Beta-endorphin is the key endogenous opioid influencing morphine μ-opioid receptor occupancy in rat hypothalamus: a binding kinetic model analysis

Budda, D.; Zhang, Z.; Hasselt, J.G.C. van; Lange, E.C.M. de

European Journal of Pharmaceutical Sciences 212 (2025) 107195

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Beta-endorphin is the key endogenous opioid influencing morphine μ-opioid receptor occupancy in rat hypothalamus: a binding kinetic model analysis

Divakar Budda, Zhicheng Zhang [1], J.G. Coen van Hasselt, Elizabeth C.M. de Lange [*]

Division of Systems Pharmacology and Pharmacy, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

ARTICLE INFO

Keywords:
Morphine
μ-opioid receptor
Receptor occupancy
Receptor internalization
Endogenous opioids
Chronic pain
Hypothalamus
Binding kinetic model

ABSTRACT

The μ-opioid receptor (μ-OR) is the key target for morphine in the treatment of (chronic) pain. Endogenous opioids at the μ-OR have been implicated in multiple physiological processes, including pain relief. However, the extent to which μ-OR occupancy of morphine is influenced by endogenous opioids and μ-OR internalization is unclear. The aim of this study was to investigate the impact of endogenous opioids and μ-OR internalization on morphine μ-OR occupancy. To this end we developed a mathematical binding kinetic model incorporating information on binding kinetic parameters, μ-OR expression, μ-OR internalization rates, and levels of multiple endogenous opioids, in rat hypothalamus. μ-OR occupancies were simulated using morphine brain extracellular fluid concentration data, for different static levels of the endogenous opioids, including beta-endorphin, Dynorphin-A 1–17, Endomorphin-2, Leu-enkephalin, and Met-enkephalin. Simulations were performed in absence or presence of μ-OR internalization. We show that beta-endorphin has a strong impact on morphine μ-OR occupancy, followed by limited impact of met-enkephalin. Other endogenous opioids did not demonstrate any significant effect. μ-OR internalization reduced the impact of beta-endorphin on morphine μ-OR occupancy to a limited extent. We conclude that beta-endorphin plays an important role in morphine μ-OR occupancy, which could therefore play a key role in explaining interindividual variability in the analgesic effects of morphine. Finally, this study demonstrates the utility of a mathematical model workflow in deciphering the role of endogenous ligands in morphine's μ-OR occupancy.

Introduction

Morphine is an essential agent for the treatment of (chronic) pain (Berland and Dodgers 2012; Dowell et al., 2022). The analgesic effects of morphine are mediated by the μ-opioid receptor (μ-OR), which is abundant in several CNS regions responsible for sensory, emotional and cognitive processing of pain, referred to collectively as the pain matrix (Garcia-Larrea and Peyron 2013; Goel M et al., 2024).

Endogenous opioids such as enkephalins, endorphins, endomorphins and dynorphins are naturally occurring opioid peptides in the body. Endogenous opioids have a role in both emotional control and sensory pain relief, primarily mediated through the μ-OR (Zubieta et al., 2005). The role of endogenous opioids in morphine analgesia (Bergmann et al. 1978) and cross-tolerance with morphine (Székely et al., 1977; Waterfield et al., 1976) suggests a potential role of competitive binding of endogenous opioids with morphine at the μ-OR. Endogenous opioids levels can vary between individuals and may depend on patient's age and sex (Corder et al., 2018; Fricker et al., 2020), based on their precursors pro-dynorphin (PDYN), pro-enkephalin (PENK) in hypothalamus (Sjöstedt et al., 2020). Studying the impact of endogenous opioid levels on morphine's μ-OR occupancy may improve our understanding of factors that might underlie the substantial variability in treatments effects of morphine.

Morphine or any endogenous opioids present in the brain extracellular fluid (brainECF) can bind and activate the μ-OR. The resulting ligand-receptor complex will undergo internalization, which is a crucial step in regulating the receptor function (Williams et al., 2013). Internalization involves endocytosis of the ligand-receptor complex via

* Corresponding author.
E-mail address: ecmdelange@lacdr.leidenuniv.nl (E.C.M. de Lange).
[1] Current address: Department of Environmental and Global Health, College of Public Health & Professions, University of Florida, Gainesville FL 32610, United States.

https://doi.org/10.1016/j.ejps.2025.107195
Received 11 March 2025; Received in revised form 27 June 2025; Accepted 7 July 2025
Available online 10 July 2025

Table 1
μ -OR endogenous opioids binding kinetic model information.

Compound	Parameter	Value	Units	Species	Location	Reference
Endogenous opioid levels (E)						
Beta-endorphin	Concentration	4941[a]	ng. g^{-1}	Rats	Hypothalamus	Ogawa et al., 1979
Dynorphin-A 1–17	Concentration	10.3	pmol. g^{-1}	Rats	Hypothalamus	Corbett et al., 1982
Endomorphin-2	Concentration	12.4	pmol. g^{-1}	Bovine	Hypothalamus	Zadina et al., 1997
Leu-enkephalin	Concentration	220	pmol. g^{-1}	Rats	Hypothalamus	Giraud et al., 1983
Met-enkephalin	Concentration	2169	pmol. g^{-1}	Rats	Hypothalamus	Giraud et al., 1983
Dissociation rate constants (K_{off})						
Beta-endorphin	K_{off_1}	0.077	min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Beta-endorphin	K_{off_2}	0.018	min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Dynorphin-A 1–17	K_{off}	7.6	s^{-1}	Rats	Trigeminal neurons	Chen et al., 1995
Endomorphin-2	K_{off}	0.085	min^{-1}	Rats	Brain membranes	Spetea et al., 1998
Morphine	K_{off}	1.388	min^{-1}	Flp-In T-REx293 cells	Flp-In T-REx293 cells	Pedersen et al., 2020
Leu-enkephalin	K_{off_1}	0.063	min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Leu-enkephalin	K_{off_2}	0.014	min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Met-enkephalin	K_{off_1}	0.091	min^{-1}	Rats	Synaptosomes	Nagy et al., 1983
Met-enkephalin	K_{off_2}	0.016	min^{-1}	Rats	Synaptosomes	Nagy et al., 1983
Association rate constants (K_{on}), Equilibrium dissociation constant (K_d)						
Beta-endorphin	K_{on}	330,000,000	mol^{-1}. min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Dynorphin-A 1–17	K_{on}	4900,000	mol^{-1}. s^{-1}	Rats	Trigeminal neurons	Chen et al., 1995
Endomorphin-2	K_{on}	0.088	$nmol^{-1}$. min^{-1}	Rats	Brain membranes	Spetea et al., 1998
Morphine	K_{on}	17,800,000	mol^{-1}. min^{-1}	In vitro	Cell membranes	Pedersen et al., 2020
Leu-enkephalin	K_{on}	9300,000	mol^{-1}. min^{-1}	Rats	Brain membranes	Ferrara et al., 1981
Met-enkephalin	K_d	8.33	nmol	Rats	Synaptosomes	Deliconstantinos et al., 1995
Ligand bound μ-OR internalization rates (proportions (%))						
Beta-endorphin	K_{int}	89.25	%	Rats	Spinal cord slices	Song and Marvizón, 2003
Dynorphin-A 1–17	K_{int}	21.51	%	Rats	Spinal cord slices	Song and Marvizón, 2003
Endomorphin-2	K_{int}	100	%	Rats	Spinal cord slices	Song and Marvizón, 2003
Leu-enkephalin	K_{int}	8.82	%	Rats	Spinal cord slices	Song and Marvizón, 2003
Met-enkephalin	K_{int}	1.08	%	Rats	Spinal cord slices	Song and Marvizón, 2003
Morphine	K_{int}	13	%	AtT20 cells	AtT20 cells	Borgland et al., 2003
Unbound μ-OR	K_{int}	6.022	%	Rats	Spinal cord slices	Song and Marvizón, 2003

K_{on}: association rate constant; K_{off}: dissociation rate constant; K_d: Equilibrium dissociation constant; K_{int}: Internalization rate; μ-OR: μ-Opioid receptor. a: Baseline value was selected due to the microwave fixation method's superior preservation of endogenous opioid peptides (Che et al., 2005 ; Juras et al., 2023 ;Murphy, 2010 ; Nylander et al., 1997)

endosomes, removing it from the cell surface and allowing for receptor resetting and recycling to the membrane for subsequent binding and activation cycles (Williams et al., 2013). This affects the available μ-OR for binding and thereby signal transduction, as was shown by enhanced internalization of morphine preventing development of analgesia attenuation (Sternini et al., 2000). While multiple studies (Bronstein et al., 1990 &1993; Nieto et al., 2002; Przewłocki et al., 1979; Rattan and Tejwani, 1996 & 1997; Takemori et al., 1993; Tejwani and Rattan 1997; Turchan et al., 1997; Yukhananov et al., 1993) have examined morphine's effect on endogenous opioid levels, the competitive binding between endogenous opioids and morphine, as well as their μ-OR internalization role in μ-OR occupancy, has not been explored.

Investigating the role of endogenous opioids and morphine in μ-OR receptor binding kinetics presents significant methodological challenges for in vivo studies. In humans, non-invasive imaging techniques including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) can be used. PET studies using [^{11}C]-Carfentanil have been used to indirectly measure a stimulus-induced endogenous opioid release (Mick et al., 2014), but not morphine brainECF concentrations, nor μ -OR expression. In animals, quantifying changes in morphine pharmacokinetics (PK) in brain extracellular fluid (ECF) is possible by microdialysis (Groenendaal et al., 2007). This technique can also be used to monitor brainECF endogenous opioids (Al-Hasani et al., 2018; Mikati et al., 2025; Nieto et al., 2002). However, obtaining additional information on μ-OR expression, endogenous opioid levels, and endogenous opioid specific internalization fractions of μ-OR is challenging, resource-intensive, and requires extensive animal use. Mathematical modelling can be used to provide a "digital twin" of the in vivo setting, simulating mechanistic processes across treatment conditions and biological or pharmacological conditions to explain their interrelationships. Model-based approaches represent a relevant strategy to address questions around the role of endogenous opioids and morphine in μ-OR receptor binding kinetics.

