A THREE-COMPARTMENT PHARMACOKINETIC MODEL OF FENTANYL AND NALOXONE: A STUDY OF THE EFFICACY OF NALOXONE ON SURVIVAL AFTER FENTANYL OVERDOSE

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ABSTRACT

The high rates of fatalities due to opioid overdoses has created a need for precise modes of opioid antagonist administration to counteract overdoses. We developed a systems pharmacology model to simulate fentanyl and naloxone interactions with the mu opioid receptor (μOR). This 3 compartment model examines the concentration of fentanyl and naloxone in the central nervous system (CNS), the blood, and the body. We conducted four different simulations: repeated dosing of fentanyl, repeated dosing of fentanyl and naloxone, missed dosing of fentanyl, and survivability based on naloxone administration. We described a survivability metric to compare the effects of different naloxone dosing regimens to the probability of survival after a fentanyl overdose. We also analyzed varying user populations such as those with a previous history of smoking and habitual drug users with nasal abrasion or users with mucus buildup (nasal congestion). Our model shows the effects of naloxone on decreasing fentanyl μOR occupancy in the CNS. The results from our model also suggest that lower concentrations of μOR receptors increase survivability.

I. INTRODUCTION AND BACKGROUND

In 2017 there were over 28,000 fatal overdoses on fentanyl in the US.[1] In Texas alone, the Drug Enforcement Administration collected 8.1 million illegal doses of fentanyl in 2020.[1] The city of Baltimore, known for its high homicide rate had 318 deaths due to homicide in comparison to 419 deaths due to fentanyl in 2016.[2] These statistics illuminate the severity of the opioid epidemic with fentanyl being the top drug responsible for overdoses on the east coast.[3-6] Fentanyl, a synthetic drug discovered by Paul Janssen in 1960, is an analgesic used to relieve pain.[1] It functions by binding to the μOR, activating the μ_1 and μ_2 transmembrane proteins.[7] Activation of μ_1 leads to the opening of the G protein-coupled inwardly-rectifying potassium channel (GIRK) receptor, causing a flood of potassium ions out of the cell.[7] This results in a repolarization of the cell, depressing the membrane potential. Activation of μ_2 further activates β-arrestin, a set of proteins responsible for respiratory depression and desensitization.[7] Fentanyl, with a fatal dose of just 2 mg, is 50-100 times stronger than morphine.[8] Just a minimal exposure to fentanyl can lead to severe complications and fatalities.[8] The potency of fentanyl is attributed to its rapid binding and occupancy of the μOR in the CNS.[8]

Naloxone is a drug used to counteract opioid overdoses by competitively binding to the μOR to reverse the effects of an overdose.[9] The common administration routes of naloxone are via a nasal spray (IN) and intramuscular injection (IM).[9] Using naloxone to counteract opioid overdoses has become more challenging due to the illicit manufacturing of more potent fentanyl and other synthetic opioids.[8] Assessing accurate doses of naloxone for outcompeting fentanyl and reversing toxicity is a pressing issue as it has been found that current approved doses of naloxone may not be adequate.[8]

Moss *et al*. developed a quantitative systems pharmacology model predicting naloxone-fentanyl competition at the μOR.[8] They conducted simulations to examine the effects of naloxone dosing for three fentanyl exposure levels. The results of this model demonstrated that the current approved doses of naloxone (2 mg IM and 4 mg IN) are not adequate for rapid reversal of fentanyl overdoses.[8] A dosage of 5 mg or 10 mg of naloxone for rapid effect was suggested based on this model's results.[8] Despite these significant results, this model is limited in that it only looks at the habitual opioid user and does not take into account repeated fentanyl dosing or patient variability.

We developed a fentanyl-naloxone three compartment (blood, brain, body) pharmacokinetic-pharmacodynamic modeling framework to analyze the interactions between fentanyl and naloxone at the μOR for various concentrations of fentanyl and naloxone. This model considers timing between dosing and various population

parameters such as availability of μOR. Our model analyzes the effect of fentanyl through oral administration. Our system focuses on the IN route of naloxone as its bioavailability is 45% greater than that of IM.[8] We analyzed the effects of missed dosing on the concentration profiles for fentanyl to aid our understanding of the drug interactions. We also looked at different populations of fentanyl users such as those with a history of smoking and drug users with nasal congestion or habitual users who have risks for nasal abrasion. Previous studies have shown that a history of smoking reduces the amount of available μOR sites.[10] Habitual drug users often use snorting as an ingestion pathway causing abrasion to nasal stream which can affect IN drug bioavailability.[18] We correlated the fentanyl receptor occupancy AUC with survival probability to analyze effective naloxone schemes to counteract overdoses.

II. METHODS AND MODEL

We modeled fentanyl and naloxone using a three compartment model: the blood plasma (C1), the body (C2), and a CNS subvolume (C3). C1 and C2 were both different for naloxone and fentanyl due to their varying volumes of distributions. C3, the subvolume where the μOR are located, was a shared compartment between the two drugs because they both interact and bind with these receptors.

All the parameters for the model are listed with their respective values and literature references in Table 1. Fentanyl was injected intravenously as a bolus, while naloxone was administered intranasally. Immediately when fentanyl enters C1, 80% of the drug binds to plasma proteins, effectively leaving 20% of the initial bolus dose of fentanyl circulating through all three compartments.[7] The bioavailability of intranasal naloxone is 50%, meaning that only 50% of the initial intranasal dose of naloxone will enter the bloodstream.[8] Once in the bloodstream, fentanyl and naloxone can independently enter the C2 and C3. In C3, both drugs can bind with the μOR. It is important to note that clearance only occurs in C1.

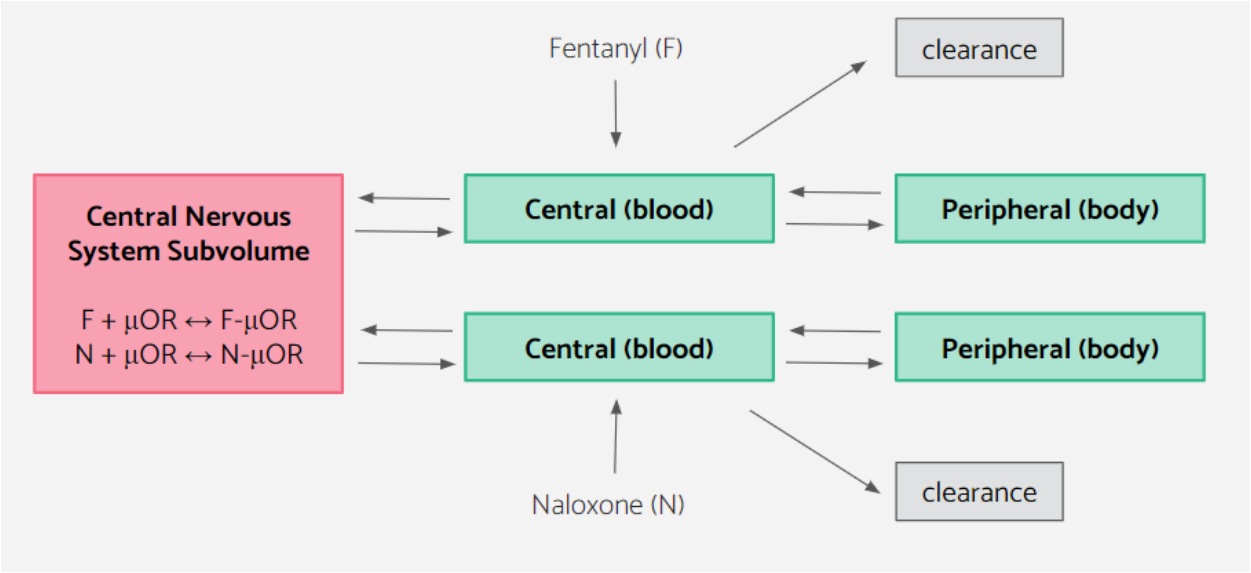

Figure 1. Fentanyl and Naloxone Compartment Models - A three compartment model (blood, body, and CNS subvolume) for fentanyl and naloxone administration.

