REVIEW ARTICLE

Molecular Basis for Morphine Addiction

*Noor Azuin Suliman, Noor Azlina Abu Bakar, Zaidatul Akmal Othman

Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu Darul Iman, Malaysia.

ABSTRACT

Opioids are known to be used medically as analgesia and illegally as recreational drugs. Morphine, a semi-synthetic opioid is used widely in managing pain. Despite knowing the side-effect of the usage, the number of illegal users of opioids or morphine, specifically, is statistically still growing. Long-term usage of opioids, especially morphine, induces addiction that is expressed as dependence, tolerance, and withdrawal behaviour. Currently, with expanding research on anti-addiction studies, many loopholes in the basic mechanism of addiction were found, providing a setback for the researchers to overcome the problem. Thus, this review is aimed to present the latest update on the cellular modifications caused by chronic morphine treatment. By understanding and updating the knowledge, research can focus on the recent postulation and suggestions.
Malaysian Journal of Medicine and Health Sciences (2023) 19(SUPP12): 89-97. doi:10.47836/mjmhs.19.s12.11

Keywords: Chronic morphine; Morphine addiction; Morphine dependence; Opioid receptor; Molecular adaptation

Corresponding Author:
Noor Azuin Suliman, PhD
Email: azuinsuliman@unisza.edu.my
Tel: +60197764007

INTRODUCTION

Opioid or interchangeably called narcotic is a class of drug that was medically prescribed as a pain reliever agent that interacts with opioid receptors in the human body. Natural opioid, such as morphine, is derived from the opium poppy plant. There is also a synthetic opioid, such as methadone, and a semi-synthetic opioid, such as heroin. Opioids are used mainly as analgesic agents in treating medical conditions. The most prescribed opioids for reducing pain are morphine [1], methadone [2] and oxycodone [3]. Morphine, a common pain-relieving drug, is used in managing moderate to severe pain conditions such as major trauma, surgery and cancer [1, 4]. However, in recent years, there is a global problem arising from the recreational use of morphine that leads to the misuse of the drug.

Opioids have been used medically as analgesia with known adverse effects on addiction, dependence, tolerance, and withdrawal. The risk of its adverse effects has overshadowed the potential therapeutic purposes [1]. Chronic opioid user experience pain tolerance whereby the analgesic effects of the drug are decreased, thus triggering the user to increase the dose. Increased doses ensure the desirable anti-nociceptive effects accompanied by a series of adverse effects such as constipation and over-sedation [5]. Abrupt termination from the usage after pro-longed and high-dose opioid treatment causes withdrawal behaviour [6].

Morphine is categorised as a schedule II-controlled substance. Giving the same therapeutic effects as opioids, morphine is widely used during intra-operative, post-operative and chronic pain [1, 4]. Chronic morphine treatment stimulates adaptation and modification of homeostatic neuronal and synaptic processes, affecting different parts of the brain [4]. Morphine addiction causes multiple molecular adaptations and alterations, as discussed in detail in the content [7-11].

Morphine on multiple receptors

Activation of μ-opioid receptor by morphine treatment affects a different part of the brain, such as the thalamus [12], midbrain and brainstem [13] expressing the therapeutic effects of morphine as an analgesic agent. Comorbid health conditions and ageing people have less effect on morphine at the same dose as healthy and young people [14]. Therefore, the dose of morphine required by those populations is higher to ensure the same analgesic effect as others. However, overactivations of the μ-opioid receptor after a prolonged and high dose of morphine lead to addiction [15].

Activation of a κ-opioid receptor, located in the limbic system [16] and spinal cord [17], increases the stress level [17] and nociceptive performance [18]. To facilitate the action of morphine on the μ-opioid receptor, the κ-opioid receptor is inactivated [19].

Thus, it suggests, the activation of the κ-opioid receptor promotes the adverse effects of morphine, as shown in the overactivation of the μ-opioid receptor [20]. Rau [20] observed that blockage of the κ-opioid receptor had produced the therapeutic desire for morphine in the postoperative patient. However, recent reports reveal the activation of the receptor by morphine exhibits pain relief, sedation, and dependence effects [21].

Activation of δ-opioid receptors in the amygdala [22] mediates respiratory depression by modulating the μ- and κ-opioid receptors [23]. Activated μ- and δ-opioid receptors are responsible for the withdrawal symptoms observed in the user that terminate the morphine usage after prolonged use. Interestingly, blockage of μ- and δ-opioid receptors are observed in morphine-dependence users [24-25].

Forecasting the effects of morphine is challenging if only observing the involvement of the opioid receptor since reports claim the antagonist, agonist, and spatial agonist properties of this substance [25, 26]. Activation of different type of opioid receptor on different parts of the brain express different desired and adverse effects. Table I simplifies the involvement of different subtypes of G-proteins after morphine treatment with its effects.

Regulation of μ-opioid receptor/ G_i-protein coupling

Analgesia induced by opioids is mediated via the opioid receptors. The μ-opioid receptor initiates the effects of morphine to induce an analgesic reaction [15]. The opioid receptor is classified under the G-protein family. Upon binding of compounds to the G-protein-coupled receptor (GPCR), the G-protein heterotrimeric is associated with the receptor. It later leads to the change of guanosine triphosphate (GTP) from guanosine diphosphate (GDP) and dissociates the βγ subunit from the α-subunit (Fig 1).

