Thesis title: Mechanisms underlying Signal Filtering at a Multisynapse Contact

Author: Timotheus Budisantoso

Summary

Neurons communicate with each other via specialized structures called synapses. They are crucial to the biological computations that underlie perception and thought. These connections have the ability to change in strength within a short-time interval in response to transmission over synaptic pathways, hence called short-term plasticity. This fundamental phenomenon of information processing modification is strikingly apparent in the retinogeniculate (RG) synapse between the optic fiber and the relay cell in the dorsal lateral geniculate nucleus (LGN). This synapse receives visual signals created in the retina and passes them onto the visual cortex. As a result of short-term depression where the strength of the second response is strongly reduced at short time intervals, postsynaptic relay cells (RC) produce much less action potentials than the retinal ganglion cells, and profound filtering and processing of visual signal is realized. However, mechanisms underlying short-term depression are unclear.

In most cases synaptic transmission is reflected by the same general sequence of events leading to neurotransmitter release: An action potential is initiated in the axon near the cell body, it propagates down the axon, voltage-gated calcium channels in the presynaptic terminal open and admit calcium, and this triggers vesicle fusion. Liberated neurotransmitter diffuses in the synaptic cleft and binds to postsynaptic AMPA receptors (AMPARs) and this influences the firing of the postsynaptic neuron.

A feature I focused on, as a source of the filtering, is the morphological constriction of transmitter diffusion in the synaptic cleft. A single RG fiber makes multiple contacts with a RC dendrite, of which each terminal consists of a large presynaptic bouton that makes a broad contact onto the postsynaptic dendrite of a RC with multiple synaptic sites within the contact. Transmitter released into the RG synaptic cleft would have nowhere else to go apart from diffusing laterally in two dimensions for long distances.

My hypothesis is that this could lead to intersynaptic spillover occurring in the range of a few hundred nm to a couple of μm. Because of the broad contact area of the RG terminal without much intervention of glial processes, spillover of glutamate to the neighboring synapse likely occurs at sufficient concentration and duration to promote desensitization of AMPARs. This mechanism of short-term depression implies that AMPARs enter a liganded (bound to glutamate) but closed (desensitized) state with a characteristic time course, resulting in a decrease of the strength of the second response.

To demonstrate this, I integrated electrophysiology to assess the properties of signal transmission, SDS-digested freeze-fracture replica labeling to quantify the receptor and transporter distribution, EM reconstruction to describe the 3D structure, and simulations to analyze the impact of transmitter diffusion on the activity of the receptors. Relevant for the simulations and our understanding of the effect of transmitter diffusion on signal transmission, I established the spatiotemporal concentration profile of glutamate diffusion reflected by the number of glutamate in a vesicle (N_{Glu}) and its diffusion coefficient (D_{Glu}) in the extracellular space, using the calyx of Held synapse as a model.

I identified that a large contributor to the filtering is the marked paired-pulse depression at this synapse which is intensified by the morphological characteristics of the contacts. Reconstruction of the three dimensional ultrastructure of the RG terminal shows a broad contact area of the RG terminal where multiple synapses are concentrated without much intervention of glial processes. Such morphology suggests that diffusion of glutamate in the extracellular space is restricted to two dimensions for some distances, and spillover of glutamate to the neighboring synapse likely occurs at sufficient concentration and duration to promote desensitization of AMPARs. CTZ that blocks desensitization of AMPARs, significantly relieved paired-pulse depression (PPD) for interstimulus interval (ISI) of up to 500 ms. CTZ elevated the degree of PPD especially at ISI < 150 ms, which clearly resulted in a faster recovery time course. Replica data demonstrated an average nearest neighbor distance of 569 nm, which is so close that intersynaptic spillover is likely to take place. At this distance glutamate spillover was demonstrated by simulations that also showed that spillover from single or multiple vesicle release at individual synapses causes significantly more depression in the response than when spillover does not occur. In support,

experiments were done where the decay of the AMPA EPSC in a situation of multiple vesicle release (MVR; 2 mM extracellular [Ca]) is compared with that in a situation of single vesicle release (0.5 mM extracellular [Ca]) in the presence of a desensitization blocker called Aniracetam. Decay was more prolonged in a MVR situation, suggesting glutamate spillover. More evidence derived from experiments where glial transporters were blocked by applying TBOA or DHK (blocker of GLT-1) which resulted in more depression of the response up to ISI 150 ms. 3D reconstructions showed that bouton contacts from the same axon are often closely located to each other but separated by glial processes. Thus, blocking glial transporters causes more desensitization resulting from diffusion between RG bouton contacts. This implies the occurrence of intersynaptic spillover within a bouton contact.

