Supplemental data

Materials and Methods

Strains

Standard methods were used for culturing and handling animals (Brenner, 1974). *hpIs3*, *hpIs61*, *hpIs49*, *juIs1* and *oxIs22* marker strains, and the mutagenesis screen that identified *hp121* mutant have been described previously (Bamber *et al.*, 1999; Yeh *et al.*, 2005; Yeh *et al.* 2008; Zhen and Jin, 1999). The *hpIs54* marker strain contains an integrated array consisting of plasmid pJH378, that was co-injected with *lin-15* marker into *lin-15(n765)* animals with *lin-15* mutation later outcrossed. *unc-9(fc16) unc-7(e5)* strain with *hpIs3* was generated by crossing *unc-9(fc16) daf-6 unc-7(e5)* strain (a gift from Drs. Starich and Shaw) with *unc-7(e5) hpIs3* males carrying the rescuing array for *unc-7*. *hpIs3*, which is mapped to left end of LGX, was used as a recombination marker. Unc animals with *hpIs3* markers (and the UNC-7 rescuing array) were isolated and checked for dye-filling defects. Animals with no dye-filling defects represent *hpIs3 unc-9(fc16) unc-7(e5)* animals. Animals were then selected for loss of UNC-7 rescuing array. All strains were sequenced to confirm presence of *fc16*. Transgenic lines used for tissue-specific rescuing experiments were generated as follows: pJH371, pJH1608, pJH621, pJH623, pJH641 and PU7S were individually co-injected with P*odr-1*-GFP marker (C. Bargmann, Rockefeller University) into *unc-7(hp121)* animals to generate ZM2150 *unc-7(hp121);hpEx527*, ZM1914 *unc-7(hp121);hpEx431*, ZM1918 *unc-7(hp121);hpEx425*, ZM1921 *unc-7(hp121);hpEx434*, ZM1917 *unc-7(hp121);hpEx428* and ZM2152 *unc-7(hp121); hpEx539* respectively for behavioral test; the same plasmids were individually injected into *unc-7(121) hpIs49 hpIs3* animals to generate ZM1944 *unc-*

7(hp121)hpIs49hpIs3;hpEx438, ZM1949 *unc-7(hp121)hpIs49hpIs3;hpEx443,* ZM1952

unc-7(hp121)hpIs49hpIs3;hpEx446, ZM1955 *unc-7(hp121)hpIs49hpIs3;hpEx449,*

ZM1946 *unc-7(hp121)hpIs49hpIs3;hpEx440* and ZM2153 *unc-7(hp121) hpIs49 hpIs3;*

hpEx540 respectively for active zone marker quantification analyses. pJH621, pJH442

and P*odr-1*::GFP marker were co-injected into *hpIs3 unc-7(hp121)* animals to generate

ZM2151 *hpIs3 unc-7(hp121)*; *hpEx525* that was used for the mosaic analysis. EH567

unc-7(e5);lwIs48 carries an integrated and functional array of *Punc-7*-UNC-7s::GFP.

lwIs48 was generated by a plasmid pU7S::GFP, a cosmid F56B12 (both injected at

75ng/µl) and the UNC-36(+) injection marker (at 50ng/µl) into *unc-7(e5)* animals. All

double or triple mutant strains generated this study were confirmed by backcrossing to

the parental single mutants.

Constructs

The genomic *unc-7* rescuing construct, pJH371, was generated by subcloning a 9.2kb

HpaI fragment from cosmid R07D5 into the HpaI site of pBluescript SK. The *acr-2*

promoter (P*acr-2*) used to express *unc-7* specifically in cholinergic neurons includes a

genomic DNA fragment that spans 3387bp upstream of *acr-2* (Squire *et al.*, 1995). The

GABAergic *unc-25* promoter (P*unc-25*) used in this study has been described previously

(Eastman *et al.*, 1999; Jin *et al.*, 1999). pPD96.52 (A. Fire) was used to create pJH623

that drives the expression of *unc-7* in the muscle under the *myo-3* promoter (P*myo-3*)

(Okkema *et al.*, 1993). The panneuronal F25B3.3 promoter (P*F25B3*.3) fragment

contained 3451bp upstream of the predicted translation start condon plus the first 12 bp

of the coding sequence (Altun-Gultekin *et al.*, 2001). pJH1608 (P*acr-2-unc-7*), pJH621

(P*unc-25-unc-7*), pJH623 (P*myo-3-unc-7*) and pJH641 (P*F25B3.3-unc-7)* were generated

by placing the specified tissue specific promoter upstream of the 1.54kb isoform *unc-7*

cDNA that was amplified from the M. Vidal cDNA library (Walhout *et al.*, 2000).

pJH378 (P*unc-129* GFP::SYD-2) was generated by replacing the *unc-25* promoter in the

previously described pJH23 (P*unc-25* GFP::SYD-2) (Yeh *et al.*, 2005) with the 2.7kb

unc-129 promoter fragment from pAC12 (Colavita *et al.*, 1998). pJH442 (*Punc-25*-RFP)

contains tdTomato (R. Tisen, University of California, San Diego) driven by P*unc-25*.

pJH505 (*Punc-25*-SNB-1::mRFP) contains the SNB-1 cDNA fused with C-terminal

mCherry driven by P*unc-25*. pJH484 (*Punc-25*-UNC-7::GFP) contains a 1.5kb UNC-7

cDNA with GFP inserted in its XbaI site driven by P*unc-25*.

