulating new DG neurons in the absence of substantial stress, showing an anti-anxiety effect after the stimulation of mature DG neurons[121].

The KOP exemplifies one of the most extensively studied systems involved in both mood disorders and adult neurogenesis[107]. KOP agonists and antagonists elicit depressive and antidepressive-like effects, respectively[122,123]. Various mechanisms and circuits involving monoamine transmitters have been pro-posed to account for these phenotypes, one of which is the involvement of KOP in adult neurogenesis. Buprenorphine, an antagonist of KOP, is a negative modulator of NSPC proliferation[112]. The antidepressant effects of KOP antagonist nor-BNI have been proposed to be due partly to an increase in BDNF expression in the hippocampus[124], which is well known to be a mechanism associated with the antidepressant effects of selective serotonin reuptake inhibitors (SSRIs). The DOP system also regulates affective-like behaviour, where constitutive knockout ofthe DOP leads to an anxiogenic phenotype[107]. Like KOP antagonists, DOP agonists produce antidepressant effects in rodent models, and this phenotype has been associated with increases in BDNF expression[125].

The role of the endogenous opioid system, especially the human MOP system, in depressive disorder has been underlined by a growing number of studies in recent years. For example, patients with depression showed MOP deactivation in the amygdala during social rejection, whereas healthy controls showed MOP activation in multiple brain regions[126]. This observation suggests the involvement of altered endogenous opioid activity in depression, which may help us yield novel therapeutics by using MOP agonists with a low risk of abuse[127]. Thus, we hypothesize the association between the endogenous opioid system, adult neurogenesis and mood and consider opioid-regulated neurogenesis and gliogenesis as novel targets for the treatment of psychiatric disorders.

Conclusion and perspectives

Knowledge of the impact of long-term opioid use on multiple biologic functions gives insight into how complex and challenging the treatment of the adverse effects of opioids can be. The most effective current medical intervention for individuals with opioid addiction is to substitute short-acting, euphoric opioids (for example, heroin) with long half-life and partial agonists such as buprenorphine or methadone to keep patients in a state where they are prevented from experiencing the reward that occurs following the administration of short-acting opioids, as well as alleviating withdrawal[128]. However, opioid maintenance therapy is defined as a harm reduction strategy aimed at relieving the cravings and symptoms of withdrawal rather than the prevention of opioid use[128]. The problem of relapse remains. Long-term exogenous opioid use alters the communication between the endogenous opioid system and the circuits involved in learning, memory and affect and the process of neurogenesis. The fact that addiction involves multiple brain functions and their underlying circuits is important when considering therapy. The treatment of addiction cannot be undertaken by only one modality.

A novel strategy that targets simultaneously opioidassociated maladaptive learning and memory, mood alteration and opioid reward via a common mechanism such as neurogenesis is worth studying for its antiaddiction therapeutic potential. Newborn healthy cells have the advantage of being able to rebuild a healthy environment, which may help to rebalance brain chemistry in patients with addiction[106,129] and lead to complete healing. Moreover, therapies based on promoting in situ neuronal generation may offer more healing potential than

strategies based on modulators of neurotransmitters such as memantine and D-cycloserine. NSPC transplantation[106] (that is, neural stem cell therapy) into specific brain areas involved in addictive behaviours could hypothetically have the same benefits as neurogenesis-based approaches. Indeed, in contrast to ESCs, the risk of tumour formation with NSPCs is reduced because NSPC differentiation is restricted to the neuronal and glial lineages[130]. The only limitation of both the neurogenesis-based strategy and NSPC transplantation is the possibility of propagation of the altered brain chemistry due to chronic opioid use from host to new cells or grafts such as in the case of Parkinson disease treatment[130]. Overall, cell replacement therapy has the potential to be the future of addiction therapy practice. Additionally, a holistic approach integrating personalized counselling, nutritional support, lifestyle modifications and/or mindfulness-based interventions[131] is required to avoid psychological and environmental causes and risk factors for relapse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Opioid
A broad term used to designate all substances, natural (for example, morphine) and synthetic (for example, fentanyl), that bind to opioid receptors in the nervous system

