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Development of a Heuristic Machine Analogy Method for Model Simplification With an Application to a Large-Scale Model of Gi/Gs Signaling

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ABSTRACT

Model simplification is a process to simplify large-scale mathematical models to enable easy applications such as simulation and parameter estimation. A novel heuristic machine analogy method of model simplification was developed and applied to a motivating example of a model for cAMP signaling switch induced by Gi/Gs pathway competition for the CB_1 receptor (consisting of 31 species and 76 parameters) to enable its use in estimation. The method first acquired an understanding of the mechanism by full model simulation, and then the mechanism was abstracted to a machine analogy. The machine analogy included signal start, signal mode selector, signal size regulator, and final effector, representing functions of different parts of the full model. The simplified minimal model (consisting of 11 species and 13 estimated parameters) was used for parameter estimation for Gi/Gs signaling of six CB_1 agonists. The results of the minimal model suggested that six CB_1 agonists have similar ratios of Gi/Gs activation, indicating Gi/Gs preference was more of a system effect rather than a ligand-specific effect. In conclusion, the novel machine analogy method can be used to heuristically simplify a larger-scale model while maintaining the important mechanisms. In the example here, the full Gi/Gs model of CB_1 was successfully simplified, and the results indicated Gi/Gs preference is a system-dependent effect.

1 | Introduction

Biological systems are highly complex; they involve large numbers of molecules and pathways, and mathematical models to describe them tend to be large-scale and cumbersome to use practically. It is therefore of benefit to consider applying a technique to simplify the model. Such techniques are widely used to reduce the dimensionality of a model. These techniques can be classified into parametric and nonparametric methods [1]. The utility of these techniques is dependent on the setting, and no one method provides a universal solution for all problems.

In this work, we propose a novel, heuristic method of model simplification based on machine analogy and apply it to an existing mathematical model of a receptor signaling system [2]. Signaling pathways of biological systems are akin to machines where each step (process in a pathway) has a relatively independent function (albeit with many interactions). The concept of machine analogy (or machine metaphor) has been used in philosophical approaches to biology [3–6]. Levy outlined the machine likeness of a system is based on an internal division of labor, and analogy is "as a means for structuring and organizing inquiry, especially the search for and interpretation of causal-explanatory

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Summary

- What is the current knowledge on the topic?
  - Model simplification is important for large-scale model use-reuse, allowing subsequent intensive computational processes such as simulation, optimization& estimation. G-protein-coupled receptors show diverse functionality, eliciting both inhibitory (Gi) and stimulatory (Gs) activity. A system model of Gi/Gs processes has been developed for CB_1.
- What question did this study address?
  - This study aimed to provide a solution to simplify large-scale models in a heuristic manner that preserves important mechanisms. Inference from using this methodology was understanding the influence of ligands on biased GPCR signaling.
- What does this study add to our knowledge?
  - This study described the development and application of a mechanistically meaningful model simplification process (a machine analogy method) and applied this to a large-scale pharmacology model of CB_1 Gi/Gs signaling. The estimation revealed the signaling bias is more likely to arise from the system rather than be ligand-specific.
- How might this change drug discovery, development, and/or therapeutics?
  - The machine analogy method would seem to be suited to complex and mechanism-based models with functional divisions, like QSP models of signaling, facilitating model-informed drug discovery and development.

models" [4]. In the signaling example we explore in this work, the receptors are signal receivers, downstream proteins are signal transducers, and further downstream factors are effectors or amplifiers [7–9] ultimately yielding some cellular response that can be measured.

A quantitative system pharmacology (QSP) model for the GPCR cannabinoid type 1 (CB_1) receptor was developed previously to understand the inhibitory and stimulatory effects of different ligands in terms of production of the second messenger cAMP [2]. The inhibitory and stimulatory effects are mediated by coupling the receptor with Gi and Gs proteins, respectively. The QSP model was able to describe the seemingly counterintuitive observed switch in pathways from inhibition to stimulation that occurs at either different levels of receptor expression [10] or during co-stimulation with D_2 agonists [11]. The relative signaling via the Gi/Gs pathways and how this is influenced by the experimental setting is of particular importance for assessing future agonist activity.

The aim of this work was to develop a novel generalized method for model simplification and illustrate this with an application to simplify the full Gi/Gs model to provide a setting for estimation of pathway preference for additional CB_1 agonists. The minimal model was used to model new experimental data to determine the influence of the ligand on Gi/Gs preference.

2 | Methods

MATLAB (2018b, Math Works Inc) was used for all simulations, estimations, and graphical applications.

2.1 | Application of the Machine Analogy to Develop and Extract a Simplified Model

A novel semi-parametric method was developed to simplify complex models. The approach is based on the concept of mapping the model to a machine; the features of the machine are retained, and a minimal model is extracted, then relevant parameters are determined.

The principle of the minimal model development is that the machine analogy forms the link in mechanism between full and minimal models and the minimal model can recreate relevant output data from input variables that closely approximate the full model (Figure 1).

The key to the method is to understand the mechanism of the system and divide the system into functional parts. This could be achieved by heuristics, or more formal methods based on simulations and sensitivity analyses. In our setting the information flow was determined according to the causal nature of the system, where the information flow was ligand and receptor → G protein → downstream molecules e.g., adenylyl cyclase → effectors, cAMP. Preservation of signal direction was ensured by simulation of kinetic curves and the areas under curve (AUCs) for important species (e.g., receptor-Gi(Gs) complex, activated Gi (Gs), Gi(Gs)-coupled adenyl cyclase), species of quasi-equilibrium were found by simulation of kinetic curves, and ligand concentration determined the signaling extents was simply found by the observation profiles (details in the full model paper [2]). The above functions were mapped to the machine as below.