The *unc-7* locus encodes a long (UNC-7L, accession #Q03412) and a short

(UNC-7S) isoform (Starich and Shaw, unpublished). The interneuron-specific expression

of UNC-7S construct PU7S was generated by cloning an 8.6-kb SalI/BamHI fragment of

the *unc-7* genomic region (nt 14412-5788 of cosmid R07D5) into pBluescript SK.

Supplemental Figure legends

Supplemental Figure S1 A) *unc-7(e5)* and *unc-9(fc16)* mutants show normal

morphology (top panels) and total number of fluorescent puncta (graph) expressed by a

vesicle marker *juIs1* (P*unc-25*-SNB-1::GFP). The total number of animals scored for

each genotype is shown within each bar. B and C) Normal synaptic morphology is seen

in *unc-7* mutant animals. Morphology of lateral cord synapses was illuminated by

synaptogyrin::GFP (SNG-1::GFP) marker *jsIs219* (B), which expresses pan-neuronally.

The post-synaptic morphology of GABAergic neurons in *unc-7* animals was detected by UNC-49::GFP marker (*oxIs22*) (C).

Supplemental Figure S2 –Robust and tissue-specific expression of UNC-7 protein driven by various tissue-specific promoters in *unc-7(hp121)* protein null mutants was confirmed by UNC-7 immunostaining. A, A') UNC-7 expression and localization along the dorsal and ventral nerve processes driven by a panneuronal promoter P*FB23.3* (arrows). B, B') UNC-7 expression driven by the cholinergic neuron-specific promoter P*acr-2* along the dorsal and ventral nerve cords (arrows). C, C') UNC-7 expression driven by a GABAergic neuron-specific promoter P*unc-25* (arrows). D-D') Expression of UNC-7 driven by a muscle-specific promoter P*myo-3* outlines the muscle bands (arrow). *: Cell bodies of cholinergic (B') and GABAergic (C') neurons. Scale bar: 5 μm.

Supplemental Figure S3 – Example of a mosaic animal expressing UNC-7 in an uncoupled DD neuron. A) Schematic representation of DD and VD neuron cell bodies situated along the ventral nerve cord near the head. The location of cell bodies is stereotyped among individual animals. The region of the dorsal cord where *hpIs3* (GFP::SYD-2) puncta was scored for DD1 neurons is indicated by the arrows. B) Picture of a mosaic animal in the Cy3 channel, where the expression of tdTomato is observed in DD1 (+) but not the neighboring DD2 (-) neuron. Cell body locations are shown by dashed circles. C) Picture of the same animal in the FITC channel that shows *hpIs3* expression in all GABAergic DD and VD neurons. Scale bar: 10 μm

Supplemental Figure S4 – Abnormal active zone distribution and morphology in *nca(gf)* mutants. A) A serial EM micrographs of a typical wild-type GABAergic NMJ with a single active zone region of defined size. B, C) the serial micrographs of two GABAergic NMJs with abnormal active zone structures in *nca(gf) (unc-77/nca-1(hp102))* mutants. The GABAergic NMJ either contains multiple active zones (B) or no prominent active zone density (C). Arrowheads: active zone region. Notice that in C, the vesicle accumulation and the appearance of cadherin-like junctions (*) with the muscle surface are not affected. D-E) The distribution curve of the active zone volume in the cholinergic (D) and GABAergic (E) NMJs of wild-type and *nca(gf)* mutants. X axis represents the calculated active zone volume in μm^3. Y axis represents the density of active zones. Compared to wild-type NMJs (black lines), the sizes of active zones in *nca(gf)* mutants (red lines) are variable.

Supplemental Movies

S1: wild-type; S2: *unc-7(hp121)*; S3: *unc-7(e5)*; S4: *unc-7(hp121); hpEx527* (genomic *unc-7* rescue); S5: *unc-7(hp121); hpEx428* (*unc-7* expressed panneuronally); S6: *unc-7(hp121); hpEx434* (P*myo-3* driven *unc-7*); S7: *unc-7(hp121); hpEx421* (P*unc-25* driven *unc-7*); S8: *unc-7(hp121); hpEx431* (P*acr-2* driven *unc-7*); S9: *unc-7(hp121); hpEx* (U7S isoform expression in interneurons); S10: *unc-9(e101)*; S11: *unc-9(fc16) unc-7(e5)*

Supplemental References

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