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Influence of Ionotropic Receptors Localization on Glutamatergic Synaptic Response to Paired-Pulse Stimulation Protocol

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***Abstract*— Paired-pulse protocol is a stimulation pattern that is often used to characterize short-term changes in synaptic potency. Responses to such protocol often yield varying results, going from a depressing response to a facilitated one following the second pulse. Similarly, experimental results have shown that synaptic structures are dynamic and receptors move along the postsynaptic membrane. The present study provides insights on the impact of glutamatergic receptors localization with respect to the neurotransmitters release site on the postsynaptic currents measured; it also proposes an explanation on the diversity of responses observed experimentally. The platform we used is the EONS/RHENOMS modeling platform widely described in the literature, which encompasses a multitude of highly detailed subsynaptic elements to most faithfully replicate synaptic function.**

I. INTRODUCTION

Synapses constitute the obligatory bridge on which information is transferred in between neurons. The weight (or potency) of this connection and its dynamic changes over time strongly influence network communication and inherently shape information processing. The major excitatory synapses in the central nervous system (CNS) use glutamate as neurotransmitter which 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 synaptic transmission. It has already been established that the location of these receptors has a significant impact on the amplitude of the postsynaptic signal (current and voltage) following a single pulse stimulation [1]. In this study, we propose to evaluate the influence of both AMPA and NMDA postsynaptic receptors locations on responses to paired-pulse stimulation protocol of varying input time intervals.

II. BACKGROUND

Recent advances in imaging tools and immunogold labeling methods have shown that spines are highly dynamic structures which contain a non-homogenous distribution and density of ionotropic receptors at CA3 / CA1 synapses [2]. These dynamic changes occur in response to normal development, synaptic maturation, neurodegeneration, but also depending on activity levels [3].

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]. It is also established that specific sub-types of AMPA receptors are distributed near the edge of the post-synaptic specialization, while others, especially GluR1-containing AMPA receptors exhibit a supralinear relationship with PSD area [2]. In contrary, the number of NMDA receptors is only weakly correlated with the PSD area at hippocampal CA3/CA1 synapses [5]. 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 these synapses are mostly voltage-independent Na+ channels and exhibit very rapid kinetics of activation/deactivation/desensitization. In contrast, NMDA receptors are also calcium permeable channels, exhibit a voltage-dependent magnesium blockade of the channels, have slower kinetics and require a longer time to recover from desensitized states.

It has long been observed that neuronal transmission was not a passive ‘cable-like’ mechanism. Instead, it displays very dynamic properties that shape and inherently control how information is transmitted across neuronal networks. One of these dynamic properties consists of the ability displayed by synapses, when stimulated by two identical pulses separated by a short interval, to induce a response that is different for the second pulse compared to the first one. Depending on the synapses studied and the stimulation

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conditions, the second response can be increased (facilitated) or decreased (depressed) relative to the first one.

This short–term synaptic facilitation called paired-pulse facilitation (PPF) is a phenomenon that is commonly observed and studied. The earliest hypothesis proposed a presynaptic mechanism consisting of calcium accumulation in presynaptic terminal (residual calcium hypothesis) [6]. Tsodyks and Markram [7] propose a general synaptic model that quantifies and predicts the amplitude of short-term changes of the synaptic responses based on changes in neurotransmitter release probability. Similarly, a significant number of other mechanisms, also mostly presynaptic, have been discovered that each contribute to the changes in responses observed during the paired-pulse stimulation paradigm [8]. In the present study, we propose to focus on a postsynaptic mechanism that could also affect paired-pulse response. More specifically, we propose to vary the location of AMPA and NMDA receptors along the postsynaptic membrane and determine the subsequent impact on postsynaptic current in response to a paired-pulse stimulation protocol.

III. MODELING FRAMEWORK

The synaptic modeling platform we used is the EONS (Elementary Objects of the Nervous System)/RHENOMS (Rhenovia Modeling and Simulation) simulation platform [4]. This platform consists of a complex parametric model of a generic glutamatergic synapse that takes into account presynaptic 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, and more specifically the ionotropic AMPA and NMDA receptors on the postsynaptic membrane which mediate rapid glutamatergic transmission.