The possible competition of endogenous opioids in morphine μ-OR binding, and potential implications for their impact on the analgesic effect of morphine, motivated us to evaluate the role of endogenous opioids competition and their specific μ-OR internalization factors on μ-OR occupancy, using a mathematical binding kinetic modelling approach.

Methods

The impact of endogenous opioids competition and μ-OR internalization on morphine's μ-OR occupancy was simulated using a mathematical modeling framework. Model parameter values for binding kinetics, μ-OR expression, endogenous opioid levels in specific CNS regions, as well as information for morphine brain ECF concentrations, were derived from literature.

Comprehensive literature search

A comprehensive literature search (Table S1) was conducted to identify the endogenous opioid concentrations (amounts per gram brain tissue) in the pain matrix, or broader representative regions, i.e. cerebral cortex, thalamus, hypothalamus, midbrain, pons, medulla, spinal cord, and their corresponding binding kinetic parameters. The literature search was conducted for humans, rats, and mice, and is summarized in detail in Table S2. We found that rat hypothalamus data was most comprehensive, and therefore this region was selected for further analyses, with the exception of bovine endomorphin-2 levels information that was lacking in rats, and assumed to be equal to that in bovine. Table 1 summarizes key information used for mathematical modelling, including the levels of endogenous opioids, binding kinetic parameters, μ-opioid receptor expression and ligand -μ-opioid receptor complex

internalization.

Data transformation & parameter derivation for the model

μ-OR expression in rat hypothalamus

μ-OR expression in the rat hypothalamus (Table 1) was obtained from literature as 197 fmol/mg (Piva et al., 1987), which was then scaled by the weight of the hypothalamus region, being 0.38 g (Welniak et al., 2019) resulting in a final value of 0.075 nM. The μ-OR expression was assumed to be constant without fluctuations in synthesis and degradation.

Endogenous opioid amounts in the rat hypothalamus

Endogenous opioid levels (Table 1) were calculated for the total hypothalamus weight of the rat (0.38 g (Welniak et al., 2019)). Endogenous opioid amounts were assumed to be constant. Data on the levels of endomorphin-2 in rats were unavailable, and only available for bovine species. Assuming comparable levels in bovine and rats, endomorphin-2 concentrations in the rat hypothalamus were calculated using bovine values and adjusted for rat hypothalamus weight.

Binding kinetic parameters

For the endogenous opioids, binding kinetic model information was only available for leu-enkephalin, met-enkephalin, beta-endorphin, dynorphin-A 1–17 and endomorphin-2 (Table S2). Dynorphin-A 1–17 and endomorphine-2 have monophasic dissociation constants while Leu-enkephalin, Met-enkephalin, and beta-endorphin were reported to have biphasic dissociation rate constants, represented by $K_{off,1}$ and $K_{off,2}$. The bi-phasic dissociation of the endogenous opioid-μ-OR (ER) complex has a magnitude of A for phase 1 and magnitude of B for phase 2. The net dissociation of the ER complex [ER] at time t, relative to time 0, can be described based on (Witt and McConnell, 1994; and Anderson and McConnell, 1999) as:

$$\frac{[ER]_t^{diss}}{[ER]_0^{diss}} = A.\ e^{-K_{off,1}\cdot\ t} + \ B.\ e^{-K_{off,2}\cdot\ t} \tag{1}$$

Information about the magnitude of phases of dissociation was not available for Leu-enkephalin, Met-enkephalin, and beta-endorphin having this bi-phasic dissociation. Hence, to model the dissociation of the complex, we used an overall dissociation rate constant (K_{off}):

$$\frac{[ER]_t^{diss}}{[ER]_0^{diss}} = e^{-K_{off}\cdot\ t} \tag{2}$$

where K_{off} is the sum of $K_{off,1}$ and $K_{off,2}$. For Met-enkephalin, only K_{off} and K_d were available. K_{on} was calculated as follows:

$$K_{on} = \frac{K_{off}}{K_d} \tag{3}$$

Internalization rate constants

Internalization rate constants (K_{int}) were derived from the fraction internalized (F_{int}) after exposing ligand for a duration of time "*t*" to the μ-OR using the Eq. 4 below based on first-order reaction rate constants. The complete derivation can be found in supplementary section 6.3.

$$K_{Int} = \frac{-[\ln(1 - F_{int})]}{t} \tag{4}$$

Morphine rat brain ECF concentrations

The population pharmacokinetic (PopPK) model on nonlinear brain distribution of morphine was based on plasma and microdialysis data obtained from rats (Groenendaal et al., 2007). It was used to predict morphine brainECF concentrations for a time-period of 18 h (~ steady-state) following intravenous infusion dose ranges of 0 – 40 mg/kg/h. This range fully covers the dose ranges used for morphine analgesia in rats (5–10 mg/kg/h) (Cox et al., 1968; Jacquet and Lajtha 1973; Johanneson et al., 1972).

Binding kinetic model and simulations

Binding kinetic models were developed to predict μ-OR occupancy using the morphine brainECF concentrations, incorporating competition with endogenous opioids (see section 2.3.1). To analyse the competitive impact of each endogenous opioid with morphine at the μ-OR, models were developed with: (1) a single ligand: either morphine or an endogenous opioid (i.e. no competition), (2) two ligands: morphine and an endogenous opioid (pairwise competition), and (3) all ligands: morphine and all endogenous opioids (i.e. Full competition). The internalization rate of the ligand-μ-OR complex (for all ligands) was also used to understand its role in the μ-OR occupancy of morphine. The value used for the μ-OR internalization rate (Table 1) reflects the ligand + μ-OR complex internalized (i.e. μ-OR removed from the cell membrane surface, reducing the μ-OR available for binding. The ordinary differential equations combining rat brainECF concentrations with competitive binding kinetic model are described in section 2.3.1.

Binding kinetic model equations

The nonlinear morphine blood-brain barrier (BBB) transport model equations based on plasma and brainECF concentrations as determined from rat microdialysis data ((Groenendaal et al., 2007), (Eq (5)), along with competitive binding kinetics for each endogenous opioid as used in the model, are presented in the Eqs. (5)–12 below.

$$\frac{d(C_{ecf})}{dt} = [K_{diff}].[C_{blood} - C_{ecf}] - \left[\frac{\frac{N_{max}}{V_{ecf}}.\ C_{blood}}{C_{50} + C_{blood}}\right].[K_{eff}].[C_{ecf}] \tag{5}$$

$$\frac{d(MR)}{dt} = [K_{On_M}].[C_{ecf}].[R] - [K_{Off_M}].[MR] - [K_{Int_M}].[MR] \tag{6}$$

$$\frac{d(ER_i)}{dt} = [K_{On_i}].[E_i].[R] - [K_{Off_i}].[ER_i] - [K_{Int_i}].[ER_i] \tag{7}$$

$$\frac{d(E_i)}{dt} = -\left([K_{On_i}].[E_i].[R] - [K_{Off_i}].[ER_i] - [K_{Int_i}].[ER_i]\right) \tag{8}$$

$$\frac{d(R)}{dt} = -\left([K_{On_M}].[C_{ecf}].[R] - [K_{Off_M}].[MR] - [K_{Int_M}].[MR]\right) - \sum_{i=1}^{5}\left([K_{On_i}].[E_i].[R] - [K_{Off_i}].[ER_i] - [K_{Int_i}].[ER_i]\right) \tag{9}$$

$$R_{total} = R + MR + \sum_{i=1}^{5} ER_i \tag{10}$$

$$RO_i = \frac{ER_i}{R_{total}} * 100\% \tag{11}$$

Table 2
Nonlinear saturable influx transporter mediated plasma (ng/ml) and brainECF (ng/ml) steady state concentration of morphine in rats across multiple doses (mg/kg/h).

Morphine continuous infusion rate (mg/kg/h)	Morphine steady state concentration (ng/mL)	
	Plasma	Brain ECF
0	0	0
2.5	147.8	169.5
5	295.7	216.8
10	591.3	305.4
12.5	739.1	348.9
17.5	1034.8	435.8
20	1182.6	479.2
22.5	1330.4	522.5
25	1478.3	565.8
32.5	1921.7	695.5
35	2069.6	738.8
37.5	2217.4	781.9
40	2365.2	825.2

$$RO_M = \frac{MR}{R_{total}} * 100\% \quad (12)$$

where subscript i stands for each individual endogenous opioid (Leu-enkephalin, met-enkephalin, dynorphin-A 1–17, beta-endorphin, and endomorphin–2); K_{diff} represents the rate constant for passive BBB diffusion between blood and brain ECF; K_{eff} represents the rate constant for active BBB efflux by Pgp out of brain ECF; N_{max} represents the maximum active BBB influx from blood into brain ECF; V_{ecf} represents the volume of the brain ECF compartment; C_{50} represents morphine's blood concentration at which 50 % of maximum active influx is reached; C_{blood} represents morphine's total blood concentration in rats; C_{ecf} represents morphine's unbound brainECF concentration; K_{on_M} represents morphine's association rate constant for the μ-OR; K_{off_M} represents morphine's dissociation rate constant from the μ-OR; MR represents morphine bound μ-OR; K_{Int_M} represents the internalization rate constant for morphine + μ-OR complex; K_{on_i} represents the endogenous opioid's association rate constant for the μ-OR; K_{off_i} represents the endogenous opioid's dissociation rate constant from the μ-OR; ER_i represents the endogenous opioid's bound to the μ-OR; E_i represents the endogenous opioid's concentration in rat hypothalamus; K_{Int_i} represents the endogenous opioid + μ-OR complex internalization rate constant; R represents the unbound μ-OR concentration; R_{total} represents the unbound + bound μ-OR; and RO represents the μ-OR occupancy.