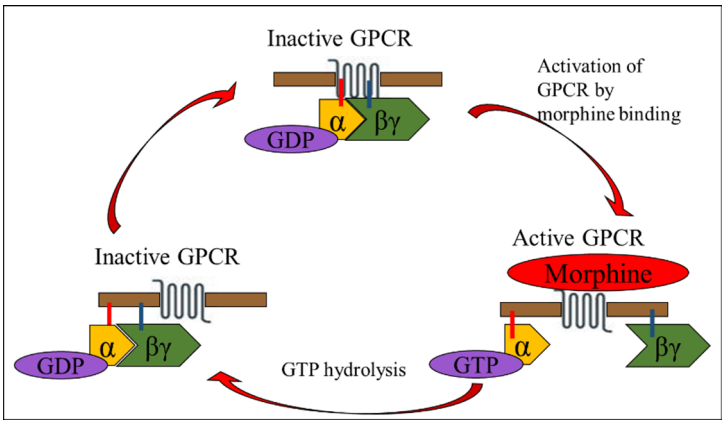

Fig. 1 : Schematic of G-protein cycle. During inactive G-protein-coupled receptor (GPCR), there receptor forms heterotrimer with Gαβγ subunit. The binding of morphine on the receptor causes the promotion of GTP from GDP, thus dissociating the Gα GTP-bound from the Gβγ subunit. Gα is responsible for hydrolysing GTP to GDP, thus subsequently re-binds to Gβγ and returns to its inactive form [87].

Activated G-protein α and βγ subunits interact with multiple cellular effector systems, which consequently causes the inhibition of adenylyl cyclase (AC), blockage of voltage-gated calcium ion (Ca^{2+}), and stimulation of G-protein-activated inwardly rectifying potassium ion (K^+) channel (GIRKs) and phospholipase Cβ (PLCβ) [27]. The coupling of opioid receptors to G-protein implicates tolerance and dependence exhibitions [28]. Tolerance to morphine is postulated to be related to opioid receptor desensitisation and up-regulation of G-protein expression [29]. The current study establishes the elevation of Gβ expression upon morphine tolerance with no changes in G_i or G_o subunits [9].

Treatment of morphine triggers the significant up-regulation of μ-opioid binding responsible for expressing the analgesia and tolerance effects [9]. The

Table I : Activation of different G-proteins upon treatment of morphine

G-protein	Reactions upon activation	Clinical expression	Unwanted clinical expression
$G_{αi}/G_{αo}$	Antagonist effect (Wang et al., 2016) - Inhibit AC - Inhibit production of cAMP	- Analgesic effect (Raffaeli and Indovina, 2015)	- Heart failure (Li et, 2016)
$G_{αq}$	Agonist effect (Ozdemir, 2017) - Increase the hydrolysis of PIP2 to DAG and IP3 - Activate PKC (Iftinca and Altier, 2020)	- Anti-hypertension (Zheng et al., 2011)	- Dilated cardiomyopathy (Movsesian and Bristow, 2005)
$G_{αs}$	Agonist effect (De Oliveira et al., 2022) - Stimulate AC - Increase production of cAMP	- Analgesic effect (Wang et al., 2016)	- Memory impairment (Liu et al., 2015)

AC= Adenylyl cyclase; cAMP= Cyclic adenosine 3′, 5′-monophosphate; PIP2= Phosphatidylinositol 4,5-bisphosphate; DAG= Diacylglycerol; IP3= Inositol triphosphate

up-regulated μ-opioid receptor induced by morphine is associated with $G_{i/o}$ protein interaction. Elevation of the μ-opioid receptor after morphine exposure leads to alteration in receptor internalisation cascade, including an adaptation of receptor trafficking, inhibition of receptor degradation, promotion of receptor synthesis and alteration in receptor conformation. The chronic treatment of morphine causes changes in μ-opioid receptor concentration [11, 30], depending on the regions of the brain.

Uncoupling of G protein from the opioid receptor

Phosphorylated receptor, upon binding with the agonist, recruits β-arrestin in which its complex promotes the receptor dissociation that causes receptor desensitisation [31]. Lacking of β-arrestin 2 upon chronic treatment of morphine prevents or decreases the desensitisation of the opioid receptor [32] that is responsible for tolerance symptoms [33].

β-arrestin and protein kinases affect receptor endocytosis, mediated by clathrin-coated pits [31]. The involvement of these protein play roles in initiating the internalisation of the receptor [34]. Chronic morphine treatment affects the activity of β-arrestin 2 which leads to μ-opioid receptor desensitisation which consequently induces an analgesic effect, as observed in tolerance symptoms [35].

The binding of morphine to an opioid receptor also influences the secondary messenger. The activation of the receptor by morphine activates the PKC cascade which is associated with the behavioural changes observed in the treated group [36]. The involvement of PKC in mediating the development of morphine addiction symptoms [37] is accompanied by the phosphorylation of ERK [38] and desensitisation of receptors [39].

Synaptic plasticity: LTP and LTD

Alterations of molecular, neuronal, and synaptic plasticity are observed during the development of drug addiction. The changes in synaptic plasticity are found to be similar to the changes observed in learning and memory physiological processes. This finding brings the mesolimbic reward system as the central role [40]. Main synaptic plasticity mechanisms altered by morphine exposure include long-term depression (LTD) and long-term potentiation (LTP) [41].

As LTP is a cellular model for memory study, studies are focused on the ERK cascade to observe cognitive performance during addiction. ERK, an important node for neuronal and synaptic alteration, phosphorylates the intracellular targets and is responsible for brain plasticity [42]. Activation of ERK cascades is influenced by the activation of other synaptic proteins such as CREB. Studies showed the upregulation of p-ERK and ERK after morphine exposure influences brain activity consistent with addiction behavioural alteration [43].

As morphine affects cognitive functions, the alteration might be contributed by the glial cell that is involved in the process of synaptic pruning and development. Repeated administration of morphine shows to improve LTP and delay LTD [44]. Those activities might involve the glial cell responsible for it playing an essential role in synaptic remodelling [44]. Activation of opioid receptors on glial cells triggers the release of signalling molecules, such as tumour necrosis factor-alpha (TNF-α) and interleukin-1b (IL-1b), subsequently modulating the synaptic activities [45].