The stimulation protocol we used consists of a series of two presynaptic pulses with an interpulse interval varying between 10 and 2000 msec. Each pulse induces one presynaptic release event with an equal number of neurotransmitters per release, making the PPF or PPD solely dependent on the postsynaptic component.

For this study, we used the glutamate diffusion model developed by Savtchenko et al. [9] which provides a good approximation of 2D diffusion with respect to monte carlo simulations using a one-dimensional radial extent and an optimal height of the cleft. We assumed that 3000 molecules were released simultaneously; the width of the cleft is maintained constant throughout the simulations at 20nm. Glutamate diffusion coefficient was set at $0.4\mu m^2/ms^2$ and the distance separating the release site from the receptors was varied from 0 nm to 300 nm in increments of 20 nm.

AMPA receptors mediate fast excitatory transmission and have four binding sites for glutamate [10]; the AMPA receptor model we used is described in [11]. Following numerous tests, this model captures extremelly well the receptor dynamics using 16 transition states in a large number of experimental conditions. Our model of NMDA receptor is also a detailed kinetic model and was described in [12]. It consists of a 15 states model, which includes interactions due to the binding of glutamate and a co-agonist glycine. 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 postsynaptic current. Both models have been validated with experimental results, and the details of the kinetic constants of the hidden markov processes are reported in [11] and [12]. Those models are coupled to a presynaptic terminal and integrated in the postsynaptic membrane of our EONS/RHENOMS synaptic modeling platform as illustrated in Fig. 1.

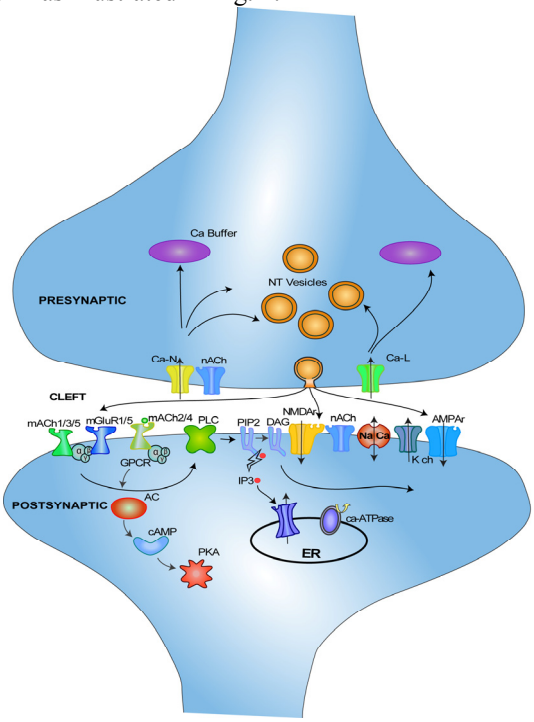

Figure 1. Simplified illustration of the main mechanisms comprised in the EONS/RHENOMS synaptic modeling platform. Ca-N and Ca-L refer to voltage-dependent calcium channels N and L types respectively; nACh stands for nicotinic receptor; mACh refers to muscarinic receptor; mGluR stands for metabotropic receptor. GPCRs (G protein coupled receptors) trigger a cascade of molecular mechanisms involving AC (adenyl cyclase), cAMP (cyclic AMP), PKA (protein kinase A, and PLC (phospholipase C), PIP2 (Phosphatidylinositol 4,5-bisphosphate), DAG (diacyl glycerol) and IP3 (inositol 1,4,5-trisphosphate). ER stands for endoplasmic reticulum.

