**SUPPLEMENTARY MATERIAL**

**Sex and seasonal differences in neural steroid sensitivity predict territorial aggression in Siberian hamsters**

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**MATERIALS AND METHODS**

***Seasonal phenotypes***

Body mass was measured weekly throughout the study, and reproductive tissues (paired testes for males, uterine horns and ovaries for females) were collected and weighed at the end of the study. Female reproductive tissue mass is reported as reproductive mass, which is the total mass of the uterine horns and ovaries (in g). Body and reproductive tissue mass were the primary criteria used to classify hamsters as responsive or non-responsive to photoperiodic treatment. For all hamsters in the LD and SD groups, each of the two variables used for classification agreed. LD hamsters had functional reproductive tissues (i.e., males had a paired testes mass between 0.500-0.900 g, females had a reproductive mass between 0.060-0.200 g) and exhibited no significant change in body mass. Conversely, LD-M and SD hamsters that were responsive to photoperiodic treatment had regressed reproductive tissues [i.e., had a paired testes mass (for males) or reproductive mass (for females) that was > 2 standard deviations less than the average reproductive tissue mass of LD hamsters) and displayed a significant reduction in body mass [≥ 5%; percent change in body mass (means ± SEM) – LD-M males: -6.20 ± 1.77%, SD males: -11.6 ± 1.45%, LD-M females: -12.3 ± 6.74%, SD females: -10.1 ± 2.05%].

***Behavioral Testing***

Staged dyads were formed for behavioral testing, which consisted of an experimental (i.e., resident) animal and a stimulus animal (i.e., intruder) of approximately the same age and body mass (± 5%) and with different parents from the experimental animal with which they were paired. The intruder was placed into the resident’s home cage, which had not been changed for 7 d prior to behavioral testing to allow the experimental (resident) animal to establish its territory (Brain, 1975; Brain and Poole, 1974). All trials were performed under low red-light illumination, and intruders had small, shaved patches on their dorsa for the purpose of identification. Each intruder was used no more than twice per testing period (e.g., group of behavior trials conducted in a single day, which was approximately 2-3 h in duration and consisted of 13-15 trials). If an intruder was used twice during a single testing period (*n* = 6 occurrences), the behavior trials for which this hamster was used were separated by ≥ 1.5 h. Hamsters used as intruders (males: *n* = 14, females: *n* = 14) were housed in pairs with a same-sex sibling and maintained in LDs prior to behavioral testing and throughout the study.

Behavioral interactions were video recorded and scored for measures of aggression, investigation, and grooming. Six hamsters (males – LD: *n* = 1, LD-M: *n* = 1; females – LD: *n* = 3, LD-M: *n* = 1) did not display attacking behavior during the testing period and were assigned an attack latency of 300 s for the purpose of statistical analysis. For the separate principal component analyses (PCAs) used to generate a composite ‘aggression score’ (PCAgg) and a composite ‘investigation score’ (PCInv) for each sex, the set of variables included in each PCA was evaluated for suitability of factor analysis with the Kaiser-Meyer-Olkin measure of sampling adequacy and Bartlett's test of sphericity (Williams et al., 2010) using the *KMO* and *cortest.bartlett* functions of the *psych* package in R version 4.2.1 (Revelle, 2019; R Core Team, 2022). The data that comprised each PCA had a Kaiser-Meyer-Olkin measure of sampling adequacy > 0.500 and a significant *p*-value (*p* < 0.05) for Bartlett’s test of sphericity and, thus, were considered appropriate for factor analysis (males – PCAgg: Kaiser-Meyer-Olkin measure of sampling adequacy = 0.670, Bartlett’s test of sphericity: χ2 = 82.185, d.f. = 10, *p* < 0.001; males – PCInv: Kaiser-Meyer-Olkin measure of sampling adequacy = 0.540, Bartlett’s test of sphericity: χ2 = 56.703, d.f. = 6, *p* < 0.001; females – PCAgg: Kaiser-Meyer-Olkin measure of sampling adequacy = 0.690, Bartlett’s test of sphericity: χ2 = 117.699, d.f. = 10, *p* < 0.001; females – PCInv: Kaiser-Meyer-Olkin measure of sampling adequacy = 0.520, Bartlett’s test of sphericity: χ2 = 83.813, d.f. = 6, *p* < 0.001; Dziuban and Shirkey, 1974; Kaiser, 1974). PCAs were conducted using the *prcomp* function of the *stats* package (R Core Team, 2022), and data were standardized using Z-scores to account for differences in scaling between variables (Jolliffe and Cadima, 2016).

***Statistical analyses***

Statistical outliers were examined with Grubbs’ Tests using the *grubbs.test* function of the *outliers* package (Komsta, 2011), and data points that affected the conceptual conclusions of the study were excluded from statistical analysis (stated in the footnotes of the statistical analysis summary tables in the Supplementary Material). For multivariate analyses, normality was assessed with Royston’s H tests using the *mvn* function of the *MVN* package (Korkmaz et al., 2014), and homogeneity of covariance matrices was assessed with Box’s M tests using the *boxM* function of the *heplots* package (Fox et al., 2021). For univariate analyses, normality of linear model residuals was assessed with Shapiro-Wilk tests using the *shapiro.test* function of the *stats* package (Team, 2022), and homogeneity of variances was assessed with Levene’s tests using the *leveneTest* function of the *car* package (Fox and Weisberg, 2019). qPCR data were log2-transformed prior to statistical analysis. Data that exhibited a non-normal distribution were visualized with Cullen and Frey plots using the *descdist* function of the *fitdistrplus* package, and the best-fit model was selected on the basis of Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values generated using the *gofstat* function of the *fitdistrplus* package (Delignette-Muller and Dutang, 2015).