Hippocampus
A major anatomical structure located in the medial temporal lobe of the mammalian brain that processes a unidirectional flow of information via a trisynaptic loop

Learning
The process by which we integrate sensory information from our interaction with our environment for behavioural adaptation

Memory
The record left by a learning process

Affect
A broad range of feelings that people can experience, embodying both emotions and moods

Emotion
An intense feeling that is short term and is typically directed at a source, often with facial expressions and body language

Opiates
The natural alkaloid compounds found in the opium poppy plant *Papaver somniferum*

Neurogenic brain regions
in the adult mammal, these include the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone-oflactory bulb system

Mood
A less specific and less intense state of mind than emotion that is less likely to be provoked by a particular event but lasts longer

G2/M phase
A period of protein synthesis and rapid cell growth (C2) transitioning into division (M)

Cell cycle
A series of consecutive phases —Cap 1 (C1) phase, DNA synthesis (S) phase, gap 2 (C2) phase (growth) and mitosis or meiosis (M) phase — that lead to the duplication and division of genetic information into two daughter cells

5-bromo-2'-deoxyuridine
(BrdU). A synthetic analogue of thymidine and marker of proliferating cells

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Box 1 |

Opioid-associated and non-opioid-associated learning and memory tasks

Opioid-associated learning

Associative learning in opioid and drug abuse in animals is measured by conducting either classical (Pavlovian) or operant (instrumental) conditioning with opioids as reinforcers[132].

Classical conditioning.

The conditioned place preference assesses drug (including opioid) contextual-associated memoryand is usually utilized to examine drug reward. The animal learns to associate an environment or context (conditioned stimulus) with opioid taking (unconditioned stimulus). Through this association, if the drug is hedonic, the animal is expected to spend more time in the drug-paired environment to seek the drug (conditioned response)[133]. If withdrawal is associated with the context, such as in the conditioned place aversion task, the subject will avoid spending time in that context[134].

Operant (instrumental) conditioning.

Self-administration is the traditional model of drug abuse used to investigate the associative habit learning of drug taking. Self-administration is based on the reinforcing property of a drug to strengthen the learning of a stimulus (pressing a lever) and response (drug taking) habit that will gradually become a maladaptive, drug-directed form of habitual behaviour[135].

Non-opioid-associated (or any addictive drug) learning

Non-opioid-associated (or any addictive drug) learning refers to learning that is motivated by reinforcers other than opioids (or any other addictive drugs). For example, the measurement of spatial learning with the Barnes maze, radial arm maze, T-maze or Y-maze involves a positive reward, such as food, as an incentive to learn the area layout[136]. The Morris water maze also assesses spatial learning and relies on the water semi-aversive property to motivate learning the location of an escaping platform thanks to distant spatial cues[136]. Passive and active avoidance tests and fear conditioning are fear-motivated associative avoidance tasks during which subjects learn to avoid an environment by associating a cue or the environment itself with an aversive stimulus (for example, foot shock) previously delivered[136]. The novel object recognition task involves exploiting rodents' innate preference for novelty to evaluate recognition memory[136].

Box 2 |

Transient dysregulation of the endogenous opioid system homeostasis

Long-term opiate consumption and cessation cause transient disruption of the endogenous opioid system homeostasis. Therefore, the organism initiates allostatic changes in order to re-establish the neuronal systems[7,137]. Indeed, continuous circulation of exogenous opioids during chronic use overstimulates the endogenous opioid system. Consequently, inhibitory mechanisms take place to physiologically and psychologically oppose the maladaptive hedonic opioid effects and maintain homeostasis[7,137]. upon opiate removal, the compensatory actions against the endogenous opioid system effects become particularly important and unopposed and may interfere with the endogenous opioid system inhibitory control of, for example, aversive learning or stress, which can render abstinent patients with opiate dependence more susceptible to relapse.

