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Influence of Ionotropic Receptor Location on their Dynamics at Glutamatergic Synapses

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***Abstract*—In this paper we study the effects of the location of ionotropic receptors, especially AMPA and NMDA receptors, on their function at excitatory glutamatergic synapses. As few computational models only allow to evaluate the influence of receptor location on state transition and receptor dynamics, we present an elaborate computational model of a glutamatergic synapse that takes into account detailed parametric models of ionotropic receptors along with glutamate diffusion within the synaptic cleft. Our simulation results underscore the importance of the wide spread distribution of AMPA receptors which is required to avoid massive desensitization of these receptors following a single glutamate release event while NMDA receptor location is potentially optimal relative to the glutamate release site thus, emphasizing the contribution of location dependent effects of the two major ionotropic receptors to synaptic efficacy.**

I. INTRODUCTION

Synapses are the major sites of information processing in brain and exhibit a wide diversity of shape and function throughout the central nervous system (CNS). Glutamate is the major excitatory neurotransmitter and interacts with two major types of ionotropic receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and the N-methyl-D-aspartate (NMDA) receptors to mediate rapid synaptic transmission. In addition, certain patterns of activation of these two receptor types along with metabotropic (m-GluR) receptors and other secondary messenger mechanisms plays a key role in short-term and long-term regulation of synaptic transmission. Synapses exhibit a wide range of functional diversity, which arises due to their morphology, receptor sub-type composition and a spectrum of other regulatory mechanisms. Both simulation and experimental studies have demonstrated a potential role for changes in receptor localization in the transition from short-term to long-term potentiation (LTP) [1]. In this computational study, we further explore the role of synaptic geometry on glutamate receptor dynamics. More specifically, we examine the effect of changing the postsynaptic location of AMPA and NMDA receptors relative to the glutamate release site, on receptors dynamics and subsequent post-synaptic responses.

II. BACKGROUND

In the last few decades several imaging tools and immunogold labeling methods have shown that spines are highly dynamic structures and revealed a non-homogenous distribution and density of postsynaptic AMPA and NMDA receptors at CA3/ CA1 synapses [2]. These receptors are concentrated in the post-synaptic density opposite to the site of glutamate release. Several factors, including the amount of neurotransmitter inside the synaptic cleft, receptor number, spine neck and head geometry, contribute to and regulate synaptic efficacy. Some sub-types of AMPA receptors are distributed near the edge of the post-synaptic specialization [2], while others, especially GluR1-containing AMPA receptors exhibit a supralinear relationship with PSD area [2]. The number of NMDA receptors is only weakly correlated with the PSD area at hippocampal CA3/CA1 synapses [3]. When glutamate binds to AMPA and NMDA receptors, transient changes in their conformations determine the amount of ions that flow through their associated channels. AMPA receptors at CA3/CA1 synapses are mostly voltage-independent Na+ channels and exhibit very rapid kinetics of activation/deactivation/desensitization. In contrast, NMDA receptors are also calcium channels, exhibit a voltage-dependent magnesium blockade of the channels, have slower kinetics and require a longer time to recover from desensitized states. In the present study, we were particularly interested in studying the effects of AMPA and NMDA receptor distribution in the postsynaptic membrane

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on EPSC and EPSP amplitudes, as well as on their transition states.

III. COMPUTATIONAL MODEL OF A GENERIC GLUTAMATERGIC SYNAPSE

The synaptic modeling platform we used is the EONS simulation platform (Elementary objects of the Nervous System) [4]. This platform is a parametric model of a generic glutamatergic synapse that takes into account pre-synaptic mechanisms, such as calcium buffering, neurotransmitter release and diffusion, and postsynaptic elements, such as ionotropic AMPA and NMDA receptors, their distribution and synaptic geometry, as well as metabotropic glutamate receptors. The focus of the present study is the postsynaptic component, in particular the postsynaptic membrane where AMPA and NMDA receptors are co-localized. Immunogold labeling studies have indicated the presence of NMDA receptors more closely to the center of the postsynaptic density while AMPA receptors are distributed more uniformly across the PSD [4]. In contrast, the metabotropic glutamate receptors type I (type I mGluRs) seem to be preferentially localized farther away from the release site and are sometimes excluded from the PSD [3]. Activation of these receptors depends on several factors, including the activity of a variety of glutamate transporters present on neuronal [5] and glial membranes nearby and neurotransmitter spillover from neighboring synapses. Since rapid glutamatergic transmission is mediated by AMPA and NMDA receptors, they were the focus of our study and these simulations were conducted within the scope of a single vesicle source of neurotransmitter release.