Data were analyzed using the *adonis2* function of the *vegan* package (PERMANOVAs; Oksanen et al., 2019), the *manova*, *glm*, and *lm* functions of the *stats* package (MANOVAs, GLMs with a Poisson distribution, and ANOVAs, respectively; R Core Team, 2022), the *glm.nb* function of the *MASS* package (GLMs with a negative binomial distribution; Venables and Ripley, 2002), the *glht* function of the *multcomp* package (Tukey’s HSD post-hoc tests; Hothorn et al., 2008), the *dunn.test* function of the *dunn.test* package (Dunn’s post-hoc tests; Dinno, 2017), the *corr.test* and *cor.mtest* functions the *psych* package (Spearman’s rank correlations; Revelle, 2019), the *corrplot* function of the *corrplot* package (correlation plots; Wei and Simko, 2017), the *summ* function of the *jtools* package (Wald χ2 and Nagelkerke's pseudo-*R*2 values for GLMs with a Poisson distribution; Long, 2020), the *poisgof* function of the *epiDisplay* package and the *RsqGLM* function of the *modEvA* package (Wald χ2 and Nagelkerke's pseudo-*R*2 values for GLMs with a negative binomial distribution; Barbosa et al., 2013; Chongsuvivatwong, 2022), and the *etasq* function of the *heplots* package (partial η2 values for MANOVAs and ANOVAs; Fox et al., 2021).

**TABLES**

**Table S1.** Principal component loading values and eigenvalues for variables that were used to generate composite aggression and investigation scores via principal component analysis.

|  |  |
| --- | --- |
| **Males** | **Females** |
| **Variables** | **PCAgg** | **Variables** | **PCInv** | **Variables** | **PCAgg** | **Variables** | **PCInv** |
| Number of Attacks | **0.528** | NTN Frequency | **0.535** | Number of Attacks | **0.503** | NTN Frequency | **0.522** |
| Attack Duration | **0.411** | NTN Duration | **0.413** | Attack Duration | **0.439** | NTN Duration | **0.465** |
| Number of Chases | **0.505** | AGI Frequency | **0.535** | Number of Chases | **0.492** | AGI Frequency | **0.535** |
| Chase Duration | **0.447** | AGI Duration | **0.507** | Chase Duration | **0.429** | AGI Duration | **0.475** |
| Latency to First Attack | **-0.313** |  |  | Latency to First Attack | **-0.359** |  |  |
| **Eigenvalues** | 3.048 |  | 2.519 | **Eigenvalues** | 3.448 |  | 2.710 |

Composite aggression and investigation scores (PCAgg and PCInv, respectively) were used to determine the effects of melatonin and photoperiodic treatment on aggressive and investigative social behaviors in male and female hamsters. For male hamsters, PCAgg accounted for 61.0% of the total variance, and PCInv accounted for 63.0% of the total variance. For female hamsters, PCAgg accounted for 69.0% of the total variance, and PCInv accounted for 67.8% of the total variance. **Boldface** indicates variables that loaded strongly (< -0.3 or > 0.3) onto a given principal component. *Abbreviations: AGI, anogenital investigation; NTN, nose-to-nose investigation.*

**Table S2.** Primers and quantitative polymerase chain reaction (qPCR) parameters used tomeasure aromatase, estrogen receptor 1, and steroid 5α-reductase type 3 expression in the brain of male and female hamsters.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Direction** | **Sequence (5’-3’)** | **Amplicon Size (bp)** | **Ta (°C)** | **Tm (°C)** | **Efficiency** |
| **Steroid-related genes** |  |  |  |  |  |  |
| *cyp19a1* | Forward | TTTCAGCCATTTGGCTTTGG | 112 | 62 | 80 | 99.38 |
|  | Reverse | GCAACGTCTTCACATGGAATC |  |  |  |  |
| *esr1* | Forward | GGCATGGAGCATCTCTACAA | 81 | 62 | 77 | 103.57 |
|  | Reverse | GTGAGCGTCCAGCATCTC |  |  |  |  |
| *srd5a3* | Forward | TGTTTCGGGCTTGTCTACTAT | 109 | 62 | 79 | 91.96 |
|  | Reverse | AGCTTGTGCCAGCAGATT |  |  |  |  |
| **Reference genes** |  |  |  |  |  |  |
| *18srrna* | Forward | GCTCCTCTCCTACTTGGATAACTGTG | 111 | 62 | 80 | 102.59 |
|  | Reverse | CGGGTTGGTTTTGATCTGATAAATGCA |  |  |  |  |
| *hrpt* | Forward | AGTCCCAGCGTCGTGATTAGTGATG | 141 | 62 | 76 | 90.52 |
|  | Reverse | CGAGCAAGTCTTTCAGTCCTGTCCA |  |  |  |  |

Abbreviations: *18srrna*, 18s ribosomal RNA; *cyp19a1*, aromatase; *esr1*, estrogen receptor 1; *hrpt*, hypoxanthine-guanine phosphoribosyltransferase; *srd5a3,* steroid 5α-reductase type 3; Ta, annealing temperature; Tm, melting temperature.