In response to its role in synaptic strength, the glial cell promotes the release of glutamate that was triggered by exposure to morphine [46]. Chronic exposure to morphine modulates the adaptation of glutamatergic transmission in the brain, altering the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors [47]. Alteration of the NMDA receptor subsequently affects the LTD, suggesting the involvement of ionotropic glutamate receptors in morphine usage. Despite that, morphine withdrawal after chronic usage downregulates the activity of metabotropic glutamate receptors [48] and promotes the release of extrasynaptic glutamate in the nucleus accumben [49].

Activation of glial with increased inflammatory cytokine expression

A study done by Liu and colleagues [50] has demonstrated the involvement of microglial, astrocytes, and inflammatory cytokines prior to morphine introduction. Chronic morphine exposure activates microglia and astrocytes, binds to the innate immune receptor toll-like receptor 4 (TLR4) [51] and subsequently elevates the release of inflammatory cytokines and other mediators. Stimulation of the glia induces the synthesis and release of pro-inflammatory cytokines and facilitates the transmission of pain [52]. These cytokines lead to several events; elevate the neuronal excitability, sensitise the pain transmission neuron, increase the blockade of glutamate transporters, and up-regulate the AMPA and NMDA receptors [53]. These events subsequently induce morphine tolerance and dependence [51].

The inflammatory mediators such as nitric oxide (NO) could either protect the brain tissues from bacterial infection [54] or trigger neuronal damage [55]. Activation of microglial is postulated to involve the protein kinase Cε (PKCε), Akt, and mitogen-activated protein kinase (MAPK) as a mediator [56] (Fig 2). Reports have proven the effects of anti-inflammatory agents in suppressing opioid nociceptive tolerance. The agents are observed to suppress the activation of the glial and diminution of morphine-induced tolerance [50].

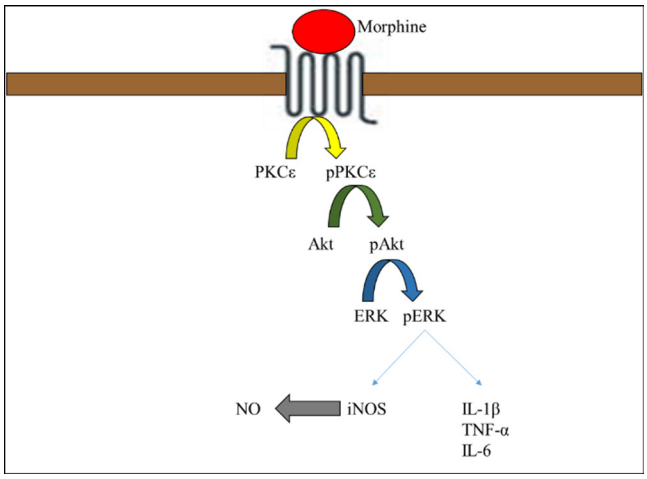

Fig. 2 : Schematic summary of the morphine binding to the μ-opioid receptor triggers the signal transduction cascade in microglial. The activation of receptors induced by morphine stimulates the kinases of PKCε, Akt, and ERK, thus consequently leading to the expression of pro-inflammatory chemokines and NO [56].

Activation of apoptosis cascade

Exposure to opioid receptor agonists, such as morphine, increases the susceptibility to death following the apoptotic mechanisms. Neuronal apoptosis shares a similar mechanism as morphine addiction [57]. An increased level of cholesterol damages the neuron, mediated by NMDA [1]. The stimulated NMDA receptor influences the intracellular pathways that subsequently cause apoptotic cell death. The excitatory amino acid and NMDA initiate the influx of Ca^{2+}, activate the Ca^{2+}-dependent enzymes and subsequently produce toxic oxygen radicals [58]. These events lead to cell death [59].

Morphine withdrawal and tolerance symptoms are reduced upon the inhibitory action of the NMDA receptor or the release of glutamate inhibitors [47, 51]. Studies proposed the usage of NMDA receptor antagonists and neuroprotective agents to prevent opioid tolerance [60-61] and apoptosis [62-63] by preserving the AKt and stimulating cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) phosphorylation [64-65].

Chronic morphine usage generates NO and oxidative stress (OS) that trigger addictive behaviour. Production of these compounds increases the concentration of Ca^{2+} and inflammation and apoptosis events via the TRPM2 channel [10]. NO, with modulatory effects, involves morphine-induced neurotoxicity that alters the activities in the brain, and leads to the development of addictive behaviours [66]. The NO-induced behavioural changes are mediated by the increased Ca^{2+} concentration and NMDA receptor activation [10].

The effects of morphine are mediated by the activation of opioids [67]. The analgesic effects of morphine influenced by δ-opioid receptors are associated with the modulation of the glutamatergic synaptic pathway at both pre- and post-synaptic [68]. The involvement of δ-opioid receptors in the glutamatergic cascade produces neuroprotective activities against glutamatergic stresses. Despite the influence of μ-opioid receptors on morphine addiction, δ-opioid receptors play an essential role in morphine adaptation activities through interaction with a μ-opioid receptors-dependent pathway [69].

Alteration of neurotransmission

Behavioural and neurochemical changes due to morphine usage are influenced by the adaptation in the neurotransmission process. Chronic morphine usage induces the cholinergic signalling pathways by significantly altering the level of acetylcholine (ACh) (depending on the region of the brain) [70] while its acute treatment reduces [71] or has no effect on its level [72].

The role of acetylcholinesterase (AChE) to break down the ACh becomes the suggested reason for the alteration in the neurotransmission process. Chronic and repeated morphine usage modifies the activity of AChE, thus subsequently influencing the level of extracellular Ach [73-74]. Tolerance to morphine after chronic morphine treatment reveals to increase the AChE activity, thus leading to tolerance symptoms and apoptotic brain cells [75] supporting the roles of AChE and ACh in inflammation. Morphine dependence and withdrawal after chronic treatment increase the activity of AChE leading to low levels of extracellular ACh in the brain, explaining the behavioural changes [74].

