Figures and figure supplements

Synaptic organization of the *Drosophila* antennal lobe and its regulation by the Teneurins

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Figure 1. Measuring CNS synapses using Bruchpilot-Short. (A) Diagram comparing full-length Bruchpilot and Bruchpilot-Short. Numbers denote amino acids and orange represents coiled coil regions (after Wagh et al., 2006). The positions of Brp and Brp-Short in relation to known active zone proteins are also shown in a simplified format (after Liu et al., 2011). (B–D) High magnification confocal stacks of a single DA1 glomerulus with putative synapses labeled by Brp-Short (B), neurites labeled by mCD8-GFP (C), and the merge (D). Insets show higher magnification of a single optical section to demonstrate the punctate nature of Brp-Short-mStraw. (E–G) Screenshots of three-dimensional renderings show conversion of Brp-Short into ‘Spots’ (E) and mCD8-GFP into a volumetric surface rendering (F). For (F) and (G), the transparency of the surface rendering is at 60% to highlight internal structure and make Brp-Short puncta visible. (H) Quantification of Brp-Short puncta (red, left axis) and neurite volume (black, right axis) in males and females for five ORN classes. Statistical comparisons between males and females of a single genotype were done by student’s t-test. Significance across all genotypes for density was assessed with a one-way ANOVA and corrected for multiple comparisons by a posthoc Tukey’s multiple comparisons test. ***p < 0.001. In all cases, n ≥ 9 brains, 18 antennal lobes for each genotype. (I) Quantification of synaptic density (Brp-Short puncta/volume of neurites) in males (blue) and females (pink) for five ORN classes. Despite considerable differences in Brp-Short puncta number, all classes of ORNs display nearly identical synaptic densities. Scale bar = 5 µm for all images. DOI: 10.7554/eLife.03726.003
Figure 1—figure supplement 1. Validation of Brp-Short. (A) Representative high magnification confocal single sections of Or67d-positive ORNs innervating the DA1 glomerulus expressing Brp-Short-mStraw and DSyd1-EGFP and stained with antibodies against mStraw (red) and GFP (green). Note the significant overlap between Brp-Short and DSyd-1, a known presynaptic marker. (B) Representative high magnification confocal single sections of Or67d-positive ORNs innervating the DA1 glomerulus expressing Brp-Short-mStraw and mCD8-GFP in a control animal. (C) Representative high magnification confocal single sections of Or67d-positive ORNs innervating the DA1 glomerulus expressing Brp-Short-mStraw, mCD8-GFP and an RNAi transgene against Brp. As endogenous Brp has been impaired, Brp-Short-mStraw is greatly diminished at the synapse. Scale bar = 5 μm. (D) A single electron dense region as imaged by immunoelectron microscopy in an animal expressing Brp-Short-EGFP in all ORNs. 5-nm Nanogold dots (arrowheads) decorate electron-dense structures characteristic of active zones following immunostaining against the GFP epitope, suggesting that Brp-Short localizes to endogenous active zones. Scale bar = 25 nm.
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Figure 1—figure supplement 2. Additional examples of the Brp-Short assay. Representative high magnification confocal z-stack images of ORNs innervating the VA1d (Or88a-GAL4; [A]), VA1lm (Or47b-GAL4; [B]), DL4 (AM29-GAL4; [C]), and DM6 (AM29-GAL4; [D]) glomeruli expressing Brp-Short-mStraw and stained with antibodies to mStraw (red) and N-Cadherin (blue). In all cases, puncta are clearly visible; these glomeruli were quantified in Figure 1H. Scale bar = 5 μm.
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Figure 2. Clonal analysis of ORN synapse number. Representative high magnification confocal stacks of small clones of DL4 ORNs (A) and DM6 ORNs (B) expressing Brp-Short-mStraw (red) and mCD8-GFP (green). (C) and (D) histograms of Brp-Short puncta counts for DL4 and DM6 ORNs. (E) and (F) histograms of puncta density for DL4 and DM6 ORNs. Figure 2. Continued on next page.
Figure 2. Continued

