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Genetic Rescue of Functional Senescence in Synaptic and Behavioral Plasticity

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Study Objectives: Aging has been linked with decreased neural plasticity and memory formation in humans and in laboratory model species such as the fruit fly, *Drosophila melanogaster*. Here, we examine plastic responses following social experience in *Drosophila* as a high-throughput method to identify interventions that prevent these impairments.

Patients or Participants: Wild-type and transgenic *Drosophila melanogaster*.

Design and Interventions: Young (5-day old) or aged (20-day old) adult female *Drosophila* were housed in socially enriched (n = 35-40) or isolated environments, then assayed for changes in sleep and for structural markers of synaptic terminal growth in the ventral lateral neurons (LN_Vs) of the circadian clock.

Measurements and Results: When young flies are housed in a socially enriched environment, they exhibit synaptic elaboration within a component of the circadian circuitry, the LN_Vs, which is followed by increased sleep. Aged flies, however, no longer exhibit either of these plastic changes. Because of the tight correlation between neural plasticity and ensuing increases in sleep, we use sleep after enrichment as a high-throughput marker for neural plasticity to identify interventions that prolong youthful plasticity in aged flies. To validate this strategy, we find three independent genetic manipulations that delay age-related losses in plasticity: (1) elevation of dopaminergic signaling, (2) over-expression of the transcription factor *blistered* (*bs*) in the LN_Vs, and (3) reduction of the *Imd* immune signaling pathway. These findings provide proof-of-principle evidence that measuring changes in sleep in flies after social enrichment may provide a highly scalable assay for the study of age-related deficits in synaptic plasticity.

Conclusions: These studies demonstrate that *Drosophila* provides a promising model for the study of age-related loss of neural plasticity and begin to identify genes that might be manipulated to delay the onset of functional senescence.

Keywords: aging, *Drosophila*, plasticity

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INTRODUCTION

Aging is associated with deficits in cognitive processes, including memory formation in humans[1] and flies.[2,3] Although sleep facilitates memory formation in young people,[4,5] sleep-dependent memory-consolidation is impaired with aging.[6] Thus, age-related deficits in sleep-dependent plasticity provide an example of functional senescence: the age-related deterioration in physiological status that interferes with the ability to maintain youthful functioning during aging.[7]

To evaluate functional senescence of neural plasticity in *Drosophila*, we have measured the effects of social enrichment on synapse number and on sleep over the following days.[8,9] Exposure to novel stimuli in an enriched environment activates Hebbian mechanisms and leads to synaptic elaboration in a variety of different species, including the fruit fly.[10-12] When housed in a socially enriched group for several days, young *Drosophila* exhibit an increase in synaptic terminals contained within projections from the *Pigment dispersing factor* (*Pdf*)-expressing ventral lateral neurons (LN_Vs),[9] a cluster of wake-promoting cells[13-15] that are involved in the consolidation of long-term memories.[9] While the precise neural functions of sleep remain unknown, the neural plasticity that occurs during enrichment is tightly correlated with an increase in sleep over the following days.[9] The magnitude of synaptic elaboration and consequent increases in sleep are proportional to the density of the social group during enrichment, and recovery sleep permits synaptic terminal number to decrease back to baseline levels after enrichment,[9,16] supporting the hypothesis that sleep is increased after social enrichment to homeostatically downscale synaptic connections.[17]

Although the fly is a genetically tractable model that can be efficiently used for genetic and pharmacological screens, directly measuring the effects of aging on neural plasticity is a difficult task that limits the practicality of large scale studies. To better study the effects of aging, we have begun to characterize behavioral correlates that can be used to measure plastic responses following novel experience. Because genetic mutations that block synaptic elaboration during social enrichment also prevent subsequent increases in sleep,[9,16] detecting increased sleep after social enrichment may provide a reliable marker of neural plasticity. In the current study, we find that both synaptic elaboration and increases in sleep following social enrichment decline with age in *Drosophila*. This age-related loss in plasticity seems to be connected with a loss of dopaminergic signaling with age; reducing dopamine signaling through *dDA1* receptors in young flies prevents plastic responses and supplementing dopamine signaling in aged flies restores youthful plasticity. Targeting these interventions to a specific neural cluster, the LN_Vs, is sufficient to postpone functional senescence, suggesting that targeted modifications of specific neuromodulators can be sufficient to delay the loss of neural plasticity with age. To validate that this strategy can identify novel interventions for prolonging youthful plasticity, we use qPCR gene profiling to identify independent genetic mechanisms that might provide

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other targets for intervention and confirm the roles of candidate genes using behavioral genetic studies. Our results find that age-related deficits in plasticity can be delayed via two additional genetic manipulations: (1) elevated expression of the transcription factor *blistered* (*bs*) in the LN_vs and (2) reduced activity of the *Imd* immune signaling pathway. Together, these data suggest that using sleep measurements after social enrichment provide a reliable marker for neural plasticity and that the fly can provide a productive model for identifying mechanisms of plasticity that degrade with age.

METHODS

Flies

Drosophila melanogaster were cultured at 25°C with 50% to 60% relative humidity and kept on a diet of yeast, dark corn syrup, molasses, sucrose, and agar under a 12-hour light:12-hour dark cycle. Flies were transferred to fresh food vials every 4-5 days during aging experiments. Canton-S flies were obtained from T. Zars (University of Missouri, Columbia), *dumb*^{2} (f02676) and *dumb*^{3} (PL00420) flies were generated by the Harvard Exelexis Library, UAS-*dDA1*^{RNAi} (107058KK) flies were generated by the Vienna *Drosophila* Resource Center (Vienna, Austria), *ird7 (PGRP-LC),* and *PGRP-LC,-LE* flies[18] were obtained from Bloomington Stock Center, *Pdf*-GAL4 flies[19] were obtained from P. Taghert (Washington University, St. Louis MO), UAS-*dlg*^{WT}-GFP flies[20] were obtained from B. Lu (Stanford University), UAS-*bs*(II) flies[21] were obtained from Z. Han (University of Michigan), and *S106 > UAS-PGRP-LE* flies[22,23] were obtained from S. Pletcher (University of Michigan).

Behavioral Analysis

Drosophila sleep and activity patterns were assessed as described previously.[24] In summary, flies were placed into individual 65-mm tubes and all activity was continuously measured through the Trikinetics Drosophila Activity Monitoring System (www.trikinetics.com). Locomotor activity was measured in 1-min bins, and sleep was defined as periods of quiescence lasting ≥ 5 minutes.

Social Enrichment

To standardize the environmental conditions during critical periods of brain development, all flies were collected upon eclosion and maintained in same-sex vials containing 30 flies. Flies were divided into a socially isolated group, which were individually housed in 65-mm glass tubes, and a socially enriched group, consisting of 35-45 female flies housed in a single vial as adapted from previous experiments.[8] Group locomotor activity during social enrichment was measured using a *Drosophila* Population Monitor (Trikinetics, Waltham MA). After 5 days of social enrichment/isolation, flies were placed into clean 65 mm glass tubes and sleep was recorded for 3 days as described above. To calculate the effect of social enrichment on subsequent sleep, we first calculate the population mean value for daytime sleep in the isolated group, averaged over 3 days, and then subtracted this isolated group mean from the daytime sleep observed for each individual socially-enriched sibling. The difference is referred to as ΔSleep.

Pharmacology

3-iodo L-tyrosine (3IY, 10 mg/mL), SCH23390 (1 mg/mL), and L-3,4-dihydroxyphenylalanine (L-DOPA, 2 mg/mL) were dissolved in standard lab media and fed to flies for 5 days while they were housed in social isolation or enrichment. Following social enrichment or isolation, flies were returned to standard media with no drug while ΔSleep following social enrichment was measured. Behavioral changes during drug administration were examined using Trikinetics Population Monitors to measure pooled locomotor activity in socially enriched groups and, in parallel, using Trikinetics Activity Monitors to measure sleep in socially isolated individuals.

Quantitative PCR

Total RNA was isolated from fly heads by using TRIzol following the manufacturer's protocol. Reverse-transcription (RT) reactions were carried out in parallel on *Dnase* I-digested total RNA as previously described.[25] RT products were stored at −80°C until use. PCRs to measure levels of artificial transcript were performed to confirm uniformity of RT within sample groups and between samples. All RT reactions were performed in triplicate. At least two quantitative PCR replications were performed for each condition. Sequences for each of the primers used were as follows:

rp49 F – aagaagcgcaccaagcacttcatc, R – tctgttgtcgataccccttgggctt,
dDA1 F – agcgattgcggatctctt, R – caaaattgcgctccaaag,
bs F – gacggagctcagctacaaca, R – gaggtaggcgatcggtcat,
AttB F – ggcaatcccaaccataatgt, R – aggcaccatgaccagcat,
CecA1 F – agctgggtggctgaagaaa, R – attgtggcatcccgagtg,
def F – ggatcatgtcctggtgcat, R – cttctggcggctatgctg,
Dro F – ccatcgaggatcacctgact, R – ctttaggcgggcagaatg,
Drs F – aagtacttgttcgccctcttcgct, R – gtatcttccggacaggcagt,
IM10 F – acgatcaggcaggaatttgggaca, R – tatcgattgtgacagtgcgtccgt,
mtk F – tcatcgtcaccagggacccattt, R – acccggtcttgggttaggatt.

Values were expressed as a percentage of socially isolated animals and were evaluated using either *t*-tests or ANOVAs with post hoc pairwise comparisons. Data are presented as mean ± SEM.

Immunohistochemistry

Brains were removed from the head casing and fixed in 4% paraformaldehyde in phosphate buffer solution (PBS) (1.86 mM NaHPO, 8.41 mM NaHPO, and 175 mM NaCl) for 1 h and washed in PBS. Following a 2-h pre-incubation in 3% normal goat serum in PBS-TX (PBS containing 0.3% Triton X-100), brains were washed in PBS-TX. Brains were incubated in 1:1000 Rabbit anti-GFP (Sigma), washed in PBS-TX and incubated in the appropriate fluorescent secondary antibodies. All samples that were directly compared were processed in parallel after dissection.

Confocal Microscopy

Confocal stacks were acquired with a 1μm slice thickness using a laser scanning confocal microscope and processed using ImageJ. All samples that were directly compared were imaged using identical microscope settings. Quantitative analysis of pre- and post-synaptic terminals was conducted on socially

isolated and socially enriched flies expressing UAS-dlg^{WT}-GFP under control of the *Pdf*-GAL4 driver. Immunopositive terminals were counted using the ImageJ binary thresholding algorithm. The number of synaptic terminals for all of the socially isolated flies was used to generate a grand mean. The grand mean of the isolated flies was used to normalize each individual enriched brain. The individual normalized values were then used to calculate the mean and standard error for the group. The mean and standard error for socially isolated flies were calculated by normalizing to their own group mean. The normalized values for each group were then evaluated using a independent sample *t*-test.

