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REVIEW

Signaling mechanisms of μ-opioid receptor (MOR) in the hippocampus: disinhibition versus astrocytic glutamate regulation

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Abstract

μ-opioid receptor (MOR) is a class of opioid receptors that is critical for analgesia, reward, and euphoria. MOR is distributed in various brain regions, including the hippocampus, where traditionally, it is believed to be localized mainly at the presynaptic terminals of the GABAergic inhibitory interneurons to exert a strong disinhibitory effect on excitatory pyramidal neurons. However, recent intensive research has uncovered the existence of MOR in hippocampal astrocytes, shedding light on how astrocytic MOR participates in opioid signaling via glia-neuron interaction in the hippocampus. Activation of astrocytic MOR has shown to cause glutamate release from hippocampal astrocytes and increase the excitability of presynaptic axon fibers to enhance the release of glutamate at the Schaffer Collateral-CA1 synapses, thereby, intensifying the synaptic strength and plasticity. This novel mechanism involving astrocytic MOR has been shown to participate in hippocampus-dependent conditioned place preference. Furthermore, the signaling of hippocampal MOR, whose action is sexually dimorphic, is engaged in adult neurogenesis, seizure, and stress-induced memory impairment. In this review, we focus on the two profoundly different hippocampal opioid signaling pathways through either GABAergic interneuronal or astrocytic MOR. We further compare and contrast their molecular and cellular mechanisms and their possible roles in opioid-associated conditioned place preference and other hippocampus-dependent behaviors.

Keywords μ-opioid receptor · Hippocampus · Astrocyte · Disinhibition · Glutamate · LTP

Introduction

Opioids such as morphine have been widely used for effective treatment of severe pain. Opioids are also frequently used non-medically for their euphoric effects, and the recreational use of opioids typically results in addiction [1, 2]. The opioids act upon their specific opioid receptors, i.e., μ-opioid receptor (MOR), δ-opioid receptor (DOR), κ-opioid receptor (KOR), and nociceptin receptors (NOR) [3]. Among these four types of opioid receptors, the prototypical agonist of MOR is morphine [4]. The identity of opioid receptors including MOR was first revealed in the 1970s [5, 6], whereas opiates have been conventionally used for remedial and recreational purposes for the past several thousand years. Furthermore, the crystal structure of G-protein-coupled MOR was elucidated very recently [7, 8], which gave significant insights into the design of new MOR ligands with improved pharmacological properties targeting the MOR. There are several well-known exogenous MOR agonists, including morphine, oxycodone, and oxymorphone, which are widely used as analgesics and are also highly addictive substances [9]. In addition to these exogenous agonists, there are endogenous MOR agonists such as beta-endorphin and enkephalin [10, 11], which were firstly isolated by groups led by Chung and Morris, respectively

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[12–16]. MOR has a widespread but selective distribution in the major circuits of pain, reward, and addiction, particularly in the periaqueductal gray region, nucleus accumbens, amygdala, and hippocampus [17, 18].

In the hippocampus, MOR is broadly expressed on GABAergic interneurons [10, 19–22]. MOR is a $G\alpha_i$-protein-coupled receptor (Gi-GPCR), which is classically regarded as an inhibitory GPCR. Therefore, MOR activation by exogenous opioids of small molecular structures (e.g., morphine and oxycodone) and opioid peptides called exorphins (e.g., casomorphine and soymorphine) has been believed to excite neurons via suppression of presynaptic release of γ-aminobutyric acid (GABA) [23–25]. In addition to this traditional view, it has been recently demonstrated that a new surprising player of astrocytes in the CA1 hippocampus exhibits high expression of MOR [26], the activation of which elicits a significant release of glutamate from astrocytes through two-pore potassium (K2P) channels containing tandem of pore domains in a weak inward rectifying K^+ channel-1 (TWIK-1) and TWIK-related K^+ channel-1 (TREK-1) [27, 28]. These new findings suggest that astrocytic glutamate by MOR activation exerts a non-canonical excitatory effect of Gi-GPCR onto neighboring neurons.

In addition to the classical analgesic effect of opioids, several recent studies have focused on the relationship between MOR and diverse hippocampal functions. For example, MOR agonists affect cognitive performance such as spatial learning and memory by modulating the excitatory synaptic transmission in the hippocampus [29–31]. Moreover, MOR agonists are known to be engaged in adult hippocampal neurogenesis [32, 33], seizure [34–36], and stress-induced hippocampal changes [37, 38]. These studies allude to the possibility that hippocampal MOR plays key roles in many cognitive processes and pathological conditions, which might be under-appreciated and needs to be highlighted. However, there is not yet a comprehensive review on this important topic. Therefore, we have reviewed previous literature on the cellular localization of MOR, signaling pathways in interneurons and astrocytes, its role in synaptic transmission and plasticity, and the behavioral and cognitive functions of hippocampal MOR.