Table 1: Parameter Values for Compartment Models

Parameter	Description	Value	Units	Reference
weight	Average weight of a male	70	kg	Estimate
p_f	Fraction of fentanyl not bound to plasma protein	0.2	unitless	7
abs_n	Bioavailability of naloxone via intranasal administration	0.5	unitless	11
k_{12f}	Transport rate of fentanyl from blood to body	0.373	1/min	12
k_{12n}	Transport rate of naloxone from blood to body	0.4967	L/min	13
k_{21f}	Transport rate of fentanyl from body to blood	0.103	1/min	12
k_{21n}	Transport rate of naloxone from body to blood	0.4967	L/min	13
k_{13f}	Transport rate of fentanyl from blood to brain	0.0367	1/min	12

k_{13n}	Transport rate of naloxone from blood to brain	0.8*0.0367 = 0.02936	L/min	Estimate
k_{31f}	Transport rate of fentanyl from brain to blood	0.0124	1/min	12
k_{31n}	Transport rate of naloxone from brain to blood	1.2*0.0124 = 0.01488	L/min	Estimate
k_{cl_f}	Clearance rate of fentanyl from blood	0.7	L/min	7
k_{cl_n}	Clearance rate of naloxone from blood	91/60	L/min	13
k_{on_f}	Binding rate of fentanyl to μOR	0.026	1/(min*nM)	8
k_{on_n}	Binding rate of naloxone to μOR	1.073	1/(min*nM)	8
k_{off_f}	Dissociation rate of fentanyl from μOR	0.035	1/min	14
k_{off_n}	Dissociation rate of naloxone from μOR	1.196	1/min	15
Vd_{f1}	Volume of distribution of fentanyl in blood	13	L	16
Vd_{f2}	Volume of distribution of fentanyl in body	295	L	16
Vd_{n1}	Volume of distribution of naloxone in blood	0.408	L	17
Vd_{n2}	Volume of distribution of naloxone in body	1.637	L	17
Vd_{brain}	Subvolume of the brain that both fentanyl and naloxone interact with	1.260 * 0.01 = 0.01260	L	Estimate
$amt_{\mu OR}$	Amount of mOR receptors in brain	0.1	nmol	Estimate

We built a system of 11 ordinary differential equation (ODE) equations to describe our three compartment model for fentanyl and naloxone. Each variable is listed in **Table 2** and the 11 equations are listed in **Table 3.**

Table 2. Variable definitions for the 11 ODEs

Variable	Description	Units
f_{blood}	Concentration of free fentanyl in blood	nM
f_{body}	Concentration of free fentanyl in body	nM
f_{brain}	Concentration of free fentanyl in brain	nM
n_{blood}	Concentration of free naloxone in blood	nM
n_{body}	Concentration of free naloxone in body	nM
n_{brain}	Concentration of free naloxone in brain	nM
μ_{free}	Concentration of free μOR in brain	nM
μ_f	Concentration of μOR bound to fentanyl in brain	nM
μ_n	Concentration of μOR bound to naloxone in brain	nM
f_{cl}	Amount of cleared fentanyl	mg
n_{cl}	Amount of cleared naloxone	mg

Table 3: Model Equations

Concentration of free fentanyl in blood [nM] (1)

$$\frac{df_{\text{blood}}}{dt} = -k_{12_f} \times f_{\text{blood}} \times p_f + k_{21_f} \times f_{\text{body}} \times \left(\frac{V_{d_{f2}}}{V_{d_{f1}}}\right) - k_{13_f} \times f_{\text{blood}} \times p_f + k_{31_f} \times f_{\text{brain}} \times \left(\frac{V_{d_{f3}}}{V_{d_{f1}}}\right) - k_{\text{cl}_f} \times f_{\text{blood}} \times p_f$$

Concentration of free fentanyl in body [nM] (2)

$$\frac{df_{\text{body}}}{dt} = +k_{12_f} \times f_{\text{blood}} \times p_f \times \left(\frac{V_{d_{f1}}}{V_{d_{f2}}}\right) - k_{21_f} \times f_{\text{body}}$$

Concentration of free fentanyl in brain [nM] (3)

$$\frac{df_{\text{brain}}}{dt} = +k_{13_f} \times f_{\text{blood}} \times p_f \times \left(\frac{V_{d_{f1}}}{V_{d_{f3}}}\right) - k_{31_f} \times f_{\text{brain}} + k_{\text{off}_f} \times \mu_{\text{f}} - k_{\text{on}_f} \times f_{\text{brain}} \times \mu_{\text{free}}$$

Concentration of free naloxone in blood [nM] (4)

$$\frac{dn_{\text{blood}}}{dt} = -k_{12_n} \times n_{\text{blood}} + k_{21_n} \times n_{\text{body}} \times \left(\frac{V_{d_{n2}}}{V_{d_{n1}}}\right) - k_{13_n} \times n_{\text{blood}} + k_{31_n} \times n_{\text{brain}} \times \left(\frac{V_{d_{n3}}}{V_{d_{n1}}}\right) - k_{\text{cl}_n} \times n_{\text{blood}}$$

Concentration of free naloxone in body [nM] (5)

$$\frac{dn_{\text{body}}}{dt} = +k_{12_n} \times n_{\text{blood}} \times \left(\frac{V_{d_{n1}}}{V_{d_{n2}}}\right) - k_{21_n} \times n_{\text{body}}$$

Concentration of free naloxone in brain [nM] (6)

$$\frac{dn_{\text{brain}}}{dt} = +k_{13_n} \times n_{\text{blood}} \times \left(\frac{V_{d_{n1}}}{V_{d_{n3}}}\right) - k_{31_f} \times n_{\text{brain}} + k_{\text{off}_n} \times \mu_{\text{n}} - k_{\text{on}_n} \times n_{\text{brain}} \times \mu_{\text{free}}$$

Concentration of free μOR in brain [nM] (7)

$$\frac{d\mu_{\text{free}}}{dt} = + k_{\text{off}_f} \times \mu_{\text{f}} \quad - \quad k_{\text{on}_f} \times f_{\text{brain}} \times \mu_{\text{free}} + k_{\text{off}_n} \times \mu_{\text{n}} \quad - \quad k_{\text{on}_n} \times n_{\text{brain}} \times \mu_{\text{free}}$$

Concentration of bound μOR bound to fentanyl in brain [nM] (8)

$$\frac{d\mu_{\text{f}}}{dt} = + k_{\text{on}_f} \times f_{\text{brain}} \times \mu_{\text{free}} \quad - \quad k_{\text{off}_f} \times \mu_{\text{f}}$$

Concentration of bound μOR bound to naloxone in brain [nM] (9)

$$\frac{d\mu_{\text{n}}}{dt} = + k_{\text{on}_n} \times n_{\text{brain}} \times \mu_{\text{free}} \quad - \quad k_{\text{off}_n} \times \mu_{\text{n}}$$

Amount of cleared fentanyl [mg] (10)

$$\frac{df_{\text{cl}}}{dt} = + k_{\text{cl}_f} \times f_{\text{blood}} \times p_f \times V_{d_{f1}}$$

Amount of cleared naloxone [mg] (11)

$$\frac{dn_{\text{cl}}}{dt} = + k_{\text{cl}_n} \times n_{\text{blood}} \times V_{d_{n1}}$$

Repeated Dosing of Fentanyl

The first simulation we ran was a repeated dosing scenario in which 25 doses of fentanyl (1 mg) were administered every 45 minutes. For this simulation, the concentrations of fentanyl in C1, C2, and C3 were analyzed as well as the binding of fentanyl with the μOR. This simulation is only to show the functionality of our model; it most likely has little clinical relevance.

Repeated Dosing of Fentanyl and Naloxone

The second simulation we ran was a repeated dosing scenario in which 5 consecutive doses of fentanyl (1 mg) were taken 45 minutes apart followed by the administration of 3 consecutive doses on naloxone (4 mg) taken 3 minutes apart. The time delay between the last dose of fentanyl and first dose of naloxone was 7 minutes. For this simulation, the concentrations of fentanyl and naloxone in C1, C2, and C3 were analyzed as well as the binding of fentanyl and naloxone to the μOR.

Missed Dose

The third simulation we ran was a missed dose scenario in which a user theoretically takes 6 doses of fentanyl (4 mg) spaced 30 minutes apart (180 minutes total with the first dose at t = 0 minutes). However, the user forgets to take the 4th dose at the proper time. We modeled how this would affect the fentanyl concentration in C1, C2, and C3, as well as the μOR occupancy in C3.

Furthermore, we modeled an extension of this scenario by shifting the time when the missed dose was taken: 6 minutes, 12 minutes, 18 minutes, and 24 minutes after they were intended to take it. (The user forgets to take the dose, but then remembers.) We analyzed how taking the dose late affects the fentanyl concentration in C1, C2, and C3, as well as the μOR occupancy in C3.