For instance, following chronic morphine treatment in rodents, preproenkephalin transcription is decreased in structures responsible for reward and habit learning, such as the caudate-putamen (CPu) and nucleus accumbens (NAc), and increased in the hippocampus and cortex, both of which are involved in declarative learning and memory[61]. Endogenous opioids attenuate maturing neuron survival[12]. Hippocampal preproenkephalin upregulation may interfere with neurogenesis and thus cue-drug association learning. Under morphine withdrawal, preproenkephalin is downregulated in the CPu, NAc and pons, which are components of the basal ganglia, which controls voluntary motor movements and procedural learning[61]. By contrast, the dysphoric prodynorphin is upregulated following withdrawal from chronic intermittent injections in the CPu and rostral NAc[61]. Both enkephalin and β-endorphin inhibit the hypothalamic-pituitary-adrenal (HPA) axis response to stress[88,138]. Enkephalin may also mediate a basal hedonic tone and basal-ganglia-mediated motor control[65]. Therefore, both the preproenkephalin downregulation and prodynorphin upregulation during opioid abstinence and withdrawal could lead to a decrease in enkephalin's inhibitory control over the HPA axis and increased anxiety, respectively[88]. These phenomena may contribute to stress-related impulsive behaviour, which in turn may impair the executive function, working memory and fluid intelligence observed in early abstinence in patients with opioid dependence[79].

Box 3 |

Risks associated with opioid misuse

Risks associated with opioid misuse

- High catastrophizing scores in chronic pain are associated with craving
- Distress intolerance (the perceived or actual inability to manage negative emotional and somatic states)
- Previous history of illicit drug use
- Anxiety, depression or anger
- Aberrant drug-related behavior
- Sleep disturbance/low average number of hours of sleep
- Sex (males)

Factors that decrease opioid misuse

- For every 1-hour increase in the average number of hours of nightly sleep, the risk of misuse decreased by 20%[139]

Patients with chronic pain with notable psychopathology are more likely to report the following:

- Heightened pain intensity
- Increased pain-related disability
- More negative mood
- Reduced effectiveness of opioid analgesics compared with the effectiveness in patients with pain and no psychopathology

