# Modeling of *N*-Methyl-*D*-Aspartate Receptors


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**Abstract** Several reduced kinetic models for *N*-methyl-*D*-aspartate receptors were derived in order to suit different experimental protocols. Their simultaneous application allows for a step-wise estimation of parameters of a conventional model that is otherwise overparameterized with respect to the existing data.

## 1 Introduction

One of the major subtypes of glutamate receptors on neurons is the *N*-methyl-*D*-aspartate receptor (NMDAR). The receptor plays critical role in neural plasticity, development, learning, and memory. Disrupted function is associated with disorders including epilepsy, depression, schizophrenia, ischemic brain injury, and others. NMDARs have been targets of numerous studies, and several models have been proposed and published over the last two decades to explain the dynamics of the currents mediated by the NMDAR ion channel, e.g., [1, 2]. However, conclusions about receptor kinetics based on these Markov models are typically limited by model overparameterization with respect to the available data. Such obstacles cannot be resolved by switching fitting methods; rather the model and the experiments must be adjusted to be in line with each other. In this work, we design the experiments alongside with model development to resolve this issue of overparameterization.

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## 2 NMDAR Desensitization

For common NMDARs are commonly heterotetramers composed of two NR1- and two NR2-subunits [2]. For NMDARs to signal by ion channel opening, they must bind glutamate at each of two NR2 subunits as well as co-agonist (either *D*-serine or glycine) at each of two NR1-type subunits. In response to prolonged agonist pulses, NMDARs desensitize: a process in which the response amplitude decays over time. The desensitization effect increases and speeds up in the presence of limiting co-agonist [1]. This phenomenon could potentially be explained by different mechanisms or a combination of mechanisms. One possibility is that co-agonist already bound to the NMDAR could experience a reduction in affinity following glutamate binding ("glycine (or *D*-serine)–dependent desensitization"; see [1]). Alternatively, the effect of co-agonist concentration on desensitization may not depend on agonist–co-agonist site interactions: upon binding all four molecules, some fraction of the receptors transfers into a long-lived nonconductive state instead [3]. The general chemical kinetic model of the process is shown in Fig. 1. Estimating the reaction rates for state transitions reflecting these processes will answer the question of the nature of NMDAR desensitization.

## 3 Modeling and Experiments

The activation of NMDAR receptors by agonist and co-agonist binding facilitates flow of ions across the cell membrane and this can be recorded as a current in a patch clamp experiment. Piezoelectric switching of solutions bathing an excised outside-out patch allows for fast agonist application. During the experiment, one substrate is chronically present while other one is supplied in a short pulse manner. Varying

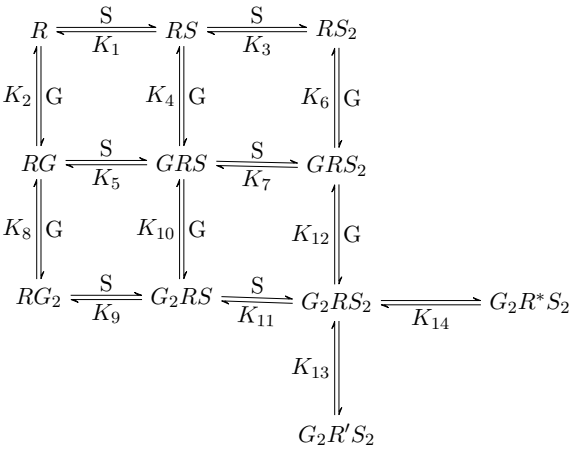


**Fig. 1** General model of NMDA receptor with two binding sites for *L*-glutamate and *D*-serine agonists. *R* denotes the receptor, *S* denotes *D*-serine, and *G* denotes *L*-glutamate. $G_2R'S_2$ is a long-lived nonconductive state and $G_2R^*S_2$ is a conductive state. Each $K_i$ is an equilibrium constant for the corresponding reaction: $K_i = k_i^+/k_i^-$, where $k_i^+$ and $k_i^-$ are the forward and reverse reaction rate constants, respectively

the concentrations of $D$-serine and $L$-glutamate from saturating to relatively low, we can accelerate or slow down reactions in particular directions. Applying Boundary Function Method [4] to the model depicted in the Fig. 1 will yield different results for different scenarios. Here, we show that the appropriate choice of an experimental design allows for a reliable step-wise parameter estimation: the resulting models have different subsets of parameters of the original model, and some parameter estimates obtained in one experiment can be used in the other ones.

