
Figures and figure supplements

Sleep deprivation suppresses aggression in *Drosophila*

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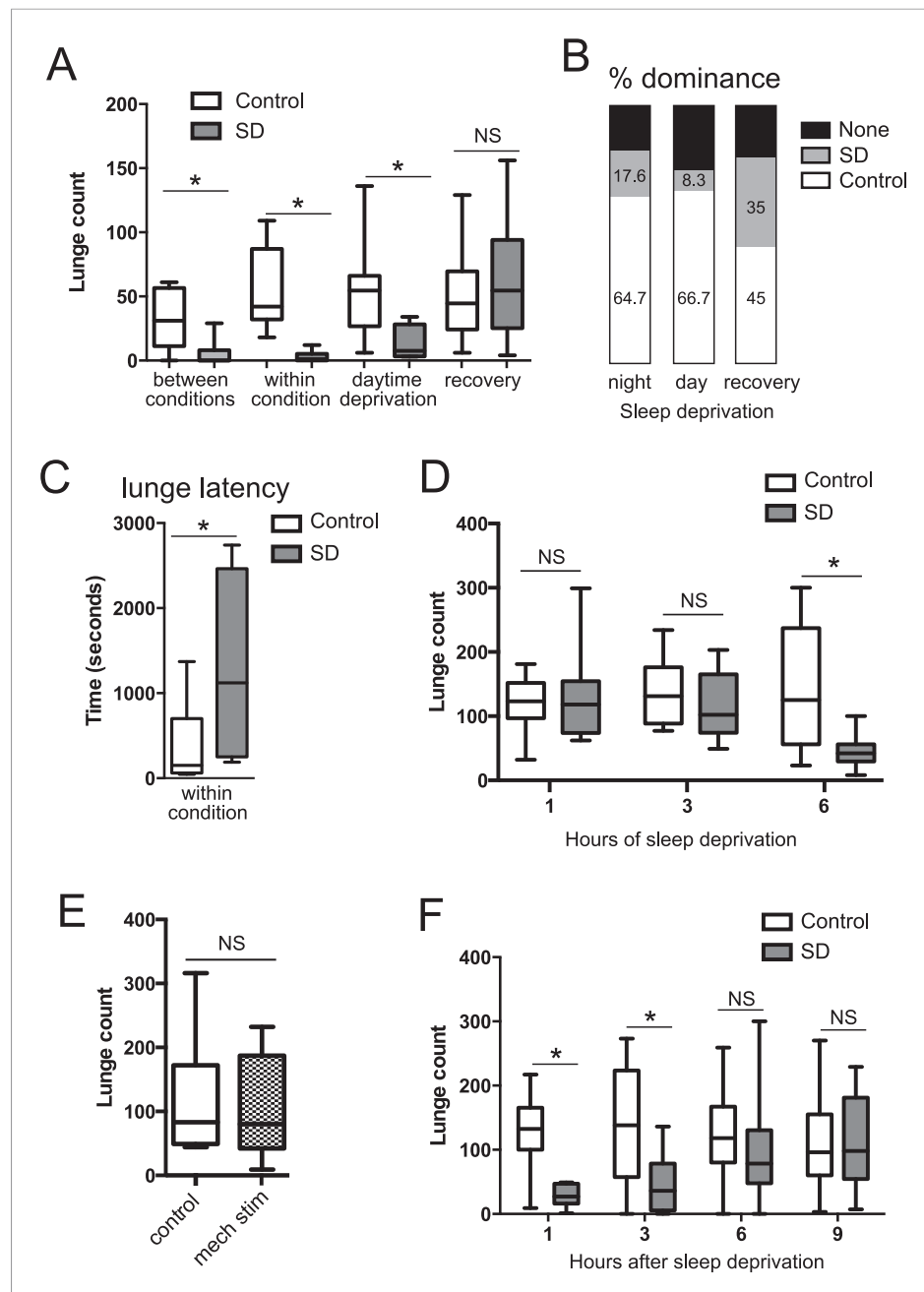


