Astrocyte-to-neuron transportation of Enhanced Green Fluorescent Protein in cerebral cortex requires F-actin dependent Tunneling Nanotubes

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Research Article

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Abstract

Tunneling Nanotube (TNT) , a dynamic cell-cell contact, is dependent on actin polimerization. TNTs are efficient in transporting ions, proteins and organelles intercellularly, which are important mechanisms in physiological and phathological precesses. Reported studies on the existence and function of TNTs among neural cells focus on cultured cell for the convenience in detecting TNTs' ultrastructure. In this study, the adeno-associated virus (AAV-GFAP-EGFP-p2A-cre) was injected in the cerebral cortex of knock-in mice ROSA26 GNZ. GFAP promoter initiated the expression of Enhanced Green Fluorescent Protein (EGFP) in infected astrocytes. At 10 days post injection (10 DPI), we found that EGFP could transfer from astrocytes in layer□-□ to neurons in layer □. The dissemination of EGFP was not through endocytosis or exosome. And the intercellular transportation of EGFP was F-actin dependent. Therefore, we concluded EGFP transported from astrocytes to neurons in coetex via F-actin dependent TNTs. Although it is hardly to detect the ultrastrucute of TNTs in brain for its transiency and the noisy background, we established an animal model and indirect experimental methods to explore TNTs in vivo.

