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ORIGINAL PAPER

A quantitative systems pharmacology model of colonic motility with applications in drug development

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Abstract

We developed a mathematical model of colon physiology driven by serotonin signaling in the enteric nervous system. No such models are currently available to assist drug discovery and development for GI motility disorders. Model parameterization was informed by published preclinical and clinical data. Our simulations provide clinically relevant readouts of bowel movement frequency and stool consistency. The model recapitulates healthy and slow transit constipation phenotypes, and the effect of a 5-HT_4 receptor agonist in healthy volunteers. Using the calibrated model, we predicted the agonist dose to normalize defecation frequency in slow transit constipation while avoiding the onset of diarrhea. Model sensitivity analysis predicted that changes in HAPC frequency and liquid secretion have the greatest impact on colonic motility. However, exclusively increasing the liquid secretion can lead to diarrhea. In contrast, increasing HAPC frequency alone can enhance bowel frequency without leading to diarrhea. The quantitative systems pharmacology approach used here demonstrates how mechanistic modeling of disease pathophysiology expands our understanding of biology and supports judicious hypothesis generation for therapeutic intervention.

Keywords Quantitative systems pharmacology · 5-HT_4 receptor · GI motility · Colon · Bowel dysfunction · Constipation · Enteric nervous system

Introduction

Gastrointestinal (GI) motility is the process of mixing and transport of contents unidirectionally through the digestive tract from ingestion to digestion and defecation. Disorders in GI motility can occur at all points along the GI tract. In this study, we focus on motility of the colon, the terminal part of the GI tract. One major disorder affecting motility of the colon is constipation. Chronic constipation affects between 2 and 27 percent of the western population [1]. The percentage of individuals suffering from chronic constipation is even higher in some rare diseases such as multiple sclerosis (MS), a chronic inflammatory neurodegenerative disorder. Treatment options for severe constipation are limited making it an area of active drug development by pharmaceutical companies [2]. Here, a quantitative systems pharmacology model of colonic motility was developed using published data from healthy and constipated individuals to support drug discovery and development.

The efficiency of colonic mass transport is governed by two factors: directional pressure waves that move colonic content, and the viscosity of chyme in the colon. Manometry studies have established that large scale mass transport in the colon is due to discrete events called high amplitude propagating contractions (HAPCs). These traveling waves of muscle contraction propel chyme in the oral to anal direction, and often precede the urge to defecate [3–5]. HAPC frequency, duration and propagation speed have been measured in healthy and constipated individuals [4–8].

The viscosity of chyme is related to its liquid content [9]. The liquid fraction is affected by liquid secretion in the

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small intestine and absorption through the colon [10]. Liquid fraction of chyme entering the colon is difficult to measure directly, but ileal efflux data from ileostomized patients suggest that there is a constant influx into the colon, and the liquid content of chyme does not appear vary due to food composition and liquid intake [11]. Direct measurement of liquid fraction in stool is possible and data is available for both healthy and constipated individuals.

Colonic muscle contractions and secretomotor activity in the GI tract are regulated by the enteric nervous system (ENS). The ENS is an extensive and interconnected system of ganglia that enmeshes the GI tract [12, 13]. A key signaling neurotransmitter of the ENS is 5-hydroxy tryptophan (5-HT, serotonin). Enterochromaffin (EC) cells of the intestinal mucosa synthesize 5-HT and release it in response to mechanical and chemical stimuli produced by content in the lumen. Released 5-HT binds to a multitude of 5-HT receptors on primary afferent neurons including 5-HT_{1A}, 5-HT_{1P}, 5-HT_3, 5-HT_4, 5-HT_7 receptor subtypes [2]. The 5-HT_4 receptor is a known drug target capable of stimulating colonic motility in humans [14]. Binding of 5-HT to 5-HT_4 receptor leads to acetylcholine (ACh) release. ACh binds to muscarinic receptors on submucosal and myenteric neurons and muscle cells to induce contractile and secretomotor activity [2, 12, 13, 15, 16]. Thus, 5-HT_4 signaling in the ENS is an important regulator of GI motility and a target for drug developement to treat constipation in multiple disorders including MS.

Multiple sclerosis (MS) is a rare chronic inflammatory neurodegenerative disorder impacting as many as 1 to 2 million people worldwide. Approximately 400,000 young adults in the United States suffer from MS [17–19]. Nearly half (43%) of individuals diagnosed with MS suffer from constipation [17, 20, 21]. This represents a much greater prevalence of constipation in MS patients as compared to the general population. It is also known that in MS, constipation symptoms worsen with disease severity. However, data on constipation in MS to inform the development of a QSP model are limited compared to studies in another severe form of constipation, slow transit constipation (STC). This motivated the authors of this work to build the QSP model using data derived from STC and healthy individuals because to date, studies performed in MS patients suggest that the constipation phenotype in MS is consistent with the phenotype of constipation in the general population [21–23]. In silico approaches can be valuable to inform drug development in the case of rare disease were patient numbers are limited as in MS patients with severe constipation.

STC has been extensively studied and is characterized by reduced colonic motility due to abnormalities of the ENS [24]. Manometric studies have demonstrated decreased HAPC frequency but normal HAPC amplitude in the colon of STC patients [1, 24]. Another characteristic of STC is a decrease in the liquid fraction of the bowel movement resulting in hard, lumpy, infrequent stool. The STC phenotype is limited to dysregulation of the colonic transit only, without changes in small intestinal transit time [25]. The defecation response also appears to be unaltered in STC [26].

Laxatives are commonly used to treat constipation, but treatment options for more severe forms of constipation such as STC and chronic constipation in neurological disorders such as MS are limited and far from satisfactory. 5-HT_4 receptor agonist have shown promising results in the clinic. These agents are capable of eliciting an increase in colon motility due to their prokinetic and prosecretory activity, but are limited by their off target adverse effects [27]. Therefore, it is desired to develop more specific 5-HT_4 receptor agonist or new strategies to modulate the 5-HT_4 signaling pathway for treatment of colon dysmotility. But it is challenging to integrate the complex interplay of biochemical signaling in the ENS with the physiological movement of colonic mass to inform therapeutic strategies.

Quantitative systems pharmacology (QSP) modeling integrates mechanistic understanding of physiological and pathological processes and preclinical and clinical data from pharmacological modulation of the system. QSP models are valuable tools for model informed drug discovery and development [28–30]. A QSP model of colonic motility can provide a unified framework linking ENS signaling with colonic motility and drug pharmacokinetics and pharmacodynamics.

The aim of this study was to develop such a fit-for-purpose QSP model to predict the effect of modulating the 5-HT signaling pathway on clinically relevant endpoints such as defecation frequency and stool consistency. We show the QSP model can be used to identify optimal therapeutic strategies. Since STC and constipation in MS share basic physiological and pathological mechanisms impacting GI motility, a QSP model built to capture the mechanism of STC should be useful in predicting the effects of drug treatment in MS patients suffering from chronic constipation.

Methods

Model structure

The QSP model is organized into two modules: a colon physiology module and a 5-HT signaling module (Fig. 1).

