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Non-linear blood–brain barrier transport and dosing strategies influence receptor occupancy ratios of morphine and its metabolites in pain matrix

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Abstract

Background and Purpose: Morphine is important for treatment of acute and chronic pain. However, there is high interpatient variability and often inadequate pain relief and adverse effects. To better understand variability in the dose-effect relationships of morphine, we investigated the effects of its non-linear blood–brain barrier (BBB) transport on μ-receptor occupancy in different CNS locations, in conjunction with its main metabolites that bind to the same receptor.

Experimental Approach: CNS exposure profiles for morphine, M3G and M6G for clinically relevant dosing regimens based on intravenous, oral immediate- and extended-release formulations were generated using a physiology-based pharmacokinetic model of the CNS, with non-linear BBB transport of morphine. The simulated CNS exposure profiles were then used to derive corresponding μ-receptor occupancies at multiple CNS pain matrix locations.

Key Results: Simulated CNS exposure profiles for morphine, M3G and M6G, associated with non-linear BBB transport of morphine resulted in varying μ-receptor occupancies between different dose regimens, formulations and CNS locations. At lower doses, the μ-receptor occupancy of morphine was relatively higher than at higher doses of morphine, due to the relative contribution of M3G and M6G. At such higher doses, M6G showed higher occupancy than morphine, whereas M3G occupancy was low throughout the dose ranges.

Conclusion and Implications: Non-linear BBB transport of morphine affects the μ-receptor occupancy ratios of morphine with its metabolites, depending on dose

Abbreviations: BBB, blood–brain barrier; $Brain_{ECF}$, brain extracellular fluid; CF, conversion factor; CSF_{SAS}, subarachnoid space cerebrospinal fluid; fAFFBBB, factor asymmetry factor at BBB; HEK-293T, human embryonic kidney 293 cells expressing the SV40 large T antigen; HPA, Human Protein Atlas; K_d, equilibrium dissociation constant; K_{off}, dissociation rate constant; K_{off_M}, morphine dissociation rate constant; K_{off_M3G}, M3G dissociation rate constant; K_{off_M6G}, M6G dissociation rate constant; K_{on}, association rate constant; K_{on_M}, Morphine association rate constant; K_{on_M3G}, M3G association rate constant; K_{on_M6G}, M6G association rate constant; L_M, Morphine concentration predicted from LeiCNS PK3.0; L_{M3G}, M3G concentration predicted from LeiCNS PK3.0; L_{M6G}, M6G concentration predicted from LeiCNS PK3.0; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; nTPM, normalized tags per million; PKPD, pharmacokinetics-pharmacodynamics; *R*, unbound receptor available in specific region; RL_M, bound morphine-μ-receptor complex; RL_{M3G}, bound M3G-μ-receptor complex; RL_{M6G}, bound M6G-μ-receptor complex; R_{tot}, total receptor available in specific region; TLR4, toll like receptor 4; μ-1, μ-receptor subtype 1; μ-2, μ-receptor subtype 2; μ-3, μ-receptor subtype 3.

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and route of administration, and CNS location. These predictions need validation in animal or clinical experiments, to understand the clinical implications.

KEYWORDS
competitive receptor binding kinetics, morphine, morphine-3-glucuronide, morphine-6-glucoronide, pain matrix, μ-opioid receptor

1 | INTRODUCTION

Morphine is an important drug used to treat moderate to severe acute and **chronic pain**. The analgesic effects of morphine are mediated by the **μ opioid receptor**, with involvement of CNS regions such as the periaqueductal grey (Tsou K & Jang CS, 1964), amygdala, hypothalamus, rostral anterior cingulate cortex, rostroventromedial medulla, medullary dorsal horns, which are collectively is referred to as the 'Pain matrix' (Garcia-Larrea & Peyron, 2013; Melzack, 2001). The narrow therapeutic index of morphine and high interpatient variability in drug effects are associated with inadequate pain relief and adverse effects. Morphine's short-term use leads to sedation and life-threatening conditions such as respiratory depression, while its long-term use leads to cognitive impairment and abuse liability (Bachmutsky et al., 2020; Cohen et al., 2021). Enhancing our understanding of factors which contribute to the dose-effect relationships of morphine is therefore clinically relevant.

Morphine is metabolized mainly into morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). The metabolite M3G has pro-nociceptive effects (Gong et al., 1991; Morley et al., 1992; Qian-Ling et al., 1992), while M6G has anti-nociceptive effects (Hanna et al., 1990; Osborne et al., 1992; Peterson et al., 1990; Regnard & Twycross, 1984) and may contribute to tolerance development and abuse liability (Abbott & Franklin, 1991). M6G has a higher intrinsic potency than morphine, and the potency ratio of M6G:morphine differs markedly between administration routes, such that intracerebroventricular administration and intrathecal administration are associated with 90- and 650-fold potency ratios, respectively (Paul et al., 1989). The variations in potency across different administration routes may be attributed to the concentrations, at the target site, of these agents, which is influenced by the pharmacokinetics of morphine, M3G and M6G. It can also be due to competitive binding of morphine and metabolites at μ-receptors .

Exposure of the CNS to morphine and its metabolites is modulated by their respective transporters across the blood-brain barrier (BBB). For example, morphine is moderately lipophilic, and more readily crosses the BBB compared to either M3G or M6G which are hydrophilic. In addition, for morphine, transport across the BBB is non-linear, which results in different relative exposure profiles of morphine, M3G and M6G (Gülave et al., 2023). In terms of μ-receptor binding, morphine has the highest binding affinity, followed by M6G and then M3G. However, M6G is more potent than morphine, despite its low binding affinity (Abbott & Franklin, 1991). The relative μ-receptor binding of morphine, M3G and M6G may therefore influence the balance between pro and anti-nociceptive effects.

To date there have been no pharmacodynamic models or studies which analysed competitive binding of morphine with M3G and M6G. To address this, we aimed to quantify role of M3G and M6G by quantitatively characterizing the relative μ-receptor occupancy between morphine, M3G and M6G in different pain matrix regions, affected by morphine's non-linear BBB transport. We did this using multiple routes of administration, using dosing schedules that can influence the non-linear BBB transport, using an innovative physiologically-based modelling approach.

What is already known?

- Morphine is metabolized mainly into its glucuronide derivatives M3G and M6G. .
- Transport of morphine across the blood brain barrier (BBB) is non-linear.

What does this study add?

- Morphine non-linear BBB transport affects competitive binding with M3G, M6G and relative occupancy ratios.

What is the clinical significance?

- Changes in occupancy ratios of morphine and metabolites can influence their pharmacodynamic effects.

2 | METHODS

To simulate μ-receptor occupancy of morphine, M3G and M6G we first simulated CNS target site concentrations. We subsequently used μ-receptor expression in different CNS pain matrix regions to simulate μ-receptor occupancy across different clinically relevant dose regimens. All simulations were conducted using R Project for Statistical Computing (RRID:SCR_001905) (Dessau and Pipper, 2008).

2.1 | Simulation of CNS concentrations for different dosing schedules and formulations

The μ-receptors in the pain matrix are facing either the brain extracellular fluid ($Brain_{ECF}$) or, for the spinal cord dorsal horn, the subarachnoid cerebrospinal fluid (CSF_{SAS}). The relevant target site concentrations are therefore the $Brain_{ECF}$ and CSF_{SAS}. We predicted these concentrations using a validated comprehensive physiologically-based pharmacokinetic model of the CNS (Saleh et al., 2021), which was extended with a non-linear BBB transport function for morphine (Gülave et al., 2023). As previously described by Gülave et al., (2023), we simulated CNS pharmacokinetic profiles for a range of clinically relevant dose regimens including IV bolus (~28–56 mg per day) (CDER & FDA, 2016), oral immediate and oral extended-release (~60–120 mg per day) (CDER & FDA, 2021) formulations for the dosing regimens, as shown in Table 1.

TABLE 1 Morphine sulphate clinical dose regimens used for simulations of PK profiles.

Dose (mg)	Frequency	Route/formulation
0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 125, 150	1, 2, 4, 6 times a day	Intravenous bolus, Oral immediate release, Oral extended-release

2.2 | Expression of μ-receptors in human CNS locations associated with pain perception and control

Data on the expression of μ-receptor protein in human brain is not available in the public domain. To this end, mRNA expression data of the gene **OPRM1 (μ-receptor type 1)**, as copies per μg) was used from the Human Protein Atlas (HPA) V22.0 (Sjöstedt et al., 2020) to compare relative expression of the μ-receptor at different CNS locations. As pain matrix region-specific expression information was not available, broader representative regions were selected (Figure 1a), including the cerebral cortex, medulla oblongata, mid-brain, pons, thalamus and spinal cord. The weights of each brain region obtained from literature (Azevedo et al., 2009; Pakkenberg & Gundersen, 1997; *Brain Facts and Figures*, n.d.) (Table 2) were combined with expression values of one region cerebral cortex, spinal cord (Peng et al., 2012) in order to determine differences between mRNA expression values (Figure 1b) for other regions (Figure 1c).