Simulations

Simulations for 300 g rats modeled a 2-hour baseline period with static endogenous opioids concentrations to mimic a chronic pain state, followed by an 18-hour constant-rate intravenous morphine infusion (0–40 mg/kg/h). This design assesses steady-state morphine competition with endogenous opioids at constant μ-opioid receptor expression (no turnover), isolating binding dynamics in chronic pain. To that end, all levels of endogenous opioids, binding kinetic rate constants, and morphine brainECF concentrations were converted into nanomoles and rate constants to per minute values for simulations. Median values of the simulated μ-OR occupancies at steady state i.e., at 18 h, were used for the analysis of the simulation results. Furthermore, a sensitivity analysis was also performed in order to evaluate the impact of variability in endogenous opioid levels between 0 % (complete absence) to 300 % (3 times the baseline value) for their effect on μ-OR occupancies for the three competition modes (none, pairwise, full).

Results

Beta-endorphin shows the highest μ-OR occupancy in absence of competition

We evaluated μ-OR occupancy of each individual endogenous opioid alone. These simulations showed that, in the rat hypothalamus, beta-endorphin has the highest μ-OR occupancy (63 %), followed by met-enkephalin (9 %) (Fig. 2). μ-OR occupancy of leu-enkephalin, endomorphin-2, and dynorphin-A 1–17 were insignificant (0.98 %, 0.45 %, and 0.00025 %, respectively). Morphine's μ-OR occupancy was ~93 % for the 5mg/kg/h dose when used alone. Based on the significant μ-OR occupancy of beta-endorphin and met-enkephalin and the insignificant role of other endogenous opioids, further results and visualizations were focused only on beta-endorphin and met-enkephalin. (Results for all endogenous opioids can be found in the Figure S1 & S2). Plasma, brainECF steady state concentrations corresponding to each dose, and used as input for μ-OR occupancies were shown in Table 2.

Beta-endorphin shows highest μ-OR occupancy in both pairwise competition with morphine and full competition with morphine and endogenous opioids

To determine the impact of endogenous opioid competition, we simulated multiple competition conditions and compared with no competition setting. When endogenous opioids compete with morphine (IV infusion of 5 mg/kg/h), beta-endorphin μ-OR occupancy decreased to 12 % in both pairwise and full competition, underlining the strength of beta-endorphin binding to μ-OR (Fig. 3). Met-enkephalin μ-OR occupancy was reduced to ~0.5 % in pairwise competition and full competition compared to no competition (9 %). Finally, morphine μ-OR occupancy decreased from 93 % in no competition to 92 % in pairwise competition with met-enkephalin, whereas it has become 82 % in pairwise competition with beta-endorphin and 81 % in full competition. In summary, beta-endorphin is the primary endogenous opioid driving μ-OR occupancy when competing with exogenously administered morphine as well as other endogenous opioids.

Internalization of μ-OR reduced beta-endorphin μ-OR occupancy

The impact of internalization rate of μ-OR ligand-receptor complexes, and μ-OR on μ-OR occupancy, was simulated in full competition condition for all endogenous opioids. The internalization rate of beta-endorphin was quite high compared to that of morphine and led to a decrease in beta-endorphin μ-OR occupancy by 8 % (Fig. 4). Due to an increased internalization rate, beta-endorphin μ-OR occupancy gradually decreased with increased morphine doses, leading to an increase in morphine μ-OR occupancy by 96 % at dose of 40 mg/kg/h compared to 89 % at a dose of 5 mg/kg/h. The internalization rate did not affect Met-enkephalin and other endogenous opioids (Figure S1).

Sensitivity analysis of endogenous opioid concentrations

To evaluate the impact of endogenous opioid level changes on morphine μ-OR occupancy, endogenous opioid levels were varied between 0 % to 300 % (where 100 % is baseline) (see section 2.4). Beta-endorphin μ-OR occupancy was ~95 % without internalization, compared to ~85 % in the presence of internalization (Figure S2). Other endogenous opioid level changes did not result in any observable differences in μ-OR occupancy of morphine/beta-endorphin/met-enkephalin (Figure S3).

When including internalization, beta-endorphin remained the major endogenous opioid affecting morphine μ-OR occupancy (Fig. 5). Beta-endorphin μ-OR occupancy was ~4 % at its baseline level (100 %) and increased to ~11 % for 3 times its baseline levels (300 %). For morphine, the μ-OR occupancy was ~ 92 % in the absence of beta-

endorphin (0 %), 88 % for the baseline beta-endorphin level (100 %), and ~82 % at 3 times higher beta-endorphin levels (300 %).

Discussion

The possible competition of endogenous opioids in morphine μ-OR binding, and potential implications for their impact on the analgesic effect of morphine, motivated us to evaluate the role of endogenous opioids competition on morphine's μ-OR occupancy. Using a binding kinetic mathematical modeling approach, we have shown that beta-endorphin has a high impact on morphine's μ-OR occupancy. We have also shown the impact of changes in endogenous opioid levels, and μ-OR receptor-ligand complex internalizations, further supporting observations on the important role of beta-endorphin on morphine's μ-OR occupancy.

Our simulations indicated that beta-endorphin is a particularly important endogenous opioid. Its μ-OR occupancy at endogenous levels is substantially higher than that of other endogenous opioids used at clinically relevant morphine exposures. Simulations exploring various endomorphin-2 levels did not significantly affect morphine μ- μ-OR occupancy, suggesting a minimal impact of endomorphin-2 on μ-OR occupancy of morphine or other endogenous opioids, in the presence of morphine. Beta-endorphin's high μ-opioid receptor (μ-OR) occupancy is primarily attributed to its substantially higher concentration (in the range of 0.66 - 138 times greater than other endogenous opioids (Table 1)) and its significantly higher association rate constant (K_{on}), which is in the range of 1.1 – 36 times greater than that of other opioids (Table 1). This high K_{on} value underscores the importance of beta-endorphin in μ-OR binding compared to other endogenous opioids (though met-enkephalin has higher concentration). In contrast, dynorphin-A 1–17, despite having a high K_{on}, exhibits very low μ-OR occupancy due to its very high dissociation rate constant (K_{off}), which is in the range of 328 - 5900 times greater than that of other opioids (Table 1). Altogether, our simulations show that in the absence of beta-endorphin, morphine μ-OR occupancy is highest (90 %), and that beta-endorphin dominates the competition with morphine, no matter the level of endogenous opioids. This suggests a possible vital role of beta-endorphin in analgesia.

Furthermore, our simulations showed that morphine exhibits high μ-OR occupancy, which is aligned with reports of strong μ-OR binding affinity (Eshleman et al., 2020) and analgesic effects (Berland and Dodgers, 2012; Dowell et al., 2022). However, two factors needs to be considered: firstly, the simulations were based on rat models; secondly, the focus on the hypothalamus, where data was most complete, may not fully account for significant regional variations in endogenous opioid levels (Giraud et al., 1983; Law et al., 1979) and μ-OR expression (Kuhar et al., 1973; Piva et al., 1987).

Our evaluation of internalization rates demonstrated a reduction in beta-endorphin's μ-OR occupancy with increasing morphine doses, driven by its higher internalization rate (Table 1). Endogenous opioids can enhance morphine internalization, suggesting synergistic interactions that influence receptor availability (Sternini et al., 2000). Quantifying internalization rate constants under combined and isolated conditions could refine μ-OR occupancy estimates, providing deeper insights into receptor dynamics. These findings emphasize the critical role of endogenous opioids in modulating morphine analgesia.

We hypothesize that inter-individual differences in beta-endorphin levels in humans might be the most important driver in variability in morphine experienced analgesia, as observed among different human subjects, with higher levels of beta-endorphin in morphine treatment results in improved analgesic action (Chapman et al., 1980), while it could also be possible that beta-endorphin mediates analgesia independently if present at elevated levels (Akil et al., 1978; Loh et al., 1976). The role of beta-endorphin is also implicated in electrical stimulation-induced analgesia (Akil et al., 1978; Hosobuchi et al., 1979; Misra et al., 2017; Taylor et al., 2012), suggesting beta-endorphin's role in both morphine-dependent and morphine-independent analgesia. Besides analgesia, cross-tolerance between beta-endorphin and morphine (Székely et al., 1977; Waterfield et al., 1976., Tseng et al., 1976), prolonged morphine exposure reducing beta-endorphin levels (Bronstein et al., 1993; Przewłocki et al., 1979) further highlights the importance of beta-endorphin in morphine tolerance development. Furthermore, the role of beta-endorphin in analgesia is further supported by age related changes in sensitivity to pain (Hess et al., 1981; Zhang and Pasternak et al., 1981), with age associated changes in beta-endorphin levels in CSF (Almay et al., 1978;von Knorring et al., 1978; Terenius and Wahlström 1975) and plasma (Bruehl et al., 2017; Gudehithlu et al., 1991). Although quantitative validation of these observations is currently lacking, literature highlights the critical role of beta-endorphin (Loh et al. 1976; Tseng et al., 1976 in various analgesic contexts, including non-pharmacological (Choh Hao Li., 1977; Foley et al., 1979; von Knorring et al., 1978; Moroni et al., 1977; von Knorring et al., 2008, electrical stimulation-based (Akil et al., 1978; Hosobuchi et al., 1979; Taylor et al., 2012; Misra et al., 2017), and morphine-based (Bergmann et al., 1978) therapies. Research indicates that beta-endorphin is more potent than other endogenous opioids and morphine (Klee and Nirenberg 1976; Loh et al., 1976; Tseng et al., 1976), Endorphins release during nociceptive stimuli (Zangen et al., 1998), changes in endorphin levels with age and exercise (Marano et al., 2025), pain (Martikainen et al., 2013), emotional state (5-HT levels) (Soliman et al., 1986), morphine treatment (Przewłocki et al., 1979; Rattan and Tejwani, 1996), further highlights the importance of considering beta-endorphin levels for efficient therapy with morphine.