Full Text

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Figures

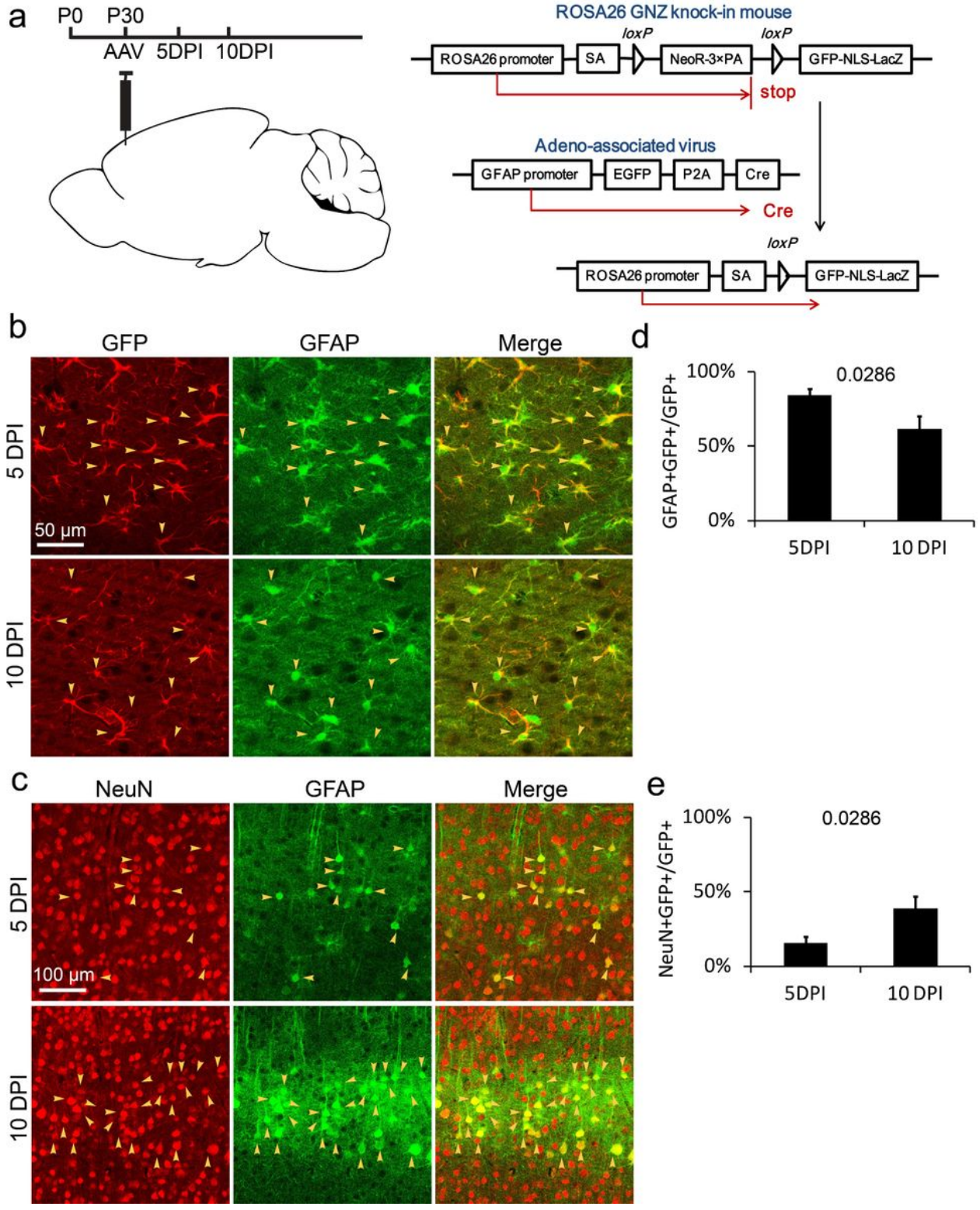

Figure 1

The existence of EGFP in the cortex. (a) Schematic illustration of stereotaxic injection with AAV-GFAP-EGFP-P2A-Cre in mouse cortex and the cre-loxP system in ROSA26 GNZ mice. AAV, Adeno-associated virus; 5 DPI, 5 days post injection of AAV; 10 DPI, 10 days post injection of AAV. (b) & (c) Detection of EGFP distribution in astrocytes and neurons 5 days and 10 days after injecting AAV. Yellow arrows, double positive cells. (d) & (e) The percentage of astrocytes and neurons in EGFP+ population at 5 DPI

and 10 DPI. Statistical analysis of significance was evaluated using unpaired two-tailed t-test, N = 3 independent mice.