Cellular localization of MOR in the hippocampus

Four major subtypes of opioid receptors, three classical and one new subtype, have been characterized, which are MOR, DOR, KOR, and NOR, respectively [3]. Each subtype of opioid receptors has distinct functional differences with varying preferences for endogenous opioid peptides and exogenous ligands. Among them, MOR has a high affinity for beta-endorphin and enkephalin. Early immunohistochemical studies have reported that enkephalin is abundant in the hippocampus [39, 40], while beta-endorphin level has been inconclusive [41–43]. The presence of beta-endorphin needs to be confirmed by advanced sensor techniques such as G-protein-coupled receptor-based (GRAB) genetically encoded fluorescent sensor or fluorescence resonance energy transfer (FRET) sensor [44, 45].

Unlike the μ-opioid peptides, the localization of MOR has been established extensively. In the hippocampus, several early studies have demonstrated a relatively abundant expression of MOR in the pyramidal cell layer, *stratum lacunosum-moleculare*, and the molecular and granular cell layers of the ventral dentate gyrus [17, 46]. Subsequent immunocytochemistry and electron microscopy studies have shown that MOR is localized in the somatodendritic and axonal compartments of GABAergic neurons in rat hippocampal formation [22]. Among many types of GABAergic interneurons, MOR is expressed in most of the parvalbumin (PV)-containing basket cells and some of the somatostatin-, neuropeptide Y-, vasoactive intestinal peptide-, and calretinin-containing interneurons in the CA1 hippocampus [19–21]. In the dentate gyrus, MOR is extensively co-localized with PV, but not with somatostatin [21]. Consistently, a recent study using MOR-mCherry transgenic mice has clearly demonstrated that the expression of MOR in the CA1 pyramidal layer belongs to the presynaptic terminals of GABAergic interneurons, but not likely to the pyramidal neurons [26]. Taken together, these previous findings have suggested that MORs are most frequently found in the GABAergic interneurons, especially in PV-containing interneurons, which phasically inhibit the activities of granule cells and pyramidal neurons (Table 1 and Fig. 1).

Meanwhile, whether non-neuronal cells, such as astrocytes, express MOR has been a controversial issue. A research group led by Hauser reported that primary cultured hippocampal astrocytes exhibit only little amounts of MOR [47]. Three years later, the same group demonstrated that primary cultured astrocytes from various brain regions, including cortex and hippocampus, express MOR [48]. Although in vivo evidence of astrocytic MOR in the hippocampus had been lacking, recent lines of evidence have clearly demonstrated the expression of MOR on astrocytes in the hippocampus, ventral tegmental area, and the nucleus accumbens by utilizing MOR-mCherry, MOR-knock-out transgenic mice, and two different antibodies validated with MOR specific-short hairpin RNA (shRNA) [26, 49] (Table 1). An ultrastructural investigation with electron microscopy further revealed that MOR is mainly localized in the soma and processes, but rarely in the microdomains of hippocampal astrocytes [26]. In addition to astrocytes, microglia are also reported to express MOR in the murine cerebral cortex, hippocampus, and striatum (Table 1) [50]. These findings have raised a possibility of neglected role of

Table 1 Cellular distribution of MOR in hippocampus

Sub-region	Layer	Species	Cell type	Subcellular location	References
CA1	SO	Rat	Interneuron (PV, SOM, NPY, VIP, and calretinin)	Axon, Dendrite, Terminal	[19]
	SP	Rat	Interneuron (PV, SOM, NPY, VIP, and calretinin)	Terminal, Axon, Dendrite	[19]
	SR	Mouse	Astrocyte	Soma, Process	[26, 28, 62]
		Rat, Mouse	Interneuron (PV, SOM, NPY, VIP, and calretinin)	Axon, Terminal, Dendrite	[19–21, 26, 29]
	SLM	Rat	Interneuron (PV, SOM, NPY, VIP, and calretinin), Pyramidal-shaped neuron	Axon, Dendrite, Terminal	[19, 22]
CA3	SP	Rat	Unidentified	Process	[22]
	Slu	Rat	Granule cell (DG)	Axon process (mossy fiber)	[111]
	SLM	Rat	Unidentified	Process	[22]
	-	Rat	-	-	[84, 112]
DG	OML	Rat	Interneuron (PV)	Dendrite, Terminal	[21]
		Rat	Granule cell (very small portion)	Dendrite	[22]
	GCL	Rat	Interneuron (PV, calretinin-containing)	Soma, Terminal, Dendrite	[21]
	Hilus	Rat	Cholinergic and GABAergic neuron (PV, SOM)	Soma, Terminal, Dendrite	[21, 113]
	-	Rat	NSCs	-	[114]
-	-	Human	Microglia	-	[50]

SO striatum oriens, *SR* stratum radiatum, *SP* stratum pyramidale, *SLM* stratum lacunosum-moleculare, *Slu* stratum lucidum, *OML* outer molecular layer, *GCL* granule cell layer, *NSCs* neural stem cells, *PV* parvalbumin, *SOM* somatostatin, *NPY* neuropeptide Y, *VIP* vasoactive intestinal peptide

