Figures and figure supplements

Assessing the effects of stress on feeding behaviors in laboratory mice

Marie Francois *et al*

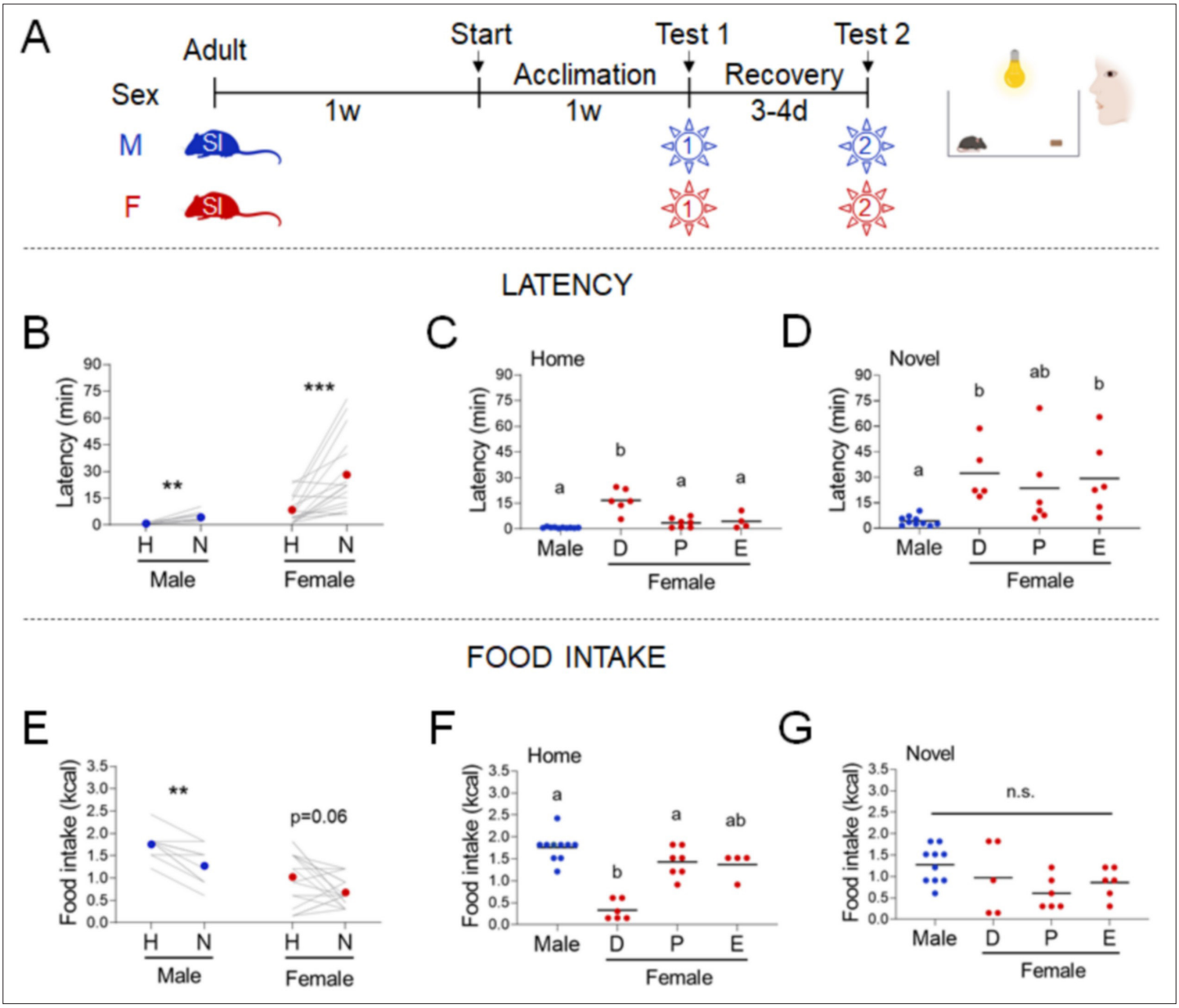

Figure 1. Effects of sex and estrous cyclicity in the standard novelty-suppressed feeding (NSF) assay. (**A**) Experimental paradigm for the standard NSF assay performed on the bench, in the morning following an overnight fast. (**B**) Latency to eat in home (H) and novel (N) tests in males (blue, $n = 10$, paired t-test: $t = 4.094$; df = 9; p = 0.027) and females (red, $n = 17$, Wilcoxon test: $W = 141$; p = 0.0002). (**C**) Latency to eat in the home test in males (blue, $n = 10$) and females (red, $n = 17$) categorized by estrous cycle stage (D: diestrus; P: proestrus; E: estrus) (one-way analysis of variance [ANOVA]: $F_{(3, 23)} = 22.52$; p < 0.0001). (**D**) Latency to eat in the novel test in males (blue, $n = 10$) and females (red, $n = 17$) categorized by estrous cycle stage (Kruskal–Wallis test: $H_{(3)} = 16.80$; p = 0.0008). (**E**) Food intake in home (H) and novel (N) tests in males (blue, $n = 10$, Wilcoxon test: $W = -36$; p = 0.0078) and in females (red, $n = 17$, Wilcoxon test: $W = -67$; p = 0.0562). (**F**) Food intake in males (blue, $n = 10$) and females (red, $n = 17$) categorized by estrous cycle stage in the home test (Kruskal–Wallis test: $H_{(3)} = 15.86$; p = 0.0012). (**G**) Food intake in males (blue, $n = 10$) and females (red, $n = 17$) categorized by estrous cycle stage in the novel test (one-way ANOVA: $F_{(3, 23)} = 2{,}370$; p = 0.0968). Significant differences denoted by different letters. **p < 0.01 and ***p < 0.001 between home and novel tests. SI, social isolation; n.s., not significant. See ***Figure 1—source data 1***.