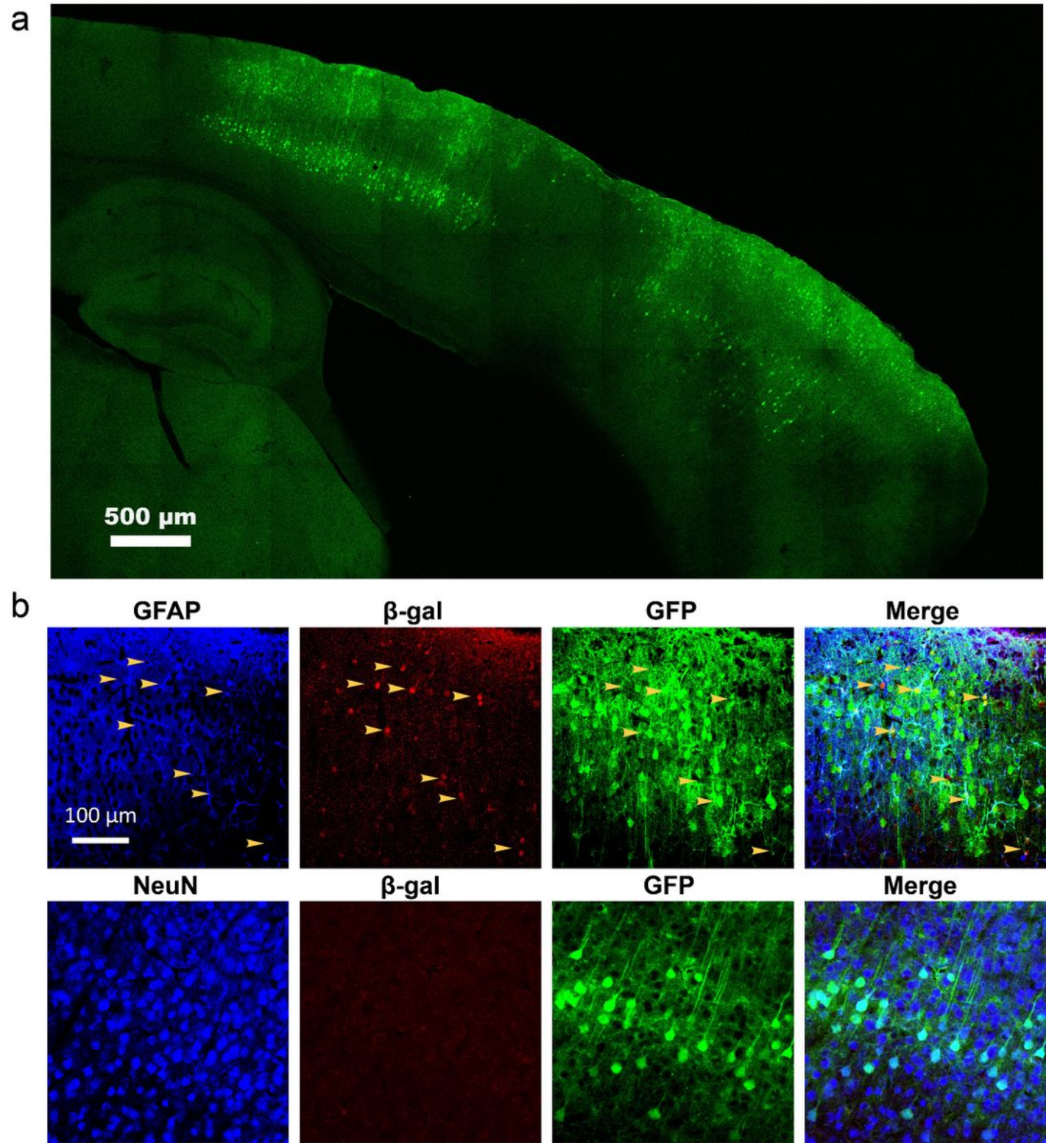

Figure 2

EGFP distribution in the cortex 10 days after injecting AAV. (a) The microscopic photo with low magnification shows EGFP+ cells in cerebral cortex. (b) Immunostainning of GFAP/NeuN, β-gal and EGFP to discriminate cells expressing EGFP or receiving transported EGFP. Yellow arrows, triple positive cells.