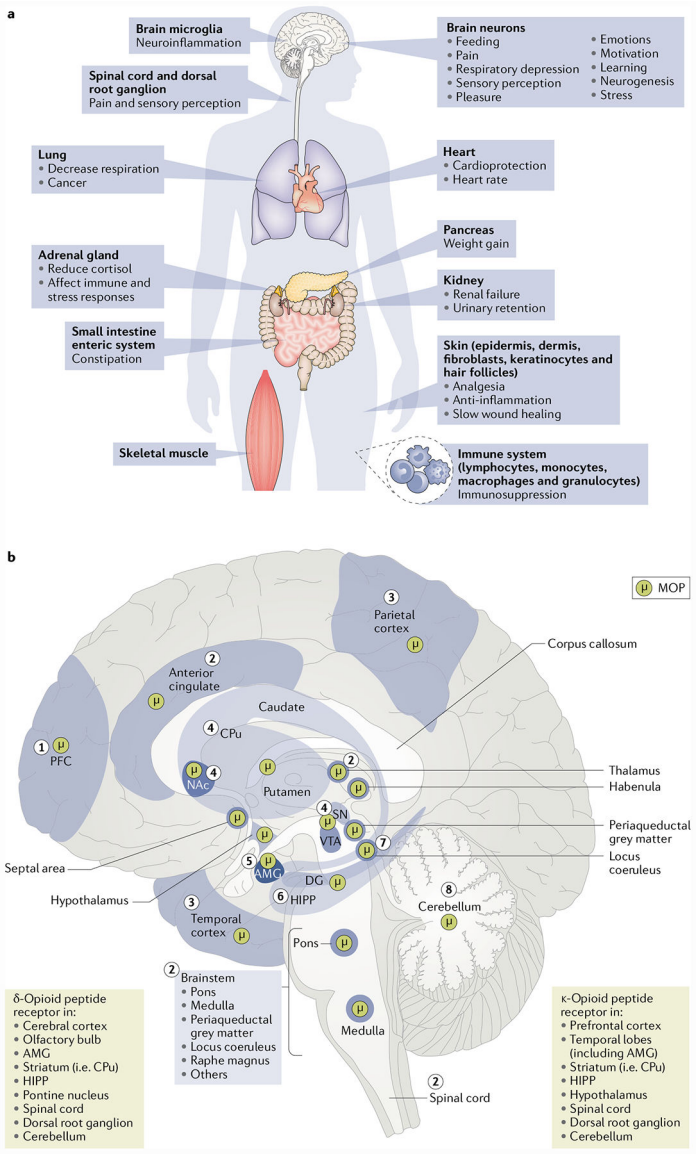

Fig. 1 |. Opioid actions throughout the body.

a | The human body naturally synthetizes opioids, which are used as neurotransmitters to regulate many vital functions. Endogenous and exogenous opioids act through receptors found peripherally on nerve terminals innervating the adrenal glands, pancreas and gastrointestinal tract and on immune, epidermal and dermal cells[140,141], thus modulating steroid production, body weight via insulin secretion, opioid-induced constipation, inflammation and wound healing[140,142–145]. **b** | Opioids also activate centrally located receptors, such as the μ-opioid peptide receptor (MOP), that play a variety of roles in brain function. Control of food and drug intake is regulated by MOP in the prefrontal cortex (PFC) (1); analgesia, slow breathing and relaxation can be induced by the activation of MOP in the anterior cingulate, thalamus, brainstem nuclei, spinal cord and dorsal root ganglia (2); sensory perception can be influenced by MOP in parietal and temporal cortices (3); motivation, desire and associative learning involve stimulation of MOP in the nucleus

accumbens (NAc), caudate-putamen (CPu) nuclei, ventral tegmental area (VTA) and substantia nigra (SN), key structures of the reward system (4); MOP is expressed in the amygdala (AMG), which is required for emotional conditioned learning and responses (5); MOP activation in the hippocampus (HIPP) can alter learningand neurogenesis (6); and MOP is present in both the locus coeruleus, a structure important in stress and drug withdrawal (7), and the cerebellum (8)[2,6,61,141,146,147]. In general, the expression of opioid peptides in projection neurons, immune cells, or epidermal and dermal cells overlaps with the location of opioid receptor expression, reflecting the autocrine, paracrine and endocrine mechanism of action of endogenous opioids[61,140]. DG, dentate gyrus.