For our study, we used the glutamate diffusion model developed by Savtchenko et al. [6] which provides a good approximation of 2D diffusion using a one-dimensional radial extent and an optimal height of the cleft using monte carlo simulations.

$$C(r,t,Q,D,\delta) = \frac{Q}{4\pi\delta Dt}\exp\frac{-r^2}{4Dt} \qquad (1)$$

Where C, r and D represent the concentration, radial distance of the receptor from the source of release and the diffusion coefficient, respectively. 'Q'corresponds to the number of glutamate molecules, and we assumed that 3000 molecules were released simultaneously. 'δ' represents the width of the cleft and is maintained constant throughout the simulations at 20nm. Glutamate diffusion coefficient 'D' was set at $0.4\mu m^2/ms^2$ and 'r' was varied from 0 nm to 200 nm in increments of 20 nm.

AMPA receptors mediate fast excitatory transmission and have four binding sites for glutamate [7]. The AMPA receptor model described in [8], which captures the receptor dynamics in 16 transition states, from resting (R) to open (O), desensitized (D) and deeply de-sensitized (E) states as shown in figure 1a, was used in this study. The index number located beside the letter corresponding to the state represents the number of glutamate molecules bound.

The NMDA receptor used in this study is also a detailed kinetic model and was described in [9]. It consists of 15 states, which include interactions due to the binding of glutamate and a co-agonist glycine (Fig. 1b). The open state conductances are also modulated by the concentration of magnesium within the extra-cellular environment. The open state transition probabilities multiplied with the conductance of the channels give an estimate of the postsyaptic current. Both models have been validated with experimental results, and the details of the kinetic constants of the hidden markov processes are reported in [8],[9].

We analyzed location-dependent changes in postsynaptic potential and current, mediated by AMPA and NMDA receptors separately.

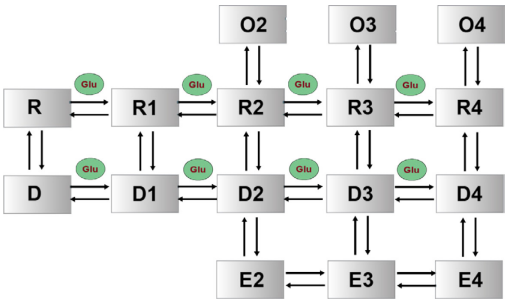

Figure 1a. 16 states kinetic model of AMPA receptor

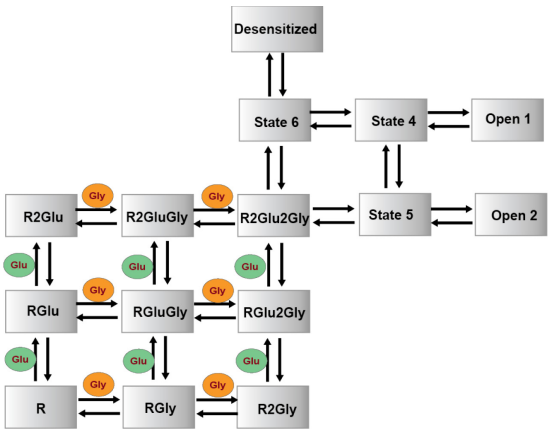

Figure 1b. 15 states kinetic model of NMDA receptor

Additionally, since the computational platform provides a unique opportunity to access each of the internal state variables, we studied the changes in receptor states when the receptors were located at different locations relative to the glutamate release site

IV. RESULTS

For the entire study, the stimulation protocol consisted in a single presynaptic pulse that elicited a successful release

event at the pre-synaptic site. Glutamate molecules released from the vesicle rapidly diffuse within the synaptic cleft. Binding affinity of free glutamate and kinetics of the ionotropic receptors determine the probability for the receptors to exist in any one of their transition states. We first studied how AMPA and NMDA receptor locations with respect to release site affected EPSP and EPSC elicited by a single release event. We then further analyzed the effects of receptor location on the distribution of transition states of the receptors.

