

Supplemental information

Serotonin distinctly controls behavioral states in restrained and freely moving *Drosophila*

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Supplemental Information

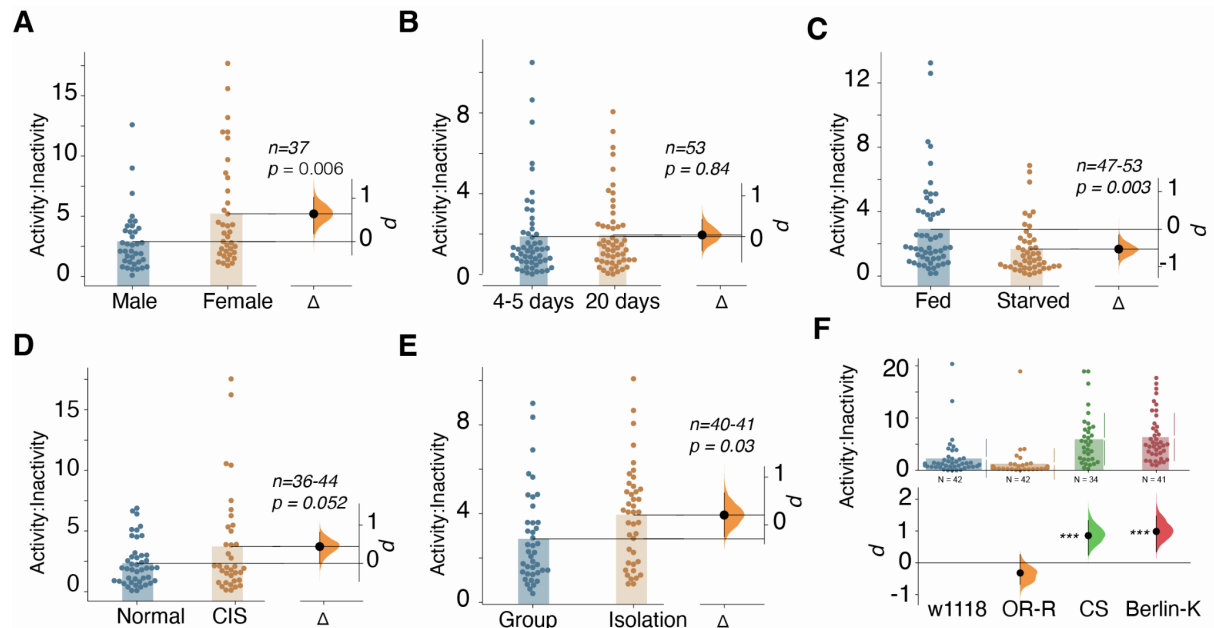


Figure S1. Potential factors influencing STRIF behavior, Related to Figure 1.

A. Activity-inactivity ratio showing that females exhibited higher levels of STRIF-activity compared to males ($n = 37$, $\Delta = 1.05$, $d = 0.7$ [95CI 0.24, 1.1], $p = 0.006$). **B.** There was no significant difference in STRIF-activity in young vs. older flies ($n = 53$, $\Delta = 0.04$, $d = 0.04$, [95CI -0.34, 0.43], $p = 0.83$). **C.** Starved flies displayed decreased STRIF response compared to fed flies ($n = 47-53$, $\Delta = 0.50$, $d = -0.58$, [95CI -0.92, -0.077], $p = 0.003$). **D.** Chronical immobilization stress (CIS) flies showed slightly increased STRIF-activity in comparison to freely moving flies ($n = 36-44$, $\Delta = 0.72$, $d = 0.42$, [95CI -0.031, 0.79], $p = 0.052$). **E.** Socially isolated flies showed increased STRIF-activity compared to grouped flies. Each dot on the scatter plot represents the STRIF response of one fly ($n = 40-41$, $\Delta = 0.47$, $d = 0.47$, [95CI 0.006, 0.96], $p = 0.03$). **F.** Activity-inactivity ratio of different genotypes, indicating that CS and *Berlin-K* flies showed more STRIF-activity compared to OR and w^{1118} flies; w^{1118} was used as a shared control ($n = 34-42$, w^{1118} -OR-R, $\Delta = -0.28$, $d = -0.31$, [95CI -0.68, 0.259], $p = 0.17$; w^{1118} -CS, $\Delta = 1.0$, $d = 0.85$, [95CI 0.248, 1.33], $p = 0.0006$; w^{1118} *Berlin-K*, $\Delta = 1.11$, $d = 0.98$, [95CI 0.34, 1.47], $p = 0.0001$). The experiment-control differences are displayed as the effect size (Cohen's d) with an error curve and 95CI. Permutation t-test P values (two-tailed) were used for testing significance.