2.2 | Development of Minimal Gi/Gs Model by Machine Analogy Method

The proposed machine method was applied to a mathematical model of CB_1 regarding Gi/Gs signaling [2].

The full Gi/Gs model consisted of four steps that control the cAMP signaling process (Figure 2): ligand binding, receptor coupling to Gi/Gs, G protein activation, and cAMP regulation. These functional units were common to both settings of high CB_1 expression and CB_1-D_2 co-stimulation [2] (Figure 2). The mechanisms and functions of the four parts of the full model were abstracted to a machine analogy (Figure 3).

2.2.1 | Step 1: Ligand Binding → Machine Analogy: Start

Ligand binding is the start of the signaling process. Ligand binding with receptors induces G protein activation. Ligand concentration determines the occupancy proportion of receptors; therefore, it affects the extent of signaling with distinct efficacy parameters for different ligands (details in Step 3).

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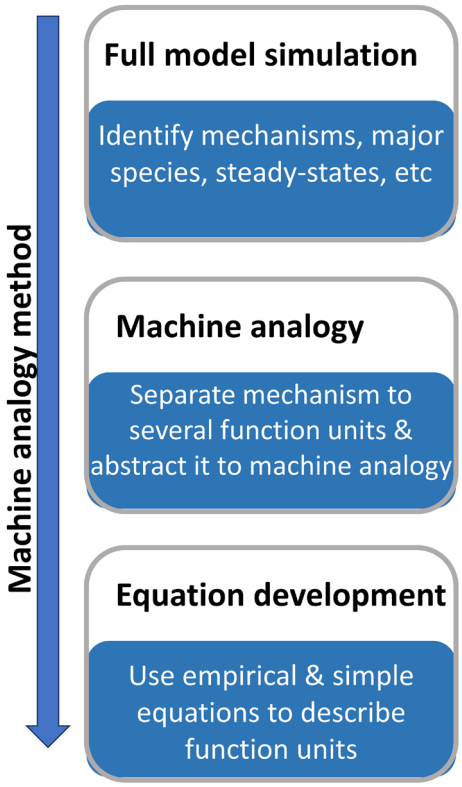

FIGURE 1 | The schematic diagram of machine analogy method.

2.2.2 | Step 2: The Competitive Coupling of Gi/Gs to Receptor → Machine Analogy: Signal Mode Selector

The full model simulation illustrated that CB_1 preferentially couples with Gi. High expression of CB_1 or D_2 coupling with Gi will deplete the finite amount of Gi, thereby increasing the amount of CB_1-Gs coupling. The ratio of Gi/Gs coupling with CB_1 determines the direction of the signaling.

Therefore, Gi/Gs competitive coupling with receptors in the full model is the signal mode selector of the machine. In this step, the relative amounts of Gi, Gs, and receptors determine the ratio of Gi/Gs coupling. In Figure 3, the color spectrum represents the variable ratio of Gi/Gs; the yellow color and the purple color on the two sides indicate pure Gi or Gs effect, respectively. The final effect (color) is the mixture of Gi and Gs effects. This ratio of Gi/Gs coupling determines whether the signal direction is biased toward the Gi or Gs pathway.

2.2.3 | Step 3: G Protein Activation → Machine Analogy: Signal Size Regulator

The coupled G proteins are activated to form guanosine triphosphate (GTP)-bound Gi(s) proteins by agonist-activated receptors or constitutively active receptors. The constitutive activity of CB_1 is weaker than its agonist-induced activity, as the receptor is

