Modeling the Effect of Cooperativity on Ligand-Driven Fluctuations of Metabotropic Glutamate Receptors

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Modeling the Effect of Cooperativity on Ligand-Driven Fluctuations of Metabotropic Glutamate Receptors

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Applied Science from The College of William and Mary

by

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Modeling the Effect of Cooperativity on Ligand-Driven Fluctuations of Metabotropic Glutamate Receptors

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Abstract

The metabotropic glutamate receptors (mGluRs) are a family of dimeric G-protein coupled receptors (GPCR) that play a significant role in the modulation of synaptic transmission and excitability in neuronal cells [10]. Upon formation of mGluR dimers, protomer binding sites interact cooperatively with ligands, leading to receptor activation [5,9,10]. The dimer typically activates with full receptor occupancy, so the noise output of the system is assumed to scale in proportion to the number of activated dimers [2]. While the characterization of noise is well appreciated, the connection between cooperativity and noise has received less attention [1,2]. We examine the effects of cooperativity on concentration fluctuations of active mGluR in synaptic complexes. The fluctuating dynamics are well-approximated by the solution to the chemical langevin equation; we derive chemical langevin equations for the fluctuating dynamics of glutamate binding to mGluRs, and use the associated stochastic system to determine the magnitude of fluctuations for different parameter values (e.g. dissociation constants and concentration). Cooperative binding increases sensitivity of the system to small changes in concentration of glutamate and can decrease active mGluR fluctuations. This raises the question of whether or not cooperativity effects on concentration fluctuations are important for modulating synaptic activity.
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0.1 Introduction

Characterization of cell signaling dynamics at small timescales is crucial to our understanding of mechanisms for synaptic transmission. Synaptic transmission consists of a series of stochastic diffusive and reactive processes, each occurring in synaptic complexes with some probability [12]. The probabilistic release of neurotransmitters underlies concentration fluctuations in the synaptic cleft. A key function of sensory systems is to reliably distinguish weak signals from random fluctuations at each step of synaptic transmission [3,12]. Reliable detection and processing of signal molecule concentrations in the Central Nervous System (CNS) depends on several evolutionary strategies, including binding cooperativity and modular architecture of receptors [12].

Glutamate, the main excitatory neurotransmitter in the CNS, targets two types of receptors in the postsynapse: ionotropic glutamate receptors (AMPA, NMDA, and Kainate receptors), and metabotropic glutamate receptors (mGluR) [9,10]. The mGluRs are a family of G-protein coupled receptors that mediate slow glutamate responses and neuronal excitability in the CNS [10]. Eight mGluR subtypes have been identified, and
are subclassified into three distinct subfamilies based on sequence homology, G-protein coupling, and ligand selectivity [10]. The widespread expression of these receptors in the CNS suggest numerous functions, and represent ideal targets for therapeutic intervention in CNS disorders [5,10].

mGluRs contain a large N-terminal binding domain, the Venus Flytrap Domain (VFD), which contains the ligand binding site [9,10]. Structural evidence suggests that VFDs dimerize to form a homodimer, and exist in three conformations depending on receptor occupancy: open-open, open-closed, and closed-closed [10]. The two VFDs exhibit allosteric interactions, allowing for binding cooperativity. Binding cooperativity refers to the extent in which binding of the first ligand facilitates the binding of the second, or alternatively, the affinity of the second ligand for the binding site is greater than the first [5]. Activation of cell surface receptors is thought to occur with full receptor occupancy [2].

For large numbers of molecules, molecular processes can be characterized by deterministic models (Michaelis-Menten Kinetics) [12]. Cooperativity affects equilibrium kinetics of ligand-receptor binding. Due to detailed balance, one can readily derive functions generating equilibrium binding curves from mass action ODES and the definition of the equilibrium dissociation constant $K_d$ [5]. Smaller $K_d$'s denote stronger ligand binding.

**Figure 2**: Schematic of VFD conformations. The open-open state is the inactive state (left). Either one (center) or two (right) VFDs can bind glutamate, resulting in active conformations. Reproduced without permission from Niswender et al [10].
to mGluR’s. Non-cooperative ligand binding yields curves fitted by functions where the $K_d$ is equivalent for the first and second binding steps. By contrast, cooperativity yields curves fitted by functions where the $K_d$ for the first binding step is greater than the $K_d$ for the second binding step, that is, cooperativity affects the relative association and dissociation rates in each binding step.

Accurate analysis of the dynamics of synaptic transmission, and in particular, receptor activation, necessitates the use of stochastic approaches [12]. Transitions between mGluR conformations depend on the fluctuating dynamics of ligands diffusing in and out of the synaptic cleft i.e. mGluR fluctuations are driven by ligand diffusion. Because downstream signals are typically initiated by ligand-induced receptor activation, we are interested in examining the concentration fluctuations of activated mGluR’s. Binding cooperativity may help amplify signal detection and decrease concentration fluctuations due to intrinsic noise, since recent experiments have shown that cell-surface receptors can exist as clusters [16].

The well-characterized structures of mGluR conformations, along with recent experimental evidence suggesting the suppression of receptor fluctuations in the presence of cooperative binding motivated us to examine the effect of cooperativity on mGluR fluctuations. The time-dependent dynamics of fluctuations can be described by two stochastic approaches [4,10]. One approach involves solving the chemical Master equation, which describes the time evolution of the probability distribution for molecules at any given time. Numerical and analytical solutions to the chemical Master equation tend to be computationally demanding, as the state space of a system is potentially huge [4,10,15]. Alternatively, the chemical Master equation can be solved by the Linear Noise Approximation (LNA) using van Kampen’s system size expansion of inverse powers. The LNA is an elegant method for probing the effects of molecular noise on small-scale chemical reaction pathways [15]. The method provides estimates of mean and variance fluctuations for ensembles of molecules, which allow for precise system descriptions [15].

