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PII: S0165-0270(18)30144-4
DOI: https://doi.org/10.1016/j.jneumeth.2018.05.014
Reference: NSM 8010

To appear in: *Journal of Neuroscience Methods*

Received date: 6-1-2018
Revised date: 19-5-2018
Accepted date: 19-5-2018

Please cite this article as: Shearin HK, Quinn CD, Mackin RD, Macdonald IS, Stowers RS, t-GRASP, a targeted GRASP for assessing neuronal connectivity, *Journal of Neuroscience Methods* (2018), https://doi.org/10.1016/j.jneumeth.2018.05.014

Research Paper

t-GRASP, a targeted GRASP for assessing neuronal connectivity

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Highlights

- split-GFP fragments were targeted to each side of the synapse.
- targeting enhanced specificity of fluorescence signal to synaptic contact sites.
- compatible with all three Drosophila binary transcription systems.
- potential use of GRASP significantly broadened for Drosophila.

Abstract

Background. Understanding how behaviors are generated by neural circuits requires knowledge of the synaptic connections between the composite neurons. Methods for mapping synaptic connections, such as electron microscopy and paired recordings, are labor intensive and alternative methods are thus desirable.
New Method. Development of a targeted GFP Reconstitution Across Synaptic Partners(GRASP) method, t-GRASP, for assessing neural connectivity is described.
Results. Numerous different pre-synaptic and post-synaptic/dendritic proteins were tested for enhancing the specificity of GRASP signal to synaptic regions. Pairing of both targeted pre- and post-t-GRASP constructs resulted in strong preferential GRASP signal in synaptic regions in Drosophila larval sensory neurons, larval neuromuscular junctions, and adult photoreceptor neurons with minimal false-positive signal.
Comparison with Existing Methods. Activity-independent t-GRASP exhibits an enhancement of GRASP signal specificity for synaptic contact sites as compared to existing Drosophila GRASP methods. Fly strains were developed for expression of both pre- and post-t-GRASP with each of the three Drosophila binary transcription systems, thus enabling GRASP assays to be performed between any two driver pairs of any transcription system in either direction, an option not available for existing Drosophila GRASP methods.
Conclusions. t-GRASP is a novel targeted GRASP method for assessing synaptic connectivity between Drosophila neurons. Its flexibility of use with all three Drosophila binary transcription systems significantly expands the potential use of GRASP in Drosophila.

Keywords: Drosophila; synapse; photoreceptor; green fluorescent protein; lamina; ventral nerve cord