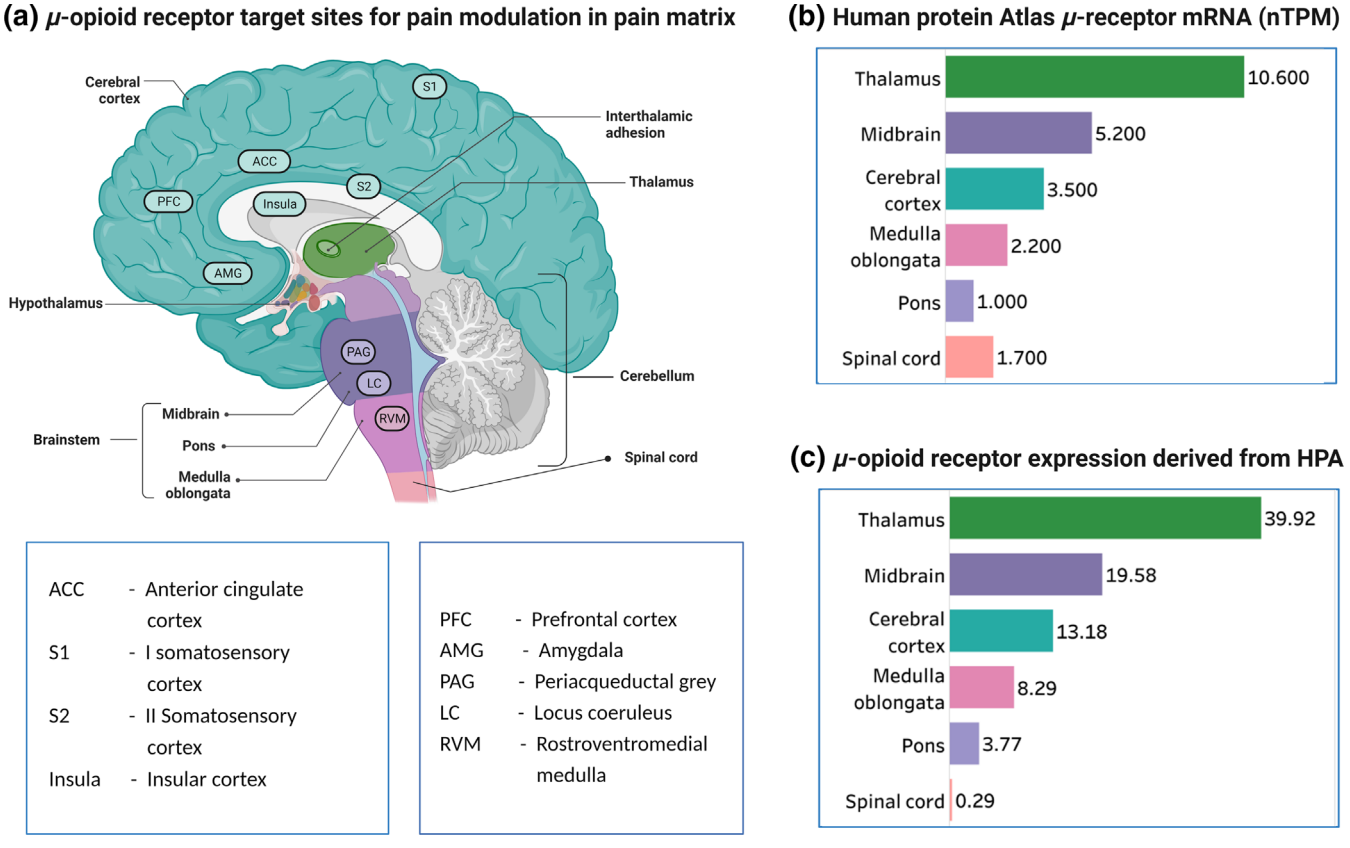

FIGURE 1 (a) Locations of μ-receptors in the pain matrix of the human CNS. (b) Human OPRM1 mRNA expression from Human Protein Atlas (HPA) V22.0 nTPM (normalized tags per million). (c) Expression of μ-receptors (in nanomoles) calculated from HPA normalized tags per million (nTPM) values (created with BioRender.com). Due to unavailability of μ-receptor expression in specific pain matrix regions, broader regions representing pain matrix regions were selected. The Figure shows the broader regions selected for calculation of receptor expression and simulation of receptor occupancy profiles.

TABLE 2 Expression of μ-receptors in human CNS regions.

CNS region	Parameter	Value	Reference
Cerebral cortex	mRNA expression	648,479 (copies per μg)	(Peng et al., 2012)
Spinal cord	mRNA expression	5,002,216 (copies per μg)	(Peng et al., 2012)
Cerebral cortex	Region weight	1232.93 g	(Azevedo et al., 2009)
Spinal cord	Region weight	35 g	(*Brain Facts and Figures*, n.d.)
Thalamus	Factor for mRNA	3.77E−9	Derived[a]
Midbrain	Factor for mRNA	3.77E−9	Derived[a]
Pons	Factor for mRNA	3.77E−9	Derived[a]
Medulla oblongata	Factor for mRNA	3.77E−9	Derived[a]

[a]Ratio of cerebral cortex total expression with its nTPM value from HPA.

TABLE 3 Mean binding kinetic (BK) parameters for morphine, M3G and M6G for binding kinetic rate constants.

K_d (μM)						
Morphine	M6G	M3G	Cell line	Drug	Assay	Reference
0.022	0.063	6.1	HEK-293T (RRID:CVCL_0063)	3H-naloxone	Radioligand binding assay	(Frölich et al., 2011)

Briefly, the following steps were followed to calculate the expression of the μ-receptor protein.

$$\mathrm{mRNA}_{\mathrm{region}} = \mathrm{mRNA} \times \mathrm{Wt} \quad (1)$$

$$\mathrm{CF} = \frac{mRNA_{region}}{nTPM_{region}} \quad (2)$$

$$R = \mathrm{nTPM} \times \mathrm{CF} \quad (3)$$

$\mathrm{mRNA}_{\mathrm{region}}$ is mRNA expression of specific CNS region, mRNA is the mRNA expression of OPRM1 in copies per μg of the specific region, Wt is weight of the region, $\mathrm{nTPM}_{\mathrm{region}}$ is the Human Protein atlas mRNA expression expressed in normalized tags per million and CF is the conversion factor and R is the final receptor expression calculated for each region.

2.3 | Binding kinetic parameters

No information of the association rate constants (K_{on}) and dissociation rate constants (K_{off}) for morphine, M3G, M6G could be found while being obtained within the same study (Table 3). Therefore, we used the Stokes-Einstein's law of diffusion to determine K_{on} values using equilibrium dissociation constant (K_d), based on assumption of diffusion limited binding to the μ-receptors at the synaptic cleft (Cruickshank, 1924). K_d values for morphine, M3G, and M6G determined under same experimental conditions by Frölich et al. (2011) were used for K_{on} and K_{off} values approximation.

K_{on} values for morphine, M3G and M6G were determined using principles of diffusion limited binding (Cruickshank, 1924; Gudowska-Nowak et al., 2017; Smoluchowski, 1918; Spiros et al., 2010). Briefly, the following steps were followed:

The effective diffusion limited association rate (K_{on}) for a particle attaching to specific point with contact radius **σ** (Smoluchowski, 1918) is given by

$$K_{on} = \frac{4\pi D\sigma}{V} \quad (4)$$

where D = translational diffusion coefficient, V = volume of container of reaction, whereas according to Stokes–Einstein's law of diffusion, diffusion coefficient (D)

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi Zr} \quad (5)$$

where R = gas constant ($\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$), T = the absolute temperature (kelvins), N = Avogadro's number, Z = the viscosity of the diffusion medium and r = the radius of the diffusing particle (picometers).

From Equations (4) and (5), diffusion coefficient and K_{on} can be related to radius of the molecule as

$$D \propto \frac{1}{r} \quad (6)$$

$$K_{on} = a.\frac{1}{r} \quad (7)$$

From Ernest Rutherford's Gold foil experiment (Rutherford, 1911), the radius (r) of a nucleus for a spherical molecule can be calculated by

$$r = R_o.\sqrt[3]{M} \quad (8)$$

where $R_o = 1.2 \times 10^{-15}$ m, and M = the atomic mass.

From Equations (7) and (8) we can derive that

$$K_{on} = a.\frac{1}{\sqrt[3]{M}} \quad (9)$$

The proportionality constant ***a*** can be determined by regressing the reciprocal of cubic root of molecular mass of ligand (i.e., $\frac{1}{\sqrt[3]{M}}$) against K_{on}. This regression analysis is conducted using data presented in a patent ((Geerts & Spiros, 2006), page 7, Table 1). The regression plot (Figure S6) gives a line that described by

$$y = ax + b \quad (10)$$

Knowing K_{on}, K_{off} is calculated from the K_d values of morphine, M3G and M6G, determined in the same study under same experimental conditions, as shown in Table 3.