Figure 1. Acute sleep deprivation suppresses aggressive behaviors. **(A)** Quantification of aggression (lunge count) in control or sleep deprived male CS flies. 'Between conditions' indicates fights between a control fly and a sleep-deprived fly; 'within condition' is a fight between 2 sleep-deprived or 2 control flies. 'Daytime deprivation' and 'recovery' refers to fights between conditions ($n = 16, 16, 9, 10, 10, 8, 14, 9$ from left to right). **(B)** Percentage of flies in each condition establishing dominance (fights between conditions). **(C)** Latency to first lunge following first social encounter (fights are within condition; $n = 9$ control, 7 deprived). **(D)** Lunging follow sleep deprivation during the final 1, 3 or 6 hr(s) of the night (fights within condition; $n = 8, 8, 13, 9, 12, 12$ from left to right). **(E)** Lunging following 6 hr of mechanical stimulation during the final 3 hr of night and first 3 hr of day (fights within condition; $n = 11$ for both). **(F)** Recovery of aggression following prior sleep deprivation for 12 hr (fights within condition; $n = 10, 10, 18, 17, 19, 18, 15, 12$ from left to right). Box plots in this figure and all others represent median value (horizontal line inside box), interquartile range (height of the box, 50% of the data within this range), and minimum and maximum value (whiskers). Bar graphs in this figure and all others are presented as mean $\pm$ s.e.m. * $p < 0.05$; 1 way ANOVA with Tukey's (**A, D**) or Sidak's (**F**) post-hoc test; unpaired two-tailed Student's t-test (**C, E**). SD = sleep deprivation.

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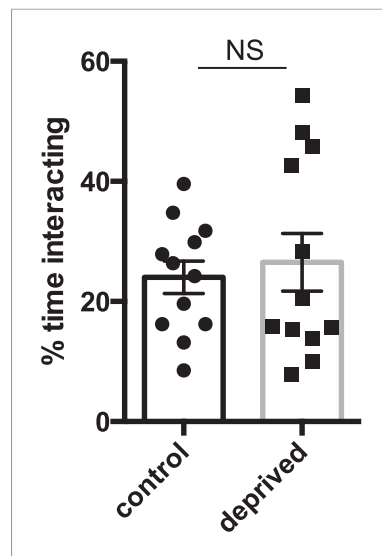


Figure 1—figure supplement 1. Quantification of percentage of time pairs of flies spent interacting, with or without prior sleep deprivation (n = 12 pairs for control and deprived). Unpaired two-tailed Student's t-test.

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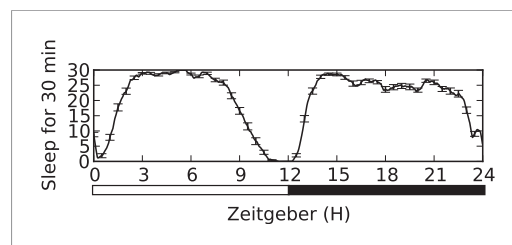


Figure 1—figure supplement 2. Sleep trace of CS male, showing high sleep amounts during both day and night (n = 22 flies).

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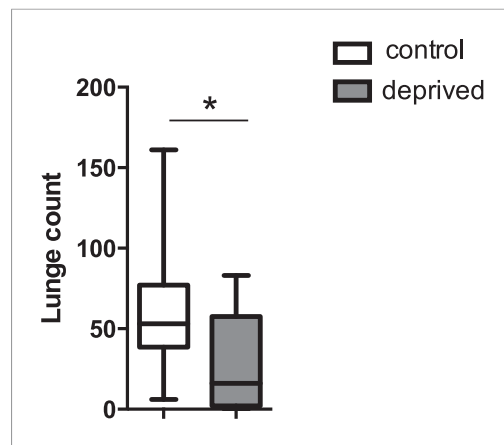


Figure 1—figure supplement 3. Quantification of aggression following 12 hr of high temperature (31°C)-induced sleep deprivation (fights within condition at 25°C; n = 21 control, 28 deprived). *p < 0.05; Unpaired two-tailed Student's t-test.