The colon physiology module has four connected compartments that represent the ascending, transverse, descending and rectosigmoid segments of the colon. Anatomically, the colon is made up of smaller functional

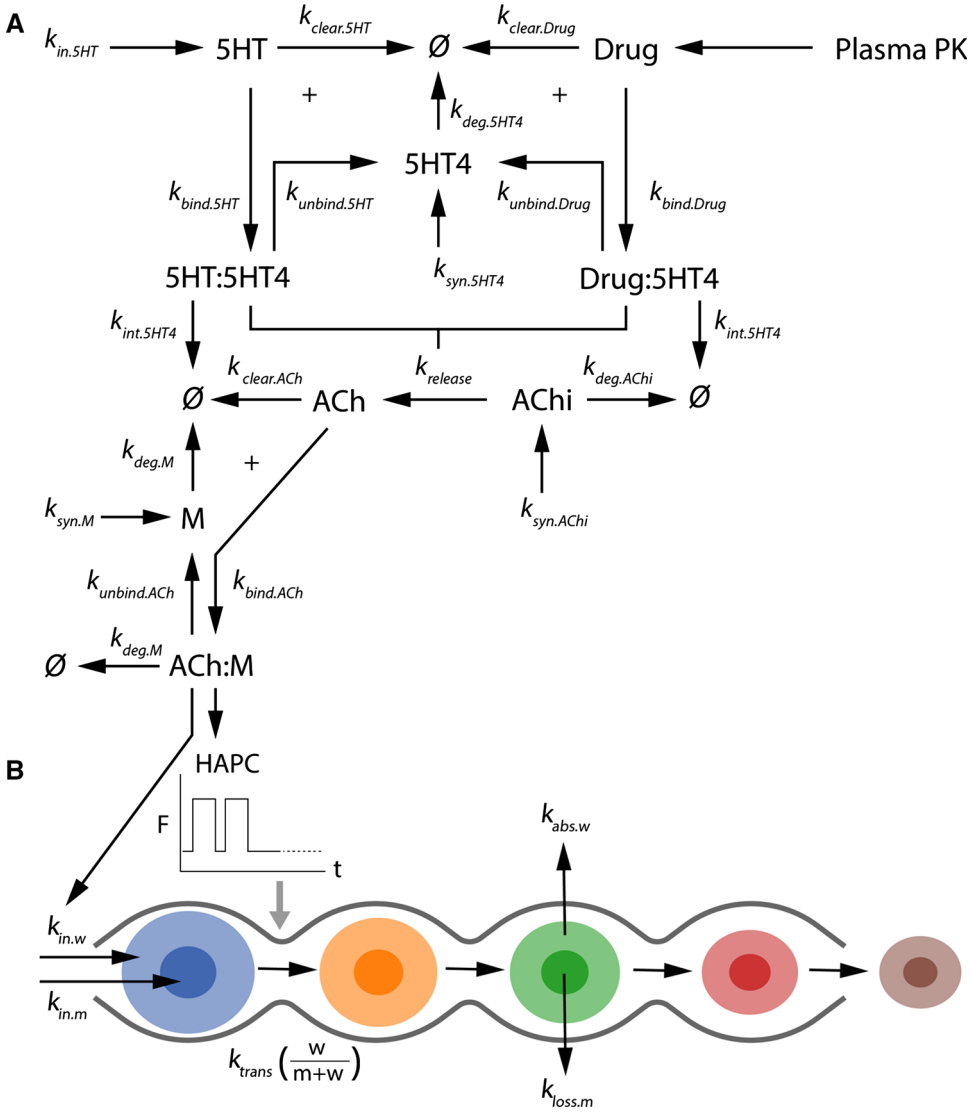

Fig. 1 QSP model diagram for colon motility and ENS signaling. The model consists of two modules: **a** 5-HT signaling module to represent 5-HT_4 receptor activation and downstream signaling, and **b** colon physiology module to represent mass transport along the colon. Muscarinic receptor occupancy in the signaling module controls HAPC frequency and liquid influx rate in the colon physiology module via mapping functions. Mass influx occurs in the first compartment, liquid absorption and solid mass loss happen in every compartment, and defecation events remove mass from the terminal compartment. See the Methods section for a detailed model description

units called haustra. Thus, each compartment in our model approximates several interconnected haustra. We assume that the contents of each compartment are well mixed by segmental contractions and ignore inter-haustral variability.

Chyme arrives into the first compartment at a continuous rate of influx. The solid and liquid fractions of chyme are treated as separate entities each with its own influx rate. Loss of liquid due to absorption through the colonic wall and loss of solid due to bacterial digestion are modeled as first-order processes that occur in all 4 compartments with rate constants k_{abs} and k_{loss}, respectively.

Chyme moves down the colon by the propulsive force generated by high amplitude propagating contractions (HAPCs). These are implemented as external forcing functions, $F_j(t)$ that switch on a first order transport term. Each HAPC persists for 1 min in a compartment before moving to the next compartment. Specifically, an HAPC propels chyme from the 1st compartment to the 2nd for one minute, then from the 2nd to the 3rd compartment for one minute and finally from the 3rd to the terminal compartment for one minute. The total duration of 3 min was derived from the mean length of the human colon ($\sim$ 160 cm), and the observed mean HAPC propagation velocity of approximately 1 cm/s [4, 6–8]. Solid and liquid components of the chyme are transported with the same first order rate constant that is assumed to be proportional to the liquid fraction of the chyme in that compartment. Between HAPCs, chyme does not move between compartments, but liquid absorption and solid loss continue to happen.

HAPCs cluster at specific times of the day, primarily after waking up in the morning, and after meals, and are infrequent between meals and at night [4–8]. Therefore, timings of HAPC initiation in the first compartment were set to happen on a schedule that reflect these trends. We

refer to a set of clustered HAPCs that occur in close succession as an "episode".

After each episode of HAPCs, the total mass (solid + liquid) in the terminal compartment was checked against a defecation threshold. If the total mass exceeded this threshold, the terminal compartment was emptied to simulate defecation. Thus, defecation is modeled as an HAPC-dependent event conditional on sufficient mass accumulation in the terminal compartment. The defecation threshold was treated as a model parameter. The threshold represents the degree of rectal distension that induces a defecation response.

Simulations record the timing of defecation, the total mass of stool and the liquid fraction of stool. The liquid fraction was converted into a discrete consistency score using the empirical mapping shown in Fig. 4b. This score approximates the Bristol stool score commonly used in the clinic to assess stool consistency. The empirical mapping was based on the correspondence between stool consistency and liquid content reported by Bliss et al. [31].

The colon physiology module is driven by the 5-HT signaling module that models the effect of a 5-HT_4 receptor agonist on acetylcholine (ACh) binding to muscarinic receptor. The ligand is either endogenous 5-HT or an exogenous drug. As shown in Fig. 1, ligand binding to 5-HT_4 receptor leads to the release of intracellular acetylcholine into the extracellular space (AChi → ACh). The released ACh binds to muscarinic receptors (M).