$$K_{off} = [K_d].[K_{on}] \quad (11)$$

Standard ODE binding equations were used, including competitive binding between morphine, M3G, and M6G (de Witte et al., 2017; Moss et al., 2020). Briefly, for these ligands (L):

$$\frac{d(RL_M)}{dt} = [K_{on\_M}].[L_M].[R] - [K_{off\_M}].[RL_M] \quad (12)$$

$$\frac{d(RL_{M3G})}{dt} = [K_{on\_M3G}].[L_{M3G}].[R] - [K_{off\_M3G}].[RL_{M3G}] \quad (13)$$

$$\frac{d(RL_{M6G})}{dt} = [K_{on\_M6G}].[L_{M6G}].[R] - [K_{off\_M6G}].[RL_{M6G}] \quad (14)$$

$$\begin{aligned}\frac{d(R)}{dt} = &-\{([K_{on\_M}].[L_M].[R] - [K_{off\_M}].[RL_M]) \\ &-([K_{on\_M3G}].[L_{M3G}].[R] - [K_{off\_M3G}].[RL_{M3G}]) \\ &-([K_{on\_M6G}].[L_{M6G}].[R] - [K_{off\_M6G}].[RL_{M6G}])\}\end{aligned} \quad (15)$$

$$Morphine\ \mu - receptor\ occupancy\ (\%) = \frac{RL_M}{R_{tot}}.100\% \quad (16)$$

$$M3G\ \mu - receptor\ occupancy\ (\%) = \frac{RL_{M3G}}{R_{tot}}.100\% \quad (17)$$

$$M6G\ \mu - receptor\ occupancy\ (\%) = \frac{RL_{M6G}}{R_{tot}}.100\% \quad (18)$$

where L_M is the morphine concentration as a function of time predicted from LeiCNS PK 3.0 model, L_{M3G} is the M3G concentration as a function of time predicted from LeiCNS PK 3.0 model, L_{M6G} is M6G concentration as a function of time predicted from LeiCNS PK 3.0 model, K_{on_M} is the K_{on} value of morphine, K_{on_M3G} is the K_{on} value of M3G, K_{on_M6G} is the K_{on} value of M6G, K_{off_M} is the K_{off} value of morphine, K_{off_M3G} is the K_{off} value of M3G, K_{off_M6G} is the K_{off} value of M6G, RL_M is the bound morphine- μ-receptor complex, RL_{M3G} is the bound M3G- μ-receptor complex, RL_{M6G} is the bound M6G-μ-receptor complex, R is the unbound μ-receptor, R_{tot} is the total μ-receptor. For this, receptor expression, binding kinetic rate constants and ligand concentrations were converted into micromoles.

2.4 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/2024 (Alexander, Christopoulos et al., 2023; Alexander, Fabbro et al., 2023a;2023b).

3 | RESULTS

3.1 | M3G showed the lowest μ-receptor occupancy despite high target site concentrations

We first simulated human CNS target site concentrations for morphine, M3G and M6G based on the analysis described by Gülave et al., (2023).

For example, the pharmacokinetic profiles in $Brain_{ECF}$, as obtained following a once daily oral immediate release dose of 30 mg (Figure 2a), shows relatively high and highly fluctuating M3G concentrations, while morphine and M6G showed similar peak and trough values, but with slower elimination for M6G. In contrast to $Brain_{ECF}$ concentrations, the resulting μ-receptor occupancy (Figure 2b) for M3G is relatively low and that for M6G is the highest. Then, also in contrast to $Brain_{ECF}$ concentrations, the μ-receptor occupancies of morphine and M6G did not reach steady state within the period of 7 days.

3.2 | Non-linear BBB transport affected the concentrations of morphine and its metabolites and μ-receptor occupancy

The effects of non-linearity across doses are shown with steady-state CNS target site concentrations on day 6–7 across 0.25–50 mg once daily doses (Table 3) for oral immediate release, oral extended release and IV bolus formulations (Figure 3a). CSF_{SAS} shows lower concentrations than $Brain_{ECF}$, with morphine's BBB non-linearity showing effects at higher doses than $Brain_{ECF}$. Figure 3b displays the resulting μ-receptor occupancy in midbrain and spinal cord. These μ-receptor occupancies increased with dose for all compounds. In the midbrain, μ-receptor occupancy of M3G was always the lowest, while M6G was often the highest. In the spinal cord, M3G μ-receptor occupancy was also the lowest, morphine μ-receptor occupancy was the highest at the lower morphine doses, whereas at higher doses, the M6G μ-receptor occupancy becomes the highest.

Morphine's proportion (fraction) relative to the total (i.e., morphine + M3G + M6G) in CNS target site concentration and occupancy is shown in Figure 4. Relative $Brain_{ECF}$ concentrations of

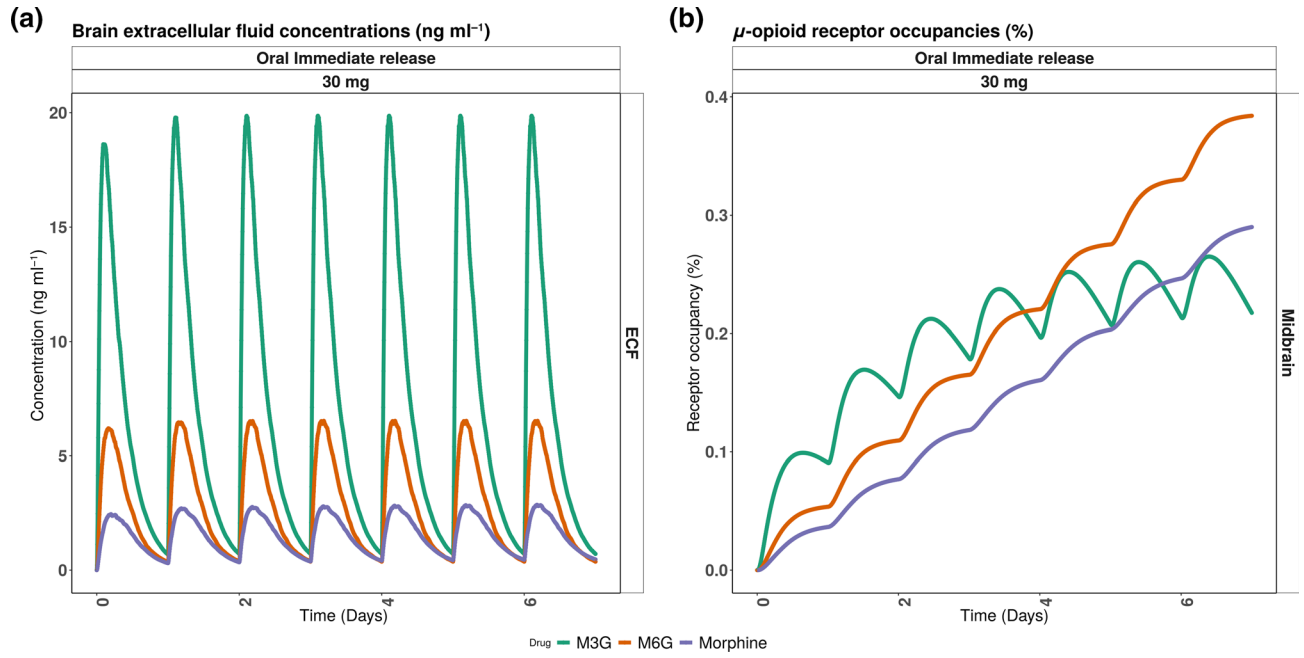

FIGURE 2 (a) Brain extracellular fluid ($Brain_{ECF}$) concentration, (b) μ-receptor occupancy of morphine, M3G, M6G in human midbrain, after dosing with the oral immediate release form of morphine (30 mg once daily). A typical profile of concentration and μ-receptor occupancy against time were simulated.

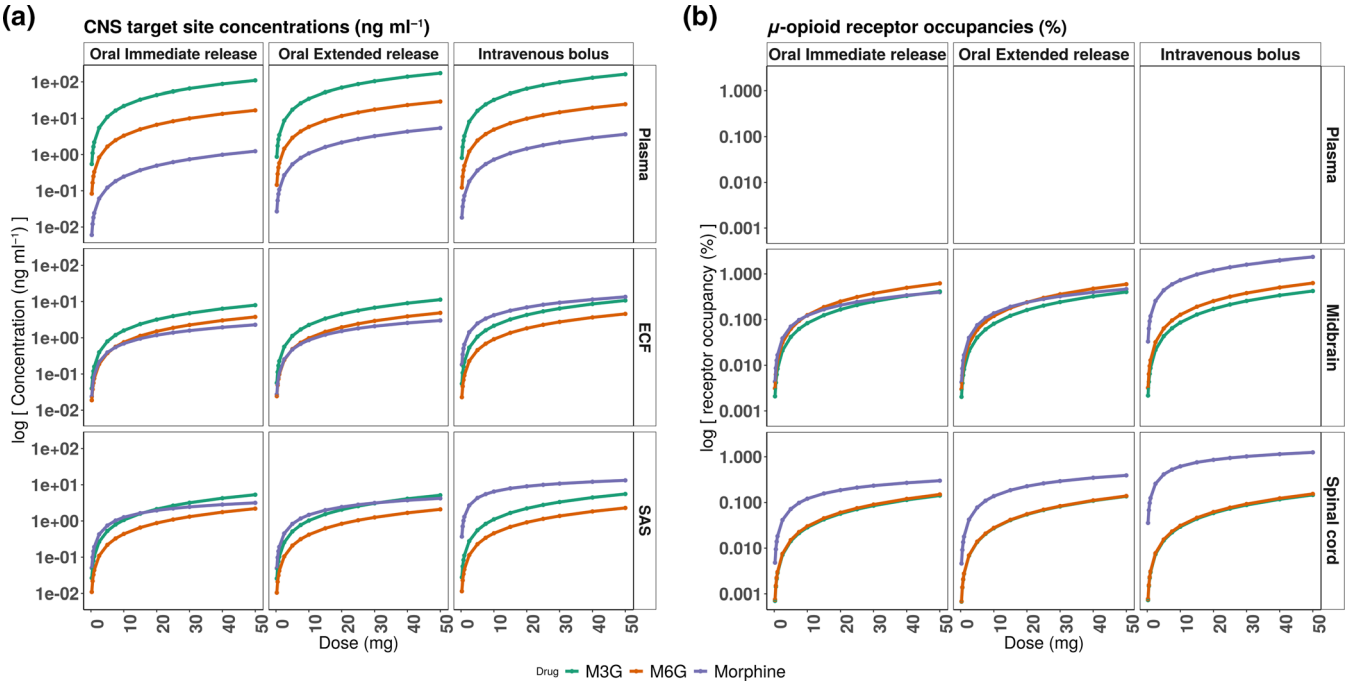

FIGURE 3 Simulation results for morphine, M3G and M6G for different once daily dosing regimens and formulations for (a) plasma, brain extracellular fluid ($Brain_{ECF}$) and cerebrospinal fluid–subarachnoid space (CSF_{SAS}) concentrations and (b) μ-receptor occupancies in the midbrain and spinal cord of human CNS. CNS target site concentrations and μ-receptor occupancies on day 6–7 were captured with the measure of median to show the trend across different doses and routes of administration. CNS target site $Brain_{ECF}$ concentrations are used as input for the midbrain, whereas the CSF_{SAS} concentrations were used for the spinal cord; hence the panels correspond to each other, whereas plasma profile is shown for making comparisons.

morphine, M3G and M6G, are different from the corresponding relative CSF_{SAS} concentrations, while both are clearly different from average plasma concentrations (Figure 4a). The resulting μ-receptor occupancy fractions for morphine were highest at low doses, while at higher doses, occupancies by M3G and especially M6G, increased (Figure 4b).