With the important impact of beta-endorphin on μ-OR of morphine, we also feel that the interpretation of PET data on μ-OR occupancy need to be reconsidered. This is because PET imaging only measures the administered PET ligands, assuming that only these bind to a receptor, without consideration of the binding of endogenous ligand competition. Changes in receptor occupancy of the PET ligands are thereby implicitly assumed to reflect changes in receptor expression. So, as an example, we question if the changes in ^{11}C-Carfentanil μ-OR occupancy in PET studies in thalamic pain patients compared to controls using ^{11}C-Carfentanil, that were interpreted as changes in μ-OR receptor density due to the pain condition (Jones et al., 2004), might have been brought about by changes in beta-endorphin levels (and/or other endogenous opioids), aside from possible μ-OR internalization or downregulation (van Waarde et al., 2014). Interestingly, this has been shown in PET studies using ^{11}C-Carfentanil and an amphetamine challenge to indirectly measure endogenous opioid release (Mick et al., 2014).

All parameters used in our simulations were derived from real-time measurements in rats, with the exception of endomorphin-2 levels, which were based on bovine data due to a lack of rat-specific information, with the explicit assumption that these bovine data are a rather good indication for rats. Furthermore, morphine brainECF concentrations used in our simulations were directly based on our previous in-house measurements using microdialysis in rats, as described by (Groenendaal et al., 2007), which give us confidence in the value our simulations.

Our study incorporated multiple factors influencing μ-OR occupancy, but did not account for the role of morphine's main metabolites, M6G and M3G, at the μ-OR. The impact of these metabolites were the focus of a parallel study, that addressed the impact of morphine's nonlinear BBB transport on the relative concentrations of morphine, M3G and M6G in brainECF, being different from those in plasma in a dosing regimen dependent fashion (Gülave et al., 2023), and their relative μ-OR binding, in which their binding affinities as well as receptor expression differences in different regions of the CNS are important (Budda et al., 2024). We have demonstrated that M6G have higher μ-OR occupancy than morphine in human simulation studies (Budda et al., 2024), while morphine shows greater occupancy than beta-endorphin in present study in rats (μ-OR occupancy: M6G > morphine > beta-endorphin). Including M6G (Ki = 63 nM; Frölich et al., 2011) in a competitive

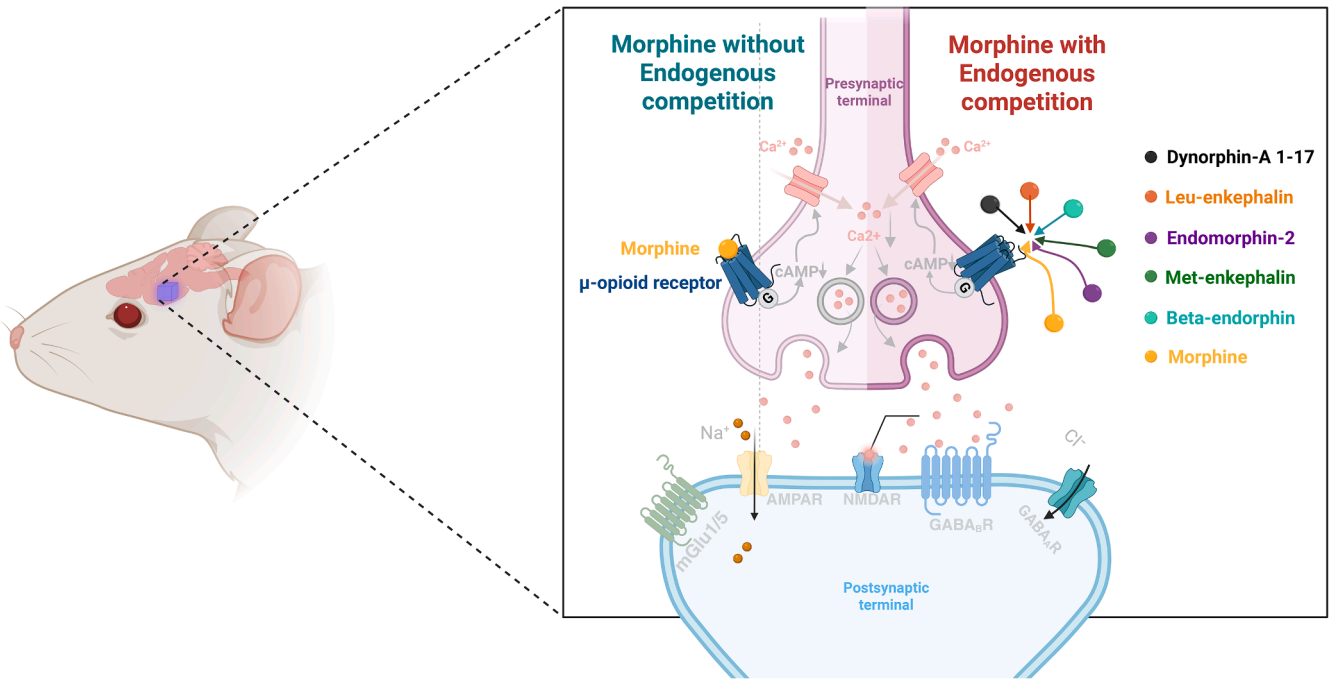

Fig. 1. Schematic illustration of endogenous opioids binding competition with morphine at μ-OR in rat hypothalamus.(Created in BioRender. Budda, D. (2025) https://BioRender.com/h90c703).

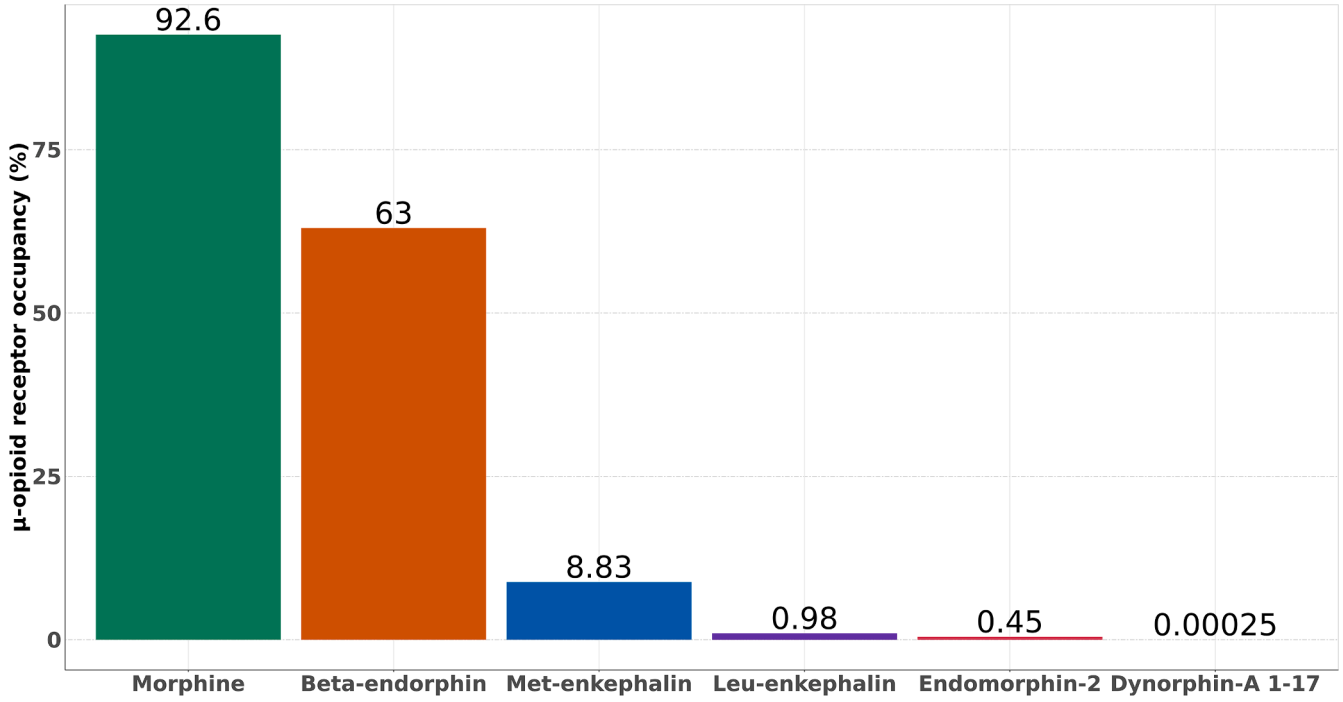

Fig. 2. Beta-endorphin shows the highest μ-OR occupancy among all endogenous opioids and morphine shows the highest -OR occupancy overall. μ-OR occupancy (%) of each sole ligand (endogenous opioid or morphine (5mg/kg/h IV infusion) at the μ-OR in the rat hypothalamus.

binding model with morphine and endogenous opioids, such as beta-endorphin (Ki = 0.28 nM; Ferrara et al., 1981), may alter μ-OR occupancy dynamics. Beta-endorphin's ~225-fold stronger binding affinity suggests it could dominate μ-OR occupancy. Morphine requires at least 18 % μ-opioid receptor occupancy for analgesia in rats (Takai et al., 2018), whereas beta-endorphin, a more potent full agonist with higher binding affinity, achieves analgesia at lower occupancy levels (Klee and Nirenberg, 1976; Loh et al., 1976; Tseng et al., 1976). This further highlights beta-endorphin role in analgesia, however further modeling is required to confirm these effects.

