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MODELING GLUTAMATERGIC SYNAPSES: INSIGHTS INTO MECHANISMS REGULATING SYNAPTIC EFFICACY

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Abstract

The hippocampal formation is critically involved for the long-term storage of various forms of information, and it is widely believed that the phenomenon of long-term potentiation (LTP) of synaptic transmission is a molecular/cellular mechanism participating in memory formation. Although several high level models of hippocampal function have been developed, they do not incorporate detailed molecular information of the type necessary to understand the contribution of individual molecular events to the mechanisms underlying LTP and learning and memory. We are therefore developing new technological tools based on mathematical modeling and computer simulation of the molecular processes taking place in realistic biological networks to reach such an understanding. This article briefly summarizes the approach we are using and illustrates it by presenting data regarding the effects of changing the number of AMPA receptors on various features of glutamatergic transmission, including NMDA receptor-mediated responses and paired-pulse facilitation. We conclude by discussing the significance of these results and providing some ideas for future directions with this approach.

Keywords

Modeling; simulation; glutamate; hippocampus; synaptic; receptor; plasticity

1. Introduction

The hippocampal formation is critically involved in the long-term storage of “declarative” memories (memories for facts/names and sequences of events), and it is widely believed that the phenomenon of long-term potentiation (LTP) of synaptic transmission, which is prevalent throughout hippocampal circuitry, is a cellular/molecular mechanism participating in certain forms of memory formation. The progress in our understanding of the mechanisms underlying learning and memory has led to the realization that there is no one “unitary” process for information storage in the brain — instead, memory evolves from multiple cellular/molecular processes interacting in complex ways. For example, the coordinated activation of AMPA and NMDA receptors is required to produce LTP; glycine site occupation must occur during the combined activation of AMPA and NMDA receptors; and a critical threshold of calcium concentration must be reached postsynaptically to elicit LTP, etc. Several LTP models have recently been developed, some of them including receptor trafficking, models of calcium diffusion and of calcium-dependent kinases and phosphatases [4–6,8,14,15] to facilitate the understanding of the roles of different processes in LTP induction and maintenance. However, none of these models provide a complete description of the events linking patterns of synaptic activity to changes in synaptic efficacy including modulation by external inputs, such as GABAergic, cholinergic or serotonergic. Moreover, no model provides for the understanding of retrograde signaling pathways, such as the endocannabinoid system, which is now

recognized as a major regulator of synaptic plasticity in hippocampus and cerebellar networks [1,2]. At the other end of the continuum spectrum between molecular level and network levels, several models of large hippocampal networks have been developed, but their lack of molecular details does not allow the understanding of the role of individual molecular mechanisms in network behavior.

It is our goal to develop a new technology of mathematical modeling and computer simulation tools to systematically explore the contribution of various molecular processes in glutamatergic synaptic transmission, and the effects of those synaptic processes on multi-synaptic cellular dynamics, and ultimately, on the behavior of a small network of hippocampal neurons. This represents a formidable challenge, as it requires the integration of elementary models of molecular processes taking place at individual synapses and their incorporation into models of individual neurons. We believe that this approach will not only provide an intimate understanding of the contribution of specific molecular events (i.e., receptor desensitization, blockade of a specific type of K^+ channels, modulation of receptors, etc.) to synaptic plasticity and ultimately overall systems function, but also will facilitate the design of better and safer therapeutic strategies for learning and memory impairments.

This paper represents a brief summary of the work going on both at the University of Southern California in Los Angeles, and at Rhenovia Pharma, which is a new start-up company in Mulhouse, France, dedicated to using this new technology to facilitate the drug discovery process. We will first summarize the features of the current platform before providing some examples of the results that illustrate the advantages of this approach to address questions regarding the contributions of the number of AMPA receptors present at a single glutamatergic synapses to responses to single as well as to paired pulse simulation.

2. Features of the Platform

This project started several years ago in Ted Berger's laboratory and was also greatly stimulated by numerous discussions with Gilbert Chauvet. The rationale was to develop a model of a glutamatergic synapse that would facilitate the testing of various hypotheses regarding the mechanisms underlying long-term potentiation. This model was referred to as EONS (Elementary Objects of the Nervous System), and has been developed as a research tool for the scientific community. The computer simulation allows studying the role of (i) presynaptic Ca^{2+} dynamics and (ii) Ca^{2+} interactions with vesicle-related proteins (VRPs) that regulate neurotransmitter release. Included in the dynamics are calcium influx through voltage-dependent Ca^{2+} channels, intracellular calcium diffusion and buffering, and transmembrane pumping. The kinetic scheme for Ca^{2+} interaction with VRPs assumes the existence of two Ca^{2+} -binding sites. Simulations using repetitive depolarization showed that an increase in single-liganded VRPs after the first depolarization is critical for the facilitation of release observed experimentally in response to repeated stimulations. Simulations also show that the time course of transmitter release slows as the distance between calcium channels and the release site increases.