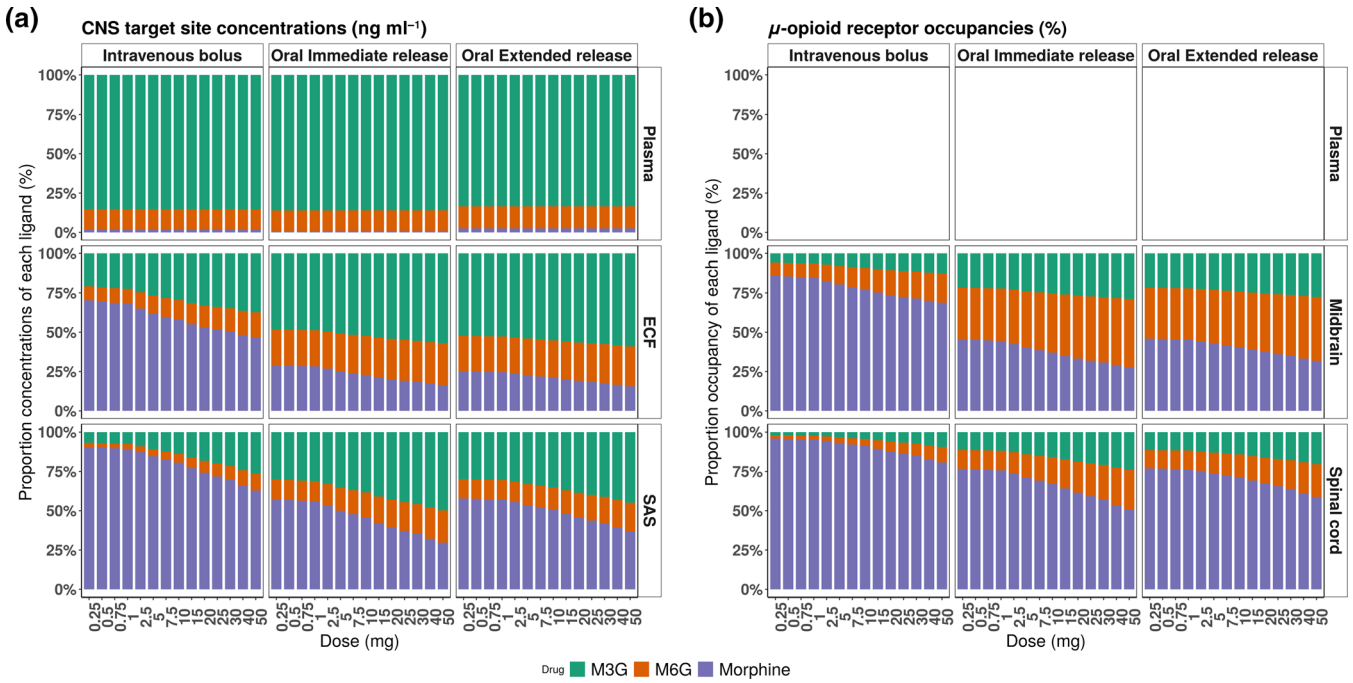

FIGURE 4 (a) Relative plasma, brain extracellular fluid ($Brain_{ECF}$) and cerebrospinal fluid–subarachnoid space (CSF_{SAS}) concentration fractions (b). Corresponding μ-receptor occupancy fractions in midbrain and spinal cord, for morphine, M3G and M6G for once daily dosing regimens and formulations. Here the same proportions of each ligand compared to the total, that is, sum of all ligands (morphine + M3G + M6G) to show how the proportions are changing with respect to increased dose due to non-linearity is shown in this figure.

3.3 | All pain matrix locations, except the spinal cord, showed similar μ-receptor occupancy ratios

All brain locations included in our simulations showed similar trends of μ-receptor occupancy, except for the spinal cord which has the lowest receptor expression, and the lowest target site concentrations (CSF_{SAS}) (Figure 5). Furthermore, the doses at which the effects of the non-linear BBB transport of morphine was observed, differed between spinal cord and other locations. It should be noted that the values for μ-receptor occupancy % values are extremely low. From Figure 5, it is also apparent that receptor occupancy is more sensitive to CNS target site concentration than differences in receptor expression, as shown by receptor occupancy profiles in the spinal cord, compared with those in other regions in the CNS.

3.4 | Formulation and dose regimen differences were reflected in μ-receptor occupancy

Morphine showed higher μ-receptor occupancy after IV dosing, compared with that after oral dosing (Figure 3). Particularly, the effects of morphine's non-linear BBB transport were observed at higher doses in IV bolus for midbrain μ-receptor occupancy, whereas for the occupancy in spinal cord, the effects of non-linear BBB transport were observed at higher doses in oral extended-release formulation. Particularly, the receptor occupancy for morphine was greater at higher doses after IV dosing, in the brain regions exposed to $Brain_{ECF}$ concentrations, whereas in the spinal cord, increased μ-receptor occupancy by morphine was observed for higher doses for the oral extended-release formulation.

The μ-receptor occupancy fractions of morphine/M3G/M6G ratio in the $Brain_{ECF}$ and CSF_{SAS} varies between IV, oral extended-release and oral immediate release formulations. In particular, after oral immediate release formulations, higher morphine occupancies in spinal cord than in midbrain were observed, compared to those of M3G. Furthermore, the dose at which non-linear morphine BBB transport affected $Brain_{ECF}$, CSF_{SAS} concentrations and μ-receptor occupancy also differed between formulations.

The ratio of fractions of morphine/M3G/M6G concentrations at CNS target sites changes with larger dosing intervals (Figure 6). Interestingly, with increase in dosing interval (24 hours) the effects of the non-linearity of morphine transport was observed at higher doses in $Brain_{ECF}$ while the non-linearity effect was observed at lower doses in CSF_{SAS}. Differences in μ-receptor occupancy between morphine and metabolites in midbrain were less influenced by dose interval changes, while the differences were reduced with more frequent administrations (6 h) compared to less frequent administrations (24 h).

4 | DISCUSSION

To better understand variability in the exposure-response relationships of morphine, we investigated the effects of its non-linear BBB