Endogenous 5-HT is synthesized by enterochromaffin cells of the gut mucosa and released in response to mechanical or chemical stimuli [2, 12, 13, 15, 16]. As the dynamics of 5-HT release are unknown, we assume a fixed steady state concentration of 5-HT, implemented using a zero-order synthesis rate and a first order degradation rate. Similarly, 5-HT_4 receptors, ACh_i and M are assumed to be synthesized with constant rates (0th order synthesis rate) and degraded/internalized with first-order kinetics. 5-HT:5-HT_4 receptor complex, drug:5-HT_4 complex and ACh:M complex also internalize and degrade with first order rates. The internalization rates of free and bound muscarinic receptors were set to be the same, to conserve total receptor concentration irrespective of receptor occupancy.

We also modeled the effect of a small molecule 5-HT_4 receptor agonist, Compound X (also referred to as the drug). As described in the Supplemental Material, PK data were fit to a two-compartment model with first order absorption from the GI. The predicted plasma concentration of free drug was used as a time-dependent model input $c(t)$. It is assumed that downstream events for the drug-bound receptor are the same as endogenous 5-HT-bound receptors.

The output of the signaling module is the 24-hour average of the bound fraction of muscarinic receptors, $f = [ACh : M]/([M] + [ACh : M])$.

This was linked to the colon physiology module using empirical Hill functions:

$$y = \frac{f^n}{(EC_{50})^n + f^n} \cdot y_{\max}$$

where y is either the daily HAPC frequency, or the liquid influx rate. A different y_{max} was used for each mapping, but the EC_{50} and the Hill coefficient n were kept the same.

Model equations

The colon physiology module is represented by the following ordinary differential equations (ODEs):

Compartment 1:

$$\frac{dm_1}{dt} = k_{in.m} - k_{loss} \cdot m_1 - F_1 \cdot k_{trans} \cdot l_1 \cdot m_1$$

$$\frac{dw_1}{dt} = k_{in.w} - k_{abs} \cdot w_1 - F_1 \cdot k_{trans} \cdot l_1 \cdot w_1$$

Compartments 2 and 3:

$$\frac{dm_j}{dt} = F_{j-1} \cdot k_{trans} \cdot l_{j-1} \cdot m_{j-1} - k_{loss} \cdot m_j - F_j \cdot k_{trans} \cdot l_j \cdot m_j$$

$$\frac{dw_j}{dt} = F_{j-1} \cdot k_{trans} \cdot l_{j-1} \cdot w_{j-1} - k_{abs} \cdot w_j - F_j \cdot k_{trans} \cdot l_j \cdot w_j$$

with $j = 2, 3$

Compartment 4:

$$\frac{dm_4}{dt} = F_3 \cdot k_{trans} \cdot l_3 \cdot m_3 - k_{loss} \cdot m_4$$

$$\frac{dw_4}{dt} = F_3 \cdot k_{trans} \cdot l_3 \cdot w_3 - k_{abs} \cdot w_4$$

where, m_j and w_j are the solid and liquid mass, and $l_i = w_j/(m_j + w_j)$ is the liquid fraction in the jth compartment. The variable F_j is an indicator for the presence of an HAPC in the jth compartment: $F_j(t) = 1$ if a contraction is present at time t in compartment j and 0 if otherwise.

At scheduled check times after every episode of the HAPCs, the total mass in the terminal compartment is checked against the defecation threshold. If $m_4 + w_4 \geq m_{thresh}$, the terminal compartment mass is reset to 0, to simulate a defecation event. The system is then integrated forward with updated initial conditions. Table 1 lists nominal parameter values for the colon physiology module used in our simulations.

The signaling module is represented by the following ODEs:

Table 1 Colon physiology module parameter definitions and nominal values used in model simulations

Parameter	Description	Value	Units	Source
$k_{in.m}$	Solid influx rate	0.033	g min^{-1}	Kramer [11], Kay [33]
$k_{in.w}$	Water influx rate	0.30	g min^{-1}	Kramer [11], Kay [33]
k_{trans}	Mass transport rate constant	0.5	min^{-1}	Model calibration
k_{loss}	Solid loss rate constant	3.3×10^{-3}	min^{-1}	Model calibration
k_{abs}	Water absorption rate constant	10^{-3}	min^{-1}	Model calibration
m_{thresh}	Defecation threshold	70	g	Model calibration

$$\frac{d}{dt}[5HT] = k_{in.5HT} - k_{clear.5HT}[5HT] - k_{bind.5HT}[5HT][5HT4] + k_{unbind.5HT}[5HT:5HT4]$$

$$\frac{d}{dt}[Drug] = c(t) - k_{clear.Drug}[Drug] - k_{bind.Drug}[Drug][5HT4] + k_{unbind.Drug}[Drug:5HT4]$$

$$\frac{d}{dt}[5HT4] = k_{syn.5HT4} - k_{deg.5HT4}[5HT4] - k_{bind.5HT}[5HT][5HT4] + k_{unbind.5HT}[5HT:5HT4] - k_{bind.Drug}[Drug][5HT4] + k_{unbind.Drug}[Drug:5HT4]$$

$$\frac{d}{dt}[5HT:5HT4] = k_{bind.5HT}[5HT][5HT4] - k_{unbind.5HT}[5HT:5HT4] - k_{int.5HT4}[5HT:5HT4]$$

$$\frac{d}{dt}[Drug:5HT4] = k_{bind.Drug}[Drug][5HT4] - k_{unbind.Drug}[Drug:5HT4] - k_{int.5HT4}[Drug:5HT4]$$

$$\frac{d}{dt}[ACh_i] = k_{syn.AChi} - k_{deg.AChi}[ACh_i] - k_{release} \cdot ([5HT:5HT4]+[Drug:5HT4]) \cdot [ACh_i]$$

$$\frac{d}{dt}[ACh] = k_{release} \cdot ([5HT:5HT4] + [Drug:5HT4]) \cdot [ACh_i] - k_{bind.ACh}[ACh][M] + k_{unbind.ACh}[ACh:M] - k_{clear.ACh}[ACh]$$

$$\frac{d}{dt}[M] = k_{syn.M} - k_{deg.M}[M] - k_{bind.ACh}[ACh][M] + k_{unbind.ACh}[ACh:M]$$

$$\frac{d}{dt}[ACh:M] = k_{bind.ACh}[ACh][M] - k_{unbind.ACh}[ACh:M] - k_{deg.M}[ACh:M]$$

Drug-free steady state concentrations of species in the signaling module are listed in Table 2, and nominal parameter values used in model simulations are listed in Table 3.

Software for model implementation

The model was implemented using KroneckerBio v0.5.2.3 (https://github.com/kroneckerbio/kroneckerbio). Simulations and model analysis were performed using MATLAB Version: 9.3 (R2017b) (MathWorks, Natick, MA). The signaling module was initialized with all species at their drug-free steady state concentrations. The colon physiology module was initialized with no mass in the colon. A model equilibration step was run untill defecation frequency and stool mass reaches steady state. Typically, a simulated 30-week run was sufficient to reach steady state. All simulations shown here were performed starting with the pre-equilibrated baseline mass distribution.

