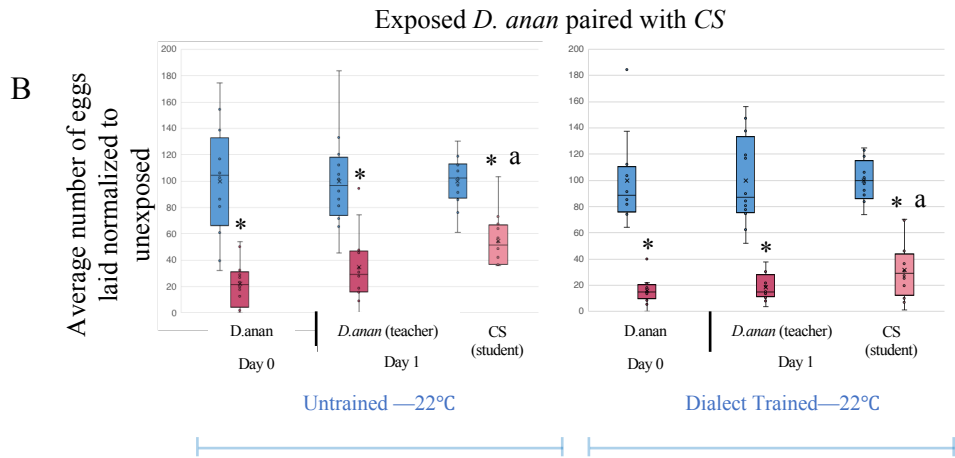
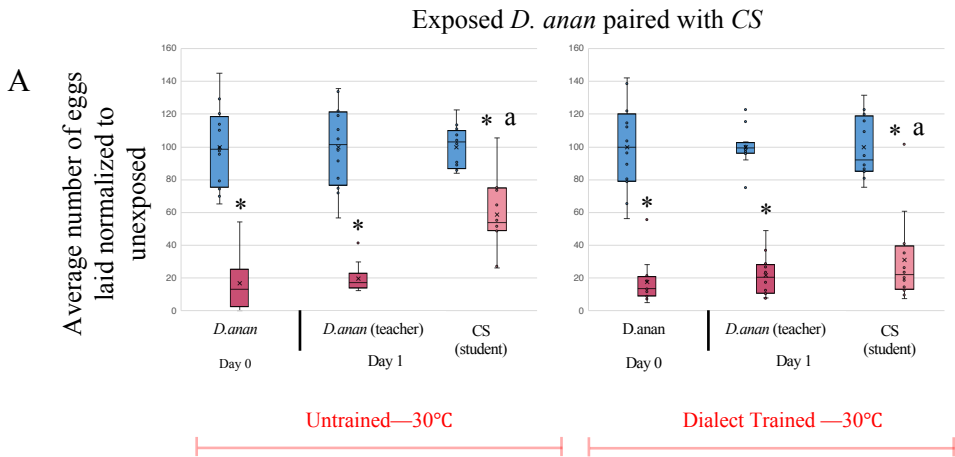


SUPPLEMENTARY FIGURES

Supplementary Figure 1. Wild-type *D. melanogaster* can be trained by *D. ananassae* at either 22°C or 30°C.

Dialect training is performed at either the permissive (22°C) or restrictive (30°C) temperature, while the wasp exposure and social learning period is performed exclusively at the permissive temperature. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. *Canton S* untrained and trained states by *D. ananassae* at either 30°C (A) or 22°C (B) show wild-type dialect acquisition. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

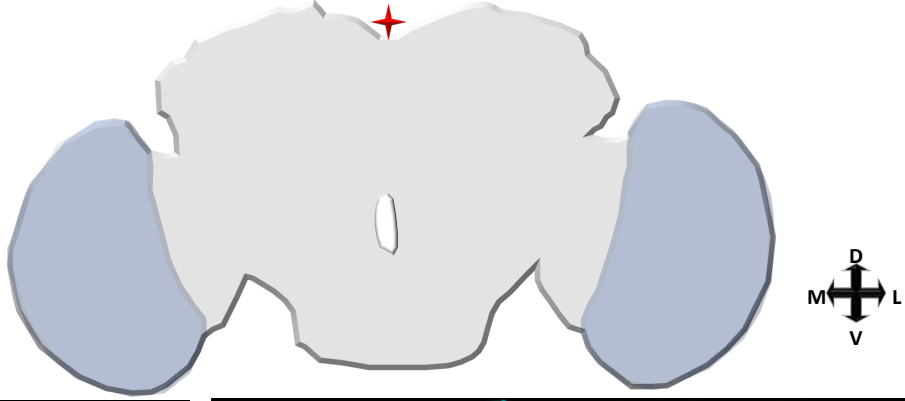


■ Unexposed ■ Exposed ■ Naïve

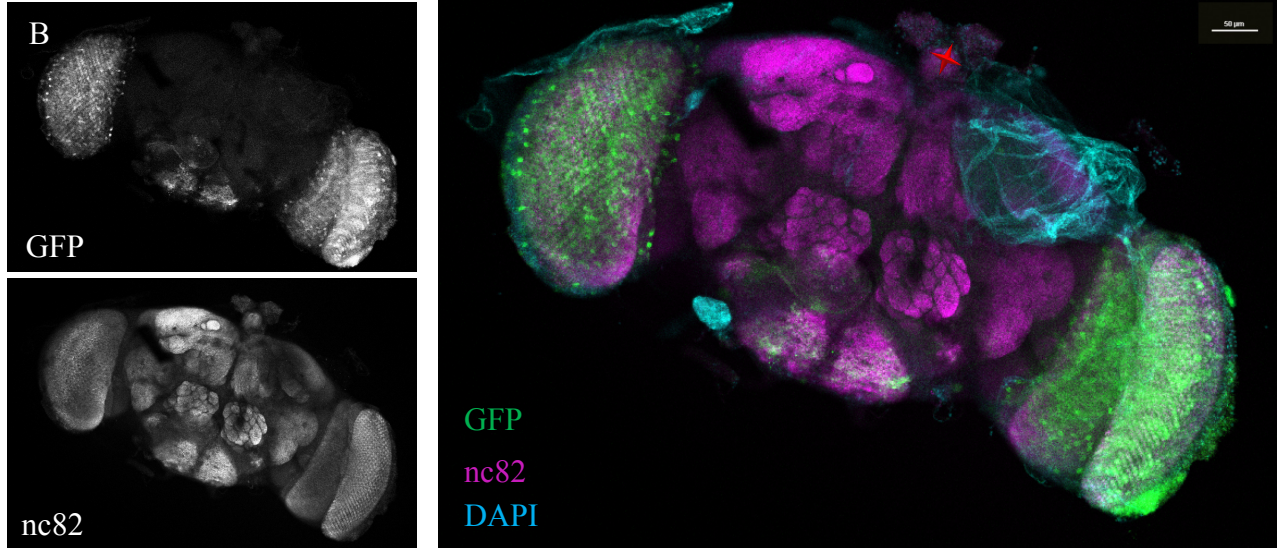
Supplementary Figure 2. The optic lobe is required for dialect training shown with R10B01 driver.

(A) Cartoon schematic of the region of interest, the optic lobe. (B) Confocal image of adult brain where R10B01^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/2 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R10B01^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

A

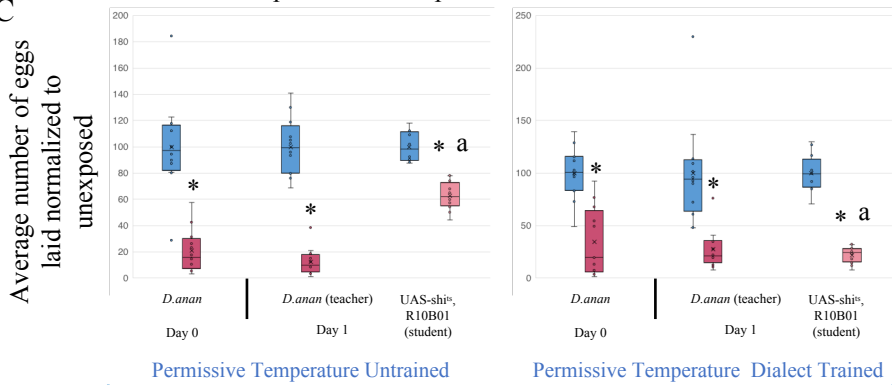


B



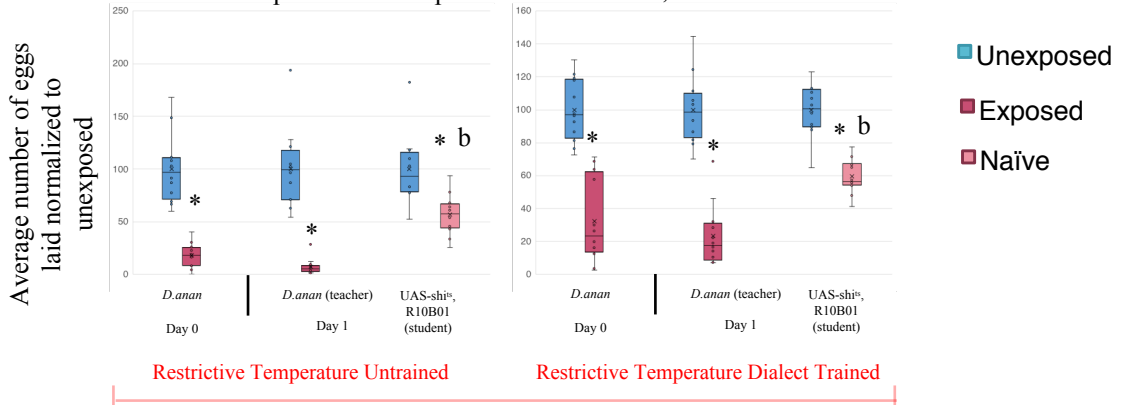
C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R10B01



D

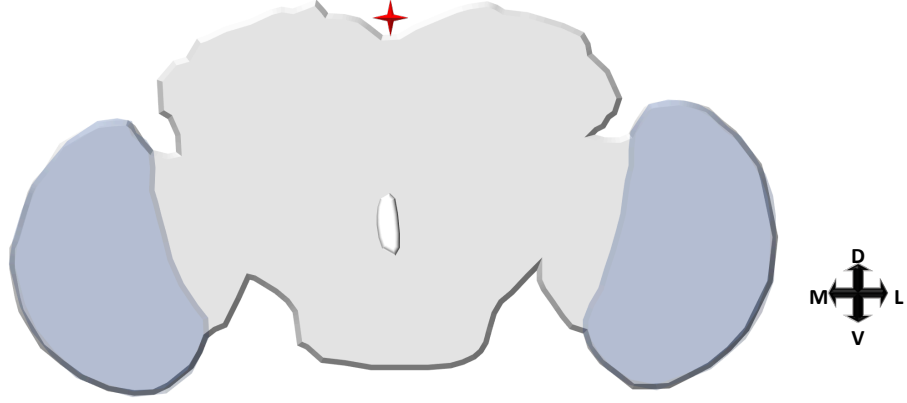
Exposed *D. anan* paired with *UAS-shi^{ts}*, R10B01



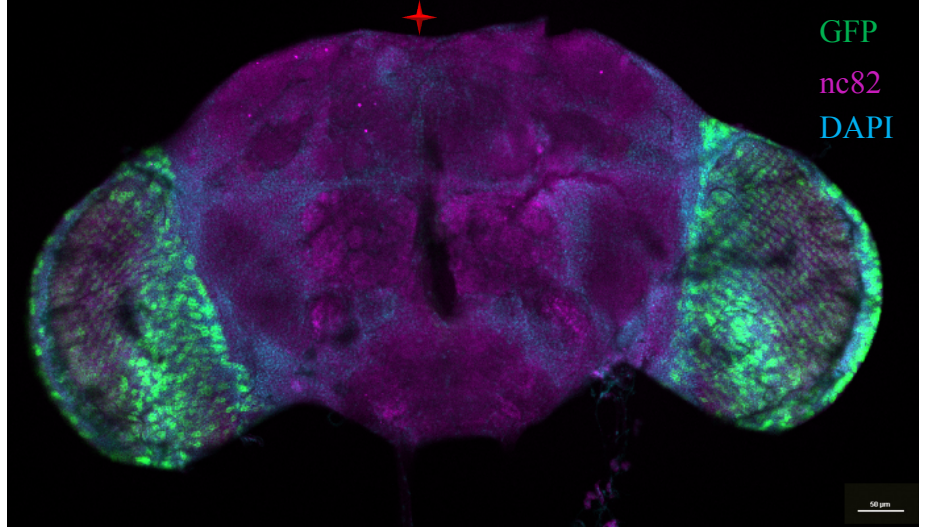
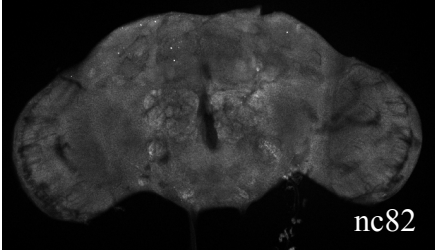
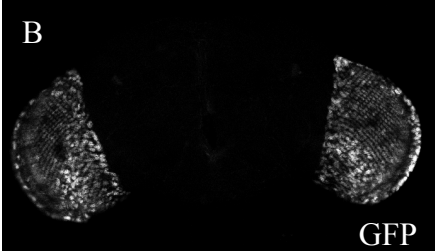
Supplementary Figure 3. The optic lobe is required for dialect training shown with R17F12 driver.

(A) Cartoon schematic of the region of interest, the optic lobe. (B) Confocal image of adult brain where R17F12^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/2 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R17F12^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

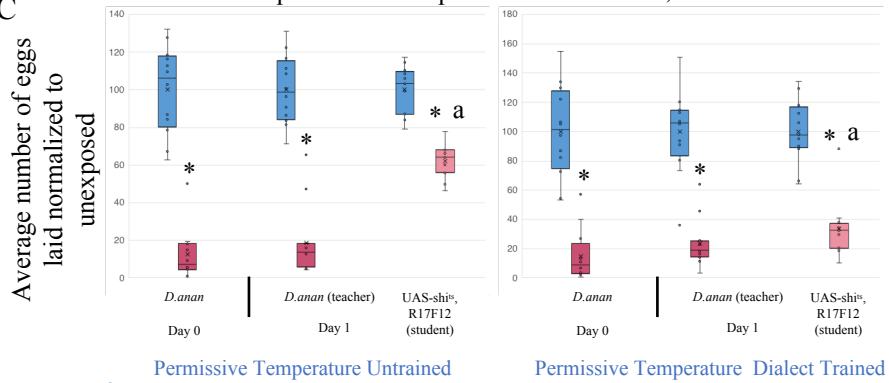
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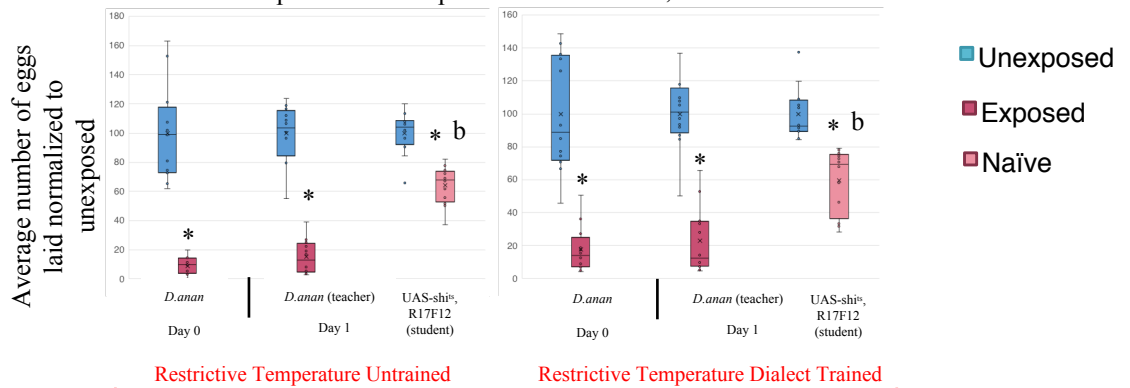
B