**Table S3.** Summary of multivariate statistical analyses performed to examine the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on social behavior in male and female hamsters.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Statistical Test** | **Dependent Variables** | **Factors** | ***F*** | **df** | ***R*2** | ***p*** | ***\**** |
| **Aggression** | PERMANOVA | Number of Attacks | Treatment | 9.656 | 1,57 | 0.192 | **0.002** | \*\* |
|  |  | Attack Duration | Sex | 3.244 | 1,57 | 0.054 | 0.120 | NS |
|  |  | Latency to First Attack | Treatment\*Sex | 6.645 | 2,56 | 0.105 | ***0.082*** | \*\* |
|  |  | Number of Chases |  |  |  |  |  |  |
|  |  | Chase Duration |  |  |  |  |  |  |
|  |  | PCAgg |  |  |  |  |  |  |
| **Investigation** | PERMANOVA | NTN Frequency | Treatment | 0.845 | 1,57 | 0.015 | 0.417 | NS |
|  |  | NTN Duration | Sex | 0.870 | 1,57 | 0.015 | 0.420 | NS |
|  |  | AGI Frequency | Treatment\*Sex | 0.831 | 2,56 | 0.029 | 0.500 | NS |
|  |  | AGI Duration |  |  |  |  |  |  |
|  |  | PCInv |  |  |  |  |  |  |
| **Grooming** | PERMANOVA | GR Frequency | Treatment | 0.222 | 1,57 | 0.004 | 0.661 | NS |
|  |  | GR Duration | Sex | 2.432 | 1,57 | 0.040 | 0.181 | NS |
|  |  |  | Treatment\*Sex | 1.717 | 2,56 | 0.058 | 0.170 | NS |

Permutational multivariate analyses of variance (PERMANOVAs) were used to assess the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on aggressive, investigative, and self-grooming behaviors in male and female hamsters (males – LD: *n* = 8, LD-M: *n* = 8, SD: *n* = 12; females – LD: *n* = 10, LD-M: *n* = 8-9, SD: *n* = 12). *F*-statistics (*F*), degrees of freedom (df), estimations of effect size (*R*2), *p*-values (*p*), and statistical significance (\*) for each analysis are shown. For tests that either showed a significant effect (*p* < 0.05, in **bold**) or trended towards a significant effect (*p* < 0.10, in **bold** and *italics*) of treatment, sex, and/or the interaction between treatment and sex, univariate analyses of variance [two-way analyses of variance (ANOVAs) or generalized linear models (GLMs)] and post-hoc testing (Tukey’s HSD tests for two-way ANOVAs or Dunn’s tests for GLMs) were conducted to examine pairwise comparisons. *Abbreviations: AGI, anogenital investigation; GR, grooming; NTN, nose-to-nose investigation; PCAgg, composite aggression score; PCInv, composite investigation score.* Symbols: NS (not significant, *p* > 0.10), \*\**p* < 0.01. *Outlier excluded from statistical analysis: one LD-M female for latency to first attack.*

**Table S4.** Summary of univariate statistical analyses performed to examine the effects of melatonin and photoperiodic treatment, sex, and/or the interaction between treatment and sex on aggressive behavior in male and female hamsters.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Statistical Test and Family** | **Coefficients** | **Estimate ± SE** | ***t or z*** | ***p*** | ***\**** |
| **Number of Attacks** | GLM, Poisson | Treatment | -0.672 ± 0.232 | -2.896 | **0.004** | \*\* |
|  | (Log link function) | Sex | 0.261 ± 0.139 | 1.877 | ***0.061*** | # |
|  |  | Treatment\*Sex | 0.097 ± 0.093 | 1.038 | 0.299 | NS |
| **Attack Duration** | GLM, Negative Binomial | Treatment | -0.129 ± 0.507 | -0.254 | 0.799 | NS |
|  | (Log link function) | Sex | -1.771 ± 0.726 | -2.440 | **0.015** | \* |
|  |  | Treatment\*Sex | 0.296 ± 0.319 | 0.929 | 0.353 | NS |
| **Latency to First Attack** | Two-Way ANOVA | Treatment | -13.24 ± 37.36 | -0.354 | **0.006** | \*\* |
|  |  | Sex | 56.79 ± 52.70 | 1.077 | 0.203 | NS |
|  |  | Treatment\*Sex | -14.86 ± 23.22 | -0.640 | 0.281 | NS |
| **Number of Chases** | GLM, Poisson | Treatment | 1.215 ± 0.425 | 2.859 | **0.004** | \*\* |
|  | (Log link function) | Sex | 0.575 ± 0.693 | 0.830 | 0.406 | NS |
|  |  | Treatment\*Sex | -0.359 ± 0.265 | -1.355 | 0.176 | NS |
| **Chase Duration** | GLM, Negative Binomial | Treatment | 1.886 ± 0.800 | 2.359 | **0.018** | \* |
|  | (Log link function) | Sex | 1.207 ± 1.220 | 0.989 | 0.323 | NS |
|  |  | Treatment\*Sex | -0.710 ± 0.490 | -1.448 | 0.148 | NS |
| **PCAgg (Males)** | One-Way ANOVA | Treatment | 0.944 ± 0.359 | 2.632 | **0.014** | \* |
| **PCAgg (Females)** | One-Way ANOVA | Treatment | 0.886 ± 0.369 | 2.402 | **0.023** | \* |