Data used to inform model

Several published observations were used to constrain model parameters to physiological values. Total colonic influx was set to a rate of 480 grams/day with a 90% liquid content to match ileal efflux measurements in ileostomized patients [11, 32]. The ileal efflux was observed to be independent of food composition, timing of meals [11] and unaltered in a constipated bowel [25]. Accordingly, we assumed a constant colonic influx rate.

The reported mean of 6 HAPC per day in healthy volunteers was used to define a healthy phenotype [4–8]. The precise timings of HAPC vary, but they are more frequent after waking up and after meals. Therefore, we used the following episodes of HAPCs to define a healthy phenotype: 3 in the morning after waking up, 2 in the afternoon (post-lunch) and 1 in the evening (post-dinner). As described below, model parameters were calibrated such that 6 HAPC/day produced one defecation per day, with an average weight of 124 grams for healthy volunteers, to match values reported in the literature [33, 34]

To simulate an STC phenotype, the HAPC frequency was lowered to 2/day [4, 5] with 1 in the morning after waking up and 1 in the afternoon post lunch. Based on the Rome III criteria for constipation, < 3 complete spontaneous bowel movements per week are expected for STC patients with a liquid fraction $< 60\%$ [24, 31]. The model

Table 2 Signaling module species and steady state concentrations for nominal parameter values listed in Table 3

Species	Description	SS concentration	Unit
5HT	Endogenous Serotonin	12.7	nM
*5HT*4	Serotonin receptor	100	nM
5HT: *5HT*4	Serotonin-bound receptor	24.9	nM
ACh_i	Intracellular Acetylcholine	899	nM
ACh	Extracellular (released) acetylcholine	251	nM
M	Muscarinic receptor	799	nM
ACh: *M*	Acetylcholine-bound Muscarinic receptor	201	nM

Table 3 Signaling module parameters and their nominal values

Parameter	Description	Value	Units	Source
$k_{in.5HT}$	Endogenous serotonin release rate constant	0.41	nM s^{-1}	Model calibration
$k_{clear.5HT}$	Serotonin clearance rate constant	0.03	s^{-1}	Adjusted to achieve baseline 5-HT4 occupancy of 20%
$k_{bind.5HT}$	Serotonin binding rate constant	10^{-3}	nM s^{-1}	Typical biochemical association rate constant for small molecules
$k_{unbind.5HT}$	Serotonin dissociation rate constant	5.0×10^{-2}	s^{-1}	Model calibration
$k_{syn.5HT4}$	$5HT_4$ receptor synthesis rate constant	0.1	nM s^{-1}	Model calibration
$k_{deg.5HT4}$	$5HT_4$ receptor degradation rate constant	7.0×10^{-4}	s^{-1}	Model calibration
$k_{int.5HT4}$	Bound $5HT_4$ receptor internalization rate constant	1.2×10^{-3}	s^{-1}	Model calibration
$k_{syn.AChi}$	Intracellular ACh synthesis rate constant	10^3	nM s^{-1}	Model calibration
$k_{deg.AChi}$	Intracellular ACh degradation rate constant	1	s^{-1}	Model calibration
$k_{release}$	ACh release rate constant	4.5×10^{-3}	nM s^{-1}	Adjusted to match Borman and Burleigh [36]
$k_{bind.ACh}$	Acetylcholine binding rate constant	10^{-3}	nM s^{-1}	Typical biochemical association rate constant for small molecules
$k_{unbind.ACh}$	Acetylcholine dissociation rate constant	1	s^{-1}	To match reported K_d in Keef [37]
$k_{clear.ACh}$	Extracellular acetylcholine clearance rate constant	0.4	s^{-1}	To ensure rapid clearance of extracellular ACh
$k_{syn.M}$	Muscarinic receptor synthesis rate constant	0.1	nM s^{-1}	Assumed synthesis rate
$k_{deg.M}$	Muscarinic receptor degradation rate constant	10^{-4}	s^{-1}	Assuming Muscarinic receptor concentration is at K_d for ACh:M binding
$k_{bind.Drug}$	Drug binding rate constant	10^{-3}	nM s^{-1}	Typical biochemical association rate constant for small molecules
$k_{unind.Drug}$	Drug:$5HT_4$ dissociation rate constant	0.03	s^{-1}	Model calibration
y_{max} (HAPC)	Maximum number of HAPCs for healthy phenotype mapping	16		Model calibration
y_{max} (liquid influx)	Maximum liquid influx rate for healthy phenotype mapping	0.77	g min^{-1}	Model calibration
n	Hill coefficient for healthy phenotype mapping	5.5		Model calibration

parameters were calibrated to reproduce these observations. The observed relationship between a qualitative consistency score and the measured liquid fraction of stool was used to map liquid fraction to a stool consistency score in our simulations [31] (Fig. 4b).

The defecation threshold mass was set to the same value for healthy and STC phenotypes, as the rectal distension volume that induces a defecatory sensation is not affected by STC [26].

Two key pieces of published data were used to inform the signaling module. Borman and Burleigh [35] measured 5-HT induced contraction of longitudinal muscle segments of human terminal ileum, and reported that maximal muscle contraction elicited by 5-HT stimulation is

approximately 40% of maximal muscle contraction due to ACh stimulation. Keef et al. measured contractile response of circular muscles of the canine proximal colon in response to ACh and determined an EC_{50} of $\sim$ 1 μM [36]. Accordingly, we set the binding K_d of ACh to muscarinic receptors to be 1 μM.

Model calibration

The colon physiology module was calibrated using a systematic parameter search strategy. The objective was to find a combination of parameters that could reproduce the following phenotypes:

- Healthy: With 6 HAPCs per day, produce a defecation frequency of 1/day and a liquid fraction of approximately 75%.
- STC: With 2 HAPCs per day, produce a defecation frequency $\leq$ 2/week and $\leq$ 60% liquid fraction.
- Healthy treated with a prokinetic drug such as Prucalopride: With $\geq$ 10 HAPCs per day, produce $\geq$ 4 defecations per day, with a liquid fraction $\geq$ 80%

A 6-dimensional grid was set to sample a range of values for each parameter listed in Table 1 (except $k_{in,m}$, which was kept fixed to literature data) [11, 32]) and different HAPC frequencies. The range of values sampled are listed in the Supporting Information (Table S2). Each point in the parameter space grid represents a unique parameter combination. For each combination, the colon physiology module was simulated for 30 weeks to ensure the model reached a steady periodic behavior. The simulation time course was then collected for one additional week to compute the defecation frequency and mean liquid. Parameter combinations that failed to match all three criteria listed above were rejected. Remaining parameter sets were tested to determine how fast the colon physiology module showed a measurable change in defecation frequency and stool consistency in response to changes in HAPC frequency or liquid influx rate for each parameterization. For the simulations in this study, we selected a parameter set that elicited a robust response within 24 h, as this is the typical time period for evaluating clinical efficacy. This parameter set is reported in Table 1.