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R17F12

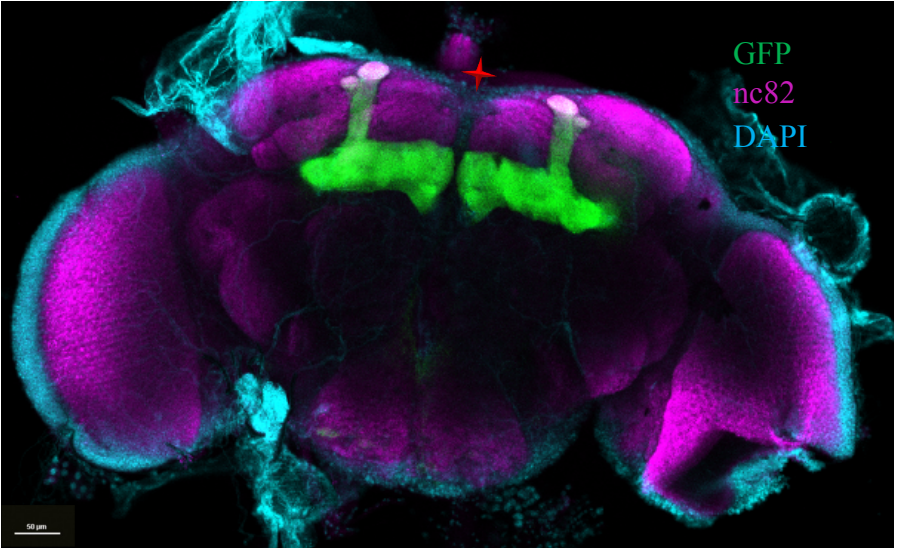
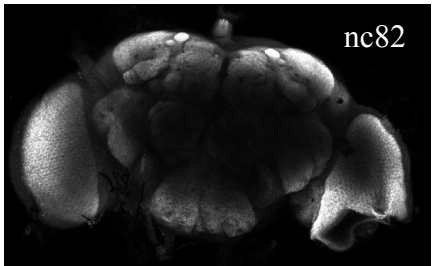
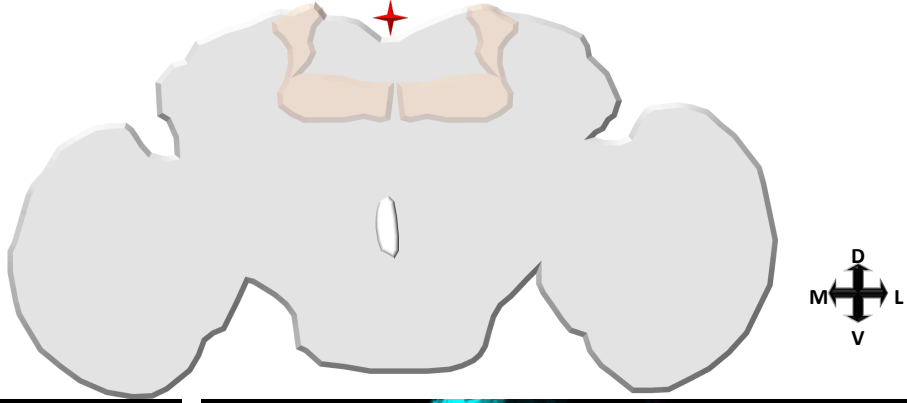
D

Exposed *D. anan* paired with *UAS-shi^{ts}*, R17F12

Supplementary Figure 4. The mushroom body is required for dialect training shown with R13F02 driver.

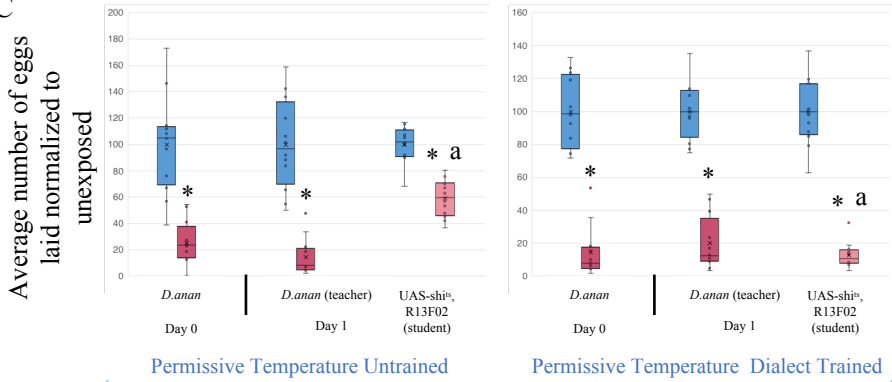
(A) Cartoon schematic of the region of interest, the mushroom body. (B) Confocal image of adult brain where R13F02^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/4 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R13F02^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

A

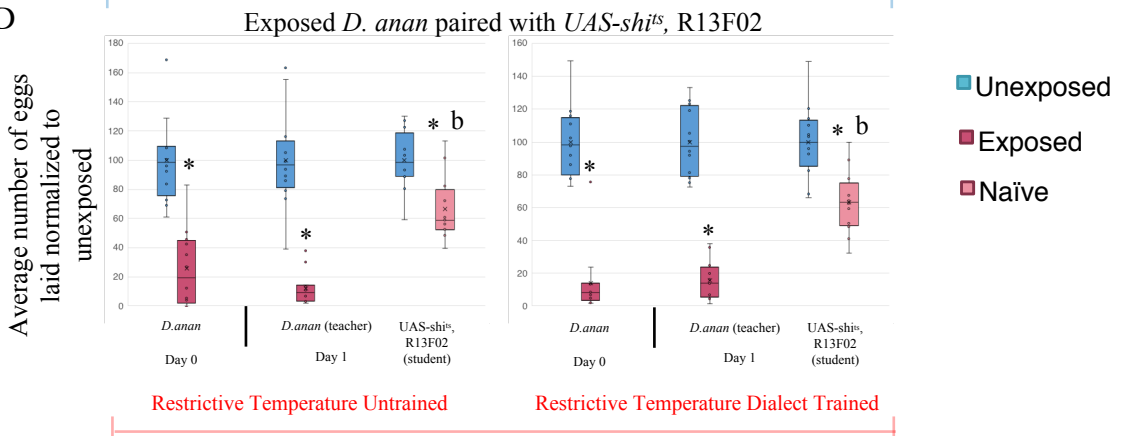


Exposed *D. anan* paired with *UAS-shi^{ts}*, R13F02

C



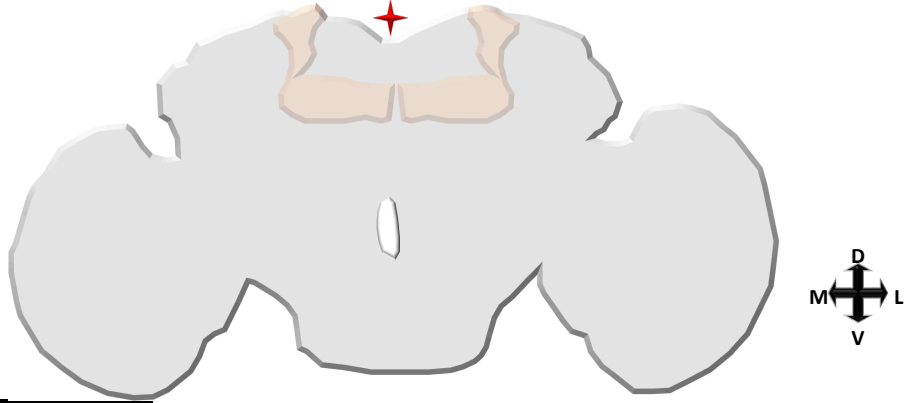
D



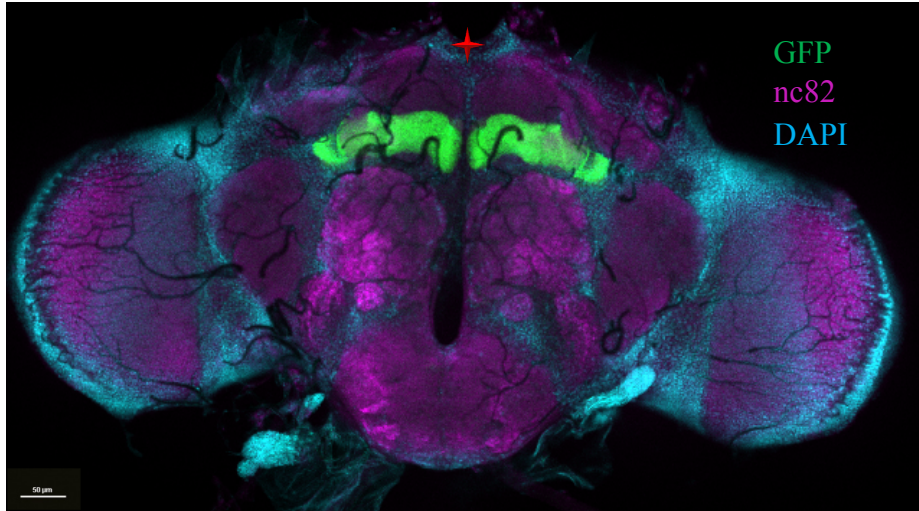
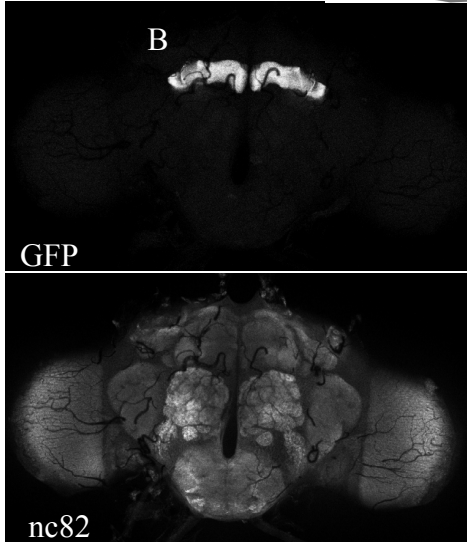
Supplementary Figure 5. The mushroom body is required for dialect training shown with R16A06 driver.

(A) Cartoon schematic of the region of interest, the mushroom body. (B) Confocal image of adult brain where R16A06^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R16A06^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

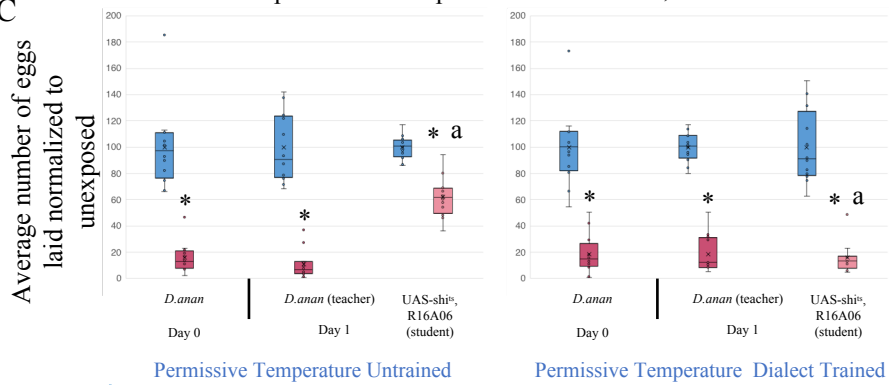
A



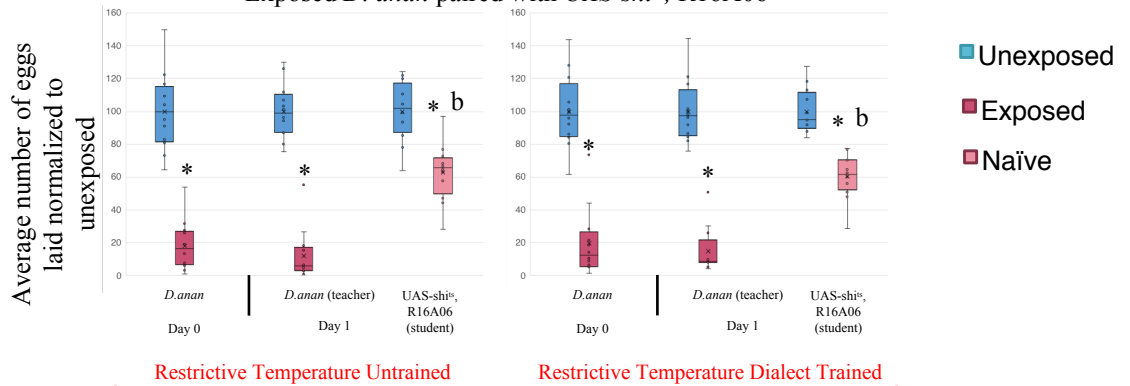
B



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R16A06

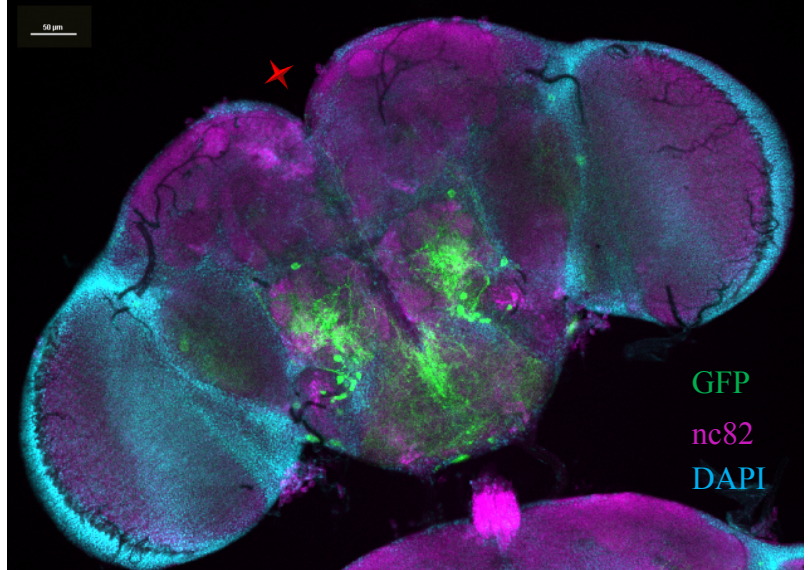
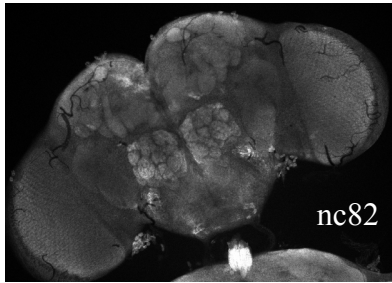
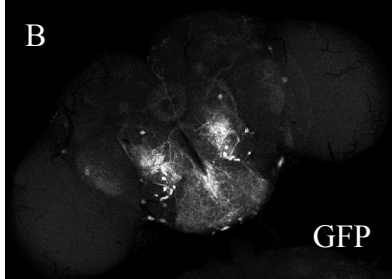
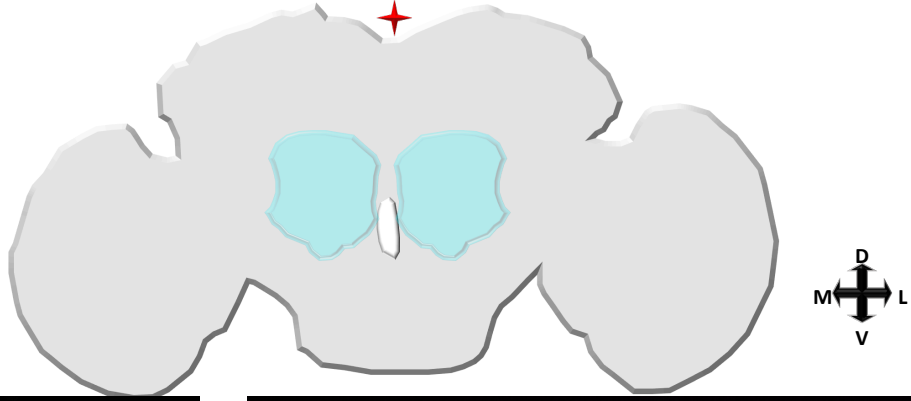
D

Exposed *D. anan* paired with *UAS-shi^{ts}*, R16A06

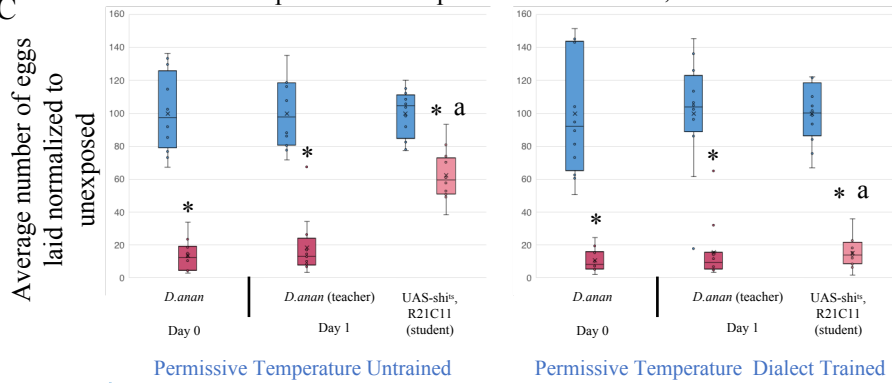
Supplementary Figure 6. The antennal lobe is required for dialect training shown with R21C11 driver.