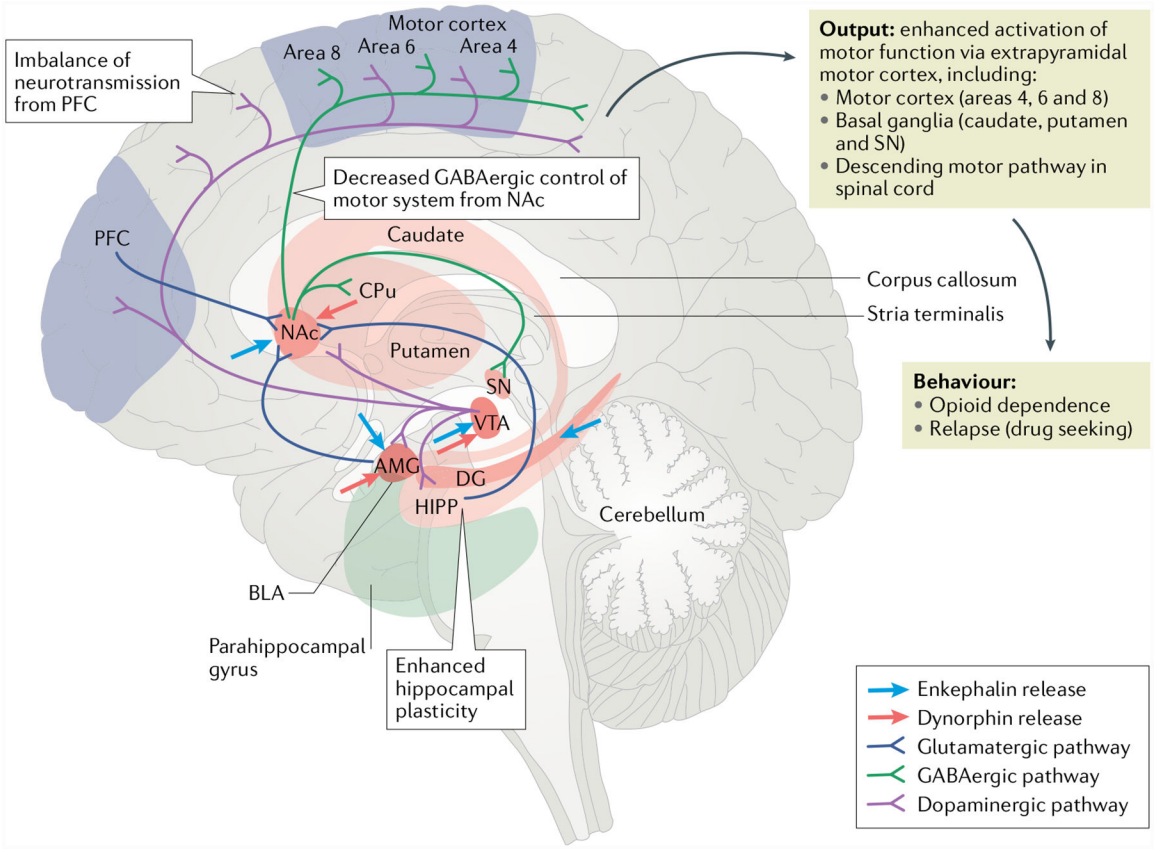

Fig. 2 |. Neural pathways involved in opioid-mediated mnesic changes.

Aberrant neuroadaptations in three associative memory systems have been described to contribute to drug addiction[6]. These systems include conditioned-incentive learning supported by the nucleus accumbens (NAc) and amygdala (AMG); habit learning depending on the caudateputamen (CPu); and declarative and contextual memory encoded by the hippocampus (HIPP)[6]. The NAc, AMG and CPu are also key components of the reward-salience-emotion circuit. The centrally localized NAc processes context and cue information from the HIPP — probably conveyed by glutamatergic projections — and is associated with the reinforcing effects of opioids, reflected by increased dopaminergic transmission from the ventral tegmental area (VTA). Opioids facilitate dopamine release in the NAc both directly and indirectly by inhibiting GABAergic control over the dopaminergic neurons in the VTA[148]. The opioid system influences the memory systems via enkephalinergic projections into the HIPP, NAc, AMG and VTA and via dynorphin projections into the NAc and/or AMG[2,6,146]. Additionally, the NAc integrates glutamatergic inputs from the basolateral amygdala (BLA), which encodes the environmental affective value during a drug experience. After long-term opioid exposure, the summed information resulting from the convergence of all these afferences[131] exits the NAc and is converted into the behaviour of opioid dependence or relapse to opioid seeking via enhanced extrapyramidal motor system activity[133,148]. Decreased GABAergic control of the motor system from the NAcand imbalance in neurotransmission in and from the prefrontal cortex (PFC) are hypothesized to contribute to the loss of inhibitory control and poor decision-making, leading to drug-induced reinstatement[6,148]. Opiate-withdrawal-related memory, reflected by enhanced

hippocampal plasticity during opiate withdrawal, can also facilitate relapse[5]. DG, dentate gyrus; SN, substantia nigra. Adapted from REF[149], CC-BY-2.0 (https://creativecommons.org/licenses/by/2.0/uk/).