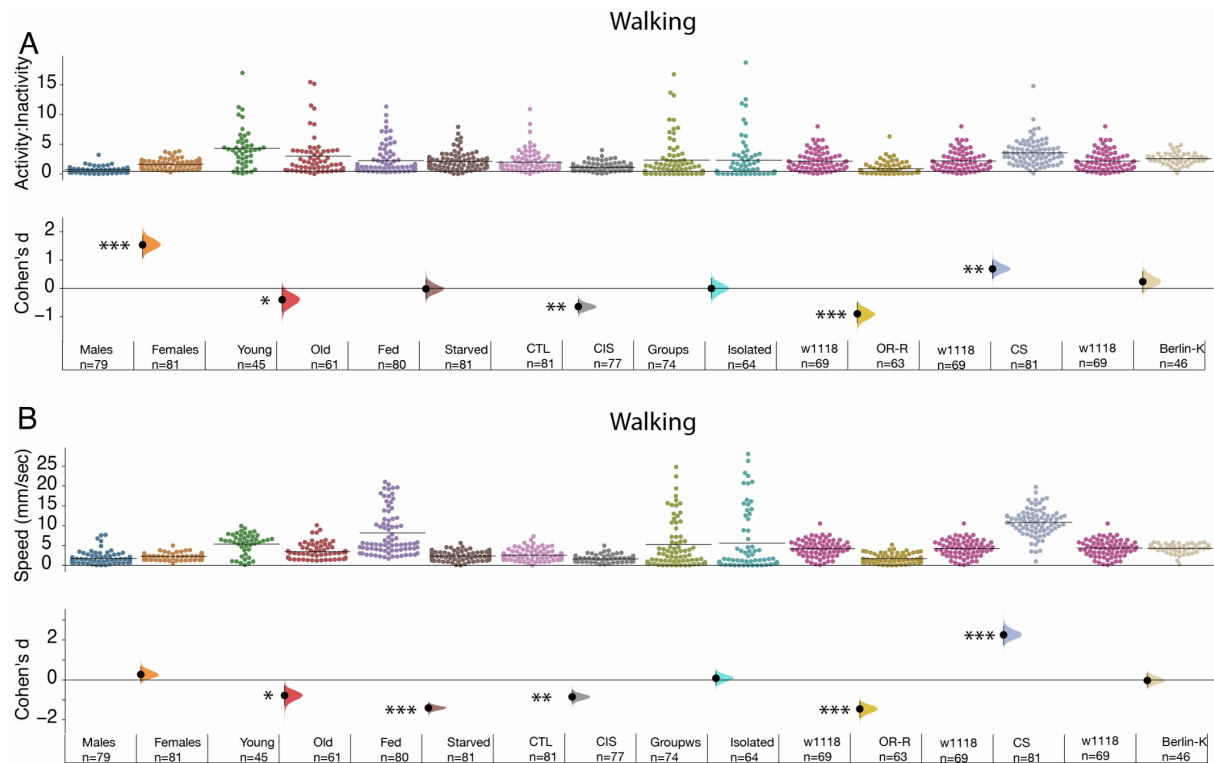


Figure S2. Potential factors influencing walking behaviors in freely moving flies, Related to Figure 1.