Survivability

In our fourth simulation, we modeled the life-saving effects that naloxone can have on fentanyl by acting as an effective competitive inhibitor. The lethal dose of fentanyl is 2 mg. We modeled four progressive scenarios to look at the interaction of naloxone and fentanyl with the μOR, varying the dose of naloxone taken and the delay between the fentanyl dose and the first dose of naloxone:

(1) **No Naloxone-Base Case:** 1 dose fentanyl (2 mg) and no naloxone.
(2) **Simulation 1 - Add Naloxone:** 1 dose of fentanyl (2 mg) followed by 3 doses of *naloxone (4 mg)* administered 3 minutes apart. *1 minute delay* between fentanyl dose and first dose of naloxone.
(3) **Simulation 2 - Increase delay time, half naloxone:** 1 dose of fentanyl (2 mg) followed by 3 doses of *naloxone (2 mg*) administered 3 minutes apart. *10 minute delay* between fentanyl dose and first dose of naloxone.
(4) **Simulation 3 - Double naloxone:** 1 dose of fentanyl (2 mg) followed by 3 doses of *naloxone (4 mg)* administered 3 minutes apart. *10 minute delay* between fentanyl dose and first dose of naloxone.

For each of these cases, we analyzed the concentration of fentanyl and naloxone in C1 and C3, as well as the concentration of bound and free μOR in C3. We were particularly interested in analyzing the area under the curve (AUC) of fentanyl receptor occupancy in each of these scenarios and correlating the ratio of the AUC of any of the naloxone cases (Simulation 1, Simulation 2, or Simulation 3) with respect to the AUC of the base case to develop a survival probability metric (**Equation 12**).

$$\text{survival rate} = 1 - \frac{\text{AUC of fentanyl receptor occupancy (simulation)}}{\text{AUC of fentanyl receptor occupancy (no naloxone-base case)}} \qquad (12)$$

Population Pharmacokinetics

To determine the effects of varying population dynamics on survivability, three populations each of 100 individuals were randomly generated. The first population models how individuals can have varying amounts of opioid receptors present in their brain due to smoking habits. Previous studies have shown that people who are heavier smokers tend to have diminished expression of opioid receptors in the brain, due to the overstimulation of these receptors by repeated consumption of nicotine. Therefore, to model this, we generated a population with varying levels of the complete amount of μOR available in the brain, ranging from 86% to 100% of this amount (**Equation 13**).[10]

$$\text{amt}_{\mu\text{OR}} = X, \text{ where } X \sim \text{Uniform } (0.86, 1) \tag{13}$$

The second population represents the change in how well naloxone is absorbed through the nasal route. Due to interindividual variations, people have varying levels of mucus buildup and congestion in their noses, which can affect the uptake of any drugs administered through the nostril. Furthermore, frequent insufflation or snorting of powdered drugs, such as cocaine or fentanyl, can result in increased inflammation of vessels lining the nasal mucosa and ultimately damage to the vasculature. Thus, we modeled this population such that the degree to which the naloxone is absorbed nasally was randomly chosen from a Normal distribution with mean 1 and standard deviation 0.1, and then multiplied by the expected 50% (**Equation 14**). This represents how individuals may have greater or lower obstruction in their nasal mucosa. Observing this population is particularly important as, although we look at fentanyl administrations via IV, many drug abusers insufflate the chemicals.[18]

$$\text{abs}_{\text{n}} = Y \times 0.5, \text{ where } Y \sim \text{Normal } (1, 0.1) \tag{14}$$

Finally, the third population sees variation in both the amount of μOR and the degree to which the naloxone is absorbed. The resulting AUC of fentanyl receptor occupancy and survivability was determined for Simulation 2.

Sensitivity

To determine the importance of different parameters used in this model, we performed a local sensitivity analysis of fentanyl receptor occupancy. This was done by finding the change in AUC when modifying the value of the parameter by 10% for the fentanyl receptor occupancy graph. Here, the base AUC is AUC for fentanyl bound before the one parameter change for S3 (**Equation 15**). We did this for all the parameters listed in Table 1 for fentanyl in order to analyze their respective effects on drug exposure.

$$\text{survival rate} = 1 - \frac{\text{AUC of fentanyl receptor occupancy after parameter change}}{\text{Base AUC in S3}} \tag{15}$$

III. RESULTS

Repeated Dosing of Fentanyl

From **Figure 2**, we can see the effects of repeated dosing of fentanyl. Within the three compartments, fentanyl saturates in the blood (~ 600 minutes) the fastest, where it is initially injected as an intravenous bolus. This is followed by fentanyl saturating in the body (~ 720 minutes) and then the brain (~ 800 minutes). This is consistent with the volume of distribution of fentanyl in each compartment as well as the transport rates between them. After approximately two doses of fentanyl (~ 90 minutes), fentanyl binding to the μOR saturates, without the presence of any naloxone.

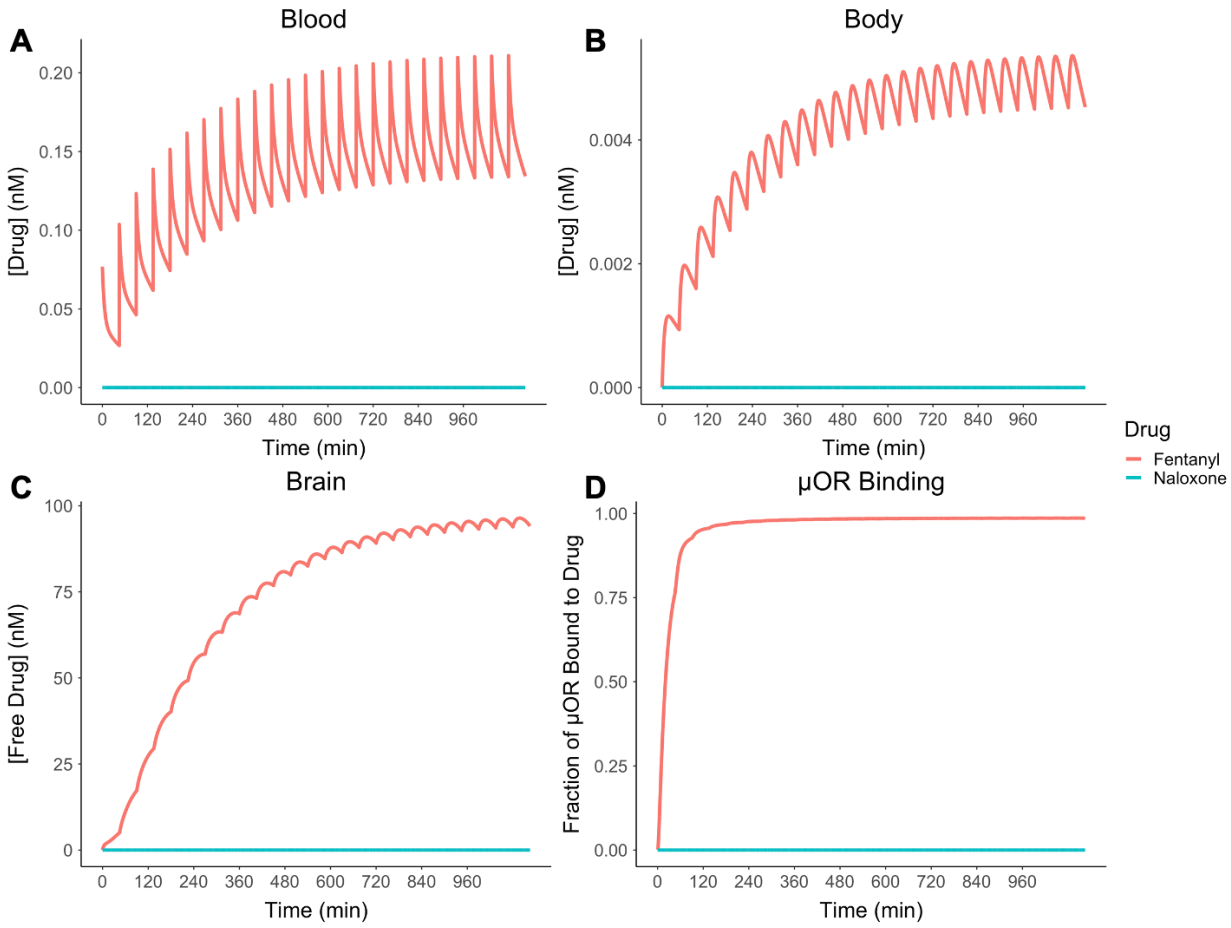

Figure 2. Effects of Repeated Dosing of Fentanyl on Fentanyl Compartment Concentrations and Receptor Occupancy - Repeated dosing scheme of fentanyl in which 1 mg of fentanyl was administered every 45 minutes for 25 doses. **(A)** Concentration of fentanyl in the blood. **(B)** Concentration of fentanyl in the body. **(C)** Concentration of free fentanyl in the brain. **(D)** Concentration of μOR bound to fentanyl. Note that no naloxone is administered in this scenario, so the concentration of naloxone is always zero.

