

Using photo-activation and two channel FRAP to quantify glutamate receptor mobility *in vivo*

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**KEY WORDS:** Living cells, *in vivo* labeling, protein turnover, FRAP, photo-activation, glutamate receptors

**Abstract:**

Both the fundamental mode of synapses' new formation as well as the regulation of glutamate receptor mobility during synapse maturation is poorly understood. This is mainly due to the reason that so far these processes could hardly be studied *in vivo*. Therefore we established protocols that allowed us to observe the formation of additional glutamate receptor fields and to measure the mobility of receptors *in vivo*. Using transgenic expression of genomic constructs of GFP-labeled glutamate receptors we were able to study these processes during the functional and structural strengthening of *Drosophila* neuromuscular junctions.

Confocal time series obtained from intact, living larvae revealed that small receptor fields form independently of preexisting synapses to then grow to a mature size. Already at a very small size these fields seem to represent the postsynaptic part of functional synapses: they colocalize with presynaptic label for the *Drosophila* active zone protein AZP180 and with presynaptic pools of recycling neurotransmitter vesicles. Using photo-activation of GFP and a two channel FRAP technique we could show *in vivo* that the glutamate receptor subunit GluRIIA is very selectively integrated into new and growing receptor fields from diffuse extrasynaptic pools. This suggests that growth of these small fields is directly driven by the entry of GluRIIA, which is consistent with previous results suggesting that GluRIIA is rate-limiting for new synapse formation. Once matured, the receptor fields of *Drosophila* neuromuscular synapses seem to be remarkably stable entities that show little entry and exit of glutamate receptors.

We will present our protocols for imaging, the image processing as well as the quantification algorithms. The described techniques make *Drosophila* neuromuscular synapses an attractive model to study many aspects of cellular neuroscience in the natural environment of an intact organism.