A second limitation in our study is the absence of connected comprehensive, species-specific data. Rat hypothalamus data was used as it offered the most complete dataset, yet still required certain assumptions. This reflects a broader issue: despite extensive studies on morphine's μ-OR occupancy and effects, most research addresses isolated aspects, limiting direct cross-comparisons (De Lange 2013). As

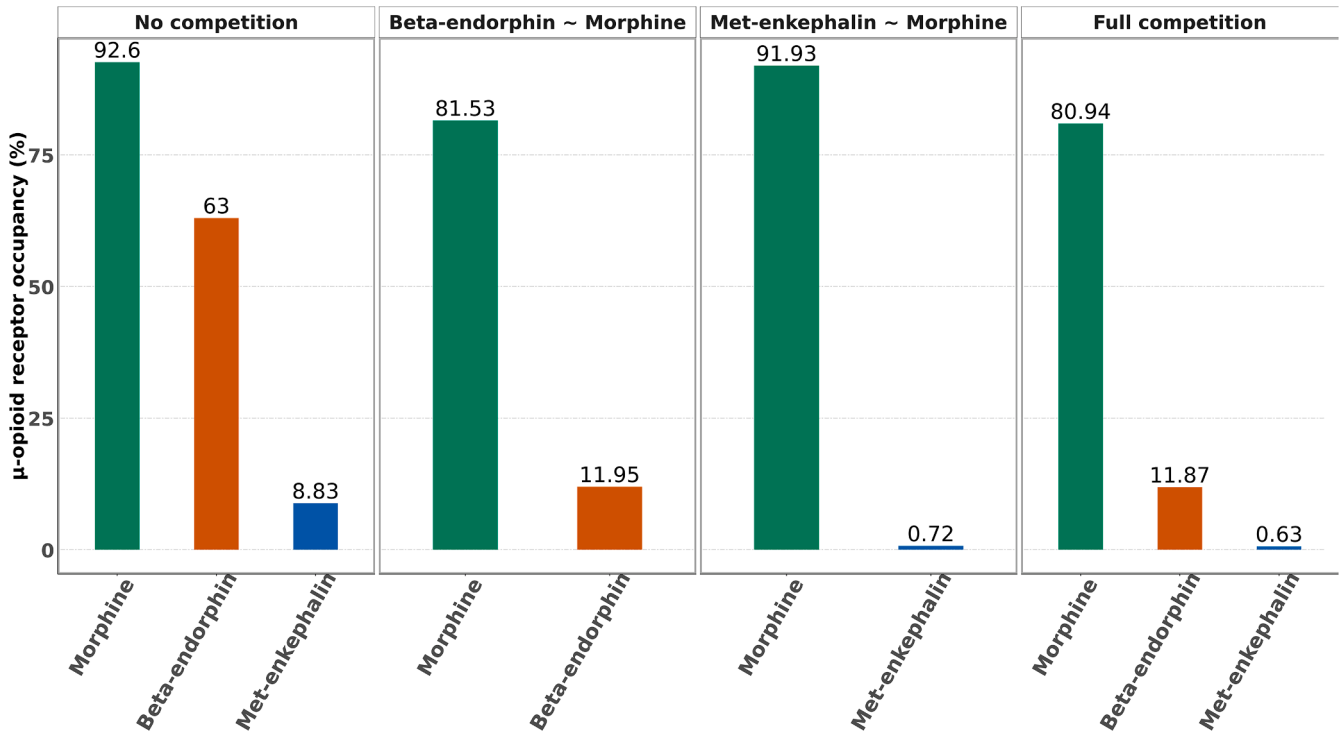

Fig. 3. Beta-endorphin shows the highest μ-OR occupancy across all endogenous opioids in competition with morphine. Morphine's μ-OR occupancy in rat hypothalamus in the absence (no competition), or presence of single endogenous opioid (i.e. pairwise), or presence of all endogenous opioids (full competition), for 5 mg/kg/h IV infusion.

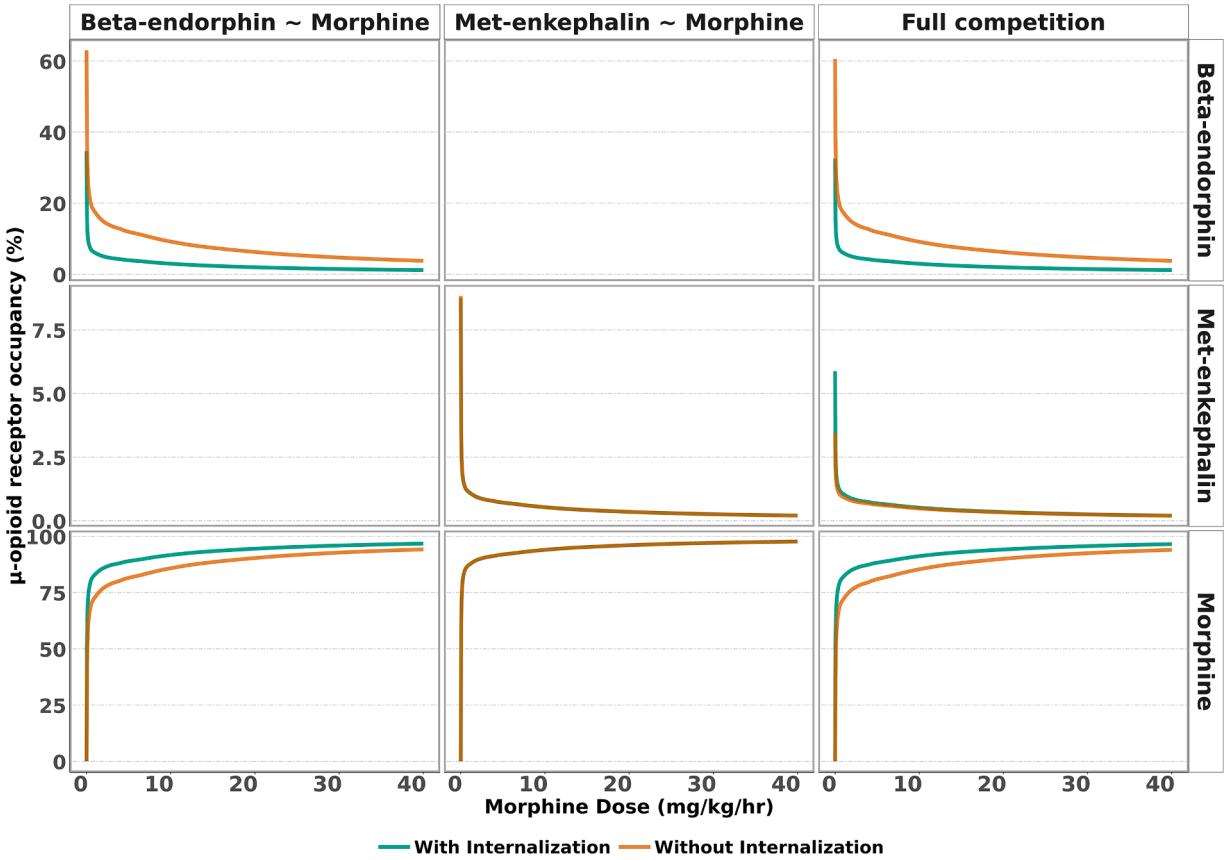

Fig. 4. Beta-endorphin μ- OR decreased due to its high internalization rate, leading to higher morphine μ- OR occupancy at higher doses**. Effect of μ- OR receptor-ligand complex internalization rate on μ- OR occupancy in rat hypothalamus by morphine and endogenous opioids across 0–40 mg/kg/h intravenous infusion doses of morphine.*Other compounds internalization rates did not affect morphine or the endogenous opioid's μ- OR occupancy.

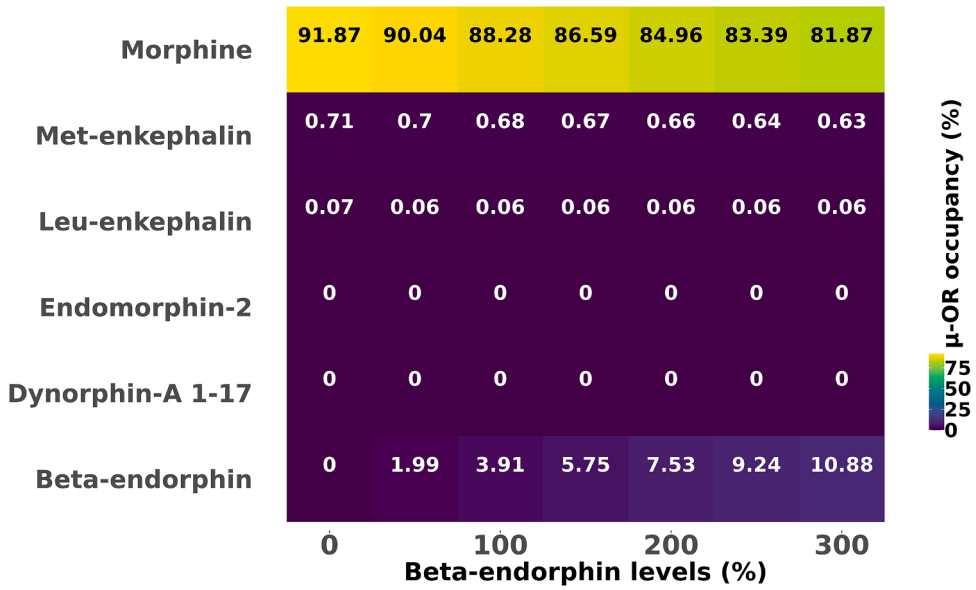

Fig. 5. Change in beta-endorphin levels show a major influence on morphine's μ- OR occupancy for simulations including internalization Sensitivity analysis for beta-endorphin concentration levels as 0 % (complete absence) to baseline (100 %) and 300 % and its effect on morphine μ-OR occupancy in rat hypothalamus for 5 mg/kg/h IV infusion dose of morphine in analysis with role of internalization.

noted in our introduction, the complexity of studying such multidimensional processes in vivo underscores the value of mathematical modeling. Our binding kinetic model offers a foundation to explore the dependency of μ-OR occupancy across various conditions, minimizing animal use laying a strong basis for future validation studies, before clinical application.