In section two, we first present a deterministic three state mGluR model with instantaneous activation. For readability, we simulate representative binding curves and scatchard plots. We then derive the corresponding stochastic model, employ the LNA,
and estimate the ensemble variances of glutamate and active mGluR as derived from the fluctuation-dissipation theorem. This calculation illustrates our approach, and provides a reference point for interpretation of results for models with closing kinetics. We then perform parameter studies to determine cooperativity effects on concentration fluctuations in the active receptor. In section three, we analyze concentration fluctuations for the four-state mGluR model with closing kinetics. In section five, we analyze concentration fluctuations in the five-state model with multiple active states and closing kinetics. We conclude with a discussion of our findings.

0.2 Three-state mGluR Model

0.2.1 Deterministic ODE Formulation

Working within the framework of the Koshland-Nemethy-Filmer model, which assumes instantaneous receptor activation upon full ligand occupancy, we derived the kinetic scheme below. L molecules are allowed to diffuse in and out of the synaptic cleft [7,16].

The empty set denotes a source and sink outside of the domain and has no effect on any reaction rates. The subscript number denotes the degree of occupancy of the mGluR dimer where $R_0$ denotes the unbound state (open-open), $R_1$ denotes the singly-bound state (closed-open), and $R_2$ denotes the doubly-bound active state (closed-closed). $k_{i}^{+}$ is a transition rate with units of $\mu M s^{-1}$, $k_{i}^{-}$, $k_{a}^{-}$, and $k_{b}^{-}$ are dissociation rate constants with units of $s^{-1}$, and $k_{a}^{+}$ and $k_{b}^{+}$ are association rate constants with units of $\mu M^{-1} s^{-1}$. $K_A$ and $K_B$ are equilibrium dissociation constants defined as the ratio of the dissociation...
The rate constant to the association rate constant. The deterministic ODEs that describes the time evolution of species concentration in the domain are

\[
\begin{align*}
\dot{L} &= k_i^+ - k_i^- L + k_a^- R_1 - 2k_a^+ LR_0 - k_b^+ LR_1 + 2k_b^- R_2 \\
\dot{R}_0 &= k_a^- R_1 - 2k_a^+ LR_0 \\
\dot{R}_1 &= 2k_a^+ LR_0 - k_a^- R_1 + 2k_b^- R_2 - k_b^+ LR_1 \\
\dot{R}_2 &= k_b^+ LR_1 - 2k_b^- R_2
\end{align*}
\]

Assuming no receptor trafficking, the total receptor concentration is conserved [8].

\[ R_T = R_0 + R_1 + R_2 \]

Thus, we have the following system of ODEs:

\[
\begin{align*}
\dot{L} &= k_i^+ - k_i^- L + k_a^- R_1 - 2k_a^+ L(R_T - R_1 - R_2) - k_b^+ LR_1 + 2k_b^- R_2 \equiv Q_0 \\
\dot{R}_1 &= 2k_a^+ L(R_T - R_1 - R_2) - k_a^- R_1 + 2k_b^- R_2 - k_b^+ LR_1 \equiv Q_1 \\
\dot{R}_2 &= k_b^+ LR_1 - 2k_b^- R_2 \equiv Q_2
\end{align*}
\]

At equilibrium, detailed balance yields the following steady-state relationships:

\[
\begin{align*}
L_{ss} &= \frac{k_i^+}{k_i^-} \\
R_{0ss} &= \frac{K_A K_B}{K_A K_B + 2K_B L_{ss} + L_{ss}^2} \\
R_{1ss} &= \frac{2K_B L_{ss}}{K_A K_B + 2K_B L_{ss} + L_{ss}^2} \\
R_{2ss} &= \frac{L_{ss}^2}{K_A K_B + 2K_B L_{ss} + L_{ss}^2}
\end{align*}
\]

The steady-state relationships derived from mass action ODEs generate equilibrium binding curves. Cooperativity yields steeper curves, indicating that ligand binding is more sensitive to small changes in glutamate concentration. At equilibrium, cooperativity also decreases the fraction of receptor occupying the singly-bound state, due to increased affinity of the ligand for the second binding site. The deterministic formulation assumes negligible concentration fluctuations in the activated mGluR. The following
section presents a stochastic formulation of the same system.

![Figure 3](image)

**Figure 3:** Cooperativity is reflected in the degree of sigmoidicity in binding curves. Cooperativity yields steeper curves, indicating receptor sensitivity to small changes in glutamate concentration. A: Binding in the absence of cooperativity. B: Binding the presence of cooperativity.

### 0.2.2 Stochastic ODE Formulation

At physiological domains, fluctuations are not negligible, but are large enough to be modeled continuously with the chemical Langevin equation, a stochastic differential equation containing a stochastic variable $\xi_L(t)$ corresponding to a fluctuating force [18]. By adding the appropriate stochastic variable to the deterministic ODEs, we obtain the Langevin equations for the time evolution of each species.

\[
\dot{L} = Q_0 + \xi_L(t) \quad (8)
\]
\[
\dot{R}_1 = Q_1 + \xi_{R_1}(t) \quad (9)
\]
\[
\dot{R}_2 = Q_2 + \xi_{R_2}(t) \quad (10)
\]
where the column vector of fluctuating forces \( \xi = (\xi_L(t), \xi_{R_1}(t), \xi_{R_2}(t))^T \), has mean zero \( \langle \xi_i(t) \rangle = 0 \) for \( i \in \{L, R_1, R_2\} \) [6,18]. The fluctuations are correlated, so the two-time covariances are most easily expressed in matrix form

\[
\langle \xi(t)\xi^T(t') \rangle = \Gamma(L, R_1, R_2) \delta(t - t'),
\]

where \( \delta \) is the dirac-delta function [18].