We first extended the model of the presynaptic terminal to include (i) the space of the synaptic cleft, and (ii) a post-synaptic membrane comprising both AMPA and NMDA receptors. The receptors model consist in kinetic models of AMPA receptor-channels and NMDA receptor-channels, with a channel-related Mg^{2+} binding site providing voltage-dependent blockade of the NMDA receptor channel. All release sites, pumps, receptors, and channels are considered with respect to their relative locations so that constraints of synaptic morphology (resolution equivalent to 10 nm) can be included in studying the dynamics of synaptic transmission. The presynaptic terminal is 400 nm wide; the cleft width can be varied from 10 to 40 nm [13,18]. Interestingly, the wider the synaptic cleft, the smaller the currents elicited by stimulation,

indicating the strong effect of glutamate diffusion on the amplitudes of synaptic responses. This result clearly demonstrates that it will be of importance to include models of glutamate transport and glutamate transporters in the general synaptic model.

This model also allows investigating the possibility that plasticity-related changes in the relative positions of presynaptic release sites and postsynaptic receptor-channels may underlie the differences in magnitude and time course of excitatory post-synaptic currents (EPSCs) [22]. Our simulations showed that AMPA receptor-mediated EPSC is about 40% higher while NMDA-mediated EPSC is less than 20% higher when the receptors are geometrically aligned with the release site than when the centers of the release sites and receptor-channels are displaced by 40 nm. Furthermore, the rising and decaying phases of AMPA receptor-mediated EPSC becomes faster, consistent with experimental data. NMDA receptors have a higher affinity than AMPA receptors, and thus, are less sensitive to such changes in relative position. If glutamate is delivered to the synapse by means of perfusion (i.e., via the extracellular space and not via the point-source of the release site), then the distribution of glutamate becomes more uniform and the influence of receptor location is reduced for both AMPA- and NMDA-mediated EPSCs, as was previously reported experimentally [21].

More recently, the model has been extended to incorporate postsynaptic metabotropic glutamate receptors type I; this has required the addition of the second messenger pathway activated by this class of receptors, i.e., the phospholipase C (PLC)/inositol-triphosphate (IP3)/IP3 receptors/calcium release from the endoplasmic reticulum (ER) in the postsynaptic structure. We have also added a calcium-ATPase located in the ER as well as a Na-Ca exchanger and a calcium-dependent K^+ channel in the postsynaptic membrane. The models for all these elements were provided in the literature [9,16,17] and the structure of EONS allowed their relatively rapid integration in the synaptic model. The goals of the project are to further extend this synaptic model to incorporate many more molecular elements, including glycine and glutamate transporters in surrounding glial cells, presynaptic postsynaptic, glial metabotropic receptors, cannabinoid receptors, muscarinic and nicotinic receptors, as well as acetylcholinesterases (Fig. 1). Ultimately, we also will incorporate a model of LTP based on calcium dynamics in the dendritic spine in order to test the current hypotheses regarding the mechanisms underlying LTP induction, maintenance and consolidation. In particular, one of us proposed many years ago that LTP was due to an increase in the number of AMPA receptors in postsynaptic membranes. There has also been some debate regarding whether or not NMDA receptors were modified as a result of LTP induction. The following sections will therefore discuss the relationships between number of available AMPA receptors and size of EPSPs recorded in dendritic spines and the links between the number of AMPA receptors and the size of NMDA receptor-mediated currents.

3. Influence of AMPA Receptor Number on Synaptic Responses

We first tested the relationship between the number of postsynaptic AMPA receptors and the corresponding postsynaptic currents they were generating in response to single pulse stimulation of the presynaptic element (Fig. 2). As expected, the total current was linearly related to the number of AMPA receptors. We then examined the relationship between the number of AMPA receptors and the EPSP generated in the dendritic spine (Fig. 2). Again, there was a linear relationship between the maximal amplitude of the EPSP and the number of AMPA receptors. It was also of interest to determine the relationship between the number of AMPA receptors and the current elicited by NMDA receptors, since this response is critically dependent on the postsynaptic membrane potential (Fig. 2). Under our basal simulation conditions and with the minimal number of AMPA receptors [9], NMDA receptor-mediated synaptic current represented about 10% of that elicited by AMPA receptors. Also, under these conditions, the size of NMDA receptor-mediated synaptic currents increased linearly with the

number of AMPA receptors. This result is interesting as it indicates that if LTP is due to an increase in AMPA receptors, LTP would be indeed expected to result in an increase in NMDA receptor-mediated synaptic currents.