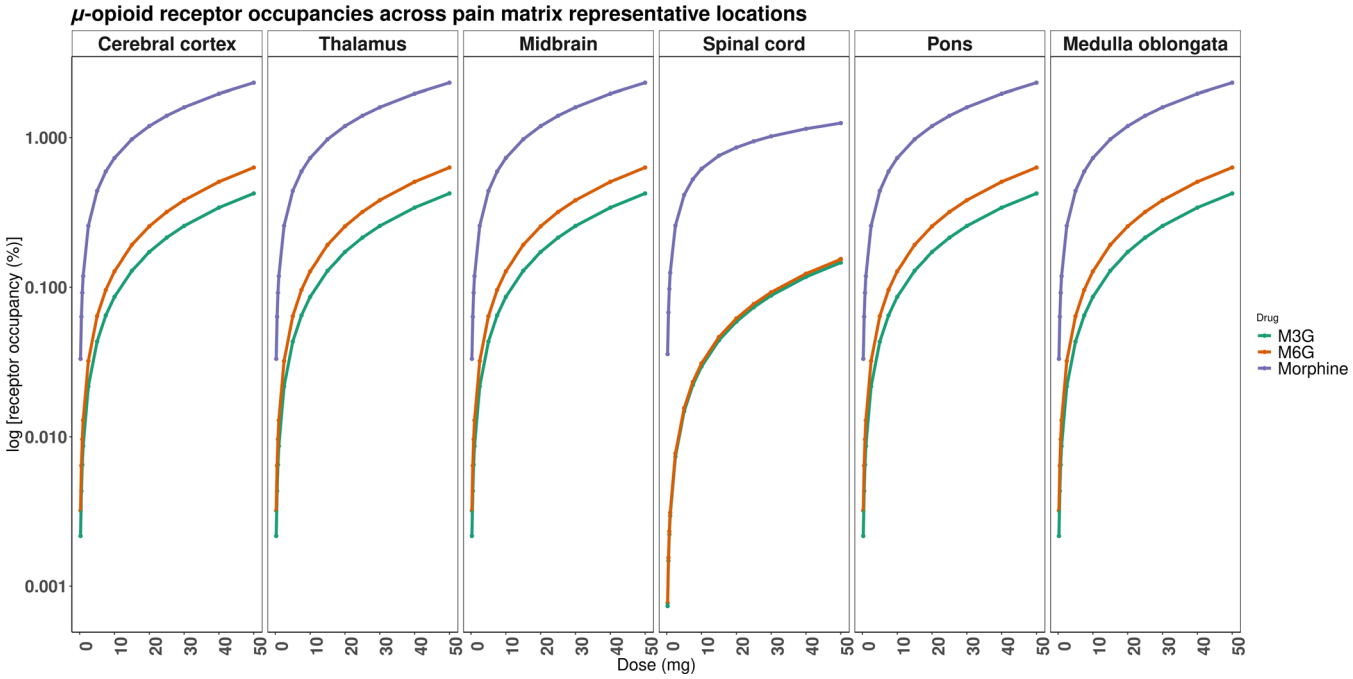

FIGURE 5 Relative μ-receptor occupancies of morphine, M3G, M6G in pain matrix representative locations (on top of panel), for IV bolus across once daily dose regimens on Days 6–7. All pain matrix regions having the $Brain_{ECF}$ concentrations showed similar occupancy ratios, whereas the spinal cord which has both lower μ-receptor expression as well the CSF_{SAS} concentration, showed a different pattern as shown in this figure. This figure is to explain the importance of considering the specific target site concentrations for PKPD analyses.

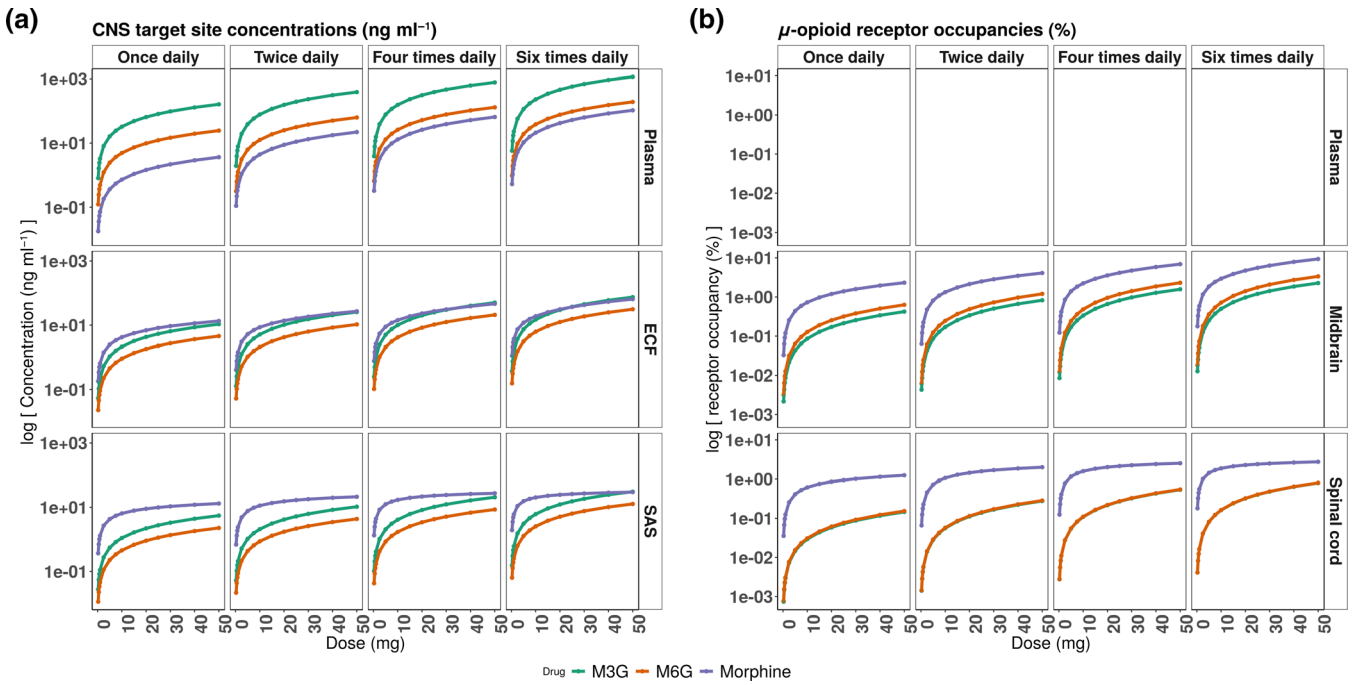

FIGURE 6 (a) Effect of dose interval (on top of panel) on non-linear morphine transport on CNS target site concentrations, (b) μ-receptor occupancies of morphine, M3G, M6G across 0.25–50 mg doses of intravenous bolus at Days 6–7. Median CNS target site concentrations and μ-receptor occupancy profiles on Days 6–7 are shown in this figure, against the dose interval (shown in the top panel), it shows the influence of non-linearity changing with dose interval, as opposed to the expected concentration differences.

transport on μ-receptor occupancy in different CNS locations, together with its main metabolites M3G and M6G. We simulated μ-receptor occupancy by morphine, M3G and M6G for a range of clinically relevant dose regimens including IV bolus (~28–56 mg per day) (CDER & FDA, 2016), oral immediate and oral extended-release (~60–120 mg per day) (CDER & FDA, 2021) formulations, including high and low doses for sensitivity analysis. We found that morphine's μ-receptor occupancy was clearly affected by its non-linear transport across BBB. Also, M3G, although it was the dominating metabolite in plasma, exhibited the lowest μ-receptor occupancy. In other words, the plasma pharmacokinetic profiles are not good predictors of μ-receptor occupancy in the CNS. Moreover, the effects of non-linearity of morphine BBB transport depends on the dose regimen, the target site location and μ-receptor binding kinetics. Based on these predictions we expect that morphine might be following non-linear dose-target μ-receptor occupancy relationship. This may improve the understanding of variability in morphine's dose–response relationships, as differences in μ-receptor occupancy are strongly correlated with the analgesic effect observed (Takai et al., 2018).

In human CNS, the target site concentrations of morphine, M3G and M6G are predicted by the LeiCNS-PK3.0 model with non-linear morphine BBB transport (Gülave et al., 2023). Gülave's predictions in rats were in line with reported observations of Stain-Texier et al. (1999), with relatively low morphine and high M6G concentrations in $Brain_{ECF}$, as measured by microdialysis (Stain-Texier et al., 1999) (note: rats do not produce M3G). For the human situation, we lack CNS target site measurements on morphine, M3G and M6G, and therefore Gülave et al., (2023) scaled from rats to humans, using the BBB **P-gp** expression ratios between rats and human (i.e., scaling factor fAFBBB) (Gülaveet al., 2023). The CNS target site concentrations were influenced by morphine non-linear BBB transport, the BBB transport characteristics of M3G and M6G, as well as dosing regimens and formulations. Here we took this information one step further and investigated the resulting μ-receptor occupancy. To that end we assumed that the μ-receptors are located on the brain cell membrane (Consortium et al., 2023) facing the $Brain_{ECF}$, and on the spinal cord cells membranes, facing the CSF_{SAS}. Potential μ-receptors inside the CNS cell cytoplasm (Gris et al., 2010) were not considered in these simulations. The results from our analysis are primarily focused on the steady state exposure and prolonged use of morphine. When considering the short-term use of morphine, the relative distribution of morphine and its metabolites could be expected to be different, but this was considered outside the scope of the current work.

Our predictions found that morphine has a higher μ-receptor occupancy, compared with that of M3G or M6G, at doses below 10 mg, while for higher and clinically relevant doses, M6G has higher μ-receptor occupancy than morphine at the CNS target sites. PKPD models (Sverrisdóttir et al., 2015), (plasma) concentration-effect studies using hysteresis loop (Ekblom et al., 1993) or indirect transfer function (Dahlström et al., 1978), highlighted the unexplained differences in inter-individual patient response to morphine, hence we were interested to study this with competitive binding between morphine, M3G and M6G. The superior potency of M6G, its role in reinforcing behaviour, and other side effects (Abbott & Franklin, 1991; Abbott & Palmour, 1988; Frances et al., 1992; Gong et al., 1991; K. Shimomura et al., 1971; Massi et al., 1994; P. B. Osborne et al., 2000; Pasternak & Wood, 1986; Paul et al., 1989; Stain et al., 1995), become more relevant for future pharmacodynamic analyses, given that the μ-receptor occupancy for M6G is higher than that for morphine, at clinically relevant doses. Also, our simulations showed that irrespective of dose or route of administration, M3G has relatively lower μ-receptor occupancy than morphine or M6G. M3G has been shown to antagonize the effect of morphine or M6G (Gong et al., 1991; Morley et al., 1992; Qian-Ling et al., 1992), with newly proposed mechanisms, such as those involving the **toll-like receptor 4 (TLR4)** (Due et al., 2012) or the interplay between μ-receptors and TLR4 (Zhang et al., 2020). Thus, studying M3G in combination with morphine and M6G might provide new insights.