We obtained EPSCs and EPSPs elicited by a single release event with AMPA and NMDA receptors located at 0, 100 or 200 nm away from the release site, and responses were normalized to the peak values resulting from receptors located at 0 nm. Figure 2a shows the normalized responses when AMPARs were clustered at 0nm, 100nm and 200nm relative to the release site.

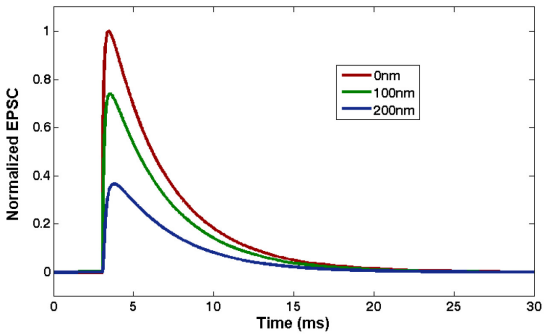

Figure 2a. Normalized EPSC responses mediated by AMPA receptors located at 0, 100 and 200 nm in the PSD. There was a 65% decrease in peak amplitude when the receptor was moved 200 nm away from the release site.

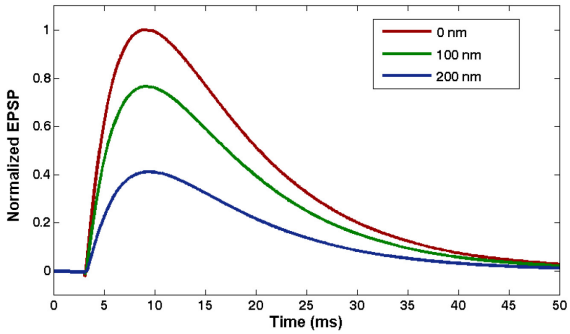

Figure 2b. Normalized EPSP responses mediated by AMPA receptors located at 0, 100 and 200 nm in the PSD. Peak amplitude was decreased 60% when AMPA receptor was located 200 nm away from the release site.

Our simulation results indicate a 30% decrease in amplitude when the location of AMPA receptors was 100nm away and a 65% decrease when AMPA receptors were placed 200nm away from the release site. Figures 2b shows the EPSP responses as a result of AMPA receptor-mediated EPSCs when they were distributed in concentric circles as mentioned above. The responses at 0nm, 100nm and 200nm were normalized to the maximum response observed at 0nm. There was a 60% decrease in the peak amplitude of the response when receptors were placed 200nm away.

The same experiment was repeated with NMDA receptors distributed in concentric circles in the PSD. Glutamate has a higher affinity for NMDA receptors than for AMPA receptors and, as observed in previous studies [10],[11], and confirmed by our simulations, there was no significant effect of receptor location on either EPSCs or EPSPs. Decreases in EPSC (figure 3a) and EPSP (figure 3b) were approximately 10% when NMDA receptors were placed 200 nm away. The changes in AMPA receptor-mediated postsynaptic currents and potentials arise due to changes in the probability of the conducting states of the AMPA receptor. However, transition to conducting states is influenced by the desensitized states of the receptor.

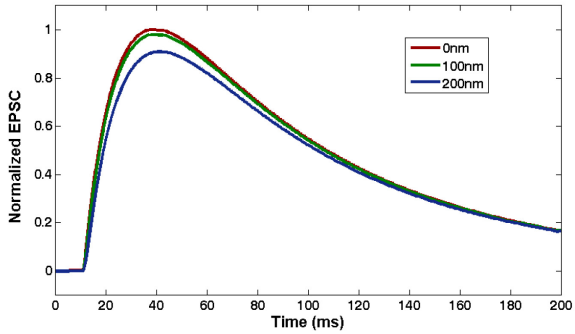

Figure 3a. Normalized EPSC mediated by NMDA receptor as a function of its location at 0 nm, 100 nm and 200 nm. There was no significant effect of location on NMDAR-mediated EPSC.

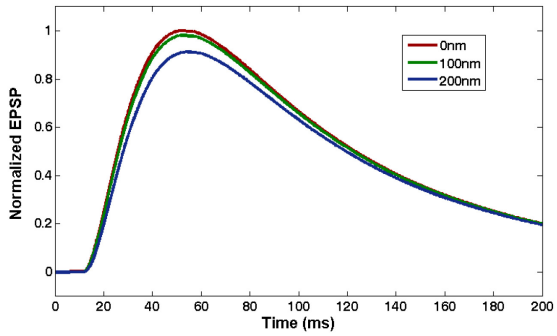

Figure 3b. Normalized EPSP response mediated by NMDA receptor as a function of its location at 0 nm, 100 nm and 200 nm away from the release site.