Repeated Dosing of Fentanyl and Naloxone

In **Figure 3**, we can see the effects of repeated doses of fentanyl followed by naloxone, thereby modeling a clinical situation of fentanyl overdose and then naloxone administration as a treatment. The concentration of the fentanyl in the blood compartment is much lower than naloxone. This is because almost 80% of the fentanyl in the bloodstream is bound and temporarily inactivated by plasma proteins. In addition, the dose of fentanyl is only 1 mg and the dose of naloxone is 4 mg. This trend is also seen in the body, however, is the exact opposite in the brain. This is due to naloxone having a lower transport rate into the brain and higher transport rate out of the brain compared to fentanyl. This also creates a delay in naloxone saturation in the brain. Despite the low concentration of naloxone in the brain, its effect as a competitive inhibitor is noticeable based on the μOR profile. Following naloxone administration, the amount of fentanyl bound to the μOR decreases as naloxone begins to bind with the receptor.

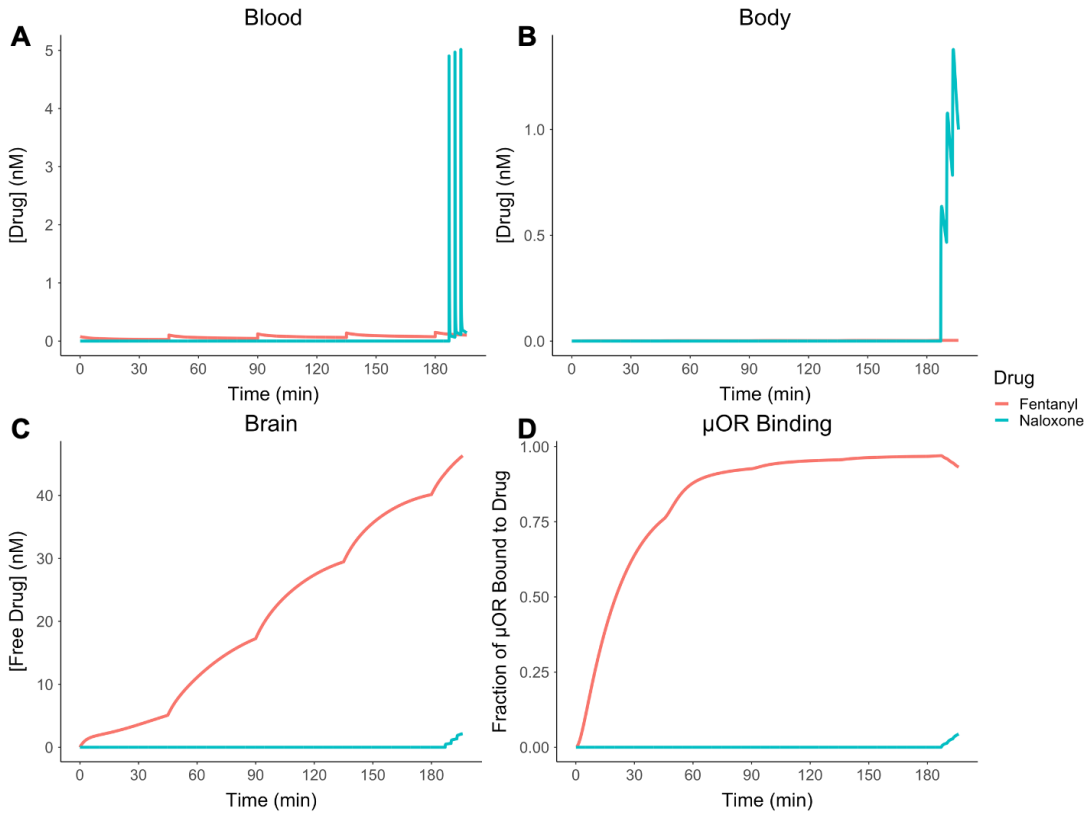

Figure 3. Effects of Repeated Dosing of Fentanyl and Naloxone on Fentanyl and Naloxone Compartment Concentrations and Receptor Occupancies - 5 consecutive doses of fentanyl (1mg) administered 45 mins apart followed by 3 consecutive doses of naloxone (4mg) administered 3 mins apart. Time to initial dose of naloxone after the last dose of fentanyl is 7 mins. **(A)** Concentration of drug in the blood. **(B)** Concentration of drug in the body. **(C)** Concentration of free drugs in the brain. **(D)** Concentration of μOR bound to fentanyl.

Missed Dose

In **Figure 4**, we can see the impact of missing a dose of fentanyl. The effect of taking a late dose does not significantly affect the peak and trough concentrations of fentanyl in the blood, body, and brain. Additionally, a late fourth dose does not affect the fraction of the μOR bound to fentanyl because it saturates after taking the second dose. This is consistent with what we saw in the repeated 25 doses of fentanyl.

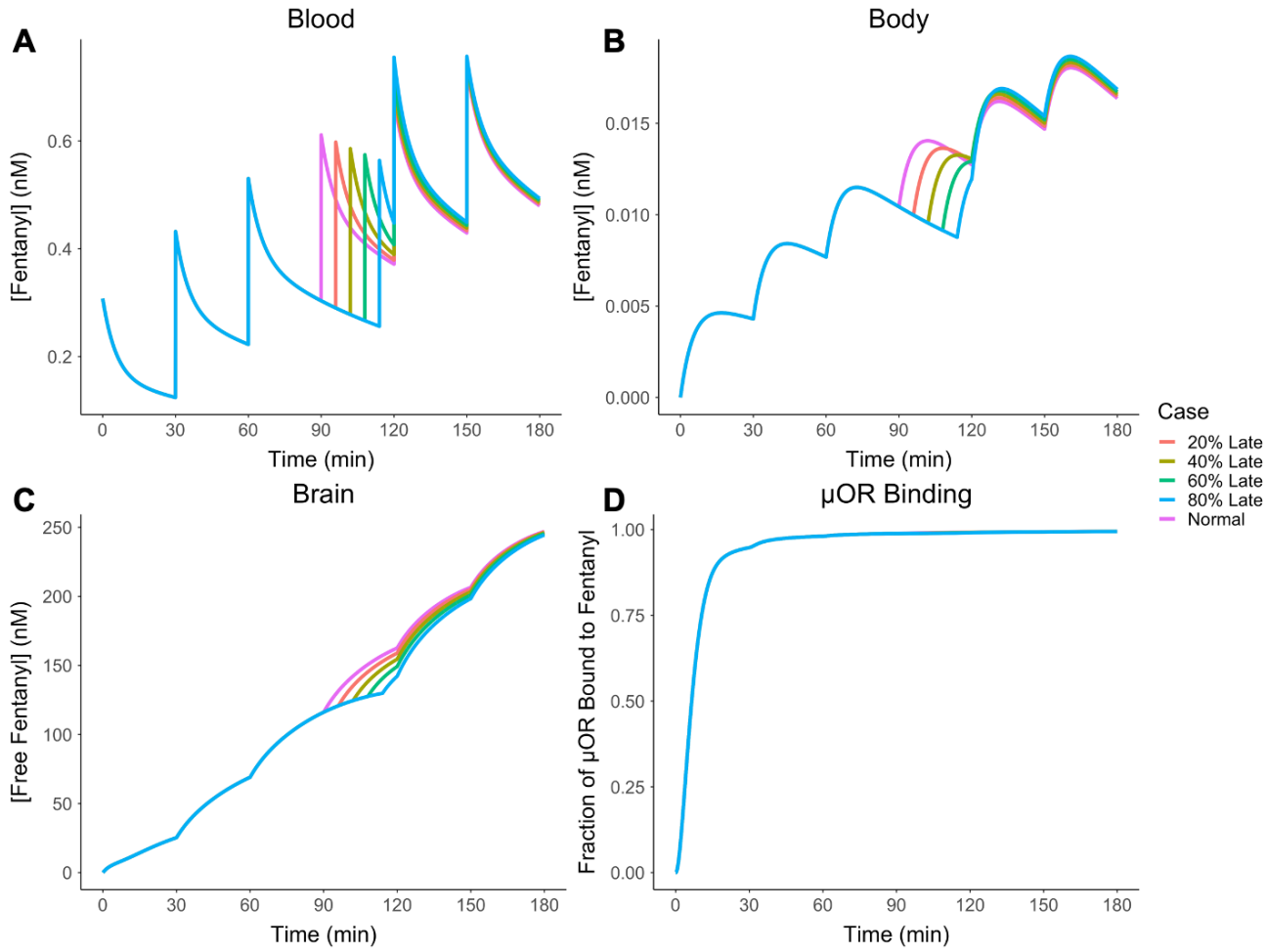

Figure 4. Effects of Missed Dose of Fentanyl on Free Fentanyl Compartment Concentrations and Receptor Occupancy - Effect of missed dose of fentanyl (1 mg) for simulations in which 6 doses of fentanyl are administered 30 minutes apart. The fourth dose is "missed" and the effect of taking the dose 6 minutes (20% late), 12 minutes (40% late), 18 minutes (60% late), 24 minutes (80% late) after the scheduled time was graphed. **(A)** Concentration of fentanyl in the blood. **(B)** Concentration of fentanyl in the body. **(C)** Concentration free fentanyl in the brain. **(D)** Concentration of μOR bound to fentanyl.