The state-dependent covariance matrix \( \Gamma \) is

\[
\begin{pmatrix}
\gamma_l + \gamma_a + \gamma_b & \gamma_b - \gamma_a & -\gamma_b \\
\gamma_b - \gamma_a & \gamma_a + \gamma_b & -\gamma_b \\
-\gamma_b & -\gamma_b & \gamma_b \\
\end{pmatrix}
\]

(12)

where

\[
\gamma_l(L) = \frac{k^+_l + k^-_l L}{\Omega}
\]

\[
\gamma_a(L, R_1) = \frac{2k^+_a L (R_T - R_1 - R_2) + k^-_a R_1}{\Omega}
\]

\[
\gamma_b(L, R_1, R_2) = \frac{k^+_b LR_1 + 2k^-_b R_2}{\Omega}
\]

\( \Omega \) is the system size in volume, and is given in units of \( L \). The linear noise approximation to Eq. (12) is as follows in matrix form.

\[
\begin{bmatrix}
\delta L \\
\delta R_1 \\
\delta R_2
\end{bmatrix} = H_{ss}
\begin{bmatrix}
\delta L \\
\delta R_1 \\
\delta R_2
\end{bmatrix} + \begin{bmatrix}
\xi_L(t) \\
\xi_{R_1}(t) \\
\xi_{R_2}(t)
\end{bmatrix},
\]

(13)

where

\[
\delta L = L - L_{ss}
\]

\[
\delta R_1 = R_1 - R_{1ss}
\]

\[
\delta R_2 = R_2 - R_{2ss}
\]
denotes concentration fluctuations around steady-state, $H_{ss}$ is the Jacobian of the full system of SDEs evaluated at steady-state,

$$H_{ss} = \begin{pmatrix}
-k_l^- - 2k_a^+(R_T - R_1^{ss} - R_2^{ss}) - k_b^- R_{1ss} & k_a^- + (2k_a^- + k_b^-)L_{ss} & 2k_b^- + 2k_a^- L_{ss} \\
2k_a^+(R_T - R_1^{ss} - R_2^{ss}) - k_b^+ R_{1ss} & -k_a^- - (2k_a^+ + k_b^+)L_{ss} & 2k_b^- - 2k_a^+ L_{ss} \\
k_b^+ R_{1ss} & k_b^- L_{ss} & -2k_b^-
\end{pmatrix}$$

$$\langle \xi_{ss}(t) \rangle = 0 \quad \text{and} \quad \langle \xi_{ss}(t)\xi_{ss}^T(t') \rangle = \Gamma_{ss}\delta(t-t')$$ is the steady-state covariance matrix

$$\Gamma_{ss} = \begin{pmatrix}
gamma_{lss} + \gamma_a + \gamma_b & \gamma_a - \gamma_{lss} & -\gamma_{bss} \\
\gamma_a - \gamma_{bss} & \gamma_a + \gamma_b - \gamma_{bss} & -\gamma_{bss} \\
-\gamma_{bss} & -\gamma_{bss} & \gamma_{bss}
\end{pmatrix},$$

where

$$\gamma_{lss}(L_{ss}) = \frac{k_l^+ + k_l^- L_{ss}}{\Omega}$$

$$\gamma_{a ss}(L_{ss}, R_{1ss}) = \frac{2k_a^+ L_{ss}(R_T - R_1^{ss} - R_2^{ss}) + k_a^- R_{1ss}}{\Omega}$$

$$\gamma_{b ss}(L_{ss}, R_{1ss}, R_{2ss}) = \frac{k_b^+ L_{ss} R_{1ss} + 2k_b^- R_{2ss}}{\Omega}.$$
0.2.3 Analysis of Concentration Fluctuations

First, we define a symmetric, positive definite, 3 x 3 covariance matrix for the fluctuating concentrations. $\Sigma(t) = (\sigma_{ij})$ for $i, j \in \{L, R_1, R_2\}$. The matrix is

$$
\Sigma(t) = \langle \delta(t)\delta^T(t) \rangle = 
\begin{pmatrix}
\langle \delta L^2 \rangle & \langle \delta L\delta R_1 \rangle & \langle \delta L\delta R_2 \rangle \\
* & \langle \delta R_1^2 \rangle & \langle \delta R_1\delta R_2 \rangle \\
* & * & \langle \delta R_2^2 \rangle
\end{pmatrix},
$$

where each * denotes a redundant entry. The time-dependent dynamics of $\Sigma(t)$ are given by Eq.22.

$$
\dot{\Sigma} = H_{ss}\Sigma + \Sigma H_{ss}^T + \Gamma_{ss}
$$

(17)

In the special case that $\Sigma(0) = 0$, the matrices $H$ and $\Gamma$ are time independent, and $L(0) = L_{ss}$, $R_1(0) = R_{1ss}$, and $R_2(0) = R_{2ss}$ i.e. $\delta L = \delta R_1 = \delta R_2 = 0$ [6]. The solution of Eq.17 with initial condition $\Sigma(0) = 0$ is,

$$
\Sigma(t) = \int_0^t \exp(H_{ss}t)\Gamma_{ss}(H_{ss}^Tt)dt
$$

(18)

The steady-state ensemble variance is the expression,

$$
\Sigma_{ss} = \int_0^\infty \exp(H_{ss}t)\Gamma_{ss}(H_{ss}^Tt)dt,
$$

(19)

but is more easily found by solving the continuous Lyapunov equation for the steady-state of Eq.17 i.e.

$$
H_{ss}\Sigma_{ss} + \Sigma_{ss}H_{ss}^T = -\Gamma_{ss}
$$

(20)

[6].

Eq. 20 can be solved numerically using the `lyap` function in Matlab. The relative magnitude of active receptor fluctuations around steady state can be characterized by
the coefficient of variation

\[
CV(R_2) = \frac{\sqrt{\langle \delta R_{2ss}^2 \rangle}}{R_{2ss}},
\]

where the steady-state concentration of receptors in the active formation \( R_{2ss} \) is determined by equilibrium dissociation constants \( K_A \) and \( K_B \) through Eq. 7 [18]. \( \langle \delta R_{2ss}^2 \rangle \) is the relevant element of \( \Sigma_{ss} \) found by numerical solution of Eq. 20.

### 0.2.4 Results

Figure 4 shows parameter studies that characterize the dependence of the relative magnitude of \([R_2]\) fluctuations on cooperativity parameters. The results were obtained by numerical integration of the continuous Lyapunov equation for a range of equilibrium dissociation constants \( K_A \). To simulate cooperativity effects, we fixed the quantity \( K_A K_B = K_\ast^2 \), where \( K_\ast \) is the geometric mean of equilibrium dissociation constants. This relation implies \( K_B = \frac{K_\ast^2}{K_A} \), that is, cooperativity depends on the relative values of equilibrium dissociation constants: a large value of \( K_A \) corresponds to a small value in \( K_B \). All studies were simulated with parameter values from Marcaggi et al [9].