To gain deeper insights into role of endogenous opioids competition, development of a detailed neurochemical map combined with systems pharmacology modeling is essential. This can be achieved in multiple steps: first, the release patterns of endogenous opioids in response to pain or opioids needs to be captured using techniques such as MRI, fMRI, molecular fMRI (wei et al., 2021), or specific sensor based imaging (Li et al., 2022). Secondly integrating the endogenous opioid level, i.e., beta-endorphin levels, with drug pharmacokinetics, physiological changes in target regions (such as receptor availability) using a systems pharmacology model would improve endogenous ligands and pharmacological PKPD analysis, paving path towards explaining interindividual differences in analgesia. Finally, PET imaging(Mick et al., 2014,van Waarde et al., 2014)' MRI, or fMRI based receptor occupancy measurements can be used to validate the systems pharmacology models, providing strong basis for clinical applications.

Conclusion

We conclude that beta-endorphin has a strong impact on the μ-OR occupancy of morphine in the rat hypothalamus, whereas the impact of other endogenous opioids studied was negligible. Our findings suggest that beta-endorphin, met-enkephalin and variability in their levels in different subjects can be useful as a guide the optimization of morphine dose for improved analgesia. However, our results serve as a good foundation and must be corroborated through human studies before considering clinical application.

Fig. 1

CRediT authorship contribution statement

Divakar Budda: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Zhicheng Zhang:** Methodology, Formal analysis, Data curation. **J.G. Coen van Hasselt:** Writing – review & editing, Validation, Supervision, Software, Resources. **Elizabeth C.M. de Lange:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors do not declare any conflicts of interest

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068. This manuscript reflects only the authors' view, and the European Commission is not responsible for any use that may be made of the information it contains.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2025.107195.

Data availability

The data used in the study has already been provided in the study, additional data and details were provided as part of supplementary files.

References

Akil, H., Richardson, D.E., Barchas, J.D., Li, C.H., 1978. Appearance of beta-endorphin-like immunoreactivity in human ventricular cerebrospinal fluid upon analgesic electrical stimulation. Proc. Natl. Acad. Sci. 75 (10), 5170–5172. https://doi.org/10.1073/pnas.75.10.5170.

Al-Hasani, R., Wong, J.-M.T., Mabrouk, O.S., McCall, J.G., Schmitz, G.P., Porter-Stransky, K.A., Aragona, B.J., Kennedy, R.T., & Bruchas, M.R. (2018). In vivo detection of optically evoked opioid peptide release. eLife, 7, e36520. https://doi.org/10.7554/eLife.36520.

Almay, B.G.L., Johansson, F., Von Knorring, L., Terenius, L., Wahlstrom, A., 1978. Endorphins in chronic pain. I. Differences in CSF endorphin levels between organic and psychogenic pain syndromes. Pain 5 (2), 153–162. https://doi.org/10.1016/0304-3959(78)90037-4.

Anderson, T.G., McConnell, H.M., 1999. Interpretation of biphasic dissociation kinetics for isomeric class II major histocompatibility complex-peptide complexes. Biophys. J 77 (5), 2451–2461. https://doi.org/10.1016/S0006-3495(99)77081-5.