A. Activity-inactivity ratio of eight factors-gender ($n = 79-81$, $\Delta = 2.06$, $d = 1.5$, [95CI 1.1, 1.9], $p = 0.0001$), age ($n = 45-61$, $\Delta = -0.39$, $d = -0.4$, [95CI -0.81, 0.02], $p = 0.05$), satiety ($n = 80-81$, $\Delta = -0.0$, $d = -0.02$, [95CI -0.34, 0.3], $p = 0.93$), chronic immobility ($n = 77-81$, $\Delta = -0.5$, $d = -0.64$, [95CI -0.85, -0.39], $p = 0.001$), isolation ($n = 64-74$, $\Delta = 0.16$, $d = 0.003$, [95CI -0.33, 0.4], $p = 0.99$), and the genotypes w^{1118} , OR-R, CS, and Berlin-K (OR-R, $n = 63-69$, $\Delta = -0.76$, $d = -0.89$, [95CI -1.2, -0.49], $p = 0.001$; CS, $n = 69-81$, $\Delta = 0.8$, $d = 0.69$, [95CI 0.38, 0.98], $p = 0.0001$; Berlin-K, $n = 46-69$, $\Delta = 0.21$, $d = 0.24$, [95CI -0.13, 0.61], $p = 0.21$). **B.** Walking speed of eight factors- gender ($n = 79-80$, $\Delta = 0.21$, $d = 0.27$, [95CI -0.08, 0.64], $p = 0.093$), age ($n = 45-60$, $\Delta = -0.7$, $d = -0.78$, [95CI -1.3, -0.3], $p = 0.005$), satiety ($n = 80-81$, $\Delta = -1.01$, $d = -1.4$, [95CI -1.6, -1.2], $p = 0.0001$), chronic immobility ($n = 77-81$, $\Delta = -0.7$, $d = -0.85$, [95CI -1.1, -0.53], $p = 0.0001$), isolation ($n = 65-74$, $\Delta = 0.01$, $d = 0.08$, [95CI -0.25, 0.42], $p = 0.64$), and the genotypes w^{1118} , OR-R, CS, and Berlin-K (OR-R, $n = 63-69$, $\Delta = -1.3$, $d = -1.5$, [95CI -1.9, 1.1], $p = 0.0001$; CS, $n = 69-81$, $\Delta = 3.08$, $d = 2.3$, [95CI 1.8, 2.7], $p = 0.0001$; Berlin-K, $n = 46-69$, $\Delta = -0.02$, $d = -0.03$, [95CI -0.4, 0.31], $p = 0.87$).

Movie S1-S9. Restrained fly exhibiting activity, immobility, abdomen thrust and wing movements and wing flapping behaviors, **Related to Figure 1.**