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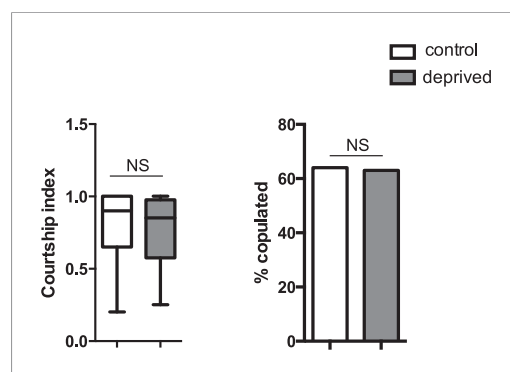


Figure 1—figure supplement 4. Courtship index and copulation frequency of control or mechanically sleep-deprived males (n = 15 control and deprived). Unpaired two-tailed Student's t-test (courtship index); Fischer's exact test (copulation frequency).

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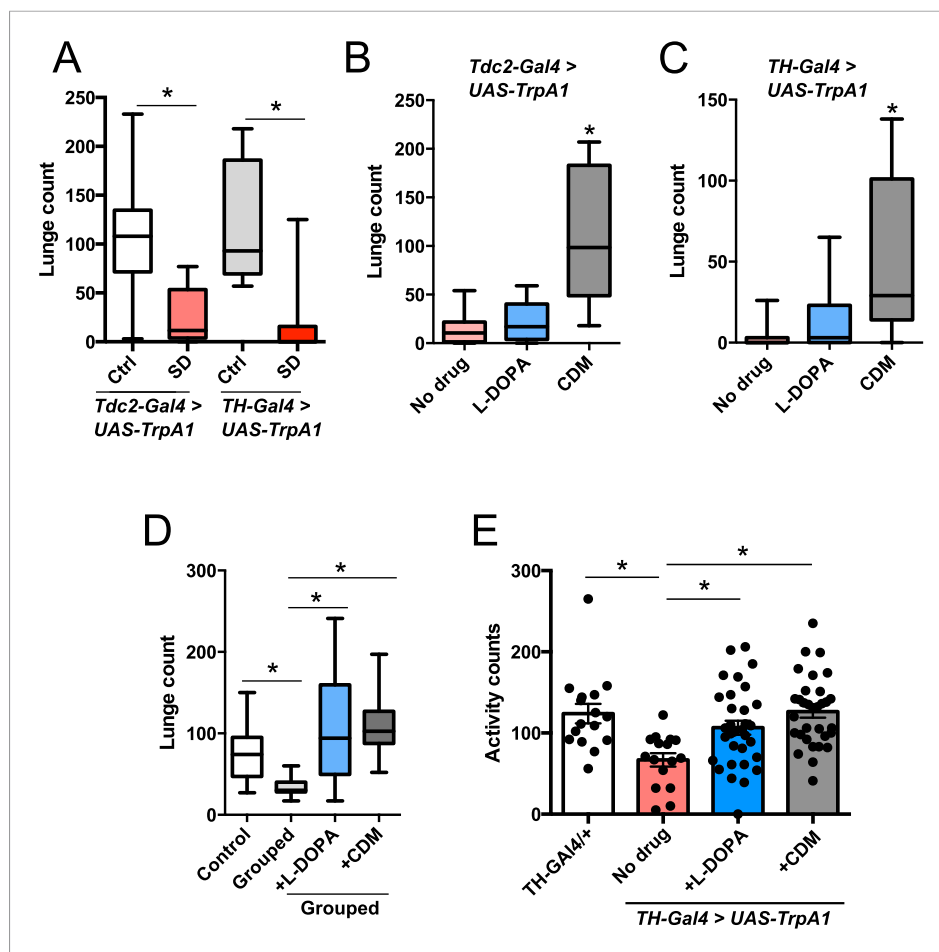