(A) Cartoon schematic of the region of interest, the antennal lobe. (B) Confocal image of adult brain where R21C11^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 4/4 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R21C11^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled under each graph. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

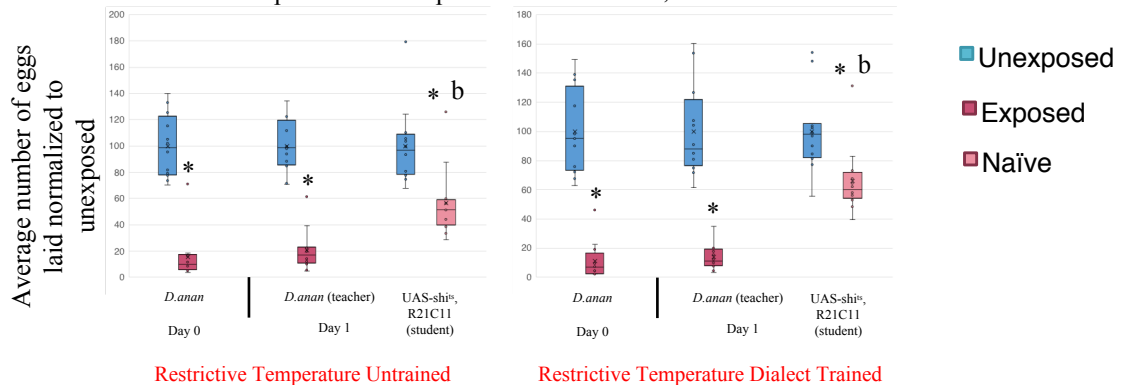
A



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R21C11

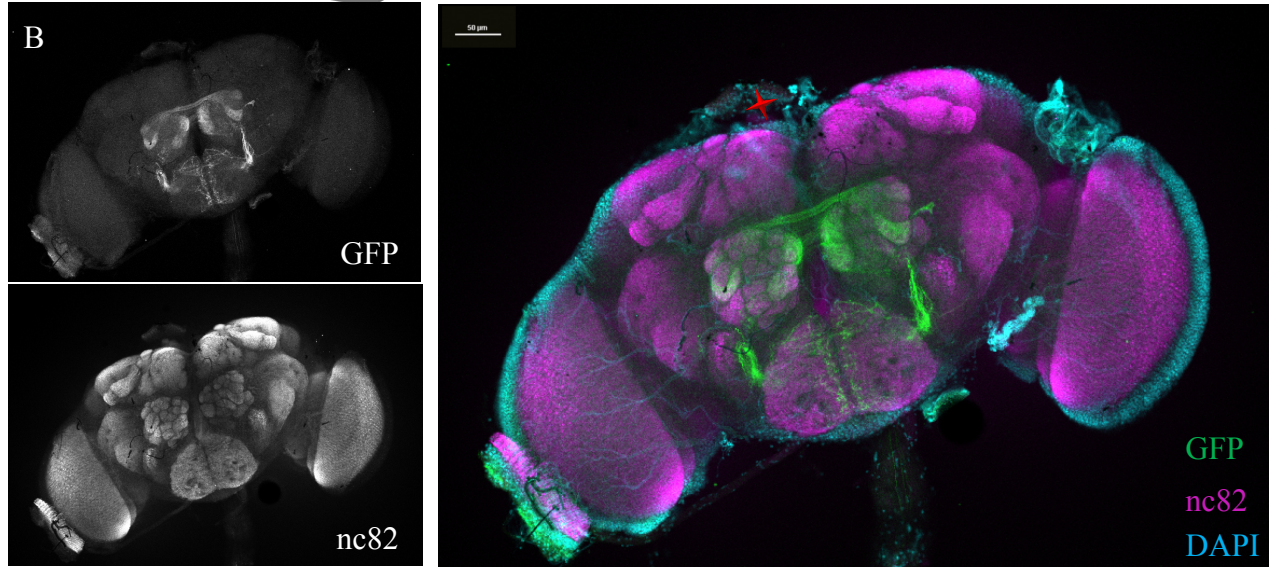
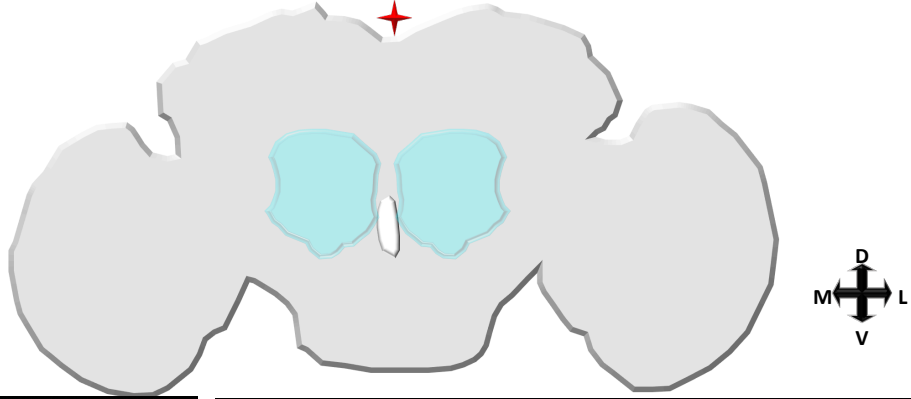
D

Exposed *D. anan* paired with *UAS-shi^{ts}*, R21C11

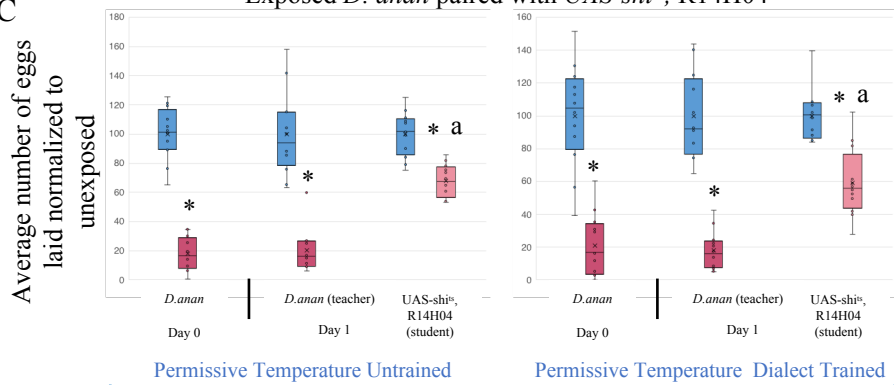
Supplementary Figure 7. The antennal lobe is required for dialect training shown with R14H04 driver.

(A) Cartoon schematic of the region of interest, the antennal lobe. (B) Confocal image of adult brain where R14H04^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/5 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R14H04^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled under each graph. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

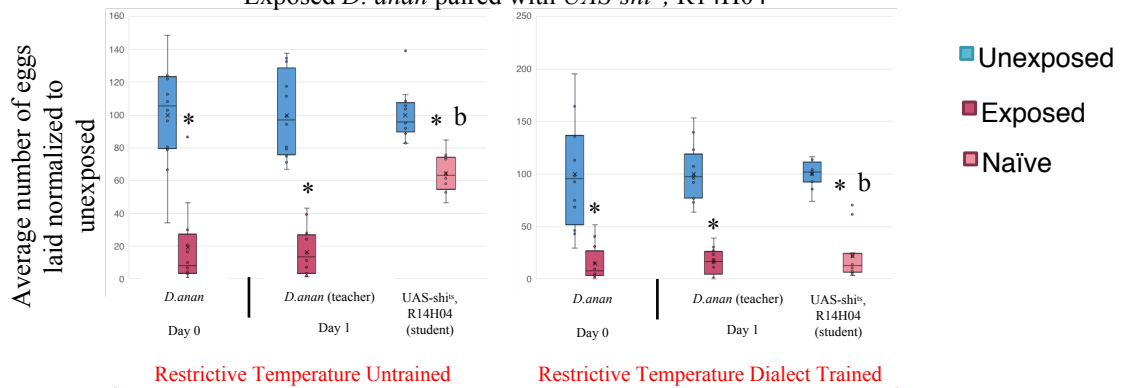
A



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R14H04

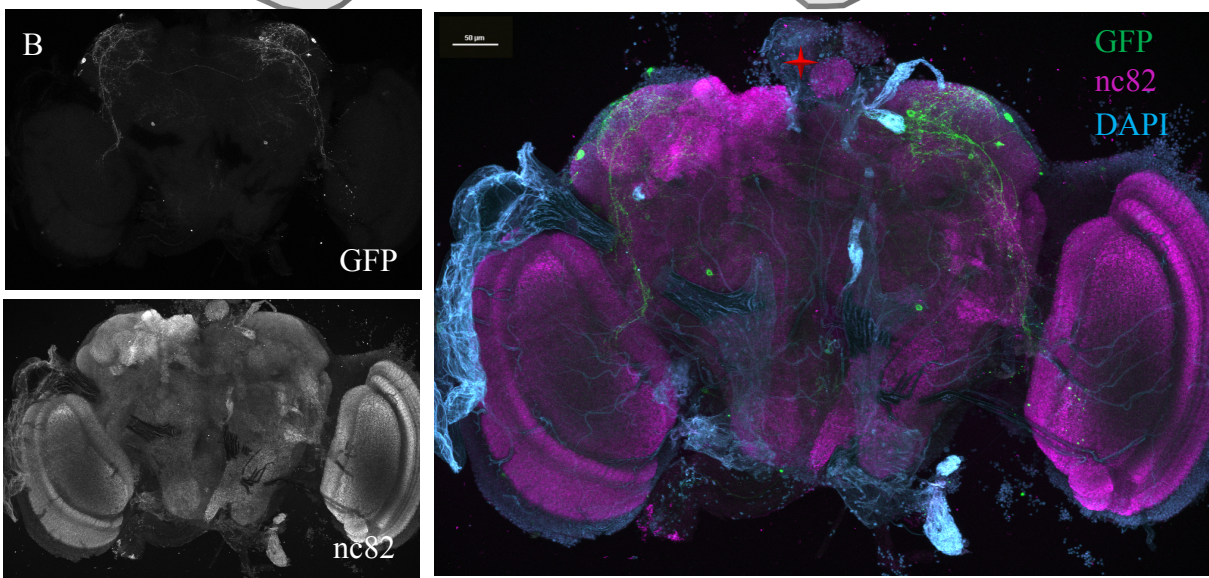
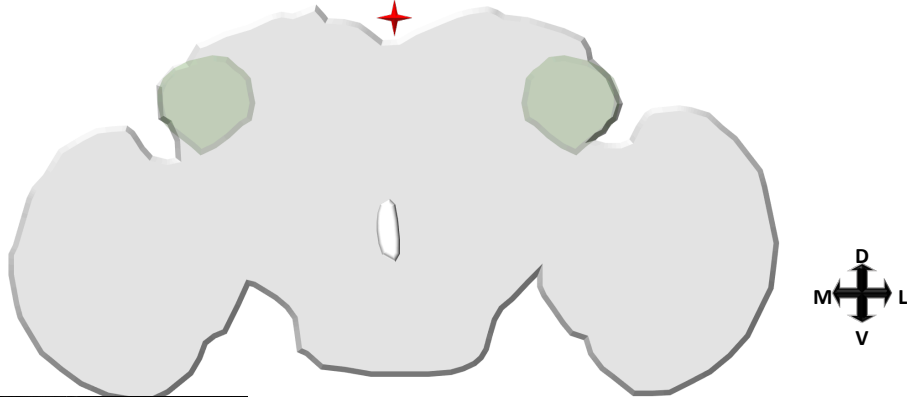
D

Exposed *D. anan* paired with *UAS-shi^{ts}*, R14H04

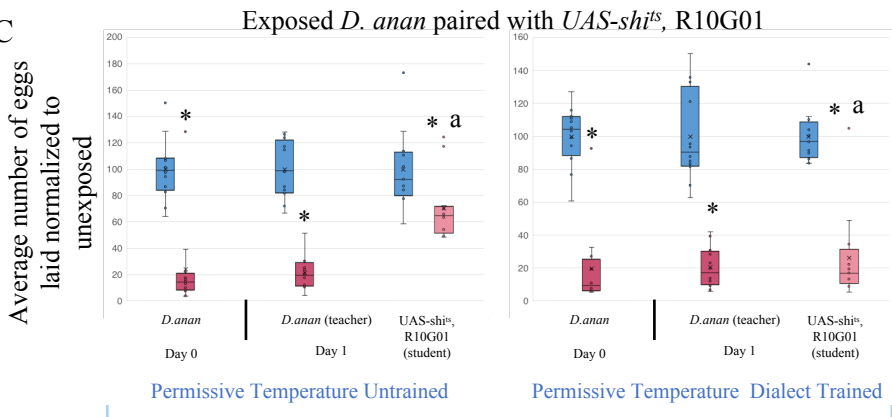
Supplementary Figure 8. The lateral horn is required for dialect training shown with R10G01 driver.

(A) Cartoon schematic of the region of interest, the lateral horn. (B) Confocal image of adult brain where R10G01^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 2/2 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R10G01^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

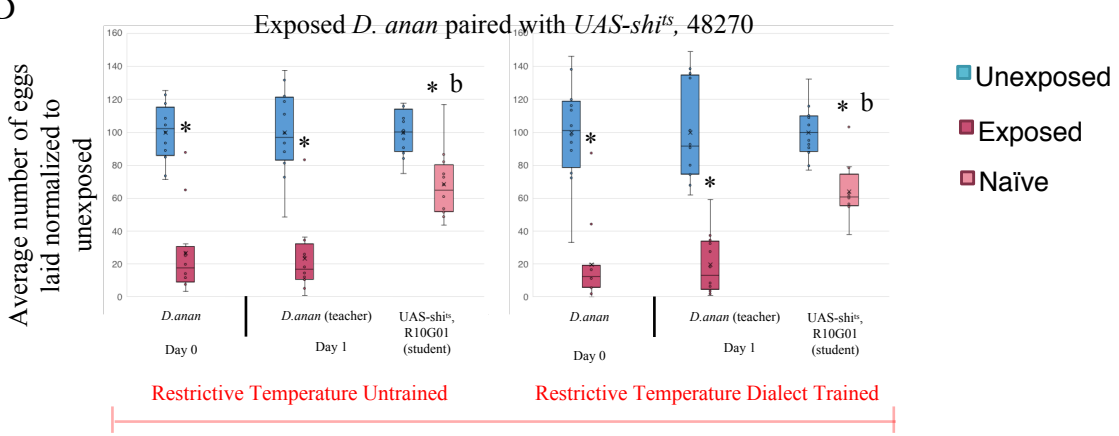
A



C



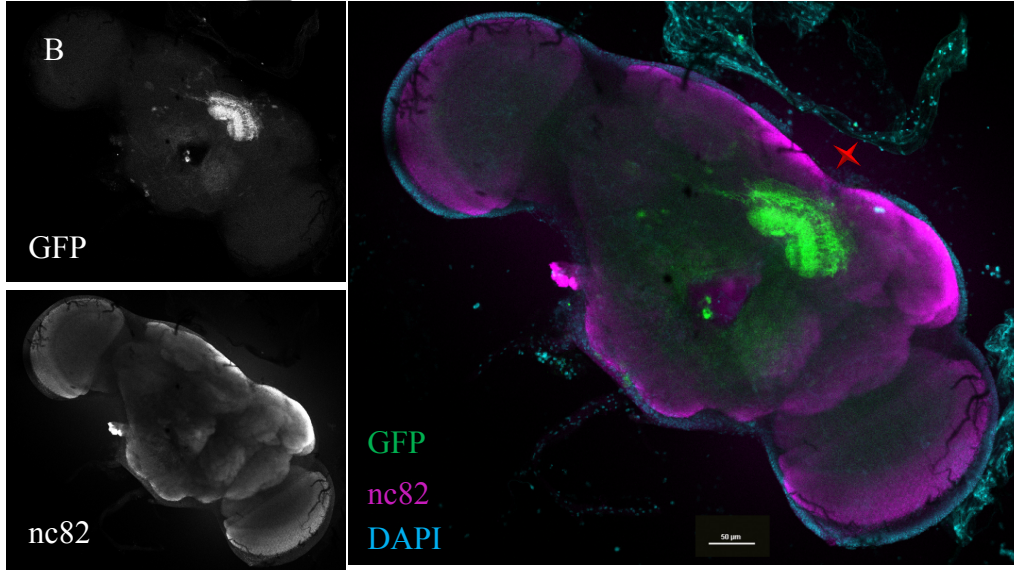
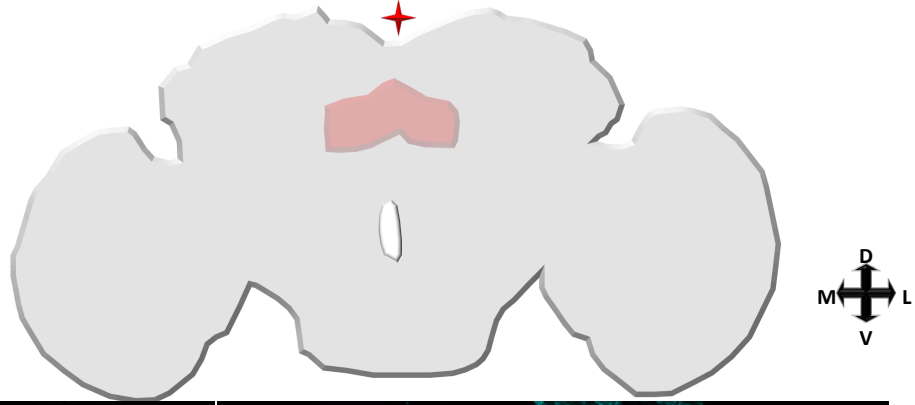
D