4. Influence of AMPA Receptor Number on Paired-Pulses Responses

We then determine the relationship between the number of postsynaptic AMPA receptors and the characteristics of postsynaptic responses elicited by paired pulse stimulation, since this paradigm is widely used to determine changes in presynaptic transmitter release. We first selected an interval of 20 msec between the first and the second stimulation of the pair. Interestingly, the maximal current elicited by AMPA receptor activation was almost identical for the first and second pulse of the paired-pulse stimulation (Fig. 3). We assume (and this was verified as well) that the lack of facilitation is due to the desensitization of AMPA receptors that is masking the observed increase in glutamate release. We then looked at the changes in EPSPs resulting from changing the number of AMPA receptors (Fig. 3). In marked contrast with the previous result, the EPSPs exhibited clear paired-pulse facilitation and in fact, the maximal EPSP amplitude increased more in response to the second stimulation than the first with increasing number of AMPA receptors. We then determined the effects of changing the number of AMPA receptors on the responses elicited by synaptic activation of NMDA receptors with paired-pulse stimulation (Fig. 3). The figure clearly shows that, as the number of AMPA receptors increases, the response of NMDA receptors to the second stimulation increases more than to the first stimulation of the pair. This is indeed not unexpected due to the kinetics of the NMDA receptors and the properties of the voltage-dependent magnesium blockade of the NMDA receptor channels. The important point that is raised by these simulation results is that modifications of AMPA receptor number will have different consequences on responses elicited by trains of stimulation as opposed to responses elicited by single pulse stimulation.

We then decided to determine the influence of changing the number of AMPA receptors on synaptic responses elicited by paired pulse stimulation with varying intervals between the first and second stimulation (Fig. 4). We first analyzed the changes in total current elicited by AMPA receptor stimulation. At short intervals, AMPA receptor-mediated currents are reduced, presumably due to receptor desensitization. The response gradually recovers with longer interstimulus interval due to the combination of recovery from receptor desensitization and increased glutamate release. Examining the changes in maximal EPSP amplitude as a function of the interval of stimulation reproduced the relatively well-described time-dependency of paired-pulse facilitation at hippocampal synapses (Fig. 4). Maximal facilitation was observed at about 20 msec interstimulus interval and decayed to no facilitation at about 150 msec interstimulus interval. Under these conditions, the increase in response to the second stimulation was indeed mostly due to the increase in the response to NMDA receptor stimulation (Fig. 4). It is interesting to note that the facilitation of the responses to NMDA receptor stimulation lasts much longer than that of the responses to AMPA receptor stimulation.

5. Simulating Patch-Clamp Responses

Finally, we decided to determine whether the simulation platform could reproduce experimental results obtained with patch-clamp recording techniques, as these techniques are widely used to study properties of synaptic transmission. Figure 5 illustrates the responses elicited by synaptic stimulation for both the AMPA and NMDA receptors recorded at different postsynaptic membrane potentials. For both AMPA and NMDA receptors, the simulation reproduced quite closely experimental data recorded by patch-clamp techniques. In particular, the responses to NMDA receptor stimulation exhibited decreased current at hyperpolarizing potentials and responses reverted to more linear at membrane potentials around -30 mV. It is

also interesting to note that the results of the simulation suggest that in the experimental conditions, about 15 synapses could have been stimulated for AMPA receptor-mediated responses, while over 100 would have been stimulated for NMDA receptor-mediated responses (the results were not obtained in the same study, so it is difficult to make any strong conclusion regarding this difference).

6. Discussion

Glutamate is the major excitatory neurotransmitter in the brain and it is therefore extremely important to have a complete understanding of the properties of glutamatergic synapses. Unfortunately, the currently available techniques do not allow direct recording of individual synapses, and most of what is known regarding glutamatergic synapses is inferred from recording synaptically-activated events most of the time in remote cell bodies or at best in large dendrites. Our objectives were therefore to develop a detailed model of such synapses that could provide a tool to better understand the roles of the different elements known to be present at these synapses first in synaptic transmission and also in synaptic plasticity and synaptic pathology. In particular, glutamatergic synapses have been extensively demonstrated to exhibit various forms of activity-dependent modifications, such as long-term potentiation (LTP) following brief trains of high frequency stimulation and long-term depression (LTD) following long trains of low frequency stimulation. These processes are widely believed to participate in information storage, hence, a second objective of our project was to use this simulation platform to better understand mechanisms underlying learning and memory. Finally, numerous neurological and neuropsychiatric diseases are associated with learning and memory impairment, and a final goal of our approach was to better understand the pathological processes underlying these cognitive deficits and potential ways to remedy these deficits.