Statistics

Statistical analyses were performed with Prism 6 (GraphPad). Pairwise comparisons used Student *t*-tests or, if appropriate, ANOVA and subsequent modified Bonferroni comparisons unless otherwise stated. All statistically different groups are defined as $P < 0.05$.

RESULTS

Enrichment-induced sleep declines with age

Although *Drosophila* can survive more than 2 months in a laboratory environment, the ability of flies to properly form memories degrades within 20 days of eclosion.[2,3] Extensive examination of age-related loss of plasticity is demanding, however, so the field might benefit from high-throughput assays for evaluating the effects of aging on plasticity. One such assay may be measuring the effects of exposing flies to either social enrichment, consisting of 35-45 flies maintained in a 50 mL vial, or social isolation, consisting of flies housed individually in small glass tubes (5 mm diameter × 65 mm long). Following 5 days of isolation or enrichment, flies are allowed a transition day and then evaluated for sleep or markers of plasticity. When young female flies expressing a fluorescent marker labeling synaptic terminals in the LN_Vs (*Pdf*-GAL4/+; UAS-dlg^{WT}-GFP/+) are placed into social isolation or enrichment for 5 days beginning on day 5 after eclosion, a significant increase in fluorescently-labeled LN_V punctae can be detected in the socially enriched group (11-day-old flies; Figure 1A, 1B).[9] This increase in synaptic punctae seems to increase the need for sleep in young female flies; wild-type Canton-S flies that experienced the same enrichment starting on day 5 after eclosion exhibit a significant increase in sleep compared to isolated siblings (Figure 1C).[8] If, however, older female flies are exposed to social enrichment for 5 days beginning on day 20 after eclosion, no change in synaptic punctae number (Figure 1D, 1E) or in sleep time can be detected (26-day-old flies; Figure 1F). Eleven-day-old and 26-day-old isolated female flies do not differ in their baseline sleep (Figure 1C, 1F) or LN_V punctae number (Figure S1, supplemental material), indicating that the observed deficits in plasticity seen during aging are not a consequence of young and old flies starting the experiment with different amounts of sleep or punctae. To more precisely analyze the effects of aging on enrichment-induced changes in sleep, we measured sleep after 5 days of social enrichment in male and female flies at ages between 5 days and 45 days after eclosion. As shown in Figure 1G, when social enrichment/isolation begins 5-10 days after eclosion, both male and female flies respond to enrichment with increased sleep over the following days. However, female flies show no change in sleep after exposure to social enrichment beginning 15 or 20 days after eclosion. Interestingly, male flies subsequently exhibit a gradual loss of social enrichment-induced sleep at 45 days of age. In young flies, social enrichment not only increases sleep time, it also increases average length of each sleep episode.[8] To determine whether this change in sleep consolidation might also be disrupted with aging, we compared the lengths of sleep bouts in young (11-day-old) and aged (26-day-old) Canton-S females after 5 days of social isolation or enrichment. Young Canton-S females exhibit increased daytime (Figure 1H, left) and nighttime bout length (Figure 1I, left) after social enrichment. These increases in bout length are not observed in aged females after social enrichment (Figure 1H, 1I, right). Together, these results indicate that the ability to respond to social enrichment in a plastic way decreases with age, and that increases in sleep time and intensity after social enrichment might be used as a marker for neural plasticity.

dDA1 signaling in the LN_Vs is required for enrichment-induced sleep in young flies

Dopaminergic signaling is vital for memory formation[26] but declines with age in *Drosophila,*[27,28] indicating that altered dopaminergic signaling might underlie age-related impairments in plasticity. Chronic disruption of dopamine synthesis beginning during development prevents increased sleep after social enrichment,[8] but it remains unclear whether more acute decreases in dopamine, such as those that occur during aging, might also reduce plastic responses to the social environment. To more closely examine whether acute changes in dopamine signaling that occur during social enrichment contribute to neural plasticity, we treated flies with the tyrosine hydroxylase inhibitor 3-iodo L-tyrosine (3IY) between day 5-10 after eclosion. During this 3IY administration, flies were housed in social isolation or enrichment and then returned to standard food in the morning of day 10. On day 10, flies were either dissected to quantify synaptic terminals or allowed to recover for ~18 h before sleep was evaluated for the following 3 days (Figure S2A, supplemental material). Delivery of 3IY prevented young flies from exhibiting enrichment-induced elaboration of LN_V synapses (Figure 2A), indicating that dopamine is required during social experience for plastic changes in neural structure. Canton-S flies that were administered the same 3IY treatment during social enrichment also showed no change in sleep over the following days (Figure 2B), consistent with the hypothesis that sleep is elevated in response to synaptic growth during enrichment. To rule out the possibility that the effects of 3IY on plasticity might be due to behavioral changes during social enrichment or isolation, we examined behavior during social enrichment using group activity monitors and found that overall locomotor activity did not differ between 3IY-fed and vehicle-fed controls (Figure S2B). Although 3IY treatment modestly increases sleep in socially isolated flies during the 5-day exposure (Figure S2C), sleep parameters were not statistically different from vehicle-fed controls during the first day of sleep recording after ~18 h on standard food (Figure S2D-S2G). It is, therefore, unlikely that the impaired response to social

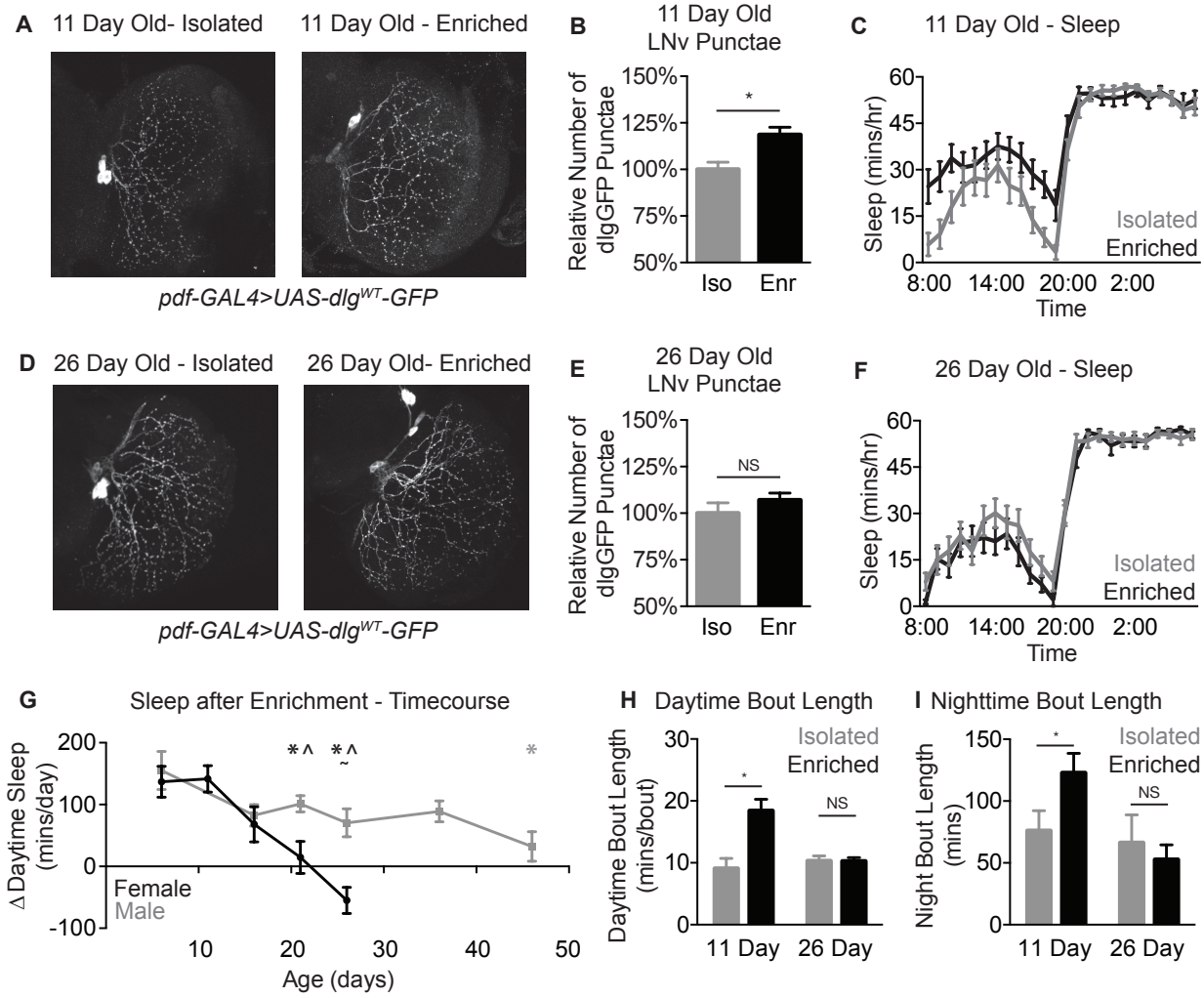

Figure 1—Aging prevents increased sleep following social enrichment. **(A,B)** LN_V synaptic punctae number is increased by social enrichment in young 11-day-old *Pdf*-GAL4/+;UAS-*dlg*^{WT}-GFP flies. Representative images from young flies are shown in **(A)**, relative quantification of terminal number in **(B)**. (Two-tailed Student *t*-test, P = 0.003, n = 11-12 each group). **(C)** Social enrichment increases sleep in young 11-day-old Canton-S flies. Two-way ANOVA finds a significant time × condition interaction ($F_{23,598}$ = 3.224, P < 0.0001, n = 13-15 each group). (D-E) Social enrichment does not change LN_V punctae number in aged 26 day old *Pdf*-GAL4/+;UAS-*dlg*^{WT}-GFP flies. Representative images from aged flies shown in **(D)**, relative quantification of terminal number in **(E)** shows no significant effect of social enrichment (Two-tailed Student *t*-test, P = 0.31, n = 13-14 each group). **(F)** Social enrichment has no effect on sleep of aged 26 day old Canton-S flies. 2-Way ANOVA finds no significant main effect for condition ($F_{1,29}$ = 0.705, P = 0.41, n = 15-16 each group) and no significant time × condition interaction ($F_{23,667}$ = 1.045, P = 0.4052, n = 15-16 each group). **(G)** Although Canton-S males show a gradual decrease in Δ Sleep between 5 and 45 days after eclosion (One-way ANOVA finds a significant effect for age, $F_{5,169}$ = 2.54, P = 0.025, n = 16-48 each group), Canton-S females show no change in sleep after social enrichment by 20-25 days of age (One-way ANOVA finds a significant effect for age, $F_{4,154}$ = 11.69, P = 2.57 × 10^{-8}, n = 32 each group). * denotes P < 0.05 vs. 6-day old, ^ denotes P < 0.05 vs. 11-day old, ~ denotes P < 0.05 vs. 16-day old. All post hoc pairwise comparisons use Holm-Sidak tests corrected for multiple comparisons. **(H)** 11-day-old Canton-S females exhibit longer daytime sleep bouts after social enrichment (left), while social enrichment does not increase daytime sleep consolidation in 26-day-old Canton-S females (right). ANOVA reveals a significant age × condition interaction ($F_{1,62}$ = 9.10, P = 0.004, *P < 0.05 modified Bonferroni post hoc test, n = 16 each group). **(I)** 11-day-old Canton-S females exhibit longer nighttime sleep bouts after social enrichment (left), while social enrichment does not increase sleep consolidation at night in 26-day-old Canton-S females (right). Two-way ANOVA reveals a significant main effect for age ($F_{1,62}$ = 5.87, P = 0.018, *P < 0.05 modified Bonferroni post hoc test, n = 16 each group).

enrichment can be attributed to effects of the drug directly on sleep or locomotion.

