

## **PRESYNAPTIC TRAFFIC OF TETANUS NEUROTOXIN IMAGED AT THE NEUROMUSCULAR JUNCTION AND ITS MODULATION BY NEUROTROPHIC FACTORS**

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The distribution, dynamics, internalization and retrograde axonal traffic of a fusion protein composed of green fluorescent protein (GFP) and the atoxic C-terminal fragment of tetanus toxin (TTC) were studied after its intramuscular injection in mice. Confocal microscopy and immunogold electron microscopy revealed that the fusion protein GFP-TTC rapidly clustered in motor nerve terminals of the neuromuscular junction. Clathrin-coated pits, and axolemma infoldings located between active zones are involved in the internalization of the GFP-TTC protein. Biochemical analysis of detergent-extracted neuromuscular preparations showed that the GFP-TTC fusion protein was associated with lipid microdomains. We suggest that GFP-TTC clustering in these lipid microdomains favours the recruitment of other proteins involved in its endocytosis and internalization in motor nerve terminals. During its retrograde trafficking, GFP-TTC accumulates in different axonal compartments from those used by cholera toxin B-subunit, suggesting that these two proteins are transported by different pathways and cargos.

The neurotrophic factors Brain Derived Neurotrophic Factor (BDNF), Glial cell line Derived Neurotrophic Factor (GDNF) and Brain Derived Neurotrophic Factor (BDNF) promote tetanus neurotoxin fusion protein internalization in motor nerve terminal determined by an increase in the relative GFP fluorescence level after GFP-TTC intramuscular injection.