Two-way analyses of variance (ANOVAs), generalized linear models (GLMs), and one-way ANOVAs were used to examine the effects of melatonin and photoperiodic treatment, sex, and/or the interaction between treatment and sex on aggression in male and female hamsters (males – LD: *n* = 8, LD-M: *n* = 8, SD: *n* = 12; females – LD: *n* = 10, LD-M: *n* = 8-9, SD: *n* = 12). Coefficient estimates ± standard error (SE), *t*-statistics (*t*, for two-way ANOVAs), *z*-statistics (*z*, for GLMs), *p*-values (*p*), and statistical significance (\*) for each model are shown. **Boldface** indicates *p* < 0.05, whereas **boldface** and *italics* indicate *p* < 0.10. Test statistics (*F*-statistic for one-way and two-way ANOVAs and Wald χ2 for GLMs) and estimations of effect size (*R*2 values for one-way and two-way ANOVAs and Nagelkerke's pseudo-*R*2 values for GLMs) for each model are provided in the Results section of the main text. *Abbreviations: PCAgg, composite aggression score.* Symbols: NS (not significant, *p* > 0.10), #*p* < 0.10, \**p* < 0.05, \*\**p* < 0.01. *Outlier excluded from statistical analysis: one LD-M female for latency to first attack.*

**Table S5.** Summary of multivariate statistical analyses performed to examine the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on the expression of aromatase (*cyp19a1*), estrogen receptor 1 (*esr1*), and steroid 5α-reductase type 3 (*srd5a3*) in the brain of male and female hamsters.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Statistical Test** | **Dependent Variables** | **Factors** | ***F*** | **df** | **η*2*** | ***p*** | ***\**** |
| **Relative *cyp19a1* Expression** | Two-Way MANOVA | MPOA | Treatment | 3.745 | 4,34 | 0.331 | **0.013** | \* |
|  |  | AH | Sex | 4.544 | 4,34 | 0.348 | **0.005** | \*\* |
|  |  | ARC | Treatment\*Sex | 1.037 | 4,34 | 0.109 | 0.403 | NS |
|  |  | PAG |  |  |  |  |  |  |
| **Relative *esr1* Expression** | Two-Way MANOVA | MPOA | Treatment | 0.961 | 4,41 | 0.087 | 0.439 | NS |
|  |  | AH | Sex | 2.552 | 4,41 | 0.199 | ***0.053*** | # |
|  |  | ARC | Treatment\*Sex | 1.288 | 4,41 | 0.112 | 0.291 | NS |
|  |  | PAG |  |  |  |  |  |  |
| **Relative *srd5a3* Expression** | Two-Way MANOVA | MPOA | Treatment | 0.702 | 4,45 | 0.059 | 0.595 | NS |
|  |  | AH | Sex | 0.192 | 4,45 | 0.017 | 0.941 | NS |
|  |  | ARC | Treatment\*Sex | 2.601 | 4,45 | 0.188 | **0.048** | \* |
|  |  | PAG |  |  |  |  |  |  |

Two-way multivariate analyses of variance (MANOVAs) were used to examine the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on the neural expression of aromatase, estrogen receptor 1, and steroid 5α-reductase type 3 in male and female hamsters (males – LD: *n* = 7-8, LD-M: *n* = 7-8, SD: *n* = 8-12; females – LD: *n* = 8-10, LD-M: *n* = 8-9, SD: *n* = 9-12). *F*-statistics (*F*), degrees of freedom (df), estimations of effect size (partial η2), *p*-values (*p*), and statistical significance (\*) for each analysis are shown. For response variables that either showed a significant effect (*p* < 0.05, in **bold**) or trended towards a significant effect (*p* < 0.10, in **bold** and *italics*) of treatment, sex, and/or the interaction between treatment and sex, Tukey’s HSD post-hoc tests were conducted to examine pairwise comparisons (response variable summaries of each model are presented in ***Table S6*** in the Supplementary Material). *Abbreviations: AH, anterior hypothalamus; ARC, arcuate nucleus;* cyp19a1*, aromatase;* esr1*, estrogen receptor 1; MPOA, medial preoptic area; PAG, periaqueductal gray;* srd5a3*, steroid 5α-reductase type 3.* Symbols: NS (not significant, *p* > 0.10), #*p* < 0.10, \**p* < 0.05, \*\*p < 0.01. *Outliers excluded from statistical analysis: one SD male and one LD female for relative* cyp19a1 *expression in the MPOA, one LD male and one SD female for relative* cyp19a1 *expression in the ARC, and one LD female, one LD-M female, and one SD female for relative* esr1 *expression in the ARC.*

