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Essential roles of the acetylcholine receptor γ -subunit in neuromuscular synaptic patterning

Development Liu et al. 135: 1957

DEV018119 Supplementary Material

Files in this Data Supplement:

- [Supplemental Figure S1](#) -

Fig. S1. Distribution of AChRs in the absence of the γ -subunit. (A) Whole-mount diaphragm muscles from E15.5 wild-type and $\gamma^{-/-}$ embryos were incubated with Texas Red-conjugated α -bungarotoxin (α -bgt). Images were collected with a low-power objective (10 \times) so the entire length of muscle fibers could be viewed under the same field. The α -bgt labeled AChRs were clustered in the central region of the wild-type muscle, but remained diffuse in the $\gamma^{-/-}$ muscle. Despite the diffused pattern, the central region (marked by the broken lines) of the $\gamma^{-/-}$ muscle was still more intensely labeled, compared with its flanking region. (B) The difference in fluorescence intensity was presented in a line-scan. The entire muscle fiber was manually traced from one end to the other (\sim 800 μ m) using NIH ImageJ software. The gray levels of each pixel were measured and plotted against its position along the muscle fiber (wild-type, black line; $\gamma^{-/-}$, grey line). The central region of the $\gamma^{-/-}$ muscle appeared more intensely labeled than its flanking region.

- [Supplemental Figure S2](#) -

Fig. S2. Innervation pattern of hind limb muscles in wild-type and $\gamma^{-/-}$ mice. (A,B) Cross-section of E18.5 hind limb, stained with Hematoxylin and Eosin (H&E); note the apparent atrophy of the $\gamma^{-/-}$ muscles (B). (C,D) Low-magnification view of hind limb sections immunostained with synaptotagmin 2 (Syt2) antibody. Note a dramatic increase of innervation in all muscles in the $\gamma^{-/-}$ embryos (D), when compared with the wild-type muscles (C). (E-H) Higher magnification views of EDL and soleus are shown in the bottom row: nerve terminals in the wild-type muscles localized to small regions of the muscle (arrowheads in E,F), whereas nerve terminals in the $\gamma^{-/-}$ muscles were broadly distributed (arrows in G,H). TA, tibias anterior muscle; EDL, extensor digitorum longus muscle; MG, medial gastrocnemius muscle; LG, lateral gastrocnemius muscle; Sol, soleus; F, fibula. Scale bars: 200 μ m in A-D; 100 μ m in E-H.

- [Supplemental Figure S3](#) -

Fig. S3. Differentiation of the presynaptic nerve terminals at E16.5. Whole-mount diaphragm muscles labeled with synaptotagmin 2 (Syt2) antibody (A,D) and Texas-Red conjugated α -bungarotoxin (B,E). AChR clusters in the $\gamma^{-/-}$ muscle (arrowheads in E) were markedly reduced, compared with those

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in the wild-type muscles (E). However, individual nerve terminals were intensely labeled by Syt2 antibody in both wild-type (arrowheads in A) and in $\gamma^{-/-}$ muscles (arrowheads in D), although the nerve terminals in $\gamma^{-/-}$ muscles were distributed across a much broader region. The majority of the nerve terminals in the wild-type muscle were closely apposed by AChR clusters (arrowheads in C), whereas the majority of the nerve terminals in the $\gamma^{-/-}$ muscles were not apposed by AChR clusters (arrows in F). Note, however, that aneural AChR clusters were still present in the wild-type muscles (arrows in B,C). Scale bar: 30 μm