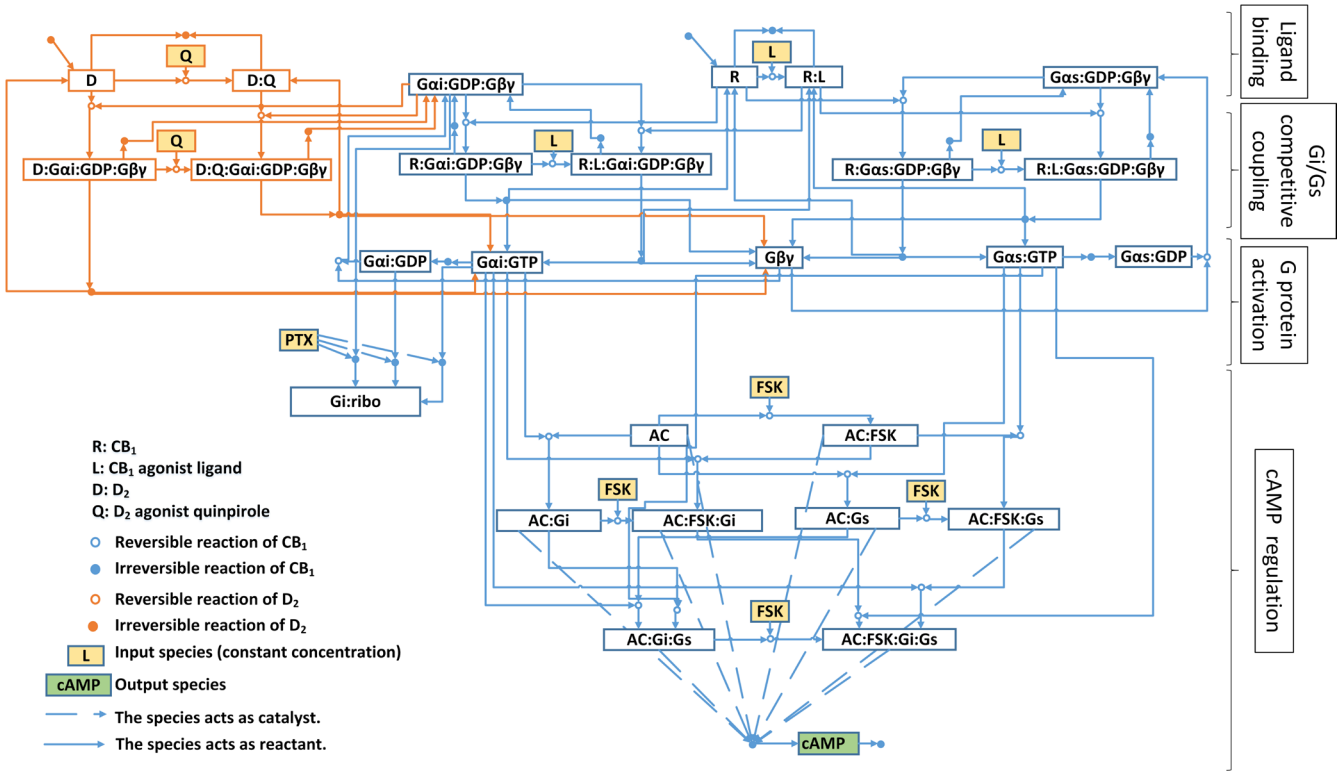

FIGURE 2 | The full model structure (revised from the paper of the full model [2]). The four mechanistic elements of the full model are noted: Ligand binding, Gi/Gs competitive coupling, G protein activation, and cAMP regulation. In the figure, the boxes are species, where the yellow boxes represent input species, and the green box is the output species cAMP. The circles linked by arrows represent reactions, where the open circles are reversible reactions, and the solid circles are irreversible reactions. The species linked by solid arrows join the reactions as reactants, and the species linked by dashed arrows join the reactions as catalysts (increasing the reaction rate). The blue part of the model is the CB_1 relevant pathway, and the orange part is the D_2 pathway. Note that D_2 shares the Gi cycle with the CB_1. The format and notations of this figure referred to the format of diagram in SimBiology (version: 5.11 (R2022b), MathWorks Inc). This figure is generated by Microsoft Visio (Microsoft Corporation, 2018).

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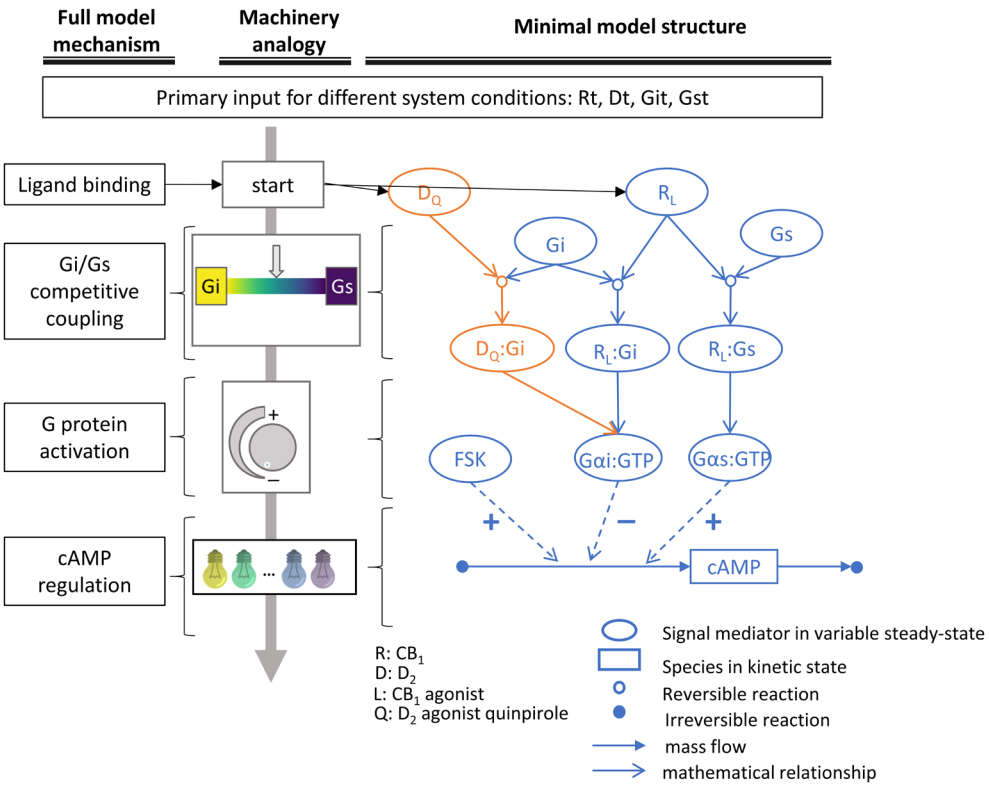

FIGURE 3 | The machine analogy and structure of the minimal model. Left and middle panel: The functions of the four parts of the full model were linked to four steps in a machine analogy. The yellow color in Step (2) and Step (4) represents pure Gi effects, and the purple color represents pure Gs effects. The intermediate color represents a mixture of Gi and Gs effects. Right panel: The model structure is divided into the four parts in the order of signaling. In the model structure, the orange part is the D_2 induced Gi pathway; the blue part is the CB_1 induced Gi/Gs pathways. Ovals represent signal mediators that connect with open arrows that indicate the direction of causality (i.e., a mathematical relationship). The cAMP response (square box) is kinetic, while all other nodes in ovals are signal mediators (mathematical variables) in steady-state.

predominantly in its inactive conformation in the absence of an agonist. Therefore, compared to constitutively active receptors, the size of the Gi/Gs signaling is primarily determined by the number of agonist-activated receptors, i.e., by agonist concentration. Different types of ligands would have different efficacy parameters.