To more specifically examine the role of dopamine in mediating plasticity in response to social enrichment, we evaluated transcript levels for the *Drosophila D1-type dopamine receptor* (*dDA1*) using qPCR. As seen in Figure 2C, *dDA1* transcripts are upregulated by ~50% in mRNA extracted from heads of socially enriched females compared to their isolated siblings. To test the extent to which the *dDA1* receptor might be involved in mediating the response to social enrichment, we evaluated sleep after social enrichment in flies fed the D1-antagonist SCH23390 (Figure S3A, supplemental material). As seen in Figure 2D, no increase in sleep was observed in socially enriched flies that had been maintained on SCH23390 for 5 days and allowed to recover for ~18 h before sleep was evaluated. As above, no changes in locomotor activity during enrichment were observed in SCH23390 fed flies compared to controls (Figure S3B). Importantly, SCH23390 did not alter

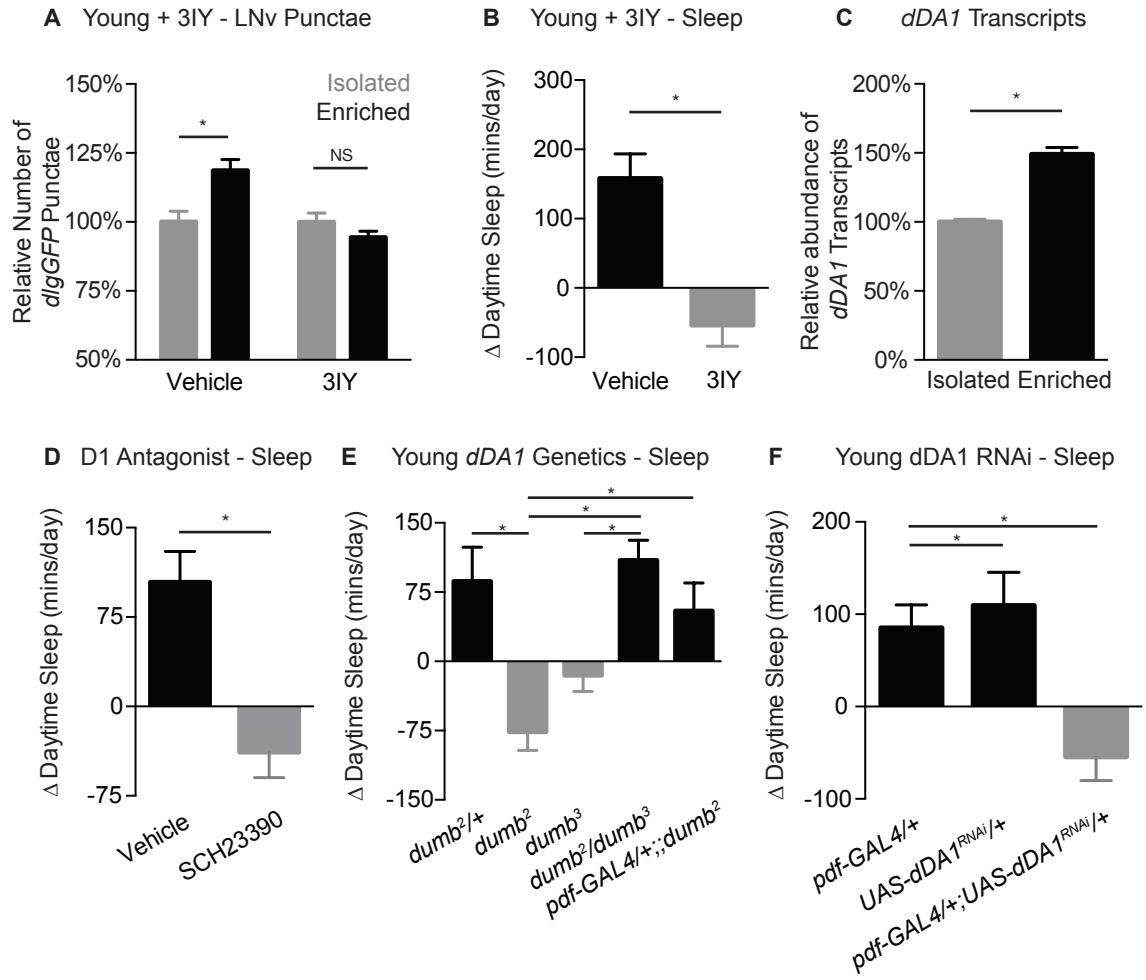

Figure 2—Dopaminergic signaling is required for increased sleep following social enrichment. **(A)** Vehicle-treated 10-day-old *Pdf*-GAL4/+; UAS-dlg^{GFP}/+ flies showed an increase in the number of dlg^{GFP}-positive varicosities (left), but siblings fed 10 mg/mL 3IY show no change in dlg^{GFP}-positive varicosities (right) following social enrichment. ANOVA reveals a significant treatment × condition interaction ($F_{1,42}$ = 17.644, P = 0.0001, * P < 0.05 modified Bonferroni post hoc test, n = 11 each group). **(B)** While vehicle-fed 11-day-old Canton-S females exhibit increased daytime sleep following social enrichment (gray), 11 day old Canton-S females fed 10 mg/mL 3IY during enrichment/isolation show no subsequent change in daytime sleep (black) (P = 8.135 × 10^{-5}, Student *t*-test, n = 16 each group). **(C)** Abundance of *dDA1* mRNA is significantly elevated in the heads of 10-day-old Canton-S females following social enrichment (P = 0.003, Two-tailed Student *t*-test, n = 2 each group). **(D)** Vehicle-fed 11-day-old Canton-S females exhibit increased daytime sleep following social enrichment (gray), but 11-day-old Canton-S females fed a D1-antagonist (1 mg/mL SCH23390) during enrichment/isolation show no subsequent change in daytime sleep (black) (P = 0.0004, Two-tailed Student *t*-test, n = 16 each group). **(E)** Although $dumb^2$ and $dumb^3$ mutants (gray) do not increase their sleep after social enrichment (gray), rescue of *dDA1* expression broadly in $dumb^2/dumb^3$ flies or exclusively in the LN_Vs (*Pdf*-GAL4;;$dumb^2$) restores increased sleep after enriched social experience (black, right). One-way ANOVA reveals a significant main effect for genotype ($F_{2,45}$ = 12.85, P < 0.001, * P < 0.05 modified Bonferroni post hoc test, n = 16-45 each group). **(F)** Flies expressing an RNAi construct for *dDA1* in the LN_Vs (*Pdf*-GAL4/+; UAS-$dDA1^{RNAi}$/+) show no increase in sleep following social enrichment (right). Parental controls (*Pdf*-GAL4/+, left, and UAS-$dDA1^{RNAi}$/+, center) exhibit increased daytime sleep following social enrichment. One-way ANOVA for genotype ($F_{2,45}$ = 6.60, P = 0.003, *P < 0.05 modified Bonferroni post hoc test, n = 16 each group).

sleep parameters in socially isolated flies either during the 5-day exposure (Figure S3C) or during the first day of sleep recording after ~18 h on standard food (Figure S3D-S3G). These data reinforce the interpretation described above that the effects of DA signaling on behavioral plasticity cannot be explained by unrelated effects of drugs on sleep or locomotion. Thus, dopaminergic signaling plays an important role in plasticity following social experience in young *Drosophila*.

Since SCH23390 has been shown to inhibit/target both the *dDA1* and the *dopamine receptor enhanced in Mushroom Bodies* (*DAMB*), we tested whether *dDA1* signaling is required for social enrichment-induced sleep in young flies by analyzing sleep after social enrichment in flies carrying the $dumb^2$ mutation, a hypomorphic allele formed by a piggyBac insertion[29] or the $dumb^3$ allele, a p-element insertion that drives the expression of GAL4.[30] While flies that are heterozygous for $dumb^2$ do show an increase in sleep after social enrichment (Figure 2E, left), mutant $dumb^2$ and $dumb^3$ homozogotes show no change (Figure 2E, gray). Rescue of *dDA1* expression by using the GAL4 driver inserted into the first intron of *dDA1* in the $dumb^3$ allele to drive expression of the UAS-element inserted into the first intron of *dDA1* in the $dumb^2$ allele in $dumb^2/dumb^3$ flies restores increased sleep following social enrichment. Given the involvement of the LN_Vs in the regulation of

enrichment-induced changes in sleep,[9] and recent findings that dopamine strongly modulates the activity of LN_Vs,[31] we tested the role of *dDA1* expression in the LN_Vs by using *Pdf*-GAL4 to rescue *dDA1* expression in a *dumb*^{2} mutant background (*Pdf*-GAL4;;*dumb*^{2}). Rescue of *dDA1* in the LN_Vs of *Pdf*-GAL4;;*dumb*^{2} flies partially restores enrichment-induced sleep compared to *dumb*^{2} mutant flies (Figure 2E, right). Because *dDA1* receptors are expressed in several areas of the brain in addition to the LN_Vs,[32] we next expressed an RNAi construct targeted against *dDA1*[33] in the LN_Vs using *Pdf*-GAL4 (*Pdf*-GAL4/+; UAS-*dDA1*^{RNAi}/+). We found that young socially enriched parental controls (*Pdf*-GAL4/+ and UAS-*dDA1*^{RNAi}/+) exhibited an increase in sleep compared to isolated siblings while *Pdf*-GAL4/+; UAS-*dDA1*^{RNAi}/+ flies did not respond to social enrichment with increased sleep (Figure 2F). Together, the data presented above indicate that disrupting *dDA1* signaling pharmacologically, with classic mutants and with RNAi blocks the response to social enrichment in young flies and that these effects are mediated, in part, through the LN_Vs.