Behavioural and neurochemical changes of chronic morphine are associated with receptor adaptation. High expression of the nicotinic ACh receptor subunit (nAChR) initiates the release of ACh in the brain [76], leading to morphine withdrawal [77] and dependence behaviours [71]. Prolonged inhibition of AChE or increased release of ACh increases the expression of nAChR in the brain. The effect of a partial agonist of chronic morphine acts on α4β2 nAChR [78] which stimulates the release of ACh that subsequently expresses the behavioural changes [79].

The binding of morphine to its receptor causes alteration at the intracellular molecular level including suppression of secondary messenger and inhibition of Ca2+ level, which subsequently affects the secretion of neurotransmitters. Other than the cholinergic signalling pathway, chronic and repeated morphine treatment affects the GABAnergic pathway that influences rewarding and addiction behaviours [80]. Activation of nAChR and the activity of AChE influences the level of dopamine in chronic morphine [81-82].

Gene expression

As explained above, the ERK cascade influences intracellular adaptation prior to drug withdrawal [83]. Alteration in the ERK cascade modifies the gene expression by adding specific epigenetic marks on histone protein. These changes subsequently alter the structure of chromatin and influence the transcription processes [80]. ERK protein initiates the neuronal periphery, activates the mitogen and stress kinase 1 (MSK1) and leads to the phosphorylation of histone H3. The latter event consequently causes alteration in the gene expression [84].

Morphine withdrawal results from the activation of the Ras/ERK signalling that consequently affects the epigenetic pathway that leads to addiction behaviours [83]. Chronic treatment of morphine causes the adaptation of histone H3 which influences the transcriptional process. Table II summarises the genetic alteration observed in the brain after chronic morphine [85].

The expression of proteins in the brain regions is different due to anatomical connections and diverse functional roles of addiction [86]. In addition, phosphorylation of MSK1 and histone H3 depend on the activation of ERK through different pathways [84]. Various impacts of chronic morphine also activate different cascades, thus, affecting different gene expressions which express differences in addiction behaviour.

Table II : Summary of the cellular changes induced by morphine (Ciccarelli et al., 2013)

	Lateral septum	Nucleus accumben shell	Nucleus accumben core
Chronic morphine			
pERK	↓	-	-
pMSK1	-	-	-
pH3	-	-	-
Morphine withdrawal			
pERK	↑	-	-
pMSK	↑	↑	-
pH3	↑	↑	↑
AcH3	-	↑	-
MeCP2	-	-	-
pMeCP2	↑	↑	-
c-fos	↑	↑	↑
ARC	↑	↑	-

↑: increase expression; pERK: phosphorylated extracellular signal-regulated kinase; pMSK1: phosphorylated mitogen - and stress - activated protein kinase; pH3: phosphorylated histone H3; AcH3: acetylated H3; MeCP2: methyl-CpG-protein 2; ARC: activity-regulated cytoskeleton-associated protein

CONCLUSION

Chronic usage of morphine mediates adaptive mechanisms, scientifically observed at molecular levels, affecting the behaviour activities of the individual. Through the administration of morphine mediates different pathways, and it expressed similarities in behavioural performances such as rewarding and cognitive activities. Depending on the purpose of the study, either on withdrawal or dependence behaviours, the specific marker can be targeted as to be proposed to have anti-addiction properties.

ACKNOWLEDGEMENT

The authors are grateful to all staff of the Department of Physiology and Unit of Examination, Faculty of Medicine, UniSZA for their support.

REFERENCES

[1] Ghasemi F, Moradi A, Izadpanah E, Moloudi MR, Hassanzadeh K, Rahimmi A, et al. Simvastatin prevents morphine antinociceptive tolerance and withdrawal symptoms in rats. J Formos Med Assoc. 2015;114(5):399-406. doi: 10.1016/j.jfma.2014.07.011

[2] Elefritz JL, Murphy CV, Papadimos TJ, Lyaker MR. Methadone analgesia in the critically ill. J Critical Care. 2016;34:84-88. doi: 10.1016/j.jcrc.2016.03.023

[3] Ruan X, Mancuso KF, Kaye AD. Revisiting oxycodone analgesia: a review and hypothesis. Anesthesiol Clin. 2017;35(2):e163-74. doi: 10.1016/j.anclin.2017.01.022

[4] Ma Q, Fu Y, Cao Z, Shao D, Song J, Sheng H, et al. A conditioning-strengthened circuit from CA1 of dorsal hippocampus to basolateral amygdala participates in morphine-withdrawal memory retrieval. Front Neurosci. 2020;14:646. doi: 10.3389/fnins.2020.00646

[5] Imam MZ, Kuo A, Ghassabian S, Smith M. T. Progress in understanding mechanisms of opioid-induced gastrointestinal adverse effects and respiratory depression. Neuropharmacol. 2018;131:238-255. doi: 10.1016/j.neuropharm.2017.12.032

[6] Kosten TR, & Baxter LE. Effective management of opioid withdrawal symptoms: A gateway to opioid dependence treatment. Am J Addict. 2019;28(2):55-62. doi: 10.1111/ajad.12862

[7] Corder G, Tawfik VL, Wang D, Sypek EI, Low SA, Dickinson JR, et al. Loss of μ opioid receptor

signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia. Nat Med. 2017;23(2):164-73. doi: 10.1038/nm.4262

[8] Drastichova Z, Hejnova L, Moravcova R, Novotny J. Proteomic analysis unveils expressional changes in cytoskeleton-and synaptic plasticity-associated proteins in rat brain six months after withdrawal from morphine. Life. 2021;11(7):683. doi: 10.3390/life11070683

