Figures and figure supplements

Insulin signaling controls neurotransmission via the 4eBP-dependent modification of the exocytotic machinery

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Figure 1. Effects of dietary protein concentrations on neurotransmission at the CM9 NMJ. (A) Diagram of Drosophila head indicating the location of the Cibarial Muscle 9 (CM9). (B) 21-day feeding paradigm used for the analysis of dietary effects on neurotransmission. Animals were raised for 21 days on a low-protein diet (1X = light gray), a high-protein diet (2X = dark gray), or subjected to a shift from a low-protein diet (1X) to a high-protein diet (2X) on day 20 (1X-2X). (C) Representative traces of evoked CM9 EPSPs and spontaneous miniature EPSPs (CM9 mEPSPs) from electrophysiological recordings of CM9 muscle fibers from flies subjected to the indicated dietary conditions. Scale = 1 mv, 10 ms. (D–F) Graphs represent the mean values for evoked EPSPs (D), mEPSPs (E), and quantal content (F) determined from recordings of CM9 muscle fibers from flies subjected to the indicated dietary condition or subjected to a diet shift (1X-2X) for 12 or 24 hr. Error bars = s.e.m. *p<0.05 determined using ANOVA. (G) Example traces of evoked EPSPs from paired-pulse experiments utilizing an inter-pulse interval (IPI) of 50 ms. (H) Graph represent the mean percent depression at indicated IPI. Error bars = s.e.m. **p<0.01, Student’s t-test. (I) Representative traces of electrophysiological recordings of hyperosmotic-induced spontaneous release events from CM9 NMJs in animals raised for 21 days on indicated diet conditions incubated in hyperosmotic recording saline. Inserts represent broader timescale of boxed regions from traces. Scale = 1 s. (J) Histogram representing the spontaneous event frequency and K, the average number of total spontaneous release events observed during 1 min of hyperosmotic recordings.

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The following source data is available for figure 1:

Source data 1. File contains the values represent the average value for the spontaneous release events per second determined in 5 s increments during the hypertonic stimulation of synaptic vesicle fusion at CM9 NMJs in animals raised on a 1X or 2X diet presented in Figure 1J.

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Figure 2. Insulin/DILP signaling negatively regulates presynaptic release at the CM9 NMJ. (A) Images from a proboscis extension reflex (PER) in response to tarsal stimulation with 0.5 M sucrose. Circle indicates location of sensory bristles tracked during the extension event resulting in an extension path (red line in panel iv) that is used for analysis of velocity. (B and C) Graphs represent the mean values for (B) average velocity and (C) max velocity for indicated genotypes and dietary conditions. All RNAi knock-downs utilize the Gal4-UAS binary expression system by combining the transgenic UAS construct (i.e. UAS-4eBPRNAi) with the CM9 motor neuron-specific E49-Gal4 driver. *p<0.05 versus 1X controls determined using ANOVA. Error bars = s.e.m. (D) Pathway represents the putative effects of insulin signaling on SV exocytosis. (E, F) CM9 EPSP traces of indicated genotype and dietary condition demonstrating the requirement for 4eBP on neurotransmission in animals raised on the 1X and 2X diets. Scale bar = 1 mV, 10 ms. (G–I) Graphs represent the average values for CM9 EPSPs (G), mEPSPs (H), and quantal content (I) determined from CM9 recordings from 21-day-old flies of indicated genotypes raised on indicated dietary conditions. Error bars = s.e.m. * indicates values significantly different from all other values determined using ANOVA (p<0.01). (J) CM9 EPSP traces of indicated genotype and dietary condition demonstrating the requirement for chico on neurotransmission in animals raised on the 1X and 2X diets. The effect of chico knock-down (chicoRNAi) on neurotransmission in animals raised on a 2X diet is suppressed by knockdown of 4eBP consistent with 4eBP functioning downstream of Chico. Scale bar = 1 mV, 10 ms. (K and L) Graphs represent the average values of EPSPs (K) and quantal content (L) determined from CM9 recordings from 21-day old flies of indicated genotypes raised on indicated dietary conditions. Error bars = s.e.m. *p<0.05 versus 2X controls determined using ANOVA.