Elevation of dDA1 signaling in aged flies restores enrichment-induced sleep

Since dopaminergic signaling is required for plasticity in young flies and dopamine levels decline with age, we asked whether we could restore youthful responses to social enrichment by increasing dopamine levels in aged flies. To test this hypothesis, 20-day old female flies were fed 2 mg/mL of L-3,4-dihydroxyphenylalanine (L-DOPA) in standard fly media for 5 days during social isolation or enrichment prior to the quantification of LN_V synaptic terminals and sleep. As seen in Figure S4A-S4F (supplemental material), neither group activity nor sleep parameters were altered during L-DOPA administration. To test whether L-DOPA administration during social enrichment might restore plasticity in aged flies, we quantified LN_V terminals in *Pdf*-GAL4/+; UAS-*dlg*^{WT}-GFP flies. Indeed, treatment with L-DOPA during enrichment restored youthful plasticity to aged flies; a significant increase in LN_V terminal number was observed in enriched flies fed L-DOPA, but no change could be detected in vehicle-treated controls (Figure 3A, 3B). Restoration of neural plasticity with L-DOPA treatment was also associated with increased daytime sleep in aged flies (Figure 3C) and sleep bout length (Figure 3D) over the following days. Administration of L-DOPA to young flies during enrichment/isolation does not enhance the effect of social enrichment on daytime sleep (Figure 3C, left) indicating that acute L-DOPA administration does not generally increase the response to social enrichment in all flies, but instead prolongs youthful responses to social experience.

As described above, dopamine seems to act through *dDA1* receptors to modulate sleep after social enrichment. To explore whether specifically increasing levels of *dDA1* signaling in the LN_Vs restores enrichment-induced sleep to the same degree as elevating dopamine levels, we over-expressed *dDA1* receptors in the LN_Vs (*Pdf*-GAL4/+;; *dumb*^{2}/+) and measured sleep in 26-day-old females after social enrichment. While aged *Pdf*-GAL4/+ and *dumb*^{2}/+ parental controls showed no increase in sleep following social enrichment, over-expression of *dDA1* in the LN_Vs of aged *Pdf*-GAL4/+;; *dumb*^{2}/+ flies demonstrate a significant increase in daytime sleep (Figure 3E) and sleep consolidation (Figure 3F) in response to social enrichment at the same age. Given these data, we conclude that enhancement of *dDA1* signaling in the LN_Vs delays the age-dependent loss of behavioral plasticity.

Increased expression of blistered delays loss of plasticity in aging flies

Plastic responses to social enrichment depend upon the expression of a variety of genes involved in synaptic plasticity, including the transcription factor *blistered* (*bs*).[9] To examine whether altered *bs* transcription is correlated with loss of plasticity during aging, we used qPCR to examine the effect of social enrichment on *bs* transcript levels. A significant increase in *bs* transcripts could be detected in socially enriched young flies compared to their age-matched isolated siblings (Figure 4A, left). However, *bs* transcripts were not increased in aged females following social enrichment compared to isolated siblings (Figure 4A, center), indicating that elevated *bs* expression after social enrichment is correlated with synaptic changes and followed by an increase in sleep. To verify this relationship, we tested the effects of L-DOPA administration during social enrichment in aged flies and found that the youthful response of *bs* transcript levels was partially restored (Figure 4A, right). Thus, deficits in plasticity may be associated with misregulation of *bs* transcription in old flies during social enrichment. We tested whether elevated expression of *bs* in the LN_Vs of aged flies might delay the loss of synaptic plasticity, and found that flies over-expressing *bs* in the LN_Vs (*Pdf*-GAL4/+; UAS-*bs*/UAS-*dlg*^{WT}-GFP) retain the ability to add LNv terminals during social enrichment even at 26 days old (Figure 4B, right). Age-matched controls (*Pdf*-GAL4/+; UAS-*dlg*^{WT}-GFP) show no growth in synaptic terminal number after enrichment (Figure 4B, left). As described above, the elaboration of LN_V synapses during social experience is followed by an increase in sleep in young flies. This response is also restored with elevated expression of *bs* in older flies; 26-day-old *Pdf*-GAL4/+;UAS-*bs*/+ flies increased daytime sleep (Figure 4C) and daytime bout length (Figure 4D) after social enrichment, while age-matched parental controls (*Pdf*-GAL4/+ and UAS-*bs*/+) showed no change in either sleep parameter. Overexpression of *bs* in young flies, however, did not cause additional increases in sleep following social enrichment (Figure 4C, left). These results suggest that elevated expression of *bs* specifically in the LN_Vs can delay age-dependent declines in behavioral and structural plasticity following social experience.

Expression of immune-related genes suppresses behavioral plasticity

To identify novel pathways that contribute to functional senescence, we have begun to use transcriptional profiling to identify candidate genes that, like *bs*, are differentially regulated by social experience in aged flies (which show no plasticity following social enrichment), compared both to young flies and to aged flies fed L-DOPA. Using this strategy, we have found that functional senescence alters the transcriptional response of a variety of antimicrobial peptides (AMPs) to social enrichment. More specifically, transcript levels of AMPs, including *AttacinB (Attb)* and *Drosomycin* (*Drs*) are strongly down regulated following social enrichment in young

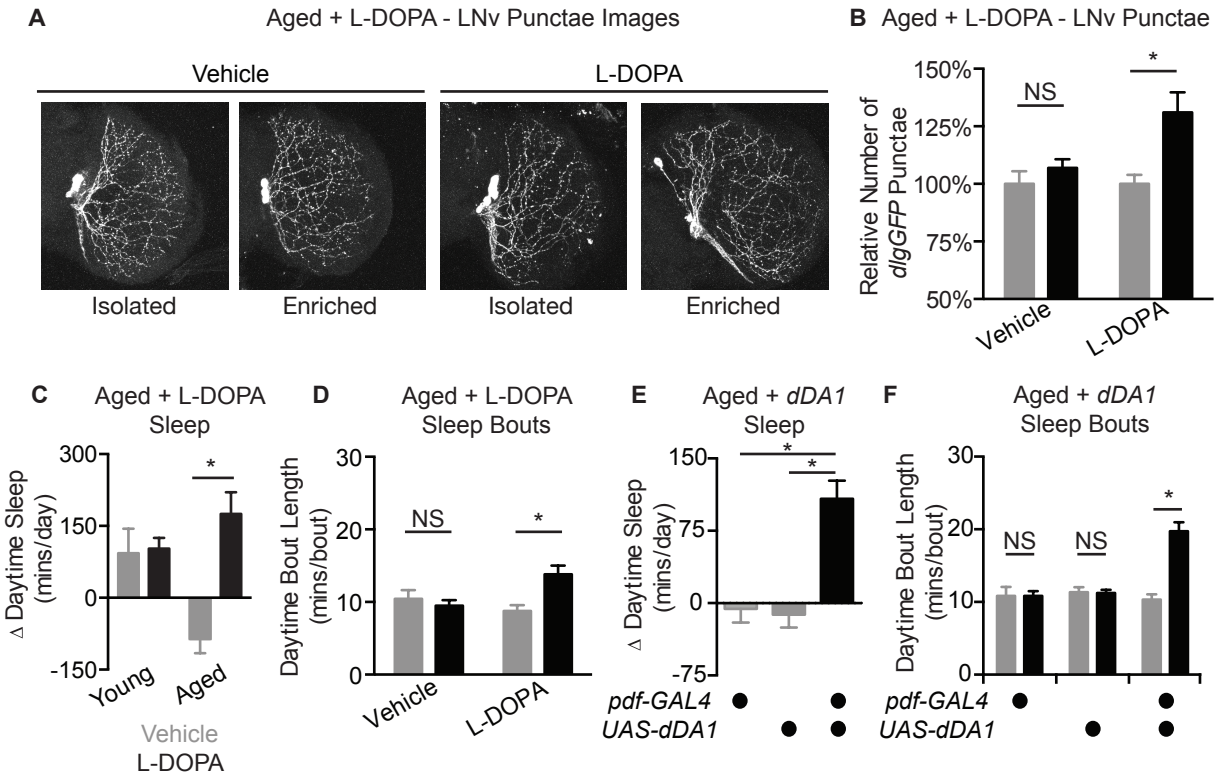

Figure 3—Elevated dopaminergic signaling restores increased sleep after social enrichment in aged flies. **(A,B)** L-DOPA administration to 20 day old *Pdf*-GAL4/+; UAS-*dlg*^{GFP}/+ flies induces an increase in LN_V terminal number following social enrichment while vehicle-treated controls show no change in LN_V terminals after social enrichment. Representative images from vehicle-fed and L-DOPA treated brains are shown in **(A)**. ANOVA reveals treatment × condition interaction in **(B)** ($F_{1,49}$ = 4.319, P = 0.042, * signifies P < 0.05 modified Bonferroni post hoc test, n = 13-14 each group). **(C)** L-DOPA administration has no effect on changes in sleep after social enrichment in 11 day old Canton-S females (left), but 26-day-old Canton-S females show a significant increase in sleep following social enrichment when fed 2 mg/mL L-DOPA during isolation/enrichment (right, black) while vehicle-fed controls show no change in sleep (right, gray). (Two-tailed Student *t*-test, P = 4.06 × 10^{-5}, n = 16 each group). **(D)** 26-day-old Canton-S females exhibit a significant increase in daytime sleep bout length following social enrichment when fed L-DOPA (right), while vehicle controls show no change in bout length (left). ANOVA reveals a significant condition × treatment interaction ($F_{1,62}$ = 8.84, P = 0.004, * P < 0.05 modified Bonferroni post hoc test, n = 16 each group). **(E)** 26-day-old flies over-expressing *dDA1* in the LN_Vs (*Pdf*-GAL4/+;; *dumb*^{2}/+, right) demonstrate a robust increase in daytime sleep after social enrichment, while 26-day-old genetic control flies show no change in sleep after social enrichment (*Pdf*-GAL4/+, left; *dumb*^{2}/+, center). One-way ANOVA ($F_{2,189}$ = 18.74, P = 3.75 × 10^{-8}, * P < 0.05 modified Bonferroni post hoc test, n = 64 each group) **(F)** Overexpression of *dDA1* in the LN_Vs induces increased daytime bout length after social enrichment in 26-day-old flies (*Pdf*-GAL4/+;; *dumb*^{2}/+, right). 26-day-old parental controls show no change in day bout length after social enrichment (*Pdf*-GAL4/+, left; *dumb*^{2}/+, center). Two-way ANOVA reveals significant genotype × condition interaction ($F_{1,340}$ = 10.81, P = 2.95 × 10^{-5}, * P < 0.05 modified Bonferroni post hoc test, n = 50-64 each group).

flies (Figure 5A, black). The transcriptional down-regulation of these peptides in response to social enrichment, however, was weakened or reversed in older animals that exhibit no plastic response (Figure 5A, light gray), but was restored in aged flies fed L-DOPA (Figure 5A, dark gray). These data suggest that increased transcript levels of AMPs during social enrichment may interfere with behavioral plasticity.

