Figures and figure supplements

Neuronal reactivation during post-learning sleep consolidates long-term memory in *Drosophila*

**Ugur Dag et al**
Figure 1. DAN-aSP13 neurons are activated during sleep. (A) (left) Luminescence of DAN-aSP13 neurons expressing Lola-LUC reporter (MB315B-GAL4>UAS-FLP; Lola>stop>LUC) normalized to luminescence of the genetic control (UAS-FLP; Lola>stop>LUC). Mean luminescence of the wild-type males trained with mated female in single pair assays as indicated (red, n = 42) and naïve males (black, n = 40) is shown as a solid line with SEM.

(Dag et al. eLife 2019;8:e42786. DOI: https://doi.org/10.7554/eLife.42786 Figure 1 continued on next page)
Figure 1 continued

indicated as thin vertical lines. (right) Total luminescence in experienced and naive males between 7–10 hr. P value is for Ho $\text{Luc}_{\text{exp}} = \text{Luc}_{\text{naive}}$; ***$p<0.001$. Student T-test. (B) (left) Sleep profile of the wild-type males that were trained for 6 hr with mated females (red, $n = 16$) and naive males (black, $n = 16$). Sleep time was plotted in 30 min bins. (right) Total sleep of the experienced and naive males between 7–10 hr. P value is for Ho $\text{Sleep}_{\text{exp}} = \text{Sleep}_{\text{naive}}$; **$p<0.01$. Student T-test. (C)(left) Sleep profile of the dopR1 mutants that were trained for 6 hr with mated females (red, $n = 16$) and naive males (black, $n = 16$). Sleep time was plotted in 30 min bins. (right) Total sleep of the experienced and naive males between 7–10 hr. P value is for Ho $\text{Sleep}_{\text{exp}} = \text{Sleep}_{\text{naive}}$, ns $p>0.05$. Student T-test. Schematic of the experimental set-up in A, B and C indicates 12 hr light and dark periods (white and black areas) and time of training and test (blue shading).

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Figure 1—figure supplement 1. Learning sufficient to induce LTM leads to the enhancement of the post-learning sleep and activation of DAN-aSP13 in the specific time window. (A) Expression pattern of MB315B-GAL4 line. (B) Luminescence of DAN-aSP13 neurons expressing Lola-LUC reporter (MB315B-GAL4>UAS-FLP; Lola>stop>LUC) normalized to luminescence of the genetic control (UAS-FLP; Lola>stop>LUC). Mean luminescence of the...
wild-type males trained in single pair assays as indicated with virgin female (red, n = 24) or naive males (black, n = 20) shown as a solid line with SEM indicated as thin vertical lines. (C) Luminescence of DAN-aSP13 neurons expressing Lola-LUC reporter (MB315B-GAL4 > UAS FLP; Lola > stop > LUC) normalized to luminescence of the genetic control (UAS-FLP; Lola>stop>LUC). Mean luminescence of the wild-type males trained in single pair assays as indicated with mated female (red, n = 22) and naive males (black, n = 19) is shown as a solid line with SEM indicated as thin vertical lines. (D) Sleep profile of the wild-type males that were trained for 6 hr with mated females as indicated (red, n = 16) and naive males (black, n = 16). Sleep time was plotted in 30 min bins. (E) SIs of wild type males trained as indicated with a mated female in single pair assays and tested 24 hr later. P value is for Ho SI = 0; ***p<0.001, ns p>0.05. Permutation test. (F) Sleep profile of the wild-type males that were trained for 1 hr with mated females (red, n = 16) and naive males (black, n = 16). Sleep time was plotted in 30 min bins. (G) CIs of the wild-type or dopR1 mutant males during 6 hr training in single pair assay w a mated female. P value is for Ho CI\text{wild-type} = CI\text{DopR1}; ***p<0.001. Mann-Whitney-Wilcoxon test. (H) SIs of the wild-type or dopR1 mutant males tested 24 hr after training for 6 hr with mated females. P value is for Ho SI = 0; ***p<0.001, ns p>0.05. Permutation test.