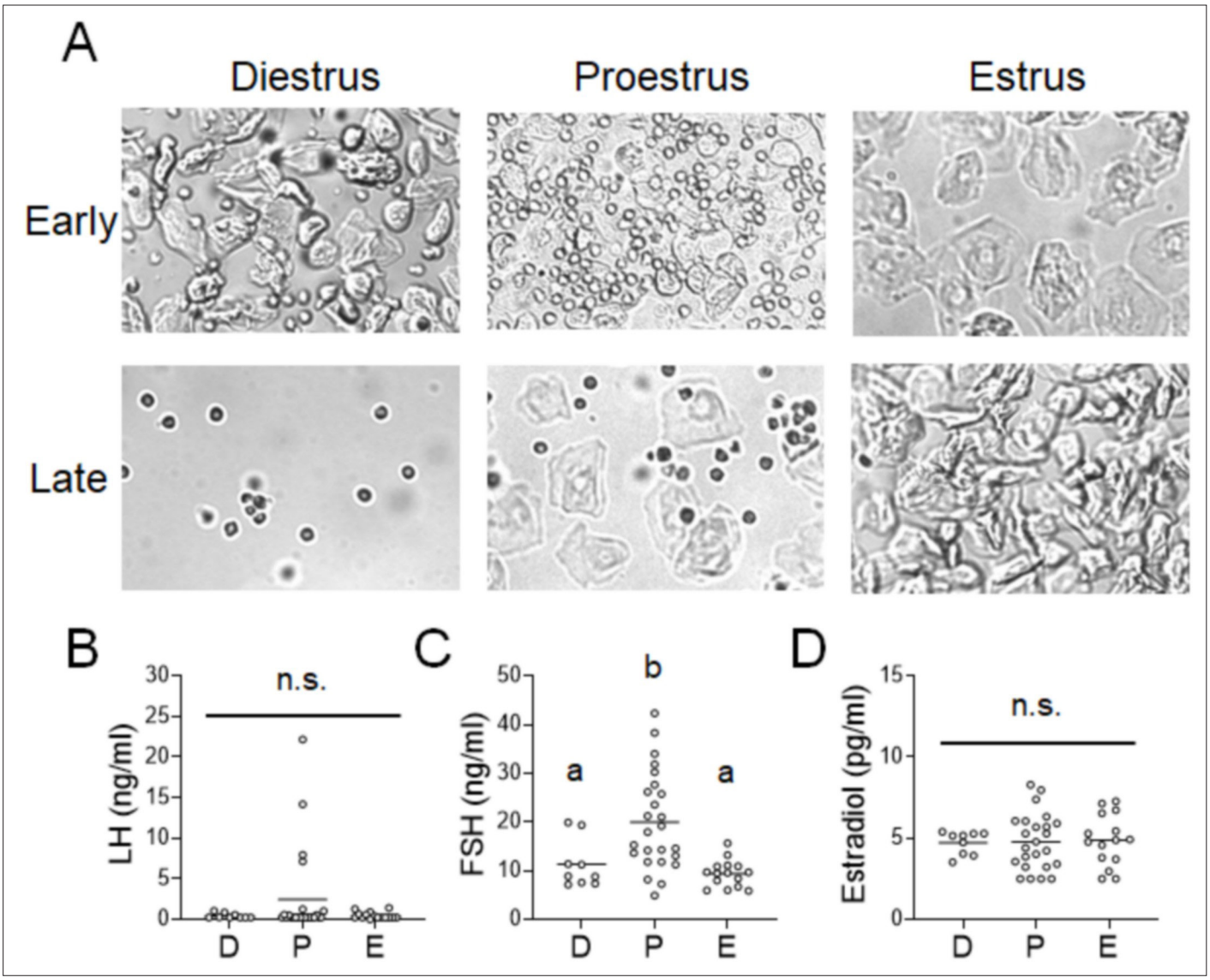

Figure 1—figure supplement 1. Evaluation of the estrous phase with cytology and hormone level measurements.
(**A**) Representative pictures of vaginal cytology used to characterize phases of the estrous cycle. (**B**) Circulating levels of luteinizing hormone (LH) across the estrous cycle (Kruskal–Wallis test: $H_{(2)} = 0.1165$; p = 9434). (**C**) Circulating levels of follicle-stimulating hormone (FSH) across the estrous cycle (Kruskal–Wallis test: $H_{(2)} = 17.20$; p = 0.0002). (**D**) Circulating levels of estradiol across the estrous cycle (one-way analysis of variance [ANOVA]: $F_{(2, 45)} = 0.02807$). Significant differences denoted by different letters. n.s., not significant. See ***Figure 1—source data 2***.