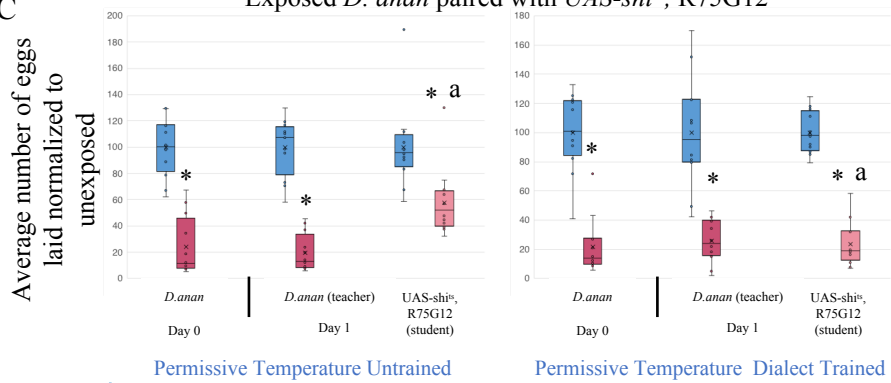
Supplementary Figure 9. The fan-shaped body is required for dialect training shown with R75G12 driver.

(A) Cartoon schematic of the region of interest, the fan-shaped body. (B) Confocal image of adult brain where R75G12^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/3 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R75G12^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

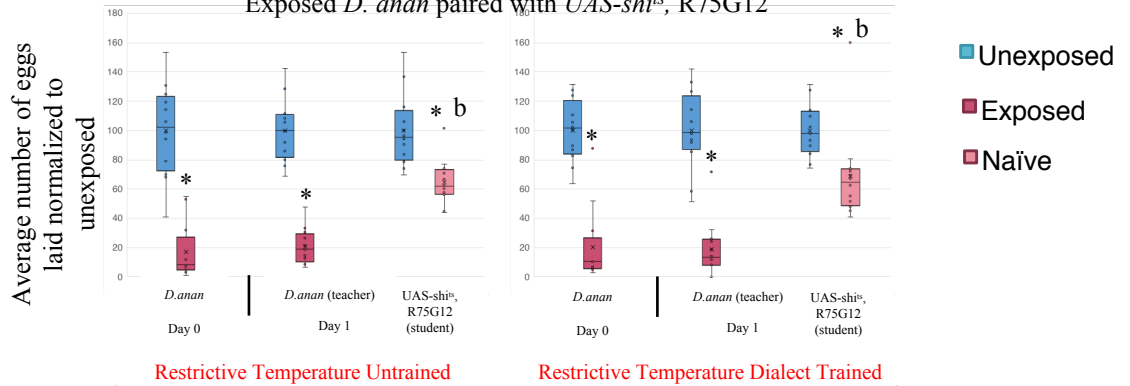
A



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R75G12

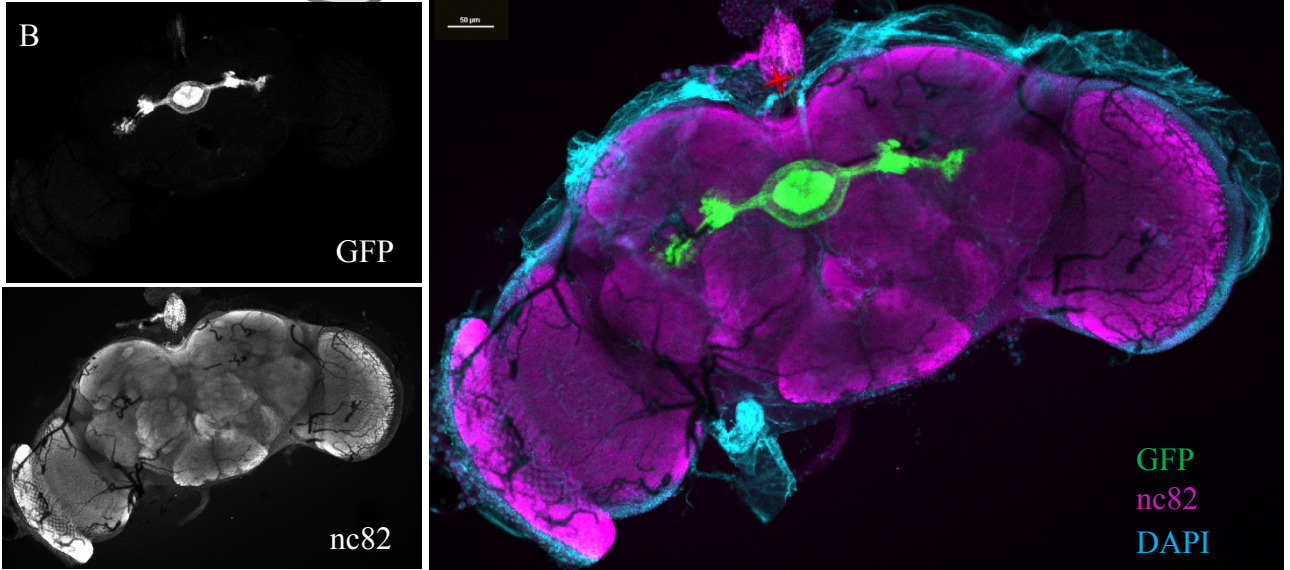
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Exposed *D. anan* paired with *UAS-shi^{ts}*, R75G12

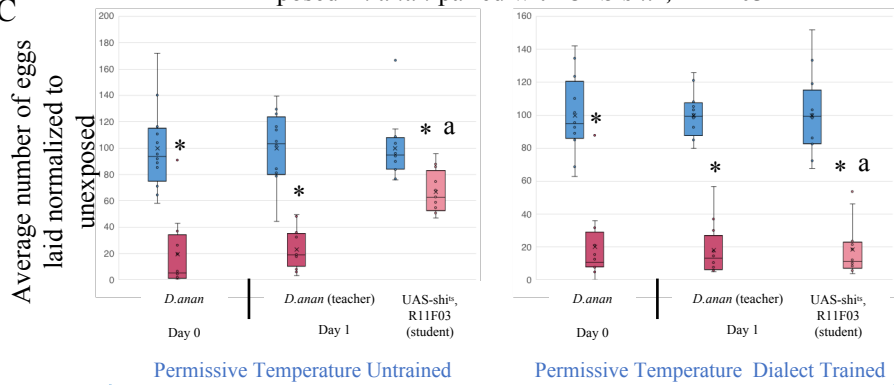
Supplementary Figure 10. The ellipsoid body is required for dialect training shown with R11F03 driver.

(A) Cartoon schematic of the region of interest, the ellipsoid body. (B) Confocal image of adult brain where R11F03^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 3/5 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R11F03^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

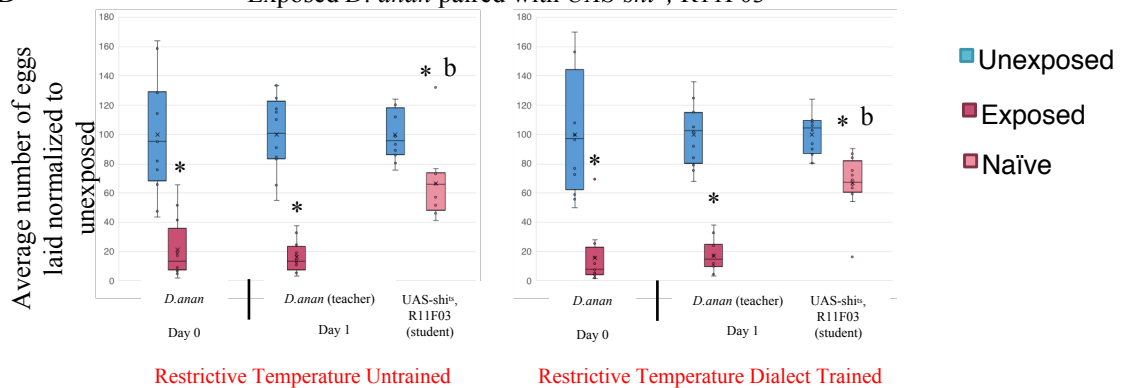
A



C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R11F03

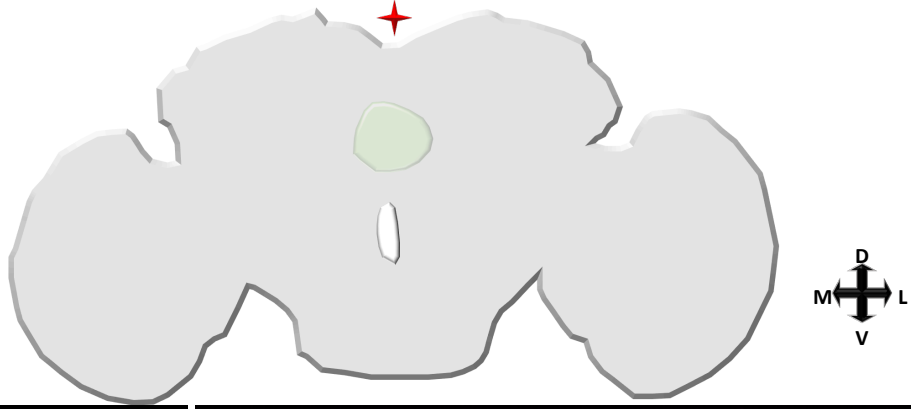
D

Exposed *D. anan* paired with *UAS-shi^{ts}*, R11F03

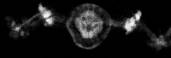
Supplementary Figure 11. The ellipsoid body is required for dialect training shown with R15F02 driver.

(A) Cartoon schematic of the region of interest, the ellipsoid body. (B) Confocal image of adult brain where R15F02^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/5 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R15F02^{GAL4} trained by *D. ananassae* at the permissive temperature shows wild-type trained state (C), but at the restrictive temperature shows defective acquisition in the trained state (D). Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