For the 5-HT signaling module, the ACh release rate was adjusted such that the maximal muscarinic receptor occupancy in response to 100% 5-HT_4 engagement was capped at approximately 40%. To link 5-HT_4 signaling module to colon physiology module, we assumed a baseline muscarinic receptor occupancy of 20% to ensure a working dynamic range. We determined Hill function parameters such that this baseline maps to 6 HAPCS/day and 432 g/day = 0.3 g/min of liquid influx for the healthy phenotype. To map to an STC phenotype, we lowered the maximal HAPC frequency such that the baseline muscarinic receptor occupancy of 20% produced 2 HAPCs/day (Fig. 4a), consistent with published data [4, 5]. We used the same liquid influx mapping for the two phenotypes with a baseline liquid influx rate of 0.3 g/min at 20% muscarinic receptor occupancy (Fig. 4b).

Pharmacokinetic (PK) data from oral and intravenous administration of Compound X in healthy volunteers were fit to a linear two-compartment model described in Supporting Information. The estimated PK parameters are reported in Table S1. We assumed that free drug diffuse freely between blood and its site of action within colonic tissue. Therefore, the plasma free drug concentration was used as the input function *c(t)* to the signaling module.

Dose response to the drug, measured by stool frequency and stool consistency in healthy volunteers treated with a single oral dose, was used to adjust the EC_{50} and Hill coefficients used in the mapping functions linking the two modules. These parameters were adjusted to match the observed dose response for Compound X.

Sensitivity analysis

Single parameter sensitivity analysis was performed for the colon physiology module and signaling module separately. For the colon physiology module, each model parameter was varied by $\pm$ 50% of its nominal value while holding the remaining parameters fixed at their nominal values. Mean defecation frequency and stool consistency were computed for each parameter set as described above. For the signaling module each parameter was varied by an order of magnitude in either direction (i.e. 10 $\times$ or 0.1x), and the model was run to steady state to determine the steady state muscarinic receptor occupancy.

Results

Calibration of colon physiology and signaling modules

We constructed a mathematical model of colonic transit driven by 5-HT signaling (Fig. 1). The colon physiology module describes the influx, transport and absorption of colonic content, and implements a threshold-dependent defecation. The signaling module implements ligand binding to 5-HT_4 receptors to trigger the release of intracellular acetylcholine (ACh). ACh binds to muscarinic receptors to stimulate contractile and secretory responses.

See "Methods" for a detailed description of the model and parameter calibration.

For the calibrated colon physiology module, a healthy phenotype with 6 HAPCs per day leads to a bowel movement (BM) once a day with an total stool mass of 123 g consisting of 96.6 g liquid (liquid fraction = 78.5%) as shown in Fig. 2a. These results are consistent with values reported in the literature [4–7, 34]. Patients with slow transit constipation (STC) show a reduction in HAPC frequency [4, 5]. In the model, decreasing HAPC frequency

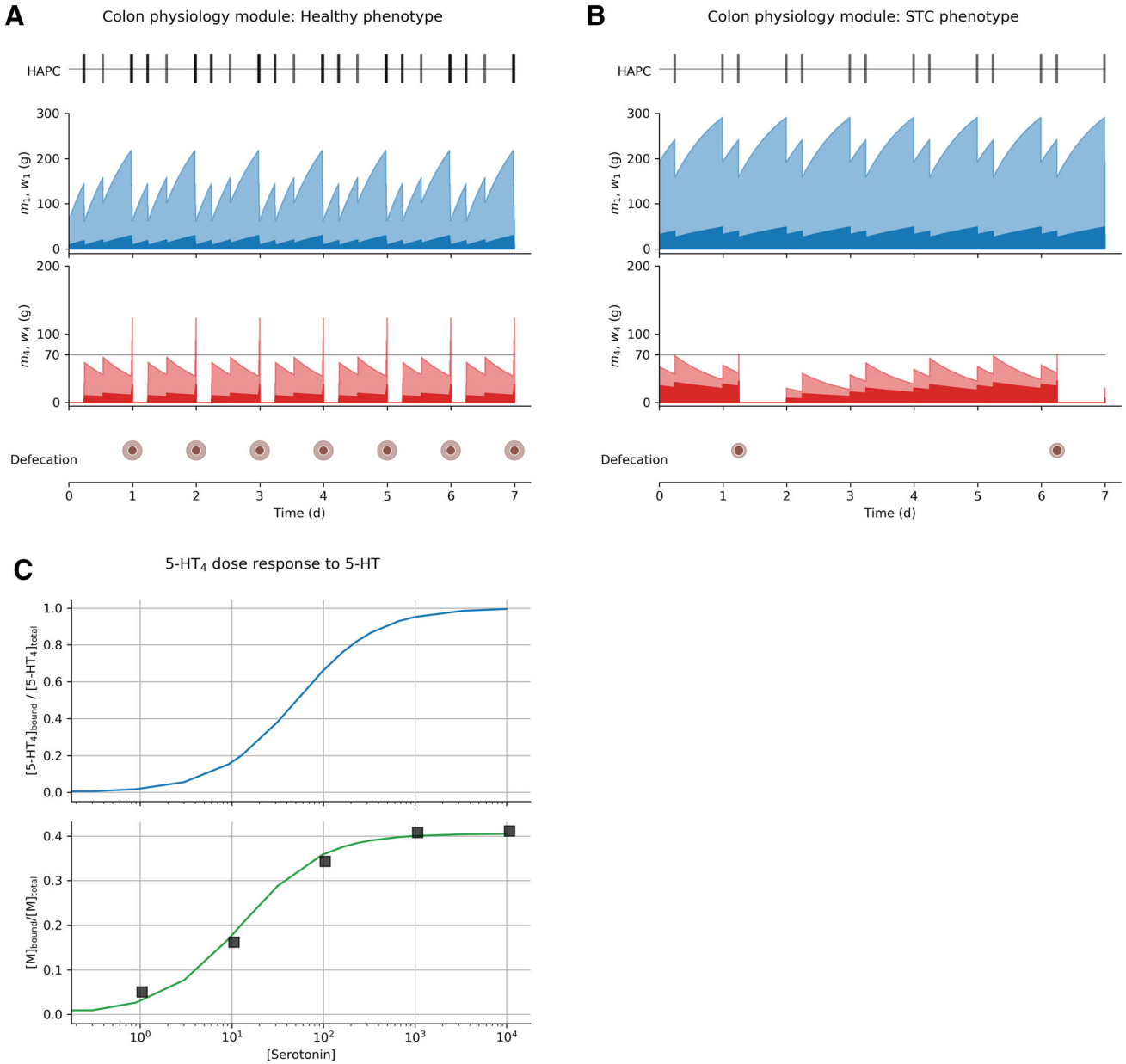

Fig. 2 Model calibration for colon physiology module and signaling module. Simulated time courses of colonic mass and defecation profiles for **a** healthy, and **b** STC phenotypes in the colon physiology module. All parameters are identical for the two phenotypes, and only the HAPC frequency varies (6/day for healthy versus 2/day for STC). The top panel shows the timing of HAPC episodes. Light tick = 1, medium tick = 2, and bold tick = 3 HAPCs per episode. The two middle panels show the time course of solid (bold shaded region) and liquid (light shaded region) mass in the first and the terminal compartments of the model. The horizontal line shows the threshold for emptying mass in the terminal compartment. Emptying of the terminal compartment leads to defecation, as shown in the bottom panels. The total mass of each defecation is proportional to the area of the full circle. The inner (dark) circle represents the solid mass, and the outer annulus represents the liquid mass. Their relative size is a visual indicator for stool consistency. **c** Dose response of the signaling module for different steady-state 5-HT concentrations. Solid lines plot 5-HT$_4$ receptor occupancy and muscarinic receptor occupancy. Observed dose response data (black squares) are the contractile response of longitudinal muscle strips of the human terminal ileum stimulated in vitro with 5-HT, relative to maximal contraction due to ACh (digitized from Fig. 1 of (27))

to 2 per day produces 2 bowel movements per week with a mass of 70 g and a much drier consistency (liquid mass = 38.7 g, liquid fraction = 59.5%) (Fig. 2b). Thus, the model recapitulates common symptoms of STC.