Bergmann, F., Alstetter, R., Weissman, B.A., 1978. In vivo interaction of morphine and endogenous opiate-like peptides. Life Sci. 23 (26), 2601–2608. https://doi.org/10.1016/0024-3205(78)90376-4.
Berland, D., Rodgers, P., 2012. Rational use of opioids for management of chronic nonterminal pain. Am. Fam. Physic. 86 (3), 252–258. https://www.aafp.org/pubs/afp/issues/2012/0801/p252.pdf.
Borgland, S.L., Connor, M., Osborne, P.B., Furness, J.B., Christie, M.J., 2003. Opioid agonists have different efficacy profiles for G protein activation, rapid desensitization, and endocytosis of mu-opioid receptors*. J. Biol. Chem. 278 (21), 18776–18784. https://doi.org/10.1074/jbc.M300525200.
Bronstein, D.M., Gutstein, H.B., Akil, H., 1993. Effects of chronic morphine treatment on β-endorphin-related peptides in the caudal medulla and spinal cord. J. Neurochem. 60 (6), 2304–2307. https://doi.org/10.1111/j.1471-4159.1993.tb03518.x.
Bronstein, D.M., Przewlocki, R., Akil, H., 1990. Effects of morphine treatment on pro-opiomelanocortin systems in rat brain. Brain Res 519 (1–2), 102–111. https://doi.org/10.1016/0006-8993(90)90066-k.
Bruehl, S., Burns, J.W., Gupta, R., Buvanendran, A., Chont, M., Orlowska, D., Schuster, E., France, C.R., 2017. Do resting plasma β-endorphin levels predict responses to opioid analgesics? Clin J Pain 33 (1), 12. https://doi.org/10.1097/AJP.0000000000000389.
Graphical abstract Created in BioRender, Budda, D., 2025. https://BioRender.com/gw51d1r.
Budda, D., Gülave, B., van Hasselt, J.G.C., de Lange, E.C.M., 2024. Non-linear blood–brain barrier transport and dosing strategies influence receptor occupancy ratios of morphine and its metabolites in pain matrix. Br. J. Pharmacol. 181 (20), 3856–3868. https://doi.org/10.1111/bph.16394.
Chapman, D.B., Hu, J., Way, E.L., 1980. Methionine-enkephalin antagonism and endorphin potentiation of narcotic-induced analgesia. Eur. J. Pharmacol. 65 (4), 369–377. https://doi.org/10.1016/0014-2999(80)90341-6.
Che, F.-Y., Lim, J., Pan, H., Biswas, R., Fricker, L.D., 2005. Quantitative neuropeptidomics of microwave-irradiated mouse brain and pituitary. Mol. Cell. Proteom. 4 (9), 1391–1405. https://doi.org/10.1074/mcp.T500010-MCP200.
Chen, L., Gu, Y., Huang, L.Y., 1995. The mechanism of action for the block of NMDA receptor channels by the opioid peptide dynorphin. J. Neurosci. 15 (6), 4602–4611. https://doi.org/10.1523/JNEUROSCI.15-06-04602.1995.
Choh, Hao Li., 1977. β-endorphin: A pituitary peptide with potent morphine-like activity. Arch. Biochem. Biophys. 183 (2), 592–604. https://doi.org/10.1016/0003-9861(77)90395-2.
Corbett, A.D., Paterson, S.J., McKnight, A.T., Magnan, J., Kosterlitz, H.W., 1982. Dynorphin1–8 and dynorphin1–9 are ligands for the κ-subtype of opiate receptor. Nature 299 (5878), 79–81. https://doi.org/10.1038/299079a0.
Corder, G., Castro, D.C., Bruchas, M.R., Scherrer, G., 2018. Endogenous and exogenous opioids in pain. Annu. Rev. Neurosci. 41, 453–473. https://doi.org/10.1146/annurev-neuro-080317-061522.
Cox, B.M., Ginsburg, M., Osman, O.H., 1968. Acute tolerance to narcotic analgesic drugs in rats. Br J. Pharmacol. Chemother 33 (2), 245–256. https://doi.org/10.1111/j.1476-5381.1968.tb00986.x.
de Lange, E.C., 2013. The mastermind approach to CNS drug therapy: translational prediction of human brain distribution, target site kinetics, and therapeutic effects. Fluids Barr. CNS 10 (1), 12. https://doi.org/10.1186/2045-8118-10-12.
Deliconstantinos, G., Villiotou, V., Stavrides, J.C., 1995. Met-enkephalin receptor-mediated increase of membrane fluidity modulates nitric oxide (NO) and cGMP production in rat brain synaptosomes. Neurochem. Res. 20 (2), 217–224. https://doi.org/10.1007/BF00970547.
Dowell, D., Ragan, K.R., Jones, C.M., Baldwin, G.T., & Chou, R. (2022). CDC clinical practice guideline for prescribing opioids for pain — United States, 2022. MMWR. Recomm. Rep., 71(3), 1–95. https://doi.org/10.15585/mmwr.rr7103a1.
Eshleman, A.J., Nagarajan, S., Wolfrum, K.M., Reed, J.F., Nilsen, A., Torralva, R., Janowsky, A., 2020. Affinity, potency, efficacy, selectivity, and molecular modeling of substituted fentanyls at opioid receptors. Biochem. Pharmacol. 182, 114293. https://doi.org/10.1016/j.bcp.2020.114293.
Ferrara, P., Li, C.H., 1981. Opiate receptor-binding activity of [D-Thr2,Thz5]-and [D-Met2,Thz5]-enkephalinamides. Eur. J. Pharmacol. 73 (1), 69–73. https://doi.org/10.1016/0014-2999(81)90146-1.
Foley, K.M., Kourides, I.A., Inturrisi, C.E., Kaiko, R.F., Zaroulis, C.G., Posner, J.B., Houde, R.W., Li, C.H., 1979. β-endorphin: analgesic and hormonal effects in humans. Proc. Natl. Acad. Sci. 76 (10), 5377–5381. https://doi.org/10.1073/pnas.76.10.5377.
Fricker, L.D., Margolis, E.B., Gomes, I., Devi, L.A., 2020. Five decades of research on opioid peptides: current knowledge and unanswered questions. Mol. Pharmacol. 98 (2), 96–108. https://doi.org/10.1124/mol.120.119388.
Frölich, N., Dees, C., Paetz, C., Ren, X., Lohse, M.J., Nikolaev, V.O., Zenk, M.H., 2011. Distinct pharmacological properties of morphine metabolites at G i-protein and β-arrestin signaling pathways activated by the human μ-opioid receptor. Biochem. Pharmacol. 81 (10), 1248–1254. https://doi.org/10.1016/j.bcp.2011.03.001.
Garcia-Larrea, L., Peyron, R., 2013. Pain matrices and neuropathic pain matrices: a review. Pain 154 (Suppl 1), S29–S43. https://doi.org/10.1016/j.pain.2013.09.001.
Giraud, P., Castanas, E., Patey, G., Oliver, C., Rossier, J., 1983. Regional distribution of methionine-enkephalin-Arg6-Phe7 in the rat brain: comparative study with the distribution of other opioid peptides. J. Neurochem. 41 (1), 154–160. https://doi.org/10.1111/j.1471-4159.1983.tb11827.x.
Goel, M., Mittal, A., Jain, V.R., Bharadwaj, A., Modi, S., Ahuja, G., Jain, A., Kumar, K., 2024. Integrative functions of the hypothalamus: linking cognition, emotion and physiology for well-being and adaptability. Ann Neurosci., 09727531241255492 https://doi.org/10.1177/09727531241255492.
Groenendaal, D., Freijer, J., de Mik, D., Bouw, M.R., Danhof, M., de Lange, E.C.M., 2007. Population pharmacokinetic modelling of non-linear brain distribution of morphine: influence of active saturable influx and P-glycoprotein mediated efflux. Br. J. Pharmacol. 151 (5), 701–712, 10.138/sj.bjp.0707257.
Gudehithlu, K.P., Tehwani, G.A., Bhargava, H.N., 1991. β-endorphin and methionine-enkephalin levels in discrete brain regions, spinal cord, pituitary gland, and plasma of morphine tolerant-dependent and abstinent rats. Brain Res. 553 (2), 284–290. https://doi.org/10.1016/0006-8993(91)90836-K.
Gülave, B., Budda, D., Saleh, M.A., van Hasselt, J.C., de Lange, E.C., 2023. Does nonlinear blood-brain barrier transport matter for (lower) morphine dosing strategies? Eur. J. Pharm. Sci. 187, 106482. https://doi.org/10.1016/j.ejps.2023.106482.
Hess, G.D., Joseph, J.A., Roth, G.S., 1981. Effect of age on sensitivity to pain and brain opiate receptors. Neurobiol. Aging 2 (1), 49–55. https://doi.org/10.1016/0197-4580(81)90059-2.
Hosobuchi, Y., Rossier, J., Bloom, F.E., Guillemin, R., 1979. Stimulation of Human periaqueductal gray for pain relief increases immunoreactive β-endorphin in ventricular fluid. Science 203 (4377), 279–281. https://doi.org/10.1126/science.83674.
Jacquet, Y.F., Lajtha, A., 1973. Morphine action at Central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose. Science 182 (4111), 490–492. https://doi.org/10.1126/science.182.4111.490.
Jóhannesson, T., Steele, W.J., Becker, B.A., 1972. Infusion of morphine in maternal rats at near-term: maternal and foetal distribution and effects on analgesia, brain DNA, RNA and protein. Acta Pharmacol. Toxicol. 31 (5–7), 353–368. https://doi.org/10.1111/j.1600-0773.1972.tb03599.x.
Jones, A.K.P., Watabe, H., Cunningham, V.J., Jones, T., 2004. Cerebral decreases in opioid receptor binding in patients with central neuropathic pain measured by [11C] diprenorphine binding and PET. Eur. J. Pain. 8 (5), 479–485. https://doi.org/10.1016/j.ejpain.2003.11.017.
Juras, J.A., Webb, M.B., Young, L.E.A., Markussen, K.H., Hawkinson, T.R., Buoncristiani, M.D., Bolton, K.E., Coburn, P.T., Williams, M.I., Sun, L.P.Y., Sanders, W.C., Bruntz, R.C., Conroy, L.R., Wang, C., Gentry, M.S., Smith, B.N., Sun, R.C., 2023. In situ microwave fixation provides an instantaneous snapshot of the brain metabolome. Cell Rep. Methods 3 (4), 100455. https://doi.org/10.1016/j.crmeth.2023.100455.
Klee, W.A., Nirenberg, M., 1976. Mode of action of endogenous opiate peptides. Nature 263 (5578), 609–612. https://doi.org/10.1038/263609a0.
Kuhar, M.J., Pert, C.B., Snyder, S.H., 1973. Regional distribution of opiate receptor binding in monkey and Human brain. Nature 245 (5426), 447–450. https://doi.org/10.1038/245447a0.
Law, P.Y., Loh, H.H., Li, C.H., 1979. Properties and localization of beta-endorphin receptor in rat brain. Proc. Natl. Acad. Sci. 76 (11), 5455–5459. https://doi.org/10.1073/pnas.76.11.5455.
Li, J., Liu, Y., Yuan, L., Zhang, B., Bishop, E.S., Wang, K., Tang, J., Zheng, Y.-Q., Xu, W., Niu, S., Beker, L., Li, T.L., Chen, G., Diyaolu, M., Thomas, A.-L., Mottini, V., Tok, J. B.-H., Dunn, J.C.Y., Cui, B., Bao, Z., 2022. A tissue-like neurotransmitter sensor for the brain and gut. Nature 606 (7912). https://doi.org/10.1038/s41586-022-04615-2, 94–11.
Loh, H.H., Tseng, L.F., Wei, E., Li, C.H., 1976. Beta-endorphin is a potent analgesic agent. Proc. Natl. Acad. Sci. 73 (8), 2895–2898. https://doi.org/10.1073/pnas.73.8.2895.
Marano, L., Tommasini, E., Missaglia, S., Vago, P., Rampinini, E., Bosio, A., Morelli, A., Pecci, C., Mordente, A., Tavian, D., 2025. Influence of age and fitness level on β-endorphin response to acute aerobic exercise in healthy men. Sport Sci. Health. https://doi.org/10.1007/s11332-025-01367-0.
Martikainen, I.K., Peciña, M., Love, T.M., Nuechterlein, E.B., Cummiford, C.M., Green, C. R., Harris, R.E., Stohler, C.S., Zubieta, J.-K., 2013. Alterations in endogenous opioid functional measures in chronic back pain. Off. J. Soc. Neurosci. 33 (37), 14729–14737. https://doi.org/10.1523/JNEUROSCI.1400-13.2013.
Mick, I., Myers, J., Stokes, P.R.A., Erritzoe, D., Colasanti, A., Bowden-Jones, H., Clark, L., Gunn, R.N., Rabiner, E.A., Searle, G.E., Waldman, A.D., Parkin, M.C., Brailsford, A. D., Nutt, D.J., Lingford-Hughes, A.R., 2014. Amphetamine induced endogenous opioid release in the human brain detected with [11C]carfentanil PET: replication in an independent cohort. Int. J. Neuropsychopharmacol. 17 (12), 2069–2074. https://doi.org/10.1017/S1461145714000704.
Mikati, M.O., Erdmann-Gilmore, P., Connors, R., Conway, S.M., Malone, J., Woods, J., Sprung, R.W., Townsend, R.R., Al-Hasani, R., 2025. Highly sensitive in vivo detection of dynamic changes in enkephalins following acute stress in mice. eLife 12, RP91609. https://doi.org/10.7554/eLife.91609.
Misra, U.K., Kalita, J., Tripathi, G., Bhoi, S.K., 2017. Role of β endorphin in pain relief following high-rate repetitive transcranial magnetic stimulation in migraine. Basic Transl. Clin. Res. Neuromodulat. 10 (3), 618–623. https://doi.org/10.1016/j.brs.2017.02.006.
Moroni, F., Cheney, D.L., Costa, E., 1977. β endorphin inhibits ACh turnover in nuclei of rat brain. Nature 267 (5608), 267–268. https://doi.org/10.1038/267267a0.
Murphy, E.J., 2010. Brain fixation for analysis of brain lipid-mediators of signal transduction and brain eicosanoids requires head-focused microwave irradiation: an historical perspective. Prostaglandins Other Lipid. Mediat 91 (3–4), 63–67. https://doi.org/10.1016/j.prostaglandins.2009.07.005.
Nagy, A., Gráff, L., Lajtha, A., 1983. Met-enkephalin binding to opiate receptors is not functionally coupled to biodegradation. Life Sci. 33 (9), 835–840. https://doi.org/10.1016/0024-3205(83)90621-5.
Nieto, M.M., Wilson, J., Cupo, A., Roques, B.P., Noble, F., 2002. Chronic morphine treatment modulates the extracellular levels of endogenous enkephalins in rat brain structures involved in opiate dependence: a microdialysis study. J. Neurosci. 22 (3), 1034–1041. https://doi.org/10.1523/JNEUROSCI.22-03-134.2002.

Nylander, I., Stenfors, C., Tan-No, K., Mathé, A.A., Terenius, L., 1997. A comparison between microwave irradiation and decapitation: basal levels of dynorphin and enkephalin and the effect of chronic morphine treatment on dynorphin peptides. Neuropeptides 31 (4), 357–365. https://doi.org/10.1016/s0143-4179(97)90072-x.