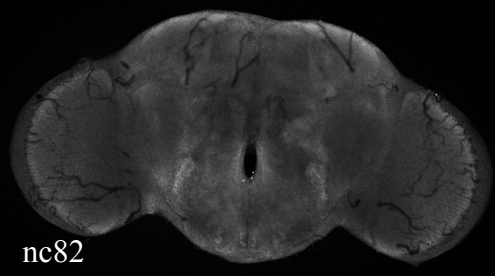
A



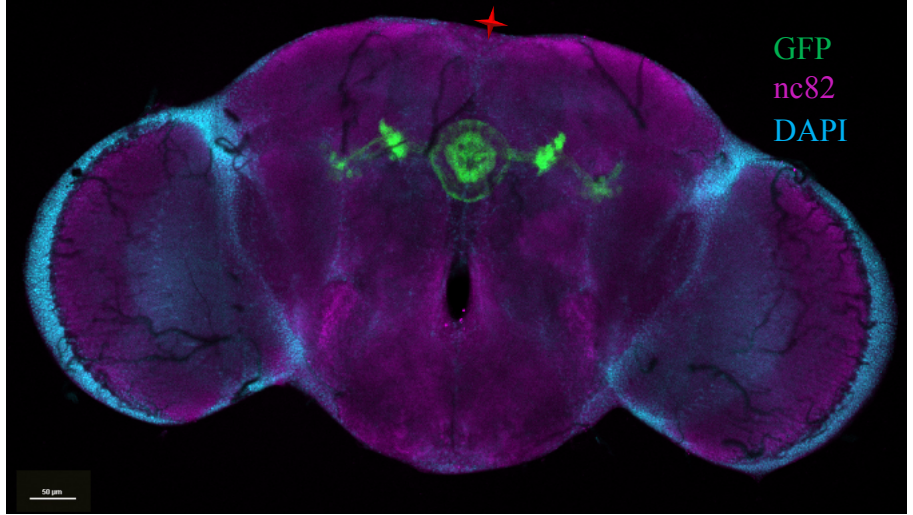
B



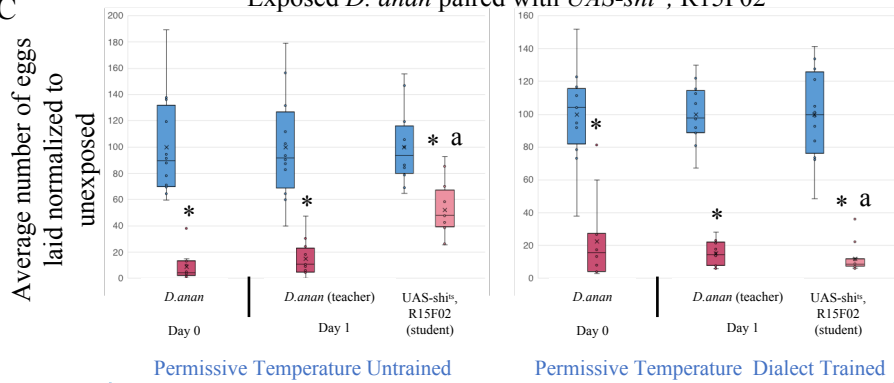
GFP



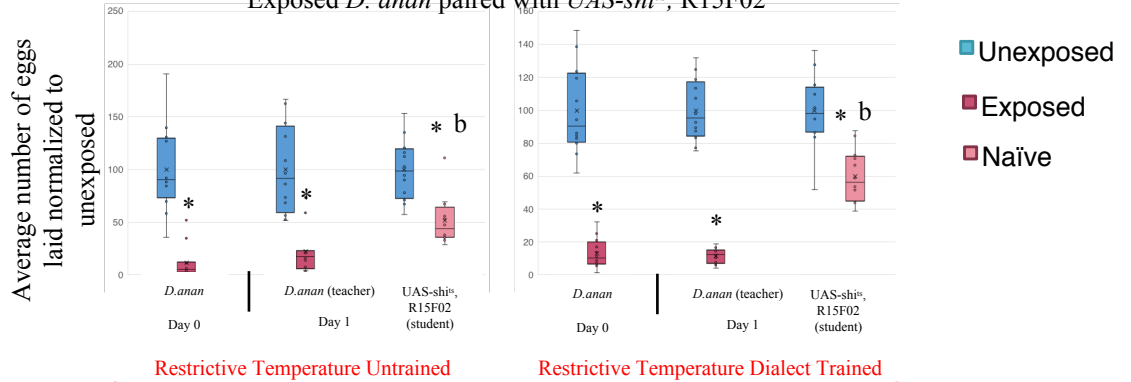
nc82

GFP
nc82
DAPI

C

Exposed *D. anan* paired with *UAS-shi^{ts}, R15F02*

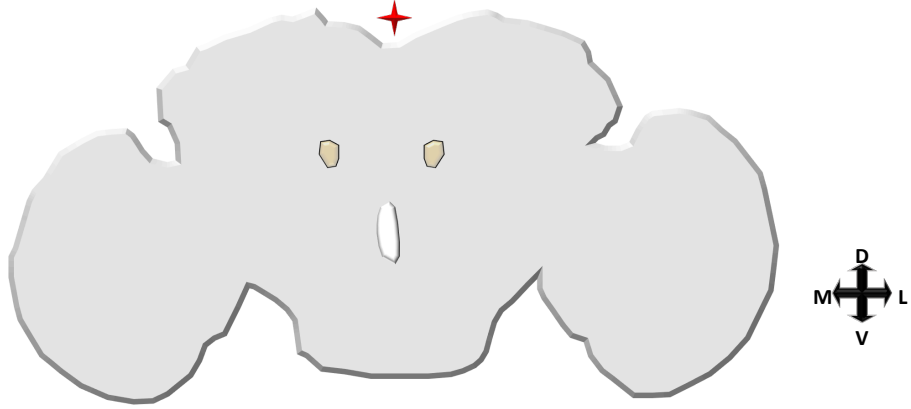
D

Exposed *D. anan* paired with *UAS-shi^{ts}, R15F02*

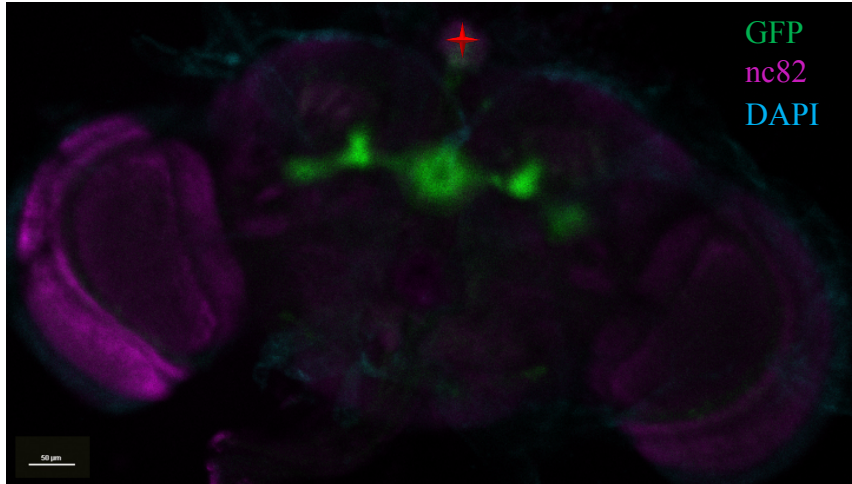
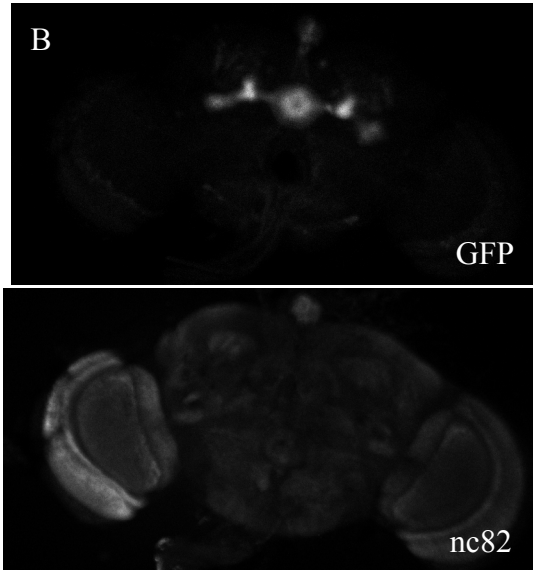
Supplementary Figure 12. The bulb is dispensable for dialect training shown with R32B03 driver.

(A) Cartoon schematic of the region of interest, the bulb. (B) Confocal image of adult brain where R32B03^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 5/4 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R32B03^{GAL4} trained by *D. ananassae* at both the permissive temperature (C) and restrictive temperature (D) shows wild-type trained state. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

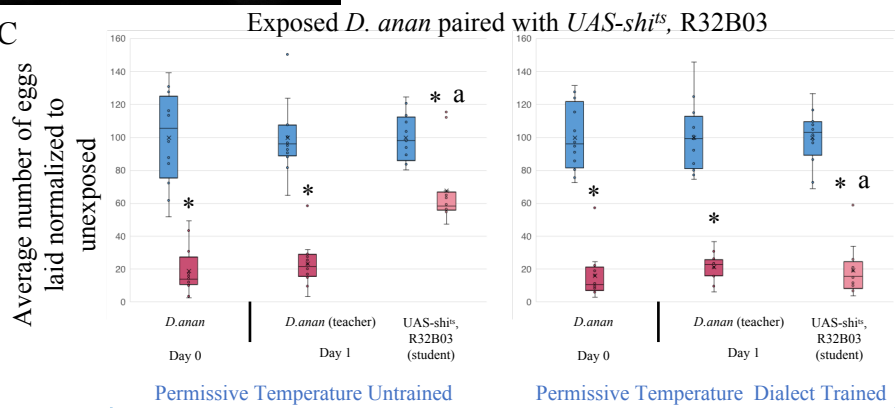
A



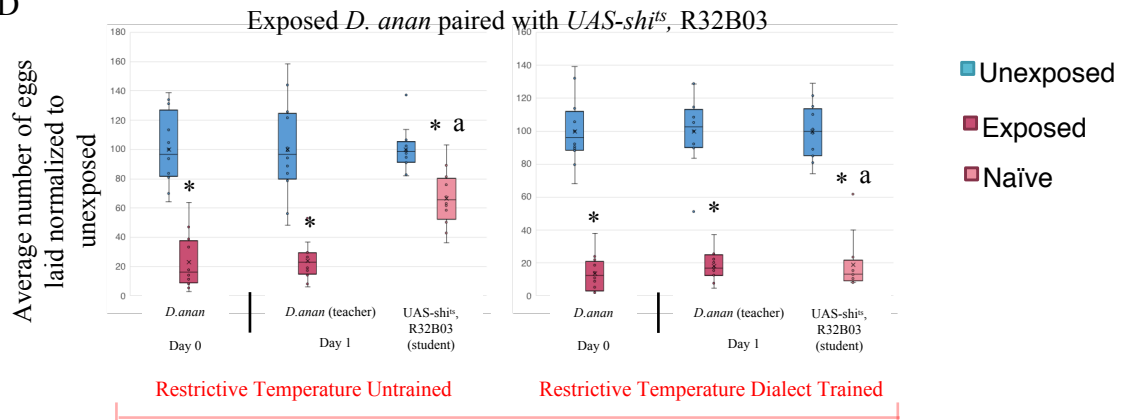
B



C



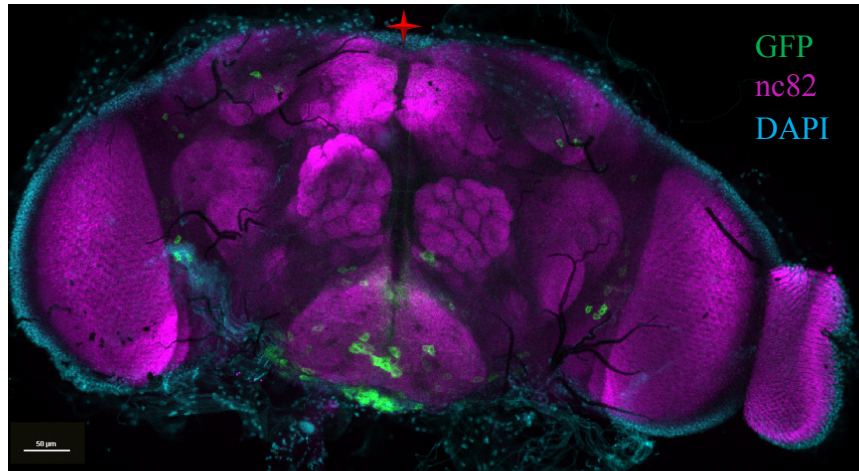
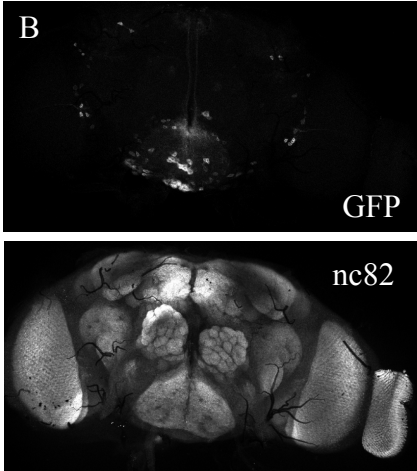
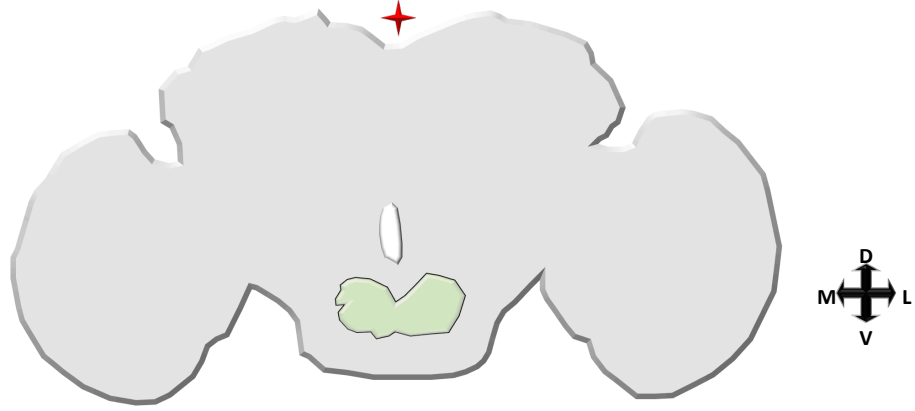
D



Supplementary Figure 13. The prow is dispensable for dialect training shown with R25G04 driver.

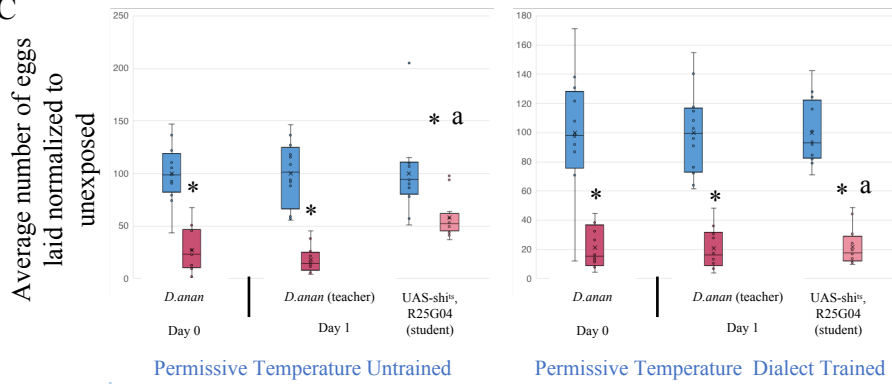
(A) Cartoon schematic of the region of interest, the prow. (B) Confocal image of adult brain where R25G04^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 4/4 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R25G04^{GAL4} trained by *D. ananassae* at both the permissive temperature (C) and restrictive temperature (D) shows wild-type trained state. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

A



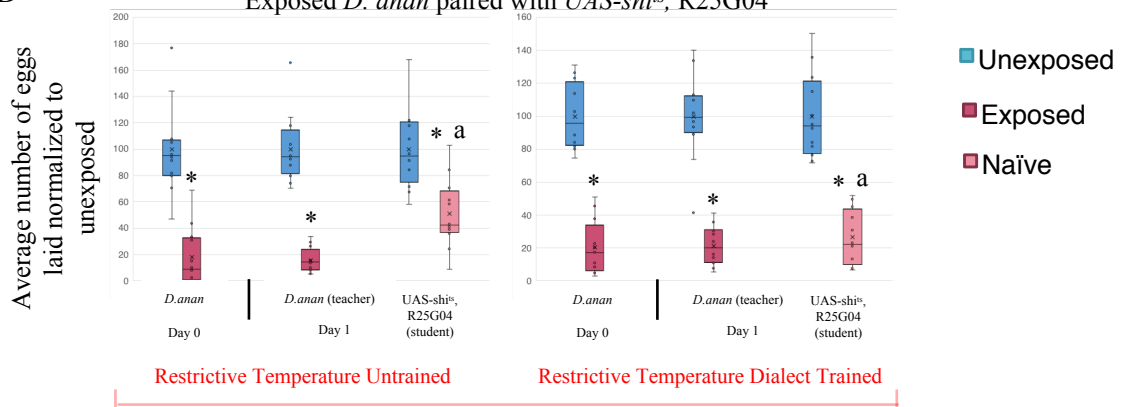
C

Exposed *D. anan* paired with *UAS-shi^{ts}*, R25G04



D

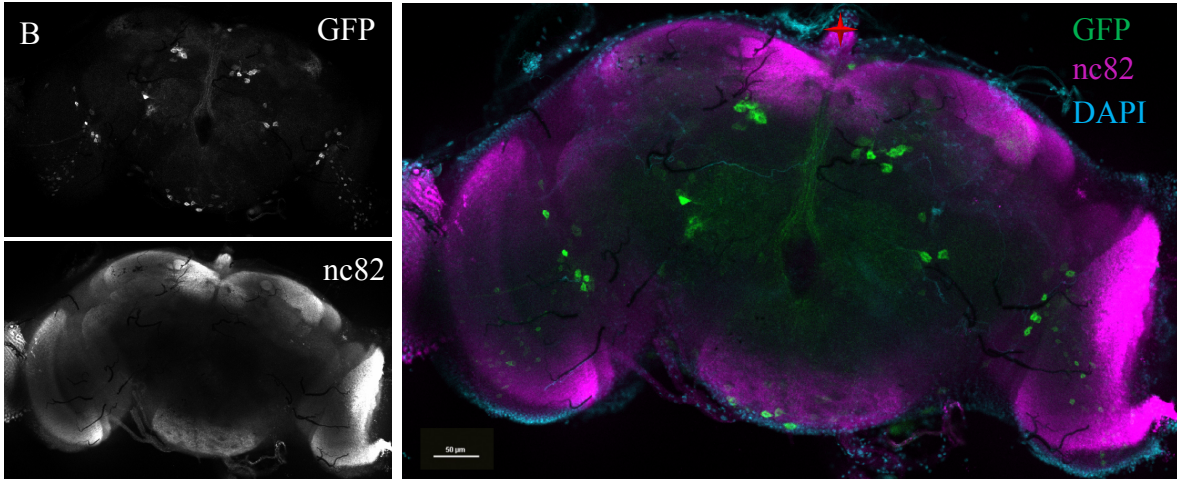
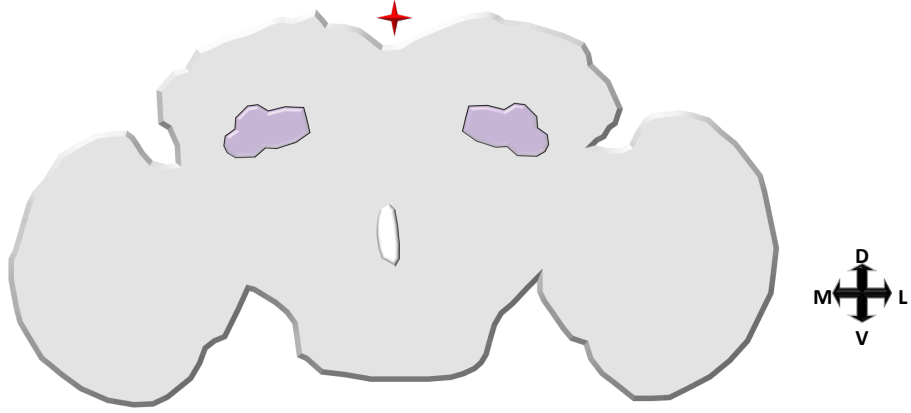
Exposed *D. anan* paired with *UAS-shi^{ts}*, R25G04



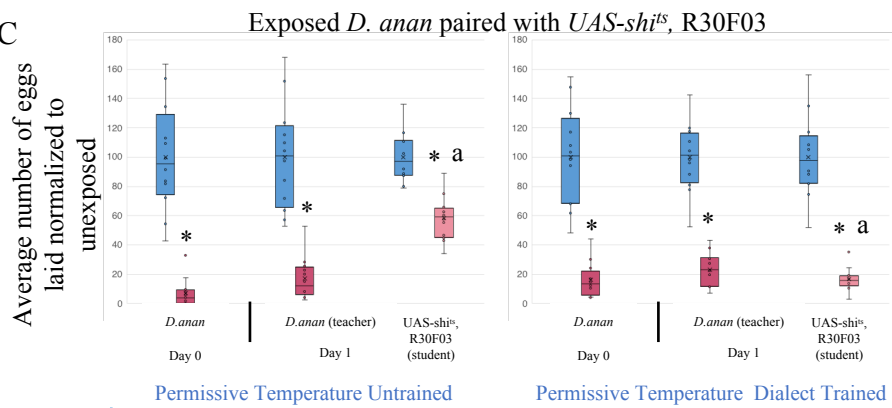
Supplementary Figure 14. The superior clamp is dispensable for dialect training shown with R30F03 driver.