The transcription of many antimicrobial peptides is under the control of the *Immune deficiency (Imd)* signaling pathway, which itself has been implicated in sleep regulation.[34-36] Previous characterization of the *Imd* signaling pathway during immune responses has identified *Peptidoglycan recognition protein* receptors (*PGRP-LC* and *–LE)* as key regulators of the downstream regulation of antimicrobial peptide transcriptions and ectoptic expression of these receptors is sufficient to drive *IMD* signaling.[37] To test whether increased *Imd* activation through *PGRP* can prevent behavioral plasticity in young animals, we expressed *UAS-PGRP-LE* under the control of the drug-inducible *S106-GAL4,* which drives *GAL4* expression in the fat bodies upon treatment with RU486 (*S106-GAL4/+*,*UAS-PGRP-LE/+*), then examined changes in sleep following social enrichment. As expected, when young EtOH-fed *S106/+*,UAS-*PGRP-LE/+* control flies were exposed to social enrichment starting 5 days after eclosion, they exhibited a significant increase in sleep compared to their isolated siblings (Figure 5B, black). However, age- and genetically-matched *S106/+,UAS-PGRP-LE/+* flies that were treated with RU486 did not increase sleep following social enrichment (Figure 5B, gray), indicating that overexpression of *PGRP-LE* mimics the effects of aging and prevents behavioral plasticity. Next, we tested whether decreased *PGRP* activity might delay the onset of functional senescence by exposing *PGRP-LC* or *PGRP-LC*, *–LE* mutants to a socially enriched environment at 20 days of age and measuring subsequent changes in sleep. As shown in Figure 5C, aged flies that were mutant for only *PGRP-LC* (Figure 5C, middle) or for both *PGRP-LC* and *PGRP–LE* (Figure 5C, right) exhibited a significant increase in sleep following social enrichment compared to isolated siblings, while wild-type Canton-S

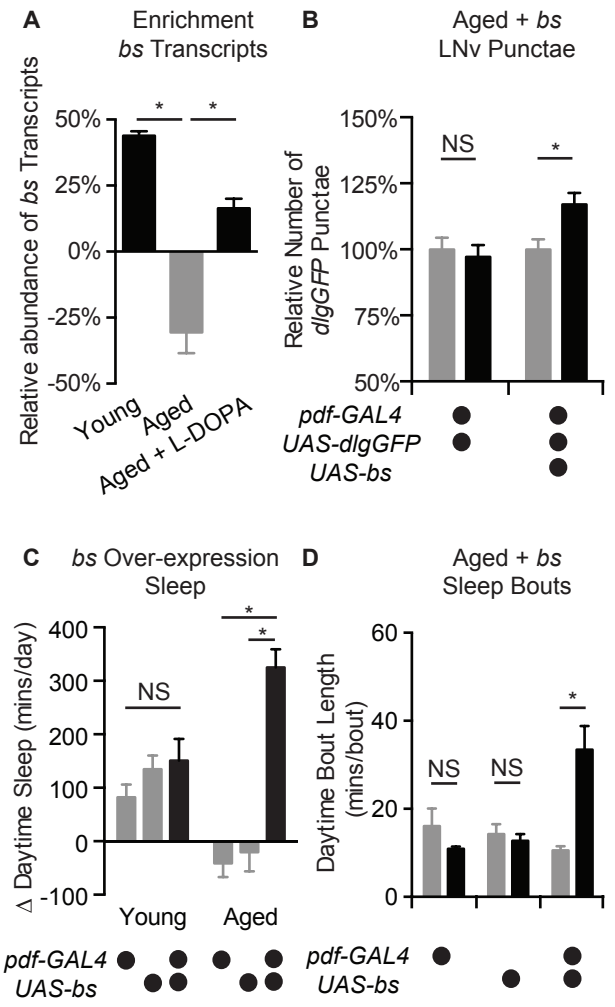

Figure 4—Overexpression of *blistered* in the LN_Vs restores plasticity in aged flies. **(A)** Abundance of *bs* transcripts is significantly elevated when Canton-S females are housed in socially enriched conditions 11 days after eclosion (left), but not when social enrichment begins at 26 days of age (center). Administration of 2 mg/mL L-DOPA during social enrichment restores elevated *bs* transcript abundance in 26-day-old Canton-S females (right). One-way ANOVA ($F_{2,3}$ = 131.76, P = 0.0012, * P < 0.05 modified Bonferroni post hoc test, n = 2 each group). **(B)** While 26-day-old control flies (*Pdf*-GAL4/+; UAS-dlg^{GFP}/+) show no change in the number of LN_V terminals following social enrichment (left), 26-day-old flies over-expressing *bs* in the LN_Vs (*Pdf*-GAL4/+; UAS-*bs*/UAS-dlg^{GFP}) exhibit a significant increase in the number of LN_V terminals (right). 2-way ANOVA reveals significant genotype × condition (Isolated vs Enriched) interaction ($F_{1,42}$ = 7.73, P = 0.008, * P < 0.05 modified Bonferroni post hoc test, n = 11 each group). **(C)** Overexpression of *bs* in the LN_Vs does not alter enrichment-induced sleep in young flies, but restores increased daytime sleep after social enrichment in 26-day-old females (*Pdf*-GAL4/+; UAS-*bs*/+, right), while 26-day-old control flies show no change after social enrichment (*Pdf*-GAL4/+, left; UAS-*bs*/+, center). Two-way ANOVA finds a significant Genotype × Age interaction ($F_{2,134}$ = 13.8, P < 0.0001, * P < 0.05 modified Bonferroni post hoc test, n = 14-32 each group). **(D)** *bs* overexpression in the LN_Vs results in increased daytime bout length after social enrichment of 26-day-old females (*Pdf*-GAL4/+; UAS-*bs*/+, right) while social enrichment has no effect on bout length in 25-day-old controls (*Pdf*-GAL4/+, left; UAS-*bs*/+, center). Two-way ANOVA reveals significant genotype × condition interaction ($F_{1,87}$ = 13.99, P = 5.1 × 10^{-6}, * P < 0.05 modified Bonferroni post hoc test, n = 14-16 each group).

flies ded not (Figure 5C, left). These data indicate that reduced signaling through the *Imd* pathway can delay the onset of functional senescence and suggest a potential link between altered immune activity and the onset of functional senescence in the aging fly.

DISCUSSION

Loss of neural plasticity is a consequence of aging that is shared between humans and fruit flies.[38] Here, we demonstrate that measuring the responses of *Drosophila* following exposure to a socially enriched environment provides a high-throughput assay for measuring deficits in plasticity that accrue across age. Social enrichment induces synaptic growth in the LN_Vs of young flies, which is followed by several days of increased sleep time and consolidation. Over the course of physiological aging, however, flies lose the ability to respond to their environment in a plastic way such that older flies no longer exhibit either synaptic plasticity or ensuing increases in sleep. While previous studies indicate that the mechanisms regulating LN_V structural plasticity and ensuing increases in sleep may overlap,[9,16] the current studies show that changes in sleep after social enrichment can be used as a reliable marker for neural plasticity. Furthermore, we provide evidence that this assay may be useful for the identification of pharmacological and genetic interventions that might preserve youthful plasticity. These interventions suggest that the architecture of circuits required for plasticity after social enrichment remains largely intact during aging, and that a state of youthful plasticity may be prolonged by adjusting the levels of specific neuromodulators.

Although dopamine production decreases with age in flies,[27,28] the architecture of dopaminergic neurons is not altered,[39] suggesting that supplementing dopamine levels within structurally latent circuits could restore youthful functioning. Our data support this hypothesis: acute dopamine depletion eliminates plasticity in young flies and, conversely, feeding L-DOPA to aged flies restores plasticity to youthful levels (Figures 2, 3). Moreover, the genetic disruption of dopaminergic signaling through the *dDA1* receptor in a cluster of wake-promoting neurons, the LN_Vs, also eliminates plasticity in young flies. Together with studies indicating that expression of *dDA1* in the LN_Vs can modulate locomotor activity,[31,40] these data suggest that dopaminergic signaling into the LN_Vs modulates arousal states and alters sleep regulation, particularly after social experience. Elevating dopaminergic signaling either by administration of L-DOPA or by overexpression of *dDA1* in the LN_Vs restores plasticity following social enrichment to aged flies, indicating that interventions targeting dopaminergic signaling may delay the onset of functional senescence. We have also begun to combine these manipulations with gene profiling to identify independent mechanisms that can restore plasticity in aged flies. For example, we found that aging results in altered transcriptional regulation of *bs* in response to social enrichment. This altered transcription is partially reversed in aged animals by L-DOPA treatment, indicating that the transcriptional response of *bs* to social experience may not be simply due to biological age alone, but rather the ability of the animal to form a plastic response. Indeed, overexpression of *bs* permits aged flies to respond to social enrichment with plastic changes in LN_V structure and sleep. Although the effects of elevated *bs* expression

are similar to those of elevated *dDA1* signaling within a common cluster of neurons (the LN_vs), it is not known how or whether the activity of the two genes may interact. The mammalian homolog of *bs*, *Serum Response Factor* (*SRF*), is known to induce transcription in response to neural activity and can be a downstream target of G-protein signaling.[41,42] Thus, it is possible that transcriptional activity regulated by *bs* may be a downstream product of *dDA1* signaling in the LN_vs. Our data, however, indicate that the induction of *bs* may not be so simple; socially isolated 25-day old flies fed L-DOPA do not exhibit increased *bs* transcription relative to age-matched controls. These data indicate that *bs* may be activated under a more complex coincidence of inputs that induces synaptic reorganization. Although *dDA1* signaling is likely a component of these pathways, further studies will be required to characterize the additional inputs.