Importantly, the smaller, drier stool produced in the STC phenotype was not engineered by changing the absorption rate constants. Rather, it was the result of a longer residence time between defecations, due to infrequent HAPCs, that led to greater water absorption and compaction of the stool.

The 5-HT signaling module was calibrated separately as described in the Methods. The dose response of the signaling module to 5-HT concentration at steady state is shown in Fig. 2c. Assuming that the fraction of bound muscarinic receptors directly correlates with muscle contraction, the dose response for muscarinic receptor occupancy agrees well with previously reported ex vivo muscle contraction response to 5-HT [35] (Fig. 2c).

Simulated treatments with colon physiology module

Two classes of drugs are commonly used to treat chronic constipation: pro-kinetic agents that increase coordinated contractions, and pro-secretory agents that increase intestinal liquid secretion. 5-HT_4 receptor agonists such as Prucalopride elicit both pro-kinetic and pro-secretory responses. Prucalopride treatment is reported to increase HAPC frequency, elevate intestinal fluid secretion, and decrease colonic transit time [7, 37, 38]. Patients treated with prucalopride experience increases in BM frequency and stool water content [39–41]. Secretagogues, such as Lubiprostone, increase fluid and mucin release in the small intestines of preclinical animal models [42, 43]. In patients, Lubiprostone increases both BM frequency and water content [44].

We tested the effect of simulating treatments that increase either the HAPC frequency or liquid influx rate in the STC phenotype described above. These perturbations simulate the effect of a pro-kinetic and a pro-secretory drug, respectively. As seen in Fig. 3a, each simulated treatment increased the defecation frequency relative to an STC phenotype. A prokinetic treatment that increased HAPC frequency from 2/day to 3/day normalized the defecation frequency to 1/day. A prosecretory treatment that increased liquid influx by 10% increases the defecation frequency to 0.5/day.

To further explore the effect of each treatment, we simulated the response to varying HAPC frequency or liquid influx rate independently over a range of values. The effect on bowel movement frequency and stool consistency are shown in Fig. 3b, c, respectively. Comparing the two treatments suggests that changes in HAPC frequency have a greater impact on BM frequency (Fig. 3b, top panel) than changes in secretion (Fig. 3c, top panel). The change in liquid fraction of the stool is comparable for both treatments (bottom panels of Fig. 3b, c).

Calibration of module mapping for a 5-HT_4 agonist and model prediction

The 5-HT signaling module was linked to the colon physiology module using empirical Hill functions that predict the HAPC frequency and liquid influx rate for a given mean muscarinic receptor occupancy (Fig. 4a). Mapping parameters (EC_{50} and Hill coefficient, n) were adjusted to capture changes in stool frequency and stool consistency in response to Compound X—an orally administered 5-HT_4 receptor agonist X [45, 46]. Observed pharmacokinetics (PK) were fit with a linear two-compartment model (see Supporting Information). The observed and predicted dose response 0–24 h after a single oral dose of Compound X are shown in Fig. 4c. Calibrated model predictions show a good match to the data from healthy volunteers treated with a single oral dose.

We next simulated treatment of STC with compound X. As shown in Fig. 4d, the model simulations predict that while a dose of 1–2 mg is sufficient to restore BM frequency, a higher dose is required to restore stool consistency to healthy levels.

Sensitivity analysis

To quantify the responsiveness of the model to changes in parameter values, we performed a single parameter sensitivity analysis for each module. For the colon physiology module, we varied each model parameter within a range of $\pm$ 50% of its nominal value for a healthy phenotype and measured the resulting changes in defecation frequency and consistency (Fig. 5a). These readouts were found to be the most sensitive to changes in HAPC frequency and, to a lesser extent, the transport rate (k_{trans}), liquid influx rate ($k_{in.w}$) and the liquid absorption rate (k_{abs}). Doubling HAPC frequency produces up to three defecations per day, with the liquid fraction approaching 85%, while doubling the liquid influx rate increased the defecation frequency to 2/day with a liquid fraction approaching 90% i.e. resembling a diarrhea-like phenotype. Changes in the solid mass influx, or mass loss had a minimal effect on defecation frequency and consistency. This is not surprising, as the solid mass influx is $< 10\%$ of the total influx. Sensitivity analysis for STC phenotype was also performed and shows similar trends (results not shown).

We assessed the sensitivity of the signaling module by increasing or decreasing each parameter by an order of magnitude from its nominal value and measured the effect on steady state muscarinic receptor occupancy (Fig. 5b).

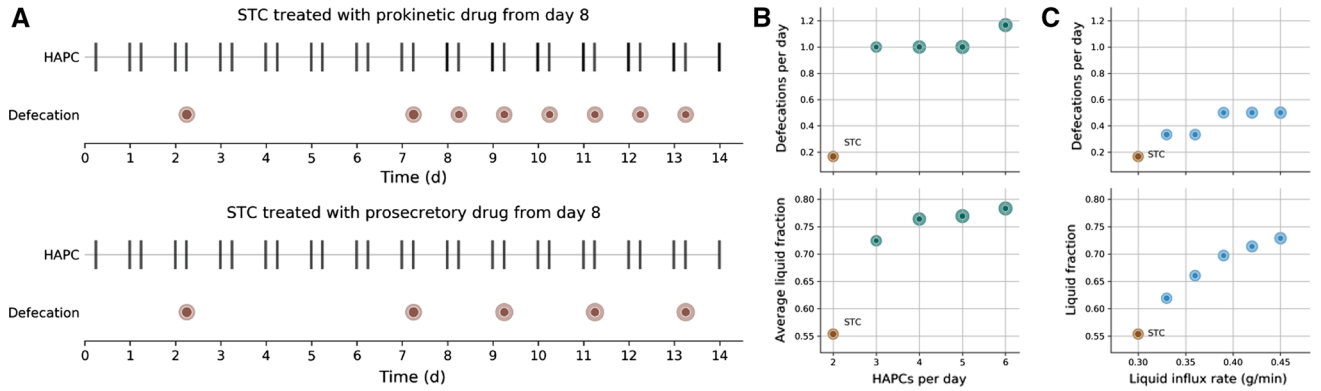

Fig. 3 Simulated treatments for STC using the colonic physiology module. **a** Simulated treatments with a prokinetic drug (top panel) and a prosecretory drug (bottom panel). The first 7 days show defecation events at pre-treatment baseline. Starting on day 8, either HAPC frequency changes from 2/day to 3/day (representing a prokinetic treatment) or the liquid influx rate increases by 10% over baseline (representing a prosecretory treatment). **b** Changes in defecation frequency and liquid fraction due to changes in HAPC frequency alone. **c** Changes in defecation frequency and liquid fraction due to changes in liquid influx rate alone