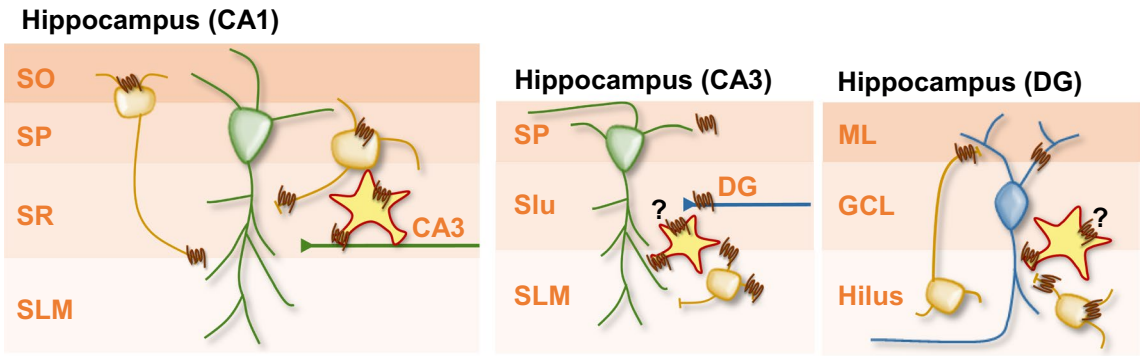

Fig. 1 Schematic diagrams of the MOR distribution in hippocampus region (CA1, CA3, and DG). Color of cell indicates cell types: gold, inhibitory neuron; green, pyramidal neuron; blue, granule cell; yellow, astrocyte. MOR is indicated in brown. *DG* dentate gyrus, *SO* stratum oriens, *SP* stratum pyramidale, *SR* stratum radiatum, *SLM* stratum lacunosum-moleculare, *Slu* stratum lucidum, *ML* molecular layer, *GCL* granule cell layer

glial, especially astrocytic, MOR in various behavioral and cognitive functions including conditioned place preference (CPP) and opioid addiction, which had been attributed solely to interneuronal MOR in the hippocampus.

The canonical signaling pathway of MOR via inhibitory neurons

MOR is found in all portions of PV-positive inhibitory interneurons, especially in axonal processes and terminals. A previous study has demonstrated that many MOR-positive terminals contact NMDAR-positive dendrites and form inhibitory synapses with each other [51]. This finding has raised a possibility that endogenous or exogenous MOR agonists (e.g., beta-endorphin, morphine, or some exorphins targeting MOR such as casomorphine) may activate MOR to suppress GABAergic activity, thus boosting excitatory action of dentate granule cells or pyramidal neurons by GABAergic disinhibition. Indeed, whole-cell patch clamp recordings of dentate granule cells showed that MOR activation reduced the amplitude of inhibitory post-synaptic currents evoked by electrical stimulation (eIPSCs) [52].

How does MOR activation suppress the GABAergic activity on the inhibitory interneurons? The activation of neuronal MOR is well documented to cause presynaptic depression through inhibition of N- and P/Q-type voltage-gated calcium channels [23]. In addition, activation of MOR causes dissociation of G-protein βγ-subunits ($G_{\beta\gamma}$) from an inactive heterotrimeric G-protein complexes ($G_{\alpha\beta\gamma}$), and the $G_{\beta\gamma}$ binds to and opens G-protein-coupled inwardly-rectifying potassium (GIRK) channels [53] (Fig. 2a, b). MOR also activates voltage-gated potassium channels [54] and increases the conductance of M-type potassium channels in hippocampus [55]. Through the potassium channels, potassium efflux leads to hyperpolarization of the cell membrane, which reduces the inhibitory action of interneurons [56]. Indeed, an endogenous MOR agonist, enkephalin was reported to hyperpolarize GABAergic interneurons [24]. This is how MOR has been thought to increase the activity of excitatory neurons through disinhibition. Furthermore, opioids are also known to exert analgesic effects through MOR-mediated disinhibition [57, 58].