**Table S6.** Response variable summaries of multivariate statistical analyses performed to examine the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on the neural expression of aromatase (*cyp19a1*), estrogen receptor 1 (*esr1*), and steroid 5α-reductase type 3 (*srd5a3*) in male and female hamsters.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Brain Region** | **Statistical Test** | **Factors** | **SS** | **df** | ***F*** | ***p*** | ***\**** |
| **Relative *cyp19a1* Expression** | MPOA | Two-Way MANOVA  | Treatment | 0.002 | 1,37 | 0.001 | 0.976 | NS |
|  |  |  | Sex | 0.933 | 1,37 | 0.358 | 0.553 | NS |
|  |  |  | Treatment\*Sex | 9.978 | 1,37 | 3.832 | ***0.058*** | # |
|  | AH |  | Treatment | 0.587 | 1,37 | 0.221 | 0.641 | NS |
|  |  |  | Sex | 18.14 | 1,37 | 6.839 | **0.013** | \* |
|  |  |  | Treatment\*Sex | 0.442 | 1,37 | 0.167 | 0.685 | NS |
|  | ARC |  | Treatment | 10.86 | 1,37 | 7.327 | **0.010** | \* |
|  |  |  | Sex | 10.98 | 1,37 | 7.408 | **0.010** | \* |
|  |  |  | Treatment\*Sex | 0.318 | 1,37 | 0.214 | 0.646 | NS |
|  | PAG |  | Treatment | 11.30 | 1,37 | 9.629 | **0.004** | \*\* |
|  |  |  | Sex | 1.427 | 1,37 | 1.216 | 0.277 | NS |
|  |  |  | Treatment\*Sex | 0.137 | 1,37 | 0.117 | 0.735 | NS |
| **Relative *esr1* Expression** | MPOA | Two-Way MANOVA | Treatment | 3.130 | 1,44 | 1.633 | 0.208 | NS |
|  |  |  | Sex | 7.308 | 1,44 | 3.813 | ***0.057*** | # |
|  |  |  | Treatment\*Sex | 0.771 | 1,44 | 0.402 | 0.529 | NS |
|  | AH |  | Treatment | 1.745 | 1,44 | 0.819 | 0.371 | NS |
|  |  |  | Sex | 0.205 | 1,44 | 0.096 | 0.758 | NS |
|  |  |  | Treatment\*Sex | 0.356 | 1,44 | 0.167 | 0.685 | NS |
|  | ARC |  | Treatment | 0.110 | 1,44 | 0.274 | 0.603 | NS |
|  |  |  | Sex | 1.838 | 1,44 | 4.564 | **0.038** | \* |
|  |  |  | Treatment\*Sex | 1.646 | 1,44 | 4.086 | **0.049** | \* |
|  | PAG |  | Treatment | 0.082 | 1,44 | 0.027 | 0.871 | NS |
|  |  |  | Sex | 0.105 | 1,44 | 0.034 | 0.855 | NS |
|  |  |  | Treatment\*Sex | 2.022 | 1,44 | 0.653 | 0.424 | NS |
| **Relative *srd5a3* Expression** | MPOA | Two-Way MANOVA | Treatment | 0.212 | 1,48 | 0.073 | 0.788 | NS |
|  |  |  | Sex | 0.494 | 1,48 | 0.170 | 0.682 | NS |
|  |  |  | Treatment\*Sex | 2.504 | 1,48 | 0.862 | 0.358 | NS |
|  | AH |  | Treatment | 1.403 | 1,48 | 0.684 | 0.412 | NS |
|  |  |  | Sex | 0.333 | 1,48 | 0.162 | 0.689 | NS |
|  |  |  | Treatment\*Sex | 1.367 | 1,48 | 3.393 | ***0.059*** | # |
|  | ARC |  | Treatment | 0.712 | 1,48 | 0.670 | 0.417 | NS |
|  |  |  | Sex | 0.284 | 1,48 | 0.267 | 0.608 | NS |
|  |  |  | Treatment\*Sex | 0.046 | 1,48 | 0.043 | 0.837 | NS |
|  | PAG |  | Treatment | 6.818 | 1,48 | 2.547 | 0.117 | NS |
|  |  |  | Sex | 0.687 | 1,48 | 0.257 | 0.615 | NS |
|  |  |  | Treatment\*Sex | 20.68 | 1,48 | 7.726 | **0.008** | \*\* |

**Table S6 (continued)**

Two-way multivariate analyses of variance (MANOVAs) were used to examine the effects of melatonin and photoperiodic treatment, sex, and the interaction between treatment and sex on the expression of aromatase, estrogen receptor 1, and steroid 5α-reductase type 3 in the brain of male and female hamsters (males – LD: *n* = 7-8, LD-M: *n* = 7-8, SD: *n* = 8-12; females – LD: *n* = 8-10, LD-M: *n* = 8-9, SD: *n* = 9-12). Sum of squares (SS), degrees of freedom (df), *F*-statistics (*F*), *p*-values (*p*), and statistical significance (\*) for response variables included in each analysis are shown. **Boldface** indicates *p* < 0.05, whereas **boldface** and *italics* indicate *p* < 0.10. Estimations of effect size (*R*2) for each response variable are provided in the Results section of the main text. *Abbreviations: AH, anterior hypothalamus; ARC, arcuate nucleus;* cyp19a1*, aromatase;* esr1*, estrogen receptor 1; MPOA, medial preoptic area; PAG, periaqueductal gray;* srd5a3*, steroid 5α-reductase type 3.* Symbols: NS (not significant, *p* > 0.10), #*p* < 0.10, \**p* < 0.05, \*\**p* < 0.01. *Outliers excluded from statistical analysis: one SD male and one LD female for relative* cyp19a1 *expression in the MPOA, one LD male and one SD female for relative* cyp19a1 *expression in the ARC, and one LD female, one LD-M female, and one SD female for relative* esr1 *expression in the ARC.*