Frequency histogram of Brp-Short puncta in DL4 clones (n = 93 clones from 64 animals). (D) Frequency histogram of Brp-Short puncta in DM6 clones (n = 90 clones from 64 animals). Both graphs exhibit notable periodicity, suggesting that each neuron contributes approximately the same number of synapses. Colored dashed lines indicate Gaussian fits for individual peaks. Solid black lines represent the best-fit sum of Gaussian relationship for each dataset. (E) Quantification of Brp-Short puncta from identified single neuron clones to DL4 (n = 16 clones) or DM6 (n = 17 clones). (F) Quantification of synapse density from all clones. In both DL4 and DM6, the clonal average density is identical to the class average density. In (E) and (F), data represent mean ± SEM. Scale bar = 5 μm.

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Figure 2—figure supplement 1. Additional DL4 and DM6 ORN MARCM clones. Representative high magnification confocal stacks of single cell clones of DL4 ORNs (A) and DM6 ORNs (B) and large clones of DL4 ORNs (C) and DM6 ORNs (D) expressing Brp-Short-mStraw and mCD8-GFP and stained for antibodies against mStraw (red), GFP (green), and N-Cadherin (blue). As previously observed (Joo et al., 2013), single cell ORN clones have a low signal-to-noise ratio while discerning larger clones is more evident. In A–B, the glomeruli are outlined (dashed line). Scale bar = 5 μm. DOI: 10.7554/eLife.03726.010
Figure 3. Antennal lobe neurons display distinct subglomerular architecture. (A–C) Single, high-magnification confocal optical sections through the center of the DA1 glomerulus in three animals where ORNs (A), PNs (B), or LNs (C) are expressing Brp-Short. A different color denotes the synapses of each class. (D) Single, high magnification confocal optical sections through the center of the DA1 glomerulus where ORNs express Brp-Short-mStraw (red) and mCD8-GFP (green). Brp-Short clusters (arrowheads) are visible, along with regions of neurite devoid of Brp-Short puncta (arrow) and voids where ORNs do not project (asterisk). (E) Single, high magnification confocal optical sections through the center of the DA1 glomerulus where PNs are expressing Brp-Short-mStraw (red) and mCD8-GFP (green). Regions of PN neurite without Brp-Short puncta are visible (arrow) as well as small voids lacking Brp-Short puncta and neurite projections (asterisk). (F) Single, high magnification confocal optical sections through the center of the DA1 glomerulus where LNs are expressing Brp-Short-mStraw (red) and mCD8-GFP (green). Figure 3. Continued on next page.
Brp-Short puncta are largely distributed evenly throughout neurites. Images in panels D–F (from three animals) demonstrate distinct patterns of synapse localization and distribution within the glomerulus. Dashed lines denote the glomerulus in (C) and (F) as defined by N-Cad staining (not shown). Scale bar = 5 μm. (G–I) Cumulative frequency histograms of the nearest neighbor distance between Brp-Short puncta in ORNs (G), PNs (H), and LNs (I). The average is indicated (μ) and the Cluster % of puncta with an NND ≤ 0.75 μm. Gray traces represent individual glomeruli. Red traces represent the aggregate average. In all cases, n ≥ 10 animals, 19 antennal lobes, and 400 (I), 800 (H), or 1400 (G) individual puncta. DOI: 10.7554/eLife.03726.011
Figure 3—figure supplement 1. Comparison of Brp-Short to endogenous Brp. (A) Representative high magnification confocal z-stack of the DA1 glomerulus in animals with an Or67d-mCD8-GFP transgene to mark its borders and stained with antibodies against GFP (red) and endogenous Brp (green). (B–D) Representative high magnification confocal single sections of Or67d-positive ORNs (B), Mz19-positive PNs (C), and NP3056-positive LNs (D) innervating the DA1 glomerulus, each expressing Brp-Short-mStraw and stained with antibodies against mStraw (red) and endogenous Brp (green). Each image was taken from different animals at a roughly equivalent Z-plane. In all cases, Brp-Short-mStraw puncta are coincident with endogenous Brp puncta. Endogenous Brp puncta that are not Brp-Short-mStraw positive are likely from other neurons not expressing the Brp-Short-mStraw transgene. Dashed lines indicate the glomerular boundary as defined by N-Cad staining (not shown). (D) Quantification of Brp-Short puncta from ORNs (green), PNs (blue), and LNs (magenta), the sum of ORNs + PNs + LNs, and the number of endogenous Brp puncta from the DA1 glomerulus (black). In all cases, data represent mean ± SEM and n ≥ 7 animals, 14 antennal lobes. Scale bar = 5 μm.