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Figure 1—figure supplement 2. Courtship Indices (CIs) of males that had undergone treatment according to Figure 1—figure supplement 1E and H. (A) Courtship indices (CIs) of the wild-type males trained in single pair assays with a mated female as indicated in Figure 1—figure supplement 1E and Table S1. (B) Courtship indices (CIs) of males of the indicated genotypes trained in single pair assays with a mated female as indicated in Figure 1—figure supplement 1H and Table S3.

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Figure 2. Sleep after learning is necessary and sufficient for LTM consolidation. (A) SIs of the wild-type males tested 24 hr after training for 6 hr with a mated female and sleep deprived at indicated time periods after training (dark purple bars). SI of the wild-type control males that were allowed to sleep (green bar). P value is for Ho SI = 0; ***p<0.001, **p<0.01, ns p>0.05. Permutation test. (B) SIs of males after training for 6 hr with a mated female and DAN-aSP13s silenced with shi at indicated time periods (dark blue bars). SI of the control males with DAN-aSP13s active (green bar). P value is for Ho SI = 0; ***p<0.001, **p<0.01, *p<0.05, ns p>0.05. Permutation test. (C) SIs of males after training for 1 hr with a mated female and 104y neurons activated with csChrimson at indicated time periods (orange bars). SI of the control males with 104y neurons not activated (green bar). SI of the wild-type males with 104y neurons activated and DAN-aSP13s silenced between 5–7 hr after training (blue bar). P value is for Ho SI = 0; ***p<0.001, ns p>0.05. Permutation test. (D) SIs of males after training for 1 hr with a mated female and DAN-aSP13 neurons activated with csChrimson at indicated time periods (orange bars). SI of the control males with DAN-aSP13 not activated (green bar). P value is for Ho SI = 0; **p<0.01, ns p>0.05. Permutation test.

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Figure 2—figure supplement 1. Activation of FB neurons induces sleep. (A) SIs of the wild-type males after 6 hr training with a mated female and sleep deprived during night at indicated time periods (dark purple bars). SI of the control males which were not deprived of sleep (green bar). P value is Figure 2—figure supplement 1 continued on next page
Figure 2—figure supplement 1 continued

for Ho SI = 0; ***p<0.001, **p<0.01, *p<0.05, ns p>0.05. Permutation test. (B) Expression pattern of VT005526-LexA line (C) (upper panel) Sleep profile of males (104y-GAL4 > UAS-TrpA1) with 104y neurons activated (30°C, red, n = 16) and not activated (20°C, black, n = 16). (lower panel) Sleep profile of the genetic control males (pBDP-GAL4 > UAS-TrpA1) at 30°C (red, n = 32) and 20°C (black, n = 32). Sleep time was plotted in 30 min bins. White and black areas indicate 12 hr light and dark periods, respectively. (D) CIs of the naive and experienced males trained for 1 hr with mated female and DAN-aSP13 activated as indicated. Ho CI_{control} = CI_{naiveact} ns p>0.05; Ho CI_{naiveact} = CI_{trainedact} ***p<0.001. Mann-Whitney-Wilcoxon test.

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Figure 2—figure supplement 2. Courtship Indices (CIs) of males that had undergone treatment according to Figure 2 and Figure 2—figure supplement 1A. (A) Courtship indices (CIs) of the wild-type males trained in single pair assays with a mated female and sleep deprived as indicated in Figure 2—figure supplement 2 continued on next page.
Figure 2—figure supplement 2 continued

*(B)* Courtship indices (CIs) of males trained in single pair assays with a mated female and DAN-aSP13s silenced as indicated in Figure 2B and Table S6. *(C)* Courtship indices (CIs) of males trained in single pair assays with a mated female and sleep induced as indicated in Figure 2C and Table S7. *(D)* Courtship indices (CIs) of males trained in single pair assays with a mated female and DAN-aSP13s activated as indicated in Figure 2D and Table S8. *(E)* Courtship indices (CIs) of males trained in single pair assays with a mated female and sleep deprived as indicated in Figure 2—figure supplement 1A and Table S5.