**Table S7.** Correlations between aggressive behavior and the neural expression of steroid-related genes in male and female hamsters.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Gene and Brain Region** | **Males** | **Females** |
| **LD** | **LD-M** | **SD** | **LD** | **LD-M** | **SD** |
| ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* |
| **Number of Attacks** | *cyp19a1*, MPOA | -0.14 | 0.74 | -0.51 | 0.20 | 0.01 | 0.98 | 0.13 | 0.75 | 0.47 | 0.24 | 0.02 | 0.94 |
| *cyp19a1,* AH | -0.43 | 0.34 | -0.46 | 0.25 | 0.41 | 0.18 | 0.51 | 0.13 | -0.01 | 0.98 | -0.11 | 0.74 |
| *cyp19a1,* ARC | 0.00 | 1.00 | -0.14 | 0.73 | -0.13 | 0.70 | 0.17 | 0.63 | -0.03 | 0.93 | 0.29 | 0.38 |
| *cyp19a1*, PAG | 0.79 | **0.04** | -0.36 | 0.42 | -0.22 | 0.58 | 0.47 | 0.24 | 0.25 | 0.55 | 0.25 | 0.52 |
| *esr1,* MPOA | 0.07 | 0.87 | -0.33 | 0.43 | -0.38 | 0.25 | -0.32 | 0.40 | 0.60 | 0.12 | 0.05 | 0.88 |
| *esr1*, AH | -0.14 | 0.76 | -0.20 | 0.63 | 0.20 | 0.54 | 0.79 | **0.01** | -0.20 | 0.60 | -0.28 | 0.40 |
| *esr1,* ARC | -0.48 | 0.23 | -0.58 | 0.13 | -0.30 | 0.35 | 0.40 | 0.33 | -0.28 | 0.51 | -0.07 | 0.84 |
| *esr1,* PAG | -0.04 | 0.94 | 0.00 | 1.00 | -0.09 | 0.79 | 0.50 | 0.14 | 0.13 | 0.76 | -0.15 | 0.65 |
| *srd5a3*, MPOA | 0.07 | 0.87 | -0.67 | ***0.07*** | -0.62 | **0.04** | -0.39 | 0.30 | 0.28 | 0.51 | 0.26 | 0.41 |
| *srd5a3*, AH | -0.14 | 0.76 | -0.05 | 0.91 | -0.32 | 0.31 | -0.51 | 0.13 | -0.40 | 0.28 | 0.48 | 0.14 |
| *srd5a3,* ARC | 0.19 | 0.65 | -0.16 | 0.71 | -0.26 | 0.42 | 0.46 | 0.19 | -0.03 | 0.93 | 0.13 | 0.68 |
| **Attack Duration** | *cyp19a1,* MPOA | -0.10 | 0.82 | -0.21 | 0.61 | 0.10 | 0.82 | 0.11 | 0.80 | 0.45 | 0.26 | 0.31 | 0.32 |
| *cyp19a1*, AH | -0.32 | 0.48 | -0.60 | 0.12 | 0.50 | 0.10 | 0.53 | 0.11 | 0.55 | 0.12 | -0.53 | 0.10 |
| *cyp19a1,* ARC | 0.21 | 0.64 | -0.24 | 0.57 | -0.23 | 0.47 | 0.13 | 0.72 | 0.17 | 0.67 | 0.14 | 0.69 |
| *cyp19a1*, PAG | 0.29 | 0.53 | -0.79 | **0.04** | -0.10 | 0.80 | 0.47 | 0.24 | 0.71 | ***0.05*** | 0.23 | 0.55 |
| *esr1,* MPOA | 0.10 | 0.82 | 0.02 | 0.96 | -0.38 | 0.25 | -0.34 | 0.37 | 0.48 | 0.23 | 0.40 | 0.20 |
| *esr1,* AH | -0.21 | 0.64 | -0.69 | ***0.06*** | 0.19 | 0.56 | 0.77 | **0.01** | 0.20 | 0.61 | -0.61 | ***0.05*** |
| *esr1,* ARC | 0.02 | 0.96 | -0.31 | 0.46 | -0.28 | 0.38 | 0.40 | 0.33 | -0.19 | 0.65 | 0.14 | 0.69 |
| *esr1,* PAG | -0.71 | ***0.07*** | -0.33 | 0.42 | 0.10 | 0.77 | 0.52 | 0.12 | 0.07 | 0.87 | -0.31 | 0.32 |
| *srd5a3,* MPOA | 0.19 | 0.65 | -0.45 | 0.26 | -0.70 | **0.02** | -0.36 | 0.35 | 0.14 | 0.74 | -0.03 | 0.93 |
| *srd5a3,* AH | -0.39 | 0.38 | 0.36 | 0.39 | -0.41 | 0.18 | -0.55 | 0.10 | 0.00 | 1.00 | 0.02 | 0.96 |
| *srd5a3,* ARC | -0.05 | 0.91 | 0.10 | 0.82 | -0.14 | 0.66 | 0.40 | 0.25 | 0.23 | 0.55 | 0.05 | 0.88 |
| *srd5a3,* PAG | 0.11 | 0.82 | -0.02 | 0.96 | 0.15 | 0.67 | 0.74 | **0.01** | 0.05 | 0.91 | -0.27 | 0.39 |
| **Latency to First Attack** | *cyp19a1*, AH | 0.68 | ***0.09*** | 0.40 | 0.32 | -0.36 | 0.26 | -0.25 | 0.48 | 0.12 | 0.78 | 0.53 | ***0.09*** |
| *cyp19a1*, ARC | 0.39 | 0.38 | 0.24 | 0.57 | 0.36 | 0.25 | -0.23 | 0.53 | 0.02 | 0.87 | 0.02 | 0.96 |
| *cyp19a1*, PAG | -0.54 | 0.22 | 0.57 | 0.18 | -0.28 | 0.46 | -0.61 | 0.11 | -0.04 | 0.94 | -0.17 | 0.67 |
| *esr1*, AH | 0.50 | 0.25 | 0.36 | 0.39 | -0.52 | ***0.08*** | -0.63 | ***0.05*** | 0.64 | ***0.09*** | 0.49 | 0.13 |
| *esr1,* ARC | 0.17 | 0.69 | 0.07 | 0.87 | 0.11 | 0.73 | -0.20 | 0.63 | -0.18 | 0.70 | 0.01 | 0.98 |
| *esr1*, PAG | 0.21 | 0.64 | 0.19 | 0.65 | -0.28 | 0.40 | -0.44 | 0.21 | 0.39 | 0.38 | 0.48 | 0.12 |
| *srd5a3*, MPOA | 0.21 | 0.61 | 0.79 | **0.02** | -0.14 | 0.69 | 0.44 | 0.24 | -0.54 | 0.22 | -0.14 | 0.66 |
| *srd5a3*, AH | 0.21 | 0.64 | -0.02 | 0.96 | 0.55 | ***0.07*** | 0.33 | 0.36 | 0.43 | 0.29 | -0.38 | 0.25 |
| *srd5a3*, ARC | 0.50 | 0.21 | -0.26 | 0.53 | 0.04 | 0.90 | -0.47 | 0.17 | -0.10 | 0.82 | -0.02 | 0.95 |
| *srd5a3,* PAG | -0.39 | 0.38 | -0.10 | 0.82 | -0.13 | 0.71 | -0.57 | ***0.08*** | 0.32 | 0.48 | -0.07 | 0.83 |