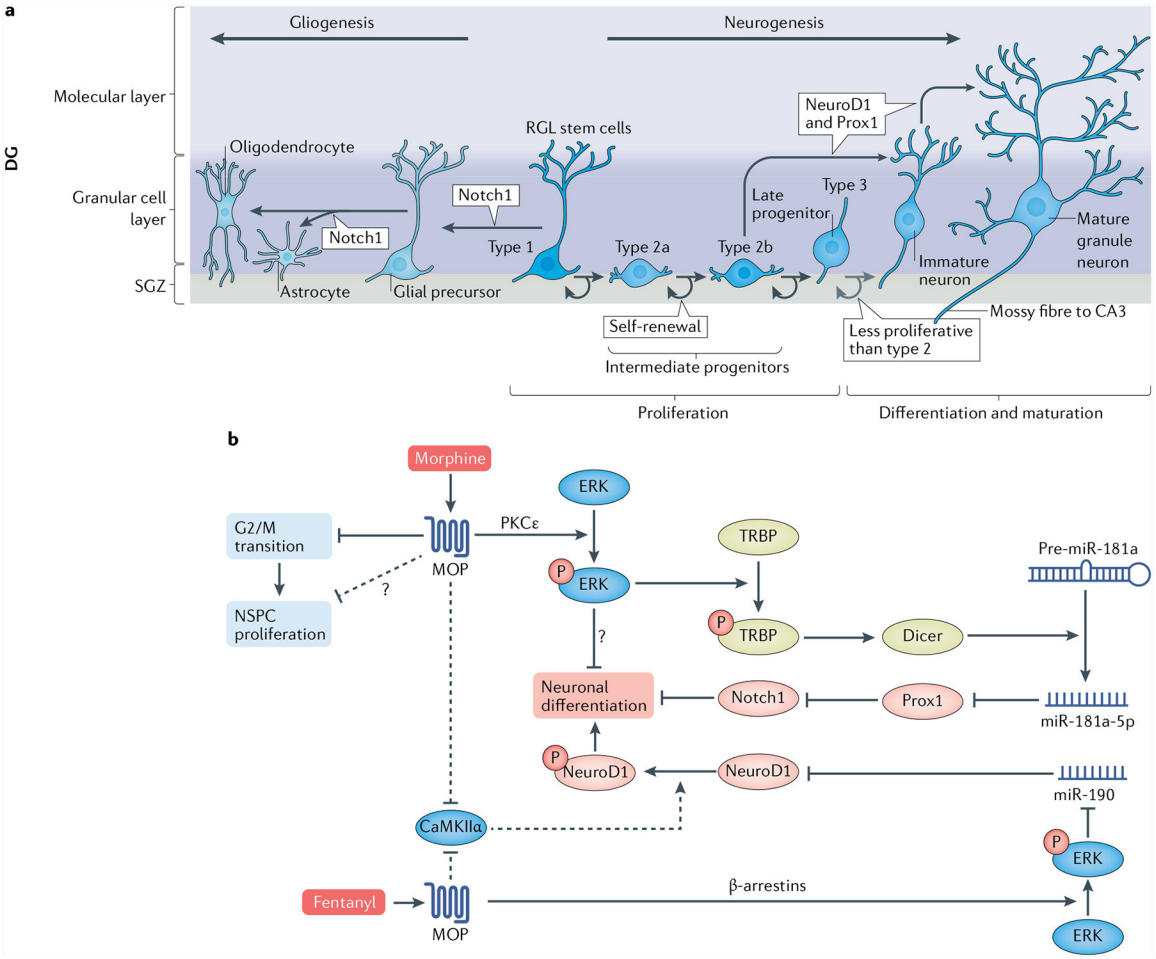

Fig. 3 |. Neurogenesis and biased MOP agonism in NSPC proliferation and differentiation.