Our gene profiling experiments have also revealed transcriptional dysregulation of antimicrobial peptides that is correlated with the loss of plasticity during aging. More specifically, young flies respond to social enrichment with a strong transcriptional repression of these peptides while older flies do not, indicating that the ability to respond to social experience in a plastic way is associated with decreased transcription of antimicrobial peptides. Our genetic experiments verify these results; over-expressing *PGRP* receptors, a signaling molecule upstream of antimicrobial peptide transcription, in young flies mimics the aged behavioral phenotype, while mutants for *PGRP* receptors retain behavioral plasticity for longer than controls. Although the neural mechanisms by which altered levels of antimicrobial peptides may influence synaptic functioning, microarray studies have found increased levels of immune-related transcripts following sleep loss[36,43-45] and a role for upstream immune signaling by *relish* has been found in sleep regulation.[36] A variety of immune-related genes are also transcriptionally elevated in aged flies.[46] These findings are consistent with the hypothesis that plasticity might be impaired, in part, by increased inflammation in the brain either after sleep loss or aging. Future studies, however, will be required to clarify the interactions between elevated immune signaling and synaptic plasticity.

Despite previous studies in humans that have identified a loss of sleep-dependent memory consolidation with age,[47] the lack of a small animal model for these effects has complicated the identification of underlying mechanisms. Given the success of using genetic model systems to identify the mechanisms of other age-related disorders,[48] we utilize the fly as a model to study the mechanisms that contribute to deficits in enrichment-induced sleep that accumulate with age. Using this model, we demonstrate that heightened dopaminergic signal or elevated expression of *bs* can delay plasticity-related senescence. Furthermore, these studies suggest a novel role for the transcription of immune response genes in plastic responses to the social environment. In combination with studies using

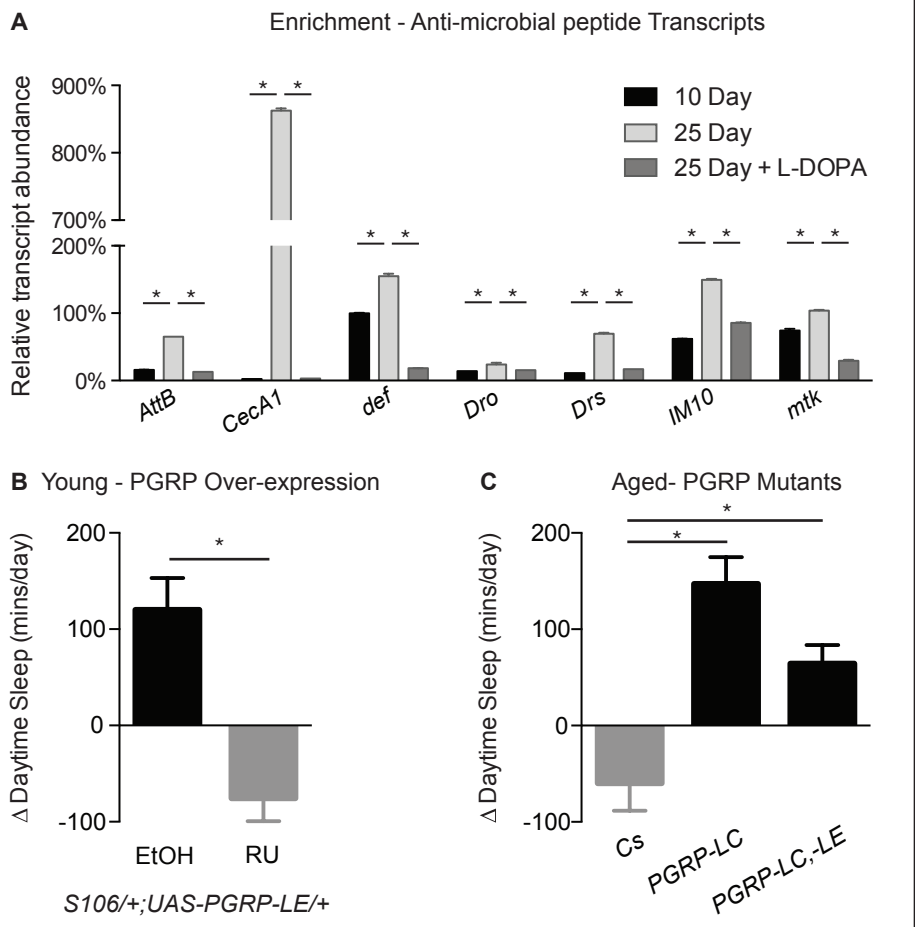

Figure 5—Expression of *PGRP* modulates behavioral plasticity. **(A)** Transcript levels of the antimicrobial peptides *AttB*, *CecA1, def, Dro, Drs, IM10,* and *Mtk* are significantly down-regulated in extracts from the heads of young (10-day-old) Canton-S females following social enrichment compared to extracts from isolated siblings (white bars). However, extracts from the heads of aged (25-day-old) Canton-S females either show a smaller decrease or an increase in the transcript levels of each of the observed antimicrobial peptides (black bars). Administration of L-DOPA to aged Canton-S females during social enrichment restores decreases in transcript levels for each peptide compared to previously isolated siblings (gray bars). ANOVA reveals a significant Gene × Condition interaction ($F_{12,29}$ = 13,961.42, P = 3.89 × 10^{-38}, *P < 0.05 modified Bonferroni post hoc test, n = 2 each group). **(B)** Young (11-day-old) EtOH-treated *S106/+ > UAS-PGRP-LE/+* flies show a significant increase in sleep following 5 days of exposure to a socially enriched environment, but age-matched *S106/+*, *UAS-PGRP-LE/+* flies fed RU beginning at eclosion do not exhibit a change in sleep time following social enrichment. *t*-test P = 4.94 × 10^{-5}, n = 15 each group. **(C)** Although aged (26-day-old) Canton-S females show no change in sleep following 5 days of exposure to a socially enriched environment, flies mutant for the *PGRP-LC* receptor (middle) or for both the *PGRP-LC* and *PGRP-LE* receptors (right) demonstrate a significant increase in sleep following 5 days in social enrichment. ANOVA reveals a significant main effect for genotype ($F_{2,52}$ = 5.35, P = 5.77 × 10^{-6}, *P < 0.05 modified Bonferroni post hoc test, n = 14-25 each group).

associative conditioning assays, examining the responses to social enrichment may contribute to future investigation of the effects of aging on sleep regulation and on plasticity.

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DISCLOSURE STATEMENT

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REFERENCES

1. Grady CL, Craik FI. Changes in memory processing with age. Curr Opin Neurobiol 2000;10:224-31.
2. Tamura T, Chiang A-S, Ito N, et al. Aging specifically impairs amnesiac-dependent memory in Drosophila. Neuron 2003;40:1003-11.
3. Yamazaki D, Horiuchi J, Nakagami Y, Nagano S, Tamura T, Saitoe M. The Drosophila DCO mutation suppresses age-related memory impairment without affecting lifespan. Nat Neurosci 2007;10:478-84.
4. Stickgold R, Hobson JA, Fosse R, Fosse M. Sleep, learning, and dreams: off-line memory reprocessing. Science 2001;294:1052-7.
5. Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron 2002;35:205-11.
6. Spencer RMC, Gouw AM, Ivry RB. Age-related decline of sleep-dependent consolidation. Learning Mem 2007;14:480-4.
7. Grotewiel M, Martin I, Bhandari P, Cook-Wiens E. Functional senescence in Drosophila melanogaster. Ageing Res Rev 2005;4:372-97.
8. Ganguly-Fitzgerald I, Donlea JM, Shaw PJ. Waking experience affects sleep need in Drosophila. Science 2006;313:1775-81.
9. Donlea JM, Ramanan N, Shaw PJ. Use-dependent plasticity in clock neurons regulates sleep need in Drosophila. Science 2009;324:105-8.
10. Volkmar FR, Greenough WT. Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 1972;176:1445-7.
11. Barth M, Hirsch HV, Meinertzhagen IA, Heisenberg M. Experience-dependent developmental plasticity in the optic lobe of Drosophila melanogaster. J Neurosci 1997;17:1493-504.
12. Barth M, Heisenberg M. Vision affects mushroom bodies and central complex in Drosophila melanogaster. Learn Mem 1997;4:219-29.
13. Sheeba V, Fogle KJ, Kaneko M, et al. Large ventral lateral neurons modulate arousal and sleep in Drosophila. Curr Biol 2008;18:1537-45.
14. Parisky KM, Agosto J, Pulver SR, et al. PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Neuron 2008;60:672-82.
15. Shang Y, Griffith LC, Rosbash M. Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the Drosophila brain. Proc Natl Acad Sci U S A 2008;105:19587-94.
16. Donlea JM, Shaw PJ. Sleeping together using social interactions to understand the role of sleep in plasticity. Adv Genet 2009;68:57-81.
17. Tononi G, Cirelli C. Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 2003;62:143-50.
18. Takehana A, Yano T, Mita S, Kotani A, Oshima Y, Kurata S. Peptidoglycan recognition protein (PGRP)-LE and PGRP-LC act synergistically in Drosophila immunity. Embo J 2004;23:4690-700.
19. Renn SCP, Park JH, Rosbash M, Hall JC, Taghert PH. A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in Drosophila. Cell 1999;99:791-802.
20. Zhang Y, Guo H, Kwan H, Wang JW, Kosek J, Lu B. PAR-1 kinase phosphorylates Dlg and regulates its postsynaptic targeting at the Drosophila neuromuscular junction. Neuron 2007;53:201-15.
21. Han Z, Li X, Wu J, Olson EN. A myocardin-related transcription factor regulates activity of serum response factor in Drosophila. Proc Natl Acad Sci U S A 2004;101:12567-72.
22. Takehana A, Katsuyama T, Yano T, et al. Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in Drosophila larvae. Proc Natl Acad Sci U S A 2002;99:13705-10.
23. Roman GW, Endo K, Zong L, Davis RL. P[Switch], a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc Natl Acad Sci U S A 2001;98:12602-7.
24. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature 2002;417:287-91.
25. Seugnet L, Boero J, Gottschalk L, Duntley SP, Shaw PJ. Identification of a biomarker for sleep drive in flies and humans. Proc Natl Acad Sci U S A 2006;103:19913-8.
26. Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. J Neurosci 2003;23:10495-502.
27. Neckameyer WS, Woodrome S, Holt B, Mayer A. Dopamine and senescence in Drosophila melanogaster. Neurobiol Aging 2000;21:145-52.
28. Imai Y, Gehrke S, Wang HQ, et al. Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila. Embo J 2008;27:2432-43.
29. Kim YC, Lee HG, Han KA. D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in Drosophila. J Neurosci 2007;27:7640-7.
30. Seugnet L, Suzuki Y, Vine L, Gottschalk L, Shaw PJ. D1 receptor activation in the mushroom bodies rescues sleep-loss-induced learning impairments in Drosophila. Curr Biol 2008;18:1110-7.
31. Shang Y, Haynes P, Pírez N, et al. Imaging analysis of clock neurons reveals light buffers the wake-promoting effect of dopamine. Nat Neurosci 2011;14:889-95.
32. Kim YC, Lee HG, Seong CS, Han KA. Expression of a D1 dopamine receptor dDA1/DmDOP1 in the central nervous system of Drosophila melanogaster. Gene Expr Patterns 2003;3:237-45.
33. Dietzl G, Chen D, Schnorrer F, S et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 2007;448:151-6.
34. Cirelli C. A molecular window on sleep: changes in gene expression between sleep and wakefulness. Neuroscientist 2005;11:63-74.
35. Kuo T-H, Pike DH, Beizaeipour Z, Williams JA. Sleep triggered by an immune response in Drosophila is regulated by the circadian clock and requires the NFkappaB Relish. BMC Neurosci 2010;11:17.
36. Williams JA, Sathyanarayanan S, Hendricks JC, Sehgal A. Interaction between sleep and the immune response in Drosophila: a role for the NFkappaB relish. Sleep 2007;30:389-400.
37. Hoffmann JA. The immune response of Drosophila. Nature 2003;426:33-8.
38. Burke SN, Barnes CA. Neural plasticity in the ageing brain. Nat Rev Neurosci 2006;7:30-40.
39. White KE, Humphrey DM, Hirth F. The dopaminergic system in the aging brain of Drosophila. Front Neurosci 2010;4:205.
40. Lebestky T, Chang JS, Dankert H, et al. Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron 2009;64:522-36.
41. Mao J, Yuan H, Xie W, Simon MI, Wu D. Specific involvement of G proteins in regulation of serum response factor-mediated gene transcription by different receptors. J Biol Chem 1998;273:27118-23.
42. Ramanan N, Shen Y, Sarsfield S, et al. SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. Nat Neurosci 2005;8:759-67.
43. Seugnet L, Suzuki Y, Thimgan MS, et al. Identifying sleep regulatory genes using a Drosophila model of insomnia. J Neurosci 2009;29:7148-57.
44. Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 2004;41:35-43.