Survivability

In **Figure 5**, we observe the change in survivability by looking at the concentrations of fentanyl for each simulation. The fentanyl concentration in the blood and the body are mostly independent of naloxone concentration. However, the amount of free fentanyl in the brain is dependent on naloxone. Greater concentrations of naloxone result in greater competition for μOR, resulting in lower fentanyl receptor occupancy and thus, greater amounts of free fentanyl. This causal relationship (lower fentanyl occupancy resulting in greater free fentanyl in the brain) is shown in panels C and D of **Figure 5**.

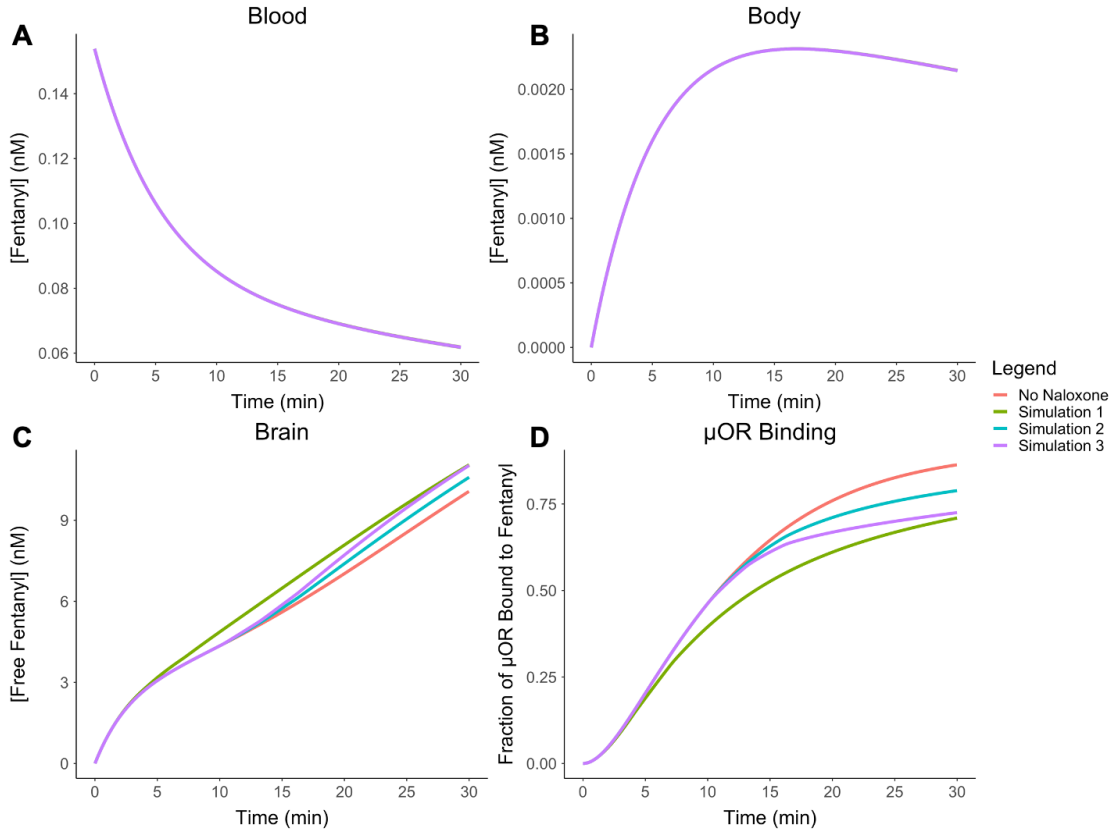

Figure 5. Effects of Naloxone on Free Fentanyl Compartment Concentration and Receptor Occupancy - Concentrations of fentanyl for varying simulations in which doses and administration times were altered. *No Naloxone-Base Case 1*: 1 dose fentanyl (2 mg) and no naloxone. *Simulation 1*: 1 dose of fentanyl (2 mg) followed by 3 doses of naloxone (4 mg) administered 3 minutes apart with a 1 minute delay between fentanyl dose and first naloxone dose. *Simulation 2*: 1 dose of fentanyl (2 mg) followed by 3 doses of naloxone (2 mg) administered 3 minutes apart with a 10 minute delay between fentanyl dose and first naloxone dose. *Simulation 3*: 1 dose of fentanyl (2 mg) followed by 3 doses of naloxone (4 mg) administered 3 minutes apart with a 10 minute delay between fentanyl dose and first dose of naloxone. **(A)** Concentration of fentanyl in the blood. **(B)** Concentration of fentanyl in the body. **(C)** Concentration free fentanyl in the brain. **(D)** Concentration of μOR bound to fentanyl.

Simulation 1 had the greatest effect on suppressing fentanyl receptor occupancy followed by Simulation 3 and Simulation 2. This makes sense because Simulation 1 has a 4 mg dose of naloxone and a delay of 1 minute between fentanyl and naloxone administration. Thus, the naloxone will have the greatest and quickest effect of binding to μOR. Simulation 3 and Simulation 2 both have a delay of 10 minutes. However, Simulation 3 has 4 mg dose of naloxone compared with 2 mg for Simulation 2, resulting in Simulation 3 having a greater effect in suppressing fentanyl receptor occupancy compared to Simulation 2.

Population Pharmacokinetics

In **Figure 6**, we observed the effects of varying μOR availability on our model's output. The AUC of fentanyl occupancy and survivability were generated based on Simulation 2. As shown in the figure, we observed that both AUC and survival rate decrease with available μOR. With lower amounts of available μOR, fentanyl receptor occupancy will saturate faster resulting in a greater AUC. This explains the negative correlation between fentanyl occupancy AUC and available μOR. It is interesting to see that the survival rate decreases with greater amounts of available μOR, given that the AUC of fentanyl receptor occupancy also decreases. This result was not expected. However, it could be because the base fentanyl receptor occupancy AUC (no naloxone) calculated is decreasing at a greater rate than that of the fentanyl receptor occupancy of Simulation 2 (**Equation 11**). This could imply that while having a lower amount of μOR increases fentanyl receptor occupancy, it is not enough to induce a deadly response.