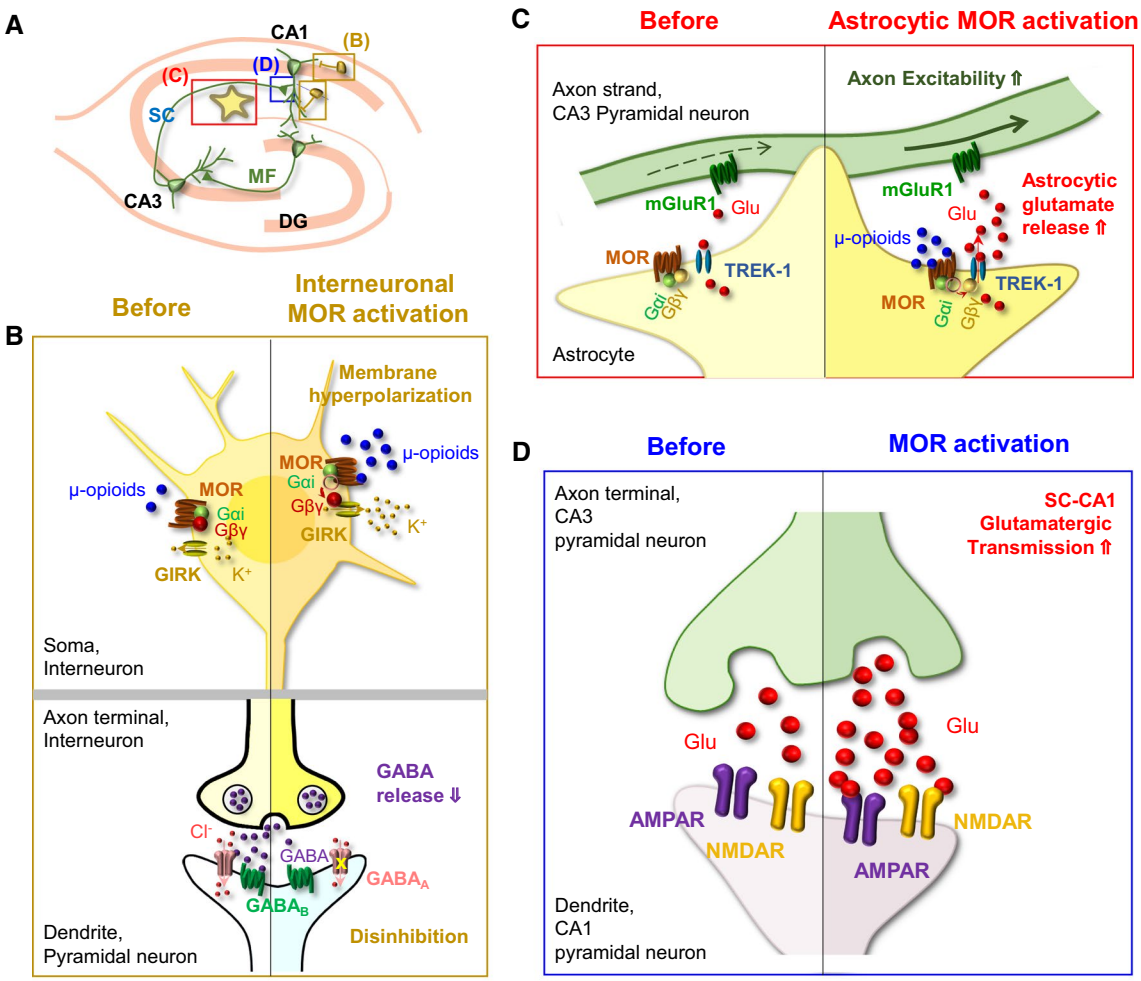

Fig. 2 Cellular mechanisms underlying how hippocampal MOR activation enhances synaptic transmission and plasticity. **a** Schematic diagram of hippocampal synapses. **b** Schematic diagram of interneuronal MOR signaling through disinhibition via membrane hyperpolarization of GABAergic interneuron. MOR activation in the interneurons dissociates $G_{\beta\gamma}$ from heterotrimeric G-protein complex, leading to the opening of GIRK by $G_{\beta\gamma}$ binding. Potassium efflux through GIRK causes hyperpolarization of the interneurons, which decreases GABA release and causes GABAergic disinhibition. **c** Schematic diagram of astrocytic MOR signaling through glutamate release. MOR activation in the astrocytes dissociates $G_{\beta\gamma}$ from heterotrimeric G-protein complex, leading to the opening of TREK-1 by $G_{\beta\gamma}$ binding. Glutamate release through TREK-1 binds to mGluR1, which is localized in the axonal process of presynaptic neurons, causing glutamatergic axonal excitability. **d** Schematic diagram of the alteration of glutamatergic synaptic transmission at SC-CA1 synapses. *CA1* cornu ammonis 1, *CA3* cornu ammonis 3, *DG* dentate gyrus, *SC* Schaffer collateral, *MF* mossy fiber, *MOR* μ-opioid receptor, $G_{\alpha i}$ G-protein alpha I subunit. $G_{\beta\gamma}$ G-protein beta gamma complex, *GIRK* G-protein-coupled inwardly-rectifying potassium channel, $GABA_A$ GABAA receptor, $GABA_B$ GABAB receptor, *Glu* glutamate, *mGluR1* metabotropic glutamate receptor 1, *TREK-1* TWIK-related potassium channel, *AMPAR* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *NMDAR* N-methyl-D-aspartate receptor

The MOR-mediated reduction in GABA release causes a suppression of both $GABA_A$ and $GABA_B$ receptor-mediated inhibitory signaling. MOR activation is also reported to facilitate the propagation of excitatory activity in CA1 hippocampus of a rat by disinhibition of all anatomical layers through a suppression of $GABA_A$ receptor signaling [59]. In addition to $GABA_A$ receptor signaling, it is also suggested that MOR activation hinders $GABA_B$-mediated hyperpolarization in CA1 hippocampus to boost the excitatory activity [60]. These findings together indicate that MOR activation in the GABAergic interneurons causes a suppression of GABAergic interneuronal activity, which in turn disinhibits the excitatory neurons in the hippocampus. So far, this mechanism, *i.e.* disinhibition through interneuronal MOR, has been accepted as the sole contributor of the MOR-mediated excitation in the hippocampus.