Our predictions found that non-linear BBB transport of morphine can and does affect the brain μ-receptor occupancy, across formulations, as shown by comparison between IV and oral routes. Multiple dose regimens lead to more fluctuating μ-receptor occupancies at CNS target sites, as shown in this simulation, which might explain differences in response between in vitro or PKPD analyses and real-time chronic pain treatment requiring multiple dose regimens. Depending on the route of administration, the time to reach specific target site varies, and then the relative competitive binding affinities between the compounds for the μ-receptor determines the μ-receptor occupancy. We show in this simulation that differences each in metabolite and morphine fractions (compared to the total) in a dose regimen-, and/or administration route-dependent manner, on top of morphine BBB transport non-linearity. The target site concentration is shown to be dependent on administration route in terms of metabolite formation, as reported as 0.48/0.08/0.10 for subcutaneous (Christian Mignat et al., 1995) and 0.53/0.16/0.11 for IV (Sjøgren et al., 1993) for morphine/M3G/M6G respectively in CSF to plasma ratios, which highlights the importance of route of administration. Furthermore, the morphine/metabolite ratios are associated with more analgesia (for more morphine/M6G plasma ratio in humans) (Portenoy et al., 1992) or less analgesia (with more M3G/morphine ratio independent of plasma concentration in rats) (Smith & Smith, 1995). Our simulations did not include inter-individual variability in systemic pharmacokinetics, as we specifically aimed to investigate the contribution of administration route, non-linear transport and competitive binding effects on receptor occupancy.

In our analysis, the binding kinetics rate constants K_{on} and K_{off} were determined using the K_d values and diffusion-limited binding (association) principles of Stokes Einstein's law (Cruickshank, 1924). We presume first that diffusion-limited binding is a valid assumption given that morphine, M3G, M6G are small molecules (<500 Da) without long side chains. Secondly, viscous $Brain_{ECF}$ fluid due to presence of drug molecules, and hindered access to the binding site due to the location of μ-receptors at the synaptic cleft, all of which adheres to the rules of diffusion-limited binding (Cruickshank, 1924). For M3G and M6G, which have the same molecular weight, we believe that

their binding affinities measured in the same experiment (Frölich et al., 2011) drives their dissociation rate and can be used for reasonable approximation of their relative competitive binding. However, while considering K_d values for simulations, we have not accounted for possible ligand depletion in these values (Hoare, 2021), but we consider the current methodology is reasonable, by using the target site concentrations to address the research question of competitive binding.

In the current simulations, competition at the same binding site of the μ-1 subtype (OPRM1, role in analgesia) was considered. The subtypes μ-2 (role in respiratory depression) (Andoh et al., 2008; Ling et al., 1985; Paul & Pasternak, 1988) and μ-3 (role in nitric oxide pathway) (Stefano et al., 1995, 2000) were outside the scope of our work. Supplementary Table S1 provides an overview on reported values for binding kinetic data for morphine, M3G and M6G, and the experimental conditions in which those were obtained. Overall, it can be seen that these data are highly variable. Among those studies, there were three in which K_d values for morphine, M3G and M6G were determined within the same experiment and, in our view, are more valuable. Chen et al., (1991) showed relative binding affinities of morphine and M6G that were extremely high compared to other results. Then, Lambert et al., (1993) used a non-selective μ-receptor antagonist **[^{3}H]-diprenorphine**. Frölich et al., (2011), however, used a selective μ-receptor antagonist **[^{3}H]-naloxone**, while also measuring the K_d values, with the same (one) binding site model. Therefore, the data of Frölich et al. (2011) were considered to be best for use in our work. It is important, though, to also investigate the results that would be obtained by using the other binding kinetic data sets. Therefore, additional simulations for the binding kinetics data sets of Chen et al. (1991) and Lambert et al. (1993) were performed and are presented in Supplementary Figure S1, showing comparable results. Surprisingly, the μ-receptor occupancies resulting from our simulations are very low. There are no other published values for μ-receptor occupancy, so no comparisons can be made. Still, we believe that our predictions provide useful insights into relative μ-receptor occupancies of morphine, M3G and M6G.

We used mRNA expression due to lack of human protein expression levels and such protein expression could be different from mRNA expression, due to loss during post translational modifications. Furthermore, μ-receptor expression for brain regions was calculated from the mRNA expression ratios, but the HPA normal consensus data (Sjöstedt et al., 2020) is based on the concept of highest expression found in the subregion of each location. Hence, the true absolute receptor mRNA expression for each region could differ from the values we have considered. But we believe that mRNA expression can still provide valuable insights into the distribution and relative binding in the brain (Koussounadis et al., 2015). There were also suggestions of OPRM1 subtypes (Dietis et al., 2011) based on splice variants, which was out of scope for this simulation study. We have accounted up until the event of drug-target interaction, however upon receptor binding, ligand and receptor go through a cascade of events such as phosphorylation, arrestin binding, desensitization, followed by recycling of receptor by internalization (for ex: endocytosis) (Williams et al., 2013). These molecular level sub-processes depend on the receptor-ligand complex, and thus can be different for morphine, M3G, and M6G at the μ-receptor, and can drive long-term use effects such as tolerance (Morley et al., 1992; Williams et al., 2013).

Many factors affecting the μ-receptor can influence the competitive binding and were not completely accounted for in the model. For example, μ-receptor expression was assumed to be constant, while the μ-receptor has an intrinsic recycling ability (Medrano et al., 2017), which is independent of the ligand-receptor complex. The availability of μ-receptors at brain target sites has been shown to depend on (patho-)physiological conditions, such as neuropathic pain (Jones et al., 2004), as well as age and sex (Kantonen et al., 2020). Also, we only considered metabolism of morphine into M3G and M6G in the periphery, and resulting plasma profiles, but published data also suggest that metabolism of morphine is also possible inside the CNS (Wahlström et al., 1988). Interestingly (Gabel et al., 2022) showed that differences in analgesia among sexes are driven by metabolism and these differences are mainly driven by morphine derivatives that are conjugates of 3-glucuronide metabolites (Peckham & Traynor 2006). Furthermore, the endogenous opioids, though probably present only in the picomolar ranges in rats (Donnerer et al., 1986) and humans (Cardinale et al., 1987), exhibit differences in strength of binding and more importantly sub-cellular signalling pathways from those of exogenous opioids (Corder et al., 2018), differences which could have implications in the responses observed. Thus, information about the receptor levels dependent on age, sex and (patho-)physiological conditions, along with any competition with endogenous opioids, should also be included to provide deeper insights into the mechanisms of inter-individual differences and to help in designing better personalized treatments.

In conclusion, our simulations on the μ-receptor occupancy by morphine, M3G and M6G, despite some uncertainties, provide initial insights into the differences in their relative μ-receptor occupancy, being dependent on the dose, the dosing route, dosing formulation and dosing frequency, and the role of non-linearity of morphine transport across the BBB. This may be part of the explanation for the inter-individual differences in response to morphine treatment, while emphasizing the need for additional studies on the binding kinetics of morphine and its metabolites. We believe these results could provide a new basis for experimental investigations of morphine, M3G and M6G together, to explain interindividual differences in morphine analgesia. The next step would be to validate the predictions with published pain biomarkers and receptor occupancy data in future research.

AUTHOR CONTRIBUTIONS

D. Budda: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); visualization (equal); writing—original draft (equal); writing—review and editing (equal). **B. Gülave:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); visualization (equal); writing—original draft (equal); writing—review

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Human Protein Atlas at, https://doi.org/10.1126/science.aay5947, reference (Sjöstedt et al., 2020).

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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REFERENCES

Abbott, F. V., & Franklin, K. B. J. (1991). Morphine-6-glucuronide contributes to rewarding effects of opiates. *Life Sciences*, *48*(12), 1157–1163. https://doi.org/10.1016/0024-3205(91)90453-I

Abbott, F. V., & Palmour, R. M. (1988). Morphine-6-glucuronide: Analgesic effects and receptor binding profile in rats. *Life Sciences*, *43*, 1685–1695. https://doi.org/10.1016/0024-3205(88)90479-1

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F.a, Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, W., Al-hosaini, K., Bäck, M., Barnes, N. M., Bathgate, R., … Ye, R. D. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. *British Journal of Pharmacology*, *178*(S1), S27–S156. https://doi.org/10.1111/bph.15538

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Beuve, A., Brouckaert, P., Bryant, C., Burnett, J. C., Farndale, R. W., Friebe, A., Garthwaite, J., Hobbs, A. J., Jarvis, G. E., … Waldman, S. A. (2023a). The Concise Guide to PHARMACOLOGY 2023/24: Catalytic receptors. *British Journal of Pharmacology*, *180*(Suppl 2), S241–S288. https://doi.org/10.1111/bph.16180

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Amarosi, L., Anderson, C. M. H., Beart, P. M., Broer, S., Dawson, P. A., Gyimesi, G., Hagenbuch, B., Hammond, J. R., Hancox, J. C., … Verri, T. (2023b). The Concise Guide to PHARMACOLOGY 2023/24: Transporters. *British Journal of Pharmacology*, *180*(Suppl 2), S374–S469. https://doi.org/10.1111/bph.16182

Andoh, T., Yageta, Y., Konno, M., Yamaguchi-Miyamoto, T., Takahata, H., Nojima, H., Nemoto, H., & Kuraishi, Y. (2008). Evidence for separate involvement of different μ-opioid receptor subtypes in itch and analgesia induced by Supraspinal action of opioids. *Journal of Pharmacological Sciences J Pharmacol Sci*, *106*, 4–670. https://doi.org/10.1254/jphs.08004SC

Azevedo, F. A. C., Carvalho, L. R. B., Grinberg, L. T., Farfel, J. M., Ferretti, R. E. L., Leite, R. E. P., Filho, W. J., Lent, R., & Herculano-Houzel, S. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology*, *513*(5), 532–541. https://doi.org/10.1002/CNE.21974