(A) Cartoon schematic of the region of interest, the superior clamp. (B) Confocal image of adult brain where R30F03^{GAL4} is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). Fly light intensity/distribution score is 3/3 and was the only region of the brain identified. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. UAS-shi^{ts} crossed to R30F03^{GAL4} trained by *D. ananassae* at both the permissive temperature (C) and restrictive temperature (D) shows wild-type trained state. Blue lines indicate permissive temperature, while red lines indicate restrictive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a→ p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b→ p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

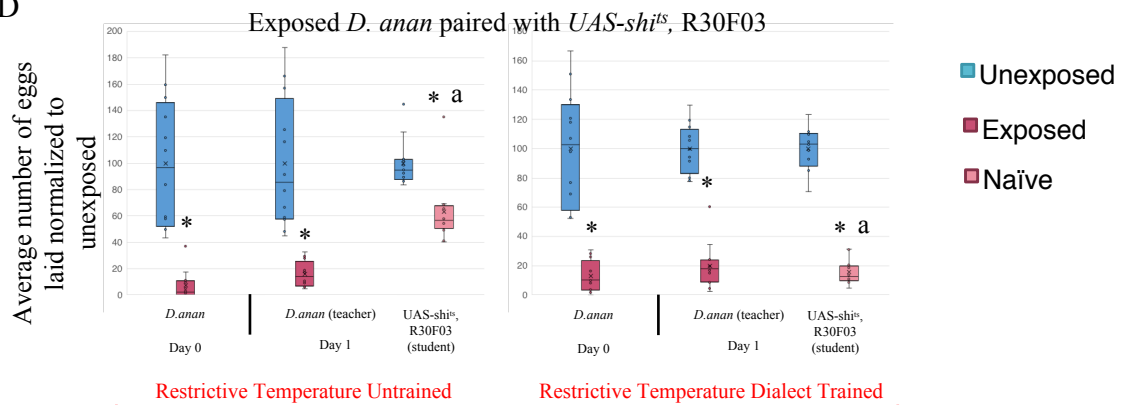
A



C

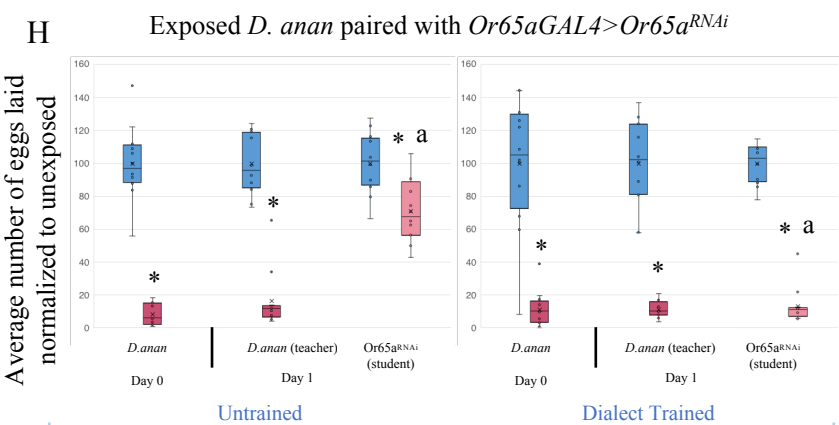
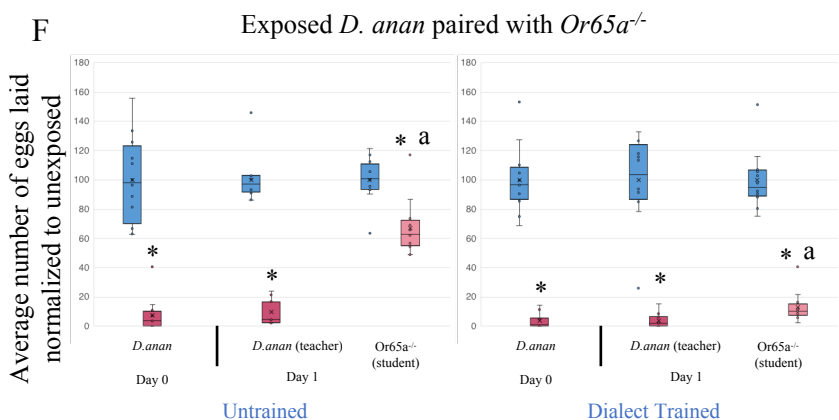
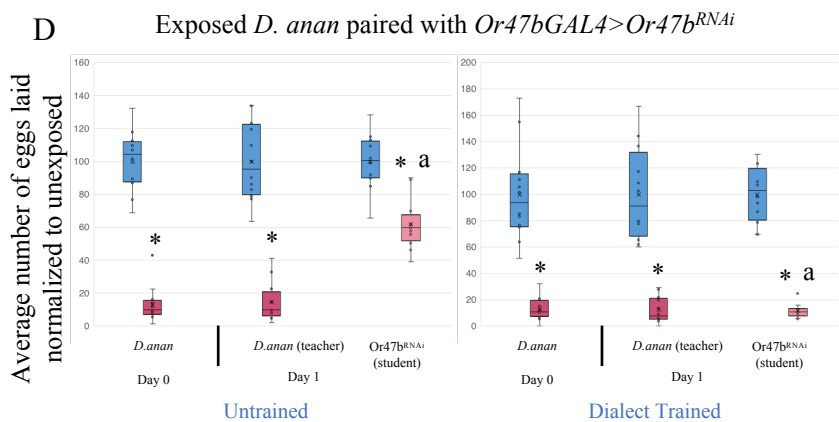
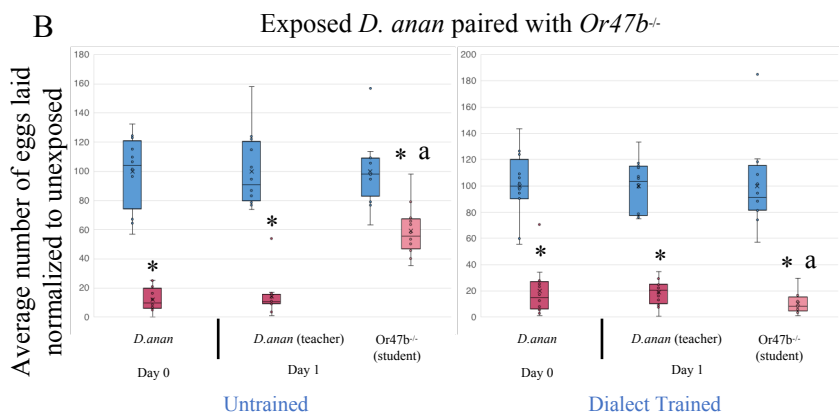
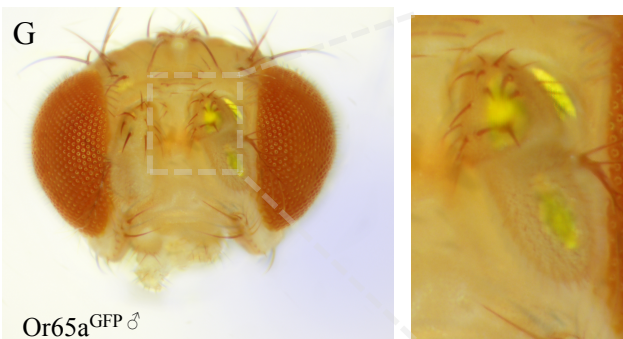
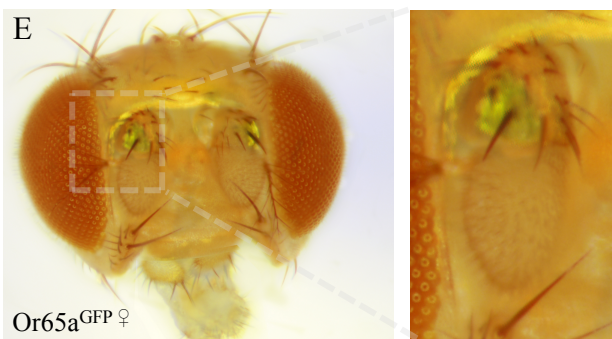
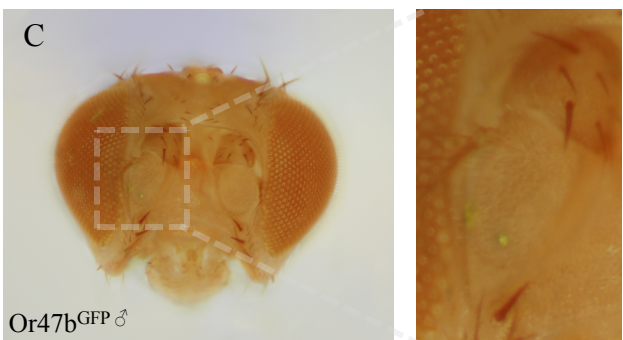
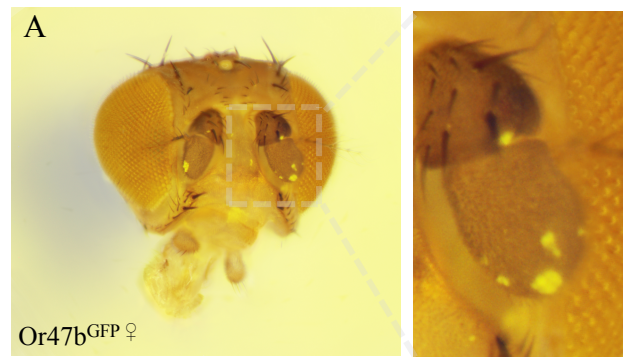


D



Supplementary Figure 15. Or47b and Or65a are dispensable for dialect training.

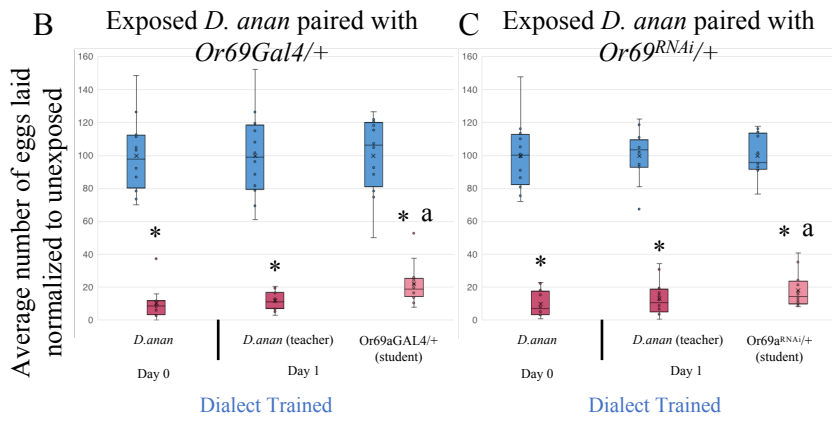
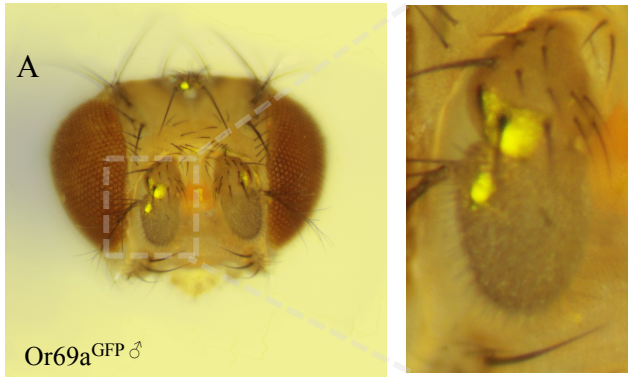
(A) Expression of Or47b in a female fly using the Or47b^{GFP} construct highlighting expression in the antenna. Dotted boxes indicate regions of magnification. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. Or47b^{-/-} show wild-type trained and untrained behavior (B). (C) Expression of Or47b in a male fly using the Or47b^{GFP} construct highlighting expression in the antenna. Or49b^{GAL4} driving Or49b^{RNAi} shows wild-type untrained behavior and can learn the dialect from *D. ananassae* following training (D). (E) Expression of Or65a in a female fly using the Or65a^{GFP} construct highlighting expression in the antenna. Or65a^{-/-} show wild-type trained and untrained behavior (F). (G) Expression of Or65a in a male fly using the Or65a^{GFP} construct highlighting expression in the antenna. Or65a^{GAL4} driving Or65a^{RNAi} shows wild-type untrained behavior and can learn the dialect from *D. ananassae* following training (H). Blue lines indicate permissive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature, c → p = ns comparing trained and untrained students in experiments with no restrictive temperature).



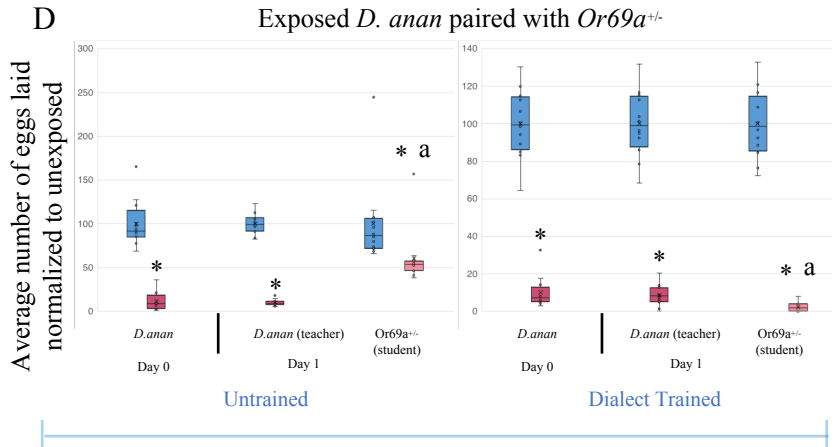
■ Unexposed ■ Exposed ■ Naive

Supplementary Figure 16. Further evidence implicating the role of *Or69a* in dialect training.

(A) Expression of *Or69a* in male flies using the *Or69a^{GFP}* construct highlighting expression in the antenna. Dotted boxes indicate regions of magnification. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. *Or69a^{GAL4/+}* and *Or69a^{RNAi/+}* show wild-type trained behavior (B-C). Outcrossed *Or69a^{-/+}* shows wild-type untrained behavior and dialect training from *D. ananassae* (D). Blue lines indicate permissive temperature. Trained and untrained states are labeled. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature).



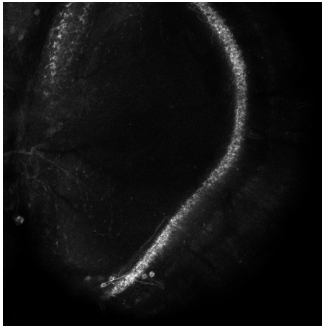
■ Unexposed ■ Exposed ■ Naïve



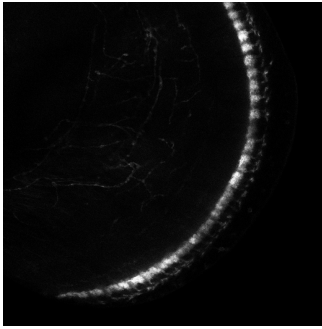
Supplementary Figure 17. Further evidence implicating the role of motion-detecting circuitry in dialect training using UAS Shi^{ts}.

Dialect training is performed at either the permissive (22°C) or restrictive (30°C) temperature, while the wasp exposure and social learning period is performed exclusively at the restrictive temperature. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. (A) UAS-shi^{ts} crossed to *L1^{GAL4}* in the untrained state at restrictive and permissive temperatures shows wild-type untrained state. Expression pattern of *L1^{GAL4}* also shown. (B) UAS-shi^{ts} crossed to *L2^{GAL4}* in the untrained state at restrictive and permissive temperatures shows wild-type untrained state. Expression pattern of *L2^{GAL4}* also shown. (C) UAS-shi^{ts} crossed to *L3^{GAL4}* in the untrained state at restrictive and permissive temperatures shows wild-type untrained state. Expression pattern of *L3^{GAL4}* also shown. (D) UAS-shi^{ts} crossed to *L4^{0987-GAL4}* in the untrained state at restrictive and permissive temperatures shows wild-type untrained state. Expression pattern of *L4^{0987-GAL4}* also shown. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested. Comparison data is located in Figure 3).