[9] Chakrabarti S, Liu NJ, Gintzler AR. Relevance of mu-opioid receptor splice variants and plasticity of their signaling sequelae to opioid analgesic tolerance. Cell Mol Neurobiol. 2021;41:855-62. doi: 10.1080/01480545.2021.1957558

[10] Osmanlıoğlu HLl, Yıldırım MK, Akyuva Y, Yıldızhan K, Nazıroğlu M. Morphine induces apoptosis, inflammation, and mitochondrial oxidative stress via activation of TRPM2 channel and nitric oxide signaling pathways in the hippocampus. Mol Neurobiol. 2020;57:3376-89. doi: 10.1007/s12035-020-01975-6

[11] Zhang P, Bu J, Wu X, Deng L, Chi M, Ma C, et al. Upregulation of μ-Opioid Receptor in the Rat Spinal Cord Contributes to the α2-Adrenoceptor Agonist Dexmedetomidine-Induced Attenuation of Chronic Morphine Tolerance in Cancer Pain. J Pain Res. 2020:2617-27. doi:10.2147/JPR.S274225

[12] Goedecke L, Bengoetxea X, Blaesse P, Pape HC, Jьngling K. μ-opioid receptor-mediated downregulation of midline thalamic pathways to basal and central amygdala. Sci Rep. 2019;9(1):1-9. doi: 10.1038/s41598-019-54128-8

[13] Khoramjouy M, Ahmadi F, Faizi M, Shahhosseini S. Optimization binding studies of opioid receptors, saturation and competition, using [3 H]-DAMGO. Pharmacol Rep. 2021;73:1390-5. doi:10.1007/s43440-021-00265-9

[14] Fullerton E. (2022). The impact of advanced age on morphine anti-hyperalgesia and the role of mu opioid receptor signaling in the periaqueductal gray of male and female rats. Dissertation. Georgia State University. doi: 10.57709/30509896

[15] Reiss D, Maduna T, Maurin H, Audouard E, Gaveriaux - Ruff C. Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice. J Neurosci Res. 2022;100(1):203-19. doi: 10.1002/jnr.24626

[16] Ona G, Sampedro F, Romero S, Valle M, Camacho V, Migliorelli C, et al. The kappa opioid receptor and the sleep of reason: cortico-subcortical imbalance following salvinorin-A. Int J Neuropsychopharmacol. 2022;25(1):54-63. doi: 10.1093/ijnp/pyab063

[17] Basu P, Custodio-Patsey L, Prasoon P, Smith BN, Taylor BK. Sex differences in protein kinase A signaling of the latent postoperative pain sensitization that is masked by kappa opioid receptors in the spinal cord. J Neurosci. 2021;41(47):9827-43. doi: 10.1523/JNEUROSCI.2622-20.2021

[18] Jacobson ML, Wulf HA, Browne CA, Lucki I. The kappa opioid receptor antagonist aticaprant reverses behavioral effects from unpredictable chronic mild stress in male mice. Psychopharmacol. 2020;237:3715-28. doi: 10.1007/s00213-020-05649-y

[19] Abraham AD, Schattauer SS, Reichard KL, Cohen JH, Fontaine HM, Song AJ, et al. Estrogen regulation of GRK2 inactivates kappa opioid receptor signaling mediating analgesia, but not aversion. J Neurosci. 2018;38(37):8031-43. doi: 10.1523/JNEUROSCI.0653-18.2018.

[20] Rau J, Hemphill A, Araguz K, Cunningham R, Stefanov A, Weise L, et al. Adverse effects of repeated, intravenous morphine on recovery after spinal cord injury in young, male rats are blocked by a kappa opioid receptor antagonist. J Neurotrauma. 2022;39(23-24):1741-55. doi: 10.1089/neu.2022.0208

[21] Brice - Tutt AC, Wilson LL, Eans SO, Stacy HM, Simons CA, Simpson GG, et al. Multifunctional opioid receptor agonism and antagonism by a novel macrocyclic tetrapeptide prevents reinstatement of morphine - seeking behaviour. Br J Pharmacol. 2020;177(18):4209-22. doi: 10.1111/bph.15165

[22] Sugiyama A, Yamada M, Saitoh A, Nagase H, Oka JI, Yamada M. Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. Psychopharmacol. 2018;235:2947-55. doi: 10.1007/s00213-018-4984-7

[23] Imam MZ, Kuo A, Ghassabian S, Cai Y, Qin Y, Li T, et al. Intracerebroventricular administration of CYX-6, a potent μ-opioid receptor agonist, a δ-and κ-opioid receptor antagonist and a biased ligand at μ, δ & κ-opioid receptors, evokes antinociception with minimal constipation and respiratory depression in rats in contrast to morphine. Eur J Pharmacol. 2020;871:172918. doi: 10.1016/j.ejphar.2020.172918

[24] Morgan MM, Peecher DL, Streicher JM. Use of home cage wheel running to assess the behavioural effects of administering a mu/delta opioid receptor heterodimer antagonist for spontaneous morphine withdrawal in the rat. Behav Brain Res. 2021;397:112953. doi: 10.1016/j.bbr.2020.112953

[25] Olson KM, Keresztes A, Tashiro JK, Daconta LV, Hruby VJ, Streicher JM. Synthesis and evaluation of a novel bivalent selective antagonist for the mu-delta opioid receptor heterodimer that reduces morphine withdrawal in mice. J Medl Chem. 2018;61(14):6075-86. doi: 10.1021/acs.jmedchem.8b00403

[26] Gastberger S, Baumgartner MR, Soyka M, Quednow BB, Hulka LM, Herdener M, et al. Concomitant heroin and cocaine use among opioid-dependent

patients during methadone, buprenorphine or morphine opioid agonist therapy. Eur Aadict Res. 2019;25(4):207-12. doi: 10.1159/000500542