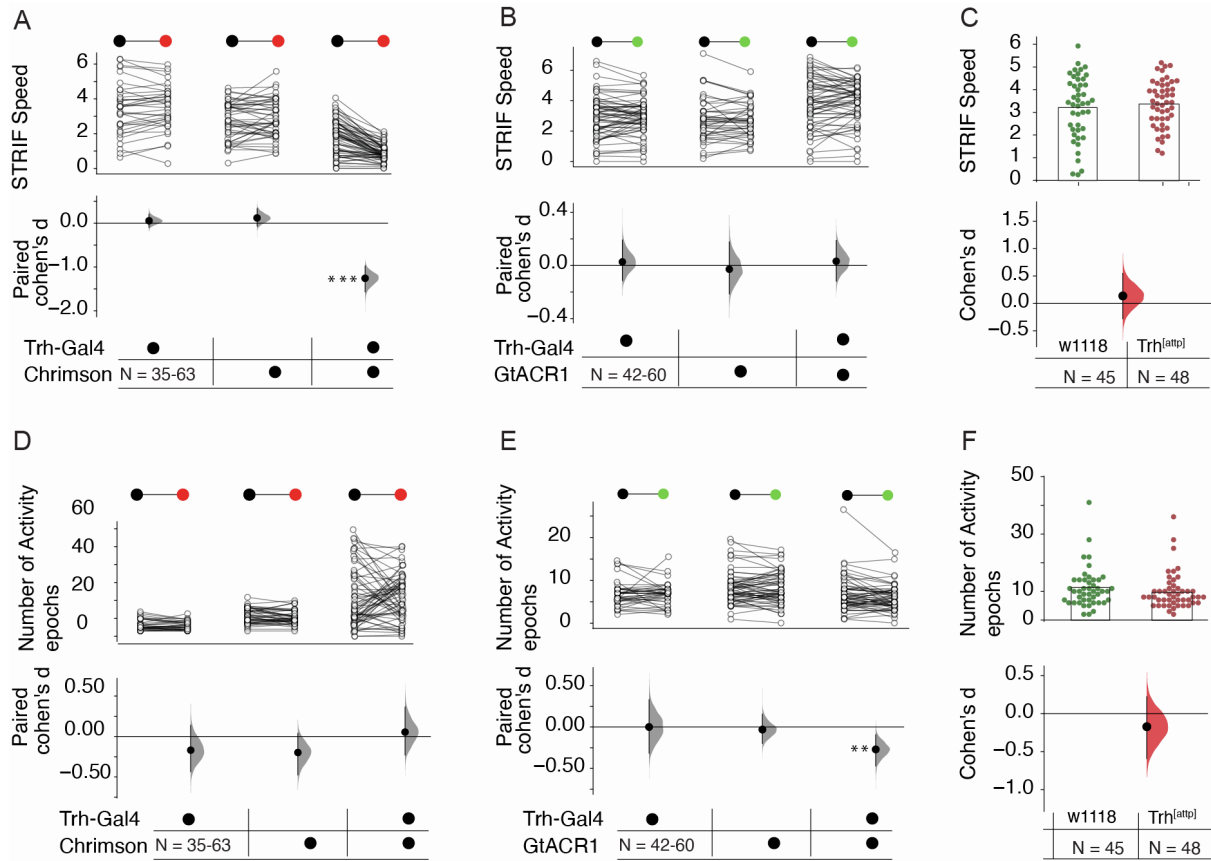


Figure S3. The effect of serotonin activation and inactivation on STRIF speed and the number of activity epochs., Related to Figure 2 and 3.

A. Optogenetic activity in the broader serotonin system (*Trh-Gal4-UAS-Chrimson*) reduced STRIF speed ($n = 65$, $\Delta = -1.09$, $d = -1.31$, [95CI -1.65, -1.0], $p = 0.0001$). **B.** Optogenetic silencing of the broader serotonin system (*Trh-Gal4-UAS-GtACR1*) did not affect STRIF speed ($n = 60$, $\Delta = 0.03$, $d = 0.03$, [95CI 0.13, 1.02], $p = 0.008$). **C.** *Trh^[attp]* homozygous mutant flies were comparable to *w¹¹¹⁸* isogenic flies ($n = 48$, $\Delta = 0.12$, $d = 0.13$, [95. CI -0.28, 0.54], $p = 0.51$). **D.** Optogenetic activity in the broader serotonin system did not affect the number of activity epochs ($n = 63$, $\Delta = 0.18$, $d = 0.18$, [95CI -0.16, 0.57], $p = 0.3$). **E.** Optogenetic inactivity in the broader serotonin system reduced the number of activity epochs ($n = 60$, $\Delta = 0.27$, $d = -0.27$, [95.0%CI -0.47, -0.09], $p = 0.008$). **F.** *Trh^[attp]* homozygous mutant flies were comparable to *w¹¹¹⁸* flies ($n = 48$, $\Delta = -0.21$, $d = -0.24$, [95CI -0.659, 0.175], $p = 0.262$).