The relative magnitude of \([R_2]\) fluctuations (CV) is a bell-shaped function of the equilibrium dissociation constant \( K_A \), where large values of \( K_A \) indicate the presence of cooperativity. For small or large values of \( K_A \), the CV asymptotically approaches the value 0. This value denotes the minimum \([R_2]\) fluctuation size i.e. fluctuations are negligible in extreme cases of cooperativity and anti-cooperativity. Intermediate values of \( K_A \), corresponding to mildly anti-cooperative or cooperative binding, amplify the relative magnitude of \([R_2]\) fluctuations. For clarity, we show the dependence of the ensemble variance (\( \langle \delta R_{2ss}^2 \rangle \)) as a function of \( K_A \).

Numerically calculated time series of species concentrations using Gillespie’s Stochastic Simulation Algorithm (Direct Method) illustrate noise reduction effects in the presence of cooperativity. These results indicate that cooperativity suppresses ligand-driven \([R_2]\) fluctuations in the synaptic cleft.
Figure 4: Dependence of the relative magnitude of $[R_2]$ fluctuations probed by plotting the coefficient of variation on cooperativity parameters. A: The solid red line indicates the numerically calculated ensemble variance ($\langle \delta R_{2ss}^2 \rangle$) for different values of $K_A$. B: The solid red line indicates the CV for different values of $K_A$ (Eq. 21). Blue lines indicate absence of cooperativity ($K_A = K_B = K_* = 30.4 \, \mu M$). When $K_A > 30.4 \, \mu M$, binding is cooperative. Parameters: $k_{a+}^o, k_{b+}^o = 4.5 \, \mu M^{-1} \, s^{-1}, K_* = 30.4 \, \mu M, k_{l+}^o = 10^5 \, \mu M s^{-1}, k_{l-} = 1000 \, s^{-1}, L_{ss} = 100 \, \mu M, R_T = 54.6 \, \mu M$ [9,13,17]. The volume of the synapse corresponds to $\Omega = 7.6 \times 10^{-19} \, L$. 
Figure 5: Influence of cooperativity on the time evolution of \([R_2]\) fluctuations. (Left) Monte Carlo simulation of \([R_0]\), \([R_1]\), and \([R_2]\) in the absence of cooperativity. Parameters: See Fig. 4 and \(R_{2ss} = 32 \mu M\). (Right) Monte Carlo simulation of \([R_0]\), \([R_1]\), and \([R_2]\) when binding is cooperative \((10 \times K_A, 10 \times K_B, K_A K_B = K_2^2)\). Cooperativity suppresses ligand-driven \([R_2]\) fluctuations and decreases steady-state \([R_1]\). Parameters: \(k_+^a = 4.5 \, \mu M^{-1}s^{-1}, k_+^b = 45 \, \mu M^{-1}s^{-1}, k_-^a = 1370 \, s^{-1}, k_-^b = 137 \, \mu M^{-1}s^{-1}, k_l^a = 10^5 \, \mu Ms^{-1}, k_l^- = 1000 \, s^{-1}, L_{ss} = 100 \, \mu M, \) and \(R_{2ss} = 47 \, \mu M\). The volume of the synaptic cleft corresponds to \(\Omega = 7.6 \times 10^{-19} L\).
0.2.5 Relationship Between \([R_2]\) and \([L]\) Fluctuations Under Rapid Equilibrium Limit

To obtain more insight into the relative magnitude of \([R_2]\) fluctuations under the rapid-equilibrium limit of ligand-receptor binding (evaluated in the limit \(k_+ \to \infty, k_- \to \infty\) for fixed \(K_*=\frac{k_-}{k_+}\)), we employed a first-order approximation to the concentration for the active form of the receptor. Analytic expressions of the CV derived under the assumption of rapid equilibrium limit, agree with numerical evaluations under the more general solution of the Lyapunov equation. Under rapid equilibrium, the concentration for the active form of the receptor is:

\[
R_{2ss} \approx \frac{R_T L_{ss}^2}{K_A K_B + 2K_B L_{ss} + L_{ss}^2}.
\]  
(22)

Linearization around the stable steady-state \((L_{ss}, R_{2ss})\) can be achieved through a Taylor series expansion,

\[
f(L_{ss} + \delta L, R_{2ss} + \delta R_2) \approx f(L_{ss}, R_{2ss}) + \frac{\partial f(R_{2ss})}{\partial R_2} \cdot \delta R_2 + \frac{\partial f(L_{ss})}{\partial L} \cdot \delta L,
\]
(23)

where we have dropped quadratic and subsequent higher-order terms. We have employed a more convenient method which involves perturbing the solution around \((L_{ss}, R_{2ss})\), dropping second-order terms and cancelling steady-state values,

\[
\delta R_2 + R_{2ss} = \frac{(\delta L + L_{ss})^2 R_T}{K_A K_B + 2K_B (\delta L + L_{ss}) + (\delta L + L_{ss})^2}
\]

\[
\delta R_2 + R_{2ss} = \frac{(\delta L^2 + 2\delta LL_{ss} + L_{ss}^2) R_T}{K_A K_B + 2K_B \delta L + 2K_B L_{ss} + \delta L^2 + 2\delta LL_{ss} + L_{ss}^2}
\]

\[
\delta R_2 = \alpha \cdot \delta L,
\]
(24)

where

\[
\alpha = \frac{2[R_T L_{ss} - (K_B + L_{ss}) R_{2ss}]}{K_A K_B + 2K_B L_{ss} + L_{ss}^2}.
\]
(25)

Using the above mentioned approach, we find that the ensemble variance is given by:

\[
\langle \delta R_{2ss}^2 \rangle = \alpha^2 \cdot \langle \delta L_{ss}^2 \rangle
\]
(26)
Numerical evaluation of this analytic solution gives the ensemble variance of the active mGluR at the rapid equilibrium limit.