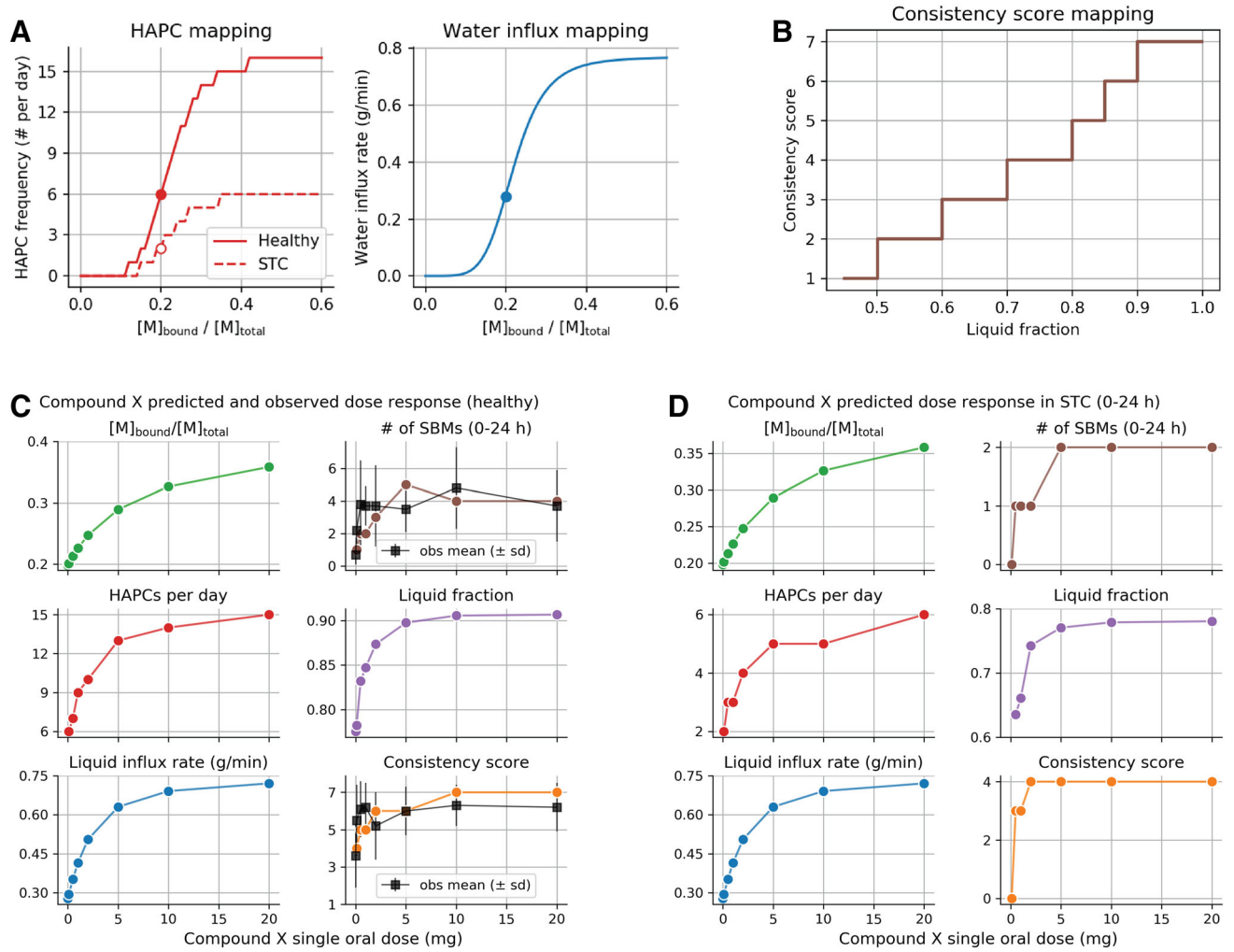

Fig. 4 Model calibration with Compound X dose–response data and prediction for STC. **a** Mapping from Muscarinic receptor occupancy to HAPC and liquid influx for Compound X. **b** Mapping from liquid fraction to consistency score. **c** Comparison of simulated (colored lines) and observed (black squares) dose response over 0–24 h following a single oral dose of Compound X given to healthy volunteers. Clinical end points shows are mean (± SD) number of BMs per day and stool consistency at each dose (black squares with error bars). **d** Predicted dose response to Compound X over 24 h after a single oral dose of Compound X given to STC patients (Color figure online)