The G protein activation step in the full model is the signal size regulator of the machine. The Gi/Gs activation ratio remains unchanged in this step; hence, *Step* (3) regulates the size of the signal but does not change the direction of the signal.

2.2.4 | Step 4: cAMP Regulation → Machine Analogy: Final Effector

GTP-bound Gi(s) proteins bind to adenylyl cyclase (AC) and regulate cAMP production.

The cAMP regulation through AC is the final effector of the machine. The catalytic efficiency of AC is determined by the amounts of both GTP-bound Gi and Gs proteins generated in *Step* (3). The coupling ratio of Gi/Gs in *Step* (2) determines the signal direction, which is represented as the color of the lightbulb, and this color is the same as *Step* (2). The size of the signal is determined in *Step* (3) and is represented as the brightness of the lightbulb (Figure 3).

Before the signaling steps of the four parts, there are system settings for different conditions due to different cell types, different expression of CB_1, and pre-treatment of pertussis toxin (PTX; Table S1) according to the experimental conditions applied.

The main aim of the model simplification is to have an estimation model, allowing evaluation of the Gi/Gs preference of multiple agonists. The model should therefore be simplified enough to allow practical estimation, maintain the main mechanisms related to Gi/Gs preference, and predict the kinetics of cAMP generation. In this regard, the simplified model maintained the interactions between Gi/Gs and ligands and receptors, cAMP regulators (Gi:GTP/Gs:GTP), and the kinetics of cAMP, but removed all other species and other kinetics (Figure 3).

2.3 | Estimation and Evaluation of Minimal Gi/Gs Model

This section aimed to evaluate the simplified model performance by comparing the predictions of the full and minimal models for the same dataset. The minimal model should be able to reproduce the model predictions of the full model.

As the minimal model is a recreation of the machine analogy based on the full model, the minimal model maintained the

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same mechanism as the full model, although not the same structure. The meanings and parameters of the minimal model therefore are largely different from those of the full model. Hence a mapping of parameter values (e.g., as if they were lumped) does not exist. The minimal model parameters were therefore obtained from estimation from the same dataset of cAMP that was used to calibrate the full model. The estimated parameters were applied to the minimal model to simulate predictions, and these predictions were aligned with full model prediction to evaluate the consistency between the two models.

The experimental data applied in this section were the same as the cAMP data applied in full model [2]. The data were observed cAMP concentrations under different scenarios: (1) the CB_1 agonist CP55940 (CP) induced cAMP response under different levels of CB_1 with or without PTX in CB_1-HEK cells [10]. PTX was used to block the Gi pathway; (2) the cAMP response under CB_1/D_2 co-stimulation in striatal neurons [11], where the data were extracted from the paper using WebPlotDigitizer-4.4 [12].

For data arising from the CB_1-HEK cell system, a two-stage method of model estimation was applied. First, the global search method (particle swarm, MATLAB 2018b) was used to determine the rough ranges of parameter initial values. Then a local search using the mixed-effects method (nlmefit, MATLAB 2018b) was applied to estimate the parameters and the between-experiment variations. For data arising from the striatal neuron cell system, the parameters were calibrated for the minimal model or sourced from the literature due to parameter unidentifiability caused by a lack of data (a detailed introduction is shown in S3: Data S1).

2.4 | Application of Minimal Gi/Gs Model to New Ligands to Explore Gi/Gs Preferences

The experimental data applied in this section were published in the format of an AUC analysis [10]. The kinetic data were available from the authors (two replicates were shown Figures S3 and S4). The data consisted of cAMP assays using six CB_1 agonists: CP55940 (CP), anandamide (AEA), Δ9-tetrahydrocannabinol (THC), 2-arachidonoylglycerol (2-AG), WIN55212-2(WIN), BAY593074 (BAY). The assays were performed under different levels of CB_1 with or without PTX in CB_1-HEK cells.

The model quantified the Gi/Gs preferences of ligands by the parameters S_{GiaRL} / S_{GsaRL}, which refer (respectively) to the Gi or Gs activation scales of each CB_1-ligand complex.

3 | Results

3.1 | Development of Minimal Gi/Gs Model by Machine Analogy Method

According to the machine analogy and the mechanism of the full model, the minimal model structure was developed (Figure 3).

The oval-connector plot of the minimal model (Figure 3, right panel) is a type of directed acyclic graph (DAG). Ovals represent signal mediators that connect with open arrows that indicate the direction of causality (i.e., a mathematical relationship). The species (ovals) in Figure 3 are mathematical variables. While they may retain molecular names, their values no longer represent the amounts of real molecules (except cAMP). Here cAMP is shown in a box and filled end-arrow schematic to imply that it remains as a real molecule in mass balance. cAMP production and elimination were depicted by a kinetic reaction. All other species were designed to be variables at steady-state (represented by ovals in Figure 3), which do not have kinetic properties.