- [Supplemental Figure S4](#) -

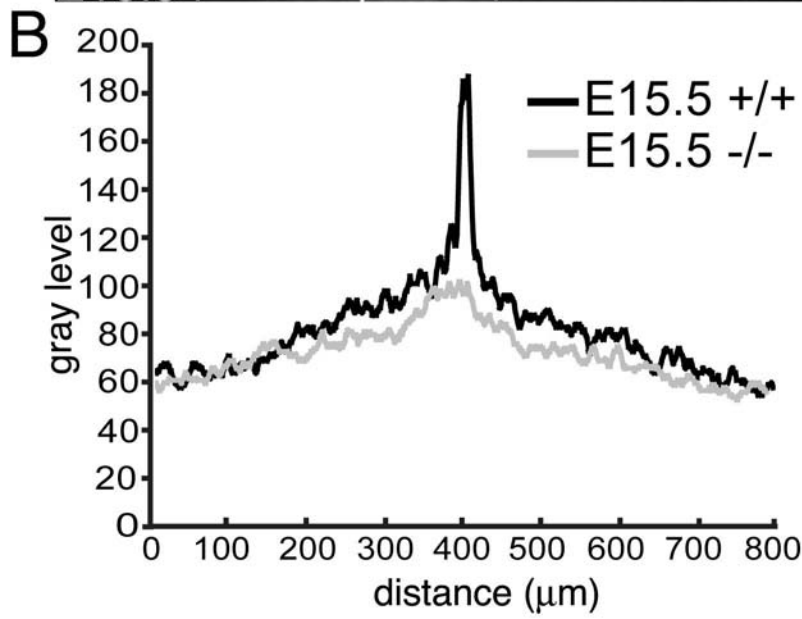
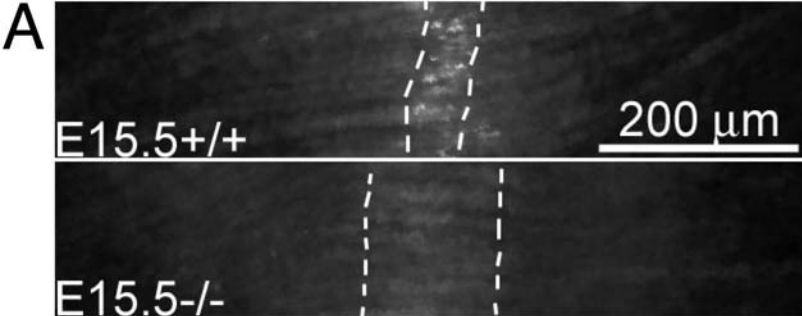
Fig. S4. Low magnification views of whole-mount diaphragm muscles immunolabeled by synaptotagmin 2 antibody. In wild-type muscle, nerve terminals (arrowhead in A) were localized along the central region of the muscle; occasionally, some nerve sprouts (arrows in A) extended beyond the central region. In the $\gamma^{-/-}$ muscle, nerve terminals (arrowheads in B) were distributed across a broad region of the muscle, especially at the dorsal quadrant of the diaphragm. Excessive nerve sprouts were seen across the entire region (arrows in B). Scale bar: 500 μm .

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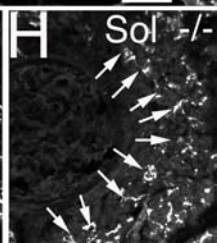
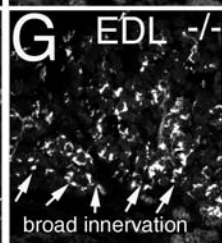
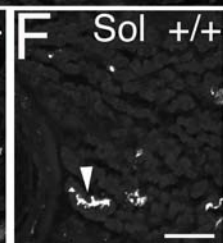
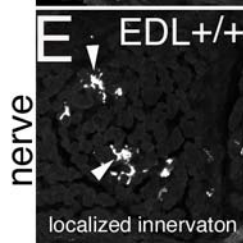
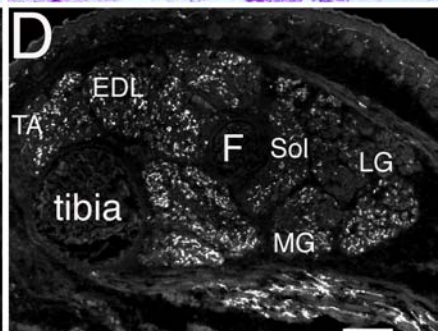
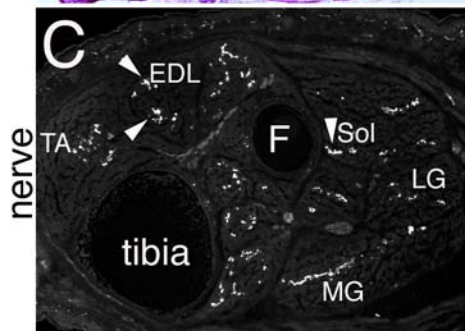
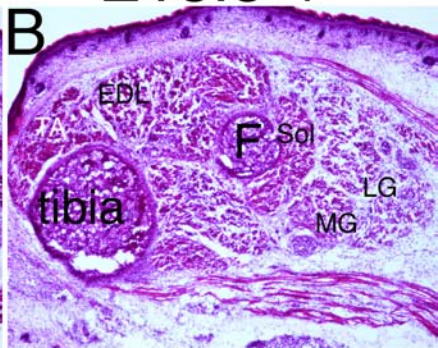
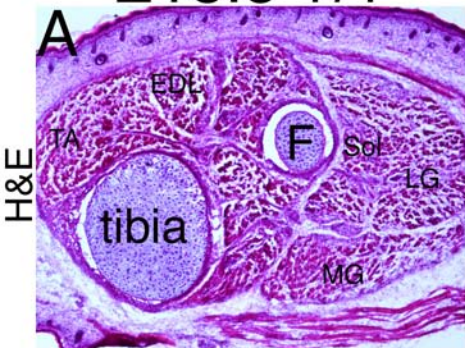
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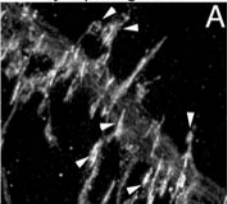
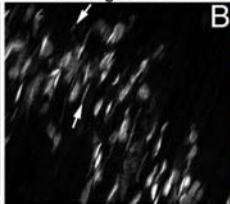


E18.5 +/+

E18.5 -/-



synaptotagmin-2

 α -bungarotoxin

merged

