Summary of experiment from Hires SA, Zhu Y, Tsien R (2008)

Optical measurement of synaptic glutamate spillover and reuptake by linker optimized glutamate-sensitive fluorescent reporters

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The popular image of neurotransmission is illustrated in **Fig. 1**: neurotransmitters are released from a nerve terminal into the synaptic cleft, to be sensed by receptors on the opposing membrane. However, the neurotransmitter glutamate (also an amino acid) has a different fate. It is known to diffuse away from the synaptic cleft and activate receptors on adjacent nerve cells (Isaacson, 1999).

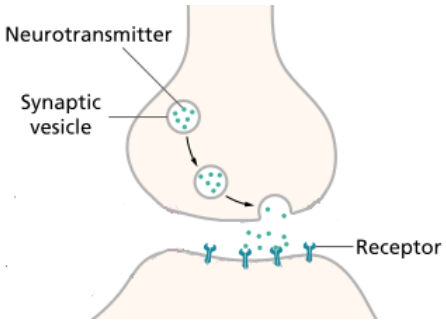

Fig. 1: Classical neurotransmitter release and perception. Image modified from Neurotransmitter (Wikipedia) by Thomas Splettstoesser [CC BY-SA 4.0]

While this phenomenon is unquestionably of physiological significance, it has proven difficult to quantitate both the amount of glutamate spilling over outside the synapse and the duration of the effect. Hires et al (2008) used a new glutamate-sensing protein to measure glutamate concentrations in real time in and around living nerve cells.

To measure glutamate near living cells, a glutamate-sensing protein was constructed (**Fig. 2**), consisting of a glutamate-binding protein from E. coli flanked by two proteins capable of fluorescence. One, cyan-fluorescent protein (CFP, derived from a naturally fluorescing protein from a jellyfish) absorbs violet light and fluoresces blue-green light. A second, yellow-fluorescent protein (YFP, derived from the same jellyfish protein) absorbs green light and emits yellow-green light. The binding of glutamate causes the two fluorescent proteins to orient such that some of the blue-green light emitted by CFP is absorbed by YFP, producing yellow-green emission. The ratio of yellow emission to blue-green emission is therefore low when glutamate is absent and high when glutamate is present. The chimeric protein was directed to the cell membrane by fusing it to growth factor receptor protein from blood platelets.

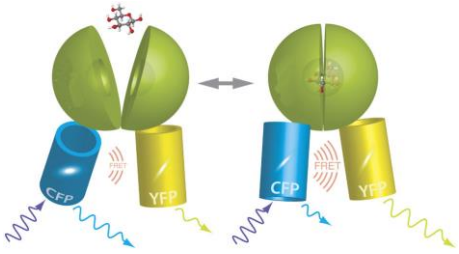

Fig. 2: Components and activity of glutamate-sensing protein. The protein consists of cyan-fluorescent protein (CFP) attached to glutamate-binding protein (green sphere) attached to yellow-fluorescent protein (YFP) attached to a membrane spanning protein (not shown). Two forms are shown: (left) not bound to glutamate (small molecule above the sphere) and (right) bound to glutamate (inside the sphere). Image modified from Hou et al (2011).

The gene encoding this construct was inserted into cultured rat neuron cells, and the neurons were electrically stimulated and imaged with violet light, measuring CFP and YFP emission. **Fig. 3** shows the combined emission from both CFP and YFP portions of the chimeric protein. Before stimulation, emission is blue-green, indicating that the chimeric protein was largely unbound. Immediately after stimulation and for 0.07 seconds thereafter, yellow fluorescence appeared over the length of the neurons, indicating that the majority of chimeric proteins had bound glutamate.

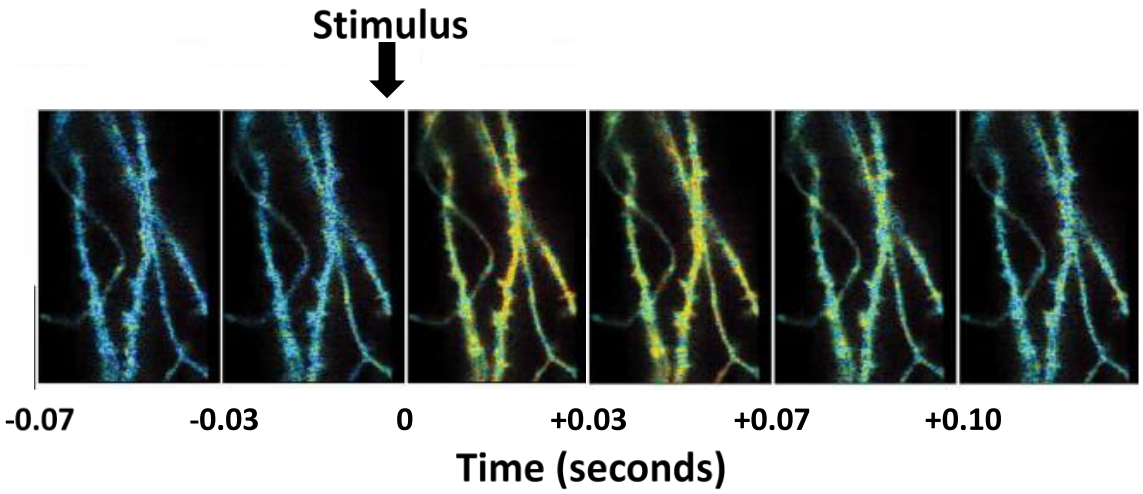

Fig. 3: Combined CFP and YFP emission before and after electrical stimulation of neurons expressing the chimeric protein on the cell surface. Modified from Figure 3c from Hires et al (2008).

This fluorescence largely disappeared 0.10 seconds after stimulation, and the neurons returned to the same state as before stimulation.

I don't know how frequently synapses occur, but if they are sparsely distributed over the neurons shown in **Fig.3**, then it is evident that spillover of glutamate from synaptic clefts is a transient phenomenon, with most of the glutamate taken up by the cells within 0.1 seconds.

REFERENCES

Hires SA, Zhu Y, Tsien R (2008). Optical measurement of synaptic glutamate spillover and reuptake by linker optimized glutamate-sensitive fluorescent reporters. Proc Natl Acad Sci USA 105:4411–4416.

Hou B-H, Takanaga H, Grossmann G, Chen L-Q, Qu X-Q, Jones AM, Lalonde S, Schweissgut O, Wiechert W, Frommer WB (2011). Optical sensors for monitoring dynamic changes of intracellular metabolite levels in mammalian cells. Nature Protocols 6:1818-1833.

Isaacson JS (1999). Glutamate spillover mediates excitatory transmission in the rat olfactory bulb. Neuron 23:377-384.