[27] Senese NB, Kandasamy R, Kochan KE, Traynor JR. Regulator of G-protein signaling (RGS) protein modulation of opioid receptor signaling as a potential target for pain management. Front Mol Neurosci. 2020;13:5. doi: 10.3389/fnmol.2020.00005

[28] Kliewer A, Schmiedel F, Sianati S, Bailey A, Bateman JT, Levitt ES, et al. Phosphorylation-deficient G-protein-biased μ-opioid receptors improve analgesia and diminish tolerance but worsen opioid side effects. Nat Commun. 2019;10(1):367. doi:10.1038/s41467-018-08162-1

[29] Nestler EJ. 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;1645:71-4.doi: 10.1016/j.brainres.2015.12.039

[30] Bai X, Wang C, Zhang X, Feng Y, Zhang X. The role of testosterone in mu-opioid receptor expression in the trigeminal ganglia of opioid-tolerant rats. Neurosci Lett. 2020;723:134868. doi: 10.1016/j.neulet.2020.134868

[31] Peterson YK, Luttrell LM. The diverse roles of arrestin scaffolds in G protein–coupled receptor signaling. Pharmacol Rev. 2017;69(3):256-97. doi: 10.1124/pr.116.013367

[32] Ugur M, Derouiche L, Massotte D. Heteromerization modulates mu opioid receptor functional properties in vivo. Front Pharmacol. 2018;9:1240. doi: 10.3389/fphar.2018.01240

[33] Grim TW, Schmid CL, Stahl EL, Pantouli F, Ho JH, Acevedo-Canabal A, et al. AG protein signaling-biased agonist at the μ-opioid receptor reverses morphine tolerance while preventing morphine withdrawal. Neuropsychopharmacol. 2020;45(2):416-25. doi: 10.1038/s41386-019-0491-8

[34] Nguyen AH, Lefkowitz RJ. Signaling at the endosome: cryo - EM structure of a GPCR–G protein–beta - arrestin megacomplex. FEBS J. 2021;288(8):2562-9. doi: 10.1111/febs.15773

[35] He L, Gooding SW, Lewis E, Felth LC, Gaur A, Whistler JL. Pharmacological and genetic manipulations at the μ-opioid receptor reveal arrestin-3 engagement limits analgesic tolerance and does not exacerbate respiratory depression in mice. Neuropsychopharmacol. 2021;46(13):2241-9. doi: 10.1038/s41386-021-01054-x.

[36] Sabzevari S, Rohbani K, Sadat-Shirazi MS, Babhadi-Ashar N, Shakeri A, Ashabi G, et al. Morphine exposure before conception affects anxiety-like behavior and CRF level (in the CSF and plasma) in the adult male offspring. Brain Res Bull. 2019;144:122-31. doi: 10.1016/j.brainresbull.2018.11.022

[37] Pena DA, Duarte ML, Pramio DT, Devi LA, Schechtman D. Exploring morphine-triggered PKC-targets and their interaction with signaling pathways leading to pain via TrkA. Proteomes. 2018;6(4):39.doi: 10.3390/proteomes6040039

[38] Gao S, Li E, Gao H. Long non-coding RNA MEG3 attends to morphine-mediated autophagy of HT22 cells through modulating ERK pathway. Pharm Biol. 2019;57(1):536-42. doi: 10.1080/13880209.2019.1651343

[39] Ram A, Edwards TM, McCarty A, McDermott MV, Bobeck EN. Morphine - induced kinase activation and localization in the periaqueductal gray of male and female mice. J Neurochem. 2021;159(3):590-602. doi: 10.1111/jnc.15506

[40] Ahmadi S, Zobeiri M, Talvar SM, Masoudi K, Khanizad A, Fotouhi S, et al. Differential expression of H19, BC1, MIAT1, and MALAT1 long non-coding RNAs within key brain reward regions after repeated morphine treatment. Behav Brain Res. 2021;414:113478. doi: 10.1016/j.bbr.2021.113478

[41] Liu Z, Zhang J, Miao L, Kong Q, Liu X, Yu L. A novel CaMKII inhibitory peptide blocks relapse to morphine seeking by influencing synaptic plasticity in the nucleus accumbens shell. Brain Sci. 2022;12(8):985. doi: 10.3390/brainsci12080985

[42] Wu D, Wu F, Lin R, Meng Y, Wei W, Sun Q, et al. Impairment of learning and memory induced by perinatal exposure to BPA is associated with ERα-mediated alterations of synaptic plasticity and PKC/ERK/CREB signaling pathway in offspring rats. Brain Res Bull. 2020;161:43-54. doi: 10.1016/j.brainresbull.2020.04.023

[43] Yang X, Wen Y, Zhang Y, Gao F, Yang J, Yang Z, et al. Dynamic changes of cytoskeleton-related proteins within reward-related brain regions in morphine-associated memory. Front Neurosci. 2021;14:626348. doi: 10.3389/fnins.2020.626348

[44] Elahi-Mahani A, Heysieattalab S, Hosseinmardi N, Janahmadi M, Seyedaghamiri F, Khoshbouei H. Glial cells modulate hippocampal synaptic plasticity in morphine dependent rats. Brain Res Bull. 2018;140:97-106. doi: 10.1016/j.brainresbull.2018.04.006

[45] Shi Y, Yuan S, Tang SJ. Morphine and HIV-1 gp120 cooperatively promote pathogenesis in the spinal pain neural circuit. Mol pain. 2019;15:1744806919868380. doi: 10.1177/174480691986838

[46] Seyedaghamiri F, Heysieattalab S, Hosseinmardi N, Janahmadi M, Elahi-Mahani A, Salari F, et al. Hippocampal glial cells modulate morphine-induced behavioral responses. Physiol Behav. 2018;191:37-46. doi: 10.1016/j.physbeh.2018.04.003