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**Figure 3.** FB neurons provide an excitatory input to DAN-aSP13. (A) (left) Expression pattern of 104y-GAL4 > UAS-Chrimson88-tdTomato with depicted dFB (magenta) and vFB (blue) layers for local activation with DMD. (right)
Excitatory response of dFB (magenta) or vFB (blue) layers in the presence of 20 uM tetrodotoxin (TTX) upon activation of dFB or vFB, respectively. The calcium response pattern evoked by stimuli was calculated by the correlation of determination. (B) (left) Expression pattern of 104y-GAL4 > UAS-Chrimson88-tdTomato with depicted 104y FB neurons (orange) for local activation with DMD. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon local activation of 104y FB neurons (104y-GAL4 > UAS-Chrimson88-tdTomato, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines and the mean trace is shown in a thick orange line with SEM indicated by shaded area. (C) (left) Expression pattern of 104y-GAL4 > UAS-Chrimson88-tdTomato with depicted dFB layer (magenta) for local activation with DMD. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon local activation of dFB (104y-GAL4 > UAS-Chrimson88-tdTomato, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines and the mean trace is shown in a thick magenta line with SEM indicated by shaded area. (D) (left) Expression pattern of 104y-GAL4 > UAS-Chrimson88-tdTomato with depicted vFB layer (blue) for local activation with DMD. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon local activation of vFB (104y-GAL4 > UAS-Chrimson88-tdTomato, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines and the mean trace is shown in a thick blue line with SEM indicated by shaded area. (B, C, D): Red line indicates the time of the light stimulus. P value in all panels represents the probability that the mean dF/F of pre-stimulation (10 s) and the mean dF/F during stimulation has the same median across flies (tested by Wilcoxon rank sum test, sample size indicated with n value).

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Figure 3—figure supplement 3. Activation of the mFB has no effect on DAN-aSP13 activity. (A) Expression pattern of the broad PAM-DANs driver (R58E02-LexA > LexAop-GCaMP6s). Manually defined DAN-γ5 (DAN-aSP13), DAN-γ4 and DAN-γ3 are depicted in white dashed circles. (B) (left) Expression pattern of 104y-GAL4 > UAS-Chrimson88-tdTomato with depicted mFB layer for local activation with DMD (yellow). (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon local activation of mFB (104y-GAL4 > UAS-Chrimson88-tdTomato, R58E02-LexA > LexAop-GCaMP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines and the mean trace is shown in a thick yellow line with SEM indicated by shaded area. Red line indicates the time of the light stimulus. P value represents the probability that the mean dF/F of pre-stimulation (10 s) and the mean dF/F during stimulation has the same median across flies (tested by Wilcoxon rank sum test, sample size indicated with n value).