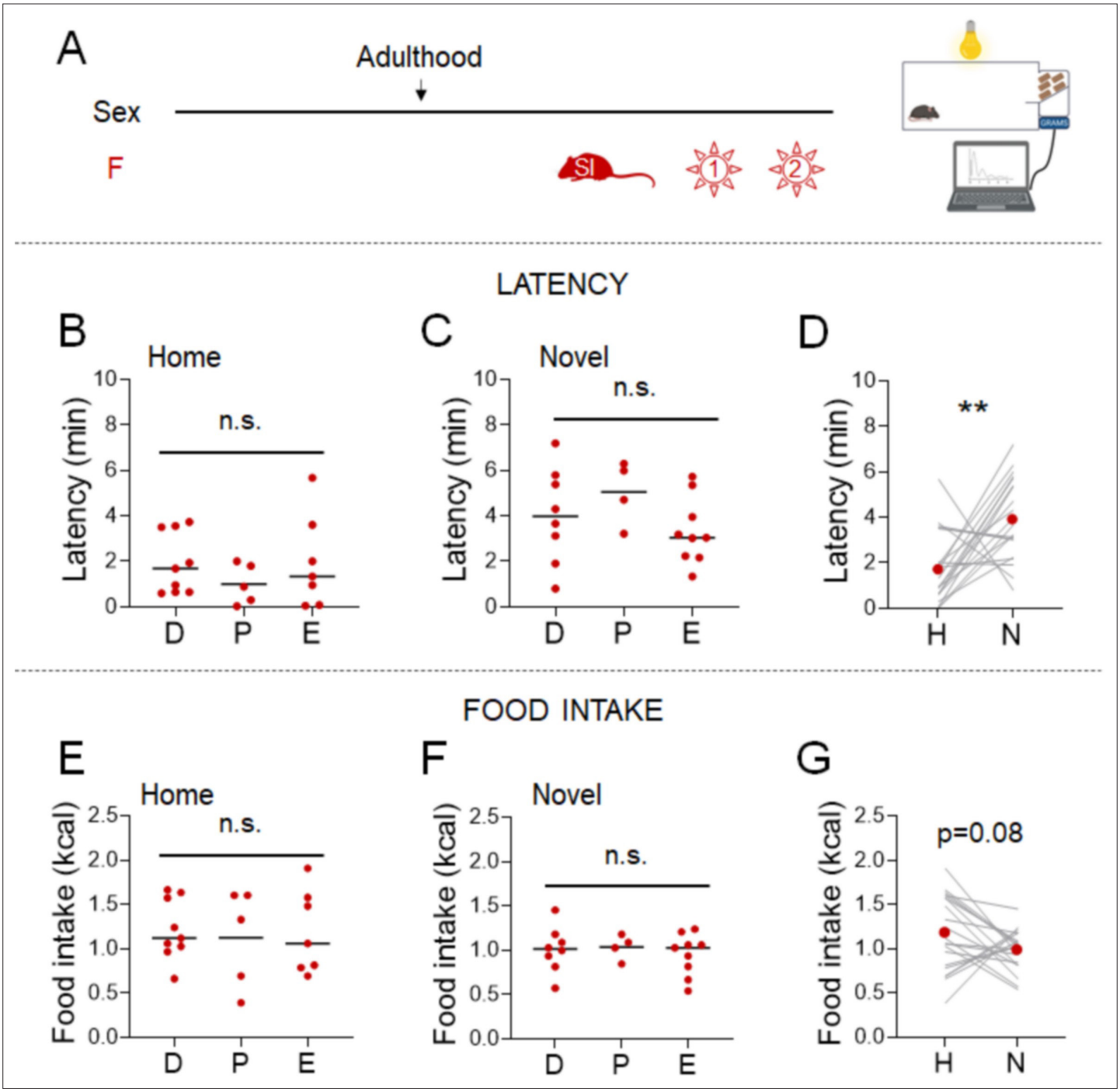

Figure 2. Effects of minimal environmental stress on the novelty-suppressed feeding (NSF) assay in females. (**A**) Experimental paradigm for the automated NSF assay performed in the automated recording system, in the morning following an overnight fast, in adult female mice socially isolated 2 weeks before the tests (n = 21). (**B**) Latency to eat in the home test in females categorized by estrous cycle stage (D: diestrus; P: proestrus; E: estrus) (Kruskal–Wallis test: $H_{(2)}$ = 1.269; p = 0.5479). (**C**) Latency to eat in the novel test in females categorized by estrous cycle stage (one-way analysis of variance [ANOVA]: $F_{(2, 18)}$ = 1.385; p = 0.2757). (**D**) Latency to eat in home (H) and novel (N) tests (Wilcoxon test: W = 151; p = 0.0071). (**E**) Food intake in the home test in females categorized by estrous cycle stage (one-way ANOVA: $F_{(2, 18)}$ = 0.0701; p = 0.9326). (**F**) Food intake in the novel test in females categorized by estrous cycle stage (one-way ANOVA: $F_{(2, 18)}$ = 0.2336; p = 0.7941). (**G**) Food intake in home (H) and novel (N) tests (paired t-test: t = 1.810; df = 20; p = 0.0854). **p < 0.01 between home and novel tests. SI, social isolation; n.s., not significant. See ***Figure 2—source data 1***.

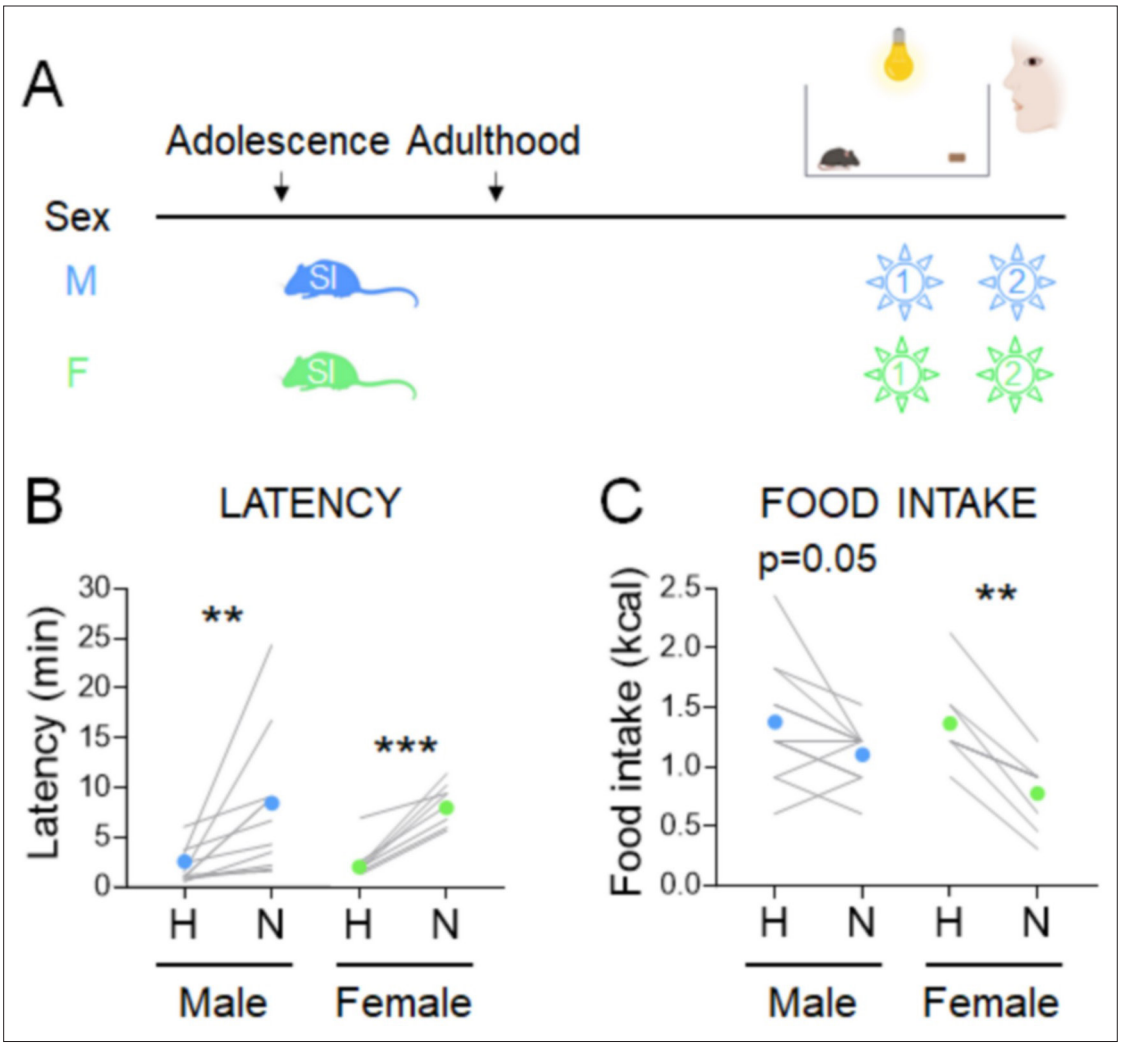

Figure 3. Effects of adolescent social isolation on the novelty-suppressed feeding (NSF) assay in males and females. (**A**) Experimental paradigm for the NSF assay performed in the morning following an overnight fast, in adult mice socially isolated at 5 weeks of age. (**B**) Latency to eat in home (H) and novel (N) tests in males (blue, $n = 11$, Wilcoxon test: $W = 66$; $p = 0.001$) and in females (green, $n = 8$, Wilcoxon test: $W = 36$; $p = 0.0078$). (**C**) Food intake in home (H) and novel (N) tests in males (blue, $n = 11$, paired t-test: $t = 2.193$; df = 10; $p = 0.0531$) and in females (green, $n = 8$, paired t-test: $t = 6.347$; df = 7; $p = 0.0004$). **$p < 0.01$, ***$p < 0.001$ between home and novel tests. SI, social isolation. See ***Figure 3—source data 1***.