Figure 6: Dependence of the relative magnitude of $[R_2]$ fluctuations as numerically evaluated under the rapid equilibrium limit of ligand-receptor binding. The dotted blue line indicates the CV for different values of $K_A$ (Eq. 26). The solid blue line indicates the case where binding is non-cooperative ($K_A = K_B = K_* = 30.4 \mu M$). When $K_A > 30.4 \mu M$, binding is cooperative. Parameters: See Fig. 4. Note that $\alpha$ is a dimensionless parameter derived under the rapid equilibrium limit of ligand-receptor binding. $\alpha = 10^{-7}$.

### 0.3 Four-State mGluR Model with Indirect Activation

In the previous section, the topology of the minimal three-state mGluR model assumes instantaneous activation upon ligand binding. In contrast, structural evidence suggests receptor activation is produced by rearrangements of the Venus Flytrap Domain (VFD) [9]. For more insight into the role of VFD rearrangements in the cooperativity-induced suppression of active receptor fluctuations, we expand our kinetic scheme to include a total of four receptor states:
The four-state mGluR model includes transitions between $R_2$ and $A_2$ to account for the kinetics of ligand-induced closing of the two VFDs, where $A_2$ denotes the doubly bound active state with both VFDs closed. As before, $R_0$ denotes the unbound state and $R_1$ denotes the singly bound state; $R_2$ now denotes the doubly-bound resting state.

In addition to the rate constants defined in the previous section, $k_c^+$ is an activation rate constant with units of $s^{-1}$, $k_c^-$ is a deactivation rate constant with units of $s^{-1}$, and $K_C = \frac{k_c^+}{k_c^-}$ is an equilibrium constant. The deterministic ODEs that describe the time evolution of species concentrations are

$$
\dot{L} = k_i^+ - k_i^- L + k_a^- R_1 - 2k_a^+ L(R_T - R_1 - R_2 - A_2) - k_b^- LR_1 + 2k_b^- R_2 \quad (27)
$$

$$
\dot{R}_1 = 2k_a^+ L(R_T - R_1 - R_2 - A_2) - k_a^- R_1 + 2k_b^- R_2 - k_b^- LR_1 \quad (28)
$$

$$
\dot{R}_2 = k_c^+ LR_1 - 2k_b^- R_2 + k_c^- A_2 - k_c^- R_2 \quad (29)
$$

$$
\dot{A}_2 = k_c^- R_2 - k_c^- A_2. \quad (30)
$$

At equilibrium, the steady-state relationships are

$$
L_{ss} = \frac{k_i^+}{k_i} \quad (31)
$$

$$
\frac{R_{0ss}}{R_T} = \frac{K_A K_B K_C}{K_A K_B K_C + 2K_B K_C L_{ss} + K_C L_{ss}^2 + L_{ss}^2} \quad (32)
$$

$$
\frac{R_{1ss}}{R_T} = \frac{L_{ss}}{K_A K_B K_C + 2K_B K_C L_{ss} + K_C L_{ss}^2 + L_{ss}^2} \quad (33)
$$

$$
\frac{R_{2ss}}{R_T} = \frac{K_C L_{ss}^2}{K_A K_B K_C + 2K_B K_C L_{ss} + K_C L_{ss}^2 + L_{ss}^2} \quad (34)
$$

$$
\frac{A_{2ss}}{R_T} = \frac{L_{ss}^2}{K_A K_B K_C + 2K_B K_C L_{ss} + K_C L_{ss}^2 + L_{ss}^2}. \quad (34)
$$
At steady-state, the column vector of fluctuating forces $\xi = (\xi_L(t), \xi_{R1}(t), \xi_{R2}(t), \xi_{A2}(t))^T$, has mean zero $<\xi_{ss}(t)> = 0$ for $i \in \{L, R1, R2, A2\}$. The fluctuations are correlated, so the two-time covariances are most easily expressed in matrix form: $<\xi_{ss}(t)\xi_{ss}(t')>$ = $\Gamma_{ss}\delta(t - t')$. The state-dependent covariance matrix evaluated at steady-state is given by

$$
\Gamma_{ss} = \begin{pmatrix}
\gamma_{ss}^L + \gamma_{ss}^R + \gamma_{ss}^A & \gamma_{ss}^B - \gamma_{ss}^A & -\gamma_{ss}^B & 0 \\
\gamma_{ss}^B - \gamma_{ss}^A & \gamma_{ss}^A + \gamma_{ss}^B & -\gamma_{ss}^B & 0 \\
-\gamma_{ss}^B & -\gamma_{ss}^B & \gamma_{ss}^A + \gamma_{ss}^C & -\gamma_{ss}^C \\
0 & 0 & -\gamma_{ss}^C & \gamma_{ss}^C
\end{pmatrix},
$$

(35)

where

$$
\gamma_{ss}^C(R_{2ss}, A_{2ss}) = \frac{k_c^+ R_{2ss} + k_c^- A_{2ss}}{\Omega}
$$
The linear noise approximation is as follows in matrix form

\[
\begin{bmatrix}
\delta L \\
\delta R_1 \\
\delta R_2 \\
\delta A_2
\end{bmatrix}
= H_{ss}
\begin{bmatrix}
\delta L \\
\delta R_1 \\
\delta R_2 \\
\delta A_2
\end{bmatrix}
+ \begin{bmatrix}
\xi_L(t) \\
\xi_{R_1}(t) \\
\xi_{R_2}(t) \\
\xi_{A_2}(t)
\end{bmatrix},
\] (36)

where

\[\delta A_2 = A_2 - A_{2ss}\]

denotes concentration fluctuations of the fully activated receptor around steady state, and \(H_{ss}\) is the Jacobian of the full system of SDEs evaluated at steady-state. The relative magnitude of active receptor fluctuations around steady state can be characterized by the coefficient of variation,

\[CV(A_2) = \frac{\sqrt{\langle \delta A_{2ss}^2 \rangle}}{A_{2ss}},\] (37)