In addition to the close morphological arrangement of synapses that invites glutamate spillover, presynaptic factors contributed to the desensitization, such as a high Pr (0.7 ± 0.03) which was determined using mean-variance analysis of the EPSC amplitude in different $[Ca^{2+}]_o$. I also found that γ-DGG block was significantly less in our control conditions (2 mM $[Ca^{2+}]_o$) compared with the conditions where Pr was reduced by decreasing the $[Ca^{2+}]_o$. This result suggests MVR in the RG terminal. A high Pr and MVR would cause a large glutamate transient in the synaptic cleft intensifying AMPAR desensitization and facilitating spillover.

Postsynaptic factors involve the properties of postsynaptic receptors. Outside-out patch experiments showed slow recovery kinetics from desensitization of the AMPARs (~500 ms). Bath application of 1-naphthyl acetyl spermine (NASPM), a subunit-specific antagonist of GluR2-lacking AMPARs, significantly blocked the first EPSC with 46.6 ± 3.1%, suggesting that glutamatergic AMPAR EPSCs were mediated by both Ca^{2+}-permeable and Ca^{2+}-impermeable AMPARs at the RG synapse with roughly a ratio of 1:1. Interestingly, I discovered that the EPSCs were significantly less depressed in NASPM at all time points studied, suggesting that the kinetic properties of the CP-AMPARs were partially responsible for the long lasting depression. Making use of the availability of GluR1 KO mice, I observed a lower degree of desensitization at ISIs of 150 and 500 ms for GluR1 KO mice compared with wild-type mice, suggesting that the GluR1 subunit is partially responsible for the particularly long-lasting desensitization.

The pre-and postsynaptic factors and, in particular, the morphological features discussed above contribute to the degradation of high temporal precision of signals created at the retinal ganglion cells. These features contrast with many other synapses where spatiotemporal spread of

transmitter is limited by rapid transmitter clearance allowing synapses to operate more independently.

In addition, I established the spatiotemporal profile of neurotransmitter concentration following synaptic vesicular release which is essential for our understanding of inter-neuronal communication. Such profile is a fundamental determinant of synaptic strength, short-term plasticity, and inter-synaptic crosstalk of signals via transmitter spillover. Although previous studies have suggested that synaptically released glutamate reaches a few mM in concentration which lasts for <1 millisecond, such estimates often treat the synaptic cleft as a single concentration compartment, whereas a huge concentration gradient of glutamate is likely created within the synaptic cleft upon exocytosis. To describe this gradient, the number of glutamate in a vesicle (N_{Glu}) and its diffusion coefficient (D_{Glu}) in the extracellular space need to be determined. Because it is technically very difficult to determine this gradient in RG synapses, the calyx of Held synapse of rat at postnatal day 12-16 was chosen as a model to estimate N_{Glu} and D_{Glu} because of the morphological advantages and electrophysiological accessibility of this synapse. It is a synapse where diffusion of glutamate occurs two-dimensionally and where the number and the distribution of postsynaptic AMPA receptors on MNTB principal cells can be relatively easily and clearly visualized using SDS-digested freeze-fracture replica labeling. To assess the performance of these receptors as glutamate sensors, a kinetic model of the receptors was constructed from outside-out patch recordings. From here, I simulated synaptic responses and compared them with the EPSC recordings. This approach narrowed down the range of N_{Glu} and D_{Glu} to an optimal value of 7000 and 0.3 μm^2 ms^{-1}, respectively. Such value would further support the occurrence of glutamate spillover between RG synapses within a bouton contact. Further simulations showed that a single vesicle did not saturate the synaptic receptors, and that spillover of glutamate did not affect the conductance amplitude at this particular synapse.

Instinctively, contacts with multiple release sites with high Pr and MVR may seem to ensure reliable transmission. It is true that having multiple synapses within a close range would guarantee the release of at least a vesicle at such a contact with almost no failure. However, as for producing reliable current input for ensuring high-fidelity of postsynaptic spiking corresponding to presynaptic spiking at high frequencies, this situation seemed to be

disadvantageous. If the number of synapses that one fiber can create on a postsynaptic cell is limited, then high Pr would be necessary to produce large enough current to drive spiking. At the RG synapse, large current input seemed to be required to produce any spike if the postsynaptic Relay cell was resting at deep potentials, and a high Pr at this synapse is therefore favorable, but at the expense of high-fidelity spiking.

My study revealed a critical role of synaptic ultrastructure for AMPAR desensitization, which affects signal filtering and ultimately visual information processing.The data are significant for our understanding that each type of synapse has specializations that result in functional differences. I show that the RG synapse is equipped with morphological specializations that are intuitively unpreferable for fast reliable signal transmission. In addition, it opens a new possible window in the mechanisms of neurological diseases that interfere with synaptic morphology. Furthermore, this study advances the field of computer modeling of synaptic transmission by incorporating detailed morphological analysis provided by FFR technique.