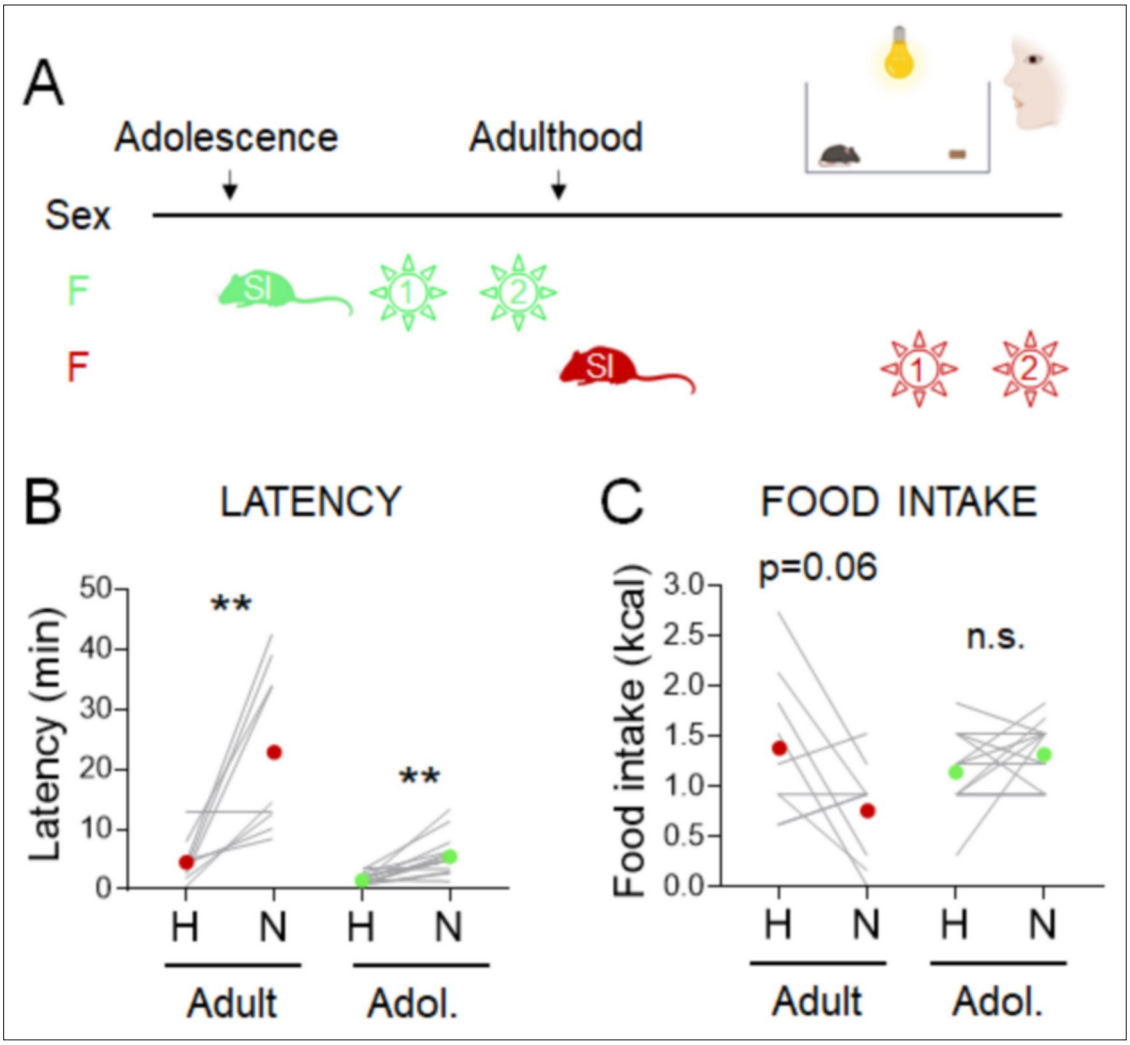

Figure 3—figure supplement 1. Parsing the effects of length vs. timing of social isolation on the standard novelty suppressed feeding (NSF) assay in females.

(**A**) Experimental paradigm for the manual novelty-suppressed feeding (NSF) assay in adolescent and adult females. (**B**) Latency to eat in home (H) and novel (N) tests in adult females socially isolated from 8 weeks of age for 7 weeks (red, $n = 9$, Wilcoxon test: $W = 43$; $p = 0.0078$) and in 7-week-old female mice socially isolated at 5 weeks of age (green, $n = 13$, Wilcoxon test: $W = 85$; $p = 0.0012$). (**C**) Food intake in home (H) and novel (N) tests in adult females socially isolated at 8 weeks of age for 7 weeks (red, $n = 9$, paired t-test: $t = 2.211$; df = 8; $p = 0.058$) and in 7-week-old female mice socially isolated at 5 weeks of age (green, $n = 13$, Wilcoxon test: $W = 18$; $p = 0.2656$). **$p < 0.01$. SI, social isolation; n.s., not significant. See ***Figure 3—source data 2***.