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Figure 4. Sleep promoting vFB neurons activate DAN-aSP13. (A) (left) Sleep profile of males (R23E10-GAL4 > UAS-TrpA1) upon activation of dFB neurons (red, n = 16) and control males with dFB neurons not activated (black, n = 16). Sleep time was plotted in 30 min bins. White and black areas
indicate 12 hr light and dark periods, respectively. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon activation of dFB (R23E10-GAL4 > UAS-Chrimson88, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines, and the mean trace is shown in thick magenta line with SEM indicated by shaded area. (B) (left) Sleep profile of males (VT03687-GAL4 > UAS-TrpA1) upon activation of vFB neurons (red, n = 16) and control males with vFB neurons not activated (black, n = 16). Sleep time was plotted in 30 min bins. White and black areas indicate 12 hr light and dark periods, respectively. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon activation of vFB (VT036875-GAL4 > UAS-Chrimson88, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines, and the mean trace is shown in thick blue line with SEM indicated by shaded area. (C) (left) Sleep profile of males (VT036875-GAL4 > UAS-TrpA1, R58E02-GAL80) upon activation of vFB neurons (red, n = 16) and control males with vFB neurons not activated (black, n = 16). Sleep time was plotted in 30 min bins. White and black areas indicate 12 hr light and dark periods, respectively. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon activation of vFB (VT036875-GAL4 > UAS-Chrimson88, R58E02-GAL80, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines, and the mean trace is shown in thick blue line with SEM indicated by shaded area. (D) (left) Sleep profile of (SS57264-GAL4 > UAS-TrpA1) males upon activation of vFB neurons (red, n = 16) and control males with vFB neurons not activated (black, n = 16). Sleep time was plotted in 30 min bins. White and black areas indicate 12 hr light and dark periods, respectively. (right) Normalized calcium levels (dF/F) in DAN-aSP13 upon activation of vFB (SS57264-GAL4 > UAS-Chrimson88, R58E02-LexA > LexAop-GCamP6s). DAN-aSP13 activity in individual flies is shown in colored thin lines, and the mean trace is shown in thick blue line with SEM indicated by shaded area. (A–D) (right panels) Red line indicates the time window of the light stimulus. P value represents the probability that the mean dF/F of pre-stimulation (10 s) and the mean dF/F during stimulation has the same median across flies (tested by Wilcoxon rank sum test, sample size indicated with n value).
Figure 4—figure supplement 1. Specific class of vFB neurons acutely induces sleep. (A) Three single FB neural cell types were manually traced in MCFO data: dFB (magenta), vFB (blue) and mFB (grey). Confocal images registered to standard brain of four FB lines in Figure 4 and superimposed on Figure 4—figure supplement 1 continued on next page.
the standard brain. (B) R23E10-GAL4 (C) VT036875-GAL4 (D) VT036875-GAL4, 58E02-GAL80 (E) SS57264 driving UAS-myrGFP or UAS-Chrimson88-tdTomato. (F) Total amount of sleep of the experienced and naive males quantified per 30 min time period upon activation with CsChrimson (activated, red, n = 8–9 and not activated, black, n = 8–9). (G) Sleep profile of the genetic control males (pBDP-GAL4 > UAS-TrpA1) (30°C, red, n = 48) and (20°C, black, n = 48). Sleep time was plotted in 30 min bins. White and black areas indicate 12 hr light and dark periods, respectively.

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Figure 5. Sleep promoting vFB neurons are sufficient and necessary for LTM consolidation. (A) SIs of males of indicated genotypes tested 24 hr after 1 hr training with a mated female and activation at the specific time interval with CsChrimson (orange bars). SI of control males with relevant neurons not

Figure 5 continued on next page
activated (green bar). P value is for Ho SI = 0; ***p<0.001, **p<0.01, *p<0.05, ns p>0.05. Permutation test. (B) SIs of males of indicated genotypes tested 24 hr after training for 6 hr with a mated female and silencing with shi<sup>th</sup> at the specific time interval (dark blue bars). SI of wild type control males with DAN-aSP13 active (green bar). P value is for Ho SI = 0; ***p<0.001, **p<0.01, *p<0.05, ns p>0.05. Permutation test.

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Figure 5—figure supplement 5. Courtship Indices (CIs) of males that had undergone treatment according to Figure 5A and B. (A) Courtship indices (CIs) of males trained in single pair assays with a mated female and with FB neurons activated as indicated in Figure 5A and Table S9. (B) Courtship indices (CIs) of males trained in single pair assays with a mated female and with FB neurons silenced as indicated in Figure 5B and Table S10.

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**Figure 6.** Post-learning activation of DAN-aSP13 neurons mediates LTM consolidation. (A) The MBγ and DAN-aSP13s are repetitively activated during 6 hr training by the olfactory and behavioral cues presented by a mated female, respectively. (B) Males display an enhanced amount of sleep after training for LTM. Enhanced sleep is mediated by the vFB neurons in response to a learning induced sleep drive while the remaining amount of sleep is regulated by dFB neurons in response to homeostatic sleep drive. Only vFB neurons activate DAN-aSP13s. Dopamine released as a result of DAN-aSP13s activation stimulates molecular processes in the γKCs neurons that involve synthesis of new proteins essential for LTM memory persistence. (C) Subsequently, experienced males suppress their courtship towards mated females for 24 hr or longer.

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