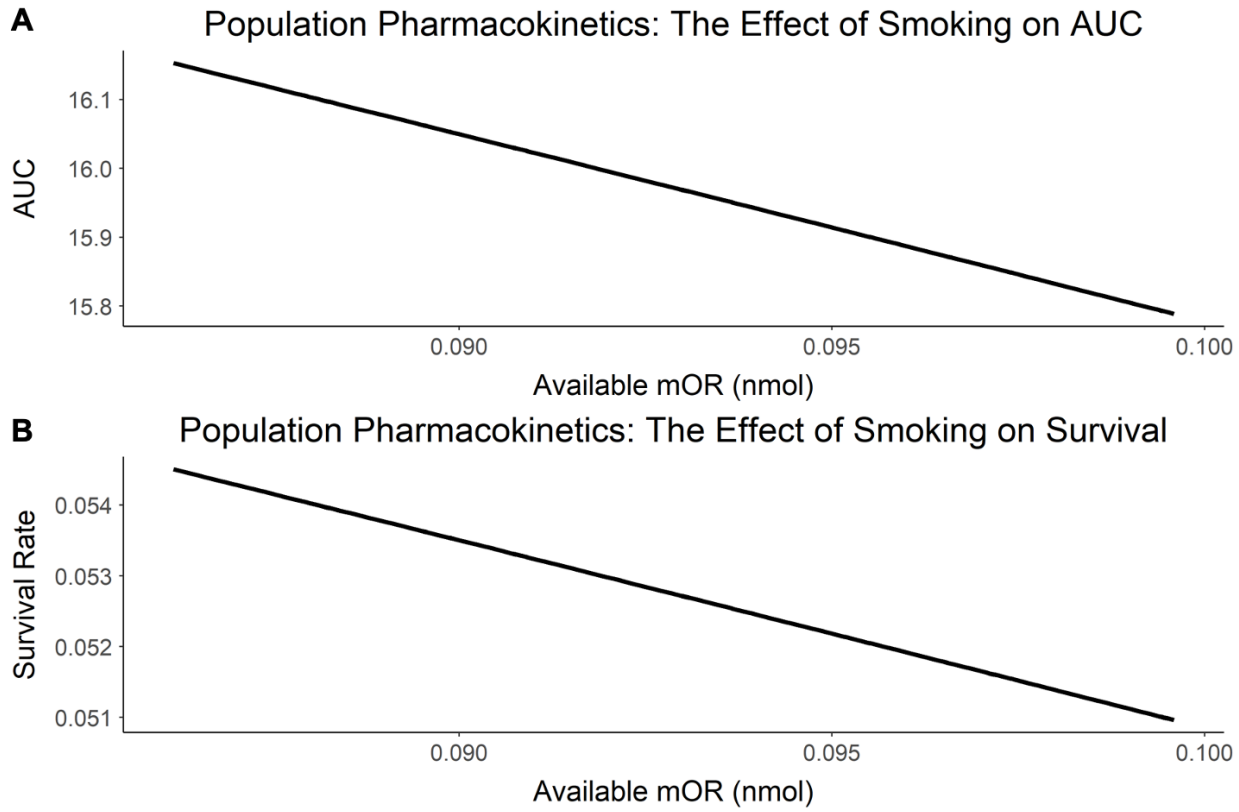

Figure 6. **PopPK of Smoking on Fentanyl Receptor Occupancy AUC -** Population pharmacokinetics with model outputs based on varying amounts of opioid receptors present in the brain. **(A)** AUC dependence on μOR. **(B)** Survival rate dependence on μOR.

In **Figure 7**, the impact of varying naloxone bioavailability in the population on our model output is shown. The AUC of fentanyl receptor occupancy and survivability were generated based on Simulation 2. As shown in the figure, we observe that the AUC decreases with increased naloxone bioavailability. This makes sense because greater bioavailability will result in an increased concentration of naloxone in the brain. As a result, fentanyl receptor occupancy will decrease, resulting in a decrease in the AUC. This consequently results in survival increasing as naloxone bioavailability increases.

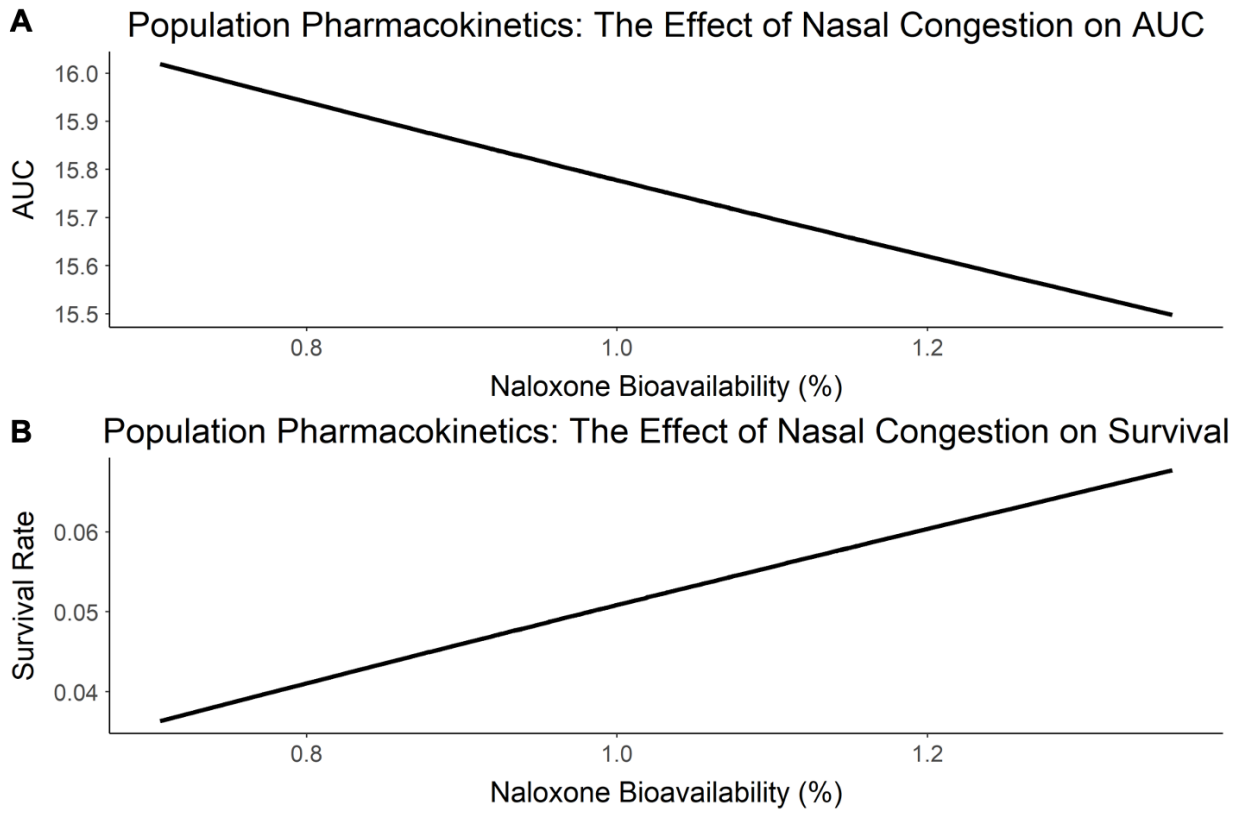

Figure 7. **PopPK of Nasal Congestion on Fentanyl Receptor Occupancy AUC -** Population pharmacokinetics with model outputs based on nasal congestion on naloxone bioavailability. **(A)** AUC dependence on naloxone bioavailability. **(B)** Survival rate dependence on naloxone bioavailability.

In **Figure 8**, we can again see the same trends in the effect of μOR availability and bioavailability of naloxone, but plotted in relation to each other.