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Figure 4. Presynaptic Ten-a is required for proper ORN synapse number and density. Representative high-magnification confocal stacks of Brp-Short puncta in VA1Im ORNs stained with antibodies against Brp-Short-mStraw (red) and N-Cadherin (blue) in control (A), ten-a null mutants (B) and ten-a null mutants where a Ten-a transgene is expressed in this specific class of ORNs using Or47b-GAL4 (C). (D) Quantification of Brp-Short puncta in the above conditions as well as following presynaptic RNAi against ten-a, presynaptic RNAi against ten-m, or in ten-a null mutants where a Ten-a transgene is expressed in 2/3 of the PNs, including those innervated by Or47b-positive ORNs. (E) Quantification of ORN neurite volume in the same genotypes. n.s. = not significant. (F) Quantification of ORN synaptic density in the same genotypes. Significance was assessed with a one-way ANOVA and corrected for multiple comparisons by a posthoc Tukey’s multiple comparisons test. **p < 0.01, ***p < 0.001. Unless otherwise noted, significance is compared to control. In all cases, the ten-a mutant and ten-a ORN RNAi phenotypes are statistically indistinguishable and, in the ten-a mutant, presynaptic Ten-a expression rescues the observed phenotypes. The disrupted glomerular shape is due to partner matching errors in the ten-a mutant (Hong et al., 2012), which is not rescued due to the late onset of Or47b-GAL4 relative to the partner matching process. Dashed lines denote the glomeruli as delineated by Brp-Short label. In all cases, data represent mean ± SEM and n ≥ 7 animals, 14 antennal lobes. Scale bar = 5 μm.
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Figure 4—figure supplement 1. Representative images for additional genetic manipulations from Figure 4. Representative high-magnification confocal stacks of Brp-Short puncta in VA1Im ORNs stained with antibodies against Brp-Short-mStraw (red) and N-Cadherin (blue) in control (A), flies expressing ten-m RNAi in Or47b-positive ORNs (B), flies expressing ten-a RNAi in Or47b-positive ORNs (C), and ten-a null mutants where a Ten-a transgene is expressed in 2/3 of the PNs of the antennal lobe using GH146-QF (D). DOI: 10.7554/eLife.03726.014
Figure 4—figure supplement 2  ten-a phenotypes in DL4, a glomerulus with no morphology defects. (A) Representative high magnification confocal stacks of ORNs innervating the DL4 glomerulus in control animals expressing Brp-Short-mStraw and mCD8-GFP and stained with antibodies to mStraw (red), GFP (green), and N-Cadherin (blue). (B) Representative high magnification confocal z-stacks of ORNs innervating the DL4 glomerulus in ten-a null mutant animals expressing Brp-Short-mStraw and mCD8-GFP and stained with antibodies to mStraw (red), GFP (green), and N-Cadherin (blue). Morphology is unaffected but synapse number is reduced. (C) Quantification of Brp-Short-mStraw puncta in control and ten-a mutant animals. (D) Quantification of ORN volume in control and ten-a mutant animals. (E) Quantification of Brp-Short-mStraw puncta density in control and ten-a mutant animals. In all cases, data represent mean ± SEM and n ≥ 7 animals, 14 antennal lobes. ***p < 0.0001. Scale bar = 5 μm.
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Figure 5. Ultrastructural analysis of active zones in ten-a mutants. Representative transmission electron microscopy (TEM) images of active zones in control (A) and ten-a null mutant antennal lobes (B). T-bar profiles are visible in both genotypes, though reduced in number in the ten-a mutant. Scale bar = 500 nm. (C) High magnification TEM of a single active zone in a control animal. Note the normal T-bar morphology. (D–H) High magnification TEM images of active zones in ten-a mutants. In some cases, normal (D) morphology is observed. In the majority of cases, defects including multiple T-bars (E), misshapen T-bars (F), detached T-bars (G), and continuous electron dense material (H) are evident. Scale bar = 100 nm. (I) Quantification of T-bars per unit perimeter of measured antennal lobe terminals. All terminals were measured, including ORNs, PNs, and LNs. ten-a mutants display a 27% reduction from control animals. Data represent mean ± SEM. (J) Distribution of T-bar defects as a percentage of the total T-bars. Here, n = 1103 T-bar per unit perimeter measurements from three animals for control and n = 847 T-bar per unit perimeter measurements from three animals for ten-a.