IV. RESULTS

The stimulation protocol used in the entire study consisted in a presynaptic paired-pulse protocol that elicited a successful release event at the pre-synaptic site for each pulse. 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

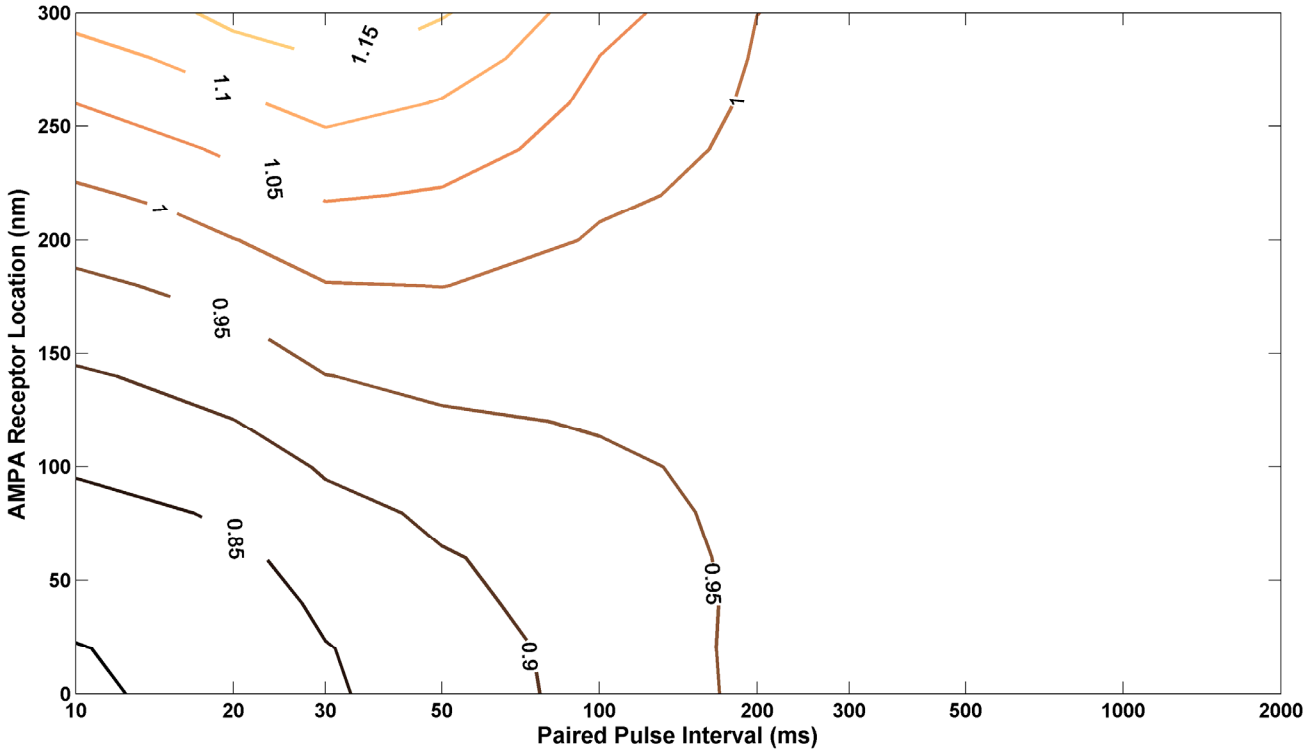

Fig. 2. Paired-pulse ratio of the EPSC response as a function of interpulse interval (X axis) and AMPA receptor location (Y axis). The figure summarizes the results of 11x16 simulations. Facilitation is observed for short interpulse intervals when receptors are located far from the release site, while depression is observed when receptors are in close proximity. No effect of location can be observed at longer inter-pulse intervals.

transition states. We studied how AMPA and NMDA receptor locations with respect to release site affected excitatory postsynaptic currents. The number of receptors used is 80 AMPA receptors, and 20 NMDA, in accordance with structural data reported in the literature for non-silent synapses in the hippocampus [13], [14]. Magnesium concentration was set to 1mM. The cleft height (distance from the release site to the postsynaptic membrane) is 20 nm. No glutamate uptake (from glial and neuronal transporters) was taken into account for these simulations.