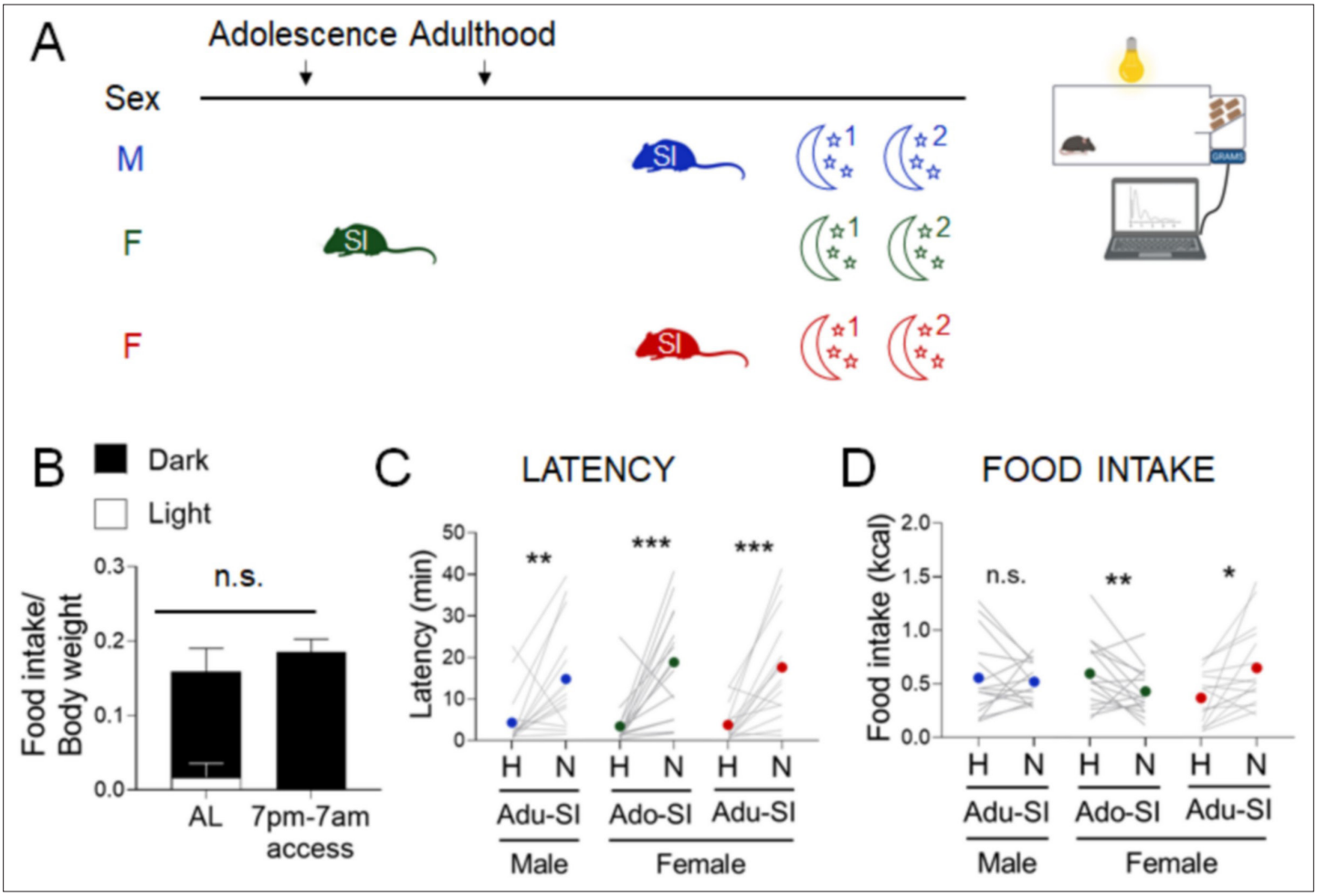

Figure 4. Effects of diurnal factors on the novelty-suppressed feeding (NSF) assay in males and females. (**A**) Experimental paradigm for the automated NSF assays performed at the onset of the dark phase in adult mice. (**B**) Food intake per g body weight across the light cycle (white bar) and dark cycle (black bar) of female mice fed ad libitum (AL) or on a 7 pm to 7 am schedule (n = 7, paired t-test: t = 1.777; df = 6; p = 1259). (**C**) Latency to eat in home (H) and novel (N) tests in males socially isolated 2 weeks before the tests (blue, n = 15, Wilcoxon test: W = 92; p = 0.0067), females socially isolated during adolescence (green, n = 16, Wilcoxon test: W = 120; p = 0.0008), and females socially isolated 2 weeks before the tests (red, n = 12, Wilcoxon test: W = 126; p = 0.0003). (**D**) Food intake in home (H) and novel (N) tests in males socially isolated 2 weeks before the tests (blue, n = 15, Wilcoxon test: W = −4.0; p = 0.9229), females socially isolated during adolescence (green, n = 16, paired t-test: t = 2.166; df = 15; p = 0.0468), and females socially isolated 2 weeks before the test (red, n = 12, Wilcoxon test: W = 106; p = 0.0038). *p < 0.05, **p < 0.01, ***p < 0.001 between home and novel tests. SI, social isolation; n.s., not significant. See ***Figure 4—source data 1***.

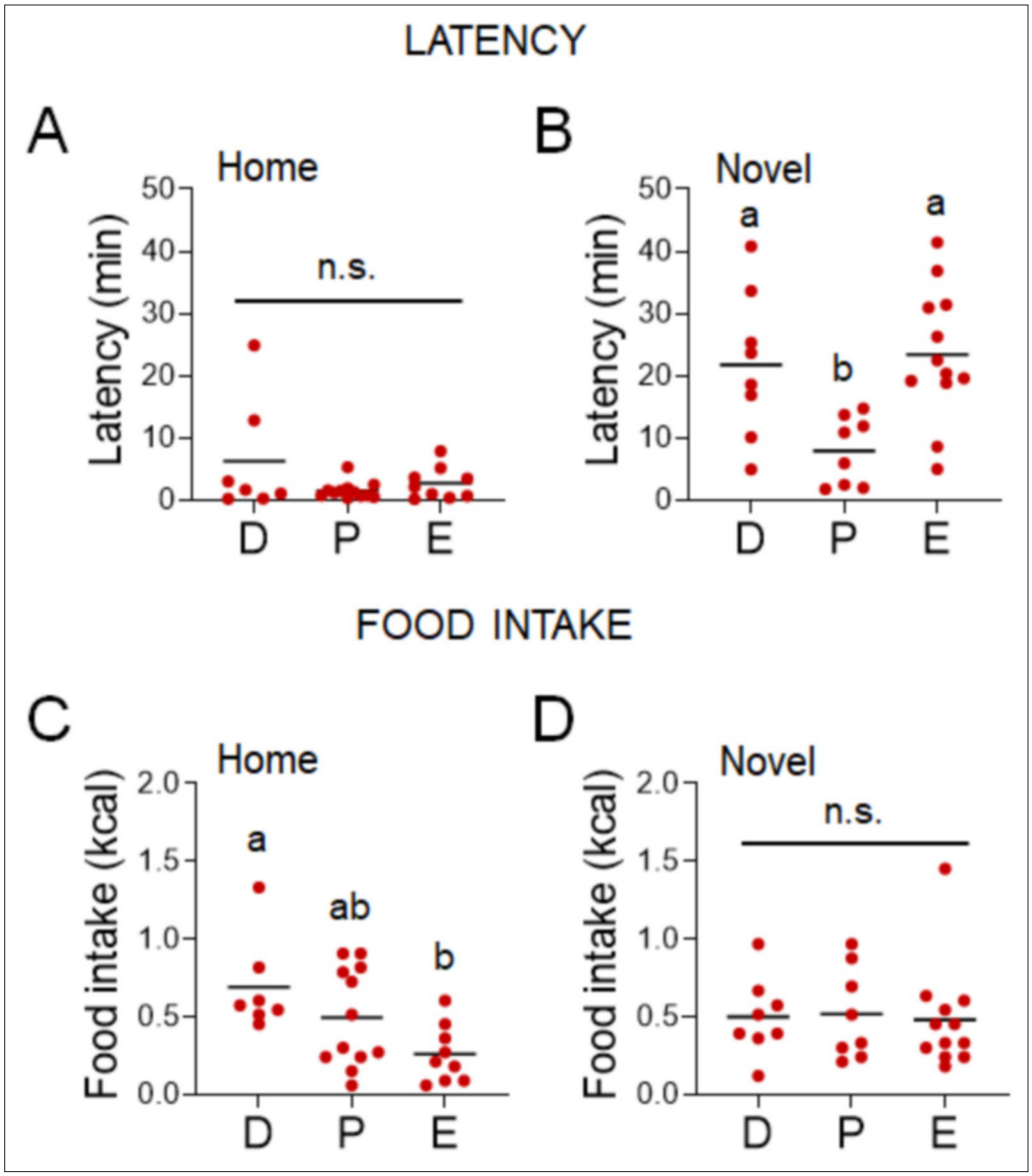

Figure 4—figure supplement 1. Influences of the estrous cycle when the stress assay is performed in the dark phase of the diurnal cycle.