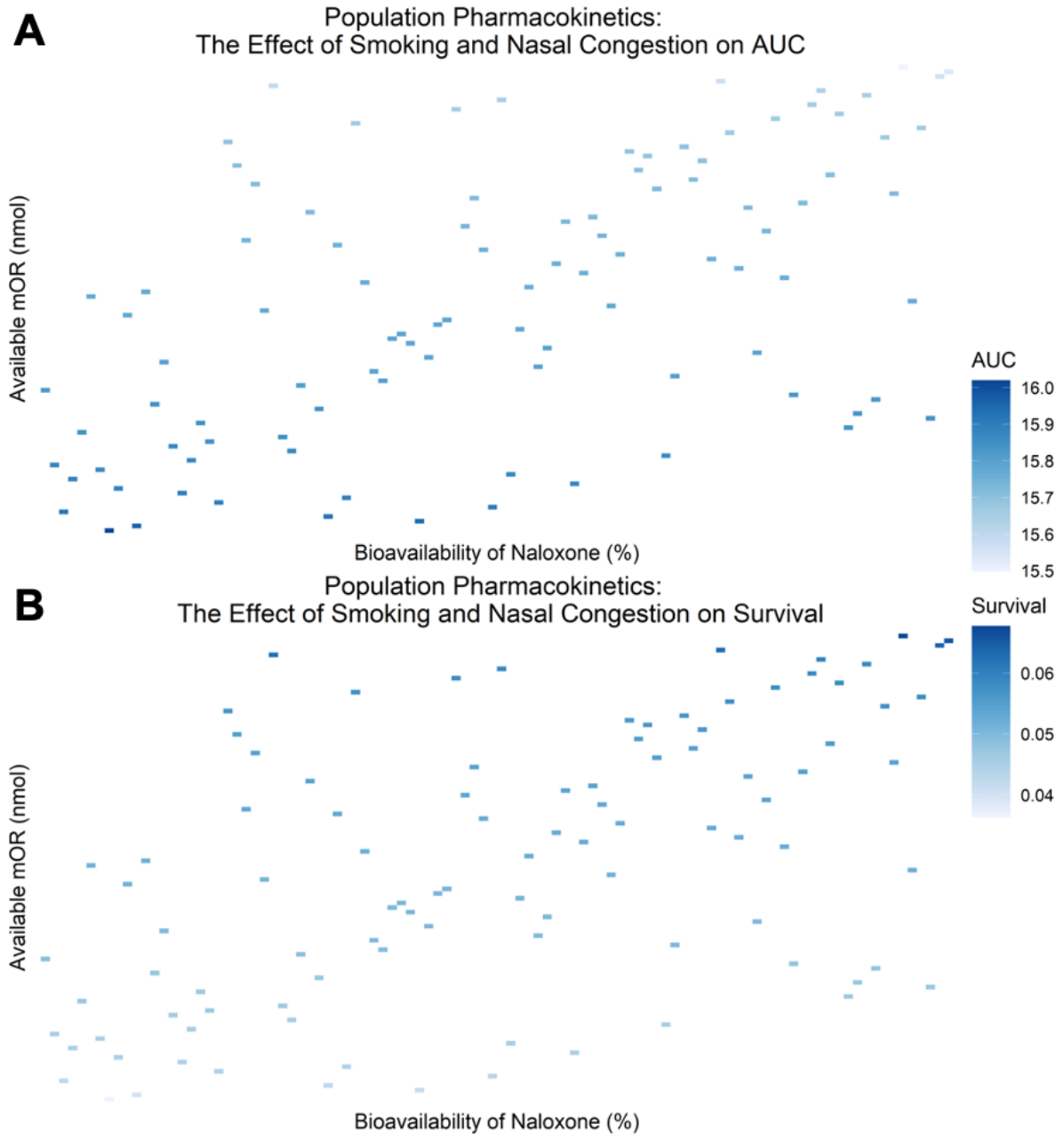

Figure 8. PopPK of Smoking and Nasal Congestion on Fentanyl Receptor Occupancy AUC - Population pharmacokinetics with model outputs based on both varying amounts of μOR in the brain due to differences in smoking habits and varying amounts of nasal congestion resulting in different naloxone bioavailabilities. **(A)** AUC dependence on available μOR and naloxone bioavailability. **(B)** Survival rate dependence on available μOR and naloxone bioavailability.

Sensitivity

Figure 9 shows the local sensitivity of fentanyl receptor occupancy AUC to the parameters used in our model. We observed k_{13f} having the greatest absolute (and positive) sensitivity to fentanyl receptor occupancy. This makes sense because a greater k_{13f} (transport rate between blood and brain) will result in more fentanyl in the brain and consequently more fentanyl binding to the μOR. This also explains why fentanyl is in a much greater amount in the brain than naloxone. In a similar manner, the sensitivity of k_{12f} is negative. By allowing less fentanyl to go into the body, there is more in the plasma which can be transported to the brain to bind with the μOR. The μOR amount had the most negative sensitivity to fentanyl receptor occupancy. A decreased amount of μOR results in a greater amount of fentanyl receptor binding, thus increasing the AUC. It also makes sense that p_f has a positive sensitivity. With greater amounts of free fentanyl circulating in the blood (because less protein binding), there will be greater concentrations of fentanyl in the brain.

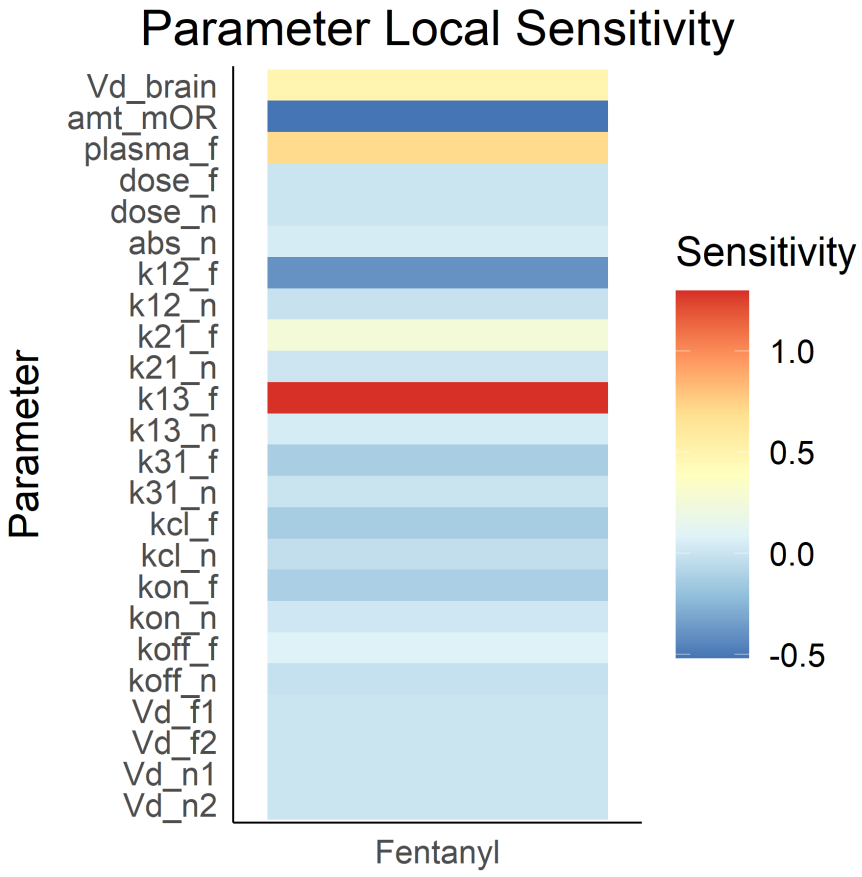

Figure 9. **Local Sensitivity Analysis with Respect to Fentanyl Receptor Occupancy AUC -** Local sensitivity analysis of all parameters used in the model. The AUC was calculated using the fentanyl receptor occupancy graph.

Interactive Visualization

Figure 10 shows screenshots of the interactive visualizations. In the first simulation (survival), the user can modify the naloxone parameters to see their effect on the fraction of μOR receptors bound to fentanyl. Specifically, there are two options for the naloxone dose, 2 mg or 4 mg, to represent different doses that a healthcare provider may have access to. The time before naloxone is first administered can also be modified by the user, and ranges from 1 minute to 10 minutes. Finally, the number of naloxone doses and the time between each dose can also be modified. We have limited the number of naloxone doses to a maximum of 4 that are given a maximum of 5 minutes apart to mimic real world conditions where naloxone is often administered multiple times a few minutes apart to a patient who has overdosed on fentanyl. In the second simulation (missed dose), the user can choose which dose (1 to 6) was missed. Additionally, they can input how late the dose was taken, from 0 minutes late (dose was not missed) to 29 minutes late (took twice the dose the next time).