In a first set of simulations, we modified the location of the AMPA receptors on the membrane from 0nm (right in front of the release site) to 300 nm away. NMDA receptors were placed at a distance of 160nm away from the release site. We repeated the simulations with NMDARs located at 60nm, but did not observe significant changes in the amplitudes of synaptic current and potential. The dynamic changes in excitatory postsynaptic current (EPSC) were recorded for the different positions, and in response to different inter pulse intervals. We performed 11x16 simulations, with input interpulse intervals varying from 10 to 2000ms (11 data points along Y-axis) and AMPAR location varying from 0nm to 300nm (16 data points along Y-axis). We then computed the paired pulse ratio (PPR) as the ratio of maximum amplitude caused by second response to the maximum amplitude of the first EPSC response. This initial set of simulations yielded the results presented in Fig. 2.

These results indicate that paired-pulse response is facilitated at small PPRs (less than 150ms) when the receptors are located far away from the release site (> 200 nm), yielding a ratio superior to 1. On the other hand, paired-pulse responses were depressed for the same inter pulse intervals when the receptors where located close to the release site (<150nm). For longer inter pulse intervals, the paired-pulse response was neither facilitated, nor depressed (consistent with previously reported data) and AMPA receptors location had no effects on paired-pulse ratio.

In a second set of simulations, we placed the NMDA receptors at three locations, 0nm, 100nm and 200nm from the release site while the location of AMPA-Rs are held constant. We also simulated varying ratios between AMPA-Rs and NMDA-Rs for two cases, 4:1 and 1:4. The number of receptors configured is 80 AMPA-R, 20 NMDA-R in one case and 20 AMPA-R, 80 NMDA-R in another. The changes in PPR were recorded and the results of these simulations are presented in Table 1. The inter pulse interval was kept at 100ms, and we simulated for 800 ms.

These results indicate that independently of location, a ratio of 20:80 for AMPA/NMDA numbers will result in paired-pulse depression. On the contrary, a ratio of 80:20 induces facilitation. In parallel, the PPR varies with NMDA-R location from 90% to 99% when the number of AMPA receptors is high compared to the number of NMDA-Rs. However it remains invariant with respect to location at 109% when the number of AMPA-Rs is 20. This result indicates that location of NMDA-Rs on the postsynaptic membrane has a relatively weak effect on PPR compared to AMPA-Rs location.

NMDA-R Location	NMDA-R/AMPA-R Ratio (20:80)	NMDA-R/AMPA-R (80:20)
0nm	0.91	1.09
100 nm	0.94	1.09
200 nm	0.99	1.09

Table 1. Values of PPR for varying numbers of receptors at different distances from the release site.

V. DISCUSSION

Changes in synaptic dynamics are believed to have a strong impact on learning and memory and brain function in general. Despite impressive technological advances over the last decade, experimentally measuring the distinct contributions of the numerous mechanisms involved in synaptic transmission is still a formidable challenge. The utilization of a computational integrated modeling platform such as EONS/RHENOMS constitutes an ideal approach that allows a more intimate access to parameters currently immeasurable by direct experimental techniques. Inherently, such integrated modeling platform also allows the characterization of potential dysfunctions in synaptic transmission, and can help identifying compounds or combinations of compounds to re-establish normal function.

Although previously reported results indicate that postsynaptic receptors play a minor role in short-term plasticity [15], [16], our results suggest that postsynaptic mechanisms do influence paired-pulse synaptic facilitation/depression. More specifically, we were able to quantify the effects of ionotropic AMPA and NMDA receptors location on the well-established paired-pulse synaptic response. We showed that, indeed, location of AMPA receptors has an effect on PPR responses. More specifically, we showed that the response to the second pulse can be depressed when receptors are close to the release site or facilitated for larger distances. These results were observed when inter pulse intervals are short. Neither facilitation nor depression was observed at longer time intervals, independently of receptors location. This study raises interesting questions to further explain the variations observed. Do we see these location-dependent effects due to saturation of receptor binding of glutamate for locations close to the release site, while such saturation is not present for longer distances? Or are the receptors undergoing deeper desensitization when closer to the release site? Further investigations are planned to evaluate these underlying mechanisms by studying the internal dynamics of the receptors. Similarly, we plan on investigating the influence of other parameters on paired-pulse response, more specifically the influence of glial and neuronal glutamate uptake, as well as other parameters that have been reported to change due to pathological conditions.

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