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Competing on the edge

Massimo Scanziani

Fast-acting neurotransmitters can exit the synaptic cleft and bind to extrasynaptic receptors. This process is modulated by transmitter uptake mechanisms (transporters). A new study focusing on glutamate-mediated transmission in the cerebellum describes the specific role of neuronal transporters in modulating the access of glutamate to extrasynaptic metabotropic glutamate receptors, and reveals important consequences of extrasynaptic signaling on synaptic plasticity.

Spatial confinement

Diffusible molecules represent a major means of communication between cells. Cells release chemical messages into the extracellular space and these reach their targets by passive diffusion or by active transport (e.g. in the blood). The extent of influence of a released substance is determined by its concentration gradient and the sensitivity of its targets. This form of communication, however, does not permit discrimination between targets within the sphere of influence, just as a speaker before an audience cannot address one individual to the exclusion of the others.

Neurons have developed a way to select their targets, namely by approaching them with an axon and releasing the transmitter substance at the point of closest contact (as if two members of the audience were whispering into each other's ears). This morphological configuration (the synapse) allows rapid and spatially confined action of the released substance, thus making neurons fast and precise communicators. The degree of spatial precision is impressive if one considers that subcellular membrane compartments as small as a fraction of 1 μm^2 (the postsynaptic density of a dendritic spine) can be selectively targeted by diffusible molecules [1]. The spatial confinement of these diffusible molecules is not absolute, however, as the compartment in which neurotransmitter is released, the synaptic cleft, forms a continuum with the extracellular space [1,2]. Thus, released neurotransmitter substances can potentially diffuse out of the cleft to also reach targets that are not juxtaposed. This escape of transmitter is mitigated by proteins responsible for the rapid degradation or sequestration of the released substances. For example, at CNS excitatory synapses, a glutamate molecule leaving the cleft is likely to be captured by specialized transporter proteins concentrated around the cleft on cell membranes of glia and neurons [3]. By acting as a sink, such transporters not only ensure the spatially restricted action of glutamate, but also might hasten the clearance of transmitter from the cleft, thus reducing the probability of rebinding to receptors or accumulation during repetitive release [4–9].

Escaping from the cleft

Do molecules of glutamate exiting the cleft ever escape sequestration by uptake proteins? And if so, what are the consequences? Immunohistochemical studies show that at least one class of receptors for glutamate, the metabotropic glutamate receptors (mGLURs), are located outside of the synaptic cleft and, hence, could represent potential targets for 'escaping' glutamate molecules [10,11]. These findings have been substantiated by physiological evidence indicating that released glutamate molecules can exit the synaptic cleft, elude glutamate transporters and bind to mGLURs [12–15]. Elucidation of the interplay between extrasynaptic receptors eagerly awaiting stray transmitter molecules and the transporters trying to restrict diffusional domains will now be necessary for a more complete comprehension of neuronal signaling.

Competing on the edge

A recent study by Gabor Brasnjo and Thomas Otis [16] provides a clear example of the interplay between extrasynaptic receptors and transporters at one of the numerically predominant excitatory synapses in the brain, the contact between granule cell axons (parallel fibers) and Purkinje cell dendrites in the cerebellar cortex. Neuronal glutamate transporters (nEAAT) expressed by Purkinje cells are located on the postsynaptic membrane surrounding the synaptic cleft [17]. Interestingly, mGLURs colocalize with glutamate transporters in this perisynaptic region [10]. This overlapping distribution suggests that the transporter proteins and mGLURs compete for glutamate molecules leaving the synaptic cleft (Fig. 1).

Brasnjo and Otis showed that when glutamate release occurred in response to a single action potential, uptake was clearly the winner in this competition (as no response to mGLUR activation could be recorded in Purkinje cells). By contrast, when glutamate release occurred in rapid succession during repetitive firing, mGLUR-mediated responses were readily detected, indicating that enough glutamate molecules had managed to

escape uptake and bind to the receptors. The threshold to detect such a response was three to four action potentials, when fired at a rate of $100 \, s^{-1}$. Thus, at high firing rates, consecutive action potentials cooperate for the activation of mGLURs.

The demonstration that glutamate transporters limit the activation of mGLURs is supported by the fact that pharmacological inhibition of glutamate transporters not only greatly enhanced mGLUR-mediated synaptic responses, but also lowered the threshold for detection of the response to two action potentials. However, the blockage of transporters by bath perfusion of the antagonist does not allow us to distinguish whether the increased mGLUR response was due to the inhibition of transporters located on the postsynaptic membrane, on glial cells surrounding the synapse, on neighboring neurons or, possibly, on the presynaptic terminal. Brasnjo and Otis elegantly addressed this question by showing that selective block of the transporters located on the recorded Purkinje cell (by dialysis of a non-transportable cation through the recording pipette) increased the mGLUR-mediated response. This indicates that most of the competition for glutamate between transporters and mGLURs occurs on the postsynaptic membrane at the edge of the cleft.

Thus, each Purkinje cell has the potential to regulate the degree of activation of its own mGLURs, by modulating the activity or the expression of its glutamate transporters. In other words, because mGLURs are located outside of the synaptic cleft, their activation can be finely tuned by uptake proteins – a principle that has also been observed for GABA-mediated transmission through G-protein-coupled GABA_B receptors [18–20]. Furthermore, extrasynaptic or perisynaptic receptors might sense transmitter originating from several adjacent presynaptic terminals, and could thereby represent a way for terminals to cooperate in producing a postsynaptic response [18–20].

Controlling LTD from the outside

Why should the activation of mGLURs surrounding parallel fiber–Purkinje cell synapses be controlled in such a subtle manner? The answer probably lies in the fact that activation of mGLURs is necessary for induction of LTD at parallel
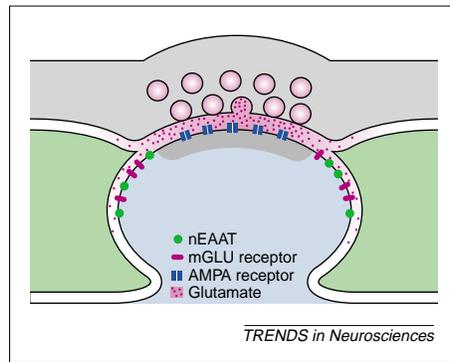

Fig. 1. Intrasynaptic and perisynaptic glutamatergic transmission at the synapse between a parallel fiber and a Purkinje cell. The spine is contacted by an *en passant* bouton and is surrounded by glial processes. The spatial segregation between ionotropic (AMPA) and metabotropic (mGLU) glutamate-receptor subtypes allows differential control of their activation by neuronal glutamate transporters (nEAAT). Using data from Refs [10,17,23].

fiber–Purkinje cell synapses [21,22]. Thus, in a final series of experiments, Brasnjo and Otis showed that shifting the competition for glutamate in favor of mGLURs (by pharmacologically blocking glutamate transporters) greatly enhanced the magnitude of LTD expressed at this synapse. Because of the likely involvement of cerebellar LTD in motor-task learning [22], this result gives an indication of how neuronal signaling at the edge of a synapse could influence the adaptive behavior of an organism.

In conclusion, besides contributing to the clarification of the specific roles of neuronal glutamate transporters at excitatory connections, the study by Brasnjo and Otis further demonstrates that synaptic transmission at the edge of the synapse is not a peripheral matter.

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