a | The subgranular zone (SGZ) of the dentate gyrus (DG) provides a unique microenvironment where adult neural stem and progenitor cells (NSPCs) undergo several consecutive developmental stages characterized by different cell types[17]. NSPCs are derived from embryonic stem cells that survived in the adult SGZ. NSPCs self-renew and include type 1 radial-glia-like (RGL) stem cells, which give rise to intermediate progenitor cells type 2a and 2b. These type 2 cells can generate type 3 progenitors (neuroblasts), which are capable of differentiating into immature neurons. During maturation, immature neurons become granule neurons and are functionally integrated into the DG pre-existing circuit. Type 2b and type 3 cells, immature and mature neurons, express the transcription factors NeuroD1 and Prox1, which are specific to granule cell development[32]. Notch 1 promotes astroglial but not neuronal differentiation[17]. **b** | μ-Opioid peptide receptor (MOP) agonists (such as morphine) inhibit NSPC proliferation mainly by slowing the cell cycle G2/M phase transition (prolonged S phase) in mouse embryonic NSPCs[28], though details regarding the mechanism remain elusive. Other pathways leading to stagnating proliferation (indicated by the dashed arrow and question mark) might exist. Morphine induces extracellular-signal-regulated kinase (ERK) phosphorylation in a PKCε-dependent manner, and the activated ERK in turn phosphorylates TRBP, which is a stabilizer of Dicer, the essential enzyme for microRNA maturation[43]. The mature miR-181a-5p then inhibits the transcription factor

Prox1, an inhibitor of another transcription factor, Notch1. Thus, increased Notch1 expression mediates the inhibition of neuronal differentiation[15]. The inhibition of neurogenic differentiation 1 (NeuroD1) phosphorylation is another pathway by which morphine affects the transition of NSPCs into immature neurons in adult mice[16]. It is inferred that although both morphine and fentanyl affect NeuroD1 phosphorylation by inhibiting CaMKIIα, a crucial enzyme that catalyses NeuroD1 phosphorylation, fentanyl was able to activate NeuroD1 via the β-arrestin-ERK-miR-190 pathway, thus reflecting the biased antagonism of MOP agonists[44]. Involvement of this pathway has been demonstrated only in cultured primary neurons[74].