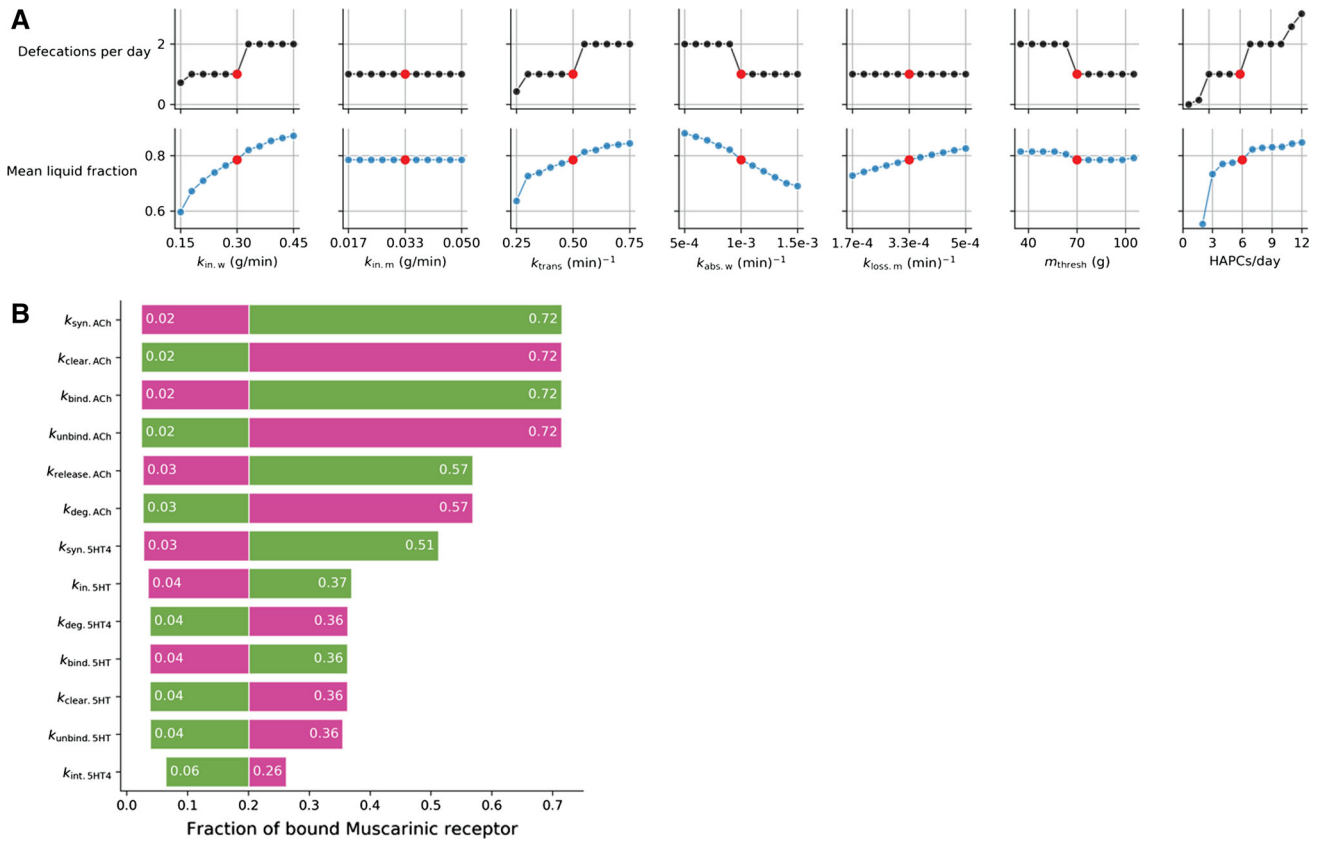

Fig. 5 Model Sensitivity analysis for healthy phenotype. **a** Colon physiology module sensitivity analysis. Each panel shows the effect of varying a single parameter (indicated on the x-axis) by ± 50% of its nominal value (indicated by the red circle) on daily defecation frequency (top row) and stool consistency (bottom row). **b** 5-HT4 signaling module sensitivity analysis. Each parameter was increased tenfold (green bars) or decreased tenfold (magenta bars) from its nominal value listed in Table 3. The change in fraction bound muscarinic receptor is plotted. Green bars represent a tenfold increase and magenta bars represent a tenfold decrease from the nominal parameter value. Numbers in the bar indicate predicted steady state fraction of bound muscarinic receptor for the new parameterization (Color figure online)

The mean muscarinic receptor occupancy is most strongly affected by parameters that control intracellular acetylcholine levels ($k_{syn.ACh}$ and $k_{deg.ACh}$), acetylcholine release and clearance ($k_{release.ACh}$ and $k_{deg.ACh}$) and muscarinic receptor binding($k_{bind.ACh}$ and $k_{unbind.ACh}$). Increasing acetylcholine synthesis, acetylcholine release or muscarinic receptor binding rates tenfold increased the muscarinic receptor occupancy by 3 to fourfold. Decreasing acetylcholine clearance or unbinding by tenfold also produced similar increases. Parameters that regulate 5-HT_4 signaling upstream of acetylcholine show a moderate range of sensitivities and result in $< 40\%$ relative change in muscarinic receptor occupancy.

Discussion

In this study, we put forth a mathematical model of colon physiology driven by serotonin signaling in the ENS. Model parameterization was informed by published preclinical and clinical data. Our simulations provide clinically relevant readouts of bowel movement frequency and stool consistency. The model recapitulates healthy and STC phenotypes, and the effect of Compound X, a 5-HT_4 agonist, in healthy volunteers. Using the calibrated model, we predicted the effects of treating STC patients with Compound X. We also performed a sensitivity analysis on the healthy phenotype and identified HAPC frequency as the most important modulator of BM frequency.

Our model includes a physiologically motivated defecation mechanism that is HAPC-driven and dependent on mass accumulation above a threshold in the last compartment. This was based on the observation that HAPCs often precede an urge to defecate [3] and that a sufficient distension of the terminal colon is required to initiate a defecation response [46]. We also implemented a consistency-dependent transport rate between compartments. Consequently, as chyme loses liquid content through absorption, its passage slows down. This approximates the process of stool compaction in constipation. Conversely, a

more liquid stool is transported and expelled faster, consistent with a diarrhea-like phenotype. Our simulations can reproduce each of these phenotypes. The liquid fraction of stool is empirically mapped to the Bristol stool scale for ease of clinical interpretation.

Sensitivity analysis of the colon physiology module suggests that a pro-kinetic treatment that solely increases HAPC frequency (Fig. 5a, far right panel) is more effective than a pro-secretory treatment that only increases the liquid influx rate (Fig. 5a, far left panel). Intuitively, this makes sense, as a prokinetic treatment directly affects mass transport, moving more mass per unit time into the terminal compartment, whereas a prosecretory treatment increases the overall mass in the system, but is limited by the rate of mass transport. In addition, the sensitivity analysis of the signaling module suggests that a muscarinic receptor agonist maybe more potent than a 5-HT_4 agonist for treatment of constipation. However, the model does not account for any increase in uncoordinated muscle contractions that do not move colonic mass.

The model focuses on control of GI motility by the ENS and does not factor in extrinsic control from the CNS. We use pre-set HAPC schedules and ignore individual-level variability in HAPC timings and frequency. The signaling module is a coarse-grained representation of a complex process involving numerous ganglia, spatially localized 5-HT secretion and coordinated neuromuscular events.

Despite these limitations, our approach creates a unified framework that captures the effect of ENS signaling on colonic motility, incorporates drug PK and produces clinically relevant endpoints. This platform is designed to enable quantitative assessment of existing drugs or experimental compounds. The model can be used to investigate optimal dosing schedules, to identify best approaches to treating constipation while avoiding diarrhea, and to enable differentiation from competitor compounds. The modular design enables model expansion to include additional signaling pathways that regulate colonic motility.

Several models of the electrophysiology of the upper GI tract and the coupling between electrophysiology and GI motility have been previously published [47–49]. These models integrate processes on different temporal and spatial scales [50]. While useful in recapitulating known biology, these studies do not directly address questions that arise during the drug development process. The structural complexity of the models can also make it challenging to simulate them and interpret the results of pharmacological modulation. In addition, these studies focus on the stomach and intestine rather than the colon. Therefore, the QSP model described here provides a unique, fit-for-purpose platform to simulate treatments of colonic dysmotility.

This large intestine motility platform with demonstrated value in slow transit constipation also has potential for applicability across many pathophysiological conditions affecting GI motility resulting in constipation or diarrhea. The current platform was originally developed to model constipation which is not considered a rare event [51]. However, this platform can be readily modified by alternative parameterization to widen its scope and utility. For example, MS can adversely affect the bowel function mainly through changes in the innervation of smooth muscles, which are controlled by ENS [52]. MS is a rare chronic inflammatory neurodegenerative disorder impacting as many as 1 to 2 million people worldwide [17, 19, 20]. The rates of constipation and fecal incontinence in MS patients are approximately 39 to 73% [53].

The QSP model presented here provides a unified framework integrating preclinical data of ENS signaling and clinically relevant endpoints to recapitulate, via simulation, physiological studies of bowel movements in healthy and constipated individuals. Simulated drug treatments of both approved drugs as well as a novel compound, compound X, are consistent with experimental observations. The model can be further extended to test novel therapeutics, based on a mechanistic understanding, for additional indications which exhibit bowel movement dysfunction.

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