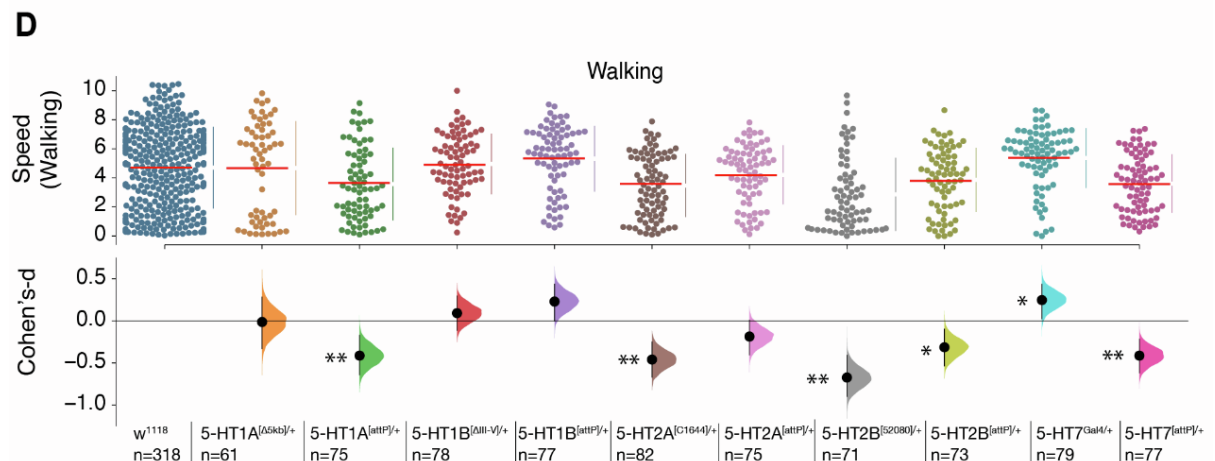
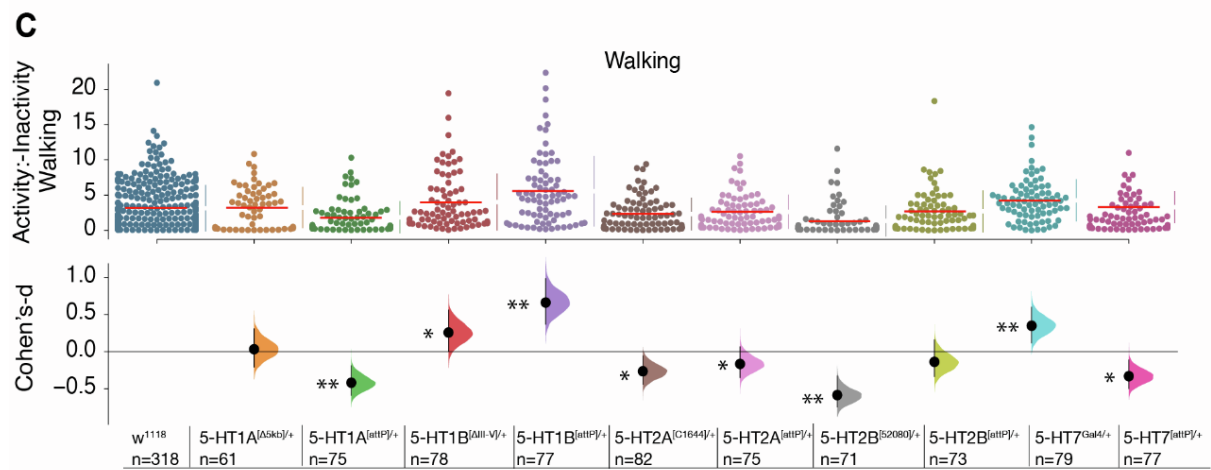
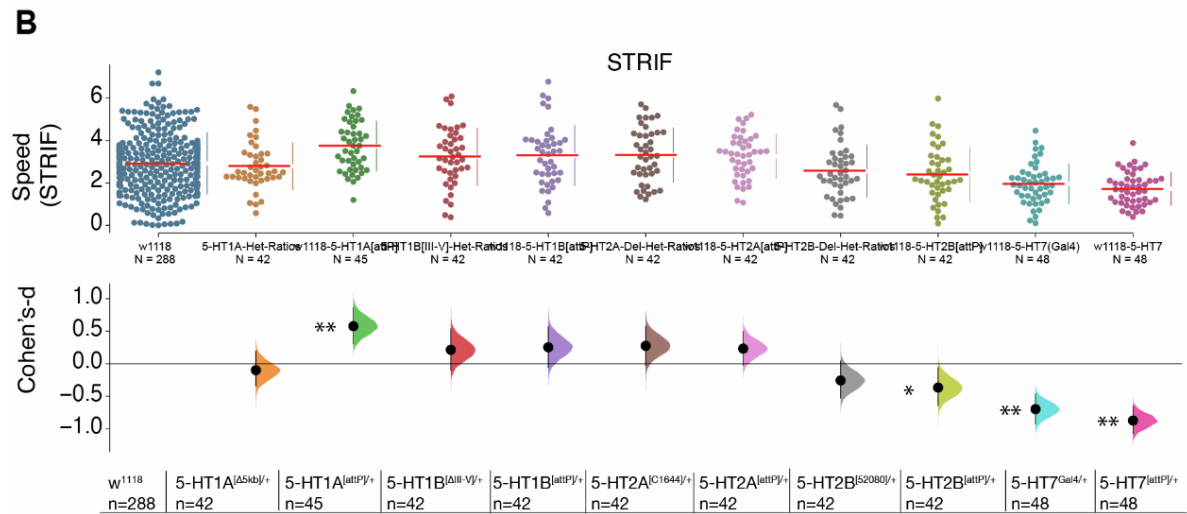
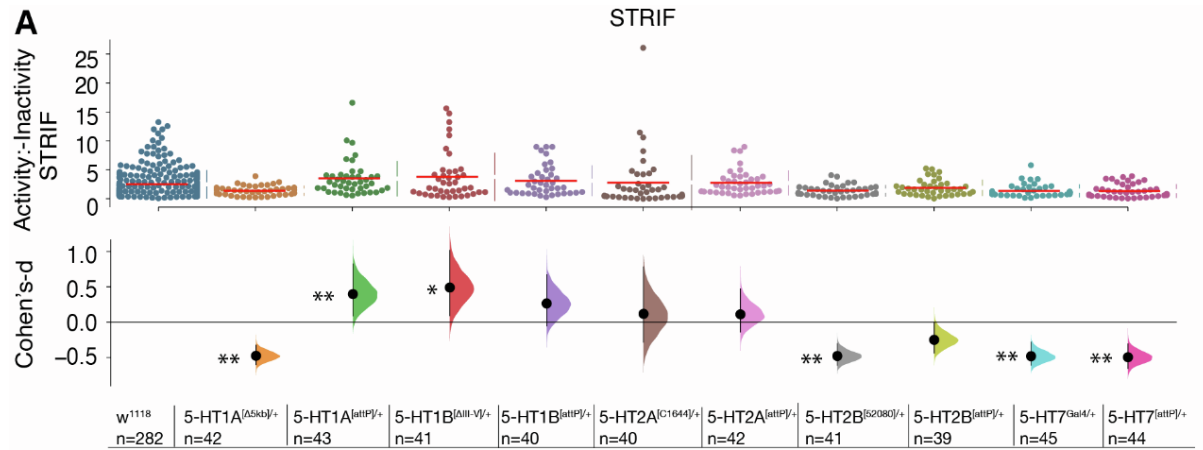