Figure 2. Octopamine agonist CDM rescues reduced aggression following sleep deprivation. Quantification of aggression at 23°C in *Tdc2-GAL4>UAS-TrpA1* or *TH-GAL4>UAS-TrpA1* males that were thermogenetically sleep-deprived the prior night at 29°C (SD, red bars) or remained at 21°C (Ctrl, white/gray bars) (n = 10, 16, 10, 12 from left to right). (**B**, **C**) Rescue of suppressed aggression in *Tdc2-GAL4>UAS-TrpA1* (**B**) or *TH-GAL4>UAS-TrpA1* (**C**) males fed either CDM or L-DOPA and thermogenetically sleep-deprived (**B**, n = 12, 10, 10; **C**, n = 12, 12, 12 from left to right). (**D**) Rescue of suppressed aggression in group-housed CS males fed either CDM or L-DOPA compared to males reared in isolation (Control) (n = 9, 9, 12, 12 from left to right). (**E**) Locomotor activity over 1 hr following exposure to 29°C the prior night (n = 16, 16, 32, 32 from left to right). All fights are within condition. *p < 0.05; 1 way ANOVA with Sidak's (**A**) or Tukey's (**B–E**) post-hoc test.

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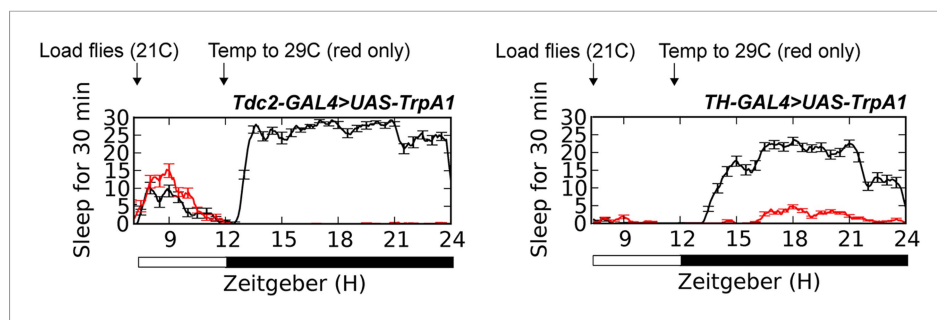


Figure 2—figure supplement 1. Sleep traces in *TH-GAL4>UAS-TrpA1* (n = 24 red, 20 black) or *Tdc2-GAL4>UAS-TrpA1* (n = 10 red, 10 black) flies loaded at ZT6 with temperature shift (red trace) to 29°C at ZT12, compared to controls (black trace) remaining at 21°C.

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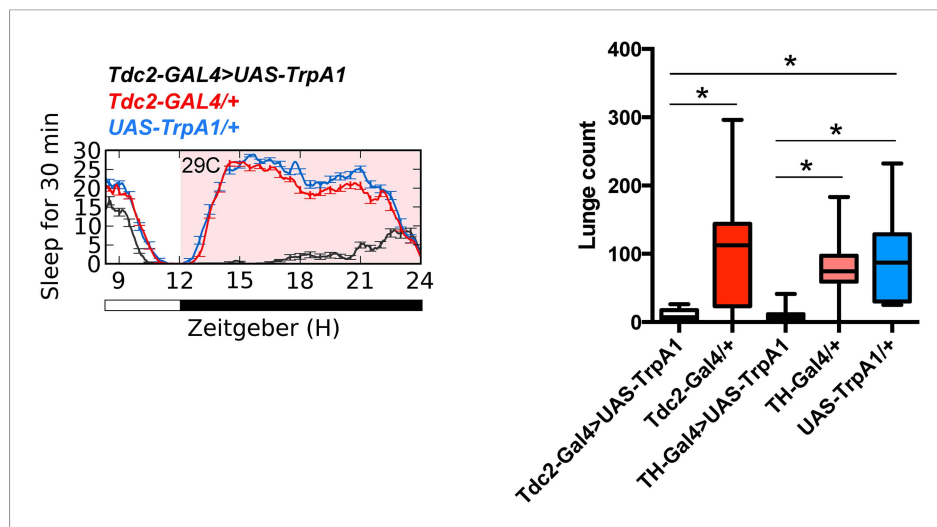


Figure 2—figure supplement 2. (Left) Sleep traces in *Tdc2-GAL4>UAS-TrpA1* (black) and GAL4 (red) and UAS (blue) controls loaded at ZT6 with temperature shift (pink box) to 29°C at ZT12. (Right) Quantification of aggression at 23°C in GAL4 and UAS controls exposed to 29°C during the prior night (n = 13, 14, 8, 8, 9 from left to right). *p < 0.05; 1 way ANOVA with Tukey's post-hoc test.