where the steady-state concentration of receptors in the active formation \(A_{2ss}\) is given by Eq.35. \(\langle \delta A_{2ss}^2 \rangle\) is the relevant element of \(\Sigma(t) = (\sigma_{ij})\) for \(i,j \in \{L, R_1, R_2, A_2\}\):

\[
\Sigma(t) = (\delta(t)\delta^T(t)) = 
\begin{pmatrix}
\langle\delta L^2\rangle & \langle\delta L\delta R_1\rangle & \langle\delta L\delta R_2\rangle & \langle\delta L\delta A_2\rangle \\
* & \langle\delta R_1^2\rangle & \langle\delta R_1\delta R_2\rangle & \langle\delta R_1\delta A_2\rangle \\
* & * & \langle\delta R_2^2\rangle & \langle\delta R_2\delta A_2\rangle \\
* & * & * & \langle\delta A_2^2\rangle
\end{pmatrix},
\]
solved at steady-state.

Figure 8 shows parameter studies that characterize the dependence of the relative magnitude of \([A_2]\) fluctuations on cooperativity parameters. The relative magnitude of \([A_2]\) fluctuations (CV) is a logistic function of the equilibrium dissociation constant \(K_A\), where large values of \(K_A\) indicate the presence of cooperativity. For small values of
Figure 8: Dependence of the relative magnitude of $[A_2]$ fluctuations probed by plotting the coefficient of variation on cooperativity parameters. A: Numerically calculated ensemble variance ($\langle \delta A_{rs}^2 \rangle$) for different values of $K_A$. B: Numerically calculated CV for different values of $K_A$ (Eq. 37). Blue lines indicate absence of cooperativity ($K_A = K_B = K_* = 30.4 \mu M$). When $K_A > 30.4 \mu M$, binding is cooperative. Parameters: See Fig. 4, $k_c^c = 50$ s$^{-1}$ and $k_c^c = 60$ s$^{-1}$.

$K_A$, the CV asymptotically approaches the minimum $[A_2]$ fluctuation size 0. Intermediate values of $K_A$, corresponding to mildly anti-cooperative or cooperative binding, amplify the relative magnitude of $[A_2]$ fluctuations. Large values of $K_A$, corresponding to extremely cooperative binding, are independent from the relative magnitude of $[A_2]$ fluctuations.
0.4 Five-State mGluR Model with Partial and Full Activation

Activation of mGluR can be partial when 1 VFD is closed or full when 2 VFDs are closed [9]. Following our notation, the five-state mGluR model allows for transitions between $R_1$ and $A_1$, where $A_1$ denotes the singly-bound active state with one VFD closed. $k^+_d$ is an activation rate constant with units of $s^{-1}$, $k^-_d$ is a deactivation rate constant with units of $s^{-1}$, and $K_D = \frac{k^+_d}{k^-_d}$ is an equilibrium constant. The deterministic ODEs that describe the time evolution of species concentrations are

\begin{align*}
\dot{L} &= k^+_i - k^-_i L + k^-_a R_1 - 2k^+_a L (R_T - R_1 - R_2 - A_2) - k^+_b LR_1 + 2k^-_b R_2 \\
\dot{R}_1 &= 2k^+_a L (R_T - R_1 - R_2 - A_2) - k^+_a R_1 - 2k^-_b R_2 - k^+_b LR_1 - k^+_d R_1 + k^-_d A_1 \\
\dot{R}_2 &= k^+_b LR_1 - 2k^-_b R_2 + k^-_c A_2 - k^+_c R_2 \\
\dot{A}_2 &= k^+_c R_2 - k^-_c A_2 \\
\dot{A}_1 &= k^+_d R_1 - k^-_d A_1.
\end{align*}
At equilibrium, the steady-state relationships are

\[
L_{ss} = \frac{k_l^+}{k_l} \tag{43}
\]

\[
R_{0ss} = \frac{K_A K_B K_C K_D L_{ss}^2 + K_C K_D L_{ss}^2 + 2 K_B K_C K_D L_{ss}}{2 K_B K_C K_D L_{ss}} \tag{44}
\]

\[
R_{1ss} = \frac{K_A K_B K_C K_D + 2 K_B K_C L_{ss} + K_C K_D L_{ss}^2 + K_D L_{ss}^2 + 2 K_B K_C K_D L_{ss}}{K_C K_D L_{ss}} \tag{45}
\]

\[
R_{2ss} = \frac{K_A K_B K_C K_D + 2 K_B K_C L_{ss} + K_C K_D L_{ss}^2 + K_D L_{ss}^2 + 2 K_B K_C K_D L_{ss}}{2 K_B K_C L_{ss}} \tag{46}
\]

\[
A_{2ss} = \frac{K_A K_B K_C K_D + 2 K_B K_C L_{ss} + K_C K_D L_{ss}^2 + K_D L_{ss}^2 + 2 K_B K_C K_D L_{ss}}{K_D L_{ss}^2} \tag{47}
\]

\[
A_{1ss} = \frac{K_A K_B K_C K_D + 2 K_B K_C L_{ss} + K_C K_D L_{ss}^2 + K_D L_{ss}^2 + 2 K_B K_C K_D L_{ss}}{2 K_B K_C L_{ss}} \tag{48}
\]

Following the previous section, \( \xi = (\xi_L(t), \xi_{R_1}(t), \xi_{R_2}(t), \xi_{A_2}(t), \xi_{A_1}(t))^T \), has mean zero \( \langle \xi_{ss}(t) \rangle = 0 \) for \( i \in \{L, R_1, R_2, A_2, A_1\} \). The two-time covariance matrix is: \( \langle \xi_{ss}(t) \xi_{ss}^T(t') \rangle = \Gamma_{ss} \delta(t - t') \). The state-dependent covariance matrix evaluated at steady-state is given