Figure S4. Serotonin receptors affect multiple aspects of STRIF and walking behaviors, Related to Figure 5.

A. Inconsistent effects in STRIF activity-inactivity ratios were observed between two mutant alleles of same receptors, except for 5-HT7 receptors, which show consistently significantly reduced activity-inactivity ratios in both alleles (5-HT7^[G]/w¹¹¹⁸, n = 45, Δ = -0.455, d = -0.482, [95.0%CI -0.611, -0.283], p = 0.004) ; (5-HT7^[attp] /w¹¹¹⁸, n = 44, Δ = -0.47, d = -0.496, [95.0%CI -0.656, -0.309], p = 0.003). **B.** Similar to activity-inactivity ratios in STRIF assay, effect on STRIF-speed was variable among receptors, except for 5-HT7, which consistent significantly reduced speed in STRIF paradigm, (5-HT7^[G]/w¹¹¹⁸, n = 48, Δ = -0.66, d = -0.69, [95.0%CI -0.92, -0.46], p = 0.0) (5-HT7^[attp] /w¹¹¹⁸, n = 48, Δ = -0.82, d = -0.87, [95.0%CI -1.07, -0.65], p = 0.0). **C.** In walking paradigm, except for 5-HT2A, two alleles of other receptors show variable results in activity-inactivity ratios (5-HT2A^[c1644]/w¹¹¹⁸, n = 82, Δ = -0.25, d = -0.26, [95.0%CI -0.44, -0.05], p = 0.02; 5-HT2A^[attp]/w¹¹¹⁸, n = 75, Δ = -0.14, d = -0.16, [95.0%CI -0.34, 0.06], p = 0.19). **D.** In walking paradigm, only 5-HT2B receptor allele show consistent reduced speed, other receptors allele show variable results 5-HT2B^[MI]/w¹¹¹⁸, n = 71, Δ = -0.66, d = -0.67 [95.0%CI -0.89, -0.40], p = 0.0; 5-HT2B^[attp]/w¹¹¹⁸, n = 73, Δ = -0.30, d = -0.31, [95.0%CI -0.53, -0.09], p = 0.01)