[47] Qian Z, Wu X, Qiao Y, Shi M, Liu Z, Ren W, et al. Downregulation of mGluR2/3 receptors during morphine withdrawal in rats impairs

mGluR2/3-and NMDA receptor-dependent long-term depression in the nucleus accumbens. Neurosci Lett. 2019;690:76-82. doi: 10.1016/j.neulet.2018.10.018

[48] Kotlinska JH, Lopatynska-Mazurek M, Gawel K, Gabka P, Jenda-Wojtanowska M, Kruk-Slomka M, et al. Impact of the metabotropic glutamate receptor7 (mGlu7) allosteric agonist, AMN082, on fear learning and memory and anxiety-like behavior. Eur J Pharmacol. 2019;858:172512. doi: 10.1016/j.ejphar.2019.172512

[49] Pillai RL, Tipre DN. Metabotropic glutamate receptor 5–a promising target in drug development and neuroimaging. Eur J Nucl Med Mol Imaging. 2016;43:1151-70 .doi: 10.1007/s00259-015-3301-5

[50] Liu DQ, Zhou YQ, Gao F. Targeting cytokines for morphine tolerance: a narrative review. Curr Neuropharmacol. 2019;17(4):366-76. doi: 10.2174/1570159X15666171128144441

[51] Eidson LN, Murphy AZ. Inflammatory mediators of opioid tolerance: Implications for dependency and addiction. Peptides. 2019;115:51-8. doi: 10.1016/j.peptides.2019.01.003

[52] Tu H, Chu H, Guan S, Hao F, Xu N, Zhao Z, et al. The role of the M1/M2 microglia in the process from cancer pain to morphine tolerance. Tissue Cell. 2021;68:101438. doi: 10.1016/j.tice.2020.101438

[53] Miranda HF, Noriega V, Moreno F, Sierralta F, Sotomayor-Zбrate R, Prieto JC. Morphine at inflammatory experimental pain: A review. World J Adv Res Rev. 2022;15(1):116-21. doi: 10.30574/wjarr.2022.15.1.0675

[54] Olivera GC, Ren X, Vodnala SK, Lu J, Coppo L, Leepiyasakulchai C, et al. Nitric oxide protects against infection-induced neuroinflammation by preserving the stability of the blood-brain barrier. PLoS pathogens. 2016;12(2):e1005442. doi: 10.1371/journal.ppat.1005442

[55] Fujikawa DG. The role of excitotoxic programmed necrosis in acute brain injury. Comput Struct Biotechnol J. 2015;13:212-21.doi: 10.1016/j.csbj.2015.03.004

[56] Merighi S, Bencivenni S, Vincenzi F, Varani K, Borea PA, Gessi S. A2B adenosine receptors stimulate IL-6 production in primary murine microglia through p38 MAPK kinase pathway. Pharmacol Res. 2017;117:9-19. doi: 10.1016/j.phrs.2016.11.024

[57] Yan X, Gong Z, Pan R, Wang H, Tang H, He H, et al. Synergistic Effect and Mechanism of Apoptosis Induction by Morphine and the HIV-1gp120V3 Loop in Hippocampal Neurons. J Neuroimmune Pharmacol. 2021:1-6. doi: 10.1007/s11481-021-09989-0

[58] Burd I, Welling J, Kannan G, Johnston MV. Excitotoxicity as a common mechanism for fetal neuronal injury with hypoxia and intrauterine inflammation. Adv Pharmacol. 2016;76:85-101. doi: 10.1016/bs.apha.2016.02.003

[59] Khanna P, Ong C, Bay BH, Baeg GH. Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. Nanomaterials. 2015;5(3):1163-80.doi: 10.3390/nano5031163

[60] Gonzalez J, Jurado-Coronel JC, Avila MF, Sabogal A, Capani F, Barreto GE. NMDARs in neurological diseases: a potential therapeutic target. Int J Neurosci. 2015;125(5):315-27. doi: 10.3109/00207454.2014.940941

[61] Pribish A, Wood N, Kalava A. A review of nonanesthetic uses of ketamine. Anesthesiol Res Pract. 2020;2020. doi: 10.1155/2020/5798285

[62] Wu QJ, Tymianski M. Targeting NMDA receptors in stroke: new hope in neuroprotection. Mol Brain. 2018;11:1-4. doi: 10.1186/s13041-018-0357-8

[63] Li V, Wang YT. Molecular mechanisms of NMDA receptor-mediated excitotoxicity: implications for neuroprotective therapeutics for stroke. Neural Regen Res. 2016;11(11):1752. doi: 10.4103/1673-5374.194713

[64] Motaghinejad M, Motevalian M, Fatima S, Beiranvand T, Mozaffari S. Topiramate via NMDA, AMPA/kainate, GABA A and Alpha2 receptors and by modulation of CREB/BDNF and Akt/GSK3 signaling pathway exerts neuroprotective effects against methylphenidate-induced neurotoxicity in rats. J Neural Transm. 2017;124:1369-87. doi: 10.1007/s00702-017-1771-2

[65] Zuo D, Lin L, Liu Y, Wang C, Xu J, Sun F, et al. Baicalin attenuates ketamine-induced neurotoxicity in the developing rats: involvement of PI3K/Akt and CREB/BDNF/Bcl-2 pathways. Neurotox Res. 2016;30:159-72.doi: 10.1007/s12640-016-9611-y

[66] Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Amirgholami N, Houshmand G, Alboghobeish S. Venlafaxine inhibits naloxone-precipitated morphine withdrawal symptoms: Role of inflammatory cytokines and nitric oxide. Met Brain Dis. 2020;35:305-13. doi: 10.1007/s11011-019-00491-4

[67] Beltran-Campos V, Silva-Vera M, Garcнa-Campos ML, Diaz-Cintra S. Effects of morphine on brain plasticity. Neurologнa (English Edition). 2015;30(3):176-80. doi: 10.1016/j.nrleng.2014.08.001