The model that correspond to the chemical kinetic scheme depicted in Fig. 1 is a system of differential equations with 11 variables, each corresponding to one state. Let us denote these variables using states notations, i.e., the variable $R(t)$ is a fraction of all receptors that are in $R$ state, etc. Therefore, the sum of all variables is 1. For all reduced models, we denote the leading order approximations in the following manner:

$$\begin{aligned} R(t) &= \alpha(t) + O(\varepsilon_s), & G_2RS(t) &= y(t) + O(\varepsilon_s), \\ RG(t) &= \beta(t) + O(\varepsilon_s), & GRS_2(t) &= \zeta(t) + O(\varepsilon_s), \\ RG_2(t) &= \gamma(t) + O(\varepsilon_s), & G_2RS_2(t) &= z(t) + O(\varepsilon_s), \\ RS(t) &= \eta(t) + O(\varepsilon_s), & G_2R'S_2(t) &= z'(t) + O(\varepsilon_s), \\ RS_2(t) &= x(t) + O(\varepsilon_s), & G_2R^*S_2(t) &= z^*(t) + O(\varepsilon_s), \\ GRS(t) &= \theta(t) + O(\varepsilon_s), \end{aligned} \tag{1}$$

where $\varepsilon$ is a small parameter, which is different for each experiment and is defined individually in each corresponding section. The current recorded during the experiments is directly proportional to the only conducting state $G_2R^*S_2$.

## 3.1 Saturating Concentration of D-Serine

In this experiment, we apply a saturating concentration of $D$-Serine and let the system reach the steady state before a short pulse of $L$-glutamate at a low concentration. The presence of saturating $D$-serine allows us to introduce a small parameter $0 < \varepsilon_s \ll 1$:

$$S \cdot k_i^+ = \frac{\widetilde{k_i^+}}{\varepsilon_s}, \quad \widetilde{k_i^+} \sim O(1),$$

$i = 1, 3, 5, 7, 9, 11$, where $S$ is a $D$-Serine saturating concentration. For the leading order approximations of the functions (1), we obtain

$$\alpha(t) \equiv 0, \quad \eta(t) \equiv 0, \quad \beta(t) \equiv 0, \quad \theta(t) \equiv 0, \quad \gamma(t) \equiv 0, \quad \zeta(t) \equiv 0,$$

$$\begin{aligned}
\frac{dx}{dt} &= -k_6^+ Gx + k_6^- y,\\
\frac{dy}{dt} &= k_6^+ Gx - k_6^- y - k_{12}^+ Gy + k_{12}^- z,\\
\frac{dz}{dt} &= k_{12}^+ Gy - k_{12}^- z - k_{13}^+ z + k_{13}^- z' - k_{14}^+ z + k_{14}^- z^*,\\
\frac{dz_0'}{dt} &= k_{13}^+ z - k_{13}^- z',\\
\frac{dz_0^*}{dt} &= k_{14}^+ z - k_{14}^- z^*,
\end{aligned}$$

with initial conditions $x(0) = 1$, $y(0) = 0$, $z(0) = 0$, $z'(0) = 0$, and $z^*(0) = 0$.