**Table S7 (continued)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Gene and Brain Region** | **Males** | **Females** |
| **LD** | **LD-M** | **SD** | **LD** | **LD-M** | **SD** |
| ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* | ρ | *p* |
| **Number of Chases** | *cyp19a1,* MPOA | -0.06 | 0.88 | -0.14 | 0.75 | 0.48 | 0.23 | -0.05 | 0.90 | 0.42 | 0.30 | 0.15 | 0.65 |
| *cyp19a1*, AH | 0.00 | 1.00 | -0.06 | 0.89 | 0.41 | 0.19 | 0.23 | 0.52 | 0.10 | 0.81 | -0.19 | 0.58 |
| *cyp19a1,* ARC | -0.13 | 0.78 | 0.02 | 0.95 | -0.07 | 0.82 | -0.19 | 0.61 | -0.04 | 0.91 | 0.33 | 0.33 |
| *cyp19a1*, PAG | -0.20 | 0.66 | -0.11 | 0.82 | 0.00 | 1.00 | 0.20 | 0.63 | 0.50 | 0.20 | 0.18 | 0.64 |
| *esr1,* MPOA | 0.22 | 0.60 | 0.10 | 0.82 | -0.25 | 0.47 | -0.46 | 0.22 | 0.42 | 0.30 | 0.05 | 0.87 |
| *esr1*, AH | 0.00 | 1.00 | -0.17 | 0.68 | 0.47 | 0.13 | 0.22 | 0.55 | -0.08 | 0.84 | -0.35 | 0.29 |
| *esr1*, ARC | -0.16 | 0.71 | -0.38 | 0.35 | -0.41 | 0.18 | -0.26 | 0.53 | -0.33 | 0.42 | -0.10 | 0.76 |
| *esr1*, PAG | -0.41 | 0.36 | -0.02 | 0.95 | 0.14 | 0.69 | 0.35 | 0.32 | 0.29 | 0.48 | -0.33 | 0.29 |
| *srd5a3*, MPOA | -0.44 | 0.28 | -0.87 | **<0.01** | -0.47 | 0.14 | -0.01 | 0.98 | 0.31 | 0.46 | 0.48 | 0.11 |
| *srd5a3*, AH | -0.20 | 0.66 | -0.09 | 0.84 | -0.02 | 0.95 | -0.47 | 0.17 | -0.14 | 0.72 | 0.20 | 0.56 |
| *srd5a3*, ARC | 0.19 | 0.66 | 0.14 | 0.75 | -0.18 | 0.57 | -0.04 | 0.92 | 0.01 | 0.98 | -0.05 | 0.89 |
| *srd5a3*, PAG | -0.20 | 0.66 | 0.09 | 0.84 | 0.24 | 0.49 | 0.53 | 0.12 | -0.02 | 0.95 | 0.18 | 0.58 |
| **Chase Duration** | *cyp19a1*, MPOA | -0.06 | 0.88 | -0.29 | 0.48 | 0.32 | 0.43 | -0.05 | 0.90 | 0.41 | 0.31 | 0.01 | 0.96 |
| *cyp19a1*, AH | 0.00 | 1.00 | 0.12 | 0.77 | 0.46 | 0.13 | 0.23 | 0.52 | 0.08 | 0.84 | -0.14 | 0.68 |
| *cyp19a1,* ARC | -0.13 | 0.78 | -0.12 | 0.77 | 0.03 | 0.93 | -0.19 | 0.61 | -0.10 | 0.79 | 0.40 | 0.23 |
| *cyp19a1*, PAG | -0.20 | 0.66 | -0.04 | 0.94 | -0.07 | 0.82 | 0.20 | 0.63 | 0.54 | 0.17 | 0.16 | 0.69 |
| *esr1*, MPOA | 0.22 | 0.60 | 0.02 | 0.95 | -0.25 | 0.46 | -0.46 | 0.22 | 0.37 | 0.37 | -0.01 | 0.97 |
| *esr1*, AH | 0.00 | 1.00 | -0.15 | 0.73 | 0.45 | 0.14 | 0.22 | 0.55 | 0.00 | 1.00 | -0.46 | 0.16 |
| *esr1*, ARC | -0.16 | 0.71 | -0.46 | 0.25 | -0.27 | 0.39 | -0.26 | 0.53 | -0.49 | 0.22 | 0.12 | 0.73 |
| *esr1*, PAG | -0.41 | 0.36 | 0.12 | 0.77 | 0.10 | 0.77 | 0.35 | 0.32 | 0.39 | 0.34 | -0.31 | 0.33 |
| *srd5a3*, MPOA | -0.44 | 0.28 | -0.88 | **<0.01** | -0.40 | 0.22 | -0.01 | 0.98 | 0.44 | 0.28 | 0.43 | 0.17 |