45. Thimgan MS, Gottschalk L, Toedebusch C, et al. Cross-translational studies in human and Drosophila identify markers of sleep loss. PLoS ONE 2013;8:e61016.
46. Felix TM, Hughes KA, Stone EA, Drnevich JM, Leips J. Age-specific variation in immune response in Drosophila melanogaster has a genetic basis. Genetics 2012;191:989-1002.
47. Daselaar SM, Veltman DJ, Rombouts SA, Raaijmakers JG, Jonker C. Neuroanatomical correlates of episodic encoding and retrieval in young and elderly subjects. Brain 2003;126:43-56.
48. Brack AS, Conboy MJ, Roy S, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007;317:807-10.

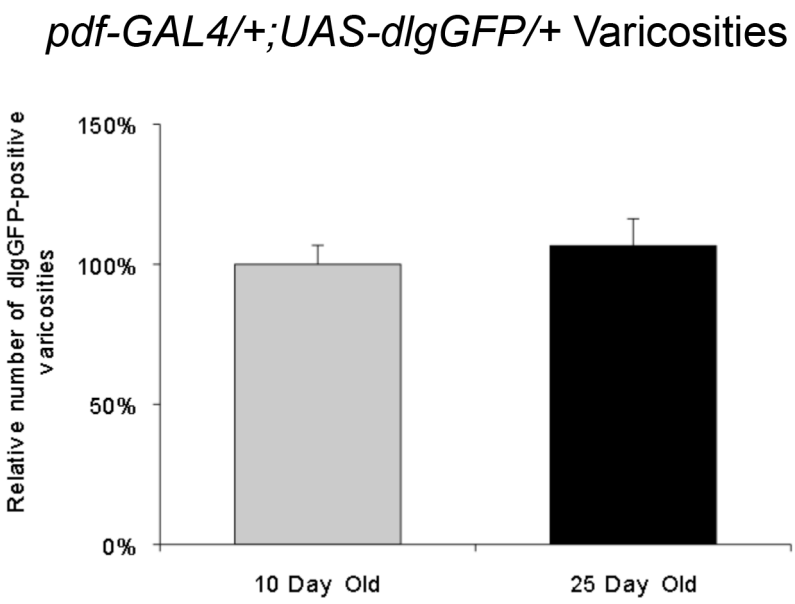
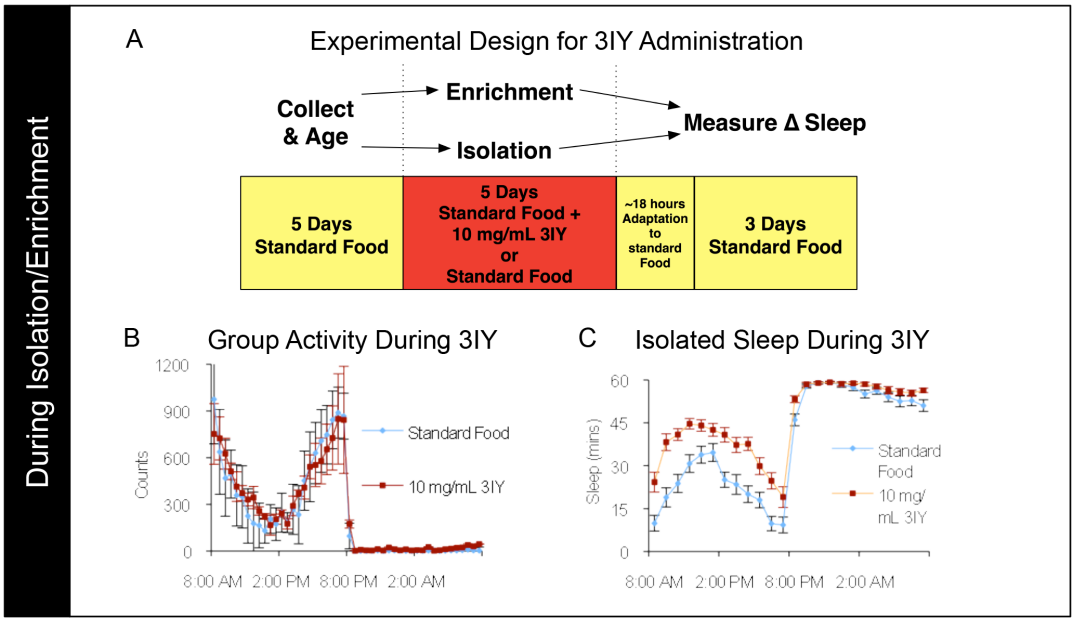


Figure S1—No effect of age on number of LN_V synaptic terminals. The number of dlg^{GFP}-positive varicosities in LN_Vs (*pdf*-GAL4/+; UAS-dlg^{GFP}/+) does not differ between 10-day-old and 25-day-old flies (P = 0.67, Two-tailed Student *t*-test, n = 9-10 each group).



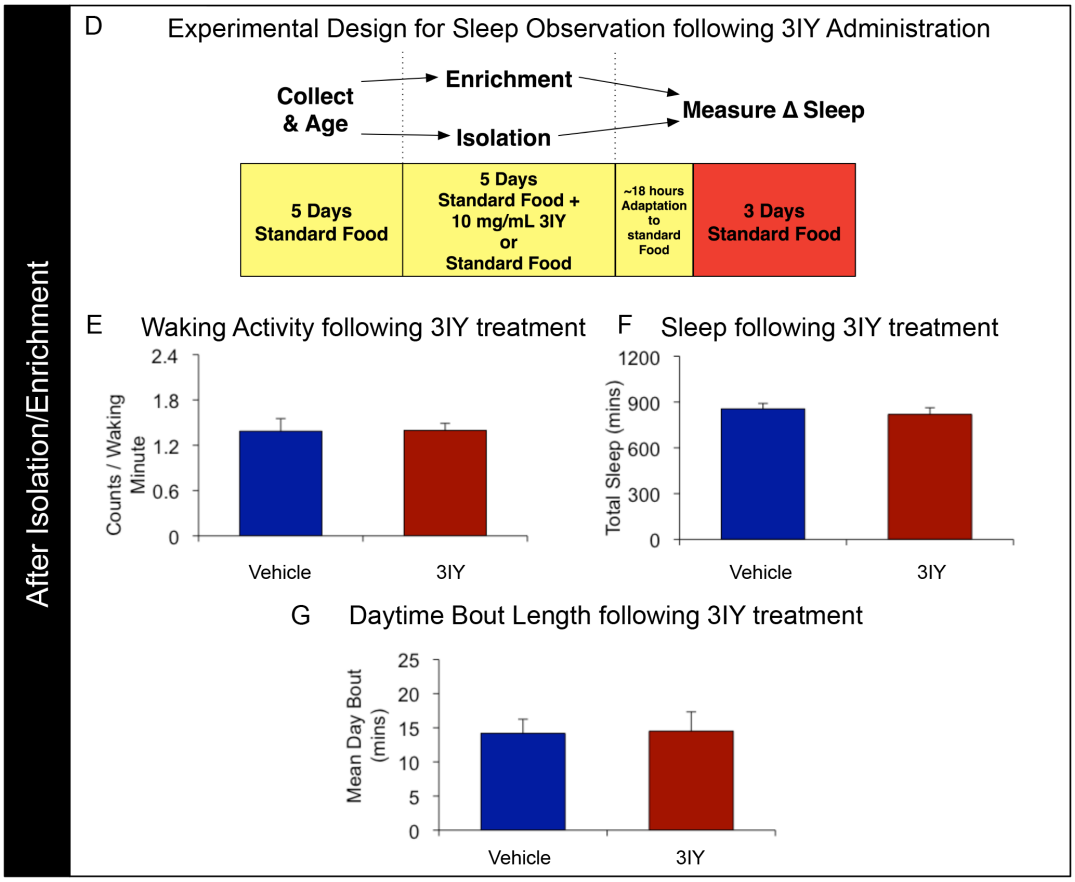


Figure S2—Sleep during and after 3IY administration. **(A)** Experimental design for dopamine pharmacology during social enrichment. Data presented in panels B-C represent sleep and activity data collected from isolated or enriched flies during pharmacological treatments. **(B)** Group activity during social enrichment is not significantly altered in flies fed 10 mg/mL 3IY. T-test for total locomotor activity P = 0.92, n = 3 each group. **(C)** Sleep is increased during social isolation in flies that are administered 10 mg/mL 3IY. Student *t*-test for total sleep time P = 4.5 × 10^{-6}, n = 30-32 each group. **(D)** Experimental design for the measurement of ΔSleep following administration of 3IY to socially isolated or enriched flies. Data presented in panels E-G was collected after all flies were allowed to adapt to fresh Trikinetics tubes containing standard food for ~18 h. **(E)** The intensity of waking activity as measured by counts/waking minute is not altered in isolated flies previously fed 3IY compared to vehicle-treated controls. Student *t*-test P = 0.44, n = 13-14. **(F)** Following ~18 h on standard food, no difference in sleep time is detected between socially isolated flies previously fed 3IY and their vehicle- treated siblings. Student *t*-test for total sleep, P = 0.53, n = 13-14. **(G)** After ~18 h of adaptation to standard food, no change in daytime sleep bout length is observed in isolated flies previously administered 3IY relative to their vehicle-fed controls. Student *t*-test n = 0.94, n = 13-14.