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Figure 2—figure supplement 1. Effects of diet and neuronal insulin signaling on SV exocytosis at larval NMJ. (A) Representative evoked EPSP and spontaneous mEPSP traces from larval muscle 6 in larval of indicated genotype and diet conditions. In these experiments, larvae were on indicated diets for the entire larval stage of development (~3 days). Genotypes: ctrl = OK6-Gal4/+; 4eBP RNAi = OK6-Gal4/+; UAS-4eBP RNAi/+; chico RNAi = OK6-Gal4/+; UAS-chico RNAi/+; chico RNAi = OK6-Gal4/+; UAS-chico RNAi/+; chico RNAi = OK6-Gal4/+; UAS-chico RNAi/+; chico RNAi = OK6-Gal4/+; UAS-chico RNAi/+. Scale bar = 10 mV, 250 ms (EPSPs); 2 mV, 50 ms (mEPSPs). (B and C) Graphs represent the mean values for EPSPs (B) and quantal content (C) recorded from the NMJs on muscle 6 in control and 4eBP knockdown larvae raised on 1X diet. Error bars = s.e.m. (D and E) Graphs represent the mean values for EPSPs (D) and quantal content (E) determined at the NMJs on muscle 6 in control and chico knockdown larvae raised on 2X diet. N = 8 animals (1 recording per animal) for each average. Error bars = s.e.m.
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Figure 2—figure supplement 2. Cycloheximide blocks the effects of diet switch on SV exocytosis. (A) Model of the translational regulation of SV exocytosis by insulin signaling via 4eBP. (B) 21-day diet shift paradigm for testing the effects of cycloheximide on neurotransmission. Flies were exposed to cycloheximide for 2 hr on 1X diet prior to switching to the 2X diet condition for 24 hr. Control flies consisted of cycloheximide treated flies that are not subjected to diet switch or treated with vehicle. (C–D) Graphs represent the mean values for EPSPs (C), mEPSPs (D), and quantal content (E) recorded from CM9 NMJs in flies from indicated treatment groups. Error bars = s.e.m. *p<0.05 versus 1X +cmx condition determined using ANOVA. DOI: 10.7554/eLife.16807.008
Figure 3. Effects of diet on the release of neurotransmitter requires FOXO. (A) Diagram depicts the regulation of 4eBP by either FOXO-dependent transcription or dTOR-dependent phosphorylation. (B) Relative mRNA expression levels of 4eBP in purified motor neurons from 21-day-old animals raised on the indicated diet conditions. (C) Graphs represent the average relative fold enrichment of 4eBP DNA in anti-dFOXO chromatin immunoprecipitations (ChIPs) from thoracic ganglions isolated from animals raised on 1X or 2X diets. (D) Graphs represent average dFOXO protein levels estimated from flies used for ChIP. Values were normalized to actin. Error bars = s.e.m. Immunoblot of dFOXO is shown below. (E) Representative CM9 EPSP traces from 14-day-old flies raised on 1X diet of the indicated genotypes. In these genotypes, the overexpression of 4eBP is restricted to the CM9 MN using the E49-Gal4 driver. (F and G) Graphs represent the mean value for EPSPs (F) and quantal content (G) for indicated genotypes raised for 14 days on indicated diets. Error bars = s.e.m. *p<0.05 versus 1X wild-type controls determined using ANOVA. DOI: 10.7554/eLife.16807.009
Figure 4. Effects of diet on the release of neurotransmitter is independent of dTOR. (A) Diagram depicts the regulation of 4eBP by either FOXO-dependent transcription or dTOR-dependent phosphorylation indicating the effects of rapamycin (Rapa) on diet-regulated SV exocytosis, animals were fed for 14 days on 1X food and then switched to a 1X food supplemented with either 200 μM rapamycin or vehicle for 6 more days. On day 20, animals were switched from a 1X to a 2X diet supplemented with rapamycin or vehicle for 24 hr prior to electrophysiological analyses. (C) Immunoblots of phosphorylated S6 kinase (P-S6K) or 4eBP (P-4eBP) from animals subjected to above rapamycin treatment demonstrating effective inhibition of dTOR kinase activity under these dietary conditions. Actin signals serves as protein loading control. (D) Quantification of intensity of P-S6K determined from immunoblots and normalized for loading. *p<0.05 determined using Student’s T-test. (E–G) Graphs represent the mean values for EPSPs (E), mEPSP (F) and quantal content (G) recorded from CM9 NMJs of 21-day-old wild-type flies of indicated dietary condition. Error bars = s.e.m. *p<0.05 versus 1X controls determined using ANOVA.