Obviously, one of the advantages of the simulation approach is the ability to rapidly test the impact of any parameter present in the model on any output of the model, something that cannot possibly be done experimentally. For this analysis to be meaningful, requires that the model is validated by comparison with experimental results. We do realize that this is a formidable task, since, as we mentioned above, the experimental data needed to validate synaptic events are not necessarily available. Nevertheless, we believe that the integration of as many data as possible will provide the necessary validation of our platform. As illustrated in this paper, the platform does reproduce many features of glutamatergic synaptic transmission. In particular, the platform produces reasonably accurate responses in a patch-clamp mode where postsynaptic membrane potential is imposed and currents generated by synaptic activation of AMPA or NMDA receptors are simulated. At this point, it is not clear whether the slight differences observed between the simulated and the experimental data are due to the choice of some parameters in the model, differences in magnesium concentration, or the distortion generated by dendritic propagation of currents.

The platform also indicated that the kinetics of the ionotropic glutamate receptors are responsible for the time-course of the EPSP at glutamatergic synapses, with the rate of receptor desensitization playing a much larger role than the rate of glutamate clearance from the synaptic cleft, as previously reported [19]. Similarly, the platform reproduced the time-course for paired-pulse facilitation reported by many authors. Interestingly, the simulation indicated that paired-pulse facilitation was mostly mediated by increased activation of NMDA receptors during the second stimulus, while the responses to AMPA receptors were mostly decreased presumably due to prolonged receptor desensitization. Experimental data analyzing paired-pulse facilitation at single synapses have indicated that both AMPA and NMDA receptor-mediated responses are increased due to increased glutamate release at the single synaptic site [12]. These results suggest that the desensitization parameters we used do not adequately reflect

the situation present at CA3-CA1 synapses, or that some other parameter(s) of the models need to be better adjusted.

Another important issue related to the mechanisms underlying LTP is related to the respective changes in AMPA and NMDA receptors resulting from tetanic stimulation of the Schaffer collateral inputs to CA1. The prevalent models of LTP all indicate that LTP is mediated, at least in part, by an increase in the number of post-synaptic AMPA receptors. However, conflicting results have been reported regarding changes in NMDA receptor-mediated responses following LTP [11,20]. Our simulation results clearly indicate that an increase in the number of AMPA receptors is associated with increased EPSP. It will be interesting to test whether the platform can reproduce a previously reported experimental result suggesting that LTP alters the modulatory effects of compounds acting on AMPA receptor desensitization [7]. Our results indicate that increasing the number of AMPA receptors increases the size of NMDA receptor-mediated synaptic responses, in the absence of modifications in the number of NMDA receptors. This effect is amplified when performing paired-pulse stimulation. This result stresses the need to be careful when determining the NMDA receptor-dependent component of synaptic responses. Using the difference between the total synaptic response and that measured in the presence of a blocker of NMDA receptors is likely to produce a different result than estimating the synaptic response measured in the presence of a blocker of AMPA receptors.

The results reported here are only brief examples of the results that can be generated by using the simulation platform. In separate studies, we have analyzed the effects of a number of compounds affecting different elements incorporated in the model and compared simulated results with available experimental data.

Future work will incorporate in the platform additional molecular elements including various transporters (glutamate and glycine transporters), other neuro-modulators (such as adenosine and endocannabinoids) that produce presynaptic modulation of glutamate release by acting on various presynaptic mechanisms, and additional second messenger pathways important for LTP induction and maintenance (cAMP, protein kinases and phosphatases). Moreover, future work will also be directed at incorporating the synaptic model into a neuron model in order to simulate a minimum network of neurons comprising both pyramidal neurons and various interneurons.

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Fig. 1. Schematic representation of the model of a glutamatergic synapse being developed at USC and Rhenovia. The model incorporates a presynaptic element, a postsynaptic element with numerous types of receptors and several second messenger pathways, and a glial element incorporating both glutamate and glycine transporters.

Fig. 2. Effects of changes in AMPA receptor number on EPSP and NMDA receptor-mediated synaptic responses. (a, b). Relationship between number of AMPA receptors and AMPA receptor-mediated synaptic responses. (c, d). Relationship between number of AMPA receptors and EPSP. (e, f). Relationship between number of AMPA receptors and NMDA receptor-mediated synaptic responses.

(a)

(b)

(c)

(d)

Fig. 3. Changes in EPSP elicited by paired pulse stimulation (20 msec interpulse interval) as a function of number of AMPA receptors. (a) Changes in EPSP; (b) Changes in AMPA receptor current; (c) Changes in NMDA receptor-mediated synaptic responses; (d) Changes in maximal EPSP.

Fig. 4. Changes in EPSP elicited by paired pulse stimulation as a function of interstimulus intervals. (a) Changes in EPSP; (b) Changes in maximal AMPA receptor-mediated synaptic responses; (c) Changes in maximal NMDA receptor-mediated synaptic responses; (d) Changes in maximal EPSP.

Fig. 5. Patch-clamp: superimposed experimental data [3] (for AMPA receptors) and [10] (for NMDA receptors) and simulation for AMPA and NMDA receptor-mediated synaptic currents as a function of postsynaptic potential.