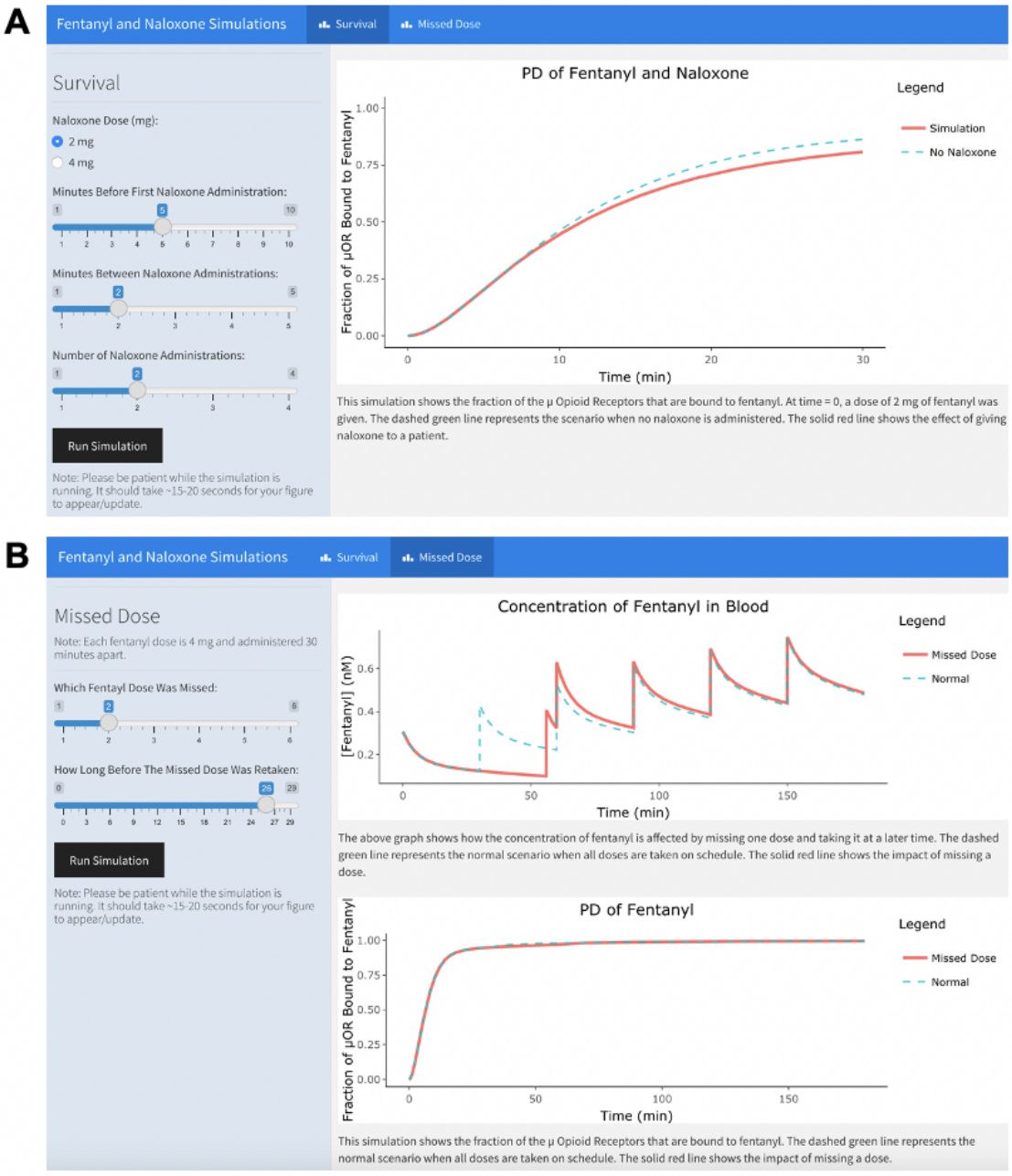

Figure 10. Interactive Visualization (Survival and Missed Dose) - Screenshots of the interactive visualization. **(A)** The survival scenario shows the effect on the binding of the μOR of administering naloxone after a patient has taken 2 mg of fentanyl. **(B)** The missed dose scenario shows the effect of taking fentanyl dose off schedule. *Note: This interactive visualization only works locally. All model simulations were conducted in MATLAB. Since the R code calls the MATLAB functions, it is not yet possible to publish this app to the Shiny servers which do not have MATLAB.*

IV. DISCUSSION

From the model simulations we ran, we observed naloxone having a visible effect on fentanyl receptor occupancy. In the base case of no naloxone being administered after a single 2 mg dose of fentanyl, the fentanyl receptor occupancy was the greatest (**Figure 5**). However, with the administration of naloxone, we observed the fentanyl receptor occupancy decreasing. It was interesting to see the effect naloxone had on changing fentanyl receptor occupancy for different types of naloxone administration. Naloxone had the greatest effect as a competitive inhibitor when the delay period between fentanyl and naloxone administration and the time between naloxone doses were minimized with the dose of naloxone being maximized. These results are reasonable as they increase the amount of naloxone in the system.

It is notable that while naloxone concentration was approximately 20-fold greater than fentanyl concentration in the blood and the body, this relationship was inverted in the brain (**Figure 3**). From our sensitivity analysis, in which we analyzed the change in the AUC of fentanyl receptor occupancy in the brain, we found that k_{13f} had the greatest positive effect on AUC (**Figure 9**). Interestingly, k_{13n} for naloxone did not have a significant effect in changing the AUC of fentanyl receptor occupancy. (We believed it would decrease it significantly). This is a possible explanation of why fentanyl had higher concentrations in the brain compared to naloxone.

In our repeated dose scenario, we found that fentanyl saturates the fastest in the bloodstream, followed by the body, and then the brain (**Figure 2**). Since fentanyl is administered as an intravenous bolus into the bloodstream, it is reasonable for it to saturate the fastest there. The transport rate of fentanyl from the bloodstream to the body, k_{12f} is approximately 13.5 times greater than the transport rate of fentanyl from the bloodstream to the brain, k_{13f}. Additionally the ratio k_{12f}/k_{21f} is approximately 4.8, whereas the ratio of k_{13f}/k_{31f} is only 1.25. This indicates that fentanyl enters the body four times the rate it leaves, while fentanyl enters the brain only 1.25 times the rate it leaves. However, the volume of distribution in the blood is 295 L for fentanyl and only 0.01260 L in the brain. This may explain why fentanyl only saturates a little faster (approximately 80 minutes faster) in the body with respect to the brain.

When analyzing the missed dose results, we found that taking the fourth dose of fentanyl at a delayed time does not significantly affect the peak and trough concentrations of fentanyl (**Figure 4**). The more delayed the dose, the smaller the maximum concentration was after the fourth dose peak and the greater the maximum concentration was after the fifth dose. This is because the interval between the fourth

and fifth dose was smaller, resulting in a greater mounted response. The inverse was true for trough concentrations.

When analyzing our population pharmacokinetics results, we found that increasing μOR in the system increased the AUC of fentanyl receptor occupancy, while decreasing survival rate (**Figure 6**). Smokers tend to have a decreased number of μOR which would suggest that they would have an increasing AUC for fentanyl receptor occupancy.[10] A lower amount of μOR also seems to suggest a greater chance of survival, possibly because the absolute number of μOR is lower (despite fentanyl occupancy saturating faster), resulting in a dampened response in the body. Additionally, when assessing the effect naloxone bioavailability has on fentanyl receptor occupancy, we observed a decreasing AUC as naloxone bioavailability increased, resulting in an increased survival rate. Naloxone bioavailability may decrease if a subject has mucus build-up or repeatedly snorts powders. For these subjects, naloxone will have less of an effect as a competitive inhibitor, resulting in a greater AUC for fentanyl receptor occupancy and consequently a lower survival rate.

Data for Development of this Model

The data used for this model largely originates from previous studies which built similar frameworks to examine the pharmacodynamics and pharmacokinetics of consumption of opioid drugs. These studies specifically focused on the middle-aged adult population, thereby neglecting to consider individuals on the more extreme ends of the age spectrum. However, although younger individuals, such as those below the age of 16 years, may not be represented well as a result, they are also less likely to be prescribed or abuse fentanyl. The case is different for the elderly, who, due to a general decline in health with age and the increased chance for various painful disorders, would be more likely to be prescribed fentanyl. These elderly individuals will likely have very different parameters than those used to develop our model, such as the level of expression of μOR or clearance rates of drugs due to poorer filtration systems. This would result in very different survivabilities.

In addition to not considering the changes in age, our model fails to consider the impact of weight, biological sex, or racial background. The parameters we have found support for in previous publications are not dependent on weight, thereby excluding this from our consideration. Weight can be important in determining the amount of drug that is considered lethal, but because this dose is already so small for fentanyl (a mere 2 mg), it may not be physiologically relevant to consider different amounts of lethal doses.

Our model does not take into account race or socioeconomic status as factors, but it can be interesting to investigate the role that they play. In 2017, the New York City

Department of Health and Mental Hygiene identified very high rates of fentanyl overdoses among the Black and Hispanic middle- and elderly-aged populations.[19] While it is not clear whether the cause is genetic or more likely social, incorporating such aspects into our model would be a future consideration.

However, our model does include the impact of varying levels of expression of opioid receptors and naloxone bioavailability. These are particularly pertinent to consider in the context of our model, as fentanyl overdoses most often occur in recreational drug users as opposed to hospital settings. These individuals often abuse other drugs as well, such as cocaine, which can lead to inflammation and damage to the blood vessels that line the nasal mucosa. Furthermore, smoking is a very prevalent habit, and is correlated with diminished expression of these receptors in the brain.[10] However, it would have been advantageous to incorporate the impact of drug tolerance into our model. Many clinicians note that some patients need several naloxone doses whereas others need only one to wake up from an overdose. However, the underlying cause for this is not well established, and so further investigation would be necessary before we can reflect this in our model.

V. Conclusion

The implementation of our results in an interactive app shows the impact our model would have in a clinical setting, providing easy and quick estimations of drug levels in a given patient based on the number of doses of fentanyl taken and the number of naloxone doses to administer. Although this model does still need improvement, taking into account greater variation in the population, it presents a preliminary framework for development of such a tool for both clinical use and investigation of drug interactions within the body.

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