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Figure 5. The role of Staufen during the regulation of neurotransmission by diet. (A) Representative traces of EPSPs from CM9 NMJs from 21 day old
staufenRNAi raised on a 1X diet or staufenRNAi and control flies subjected to a diet switch from 1X to 2X diet on day 20 and recorded on day 21. Scale
bar = 1 mv, 10 ms. (B–D) Graphs represent the mean values for EPSPs (B), mEPSPs (C), and quantal content (D) recorded from indicated genotypes.
*p<0.05 determined using ANOVA. (E) Graphs represent the fold enrichment of Staufen-bound mRNAs immunoprecipitated from FACS sorted motor
neurons. *p<0.01 versus tubulin control determined using ANOVA. (F) Diagram depicts the putative regulation of complexin mRNA translation in
response to insulin signaling in the CM9 motor neuron.
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Figure 6. Complexin levels regulate SV release in response to diet. (A) Immunofluorescent images of CM9 NMJs from animals raised on a 1X (left panels) or 2X (right panels) diet co-stained for Complexin (red-upper panels and lower panels), Discs-large (Dlg, green upper panels) and Dapi (blue-upper panels). (B) High magnification of Complexin (Cpx-upper panels) and Discs-large (Dlg-lower panels) from CM9 NMJ boutons in animals raised on a 1X (left panels) or 2X (right panels) diet conditions. Images for Cpx have been deconvolved. (C and D) Graphs represent the average value (C) and frequency histogram (D) for the maximum pixel intensity (m.p.i.) of synaptic Complexin from indicated diet conditions and genotype. *p<0.05 determined using ANOVA comparison of mean values (C) or using a Kolmogorov-Smirnov test (D). (E) Representative traces of EPSPs from CM9 NMJs from wild type (wt) or complexin heterozygotes (cpxSH1+/+) raised for 21 days on the indicated diets. Scale bar = 1 mV, 10 ms. (F and G) Graphs represent the mean values for mEPSPs (F) and quantal content (G) from CM9 NMJs 21-day-old animals of indicated genotypes raised on the indicated diet. *p<0.05 versus 1X condition determined using ANOVA. (H) Representative traces from wt and cpxSH1+/+ animals raised on 2X diet for 21 days. Graphs below traces represent the quantification of the events per second from CM9 NMJs of the indicated genotypes raised on the indicated diets. *p<0.05 versus 1X control determined using ANOVA. (I) Representative traces of EPSPs from CM9 NMJs in 21-day-old animals on the indicated diet conditions. Scale bar = 1 mV, 10 ms. (J and K) Graphs represent the mean values for mEPSPs (J) and quantal content (K) from CM9 NMJs 21-day-old animals of indicated genotypes raised on the indicated diets. *p<0.05 versus 1X control determined using ANOVA.

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The following source data is available for figure 6:

Source data 1. File contains background-corrected values of max pixel intensity from complexin (Cpx) staining at the CM9 NMJ from indicated genotypes and diet conditions.

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Figure 6—figure supplement 1. Diet effects on synaptic complexin levels at the lateral abdominal muscle NMJs. (A) Diagram represents the area of the abdomen that is being analyzed (grey box). Image shows the NMJs on the lateral abdominal muscles (LAMs) used in these analyses. Staining for Dlg is shown and dashed line indicates the ventral mid line. (B) Non-deconvolved immunofluorescent images of LAM NMJs from animals raised on a 1X (left panels) or 2X (right panels) diet co-stained for Complexin (Cpx; upper panels), Discs-large (Dlg, middle panels). A merged image is shown in the bottom panels (Cpx = green channel; Dlg = red channel). Scale bar = 10 μm. (C and D) Graphs represent the average value (C) and frequency histogram (D) for the maximum pixel intensity (m.p.i.) of synaptic Complexin from indicated diet conditions. *p<0.05 determined using ANOVA comparison of mean values (C) or using a Kolmogorov-Smirnov test (D).

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