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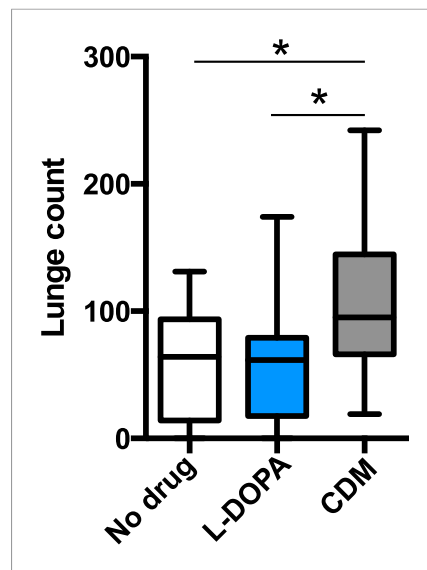


Figure 2—figure supplement 3. Quantification of aggression in CS males fed either CDM or L-DOPA and mechanically sleep-deprived ($n = 17, 20, 24$ from left to right). * $p < 0.05$; 1 way ANOVA with Tukey's post-hoc test.

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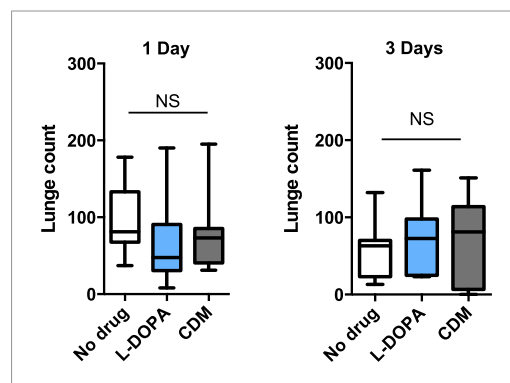


Figure 2—figure supplement 4. Quantification of aggression following 1 days or 3 days of drug exposure in isolated males. 1 way ANOVA with Tukey's post-hoc test.