The novel signaling pathway of MOR involving astrocytes

In addition to GABAergic interneurons, MOR is highly expressed in astrocytes of the hippocampus [26]. Astrocytic MOR is mainly localized in the soma and processes, but rarely in the microdomains which form a tripartite synapse [26, 28]. This subcellular distribution of MOR in the hippocampal astrocytes is coinciding with TREK-1- and TWIK-1-containing K2P channels, which is known to be a glutamate-releasing channel in the astrocytes [27, 28]. The TREK-1- and TWIK-1-containing K2P channels can be opened upon the activation of astrocytic MOR and other Gi-GPCRs ($GABA_BR$, CB_1R, and A_1R) through dissociation of $G_{\beta\gamma}$ which binds to N-terminus of TREK-1 (Fig. 2a, c) [27, 28]. Subsequently, intracellular glutamate is released through TREK-1- and TWIK-1-containing K2P channels in a Ca^{2+}-independent manner [27, 28].

The glutamate released upon the activation of astrocytic MOR exerts an excitatory action through binding to group I metabotropic glutamate receptors (mGluRs), especially mGluR1 [61, 62]. mGluR1 is predominantly expressed on the axon strands of presynaptic neurons [62]. The astrocytic glutamate released upon MOR activation binds to axonal mGluR1, leading to an enhancement of the axonal excitability and subsequent increase in the probability of presynaptic glutamate release at the Schaffer collateral-CA1 (SC-CA1) synapses of the hippocampus [62] (Fig. 2c, d). Finally, the activation of astrocytic MOR, a classical inhibitory Gi-GPCR, exerts a paradoxical excitatory action through astrocytic glutamate release and this mechanism is entirely distinct from the action of interneuronal MOR. Based on these recent studies, the novel mechanism involving astrocytic MOR has made a debut as an alternative contributor to the MOR's ability to boost excitatory signaling in the hippocampus.

Synaptic transmission enhancement through two distinct MOR signaling pathways

Since 1990s, several reports demonstrated that activation of MOR enhances glutamatergic synaptic transmission and plasticity at the hippocampal mossy fiber-CA3 synapses [63, 64], SC-CA1 synapses [62, 65], and the perforant path-dentate granule cell synapses [66, 67]. In the early 1990s, Martinez group has demonstrated that opioid receptors, especially MORs, are involved in the induction of long-term potentiation (LTP) at hippocampal mossy fiber-CA3 synapses [63, 64, 68, 69], which was also validated by other groups [70, 71]. A few years later, McQuiston group utilized voltage sensor imaging for an in-depth study of the layer-specific actions of MOR activation. They have demonstrated that MOR activation facilitates the excitatory activity more sensitively in *stratum pyramidale, oriens,* and *radiatum*, but less in *stratum lacunosum-moleculare* [59, 72]. Another group has also proposed that acute treatment of fentanyl, which is a strong agonist of MOR, dose-dependently increases the field excitatory post-synaptic potentials (fEPSPs) at the Schaffer collateral-CA1 (SC-CA1) synapses [73]. In addition to LTP, hippocampal MOR has been also implicated in the induction of long-term depression (LTD) at SC-CA1 synapses [38, 74].

This effect of hippocampal MOR in excitatory synaptic potentiation can be attributed to either GABAergic disinhibition or glutamate release from astrocyte or both. Until now, the excitatory action exerted upon MOR activation has been mostly ascribed to GABA-mediated disinhibition [73, 75, 76] based upon previous reports of the exclusive expression of MOR in the inhibitory interneurons [22]. In detail, MORs are known to act exclusively by hyperpolarizing inhibitory interneurons and suppressing inhibitory synaptic transmission, which translates into an increase in excitatory activity in the hippocampus. This was coincident with the findings that MOR-dependent augmentation of excitatory synaptic transmission is mediated by a suppression of both $GABA_A$ [59] and $GABA_B$ receptor-mediated hyperpolarization [60].

On the other hand, some contradictory data from electrophysiological experiments have been reported. A recent study reported that the enhancement of evoked EPSC (eEPSC) and evoked EPSP (eEPSP) by DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin), the MOR agonist, was observed even in the presence of $GABA_A$ and $GABA_B$ blockers, bicuculline and CGP55845, respectively, in the CA1 hippocampus [62]. This surprising result suggested that the disinhibitory action of MOR might be a minor

contributor to the enhancement of eEPSC and eEPSP. In an attempt to resolve this conflict, we performed an additional experiment of whole-cell patch clamp recording to measure eEPSC in the presence or absence of bicuculline and CGP55845 (Fig. 3). Surprisingly, we were able to recapitulate that the DAMGO-induced enhancement of eEPSC was indistinguishable between the absence and presence of bicuculline and CGP55845 (Fig. 3). These additional findings indicate that, in addition to GABAergic disinhibition, there is another critical player in the excitatory action of MOR, the astrocytic MOR [26, 28, 62]. These results raise a surprising possibility that the astrocytic MOR could be the major contributor to the excitatory action of MOR in the CA1 hippocampus.