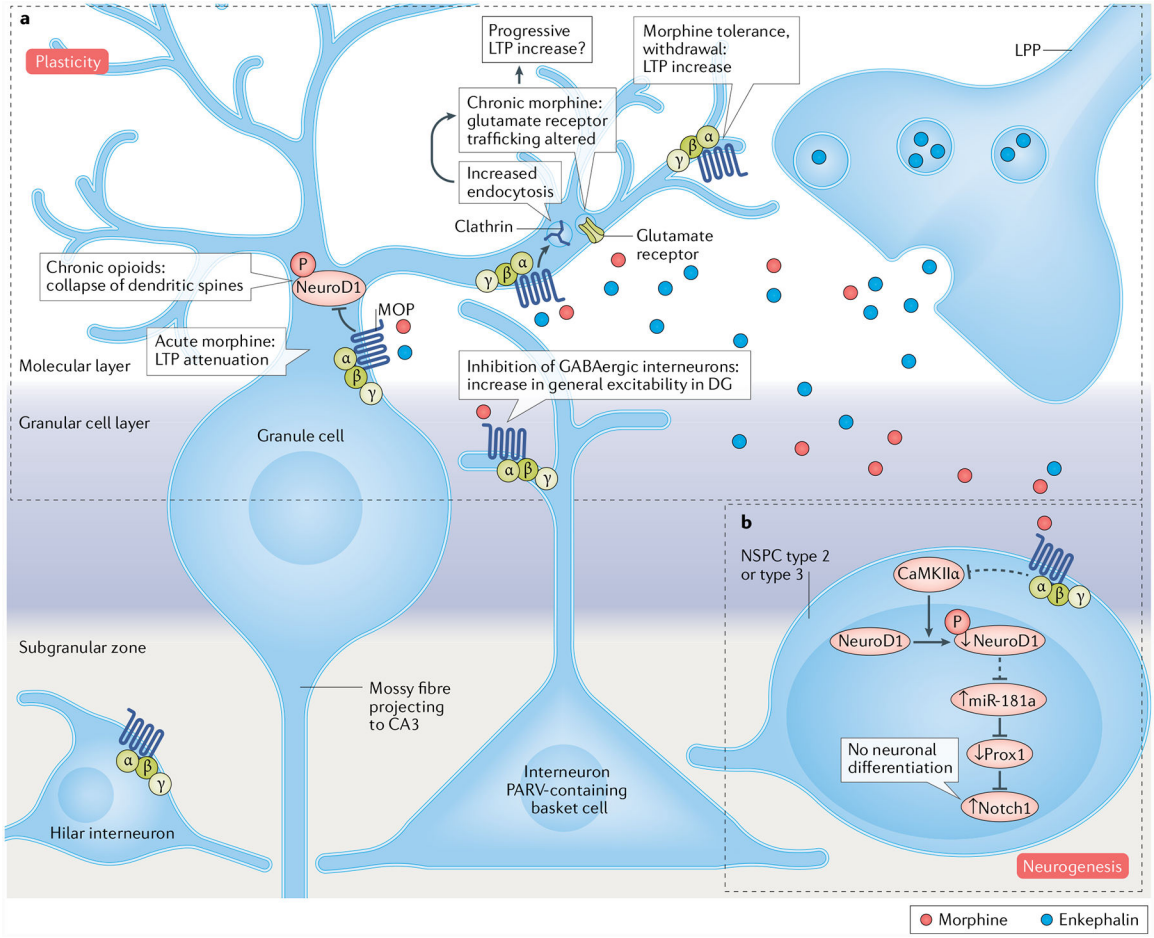

Fig. 4 |. Opioids alter normal learning by potentially regulating neurogenesis and plasticity.

a | In the dentate gyrus(DG) of the hippocampus, opioid receptors are located in the molecular layer, the granule cell layer[3,150] and the hilus[150] (not included in part **a**). The μ-opioid peptide receptor (MOP) is predominantly in distinct subpopulations of GABAergic interneurons known to inhibit granule cells: for example, the MOP is common in parvaĺbumin (PARV)-containing basket cell terminals that form inhibitory-type synapses with granule cell somata and dendrites, occurs occasionally in interneurons that innervate other interneurons (not represented) and appears in a modest number of hilar interneurons[150]. MOP immunoreactivity is also seen in some dendrites of granule cells[150]. Enkephalins are released by the lateral perforant path (LPP) projecting into the molecular layer[150]. Acute morphine infusion induces long-term potentiation (LTP) attenuation in LPP-granuĺe cell synapses[75]. Prolonged morphine exposure and the endogenous opioid tone decrease dendritic spine density possibly via reduced activity of neurogenic differentiation 1 (NeuroD1)[70,71,74,151], a neurogenic transcription factor expressed in cells such as the immature postmitotic granule neurons. Chronic morphine treatment (48 hours) also stimulates clathrin-mediated endocytosis, which alters glutamate receptor trafficking[73,78], thus leading to glutamatergic neurotransmission and LTP changes. After chronic administration (72 hours), tolerance to the effects of morphine enhances LTP in the same way as for opioid antagonists[75]. Opioid-agonist-mediated or opioid-antagonist-mediated

LTP modulations may cause learning impairment or improvement, respectively. **b** | In the sub granular zone, opioids are assumed to directly act on neural stem and progenitor cells (NSPCs) with MOP on the cell surface[39,47]. Morphine disrupts neurogenesis by attenuating the activity of NeuroD1, which is also expressed in late-stage dividing type 2b and type 3 NSPCs. Reduced NeuroD1 activity is correlated with up regulation of the microRNA miR-181a (dashed line, potential pathway), which silences Prox1 expression. The decrease in Prox1, being an inhibitor of Notch1, results in an increase in the levels of Notch1, whose role is to block neuronal differentiation[15], thus affecting normal learning.