Receptor desensitization is an important property, since receptor enters into an inactive state after binding/unbinding of glutamate and cannot respond to subsequent pulses of glutamate. For the receptor to become re-sensitized, it must slowly recover to its resting state, which enables it to respond to subsequent inputs.

AMPA receptors exhibit fast kinetics and recover from desensitization relatively rapidly. Figure 4a shows that when four glutamate molecules are bound, receptors close to the release site exhibit a higher probability of being in a desensitized state. When they are farther away they are less desensitized and have a higher probability to respond to subsequent pulses than those located close by.

Figure 4b shows the desensitization property of NMDA receptors as a function of location of the receptor relative to the release site. In contrast to AMPA receptors, NMDA

receptors distributed at various distances showed more or less and equal amount of desensitization, indicating that the desensitized state is not affected by receptor location.

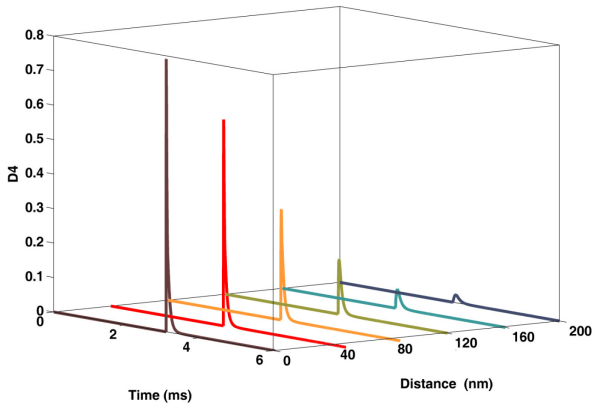

Figure 4a. Desensitization state probability of AMPA receptors when four glutamate molecules are bound as a function of location of the AMPA receptor relative to glutamate release site.

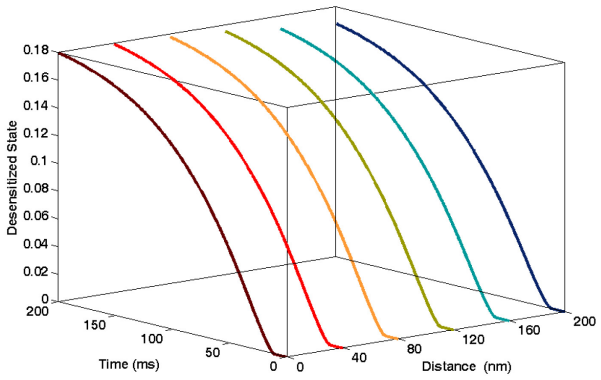

Figure 4b. Desensitization state probability of the NMDA receptors when glutamate is bound as a function of location of the NMDA receptor relative to the glutamate release site.

These results provide a clear insight into why there is a difference in distribution of AMPA and NMDA receptors within the PSD. While NMDA receptor location is potentially optimal with receptors located close to glutamate release site and previous studies have attributed this behavior of NMDA to their high binding affinity towards glutamate. A more widespread distribution of AMPA receptors is required to avoid a massive desensitization of all AMPA receptors following a single release event. Such distribution would allow repeated events to activate more distal receptors which would not have been desensitized by a prior release event.

V. DISCUSSION

The computational model used in this study was developed to explore the role of receptor location within the PSD relative to glutamate release site on receptor kinetics and function. AMPA receptor trafficking [12] has received a lot of attention throughout the years for its role in synaptic tuning. Experimental testing of such hypothesis is still a technological challenge. Our simulation results provide a unique opportunity to analyze in details the role of various transition states for AMPA and NMDA receptors on their functional properties. Our model provides a unique tool to test possible hypotheses that could explain the differential distribution of AMPA and NMDA receptors revealed under experimental conditions. The current study was limited to the analysis of the behavior and internal dynamics of the receptors in response to a single glutamate release event. Future work will be directed at studying these dynamics when input pulses with varying time intervals are administered. Such studies will provide additional information regarding the implications of differential distribution of AMPA and NMDA receptors for synaptic transmission and its plasticity.

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