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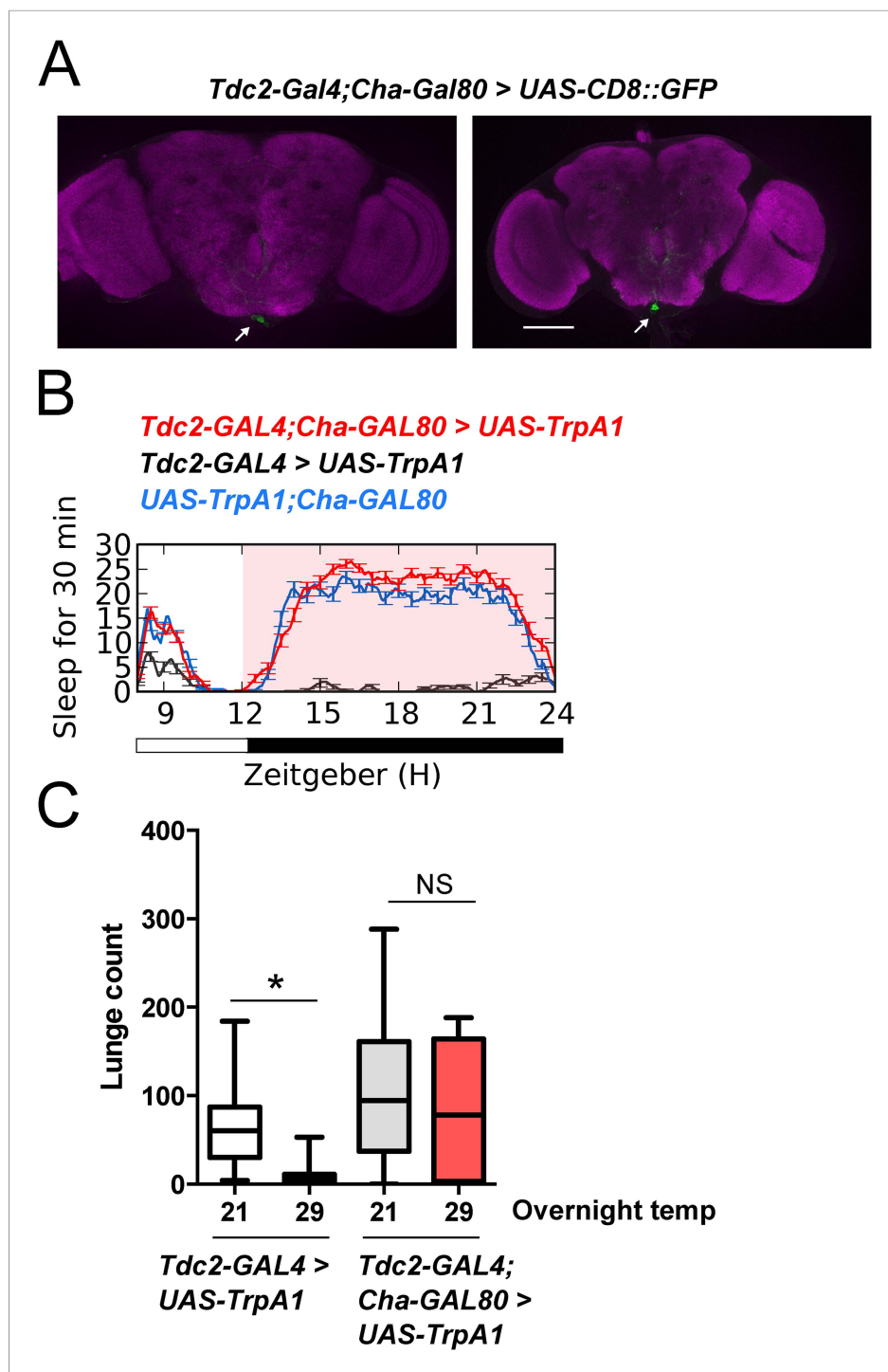


Figure 3. Sleep loss is required for suppressed aggression following octopaminergic activation. **(A)** Images of *Tdc2+* *Cha-* neurons in brains from *Tdc2-GAL4;Cha-GAL80>UAS-CD8::GFP* flies immunostained for GFP (green) and nc82 (magenta). Arrows indicate 2–4 VUM neuron cluster in posterior brain. Scale bar = 100 μ m. **(B)** Sleep traces in *Tdc2-GAL4;Cha-GAL80>UAS-TrpA1* (red), *Tdc2-GAL4>UAS-TrpA1* (black), and *UAS-TrpA1;Cha-GAL80* (blue) flies with temperature shift (pink box) to 29°C at ZT12 ($n = 12$ flies for all conditions). **(C)** Quantification of aggression in *Tdc2-GAL4>UAS-TrpA1* males exposed to elevated temperature (and sleep deprived) overnight compared to *Tdc2-GAL4;Cha-GAL80>UAS-TrpA1* males, and temperature controls (fights within condition; $n = 14, 15, 16, 15$ flies, from left to right). * $p < 0.05$; 1 way ANOVA with Sidak's post-hoc test.