The model structure was briefly described below in four parts (detailed equations in S1: Data S1). Before the four signaling steps, there are system settings for different experimental conditions (Table S1): (1) CB_1-HEK cells [10], normal or high CB_1 expression, with or without PTX (no D_2 exists); (2) Striatal neurons [11], both CB_1 and D_2 exist.

3.1.1 | Step 1: Ligand Binding → Machine Analogy: Start

In Figure 3, R_L denotes the mixture of free CB_1 and the CB_1-ligand complex, where the two species keep a constant ratio of $\frac{\text{dissociation constant}}{\text{ligand concentration}}$ (Equation S1: Data S1). The ratio was constant because ligand binding is a relatively fast reaction and assumed to be quasi-equilibrium [1]. Similarly, D_Q denotes the mixture of free D_2 and the D_2-quinpirole complex, the two also maintain a constant ratio (Equation S2: Data S1).

3.1.2 | Step 2: The Competitive Coupling of Gi/Gs to Receptor → Machine Analogy: Signal Mode Selector

The step of Gi/Gs coupling involves the two receptors (if D_2 exists in the cell system) with both Gi and Gs proteins. The competitive relationship was formed due to the limited amounts of the receptors and the G proteins, as well as the coupling of receptor–G proteins. The equations of mass conservation (i.e., the sums of all free or coupled receptors or G proteins equal to the overall amounts of receptors or G proteins) and steady-state of receptor–G protein coupling were derived, and the two together described such competitive relationship. These equations could predict different Gi/Gs coupling ratios under different CB_1 expression levels or D_2 co-stimulation. The equations of this step were derived separately for the CB_1-HEK system (Equation S3: Data S1) and striatal neuron systems (Equation S4: Data S1).

3.1.3 | Step 3: G Protein Activation → Machine Analogy: Signal Size Regulator

The activation of Gi(s)-receptor complex ($[R_L:Gi(s)]$) is induced by two parts: the constitutive activity ($S_{Gi(s)aR}$) of the free receptor and the ligand-induced receptor activity ($S_{Gi(s)aRL}$; Equations S5 and S6: Data S1). In striatal neurons with both CB_1 and D_2, the amount of $G\alpha i:GTP$ is generated from both $R_L:Gi$ and $D_Q:Gi$ (Equation S7: Data S1). The $G\alpha s:GTP$ equation in striatal neurons is the same as that in the CB_1-HEK cell.

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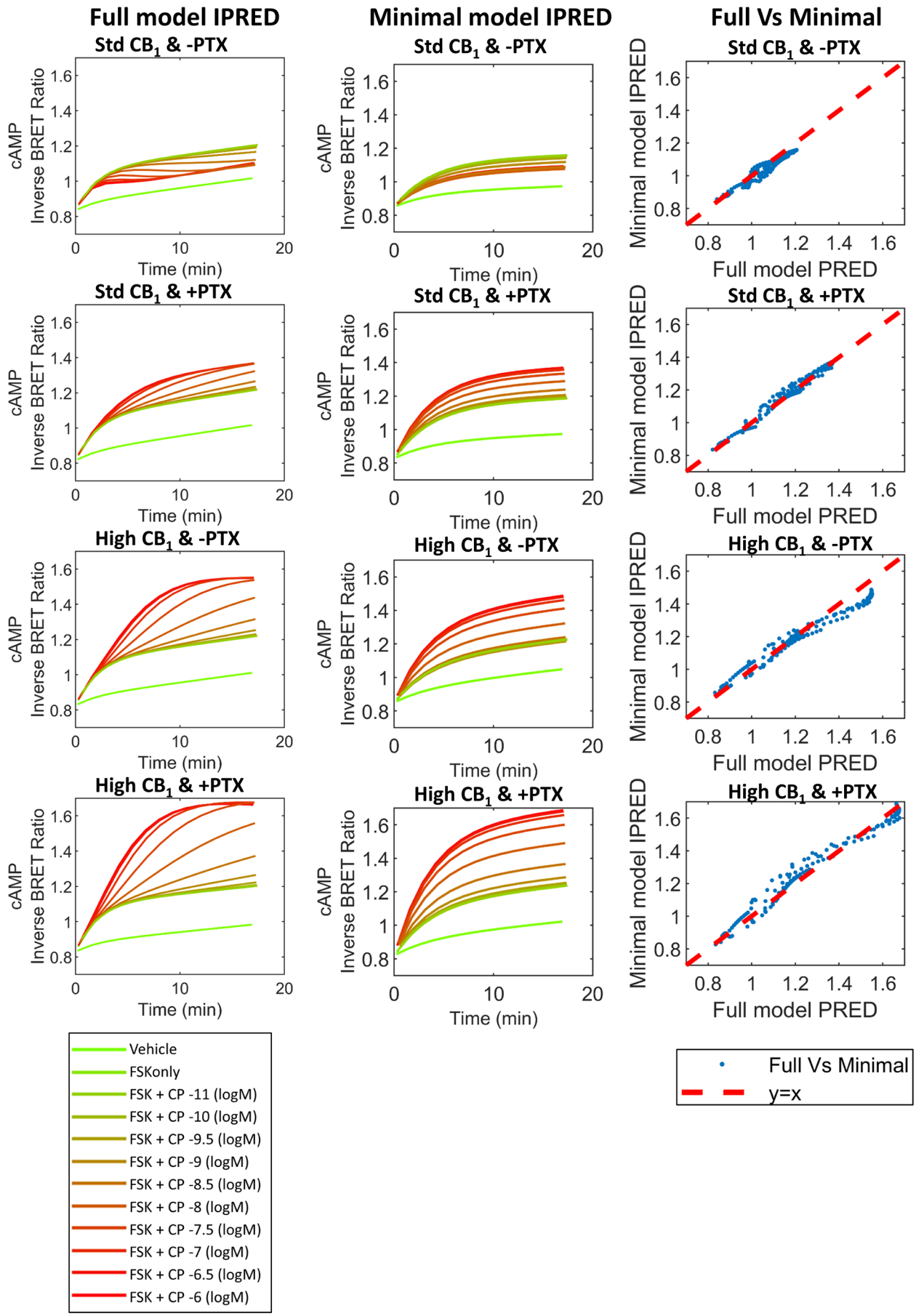

FIGURE 4 | Legend on next page.