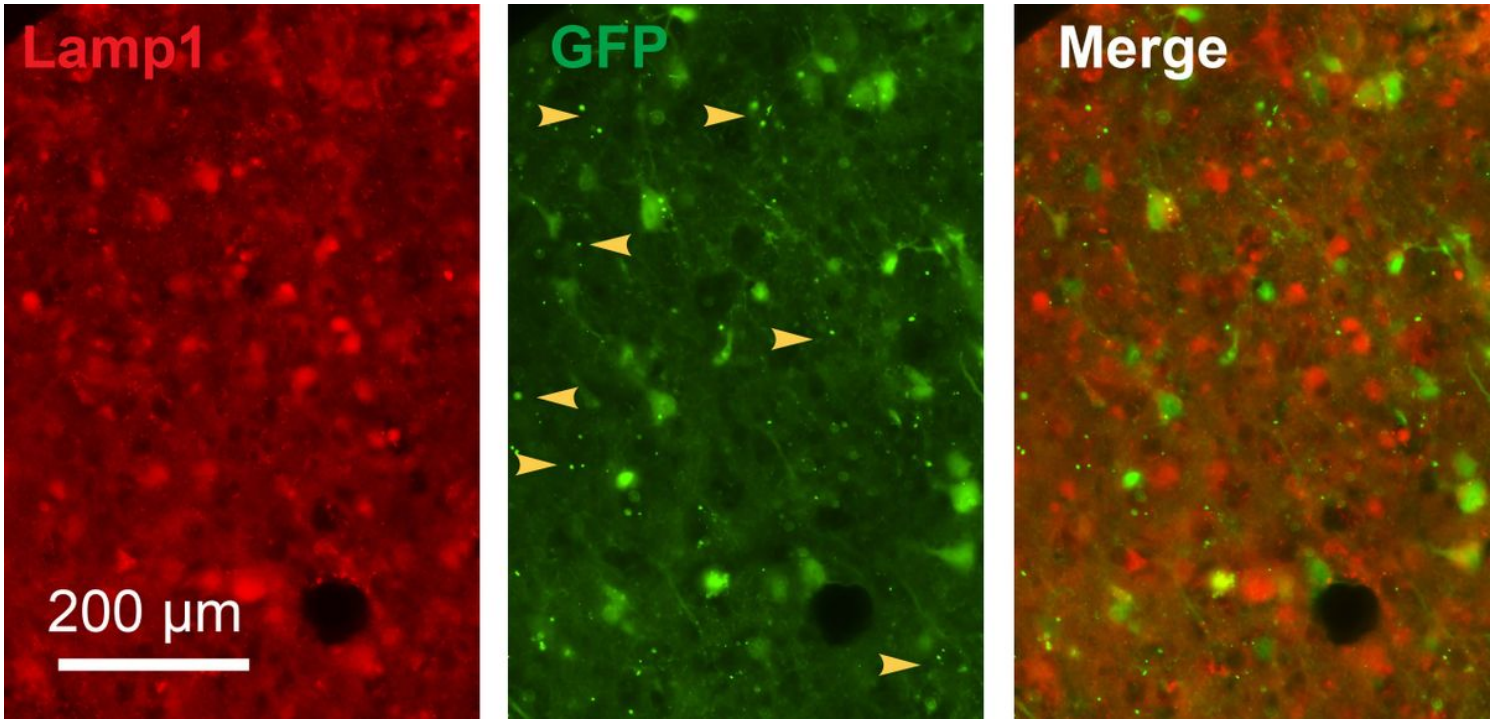

Figure 3

Immunostating of lamp1 and EGFP to demonstrate the localization of endocytotic vesicles and EGFP particles in the cortex. Yellow arrows, EGFP particles do not colocalize with lamp1.