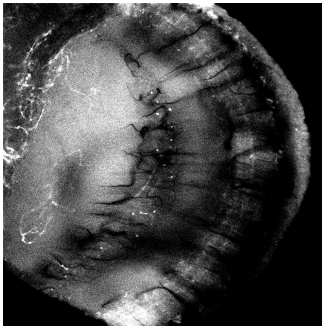
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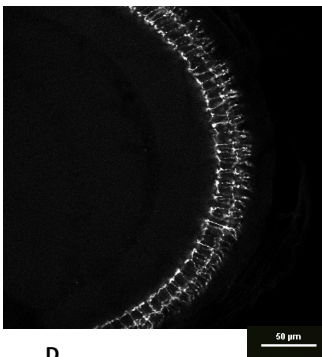
B



C



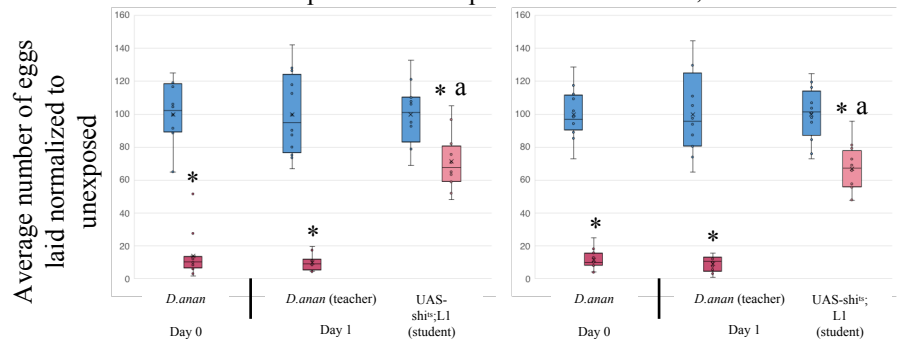
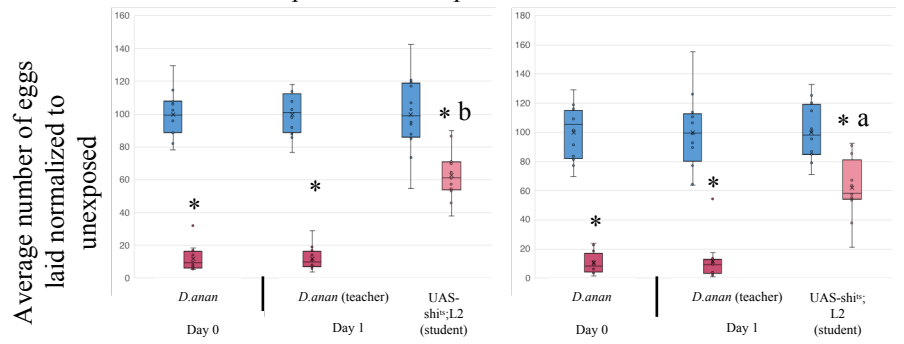
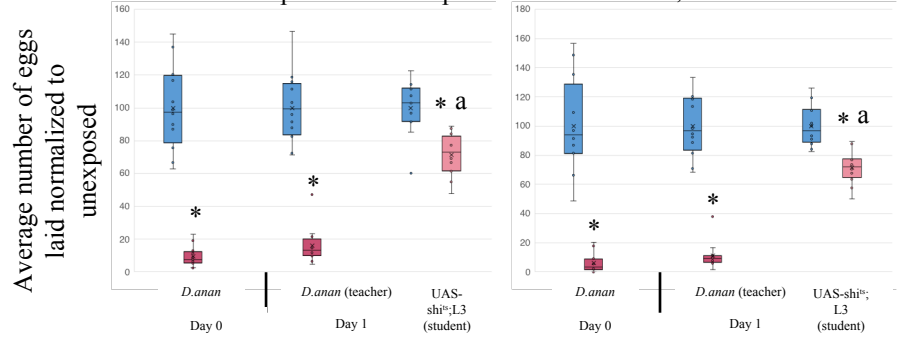
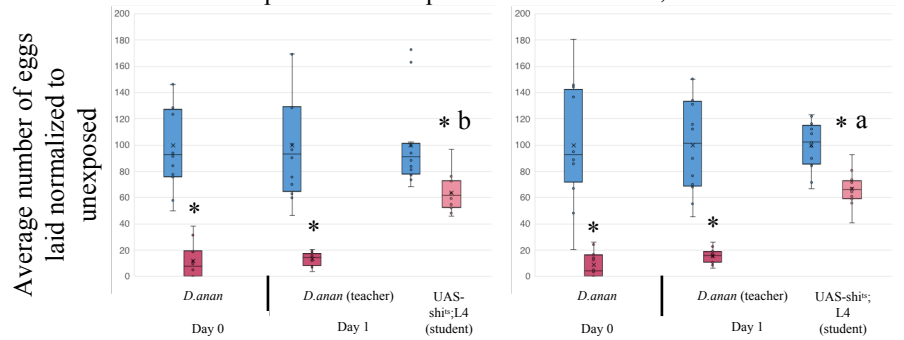
D



GFP

Restrictive Temperature Untrained

Permissive Temperature Untrained

Exposed *D. anan* paired with *UAS-shi^{ts}, L1*Exposed *D. anan* paired with *UAS-shi^{ts}, L2*Exposed *D. anan* paired with *UAS-shi^{ts}, L3*Exposed *D. anan* paired with *UAS-shi^{ts}, L4⁰⁹⁸⁷*

Restrictive Temperature Untrained

Permissive Temperature Untrained

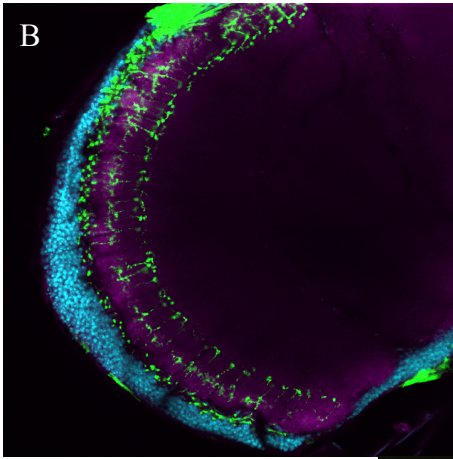
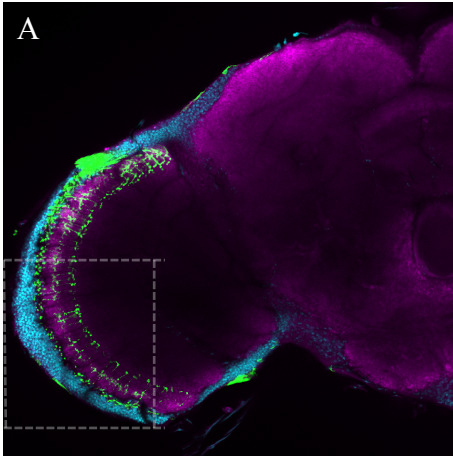
Unexposed

Exposed

Naïve

Supplementary Figure 18. L4 motion sensing neurons in the optic lobe are required for dialect training.

Dialect learning is performed at either the permissive (22°C) or restrictive (30°C) temperature, while the wasp exposure and social learning period is performed exclusively at the permissive temperature. (A) Confocal image of adult brain where *splitL4^{GAL4}* is driving UAS-CD8-GFP, stained with nc82 (magenta) and DAPI (teal). A magnification is shown of boxed area. UAS-shi^{ts} crossed to *splitL4^{GAL4}* trained by *D. ananassae* at the permissive temperature shows wild-type trained state, but at the restrictive temperature shows defective acquisition in the trained state. (B) UAS-shi^{ts} crossed to *splitL4^{GAL4}* in the untrained state at restrictive and permissive temperatures shows wild-type untrained state. Expression pattern of *splitL4^{GAL4}* also shown. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested).

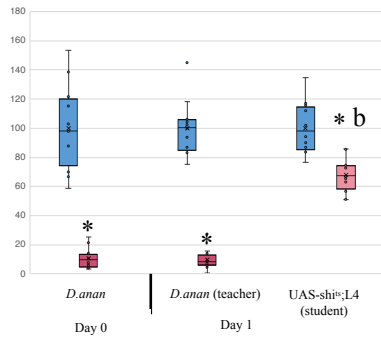


DAPI GFP nc82

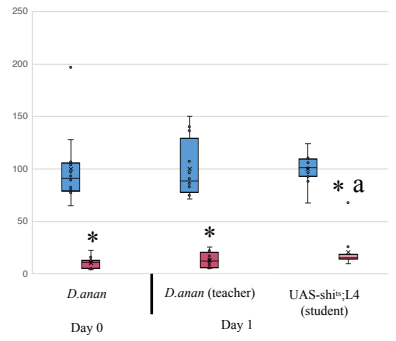


Exposed *D. anan* paired with *UAS-shi^{ts}*, *splitL4* trained by *D. anan*

Average number of eggs laid normalized to unexposed



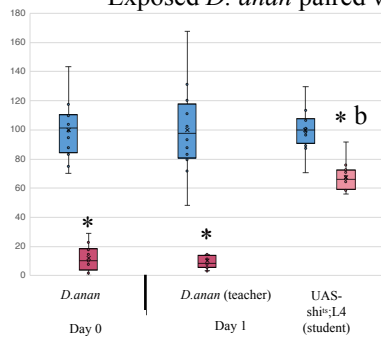
Restrictive Temperature Dialect Trained



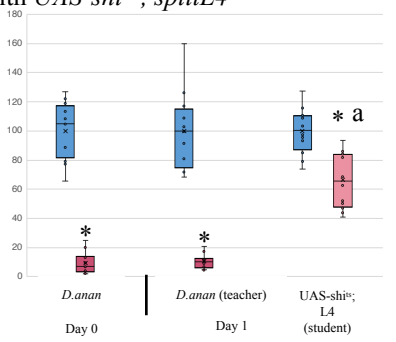
Permissive Temperature Dialect Trained

Exposed *D. anan* paired with *UAS-shi^{ts}*, *splitL4*

Average number of eggs laid normalized to unexposed



Restrictive Temperature Untrained

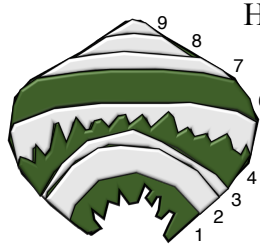
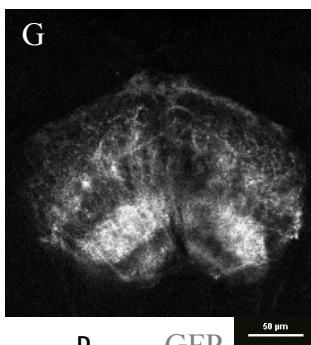
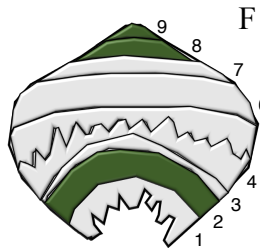
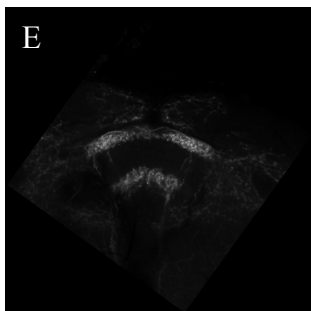
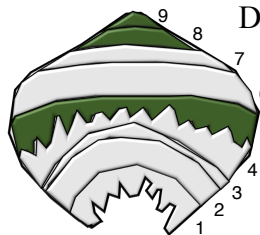
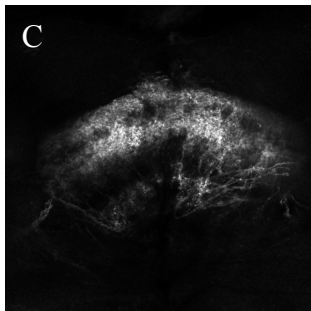
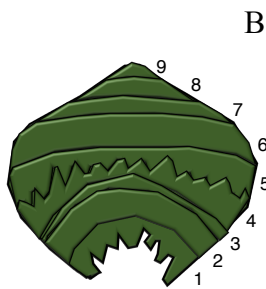
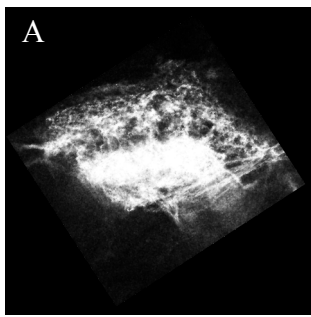


Permissive Temperature Untrained

■ Unexposed ■ Exposed ■ Naïve

Supplementary Figure 19. Further evidence indicating that Region 5 of the fan-shaped body is necessary for dialect training.

Dialect learning is performed at either the permissive (22°C) or restrictive (30°C) temperature, while the wasp exposure and social learning period is performed exclusively at the restrictive temperature. Percentage of eggs laid by exposed flies normalized to eggs laid by unexposed flies is shown. (A) Expression and schematic of expression pattern of the R75G12^{GAL4} FSB driver shows pan-FSB marking. (B) UAS-shi^{ts} crossed to R75G12^{GAL4} trained by *D. ananassae* at the permissive and restrictive temperature shows wild-type untrained state. (C) Expression and schematic of expression pattern of the R38E07^{GAL4} FSB driver shows expression in regions 5, 8, and 9. (D) UAS-shi^{ts} crossed to R38E07^{GAL4} trained by *D. ananassae* at the permissive and restrictive temperature shows wild-type untrained state. (E) Expression and schematic of expression pattern of the R89E07^{GAL4} FSB driver shows expression in regions 2, 8, and 9. (F) UAS-shi^{ts} crossed to R89E07^{GAL4} trained by *D. ananassae* at the permissive and restrictive temperature shows wild-type untrained state. (G) Expression and schematic of expression pattern of the R49H02^{GAL4} FSB driver shows expression in regions 1, 4, and 6. (H) UAS-shi^{ts} crossed to R49H02^{GAL4} trained by *D. ananassae* at the permissive and restrictive temperature shows wild-type untrained state. Plots are standard Tukey Box Plots, where the bounds of the shaded box represent the first and third quartile; whiskers indicate the minimum and maximum data point within 1.5 times the interquartile range. Mean is denoted with an 'X.' Data used to generate the plots have been superimposed on the graph as open circles (n = 12 biologically independent experiments) (*p < 0.05, a → p < 0.05 comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested, b → p = ns comparing trained and untrained students within a given temperature when both restrictive and permissive temperatures are tested. Comparison data is located in Figure 4).