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FIGURE 4 | The comparison of predictions for the CB_1-HEK system between the minimal model and the full model. The first column is the prediction of the full model for the cAMP assays in the CB_1-HEK system under four conditions: Standard CB_1 expression level without PTX, standard CB_1 expression level with PTX, high CB_1 expression level without PTX, high CB_1 expression level with PTX. The ligand concentrations are shown in the legend below. The second column is the individual prediction of the minimal model for the four cAMP assays. The third column shows the figures of the full model prediction (PRED) versus the minimal model IPRED for the four cAMP assays. The dashed red lines are lines of identity, i.e., $y = x$ line. Note, as the full model does not have between-experiment variation, the IPRED of the full model is the typical prediction of the full model.

3.1.4 | Step 4: cAMP Regulation → Machine Analogy: Final Effector

The cAMP production is regulated by both Gαi: GTP and Gαs: GTP. The full model simulation showed the kinetics of Gαi: GTP and Gαi: GTP-bound ACs remain in relatively stable plateaus over 20-min experiments [2]. Therefore, kinetics of AC is unimportant and can be replaced by a quasi-steady-state. This assumption of quasi-steady-state is based on the full model simulation of the key regulator (AC) for the final response (cAMP). As described in the full model, AC has two distinct sites for Gαi: GTP and Gαs: GTP and therefore AC can simultaneously couple with both. For simplicity, we assume that Gαi: GTP and Gαs: GTP coupling with AC are independent. The overall AC catalytic efficiency was described by the sum of the efficiency of each Gi- or Gs- coupled AC complexes (Equation S8: Data S1). FSK stimulation effect was described by a linear coefficient as there was only one FSK concentration in the data, which therefore cannot support the estimation of Emax for FSK. Equation S8: Data S1 was further simplified by removing parameter redundancies and applying parameter constraints (Equations S9 and S10: Data S1).

3.2 | Estimation and Evaluation of Minimal Gi/Gs Model

The cAMP data of CB_1-HEK were fitted by the minimal model. The CB_1-HEK system parameters and ligand-specific parameters of CP are shown in Table S2. The predictions of the full model and the minimal model show the same trend and extent of cAMP responses (Figure 4): CP inhibits the cAMP response in the standard CB_1 expression condition, but stimulates the cAMP response in the high CB_1 expression condition or following PTX treatment. The dots of the prediction of the full model versus the minimal model gathered around the red $y = x$ line, showing the two models produce similar predictions (Figure 4, the third column).

However, there are differences between the full model and the minimal model. The prediction lines of the full model show a more curved pattern than the minimal model (Figure 4). The trends of the observations are closer to the minimal model than to the full model (Figure S1A). This may be because the full model was only heuristically calibrated and not estimated, which leads to the inaccuracy of the full model parameters.

The cAMP data of the striatal neuron were calibrated by the minimal model (the overlay plots of prediction and observation are shown in Figure S1C). The striatal neuron system parameters and ligand-specific parameters are shown in Table S3. System parameter Git of neuron system were calibrated to two folds of the parameter in CB_1-HEK system. Other than this parameter, all other system parameters of striatal neuron kept the same values as for CB_1-HEK. The minimal model and the full model both correctly captured the cAMP signaling switch for the striatal neuron system: cAMP was inhibited when CB_1 or D_2 agonists perform alone (Figure S2A), but cAMP was stimulated when CB_1 and D_2 agonists perform together (Figure S2B). The minimal model predictions were quantitatively similar with the full model.

The cAMP levels of 1 μM HU in the CB_1-D_2 co-stimulation were underpredicted by the full model compared with the minimal model. This difference is possibly due to inaccurate parameter values in the full model. The minimal model also showed a better fit at this point than the full model (Figure S1C).

3.3 | Application of Minimal Gi/Gs Model to New Ligands to Explore Gi/Gs Preferences

The minimal model provided a good fit for the cAMP assays of the six CB_1 agonists in the CB_1-HEK cell system (Figure 5). The system parameters are shown in Table S4, and the ligand-specific parameters are shown in Table 1. The model was able to capture the trends of the cAMP assays under different CB_1 expression conditions, with or without PTX. For the condition of standard CB_1 expression without PTX, the model prediction showed that cAMP signaling is inhibited by all six agonists. For CB_1 high expression with or without PTX, the model prediction showed cAMP stimulation by all six agonists. The varying extents of cAMP inhibition and stimulation between the six agonists were also well described by the model. For example, the model predicted larger cAMP inhibition and stimulation effects for CP and smaller effects for BAY (Figure 5).