Ogawa, N., Panerai, A.E., Lee, S., Forsbach, G., Havlicek, V., Friesen, H.G., 1979. Beta-endorphin concentration in the brain of intact and hypophysectomized rats. Life Sci. 25 (4), 317–326. https://doi.org/10.1016/0024-3205(79)90261-3.

Pedersen, M.F., Wróbel, T.M., Märcher-Rørsted, E., Pedersen, D.S., Møller, T.C., Gabriele, F., Pedersen, H., Matosiuk, D., Foster, S.R., Bouvier, M., Bräuner-Osborne, H., 2020. Biased agonism of clinically approved μ-opioid receptor agonists and TRV130 is not controlled by binding and signaling kinetics. Neuropharmacology 166, 107718. https://doi.org/10.1016/j.neuropharm.2019.107718.

Piva, F., Maggi, R., Limonta, P., Dondi, D., Martini, L., 1987. Decrease of mu opioid receptors in the brain and in the hypothalamus of the aged male rat. Life Sci. 40 (4), 391–398. https://doi.org/10.1016/0024-3205(87)90141-X.

Przewłocki, R., Höllt, V., Duka, Th., Kleber, G., Gramsch, Ch., Haarmann, I., Herz, A., 1979. Long-term morphine treatment decreases endorphin levels in rat brain and pituitary. Brain Res 174 (2), 357–361. https://doi.org/10.1016/0006-8993(79)90862-X.

Rattan, A.K., Tejwani, G.A., 1996. Effect of chronic treatment with morphine, midazolam, and both together on beta-endorphin levels in the rat. Brain Res. Bullet. 41 (6), 335–341. https://doi.org/10.1016/s0361-9230(96)00022-6.

Rattan, A.K., Tejwani, G.A., 1997. Effect of chronic treatment with morphine, midazolam, and both together on dynorphin(1–13) levels in the rat. Brain Res. 754 (1), 239–244. https://doi.org/10.1016/S0006-8993(97)00084-X.

Sjöstedt, E., Zhong, W., Fagerberg, L., Karlsson, M., Mitsios, N., Adori, C., Oksvold, P., Edfors, F., Limiszewska, A., Hikmet, F., Huang, J., Du, Y., Lin, L., Dong, Z., Yang, L., Liu, X., Jiang, H., Xu, X., Wang, J., Mulder, J., 2020. An atlas of the protein-coding genes in the human, pig, and mouse brain. Sci.(NewYork N.Y.) 367 (6482), eaay5947. https://doi.org/10.1126/science.aay5947.

Soliman, K.F., Mash, D.C., Walker, C.A., 1986. The effect of altered 5-hydroxytryptamine levels on beta-endorphin content in rat brain. In: Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.), 182, pp. 187–193. https://doi.org/10.3181/00379727-182-42326.

Song, B., Marvizón, J.C.G., 2003. Peptidases prevent μ-opioid receptor internalization in dorsal horn neurons by endogenously released opioids. J. Neurosci. 23 (5), 1847–1858. https://doi.org/10.1523/JNEUROSCI.23-05-01847.2003.

Spetea, M., Monory, K., Tömböly, C., Tóth, G., Tzavara, E., Benyhe, S., Hanoune, J., Borsodi, A., 1998. VitroBinding and signaling profile of the novel μ opioid receptor agonist endomorphin 2 in rat brain membranes. Biochem. Biophys. Res. Commun. 250 (3), 720–725. https://doi.org/10.1006/bbrc.1998.9395.

Sternini, C., Brecha, N.C., Minnis, J., D'Agostino, G., Balestra, B., Fiori, E., Tonini, M., 2000. Role of agonist-dependent receptor internalization in the regulation of μ opioid receptors. Neuroscience 98 (2), 233–241. https://doi.org/10.1016/S0306-4522(00)00118-4.

Székely, J.I., Rónal, A.Z., Dunai-Kovács, Z., Miglécz, E., Bajusz, S., Gráf, L., 1977. Cross tolerance between morphine and β-endorphin in vivo. Life Sci. 20 (7), 1259–1264. https://doi.org/10.1016/0024-3205(77)90500-8.

Takai, N., Miyajima, N., Tonomura, M., Abe, K., 2018. Relationship between receptor occupancy and the antinociceptive effect of mu opioid receptor agonists in male rats. Brain Res. 1680, 105–109. https://doi.org/10.1016/j.brainres.2017.12.014.

Takemori, A.E., Loh, H.H., Lee, N.M., 1993. Suppression by dynorphin A and [des-Tyr1] dynorphin A peptides of the expression of opiate withdrawal and tolerance in morphine-dependent mice. J. Pharmacol. Exp. Ther. 266 (1), 121–124.

Taylor, J.J., Borckardt, J.J., George, M.S., 2012. Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. PAIN 153 (6), 1219. https://doi.org/10.1016/j.pain.2012.02.030.

Tejwani, G.A., Rattan, A.K., 1997. Met-enkephalin alteration in the rat during chronic injection of morphine and/or midazolam. Brain Res. 775 (1), 119–126. https://doi.org/10.1016/S0006-8993(97)00875-5.

Terenius, L., Wahlström, A., 1975. Morphine-like ligand for opiate receptors in human CSF. Life Sci. 16 (12), 1759–1764. https://doi.org/10.1016/0024-3205(75)90269-6.

Tseng, L.F., Loh, H.H., Li, C.H., 1976. beta-endorphin: cross tolerance to and cross physical dependence on morphine. Proc. Natl. Acad. Sci. 73 (11), 4187–4189. https://doi.org/10.1073/pnas.73.11.4187.

Turchan, J., Lasoń, W., Budziszewska, B., Przewłocka, B., 1997. Effects of single and repeated morphine administration on the prodynorphin, proenkephalin and dopamine D2 receptor gene expression in the mouse brain. Neuropeptides 31 (1), 24–28. https://doi.org/10.1016/S0143-4179(97)90015-9.

van Waarde, A., Absalom, A.R., Visser, A.K.D., Dierckx, R.A.J.O., van Waarde, A., 2014. Positron emission tomography (PET) imaging of opioid receptors. In: Dierckx, R.A.J.O., Otte, A., de Vries, E.F.J., Luiten, P.G.M. (Eds.), PET and SPECT of Neurobiological Systems. Springer, pp. 585–623, 100.1007/978-3-642-42014-6_20.

von Knorring, L., Almay, B.G.L., Johansson, F., Terenius, L., 1978. Pain perception and endorphin levels in cerebrospinal fluid. PAIN 5 (4), 359. https://doi.org/10.1016/0304-3959(78)90004-0.

von Knorring, L., Almay, B.G.L., Johansson, F., Terenius, L., 2008. Endorphins in CSF of chronic pain patients, in relation to augmenting-reducing response in visual averaged evoked response. Neuropsychobiology 5 (6), 322–326. https://doi.org/10.1159/000117698.

Waterfield, A.A., Hughes, J., Kosterlitz, H.W., 1976. Cross tolerance between morphine and methionine-enkephalin. Nature 260 (5552), 624–625. https://doi.org/10.1038/260624a0.

Wei, H., Frey, A.M., Jasanoff, A., 2021. Molecular fMRI of neurochemical signaling. J. Neurosci. Methods 364, 109372. https://doi.org/10.1016/j.jneumeth.2021.109372.

Welniak–Kaminska, M., Fiedorowicz, M., Orzel, J., Bogorodzki, P., Modlinska, K., Stryjek, R., Chrzanowska, A., Pisula, W., Grieb, P., 2019. Volumes of brain structures in captive wild-type and laboratory rats: 7T magnetic resonance in vivo automatic atlas-based study. PLOS. ONE 14 (4), e0215348. https://doi.org/10.1371/journal.pone.0215348.

Williams, J.T., Ingram, S.L., Henderson, G., Chavkin, C., von Zastrow, M., Schulz, S., Koch, T., Evans, C.J., Christie, M.J., 2013. Regulation of μ-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. Pharmacol. Rev. 65 (1), 223–254. https://doi.org/10.1124/pr.112.005942.

Witt, S.N., McConnell, H.M., 1994. Formation and dissociation of short-lived class II MHC-peptide complexes. Biochemistry 33 (7), 1861–1868. https://doi.org/10.1021/bi00173a032.

Yukhananov, R.Yu., Zhai, Q.Z., Persson, S., Post, C., Nyberg, F., 1993. Chronic administration of morphine decreases level of dynorphin a in the rat nucleus accumbens. Neuropharmacology 32 (7), 703–709. https://doi.org/10.1016/0028-3908(93)90084-G.

Zadina, J.E., Hackler, L., Ge, L.-J., Kastin, A.J., 1997. A potent and selective endogenous agonist for the μ-opiate receptor. Nature 386 (6624), 499–502. https://doi.org/10.1038/386499a0.

Zangen, A., Herzberg, U., Vogel, Z., Yadid, G., 1998. Nociceptive stimulus induces release of endogenous beta-endorphin in the rat brain. Neuroscience 85 (3), 659–662. https://doi.org/10.1016/s0306-4522(98)00050-5.

Zhang, A.-Z., Pasternak, G.W., 1981. Ontogeny of opioid pharmacology and receptors: high and low affinity site differences. Eur. J. Pharmacol. 73 (1), 29–40. https://doi.org/10.1016/0014-2999(81)90142-4.

Zubieta, J.-K., Bueller, J.A., Jackson, L.R., Scott, D.J., Xu, Y., Koeppe, R.A., Nichols, T.E., Stohler, C.S., 2005. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. Off. J. Soc. Neurosci. 25 (34), 7754–7762. https://doi.org/10.1523/JNEUROSCI.0439-05.2005.