The activation of astrocytic MOR in the dorsal hippocampus can induce a fast glutamate release through TREK-1- and TWIK-1-containing K2P channels, as revealed by sniffer patch technique [28] and astrocytic glutamate sensor (iGluSnFr) imaging in CA1 hippocampus [62]. An investigation with whole-cell patch clamp of CA1 pyramidal neurons has demonstrated that astrocytic MOR activation also causes a significant decrease in the paired pulse ratio and a dramatic increase in the frequency of spontaneous excitatory post-synaptic currents (sEPSCs) without affecting sEPSC amplitude, indicating an increased presynaptic release at the glutamatergic SC-CA1 synapses. Moreover, this enhancement of synaptic transmission by MOR activation further led to an induction of the NMDAR-dependent LTP with subthreshold stimulation at SC-CA1 synapses of the hippocampus [62]. The astrocytic MOR-dependent enhancements of synaptic transmission and LTP induction were dependent on presynaptic mGluR1. More importantly, DAMGO-induced enhancements of synaptic transmission and LTP in the hippocampal slices were not attributed to GABAergic disinhibition, as most of the experiments were performed in the presence of $GABA_A$ and $GABA_B$ blockers [62]. Furthermore, the study also revealed that DAMGO's action was much more potent in increasing the frequency of sEPSC (EC_{50} = 0.49 nM) than in reducing the frequency of sIPSC (IC_{50} = 50.67 nM) [62]. These findings indicate that DAMGO-induced enhancement of glutamatergic transmission is less likely mediated by the suppression of GABAergic transmission. Taken together, the recent lines of evidence suggest that the astrocytic glutamate release upon MOR activation is a more predominant contributor to the excitatory action of MOR agonists, compared to the disinhibitory action of interneuronal MOR.

Opioid-associated contextual memory formation by hippocampal astrocytic MOR

The MOR agonists, such as morphine and DAMGO, are well known to induce CPP through the disinhibition of mesocorticolimbic dopamine system by suppressing GABAergic transmission [77]. Although mesocorticolimbic dopamine system is critical for wanting/motivational aspect of CPP, there is another major aspect to CPP: spatial learning and memory. The precise mechanism of spatial learning and memory in CPP is not very well understood.

The hippocampus is critical to the formation of contextual memory, especially to the opioid-associated contextual memory. Several studies have demonstrated the causal relationship between the hippocampus and MOR-induced CPP. A lesion study previously demonstrated that the hippocampus is necessary for systemically administered morphine-induced CPP [78]. The necessity of hippocampal MOR for opioid-induced CPP was also demonstrated by pharmacological blockade of MOR through intra-hippocampal infusion of a specific antagonist of MOR,

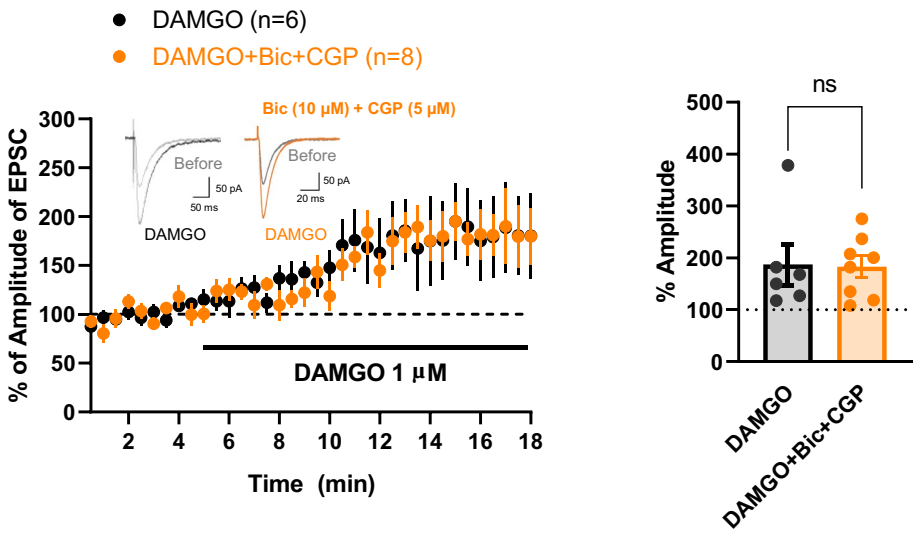

Fig. 3 DAMGO enhances eEPSC at SC-CA1 synapse, which is not mediated by GABAergic disinhibition. DAMGO-mediated enhancement of eEPSC amplitude was not further increased by treatment with bicuculline and CGP55845, which are blockers against $GABA_A$ and $GABA_B$ receptors, respectively. Orange trace is originated from Nam et al. Cell Reports (2019). *DAMGO* [D-Ala2, N-MePhe4, Gly-ol]-enkephalin, *Bic* bicuculline, *CGP* CGP55845, *EPSC* excitatory post-synaptic current, *ns* non-significant