## 3.2 Saturating Concentration of L-Glutamate

This case mirrors the experiment from the Sect. 3.1: we apply saturating concentration of $L$-glutamate and let the system reach the steady state before a short pulse of $D$-serine at a limiting concentration. We introduce a small parameter $0 < \varepsilon_g \ll 1$ in a similar manner:

$$G \cdot k_i^+ = \frac{\widetilde{k_i^+}}{\varepsilon_g}, \quad \widetilde{k_i^+} \sim O(1),$$

$i = 2, 4, 5, 8, 10, 12$, where $G$ is a $L$-Glutamate saturating concentration. For the leading order approximations of the functions (1), we obtain

$$\alpha(t) \equiv 0, \quad \eta(t) \equiv 0, \quad x(t) \equiv 0, \quad \beta(t) \equiv 0, \quad \theta(t) \equiv 0, \quad y(t) \equiv 0,$$

$$\begin{aligned}
\frac{d\gamma}{dt} &= -k_9^+ S\gamma + k_9^- \zeta,\\
\frac{d\zeta}{dt} &= k_9^+ S\gamma - k_9^- \zeta_0 - k_{11}^+ S\zeta + k_{11}^- z,\\
\frac{dz}{dt} &= k_{11}^+ S\zeta - k_{11}^- z - k_{13}^+ z + k_{13}^- z' - k_{14}^+ z + k_{14}^- z^*,\\
\frac{dz'}{dt} &= k_{13}^+ z - k_{13}^- z',\\
\frac{dz^*}{dt} &= k_{14}^+ z - k_{14}^- z^*,
\end{aligned}$$

with initial conditions $\gamma(0) = 1$, $\zeta(0) = 0$, $z(0) = 0$, $z'(0) = 0$, and $z^*(0) = 0$.

### 3.3 Saturating Concentrations of both Substrates

In this experiment, the concentrations of both agonist and co-agonist are high, therefore, a small parameter $0 < \varepsilon_b \ll 1$ can be defined as

$$S \cdot k_i^+ = \frac{\widetilde{k_i^+}}{\varepsilon_b}, \quad \widetilde{k_i^+} \sim O(1),$$

$$G \cdot k_j^+ = \frac{\widetilde{k_j^+}}{\varepsilon_b}, \quad \widetilde{k_j^+} \sim O(1),$$

$i = 1, 3, 5, 7, 9, 11$, $j = 2, 4, 5, 8, 10, 12$. The functions (1) depend on which substance is always present and which is given in a short pulse manner. Let us consider the case when $D$-serine is applied continuously throughout the whole experiment with steps of $L$-glutamate. Then we have

$$\alpha(t) \equiv 0, \quad \eta(t) \equiv 0, \quad \beta(t) \equiv 0, \quad \theta(t) \equiv 0, \quad \gamma(t) \equiv 0, \quad \zeta(t) \equiv 0,$$

$$\begin{aligned}
\frac{dz_0}{dt} &= -k_{13}^+ z_0 + k_{13}^- z' - k_{14}^+ z_0 + k_{14}^- z^* \\
\frac{dz'}{dt} &= k_{13}^+ z_0 - k_{13}^- z', \\
\frac{dz^*}{dt} &= k_{14}^+ z_0 - k_{14}^- z^*,
\end{aligned}$$

with initial conditions $z_0(0) = 1$, $z'(0) = 0$, and $z^*(0) = 0$. And

$$\begin{aligned}
x(t) &= e^{-k_6^+ Gt}, \\
y(t) &= \frac{k_6^+}{k_{12}^+ - k_6^+} \left( e^{-k_6^+ Gt} - e^{-k_{12}^+ Gt} \right), \\
z(t) &= z_0(t) + \frac{k_6^+ k_{12}^+}{k_{12}^+ - k_6^+} \left( \frac{1}{k_{12}^+} e^{-k_{12}^+ Gt} - \frac{1}{k_6^+} e^{-k_6^+ Gt} \right).
\end{aligned}$$

Let us also notice that after the $L$-Glutamate pulse stops, one should use the model from Sect. 3.1 with $G = 0$.

## 4 Conclusion

The experiments and the corresponding reduced models described in Sect. 3 can be used for the estimation of parameters of the full model depicted in Fig. 1 in a step-wise manner. The low number of parameters at each step helps to resolve the overparameterization issue. The estimates of reaction rates constants will give us the answer about the nature of NMDAR desensitization.

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