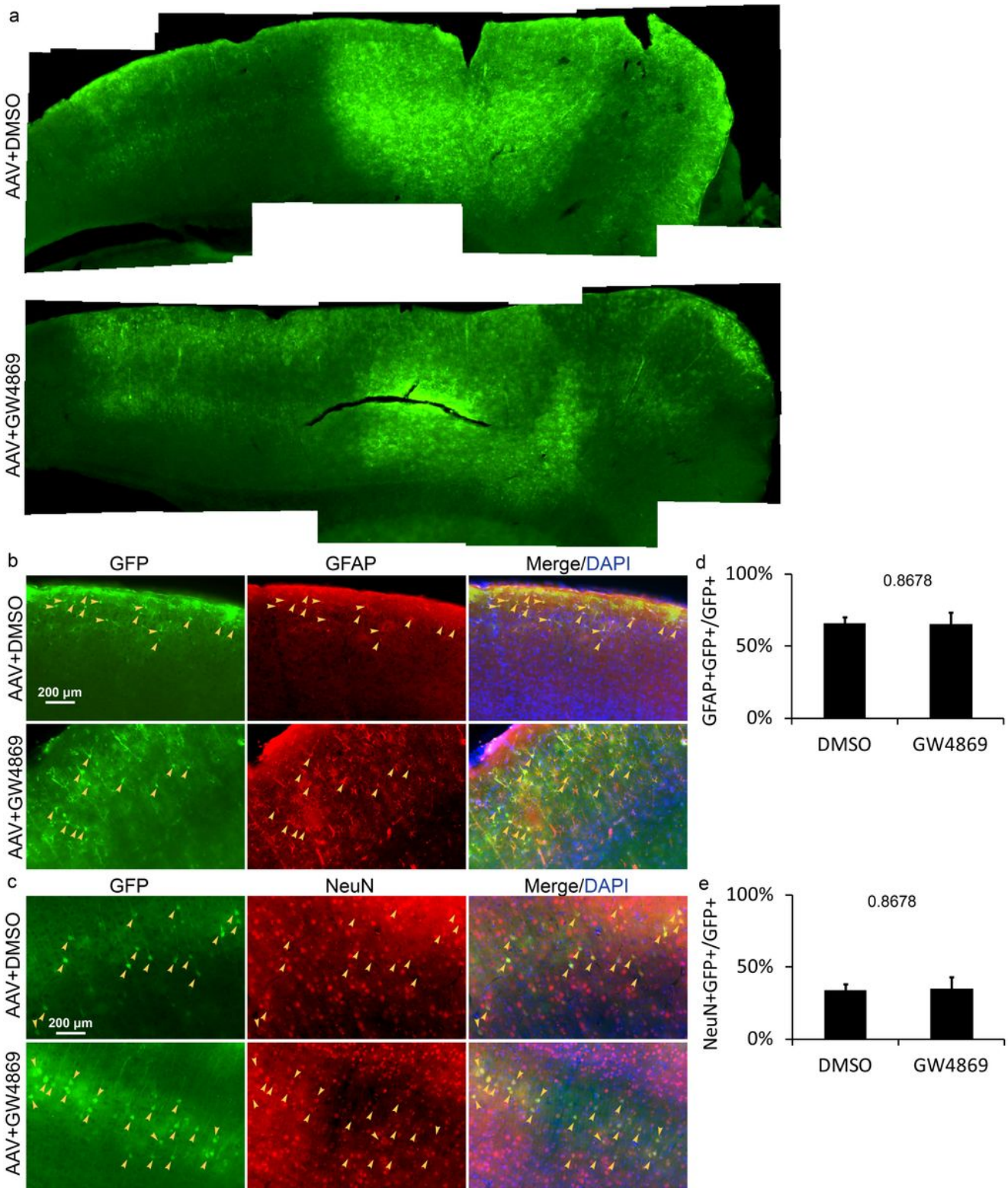

Figure 4

EFGP distribution in the cortext 10 days after injecting AAV and DMSO/GW4869. (a) The microscopic photo with low magnification shows EGFP+ cells in cerebral cortex. (b) & (c) Detection of EGFP distribution in astrocytes and neurons at 10 DPI. Yellow arrows, double positive cells. (d) & (e) The percentage of astrocytes and neurons in EGFP+ population at 10 DPI. Statistical analysis of significance was evaluated using unpaired two-tailed t test, N = 3 independent mice.