**Figure 9:** A: Equilibrium binding in the absence of cooperativity. B: Equilibrium binding in the presence of cooperativity. Cooperativity yields steeper curves and lowers the occupation of receptors in \([R_1]\). Transitions from \(R_2\) to \(A_2\) and \(R_1\) to \(A_1\) are ligand-independent.
by

\[
\Gamma_{ss} = \begin{pmatrix}
\gamma_{a}^{ss} + \gamma_{b}^{ss} + \gamma_{c}^{ss} & \gamma_{b}^{ss} - \gamma_{a}^{ss} & -\gamma_{b}^{ss} & 0 & 0 \\
\gamma_{b}^{ss} - \gamma_{a}^{ss} & \gamma_{a}^{ss} + \gamma_{b}^{ss} + \gamma_{c}^{ss} & -\gamma_{b}^{ss} & 0 & -\gamma_{d}^{ss} \\
-\gamma_{b}^{ss} & -\gamma_{b}^{ss} & \gamma_{b}^{ss} + \gamma_{c}^{ss} & -\gamma_{c}^{ss} & 0 \\
0 & 0 & -\gamma_{c}^{ss} & \gamma_{c}^{ss} & 0 \\
0 & -\gamma_{d}^{ss} & 0 & 0 & \gamma_{d}^{ss}
\end{pmatrix},
\]  

(49)

where

\[
\gamma_{d}^{ss}(R_{2ss}, A_{2ss}) = \frac{k_{1}^{+} R_{2ss} + k_{1}^{-} A_{2ss}}{\Omega}
\]

The linear noise approximation is as follows in matrix form

\[
\begin{bmatrix}
\dot{\delta L} \\
\delta \dot{R}_1 \\
\delta \dot{R}_2 \\
\delta \dot{A}_2 \\
\delta \dot{A}_1
\end{bmatrix} = H_{ss}
\begin{bmatrix}
\delta L \\
\delta R_1 \\
\delta R_2 \\
\delta A_2 \\
\delta A_1
\end{bmatrix}
+ \begin{bmatrix}
\xi_{L}(t) \\
\xi_{R_1}(t) \\
\xi_{R_2}(t) \\
\xi_{A_2}(t) \\
\xi_{A_1}(t)
\end{bmatrix},
\]  

(50)

where

\[
\delta A_1 = A_1 - A_{1ss}
\]

denotes concentration fluctuations of the partially activated receptor around steady state, and \(H_{ss}\) is the Jacobian of the full system of SDEs evaluated at steady-state. The relative magnitude of mGluR fluctuations around steady state can be characterized by the coefficient of variation, given by

\[
j.
\]  

(51)
where the ensemble variance of the summed deviations in partial and fully activated receptors is:

\[
\langle (\delta A_{1ss} + \delta A_{2ss})^2 \rangle > = \langle \delta A_{1ss}^2 \rangle + \langle \delta A_{2ss}^2 \rangle + 2\langle \delta A_{1ss}\delta A_{2ss} \rangle.
\]

The steady-state concentration of receptors in the active formation is given by the sum \(A_{1ss} + A_{2ss}\). \(\langle (\delta A_{1ss} + \delta A_{2ss})^2 \rangle \) is obtained from the relevant elements of \(\Sigma(t) = (\sigma_{ij})\) for \(i, j \in \{L, R_1, R_2, A_2, A_1\}\):

\[
\Sigma(t) = \langle \delta(t)\delta^T(t) \rangle = \begin{pmatrix}
\langle \delta L^2 \rangle & \langle \delta L\delta R_1 \rangle & \langle \delta L\delta R_2 \rangle & \langle \delta L\delta A_2 \rangle & \langle \delta L\delta A_1 \rangle \\
* & \langle \delta R_1^2 \rangle & \langle \delta R_1\delta R_2 \rangle & \langle \delta R_1\delta A_2 \rangle & \langle \delta R_1\delta A_1 \rangle \\
* & * & \langle \delta R_2^2 \rangle & \langle \delta R_2\delta A_2 \rangle & \langle \delta R_2\delta A_1 \rangle \\
* & * & * & \langle \delta A_2^2 \rangle & \langle \delta A_2\delta A_1 \rangle \\
* & * & * & * & \langle \delta A_1^2 \rangle
\end{pmatrix},
\]
solved at steady-state.

**Figure 10:** Dependence of the relative magnitude of active receptor fluctuations probed by plotting the coefficient of variation on cooperativity parameters. A-C: Numerically calculated CVs for different values of \(K_A\) (Eq. 51). Blue lines indicate absence of cooperativity \((K_A = K_B = K_c = 30.4 \mu M)\). When \(K_A > 30.4 \mu M\), binding is cooperativity. Parameters: See Fig. 8, \(k^+_c = 19 \text{ s}^{-1}\), and \(k^-_c = 20 \text{ s}^{-1}\).
Figure 10 shows parameter studies that characterize the dependence of the relative magnitude of $[A_1]$ and $[A_2]$ fluctuations on cooperativity parameters. Similar to the simulation results in the four-state mGluR model, the relative magnitude of active receptor fluctuations (CV) is a logistic function of the equilibrium dissociation constant $K_A$, where large values of $K_A$ indicate the presence of cooperativity. For small values of $K_A$, the CV asymptotically approaches the minimum $[A_1] + [A_2]$ fluctuation size. Intermediate values of $K_A$, corresponding to mildly anti-cooperative or cooperative binding, amplify the relative magnitude of $[A_1] + [A_2]$ fluctuations. Large values of $K_A$ are independent from the relative magnitude of $[A_1] + [A_2]$ fluctuations.