[68] Zhang XY, Li Q, Dong Y, Yan W, Song K, Lin YQ, et al. Mu-Opioid receptors expressed in glutamatergic neurons are essential for morphine withdrawal. Neurosci Bull. 2020;36:1095-106. doi: 10.1007/s12264-020-00515-5

[69] Beaudry H, Gendron L, Moryn JA. Implication of delta opioid receptor subtype 2 but not delta opioid receptor subtype 1 in the development of morphine analgesic tolerance in a rat model of chronic inflammatory pain. Eur J Neurosci. 2015;41(7):901-7. doi: 10.1111/ejn.12829

[70] Jiang X, Zhang JJ, Song S, Li Y, Sui N. The duration

of withdrawal affects the muscarinic signaling in the nucleus accumbens after chronic morphine exposure in neonatal rats. J Neurophysiol. 2021;125(6):2228-36. doi: 10.1152/jn.00441.2020

[71] Neugebauer NM, Einstein EB, Lopez MB, McClure-Begley TD, Mineur YS, Picciotto MR. Morphine dependence and withdrawal induced changes in cholinergic signaling. Pharmacol Biochem Behav. 2013;109:77-83. doi: 10.1016/j.pbb.2013.04.015

[72] Bajic D, Soiza-Reilly M, Spalding AL, Berde CB, Commons KG. Endogenous cholinergic neurotransmission contributes to behavioral sensitization to morphine. PloS one. 2015;10(2):e0117601. doi: 10.1371/journal.pone.0117601

[73] Cansız D, Ustundag UV, Unal I, Alturfan AA, Emekli-Alturfan E. Morphine attenuates neurotoxic effects of MPTP in zebrafish embryos by regulating oxidant/antioxidant balance and acetylcholinesterase activity. Drug Chem Toxicol. 2022;45(6):2439-47. doi: 10.1080/01480545.2021.1957558

[74] Elwy AE, Tabl G. Impact of tramadol and morphine abuse on the activities of acetylcholine esterase, Na+/K+-ATPase and related parameters in cerebral cortices of male adult rats. Electron Physician. 2017;9(3):4027. doi: 10.19082/4027

[75] Qiu S, Feng Y, LeSage G, Zhang Y, Stuart C, He L, et al. Chronic morphine-induced microRNA-124 promotes microglial immunosuppression by modulating P65 and TRAF6. J Immunol. 2015;194(3):1021-30. doi: 10.4049/jimmunol.1400106

[76] Jensen KP, DeVito EE, Yip S, Carroll KM, Sofuoglu M. The cholinergic system as a treatment target for opioid use disorder. CNS drugs. 2018;32:981-96. doi: 10.1007/s40263-018-0572-y

[77] Sal F, Prados J, Urcelay GP. Nicotine chronic tolerance development and withdrawal in the planaria (Schmidtea mediterranea). Pharmacol Biochem Behav. 2021;200:173075. doi: 10.1016/j.pbb.2020.173075

[78] Bagdas D, Ergun D, Jackson A, Toma W, Schulte MK, Damaj MI. Allosteric modulation of α4β2* nicotinic acetylcholine receptors: Desformylflustrabromine potentiates antiallodynic response of nicotine in a mouse model of neuropathic pain. Eur J Pain. 2018;22(1):84-93. doi: 10.1002/ejp.1092

[79] Lee HW, Yang SH, Kim JY, Kim H. The role of the medial habenula cholinergic system in addiction and emotion-associated behaviors. Front Psychiatry. 2019;10:100. doi: 10.3389/fpsyt.2019.00100

[80] Listos J, Łupina M, Talarek S, Mazur A, Orzelska-Gyrka J, Kotlińska J. The mechanisms involved in morphine addiction: an overview. Int J Mol Sci. 2019;20(17):4302. doi: 10.3390/ijms20174302

[81] Brynildsen JK, Yang K, Lemchi C, Dani JA, De Biasi M, Blendy JA. A common SNP in Chrna5 enhances morphine reward in female mice. Neuropharmacol. 2022;218:109218. doi: 10.1016/j.neuropharm.2022.109218

[82] Motahari AA, Sahraei H, Meftahi GH. Role of nitric oxide on dopamine release and morphine-dependency. Basic Clin Neurosci. 2016;7(4):283. doi: 10.15412/J.BCN.03070401

[83] Crespo LG, Jьnior JB, de Mello Bastos JM, Samuels RI, Coimbra NC, Carey RJ, et al. Context evoked morphine conditioned effects can be equivalent to morphine induced drug effects in terms of behavioral response and ERK activation in reward associated subcortical brain structures. Pharmacol Biochem Behav. 2022;214:173356. doi: 10.1016/j.pbb.2022.173356

[84] Gйranton SM, Tochiki KK. Regulation of gene expression and pain states by epigenetic mechanisms. Prog Mol Biol Transl Sci. 2015;131:147-83. doi: 10.1016/bs.pmbts.2014.11.012

[85] Ciccarelli A, Calza A, Santoru F, Grasso F, Concas A, Sassoи-Pognetto M, et al. Morphine withdrawal produces ERK-dependent and ERK-independent epigenetic marks in neurons of the nucleus accumbens and lateral septum. Neuropharmacol. 2013;70:168-79. doi: 10.1016/j.neuropharm.2012.12.010

[86] Barrow TM, Byun HM, Li X, Smart C, Wang YX, Zhang Y, et al. The effect of morphine upon DNA methylation in ten regions of the rat brain. Epigenetics. 2017;12(12):1038-47. doi: 10.1080/15592294.2017.1398296

[87] Urano D, Chen JG, Botella JR, Jones AM. Heterotrimeric G protein signalling in the plant kingdom. Open Biol. 2013;3(3):120186. doi: 10.1098/rsob.120186