The ratio of parameters S_{GiaRL} and S_{GsaRL} can represent the relative preference of Gi and Gs by ligands. A linear relationship between S_{GiaRL} and S_{GsaRL} was seen across the 6 ligands (Figure 6). The slope was 1.06 (p value = 0.00432, 95% CI [0.56, 1.6]), which means S_{GiaRL} is significantly linearly related with S_{GsaRL}. The intercept is 0.128 (p value = 0.418, 95% CI is [−0.27, 0.52]), which is not significantly different with 0. The result of the linear regression shows all the six agonists' ratios of S_{GiaRL} and S_{GsaRL} are similar, i.e., the Gi/Gs preferences of the six ligands are similar.

This result suggests that the Gi/Gs preference is likely to be a function of the biological system rather than the ligand.

4 | Discussion

A novel model simplification approach, a machine analogy method, was developed in this study. The method was based

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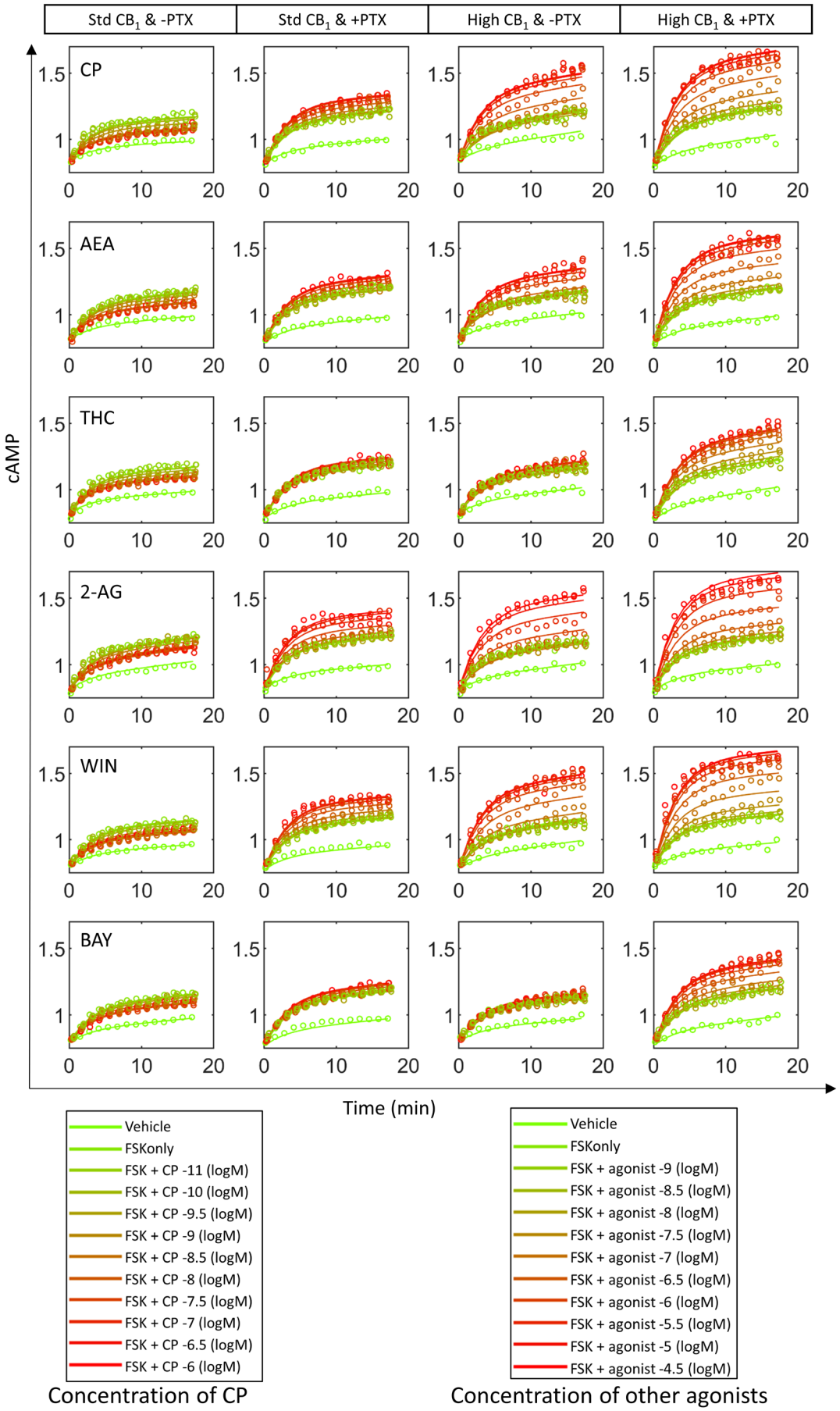

FIGURE 5 | Legend on next page.

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FIGURE 5 | The fitting results of cAMP assays of the six CB_1 agonists in CB_1-HEK cell system. The model fitting curves (lines) were overlayed with the observations (open circles) in the cAMP assays of the six CB_1 agonists (CP, AEA, THC, 2-AG, WIN, BAY) under four conditions: Standard CB_1 expression level without PTX, standard CB_1 expression level with PTX, high CB_1 expression level without PTX, high CB_1 expression level with PTX. The concentrations of CP are shown in left legend; the concentrations of the other agonists are shown in right legend. From green to red, agonist concentration increases.

TABLE 1 | The ligand-specific parameters in CB_1-HEK system.