0.4.1 Interpretation of Steady-state Fluctuations with Closing Kinetics

The elements of the two-time covariance matrix correspond to fluctuating forces (Gaussian white noise) for each species. For the four-state mGluR model, the fluctuating force for $A_2$ is given by the relevant element of the two-time covariance matrix, $\gamma_{cc}^{ss}$, evaluated at steady-state:

$$\gamma_{cc}^{ss} = \frac{2k_c^+ R_{2ss}}{\Omega}$$

Similarly, the fluctuating forces for the active receptors in the five-state mGluR model are given by $\gamma_{cc}^{ss} + \gamma_{dd}^{ss}$, where we have summed the relevant elements from Eq. 49:

$$\gamma_{cc}^{ss} + \gamma_{dd}^{ss} = \frac{2k_c^+ R_{2ss} + 2k_d^+ R_{1ss}}{\Omega}.$$  

The fluctuating forces for $A_2$ only depend on $[R_{2ss}]$ and the elementary activation rate constant, $k_c^+$. Cooperativity increases the occupation of receptors in $R_2$ and decreases the occupation of receptors in $R_1$, which corresponds with an increase in the frequency of transitions between fully activated/resting states and a decrease in the frequency of transitions between partially activated/resting states. Thus, cooperativity should increase the fluctuating force for $A_2$ while decreasing the fluctuating force for $A_1$. In contrast,
anti-cooperativity increases the occupation of receptors in $R_1$ and decreases the occupation of receptors in $R_2$. Thus in the presence of anti-cooperativity, we should expect an increase in the frequency of transitions between partially activated/resting states and a decrease in the frequency of transitions between fully activated/resting states. Consequently, anti-cooperativity should increase the fluctuating force for $A_1$ while decreasing the fluctuating force for $A_2$. Given that the four-state mGluR model only reflects transitions between the edge connecting $R_2$ and $A_2$: the relative magnitude of active receptor fluctuations can only increase or stay constant during cooperative binding. By contrast, the five-state mGluR model reflects transitions along the edges connecting partially activated/resting states and fully activated/resting states. We would expect fluctuations to exhibit a wider range of dynamic behavior when probed by cooperativity parameters.

0.5 Discussion

This project employed the Linear Noise Approximation to simulate cooperativity effects on ligand-driven fluctuations in active mGluR. This approach is the limit of Van Kampen’s system size expansion which assumes large system size $\Omega$ [4,6,11,14]. We confirm the validity of the approach by comparison with Monte-Carlo time course simulations. This approach is an elegant method for probing the effects for molecular noise on small-scale pathways, avoiding the computationally intensive task of numerically solving the chemical Master equation [14]. Using the LNA, we were able to examine the dependence of the relative magnitude of active receptor fluctuations on cooperativity parameters for three topologies: three-state mGluR (KNF) model, four-state mGluR model with closing kinetics, and five-state mGluR model with partially and fully activated states. Simulations of cooperativity effects required changing $K_A$ relative to $K_B$ such that $K_AK_B = K_*^2$, $K_B = K_*^2 / K_A$, and $K_* = 30.4 \, \mu M$. In the three-state mGluR model, the relative magnitude of fluctuations active receptor fluctuations is a bell-shaped function of the equilibrium dissociation constant $K_A$. Binding cooperativity decreased the CV and increased the signal-to-noise ratio (SNR), indicating an increase in signal detection and decrease in intrinsic noise. The active receptor fluctuations in the four-state and five-state model likely depend on the rates of activation and deactivation accounting for...
ligand-induced conformational changes. Although interpretation of cooperativity effects on active receptor fluctuations is speculative, the simulation results support my intuition presented in the above section.

Recent theoretical studies have explored cooperativity effects on intrinsic noise. Extending the work of Berg and Purcell, Bialek and Setayeshgar showed that cooperativity facilitates the sensitivity of a receptor to small changes in concentration [3]. Working with a dynamic Ising-type model, Skoge et al. showed that receptor cooperativity slows receptor activation, leading to a decrease in the SNR of active receptors [15]. Moreover, Sun et al. showed that for small clusters of cooperative receptors, receptor cooperativity can increase the SNR, but this increase depends on the dynamics of the cooperative cluster [16]. The SNR also depends heavily on the concentration of ligands diffusing in and out of the synaptic cleft. As such, the amplitude of noise and fluctuations depend on the fluctuating dynamics. For the parameter regimes chosen here, cooperativity can reduce active mGluR fluctuations.

Although the simulations were fitted with parameters from Marcaggi et al., we note that information on rate constants associated with $R_1^1$ (symmetrical to $R_1^1$) could not be derived from experimental data [9]. In addition, the kinetic scheme provided for the four-state and five-state model is a simplified version of nine-state model found in Marcaggi et al, where the additional four states correspond to sensitized states observed following prolonged application of glutamate [9]. The reported kinetics are also limited to mGluR1Beta conformational changes.

One notable difficulty encountered in this project is the Monte-Carlo time course simulation of the species in the four-state and five-state mGluR models. Results were omitted because presence of noise-reduction effects was not readily apparent for the simulated parameter regimes. Some results in this thesis may involve estimations of certain parameters; however, all parameters were carefully picked from relevant literature.
0.6 Future Directions

Several future directions for this project appear promising. One direction involves evaluating the LNA. A major assumption of the LNA rests on the convergence to a macroscopically stable solution which we denote with the subscript $ss$ [14]. When the stable steady-state converges to zero, the variance approaches infinity [11]. Ligand and receptor concentrations may not reach a steady-state in the synaptic cleft, which means the LNA may fail to give insight into the stochastic properties of similar chemical networks. The presented model framework could be employed with a more realistic model accounting for probabilistic vesicle fusion in the pre-synapse, where the transition rates for neurotransmitter release are coupled by global factors (presynaptic potentials).

Disruptions in glutamate release have been linked to numerous diseased states of the brain [1]. A more thorough understanding of neuropathology will require better characterization of synaptic processes including release, uptake, receptor activation, and sensitization. One consideration is spatial distributions of glutamate receptors in the post-synapse. mGluRs are expressed more laterally, and this organization is thought to play an important role in resolving activation over rapidly decreasing glutamate concentrations over spatial and temporal dimensions (50 – 90% exit the synaptic cleft within 10 – 70 ms of release) [1]. Extracellular glutamate is also compartmentalized into distinct microdomains [1]. Synapses neighboring astrocytes have excitatory amino acid transporters that limit overflow of glutamate in the synaptic cleft. Extrasynaptic space between two synapses may also be a critical site for glutamate transmission. Thus, multiple microdomains may exhibit different glutamatergic signaling. These physiological scenarios present interesting extensions of the LNA approach to investigate the dynamics of receptor activation and glutamate release.
References