D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) [62]. In addition to the necessity of hippocampal MOR, the sufficiency was also demonstrated by intra-hippocampal injection of morphine, which was sufficient to induce CPP in rats [79]. These reports have suggested that the hippocampus is sufficient and necessary for morphine-induced CPP. However, to date, there has been no study using cell-type specific gene-modulation of MOR, possibly due to the strong belief that MOR is exclusively expressed in GABAergic interneurons.

In 2019, a study utilizing the genetic strategy of cell-type specific gene-silencing of MOR systematically tested the possible contribution of MORs of pyramidal neurons, GABAergic interneurons, or astrocytes in the CA1 hippocampus to the DAMGO-induced CPP [62]. The study demonstrated that astrocytic MOR, but not of pyramidal neurons and interneurons, was necessary for CPP by both intra-CA1 infusion and systemic administration of DAMGO [62]. Moreover, this study also demonstrated that astrocyte-specific expression of MOR in the CA1 hippocampus recovered systemic DAMGO or morphine-induced CPP in the MOR-deficient mice [62]. These findings indicate that the astrocytic MOR in CA1 hippocampus is sufficient and necessary for CPP, establishing a causal relationship between the two.

Consistently, several other previous reports have alluded to the idea that another player besides GABAergic disinhibition is required for CPP. Subcutaneous administration of morphine (1 mg/kg) is well documented as sufficient to induce CPP in rats [80]. If this CPP is induced by GABAergic disinhibition, the concentration of morphine in the brain should be enough to inhibit sIPSCs. However, previous pharmacokinetics reports point to the fact that the concentration of morphine in the brain after subcutaneous administration may not be enough to inhibit sIPSCs. It has been reported that after intravenous injection of morphine (10 mg/kg), the maximum concentration of morphine in the brain is ~ 120 ng/mL, which is equivalent to 0.42 μM [81]. The subcutaneous administration of morphine (1 mg/kg) can be assumed to reach the brain with the concentration of under 0.042 μM after accounting for the fact that subcutaneous administration is generally less effective to reach the brain compared to intravenous administration. Another report demonstrated that such a low dose of morphine (under 0.1 μM) merely inhibits sIPSC frequency (~ 20%), and the IC_{50} of morphine to inhibit sIPSC frequency is about 2 μM [25]. Taken together, these findings suggest that low dosage morphine-induced CPP is less likely to be mediated by GABAergic disinhibition, but more likely mediated by other players such as astrocytic MOR.

Since the hippocampus contains a high density of endogenous MOR agonists such as enkephalin [40], it is possible that the hippocampal MORs contribute to the acquisition and retrieval of spatial memory. Indeed, MOR null knock-out mice showed an impaired spatial memory in eight-arm radial maze and Morris water maze tests with impaired LTP at mossy fiber to CA3 synapses [71, 82]. A few years later, another report insisted that the impaired task performance in the Morris water maze test was attributed to a motivational deficit (namely, a deficit in dopamine signaling), but not a learning deficit [83]. However, there was a report showing that the CA3-specific pharmacological blockade of MOR by β-funaltrexamine (β-FNA) caused a significant impairment in the acquisition and retrieval of spatial learning [84]. On the other hand, a recent study reported a normal spatial memory of MOR null knock-out mice in the passive avoidance test [62]. Taken together, unlike opioid-associated spatial memory, the contribution of hippocampal MOR to the non-opioid-associated spatial memory is still controversial. Future investigations are needed to establish the precise role of MOR in the acquisition of spatial memory in the hippocampus.

Other behavioral roles of hippocampal MOR

Adult neurogenesis Several previous studies have revealed that the chronic administration of opioids negatively influences adult hippocampal neurogenesis [85–87], which is correlated to the hippocampus-dependent learning ability [88, 89]. Among the multi-stage process of neurogenesis (i.e., proliferation, differentiation, migration, and maturation), the chronic exposure to morphine is reported to negatively affect proliferation [85, 90, 91], decrease the survival of newborn cells [86] and interrupt maturation [85]. Moreover, MOR null knock-out mice show an increase in the survival rate of newborn cells without affecting proliferation rate, leading to the increased number of granule cells and increased layer volume in the granule cell layer of the dentate gyrus [92]. Nonetheless, the precise mechanism of how MOR negatively influences hippocampal neurogenesis has not been fully elucidated. Meanwhile, MOR was recently reported to be expressed in hippocampal astrocytes [26, 62], with close contact with neural stem cells and an ability to affect their proliferation and differentiation [93]. Moreover, astrocytic glutamate uptake is reported to be critical for adult neurogenesis [94], implying that astrocytic glutamate release might also affect adult neurogenesis. To sum up, the role of astrocytic MOR in the adult hippocampal neurogenesis needs to be investigated in the future.