Ligand-specific parameter	*pKd* (−logM)	S_{GiaRL}	S_{GsaRL}
Parameter meaning	Dissociation constant of CB_1 agonist in negative logarithm	Gi activation scale of CB_1-ligand complex	Gs activation scale of CB_1-ligand complex
CP	8.1	1.0 FIX	1.0 FIX
AEA	6.4	0.65	0.89
THC	7.1	0.40	0.53
2-AG	5.9	1.0	1.3
WIN	6.8	0.96	1.2
BAY	6.6	0.36	0.50

Note: Some units of parameters are unknown because of the unknown units of receptor, G protein, cAMP, etc., or because the parameters are dimensionless.

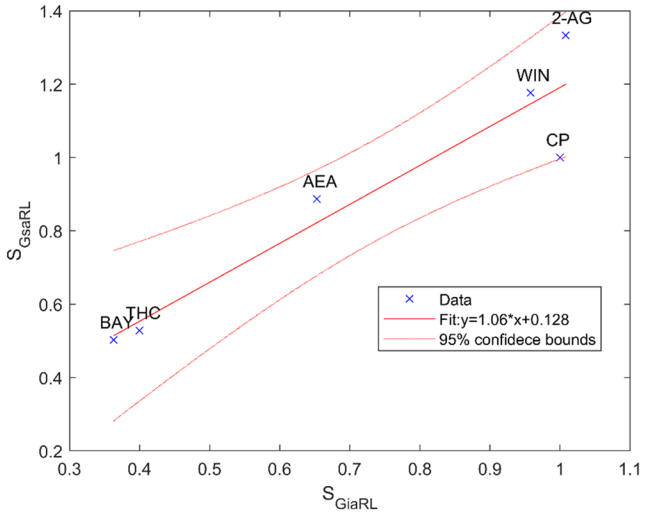

FIGURE 6 | The linear regression plot for the S_{GiaRL} and S_{GsaRL}. The blue crosses are the data points of pairs of S_{GiaRL} and S_{GsaRL} for the six ligands, where the agonists' names are labeled. The solid red line is the fit line, and the dotted red lines are the 95% confidence bounds.

on an understanding of the full model mechanism for the Gi/Gs model, which was then abstracted to a machine analogy. The key to this method is to capture the main mechanisms and to describe these mechanisms using the simplest possible machine flow and equations. The machine analogy focuses on imitating the functions of each part of the system in the target responses. Specifically, in this example, cAMP is the final response, and all the functions related to cAMP were imitated by the machine analogy, but the other functions, such as the complete Gi cycle, were ignored. This machine analogy method is suitable for complex models with relatively clear divisions of functional parts. There are two parts to the machine analogy method: (1) breaking a complicated model into functional parts and (2) simplifying each of these functional parts using any formal model reduction method or simpler (more empirical) equations. If there were a multi-scale model, then the machine analogy may be applied to each different scale, and then each functional element simplified for each scale. For example, while signaling models usually suit this method well, a metabolic model of the tricarboxylic acid (TCA) cycle is difficult to break into functional parts because several different steps can generate the final target, ATP. As such, all reactions are connected as a cycle. The signaling model is more linear and causal compared to the TCA metabolic model. We provided another example of a QSP model of CD3 bispecific antibody [13] to illustrate the application (S6: Data S1).

The study developed a minimal model for the full Gi/Gs model of CB_1, which was identifiable and supported parameter estimation. The minimal model was applied to quantify CB_1 agonist preference for Gi/Gs. The minimal model yielded additional insights into the influence of different ligands on the system and suggested that since the six CB_1 agonists had similar Gi/Gs preferences that an agonist can change the magnitude of the signaling but not change the *quality* (direction) of the signaling. Hence, the signaling preference would appear more likely to be a system-dependent rather than a ligand-dependent effect. In the GPCR field, system-dependent differences have frequently been misidentified as ligand-dependent (e.g., see [14–17]), which has resulted in studies erroneously reporting instances of agonist bias. The results of this study suggest that such conclusions should be viewed with additional skepticism.

In the CB_1-HEK cell system without the presence of D_2, the minimal model described the relationship of coupling of Gi, Gs, and CB_1 by a series of algebraic equations (Equation S3: Data S1) whose solution is the root of a cubic equation. In the more complicated striatal neuron system with the presence of both CB_1 and

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D_2, the minimal model described the relationship of coupling of four components (Gi, Gs, CB_1, and D_2; Equation S4: Data S1) whose solution is the root of a quintic equation which was solved numerically. The striatal neuron system became more complex due to the additional D_2 dimension. It could be expected that the whole biological system where all the receptors are working together or being ready for work is much more complex than this two-receptor and two-pathway system. The biological system is also expected to be highly non-linear, and simple linear analyses (like up−/down- regulation of a single molecule) cannot expect to meet the requirements of hypothesis development, and hence abstraction from isolated experiments may be misleading. This model could be generalized to competition in other GPCR pathways. For example, the competitive coupling of Gi/Gs could be changed to the competitive coupling of arrestin/Gi. More generally, more components could be written into the equations to form ternary, quaternary competition systems, etc.; however, this will increase the calculation difficulty.

In conclusion, the machine analogy method for developing a minimal model was utilized. We expect this method can generalize to other settings. In this particular case, the minimal Gi/Gs model showed the Gi/Gs preferences are similar for six CB_1 agonists, which together support the suggestion that Gi/Gs preferences may be a system rather than ligand-dependent (with ensuing suggestions about whether agonist bias is relevant for CB_1, particularly between the Gi/Gs pathways).

Author Contributions

All authors wrote the manuscript. D.F., M.G., and S.D. designed the research. L.Y. performed the research. D.F. analyzed the data.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

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