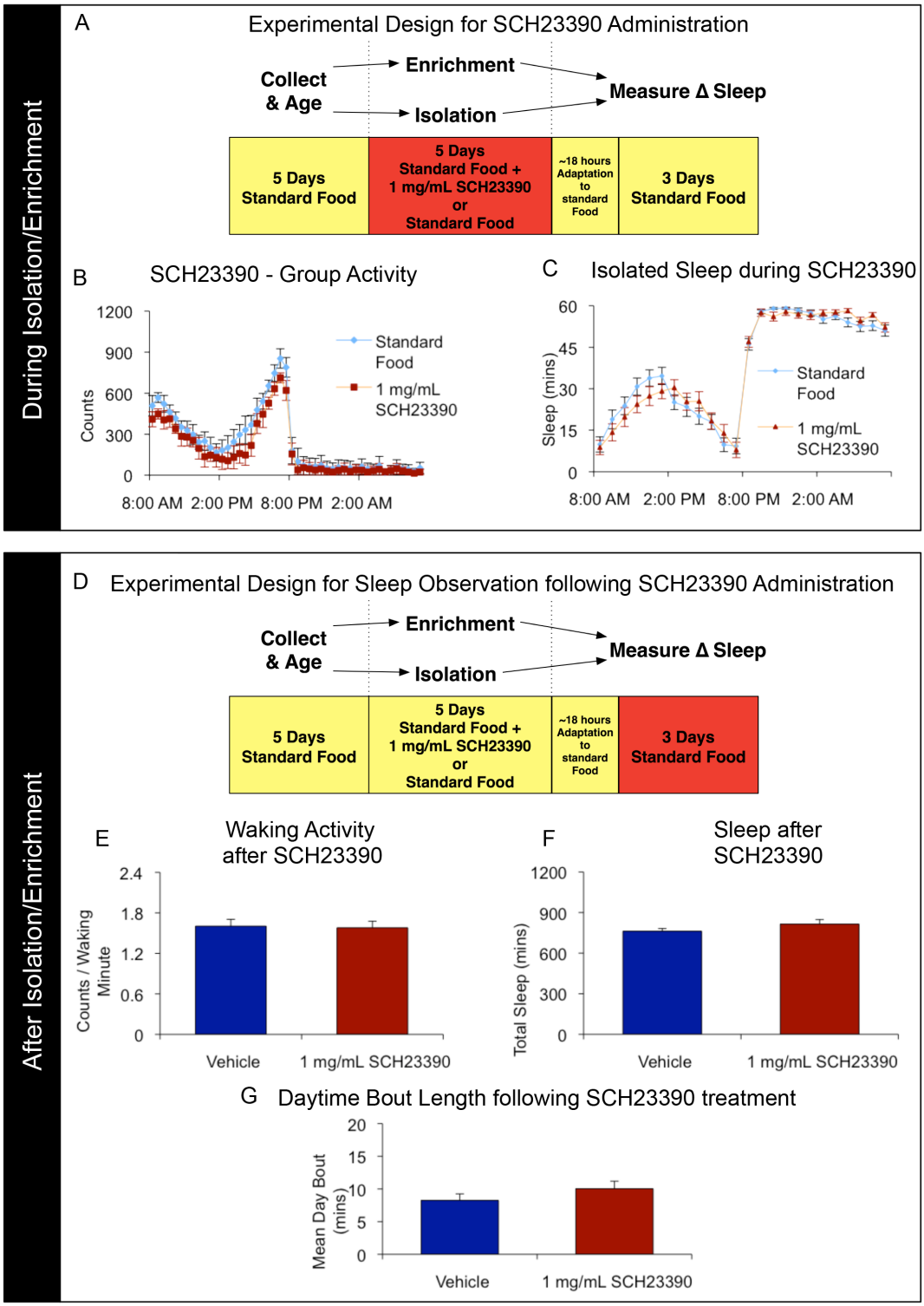


Figure S3—Sleep during and after administration of SCH23390. **(A)** Experimental design for dopamine pharmacology during social enrichment. Data presented in panels B-C were collected while flies were administered 1 mg/mL SCH23390 during social isolation or enrichment. **(B)** Administration of 1 mg/mL SCH23390 to 5-day-old *Cs* females during social enrichment does not alter locomotor activity. *t*-test for total locomotor activity P = 0.46, n = 3 each group. **(C)** Sleep time is not altered in flies during administration of SCH23390. Student *t*-test for total sleep time P = 0.80, n = 30-32 each group. **(D)** Experimental design for measuring sleep following administration of SCH23390 during social isolation or enrichment. Panels E-G represent data collected after flies adapted to fresh Trikinetics tubes containing standard food for ~18 h. **(E)** Intensity of waking activity is not altered in socially isolated flies allowed to adapt to standard food for 18 h after administration of SCH23390 compared to vehicle-treated controls. Student *t*-test P = 0.86, n = 16 each group. **(F)** No change in total sleep time can be detected in socially isolated flies fed standard food for ~18 h after administration of SCH23390 compared to vehicle-treated controls. Student *t*-test P = 0.18, n = 16 each group. **(G)** Daytime sleep bout length is not altered in socially isolated flies that are fed standard food for ~18 h after treatment with SCH23390 relative to vehicle-fed controls. Student *t*-test P = 0.25, n = 16 each group.

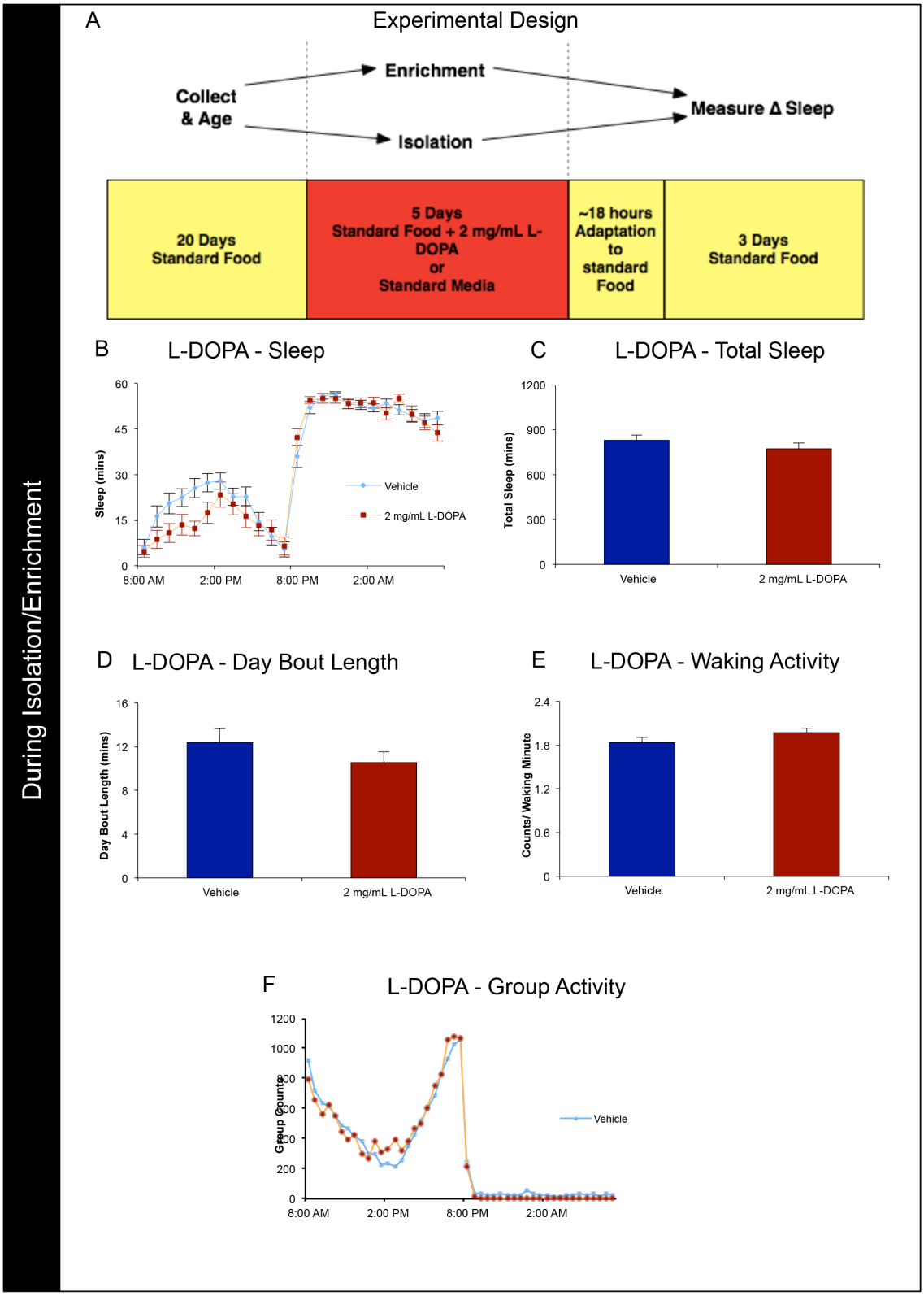


Figure S4—No effect of L-DOPA administration on sleep in aged *Cs* females. **(A)** Experimental design for L-DOPA administration during social isolation or enrichment in aged *Cs* females. Data presented in panels B-F were collected from socially isolated or enriched flies during administration of 2 mg/mL L-DOPA. **(B,C)** No change in total sleep time of 20-day-old, socially isolated *Cs* females during administration of 2 mg/mL L-DOPA compared to vehicle-fed siblings (P = 0.29, Two-tailed Student *t*-test, n = 23-25). **(D)** Administration of 2 mg/mL L-DOPA to 20-day-old *Cs* females during isolation has no significant effect on daytime bout length (P = 0.27, Two-tailed Student *t*-test, n=23-35). **(E)** Intensity of waking activity is not altered by administration of 2 mg/mL L- DOPA to 20-day-old *Cs* females during social isolation (P = 0.15, Two-tailed Student *t*-test, n = 23-35). **(F)** Amount of locomotor activity is not altered in socially enriched groups of 20-day-old *Cs* females during administration of 2 mg/mL L-DOPA. ANOVA reveals no significant main effect for treatment ($F_{46,1}$ = 0.46, P = 0.50).