Bachmutsky, I., Wei, X. P., Kish, E., & Yackle, K. (2020). Opioids depress breathing through two small brainstem sites. *eLife*, *9*. e52694. https://doi.org/10.7554/ELIFE.52694

Brain Facts and Figures. (n.d.). Retrieved March 6, 2023, from https://faculty.washington.edu/chudler/facts.html

Cardinale, G. J., Donnerer, J., Finck, A. D., Kantrowitz, J. D., Oka, K., & Spector, S. (1987). Morphine and codeine are endogenous components of human cerebrospinal fluid. *Life Sciences*, *40*(3), 301–306. https://doi.org/10.1016/0024-3205(87)90347-X

CDER, & FDA. (2016). *Morphine sulphate injection prescribing information*. CDER. FDA. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/204223s006lbl.pdf

CDER, & FDA. (2021). *Morphine sulphate tablets prescribing information*. CDER. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/022207s010lbl.pdf

Chen, Z. R., Irvine, R. J., Somogyi, A. A., & Bochner, F. (1991). Mu receptor binding of some commonly used opioids and their metabolites. *Life Sciences*, *48*(22), 2165–2171. https://doi.org/10.1016/0024-3205(91)90150-A

Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain: An update on burden, best practices, and new advances. *The Lancet*, *397*(10289), 2082–2097. https://doi.org/10.1016/S0140-6736(21)00393-7

Consortium, T. U., Bateman, A., Martin, M.-J., Orchard, S., Magrane, M., Ahmad, S., Alpi, E., Bowler-Barnett, E. H., Britto, R., Bye-A-Jee, H., Cukura, A., Denny, P., Dogan, T., Ebenezer, T., Fan, J., Garmiri, P., da Costa Gonzales, L. J., Hatton-Ellis, E., Hussein, A., … Zhang, J. (2023). UniProt: The universal protein knowledgebase in 2023. *Nucleic Acids Research*, *51*(D1), D523–D531. https://doi.org/10.1093/NAR/GKAC1052

Corder, G., Castro, D. C., Bruchas, M. R., & Scherrer, G. (2018). Endogenous and exogenous opioids in pain. *Annual Review of Neuroscience*, *41*(April), 453–473. https://doi.org/10.1146/annurev-neuro-080317-061522

Cruickshank, C. (1924). The stokes-Einstein law for diffusion in solution. *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character*, *106*(740), 724–749. https://doi.org/10.1098/RSPA.1924.0100

Dahlström, B. E., Paalzow, L. K., Segre, G., & Ågren, A. J. (1978). Relation between morphine pharmacokinetics and analgesia. *Journal of Pharmacokinetics and Biopharmaceutics*, *6*(1), 41–53. https://doi.org/10.1007/BF01066062/METRICS

de Witte, W. E. A., Vauquelin, G., van der Graaf, P. H., & de Lange, E. C. M. (2017). The influence of drug distribution and drug-target binding on target occupancy: The rate-limiting step approximation. *European Journal of Pharmaceutical Sciences*, *109*(May), S83–S89. https://doi.org/10.1016/j.ejps.2017.05.024

Dessau, R. B., & Pipper, C. B. (2008). "R"—Project for statistical computing. *Ugeskrift for Laeger*, *170*(5), 328–330.

Dietis, N., Rowbotham, D. J., & Lambert, D. G. (2011). Opioid receptor subtypes: Fact or artifact? *British Journal of Anaesthesia*, *107*(1), 8–18. https://doi.org/10.1093/BJA/AER115

Donnerer, J., Oka, K., Brossi, A., Rice, K. C., & Spector, S. (1986). Presence and formation of codeine and morphine in the rat. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(12), 4566–4567. https://doi.org/10.1073/PNAS.83.12.4566

Due, M. R., Piekarz, A. D., Wilson, N., Feldman, P., Ripsch, M. S., Chavez, S., Yin, H., Khanna, R., & White, F. A. (2012). Neuroexcitatory effects of morphine-3-glucuronide are dependent on toll-like receptor 4 signaling. *Journal of Neuroinflammation*, *9*(1), 1–12. https://doi.org/10.1186/1742-2094-9-200/FIGURES/5

Ekblom, M., Gårdmark, M., & Hammarlund-Udenaes, M. (1993). Pharmacokinetics and pharmacodynamics of morphine-3-glucuronide in rats and its influence on the antinociceptive effect of morphine. *Biopharmaceutics & Drug Disposition*, *14*(1), 1–11. https://doi.org/10.1002/BDD.2510140102

Frances, B., Gout, R., Monsarrat, B., Cros, J., & Zajac, J. M. (1992). Further evidence that morphine-6 beta-glucuronide is a more potent opioid agonist than morphine. *Journal of Pharmacology and Experimental Therapeutics*, *262*(1), 25–31.

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. *Biochemical Pharmacology*, *81*(10), 1248–1254. https://doi.org/10.1016/j.bcp.2011.03.001

Gabel, F., Hovhannisyan, V., Andry, V., & Goumon, Y. (2022). Central metabolism as a potential origin of sex differences in morphine antinociception but not induction of antinociceptive tolerance in mice. *British Journal of Pharmacology*, *180*, 843–861. https://doi.org/10.1111/BPH.15792

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

Geerts, H., & Spiros, A. (2006). Method and apparatus for computer modeling of the interaction between and among cortical and subcortical areas in the human brain for the purpose of predicting the effect of drugs in psychiatric & cognitive diseases.

Gong, Q. L., Hedner, T., Hedner, J., Björkman, R., & Nordberg, G. (1991). Antinociceptive and ventilatory effects of the morphine metabolites: Morphine-6-glucuronide and morphine-3-glucuronide. *European Journal of Pharmacology*, *193*(1), 47–56. https://doi.org/10.1016/0014-2999(91)90199-Z

Gris, P., Gauthier, J., Cheng, P., Gibson, D. G., Gris, D., Laur, O., Pierson, J., Wentworth, S., Nackley, A. G., Maixner, W., & Diatchenko, L. (2010). A novel alternatively spliced isoform of the mu-opioidreceptor: Functional antagonism. *Molecular Pain*, *6*, 33. https://doi.org/10.1186/1744-8069-6-33

Gudowska-Nowak, E., Lindenberg, K., & Metzler, R. (2017). Preface: Marian Smoluchowski's 1916 paper—A century of inspiration. *Journal of Physics A: Mathematical and Theoretical*, *50*(38), 380301. https://doi.org/10.1088/1751-8121/AA8529

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? *European Journal of Pharmaceutical Sciences*, 106482. https://doi.org/10.1016/J.EJPS.2023.106482

Hanna, M. H., Peat, S. J., Woodham, M., Knibb, A., & Fung, C. (1990). Analgesic efficacy and CSF pharmacokinetics of intrathecal morphine-6-glucuronide: Comparison with morphine. *British Journal of Anaesthesia*, *64*(5), 547–550. https://doi.org/10.1093/BJA/64.5.547

Hoare, S. R. J. (2021). The problems of applying classical pharmacology analysis to modern in vitro drug discovery assays: Slow binding kinetics and high target concentration. *SLAS Discovery*, *26*(7), 835–850. https://doi.org/10.1177/24725552211019653

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. *European Journal of Pain*, *8*(5), 479–485. https://doi.org/10.1016/j.ejpain.2003.11.017

Kantonen, T., Karjalainen, T., Isojärvi, J., Nuutila, P., Tuisku, J., Rinne, J., Hietala, J., Kaasinen, V., Kalliokoski, K., Scheinin, H., Hirvonen, J., Vehtari, A., & Nummenmaa, L. (2020). Interindividual variability and lateralization of μ-opioid receptors in the human brain: Individual differences in the μ-opioid receptor system. *NeuroImage*, *217*(April), 116922. https://doi.org/10.1016/j.neuroimage.2020.116922

Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J., & Smith, V. A. (2015). Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Scientific Reports*, *5*(1), 1–9. https://doi.org/10.1038/srep10775

Lambert, D. G., Atcheson, R., Hirst, R. A., & Rowbotham, D. J. (1993). Effects of morphine and its metabolites on opiate receptor binding, camp formation and [3h]noradrenaline release from SH-SYSY cells. *Biochemical Pharmacology*, *46*(7), 1145–1150. https://doi.org/10.1016/0006-2952(93)90462-6

Ling, G. S., Spiegel, K., Lockhart, S. H., & Pasternak, G. W. (1985). Separation of opioid analgesia from respiratory depression: Evidence for different receptor mechanisms. *Journal of Pharmacology and Experimental Therapeutics*, *232*(1), 149–155.