Seizure Seizures are known to modify hippocampal distribution of MORs and vice versa. The protein density, mRNA level, and basal binding affinity of MOR are higher in the hippocampus of human post-mortem brains with epilepsy [95]. Seizures increase the MOR immunoreactivities in the inner molecular layer where GABAergic interneurons

are mostly located, but lower than in the granule cell layer and hilus of DG where excitatory neurons are mostly located [11, 34, 35]. In addition to the alteration of the receptor, the mRNA and hormone levels of enkephalin are increased in the hippocampus of human epileptics and the hippocampus of several epileptic rodent models [39, 96–100]. Increased MOR signaling could contribute to seizure development by altering excitation/inhibition (E/I) balance towards excitation, possibly through MOR-mediated enhanced disinhibition. In contrast, several reports have demonstrated that MOR agonists such as morphine and beta-endorphin also increased the susceptibility to seizures [101, 102], while only few studies reported that systemic application of MOR agonist results in an anti-convulsant effect [103]. These results strongly suggest an interesting possibility of MOR involvement in epilepsy. In addition to MOR, KOR, and DOR are also highly implicated in seizure pathology [11]. It will be of great interest to determine whether the disinhibitory action or astrocytic MOR is critical to the role of MOR in seizures.

Stress-induced memory impairment Learning and memory is strongly affected by stress, which is known to facilitate LTD in CA1 hippocampus [104, 105]. Two recent studies have demonstrated that acute stress-induced memory impairment is mediated by GABAergic interneuronal MOR, but not by astrocytic or excitatory neuronal MOR [37, 38]. The authors showed that an acute elevated platform (EP) stress caused the activation of the GABAergic interneuronal MOR in the hippocampus, possibly by upregulation of endogenous MOR agonists [37, 38, 106]. In turn, GABAergic feedforward and feedback inhibition of CA1 pyramidal neurons is attenuated and LTD at SC-CA1 glutamatergic synapses is facilitated [38]. These results implicate the engagement of GABAergic interneuronal MOR in acute stress-induced memory impairment. As stress-induced upregulation of endogenous MOR agonists can also affect astrocytic MOR in the hippocampus, future investigations on the alteration of astrocytic MOR signaling under stressful condition could be of interest.

Sexual dimorphism of hippocampal MOR A recent study reported that the MOR-mediated response to stress is sex-dependent due to the sexual dimorphic phenotype of MOR [107]. Indeed, the MOR trafficking in PV-positive neurons and enkephalin level are positively regulated by gonadal hormones, especially ovarian hormones [108, 109]. In detail, MOR trafficking in the hippocampal PV-positive interneurons is increased in the proestrus phase (relatively high estrogens) of a female rat, as compared to diestrus phase [109]. This sexual dimorphism of hippocampal μ-opioid signaling leads to sex-dependent behavior of MOR. For example, MOR-mediated regulation of mossy fiber transmission is distinct only in females [110]. More interestingly, acute immobilization stress increased the immunoreactivity of phosphorylated MOR in the hippocampus of male, did not alter it in the diestrus female, and significantly decreased it in proestrus female rats [111]. Not only the protein level, but also the MOR trafficking is sex-differentially altered by acute stress: decreased in females and increased in males [107, 109]. These previous findings have proposed the estrogen-dependent sexual dimorphism of hippocampal μ-opioid signaling, which affects its response to acute stress. All the current studies on sexual dimorphism of MOR have only focused on MOR of GABAergic interneurons. Based on recent evidence of astrocytic MOR [26, 62], the possible sex-dimorphic alteration of the expression and trafficking of astrocytic MOR requires further investigation.

Summary

We have comprehensively reviewed the cellular expression of MOR, its signaling pathways, and its behavioral and cognitive function in the hippocampus. While MOR in the GABAergic interneurons has long been focused on, the presence and function of MOR in the astrocytes have only recently been investigated. Activation of interneuronal MOR causes membrane hyperpolarization and suppresses GABAergic synaptic transmission leading to disinhibition of pyramidal neurons in the CA1 and granule cells in the dentate gyrus. Therefore, MORs of the GABAergic interneurons are considered as the major contributors of hippocampal MOR signaling. However, recent studies have revealed that hippocampal astrocytes express MOR, and the activation of the astrocytic MOR causes glutamate release to enhance glutamatergic synaptic transmission at SC-CA1 synapses. Furthermore, astrocytic MOR activation, but not interneuronal MOR activation, mainly contributes to MOR-mediated enhancement of synaptic transmission in the hippocampus and opioid-mediated contextual memory. Lastly, hippocampal MOR signaling is engaged in hippocampal neurogenesis, seizure, and stress-induced memory impairment, which are reportedly dependent on MOR-mediated disinhibition of principal hippocampal cells. Further investigations are needed to determine the contribution of astrocytic MOR to these pathophysiological functions.

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