(**A**) Home test latency across the estrous cycle (D: diestrus; P: proestrus; E: estrus) when the assay is performed at the onset of the dark phase (n = 28, Kruskal–Wallis test: $H_{(2)}$= 0.5602; p = 0.7557). (**B**) Novel test latency across the estrous cycle when the assay is performed at the onset of the dark phase (n = 28, one-way analysis of variance [ANOVA]: $F_{(2, 25)}$ = 1.197; p = 0.0053). (**C**) Home test latency across the estrous cycle when the assay is performed at the onset of the dark phase (n = 28, Kruskal–Wallis test: $H_{(2)}$ = 7.649; p = 0.0218). (**D**) Novel test latency across the estrous cycle when the assay is performed at the onset of the dark phase (n = 28, Kruskal–Wallis test: $H_{(2)}$ = 0.4612; p = 0.7941). Significant differences denoted by different letters. SI, social isolation; n.s., not significant. See ***Figure 4—source data 1***.

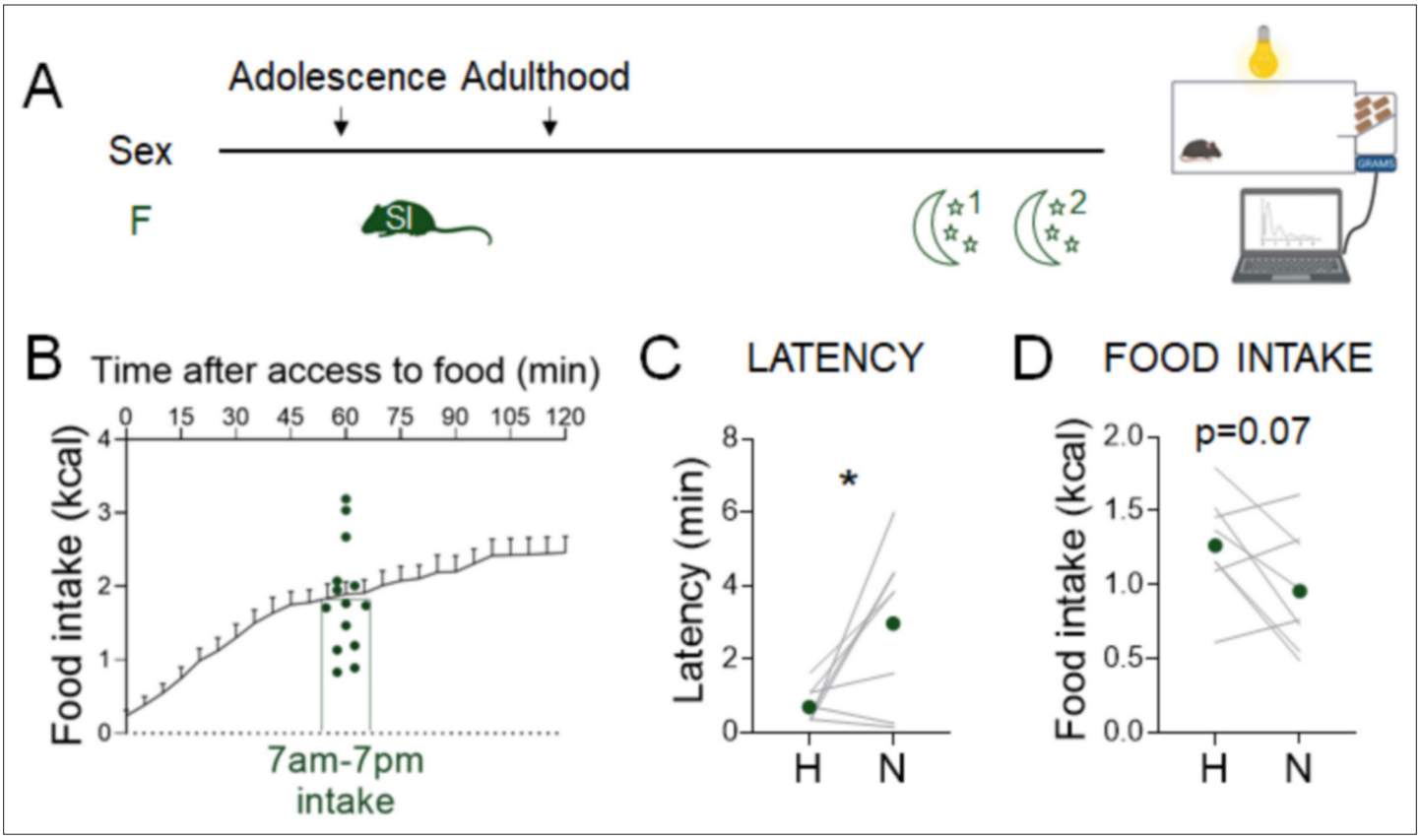

Figure 4—figure supplement 2 Parsing the effects of the time of day vs. prandial state on the effects of novel environment stress on feeding behavior. (**A**) Experimental paradigm for the automated novelty-suppressed feeding (NSF) assays performed at the onset of the dark phase following 90% calorie restriction in adult females (n = 8). (**B**) Representation of how 7 am to 7 pm average intake preceding an overnight fast was matched on a 7 pm to 7 am food access schedule. (**C**) Latency to eat in home (H) and novel (N) tests (paired t-test: t = 2.932, df = 7; p = 0.022). (**D**) Food intake in home (H) and novel (N) tests (paired t-test: t = 2.104, df = 7; p = 0.0734). *p < 0.05. SI, social isolation; n.s., not significant. See ***Figure 4—source data 2***.