Massi, P., Giagnoni, G., Basilico, L., Gori, E., Rubino, T., & Parolaro, D. (1994). Intestinal effect of morphine 6-glucuronide: In vivo and in vitro characterization. *European Journal of Pharmacology*, *253*(3), 269–274. https://doi.org/10.1016/0014-2999(94)90201-1

Medrano, M. C., Santamarta, M. T., Pablos, P., Aira, Z., Buesa, I., Azkue, J. J., Mendiguren, A., & Pineda, J. (2017). Characterization of functional μ opioid receptor turnover in rat locus coeruleus: An electrophysiological and immunocytochemical study. *British Journal of Pharmacology*, *174*(16), 2758–2772. https://doi.org/10.1111/BPH.13901

Melzack, R. (2001). Pain and the Neuromatrix in the brain; pain and the Neuromatrix in the brain. *Journal of Dental Education*, *65*(12), 1378–1382. https://doi.org/10.1002/j.0022-0337.2001.65.12.tb03497.x

Mignat, C., Janssen, R., & Ziegler, A. (1995). Plasma and Cerebrosninal fluid concentrations of morphine and morphine glucuronides in rabbits receiving single and repeated doses of morphine. *Journal of Pharmacy and Pharmacology*, *47*(2), 171–175. https://doi.org/10.1111/j.2042-7158.1995.tb05772.x

Morley, J. S., Miles, J. B., Chris Wells, J., & Bowsher, D. (1992). Paradoxical pain. *The Lancet*, *340*(8826), 1045. https://doi.org/10.1016/0140-6736(92)93067-W

Moss, R. B., McCabe Pryor, M., Baillie, R., Kudrycki, K., Friedrich, C., Reed, M., & Carlo, D. J. (2020). Higher naloxone dosing in a quantitative systems pharmacology model that predicts naloxone-fentanyl competition at the opioid mu receptor level. *PLoS ONE*, *15*(6), e0234683. https://doi.org/10.1371/journal.pone.0234683

Osborne, P. B., Chieng, B., & Christie, M. J. (2000). Morphine-6β-glucuronide has a higher efficacy than morphine as a mu-opioid receptor agonist in the rat locus coeruleus. *British Journal of Pharmacology*, *131*(7), 1422–1428. https://doi.org/10.1038/SJ.BJP.0703697

Osborne, R., Thompson, P., Joel, S., Trew, D., Patel, N., & Slevin, M. (1992). The analgesic activity of morphine-6-glucuronide. *British Journal of Clinical Pharmacology*, *34*(2), 130–138. https://doi.org/10.1111/J.1365-2125.1992.TB04121.X

Pakkenberg, B., & Gundersen, H. J. (1997). Neocortical neuron number in humans: Effect of sex and age - PubMed. *The Journal of Comparative Neurology.*, *384*, 312–320. https://doi.org/10.1002/(SICI)1096-9861(19970728)384:2<312::AID-CNE10>3.0.CO;2-K

Pasternak, G. W., & Wood, P. J. (1986). Minireview: Multiple MU opiate receptors. *Life Sciences*, *38*(21), 1889–1898. https://doi.org/10.1016/0024-3205(86)90217-1

Paul, D., & Pasternak, G. W. (1988). Differential blockade by naloxonazine of two μ opiate actions: Analgesia and inhibition of gastrointestinal transit. *European Journal of Pharmacology*, *149*(3), 403–404. https://doi.org/10.1016/0014-2999(88)90680-2

Paul, D., Standifer, K. M., Inturrisi, C. E., & Pasternak, G. W. (1989). Pharmacological characterization of morphine-6 beta-glucuronide, a very potent morphine metabolite. *Journal of Pharmacology and Experimental Therapeutics*, *251*(2), 477–483.

Peckham, E. M., & Traynor, J. R. (2006). Comparison of the Antinociceptive response to morphine and morphine-like compounds in male and female Sprague-Dawley rats. *Journal of Pharmacology and Experimental Therapeutics*, *316*(3), 1195–1201. https://doi.org/10.1124/JPET.105.094276

Peng, J., Sarkar, S., & Chang, S. L. (2012). Opioid receptor expression in human brain and peripheral tissues using absolute quantitative real-time RT-PCR. *Drug and Alcohol Dependence*, *124*, 223–228. https://doi.org/10.1016/j.drugalcdep.2012.01.013

Peterson, G. M., Randall, C. T. C., & Paterson, J. (1990). Plasma levels of morphine and morphine glucuronides in the treatment of cancer pain: Relationship to renal function and route of administration. *European Journal of Clinical Pharmacology*, *38*(2), 121–124. https://doi.org/10.1007/BF00265969/METRICS

Portenoy, R. K., Thaler, H. T., Inturrisi, C. E., Friedlander-Klar, H., & Foley, K. M. (1992). The metabolite morphine-6-glucuronide contributes to the analgesia produced by morphine infusion in patients with pain and normal renal function. *Clinical Pharmacology & Therapeutics*, *51*(4), 422–431. https://doi.org/10.1038/CLPT.1992.42

Qian-Ling, G., Hedner, J., Björkman, R., & Hedner, T. (1992). Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. *Pain*, *48*(2), 249–255. https://doi.org/10.1016/0304-3959(92)90065-J

Regnard, C. F. B., & Twycross, R. G. (1984). Metabolism of narcotics. *British Medical Journal (Clinical Research Ed.)*, *288*(6420), 860. https://doi.org/10.1136/BMJ.288.6420.860

Rutherford, E. (1911). The scattering of α and β particles by matter and the structure of the atom. *Philosophical Magazine*, *21*(6), 669–688. https://doi.org/10.1080/14786440508637080

Saleh, M. A. A., Loo, C. F., Elassaiss-Schaap, J., & De Lange, E. C. M. (2021). Lumbar cerebrospinal fluid-to-brain extracellular fluid surrogacy is context-specific: Insights from LeiCNS-PK3.0 simulations. *Journal of Pharmacokinetics and Pharmacodynamics*, *48*(5), 725–741. https://doi.org/10.1007/S10928-021-09768-7

Shimomura, K., Kamata, O., Ueki, S., Ida, S., Oguri, K., Yoshimura, H., & Tsukamoto, H. (1971). Analgesic effect of morphine glucuronides. *The Tohoku Journal of Experimental Medicine*, *105*, 45–52. https://doi.org/10.1620/tjem.105.45

Sjøgren, P., Dragsted, L., & Christensen, C. B. (1993). Myoclonic spasms during treatment with high doses of intravenous morphine in renal failure. *Acta Anaesthesiologica Scandinavica*, *37*(8), 780–782. https://doi.org/10.1111/J.1399-6576.1993.TB03809.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. *Science*, *367*(6482). https://doi.org/10.1126/science.aay5947

Smith, G. D., & Smith, M. T. (1995). Morphine-3-glucuronide: Evidence to support its putative role in the development of tolerance to the antinociceptive effects of morphine in the rat. *Pain*, *62*(1), 51–60. https://doi.org/10.1016/0304-3959(94)00228-7

Smoluchowski, M. V. (1918). Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen. *Zeitschrift für Physikalische Chemie*, *92U*(1), 129–168. https://doi.org/10.1515/ZPCH-1918-9209

Spiros, A., Carr, R., & Geerts, H. (2010). Not all partial dopamine D2 receptor agonists are the same in treating schizophrenia. Exploring the effects of bifeprunox and aripiprazole using a computer model of a primate striatal dopaminergic synapse. *Neuropsychiatric Disease and Treatment*, *6*, 589–603. https://doi.org/10.2147/NDT.S12460

Stain, F., Barjavel, M. J., Sandouk, P., Plotkine, M., Scherrmann, J. M., & Bhargava, H. N. (1995). Analgesic response and plasma and brain extracellular fluid pharmacokinetics of morphine and morphine-6-beta-D-glucuronide in the rat. *Journal of Pharmacology and Experimental Therapeutics*, *274*(2), 852–857.

Stain-Texier, F., Boschi, G., Sandouk, P., & Scherrmann, J. M. (1999). Elevated concentrations of morphine 6-beta-D-glucuronide in brain extracellular fluid despite low blood–brain barrier permeability. *British Journal of Pharmacology*, *128*(4), 917–924. https://doi.org/10.1038/SJ.BJP.0702873

Stefano, G. B., Goumon, Y., Bilfinger, T. V., Welters, I. D., & Cadet, P. (2000). Basal nitric oxide limits immune, nervous and cardiovascular excitation: Human endothelia express a mu opiate receptor. *Progress in Neurobiology*, *60*(6), 513–530. https://doi.org/10.1016/S0301-0082(99)00038-6

Stefano, G. B., Hartman, A., Bilfinger, T. V., Magazine, H. I., Liu, Y., Casares, F., & Goligorsky, M. S. (1995). Presence of the μ3 opiate receptor in endothelial cells. *Journal of Biological Chemistry*, *270*(51), 30290–30293. https://doi.org/10.1074/jbc.270.51.30290

Sverrisdóttir, E., Lund, T. M., Olesen, A. E., Drewes, A. M., Christrup, L. L., & Kreilgaard, M. (2015). A review of morphine and morphine-6-glucuronide's pharmacokinetic–pharmacodynamic relationships in experimental and clinical pain. *European Journal of Pharmaceutical Sciences*, *74*, 45–62. https://doi.org/10.1016/J.EJPS.2015.03.020

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 Research*, *1680*, 105–109. https://doi.org/10.1016/j.brainres.2017.12.014

Tsou, K., & Jang, C. S. (1964). Studies on the site of analgesic action of morphine by intrathecal micro-injection. *Scientia Sinica*, *13*, 1099–1109.

Wahlström, A., Winblad, B., Bixo, M., & Rane, A. (1988). Human brain metabolism of morphine and naloxone. *Pain*, *35*(2), 121–127. https://doi.org/10.1016/0304-3959(88)90219-9

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. *Pharmacological Reviews*, *65*, 223–254. https://doi.org/10.1124/pr.112.005942

Zhang, P., Yang, M., Chen, C., Liu, L., Wei, X., & Zeng, S. (2020). Toll-like receptor 4 (TLR4)/opioid receptor pathway crosstalk and impact on opioid analgesia, immune function, and gastrointestinal motility. *Frontiers in Immunology*, *11*, 1455. https://doi.org/10.3389/FIMMU.2020.01455/BIBTEX

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