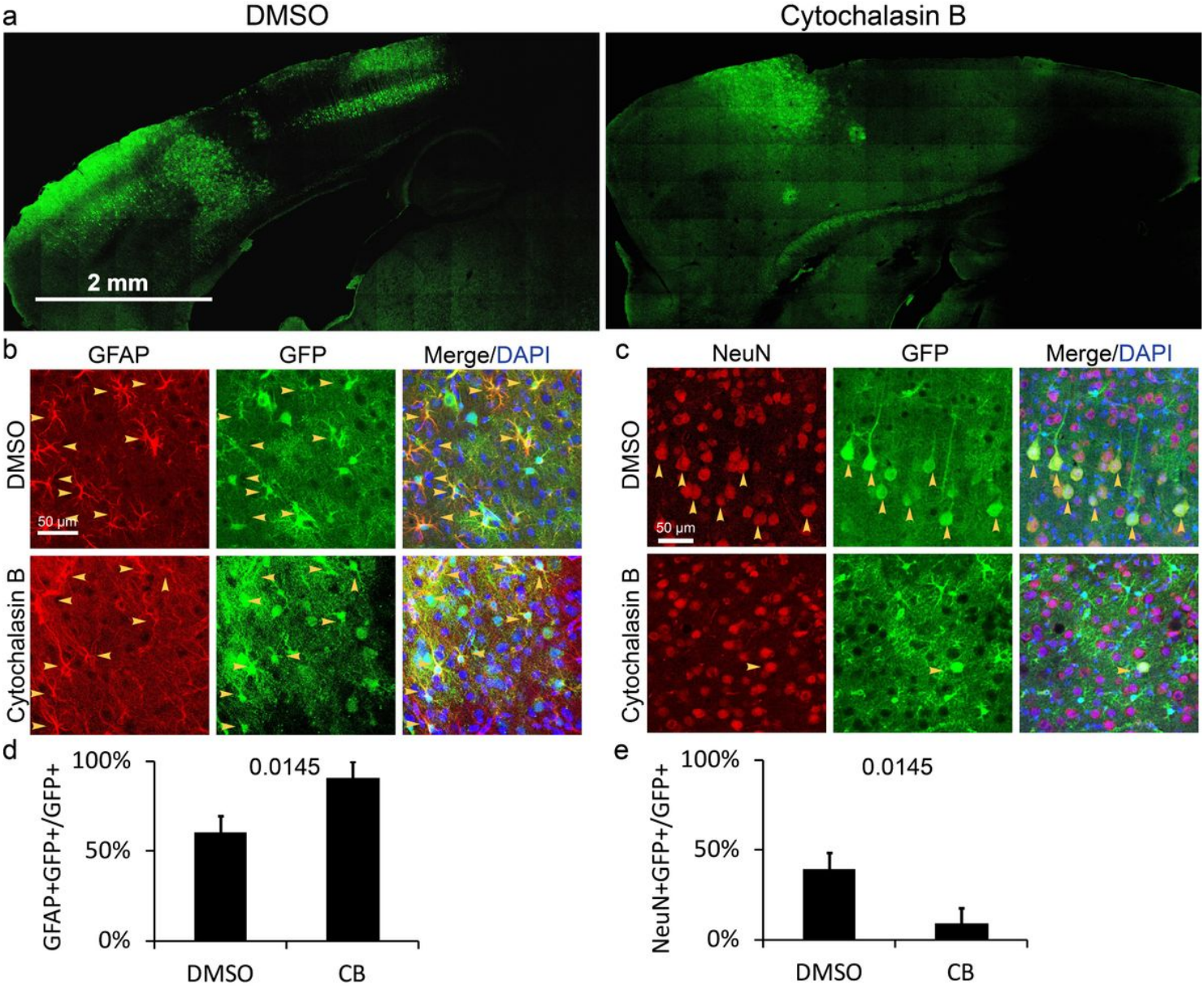

Figure 5

EFGP distribution in the cortext 10 days after injecting AAV and DMSO/cytochalasin B. (a) The microscopic photo with low magnification shows EGFP+ cells in cerebral cortex. (b) & (c) Detection of EGFP distribution in astrocytes and neurons at 10 DPI. Yellow arrows, double positive cells. (d) & (e) The percentage of astrocytes and neurons in EGFP+ population at 10 DPI. CB, cytochalasin B. Statistical analysis of significance was evaluated using unpaired two-tailed t test, N = 3 independent mice.