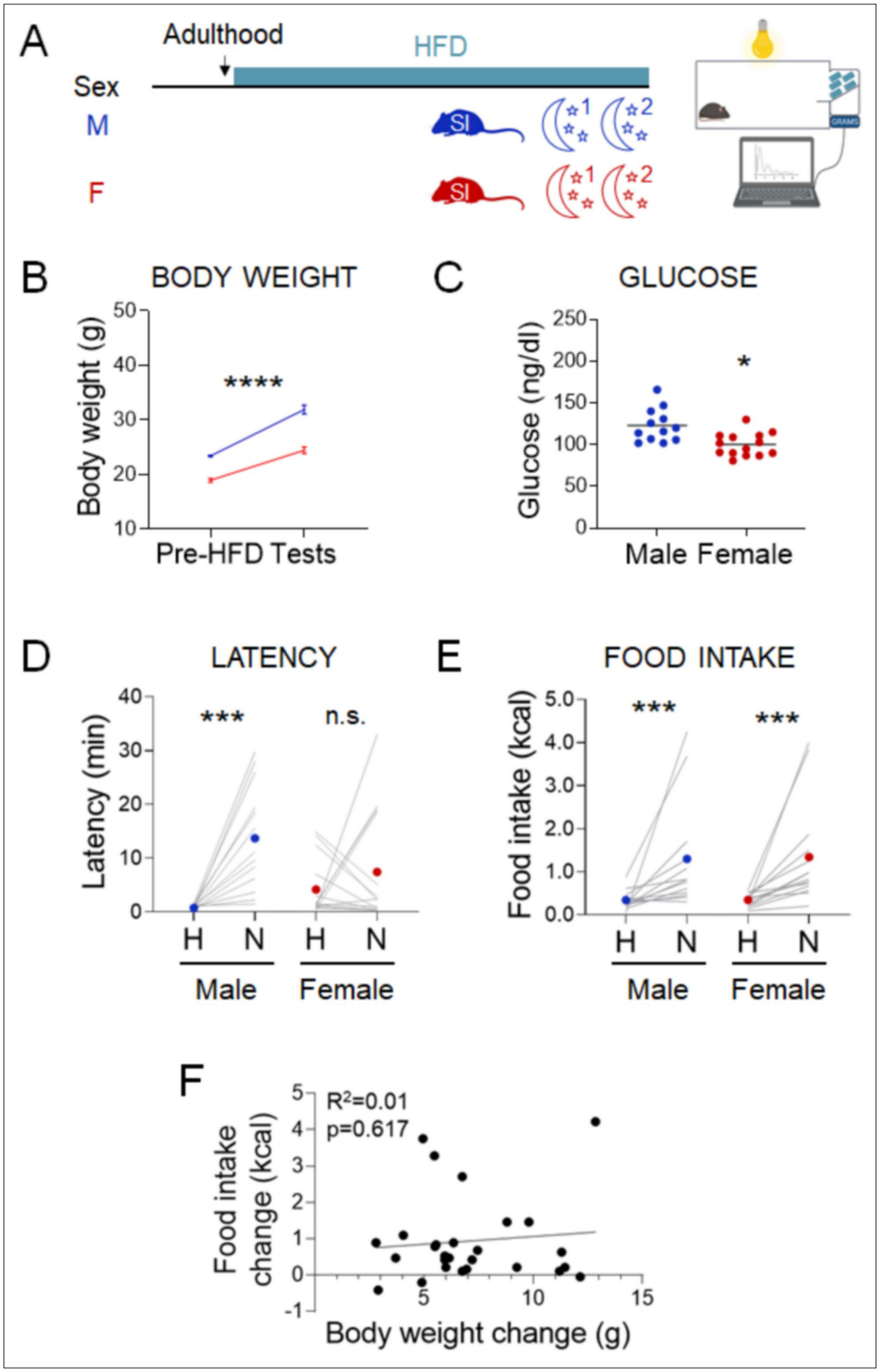

Figure 5. Effects of chronic exposure to high-fat diet (HFD) on the novelty-suppressed feeding (NSF) assay in males and females. (**A**) Experimental paradigm for the automated NSF assay performed at the onset of the dark phase in adult chronically exposed to HFD mice socially isolated 2 weeks before the tests. (**B**) Body weights before exposure to HFD and at the time of the tests, in males (blue, n = 13, paired t-test: t = 11.25; df = 12; p <

Figure 5 continued on next page

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0.0001) and females (red, $n = 14$, paired t-test: $t = 13.53$; df = 13; $p < 0.0001$). (**C**) Blood glucose levels in males (blue, $n = 13$) and females (red, $n = 14$, unpaired t-test: $t = 3.460$; df = 24; $p = 0.002$). (**D**) Latency to eat in home (H) and novel (N) tests in adult males (blue, $n = 13$, paired t-test: $t = 4.751$; df = 12; $p = 0.0005$) and females (red, $n = 14$, Wilcoxon test: $W = 5$; $p = 0.9032$). (**E**) Food intake in home (H) and novel (N) tests in adult males (blue, $n = 13$, Wilcoxon test: $W = 89$; $p = 0.0005$) and in adult females (red, $n = 14$, Wilcoxon test: $W = 105$; $p = 0.0001$). (**F**) Correlation between change in food intake in N vs. H and change in body weight before and after chronic exposure to HFD. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. SI, social isolation; n.s., not significant. See ***Figure 5—source data 1***.