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Figure 6. Ten-a and spectrin function together for normal synapse number. (A–B) Representative single confocal optical sections taken at equivalent positions of antennal lobes stained for α-spectrin and N-Cadherin in control (A) and ten-a mutant (B) adults. In ten-a mutants, α-spectrin staining is reduced compared to control. The disrupted morphology of the antennal lobe itself is due to partner matching errors evident in the ten-a mutant (Hong et al., 2012). Scale bar = 20 μm. (C–E) Representative confocal Z-stack images of the ORNs in the VA1lm glomerulus expressing Brp-Short in control animals (C), or animals expressing dsRNA against α-spectrin (D) or β-spectrin (E), and stained with antibodies against Brp-Short (red) and N-Cadherin (blue). (F) Quantification of α-spectrin (green), β-spectrin (red), and N-Cadherin (blue) immunofluorescence in control and ten-a mutants. ***p < 0.001. (G) Quantification of Brp-Short puncta in the noted genotypes. In all cases, similar reductions in puncta number are observed. Moreover, the genetic perturbations do not enhance each other, suggesting function in the same pathway. Significance was assessed with a one-way ANOVA and corrected for multiple comparisons by a posthoc Tukey’s multiple comparisons test. **p < 0.001 (compared with control). In all cases, data represent mean ± SEM and n ≥ 10 animals, 19 antennal lobes. Scale bar = 5 μm.

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Figure 6—figure supplement 1. ten-a regulates spectrin levels and spectrin regulates synapse number. Representative confocal single optical sections of control (A) and ten-a mutant (B) antennal lobes stained with antibodies to β-spectrin (red) and N-Cadherin (blue). Note the reduction (quantified in Figure 6F) of staining in the ten-a mutant. Scale bar = 20 μm. (C–E) Representative high magnification confocal z-stacks of Or67d ORNs expressing Brp-Short-mStraw in control animals (C) and animals co-expressing dsRNA against α-spectrin (D) or β-spectrin (E) and stained with antibodies against mStraw (red) and N-Cadherin (blue). Glomerular morphology is unaffected by spectrin dsRNA but synapse number is reduced. Scale bar = 5 μm. (F) Quantification of Brp-Short puncta in the genotypes from C–E. ***p < 0.0001. In all cases, data represent mean ± SEM and n ≥ 10 animals, 19 antennal lobes.