GFP



■ Unexposed

■ Exposed

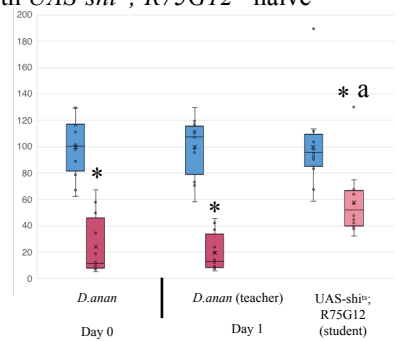
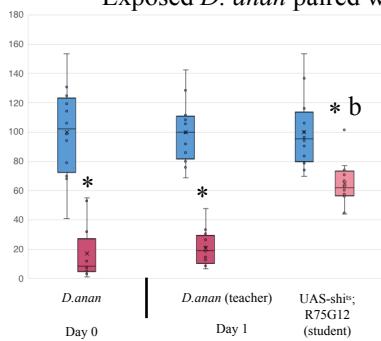
■ Naïve

Restrictive Temperature Untrained

Permissive Temperature Untrained

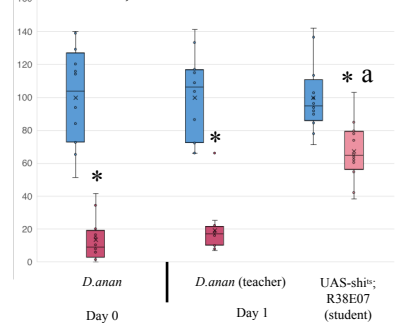
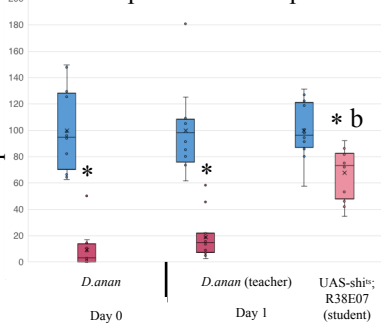
Exposed *D. anan* paired with *UAS-shi^{ts}; R75G12* naïve

Average number of eggs laid normalized to unexposed



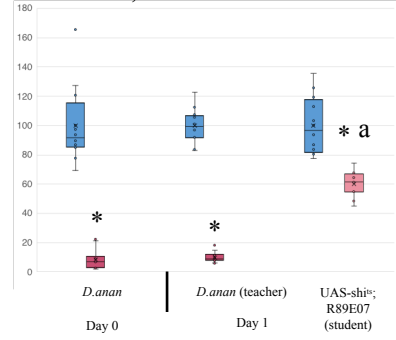
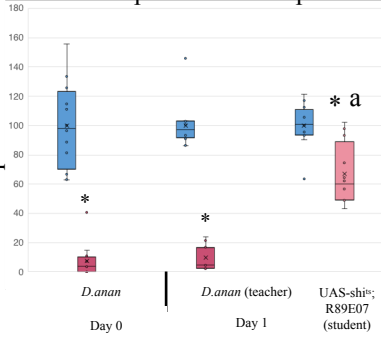
Exposed *D. anan* paired with *UAS-shi^{ts}; R38E07* naïve

Average number of eggs laid normalized to unexposed



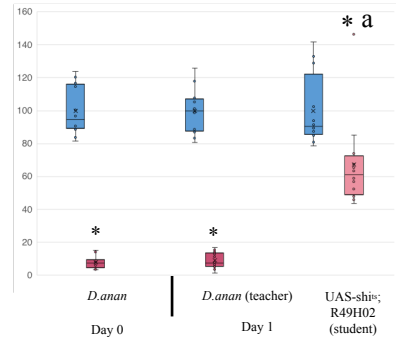
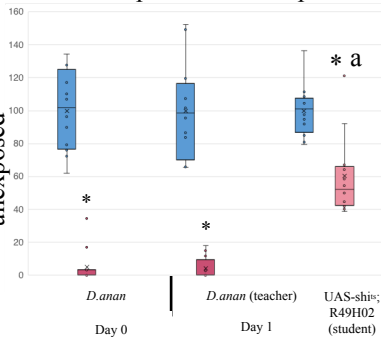
Exposed *D. anan* paired with *UAS-shi^{ts}; R89E07* naïve

Average number of eggs laid normalized to unexposed



Exposed *D. anan* paired with *UAS-shi^{ts}; R49H02* naïve

Average number of eggs laid normalized to unexposed

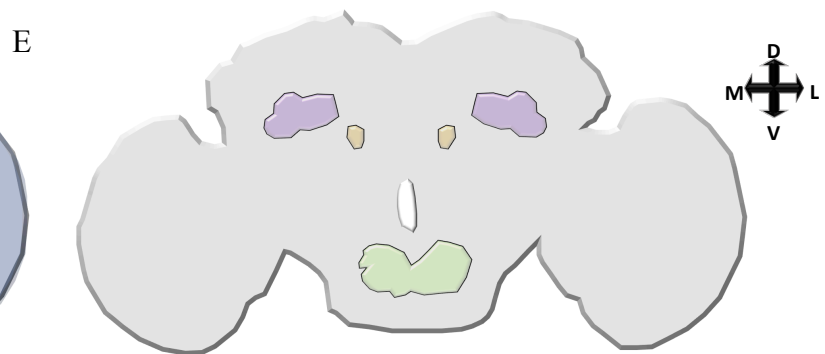
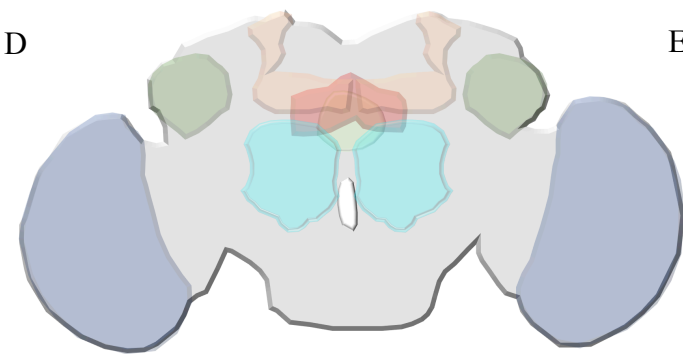
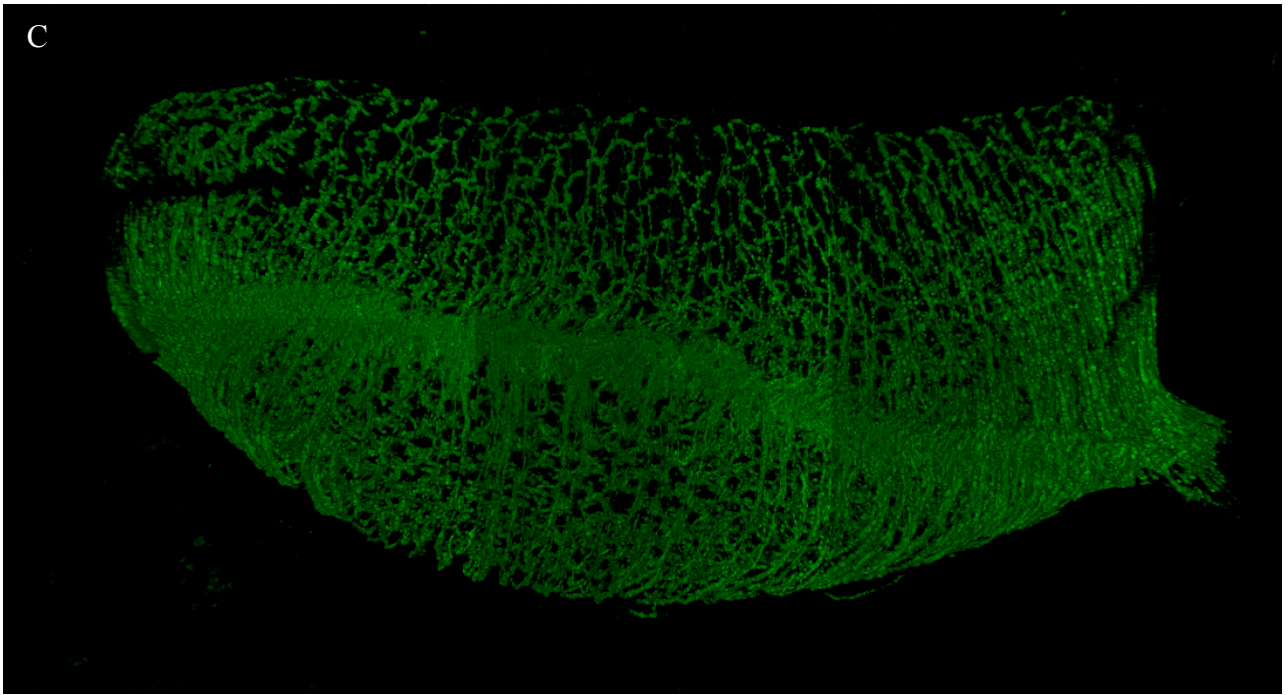
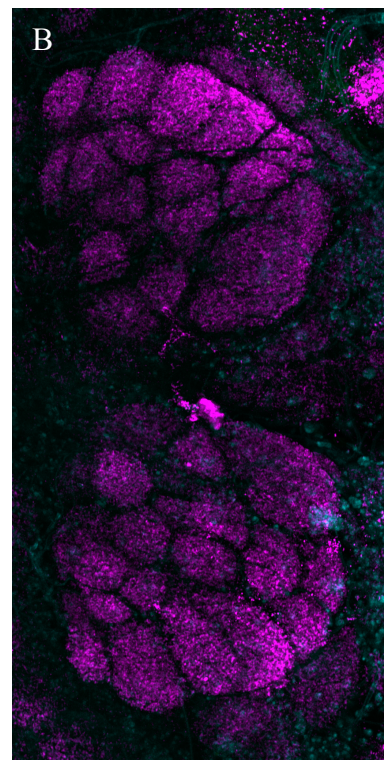
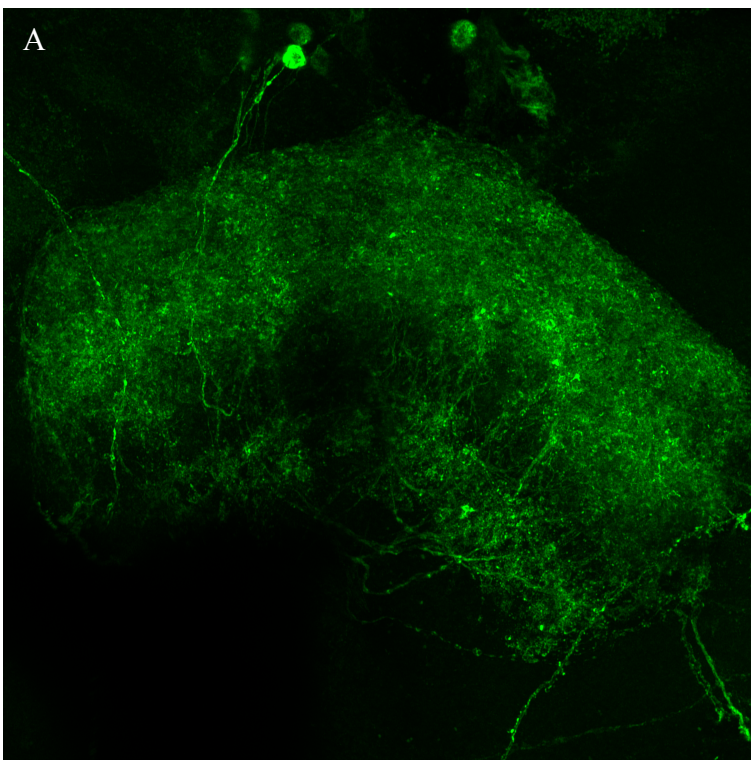


Restrictive Temperature Untrained

Permissive Temperature Untrained

Supplementary Figure 20. Super resolution microscopy of key brain regions in dialect learning.

(A) Z-stack maximum intensity project super resolution image of driver line R38E07 in conjunction with UAS-CD8-GFP. (B) Z-stack maximum intensity projection super resolution image of the antennal lobe stained with nc82. (C) Z-stack maximum intensity projection super resolution image of driver line L4⁰⁹⁸⁷ in conjunction with UAS-CD8-GFP. (D) Brain regions identified as involved in dialect learning—the optic lobe (blue), mushroom body (orange), antennal lobe (teal), lateral horn (green), fan-shaped body (red), and ellipsoid body (light green). (E) Brain regions identified as being dispensable for dialect learning—the prow (light green), bulb (yellow), and superior clamp (purple).



SUPPLEMENTARY TABLES

Supplementary Table 1. Name, genotype, acquisition location, and stock identification number (if applicable) are shown.

Gene/Allele	Genotype	Acquisition location	ID #
CS (WT control)	-	n/a	n/a
<i>D. ananassae</i>	-	Drosophila species stock center	14024-0371.13
L1 GAL4	w[+] (I); L1[c202a]Gal4 / (CyO) (isoD1)	Marion Silies--European Neuroscience Institute	n/a
L2 GAL4	w[+] (I); L2[21Dhh]-Gal4 / TM6B (isoD1)	Marion Silies--European Neuroscience Institute	n/a
L3 GAL4	w[+] (I); L3[0595]-Gal4 / TM6B	Marion Silies--European Neuroscience Institute	n/a
L4 GAL4	w (I); L4 [0987]-GAL4 / (CyO) (isoD1)	Marion Silies--European Neuroscience Institute	n/a
splitL4 GAL4	w (I); L4 [0987]-GAL4-F32.1-A30-dw (II); 0980-D33.1-A2-dw (III) (isoD1)	Marion Silies--European Neuroscience Institute	n/a
Or69a-GFP	w[*]; P{w[+mC]=Or69aA-Mmus\Cd8a.GFP}3	Bloomington Drosophila Stock Center	52636
Or65a-GFP	w[*]; P{w[+mC]=Or65a-Mmus\Cd8a.GFP}2	Bloomington Drosophila Stock Center	52631
Or47b-GFP	y[1] w[*]; P{w[+mC]=Or47b-CD2.M}3/TM3, Sb[1]	Bloomington Drosophila Stock Center	9915
Or47b-/-	y[1] w[*]; Mi{y[+mDint2]=MIC}Or47b[Mi05132]	Bloomington Drosophila Stock Center	38008
Or47b[RNAi]	y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02586}attP2	Bloomington Drosophila Stock Center	27274
Or47b[GAL4]	w[*]; P{w[+mC]=Or47b-GAL4.7.467}15.6	Bloomington Drosophila Stock Center	9984
Or69a-/-	y[1] w[*]; Mi{y[+mDint2]=MIC}Or69a[Mi00896]	Bloomington Drosophila Stock Center	32714
Or69a[GAL4]	w[*]; P{w[+mC]=Or69a-GAL4.F}81.3	Bloomington Drosophila Stock Center	9999
Or65a-/-	w[*]; P{w[+mC]=Or65a-GAL4.F}72.5	Bloomington Drosophila Stock Center	9993
Or65a[RNAi]	y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02763}attP2	Bloomington Drosophila Stock Center	27683
Or65a[GAL4]	w[*]; P{w[+mC]=Or65a-GAL4.F}72.5	Bloomington Drosophila Stock Center	9993
Or69a[RNAi]	y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC05836}attP40	Bloomington Drosophila Stock Center	64962
optic lobe-GAL4 R10B01	w[1118]; P{y[+t7.7] w[+mC]=GMR10B01-GAL4}attP2	Bloomington Drosophila Stock Center	49234
optic lobe-GAL4 R17F12	w[1118]; P{y[+t7.7] w[+mC]=GMR17F12-GAL4}attP2	Bloomington Drosophila Stock Center	48779
mushroombody-GAL4 R13F02	w[1118]; P{y[+t7.7] w[+mC]=GMR13F02-GAL4}attP2	Bloomington Drosophila Stock Center	48571
mushroombody-GAL4 R16A06	w[1118]; P{y[+t7.7] w[+mC]=GMR16A06-GAL4}attP2	Bloomington Drosophila Stock Center	48709
antenna lobe-GAL4 R21C11	w[1118]; P{y[+t7.7] w[+mC]=GMR21C11-GAL4}attP2	Bloomington Drosophila Stock Center	47898
antenna lobe-GAL4 R14H04	w[1118]; P{y[+t7.7] w[+mC]=GMR19H04-GAL4}attP2	Bloomington Drosophila Stock Center	48865
lateral horn-GAL4 R10G01	w[1118]; P{y[+t7.7] w[+mC]=GMR10G01-GAL4}attP2	Bloomington Drosophila Stock Center	48270
fan-shapedbody-GAL4 R75G12	w[1118]; P{y[+t7.7] w[+mC]=GMR75G12-GAL4}attP2	Bloomington Drosophila Stock Center	39906
fan-shapedbody-GAL4 R38E07	w[1118]; P{y[+t7.7] w[+mC]=GMR38E07-GAL4}attP2	Bloomington Drosophila Stock Center	50007
fan-shapedbody-GAL4 R89E07	w[1118]; P{y[+t7.7] w[+mC]=GMR89E07-GAL4}attP2	Bloomington Drosophila Stock Center	40553
fan-shapedbody-GAL4 R49H02	w[1118]; P{y[+t7.7] w[+mC]=GMR58F03-GAL4}attP2	Bloomington Drosophila Stock Center	38712
ellipsoidbody-GAL4 R11F03	w[1118]; P{y[+t7.7] w[+mC]=GMR11F03-GAL4}attP2	Bloomington Drosophila Stock Center	48464
ellipsoidbody-GAL4 R15F02	w[1118]; P{y[+t7.7] w[+mC]=GMR15F02-GAL4}attP2	Bloomington Drosophila Stock Center	48698
bulb-GAL4 R32B03	w[1118]; P{y[+t7.7] w[+mC]=GMR32B03-GAL4}attP2	Bloomington Drosophila Stock Center	49886
prow-GAL4 R25G04	w[1118]; P{y[+t7.7] w[+mC]=GMR25G04-GAL4}attP2	Bloomington Drosophila Stock Center	49136
superiorclamp-GAL4 R30F03	w[1118]; P{y[+t7.7] w[+mC]=GMR30F03-GAL4}attP2	Bloomington Drosophila Stock Center	49640
UAS-shi[ts]	w[*]; P{w[+mC]=UAS-shi[ts1].K}3	Bloomington Drosophila Stock Center	44222
CD8-GFP	UAS-CD8-GFP/CyO	Mani Ramaswami	n/a