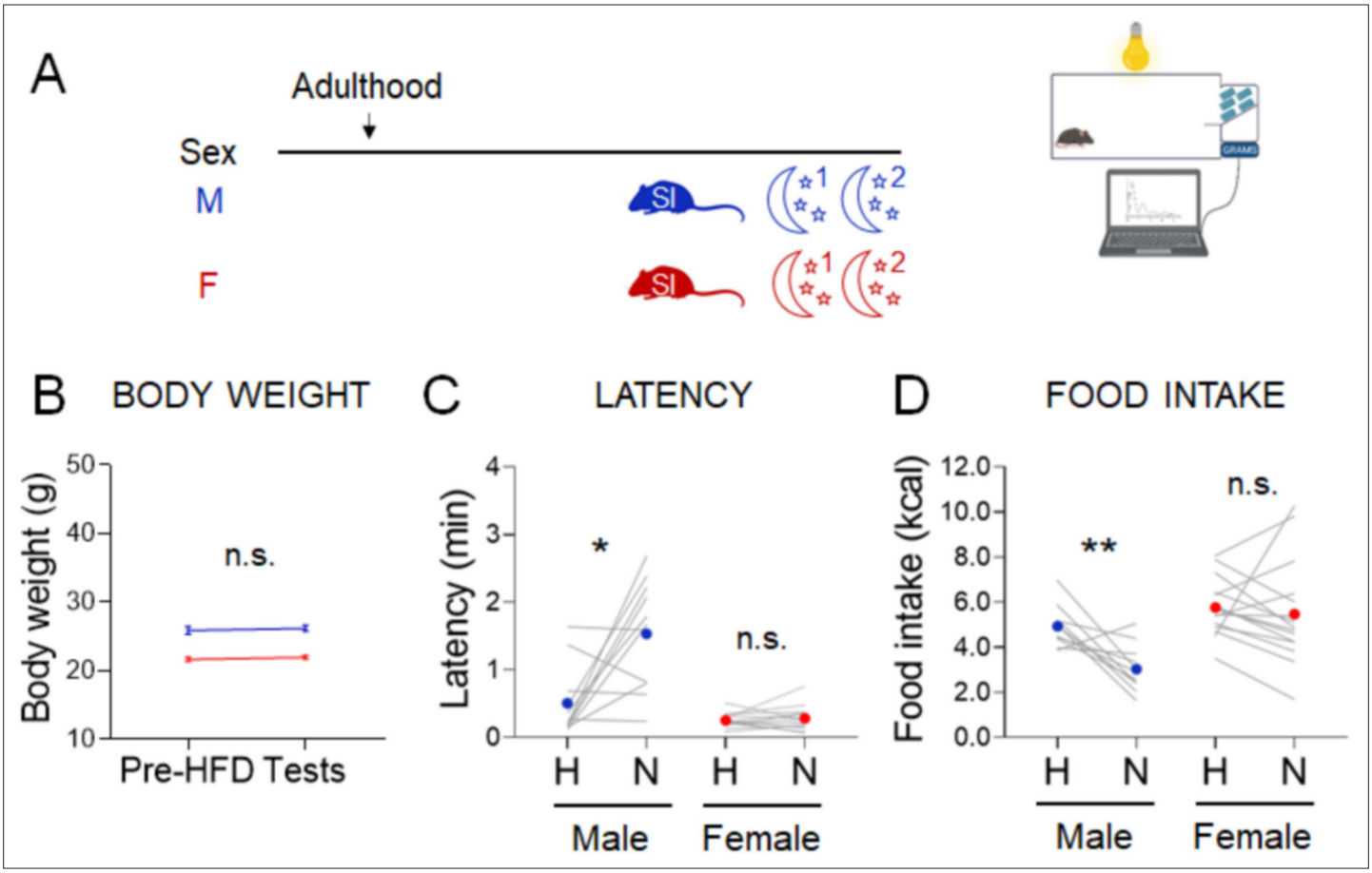

Figure 5—figure supplement 1. Effect of novel envrionment stress assessed in the dark phase of the light cycle in lean mice acutely exposed to high-fat diet (HFD).

(**A**) Experimental paradigm for the automated novelty-suppressed feeding (NSF) assay performed at the onset of the dark phase in lean adult socially isolated 2 weeks before the tests, when high-fat diet (HFD) was presented during the test. (**B**) Body weights before acclimation to HFD and at the time of the tests, in males (blue, $n = 11$, paired t-test: $t = 1.266$; df = 10; $p = 0.2343$) and females (red, $n = 15$, paired t-test: $t = 1.389$; df = 15; $p = 0.1865$). (**C**) Latency to eat in home (H) and novel (N) tests in males (blue, $n = 11$, Wilcoxon test: $W = 46$; $p = 0.04$) and females (red, $n = 15$, paired t-test: $t = 0.6366$; df = 14; $p = 0.5347$). (**D**) Food intake in home (H) and novel (N) tests in adult males (blue, $n = 11$, paired t-test: $t = 3.956$; df = 10; $p = 0.0027$) and in adult females (red, $n = 15$, paired t-test: $t = 0.5385$; df = 14; $p = 0.5987$). *$p < 0.05$; **$p < 0.01$. SI, social isolation; n.s., not significant. See ***Figure 5—source data 2***.

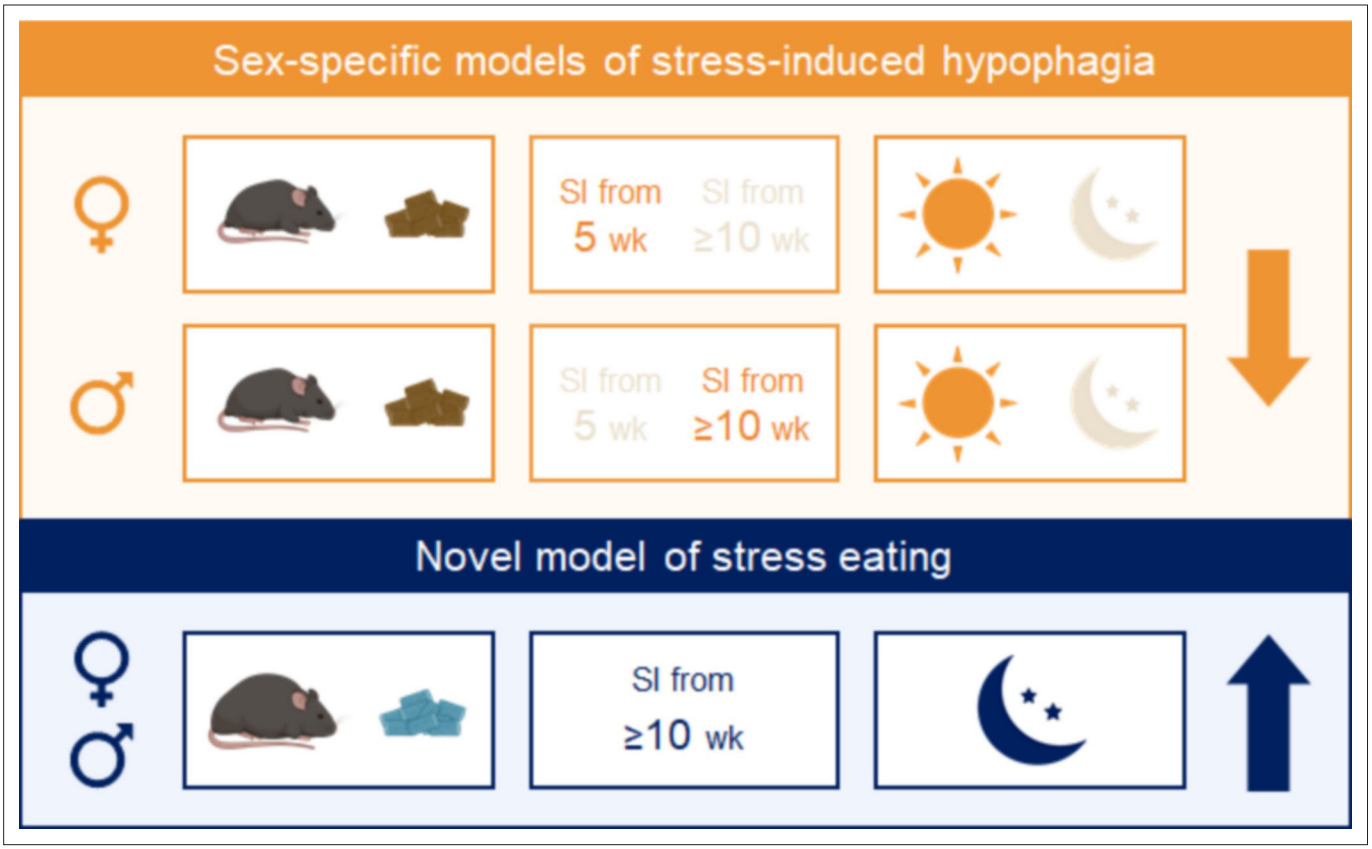

Figure 6. Summary. Combining experimental variables that influence stress-induced food intake in the same direction yields consistent and reproducible effects in males and females. Sex-specific models of stress-induced hypophagia (top panel). The lean state and performing the assay in the light phase promote hypophagic responses. A short period of social isolation in adult (≥10 weeks) males elicits hypophagic responses, but a prolonged (~6 weeks) period of social isolation starting in adolescence (5 weeks) is needed to produce the same effect in females. Sex-independent model of stress-induced hyperphagia (bottom panel). Chronic exposure to high fat diet and performing the assay in the dark phase promote hyperphagic responses in both sexes.