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Figure 7. Measuring postsynaptic acetylcholine receptor clusters with Da7-GFP. (A) Diagram of the GFP-tagged Da7 acetylcholine receptor subunit used for AChR visualization. (B) High magnification confocal z-stack images of PNs in the DA1 and VA1d glomeruli expressing Da7-GFP and mtdT, stained with antibodies against GFP (green), mtdT (red), and endogenous Brp (blue). Patches of mtdT labeling represent ascending PN axons within the plane. *Figure 7. Continued on next page*
of the glomerulus. Insets show a high magnification single optical section demonstrating the punctate nature of Da7-GFP. (C) Representative high magnification single optical sections of Mz19-positive PNs in the DA1 glomerulus expressing Da7-GFP and stained with antibodies against GFP (green) and endogenous Brp (red). The majority of GFP-positive puncta are apposed to or colocalized with endogenous Brp (yellow), consistent with their association with bona fide active zones. Insets show high magnification of a single optical section where asterisks denote apposed Da7 puncta. Brp puncta without apposition likely belong to synapses not labeled by the PN-GAL4 driver. Scale bar = 5 µm (2 µm for inset). (D) Representative high magnification single optical sections of Mz19-positive PNs and Or67d-positive ORNs in the DA1 glomerulus expressing Da7-GFP in the PNs and Brp-Short-mStraw in the ORNs using two binary expression systems. Most ORN active zones (labeled by Brp-Short, red) are apposed to or colocalized (yellow) with PN Da7 puncta (green), further supporting that these puncta label bona fide synaptic contacts. Insets show high magnification of a single optical section where asterisks denote apposed Brp-Short::Da7 pairs. Brp puncta without apposition likely belong to ORN synapses with neurons other than PNs and Da7 puncta without apposition likely correspond to PN postsynaptic sites apposed to synaptic contacts from neurons other than ORNs. (E) Quantification of Da7 AChR puncta (green, left axis) and neurite volume (black, right axis) in the DA1 and VA1d glomeruli of both male and female adult flies. Statistical comparisons between males and females of a single genotype were done by student’s t test. ***p < 0.001. (F) Quantification of AChR density in male (blue) and female (pink) adults based on the data from (C). Significance was assessed with a one-way ANOVA and corrected for multiple comparisons by a posthoc Tukey’s multiple comparisons test. *p < 0.05. n.s. = not significant. In all cases, data represent mean ± SEM and n ≥ 11 animals, 21 antennal lobes. Scale bar = 5 µm.

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Figure 8. Teneurins function to regulate acetylcholine receptor number. (A–C) Representative high magnification confocal z-stack images of DA1 and VA1d PNs expressing Da7-GFP in control animals (A), ten-a mutants (B), or animals expressing RNAi against ten-m in the same PNs (C), and stained with antibodies against GFP (green) and N-Cadherin (blue). DA1 and VA1d glomeruli are outlined together (white dashed line) and were determined using the accompanying mtdT label (not shown). Due to partner matching defects in the ten-a mutant that prevent clear delineation between the two glomeruli, the combined count was compared across genotypes. (D) Quantification of Da7-GFP puncta in the noted genotypes. (E) Quantification of PN neurite volume in all genotypes. (F) Quantification of AChR density in all genotypes. All observed phenotypes are statistically indistinguishable. Significance was assessed with a one-way ANOVA and corrected for multiple comparisons by a posthoc Tukey’s multiple comparisons test. ***p < 0.001 in comparison with control. n.s. = not significant. In all cases, data represent mean ± SEM and n ≥ 8 animals, 16 antennal lobes. Scale bar = 5 μm.

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Figure 9. Ten-m regulates presynaptic active zone number transsynaptically. (A) Diagram of the antennal lobe with the DA1 glomerulus outlined showing the experimental design. Or67d-positive ORN axon terminals that innervate DA1 are outlined in black; their active zones are labeled by Or67d-QF-driven QUAS-Brp-Short-mStraw. Mz19-GAL4-positive PNs that project dendrites to DA1 express UAS-RNAi against ten-m in experimental animals. (B–C) Representative high magnification confocal z-stack images of DA1 ORNs expressing Brp-Short in control animals (B) or in animals concurrently expressing ten-m RNAi in the DA1 and VA1d PNs (C) and stained with antibodies against mStraw (red) and N-Cadherin (blue). (D) Quantification of Brp-Short puncta in the noted genotypes. Controls 1 and 2 represent Brp-Sh puncta assayed by GAL4/UAS (as in Figure 1) and QF/QUAS binary system binary system, respectively, and are not significantly different, as assayed by student’s t test (n.s.). Significance of ten-m PN RNAi was assayed by student’s t test between it and Control 2. ***p < 0.001. In all cases, data represent mean ± SEM and n ≥ 5 animals, 10 antennal lobes. Scale bar = 5 μm.
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