| *srd5a3*, AH | -0.20 | 0.66 | -0.02 | 0.95 | -0.01 | 0.97 | -0.47 | 0.17 | -0.06 | 0.88 | 0.07 | 0.83 |
| *srd5a3,* ARC | 0.19 | 0.66 | 0.07 | 0.86 | -0.02 | 0.95 | -0.04 | 0.92 | -0.13 | 0.74 | 0.03 | 0.92 |
| *srd5a3*, PAG | -0.20 | 0.66 | 0.00 | 1.00 | 0.41 | 0.21 | 0.53 | 0.12 | 0.24 | 0.56 | 0.05 | 0.89 |
| **PCAgg** | *cyp19a1*, MPOA | 0.02 | 0.96 | -0.36 | 0.39 | 0.07 | 0.87 | 0.13 | 0.76 | 0.48 | 0.23 | 0.21 | 0.51 |
| *cyp19a1*, AH | -0.43 | 0.34 | -0.24 | 0.57 | 0.54 | ***0.07*** | 0.37 | 0.29 | 0.28 | 0.46 | -0.35 | 0.28 |
| *cyp19a1,* ARC | 0.00 | 1.00 | -0.02 | 0.96 | -0.27 | 0.40 | 0.08 | 0.83 | 0.02 | 0.97 | 0.28 | 0.40 |
| *cyp19a1*, PAG | 0.43 | 0.34 | -0.36 | 0.43 | 0.00 | 1.00 | 0.44 | 0.27 | 0.52 | 0.18 | 0.23 | 0.55 |
| *esr1*, MPOA | 0.19 | 0.65 | -0.19 | 0.65 | -0.39 | 0.23 | -0.37 | 0.32 | 0.57 | 0.14 | 0.19 | 0.56 |
| *esr1*, AH | -0.21 | 0.64 | -0.26 | 0.53 | 0.38 | 0.23 | 0.66 | **0.04** | 0.00 | 1.00 | -0.54 | ***0.09*** |
| *esr1*, PAG | -0.43 | 0.34 | -0.12 | 0.78 | 0.14 | 0.69 | 0.46 | 0.18 | 0.14 | 0.74 | -0.22 | 0.50 |
| *srd5a3*, MPOA | -0.12 | 0.78 | -0.79 | **0.02** | -0.62 | **0.04** | -0.31 | 0.42 | 0.19 | 0.65 | 0.31 | 0.32 |
| *srd5a3*, AH | -0.21 | 0.64 | -0.02 | 0.82 | -0.10 | 0.76 | -0.47 | 0.17 | -0.18 | 0.64 | 0.31 | 0.36 |
| *srd5a3*, ARC | -0.10 | 0.82 | -0.12 | 0.78 | -0.17 | 0.60 | 0.37 | 0.29 | 0.13 | 0.73 | 0.08 | 0.80 |
| *srd5a3,* PAG | 0.32 | 0.48 | 0.02 | 0.96 | 0.34 | 0.31 | 0.67 | **0.03** | -0.24 | 0.57 | -0.20 | 0.53 |

**Table S7 (continued)**

Spearman’s rank correlations between aggressive behavior and aromatase, estrogen receptor 1, and steroid 5α-reductase type 3 expression in the brain of long-day hamsters (LD), LD hamsters administered timed melatonin injections (LD-M), and short-day hamsters (SD). Correlation coefficients (ρ) and *p*-values (p) are shown for each analysis, which was performed within each treatment group and sex (males – LD: *n* = 7-8, LD-M: *n* = 7-8, SD: *n* = 8-12; females – LD: *n* = 8-10, LD-M: *n* = 8-9, SD: *n* = 9-12). **Boldface** indicates *p* < 0.05, whereas **boldface** and *italics* indicate *p* < 0.10. *Abbreviations: AH, anterior hypothalamus; ARC, arcuate nucleus;* cyp19a1, *aromatase;* esr1, *estrogen receptor 1; MPOA, medial preoptic area; PAG, periaqueductal gray;* srd5a3, *steroid 5α-reductase type 3.*

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