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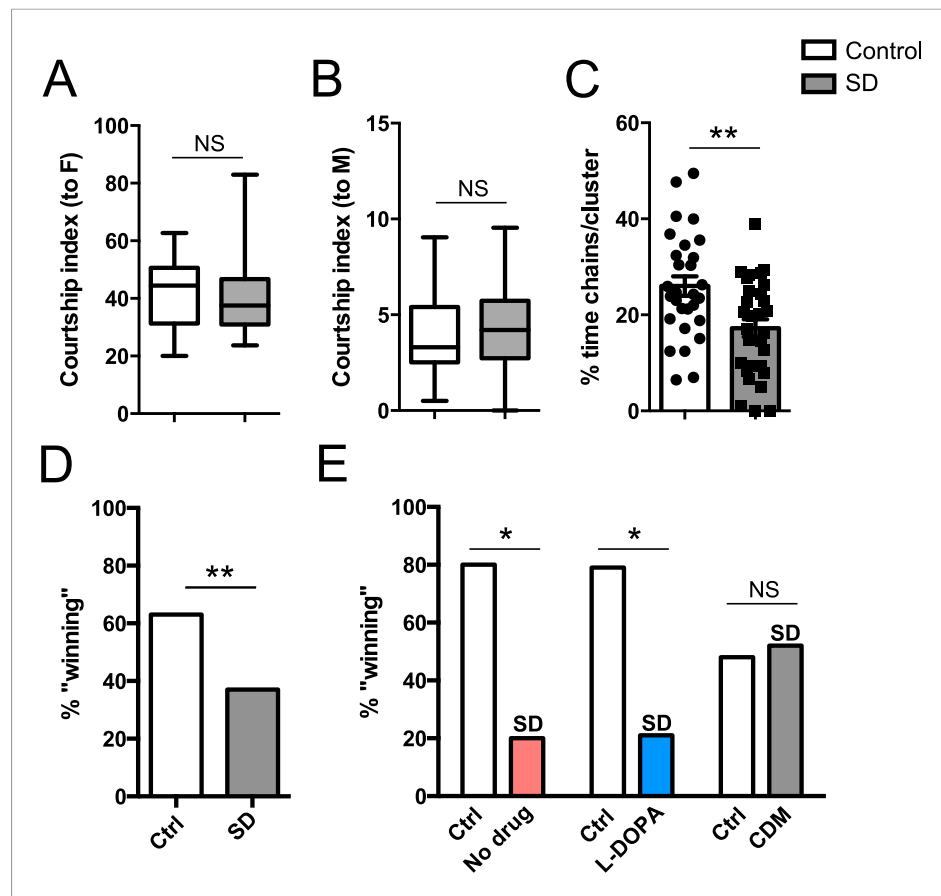


Figure 4. Suppressed aggression following sleep deprivation impairs reproductive fitness. Courtship index towards the female target (A) or male (B) in control (white, n = 32) or sleep-deprived (gray, n = 31) flies during a competitive courtship assay (within condition). (C) Percentage of time spent in chains/clusters during the competitive courtship assay with control (n = 29) or sleep-deprived (n = 30) flies (within condition). (D) Percentage of assays in which the control or sleep-deprived male first copulates with ('wins') the female target in a competitive courtship assay between conditions (n = 116 assays, 5 independent experiments). (E) As in (D) but with control vs sleep-deprived ± drug rescue males (control vs SD + no drug, n = 20 assays; control vs SD + L-DOPA, n = 28 assays; control vs SD + CDM n = 27 assays; 3 independent experiments). **p < 0.01, *p < 0.05; Unpaired two-tailed Student's t-test (A–C) or two-tailed Binomial test (D, E).

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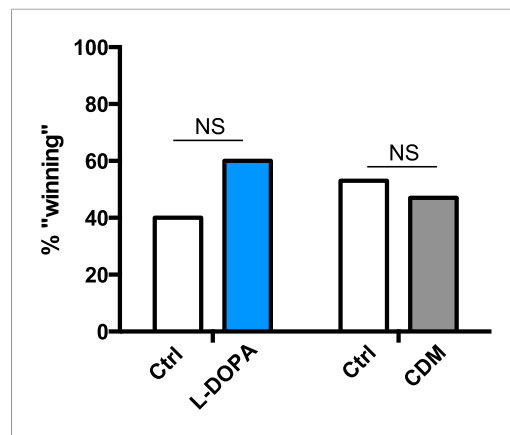


Figure 4—figure supplement 1. Percentage of assays in which the control or drug condition male first copulates with the female target in a competitive courtship assay between conditions (control vs L-DOPA, $n = 30$ assays; control vs CDM $n = 30$ assays; 3 independent experiments). $p = 0.36$ for control vs L-DOPA, $p = 0.86$ for control vs CDM; two-tailed Binomial test.

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