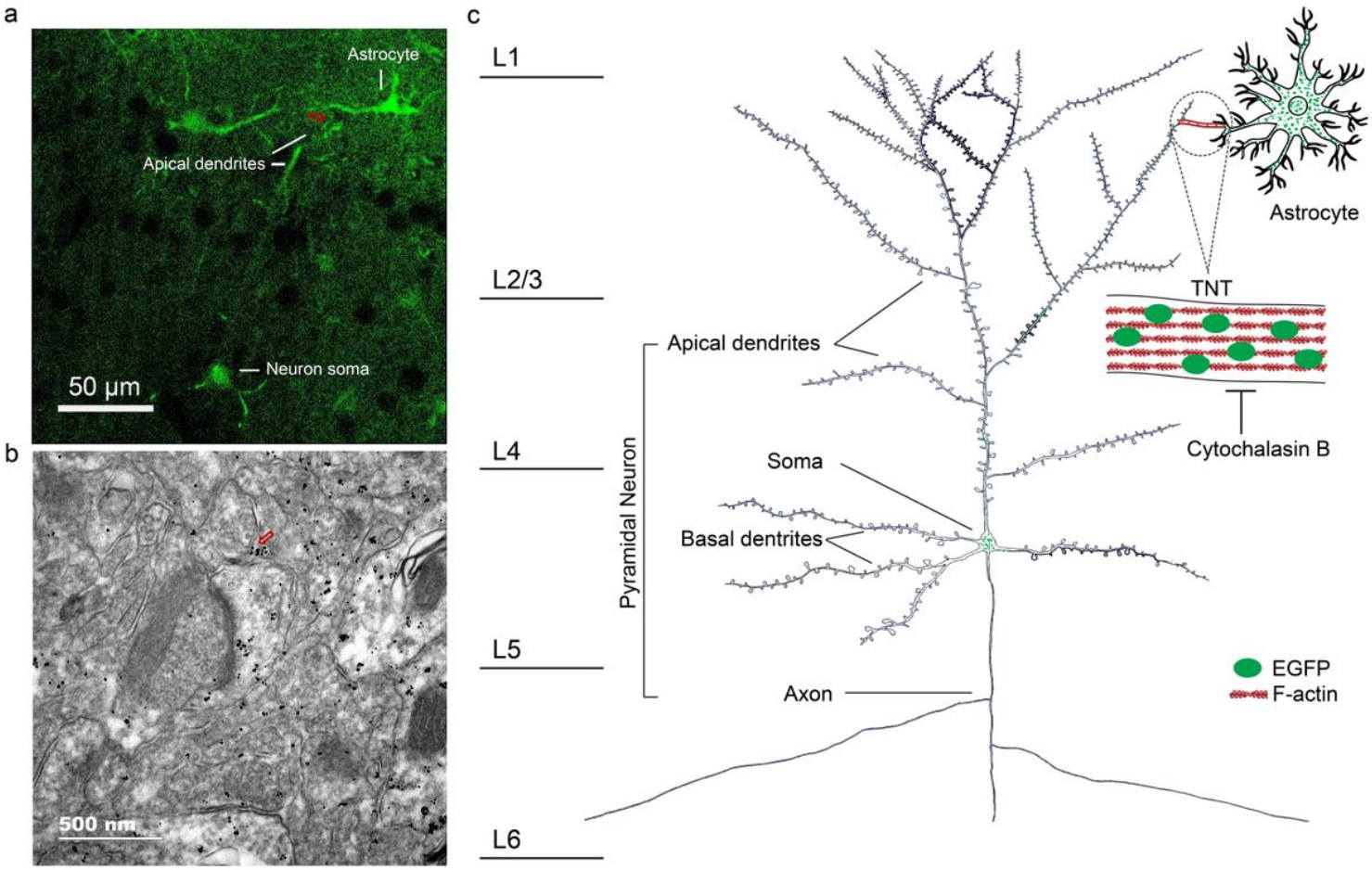

Figure 6

Illustration of the working model for EGFP transportation from astrocytes to neurons in cerebral cortex. (a) Detection of a TNT-like link between an apical dendrite and an astrocyte process by confocal microsopy. (b) Detection of a TNT-like ultrastructure in the cortex by immunoelectron microsopy. (c) Schematic illustration: Astrocytes in layer □-□express EGFP. Pyramidal neurons in layer □ strech out apical dendrites to layer □-□ with branches close to EGFP expressing astrocytes. With the assistance of F-actin which is enriched in sprouting dendrite branches, pyramidal neurons develope tunneling nano-tubules to astrocytes. EGFP disseminates from astrocytes to neurons through the tunneling nano-tubule. Red arrows, TNT. TNT, tunneling nano-tubule.

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- movie1.avi