**SUPPLEMENTARY MATERIAL**

**Adrenal MT1 melatonin receptor expression is linked with seasonal variation in social behavior in**

**male Siberian hamsters**

Kathleen M. Munley1\*, Sohini Dutta2,3, Aaron M. Jasnow2,4, and Gregory E. Demas1

*1Department of Biology and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405, USA, 2Department of Psychological Sciences, Kent State University, Kent, OH 44240, USA, 3Department of Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA, and 4Department of Pharmacology, Physiology & Neuroscience, University of South Carolina School of Medicine, Columbia, SC, 29209 USA*

**TABLES**

**Table S1.** Primers and quantitative polymerase chain reaction (qPCR) parameters used tomeasure adrenal MT1 receptor expression in male hamsters.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Direction** | **Sequence** | **Amplicon Size (bp)** | **Annealing Temp (°C)** | **Tm (°C)** |
| *18srrna* | Forward | GCTCCTCTCCTACTTGGATAACTGTG | 111 | 63 | 81 |
|  | Reverse | CGGGTTGGTTTTGATCTGATAAATGCA |  |  |  |
| *gapdh* | Forward | TTCTTGTGCAGTGCCAGCCTCG | 207 | 63 | 82 |
|  | Reverse | CTGTGCCGTTGAACTTGCCGTG |  |  |  |
| *mt1* | Forward | CTACACTGGCCTTCATCCTTATC | 102 | 63 | 80 |
|  | Reverse | AAGAAGCTCAGGAACGCAG |  |  |  |

Abbreviations: *18srrna*, 18s ribosomal RNA; *gapdh*, glyceraldehyde 3-phosphate dehydrogenase; *mt1*, MT1 melatonin receptor; Tm, melting temperature.

**Table S2.** Adrenal MT1 receptor overexpression and short days increased aggression, but decreased investigation in male hamsters.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Behavior** | **LD CON** | **SD-R CON** | **SD-NR CON** | **LD MT1** | **LD-M CON** | **LD-M MT1** |
| Attack Duration | 18.24 ± 3.23a,b | 36.48 ± 9.41a | 28.44 ± 7.37a,b | 12.59 ± 5.77**b** | 15.15 ± 2.44**b** | 21.12 ± 5.04a,b |
| Chase Duration | 3.25 ± 1.38a | 5.61 ± 2.27a | 3.10 ± 1.25a | 5.36 ± 2.53a | 1.45 ± 0.68a | 3.12 ± 0.98a |
| Aggression Frequency | 17.55 ± 4.16a,b | 30.40 ± 7.64a | 26.53 ± 7.02a,b | 26.20 ± 8.93a,b | 10.64 ± 2.12**b** | 29.44 ± 6.87a,b |
| NTN Duration | 16.83 ± 4.68a | 10.14 ± 2.32a | 18.30 ± 4.25a | 14.45 ± 4.16a | 23.58 ± 6.18a | 24.13 ± 7.65a |
| Investigation Frequency | 17.73 ± 4.24a | 6.10 ± 1.16**b** | 14.67 ± 2.34a | 9.80 ± 2.42a,b | 17.00 ± 3.20a | 18.00 ± 4.00a |
| Investigation Duration | 46.02 ± 10.43a | 19.53 ± 3.95**b** | 56.46 ± 11.09a | 37.89 ± 10.6a,b | 53.20 ± 12.02a | 55.79 ± 13.18a |

Attack duration, chase duration, aggression frequency, nose-to-nose investigation (NTN) duration, investigation frequency, and investigation duration of long-day hamsters infused with the control (CON) lentivirus (LD CON), short-day hamsters infused with the CON lentivirus that were responsive to photoperiodic treatment (SD-R CON), SD hamsters infused with the CON lentivirus that were non-responsive to photoperiodic treatment (SD-NR CON), LD hamsters infused with the MT1 receptor-expressing (MT1) lentivirus (LD MT1), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON), and LD hamsters infused with the MT1 lentivirus and given timed melatonin injections (LD-M MT1). Data are presented as mean ± SEM (LD CON: *n* = 10-11, SD-R CON: *n* = 10, SD-NR CON: *n* = 15, LD MT1: *n* = 8-10, LD-M CON: *n* = 11-12, LD-M MT1: *n* = 14-16). Different superscript letters indicate a significant difference between treatment groups (*P* < 0.05, Kruskal-Wallis one-way ANOVAs on ranks with Dunn’s post-hoc tests for multiple comparisons). *Outliers excluded from statistical analysis: one LD CON hamster, two LD MT1 hamsters, and two LD-M MT1 hamsters for attack duration and one LD-M CON hamster for aggression frequency.*

**Table S3**. Correlations between adrenal MT1 receptor (*mt1*) expression, aggression, investigation, and grooming in male hamsters.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Behavior** | **LD CON** | | **SD-R CON** | | **SD-NR CON** | | **LD MT1** | | **LD-M CON** | | **LD-M MT1** | |
| ρ | *P* | ρ | *P* | ρ | *P* | ρ | *P* | ρ | *P* | ρ | *P* | |
| Attack Duration | -0.43 | 0.21 | 0.59 | ***0.07*** | 0.15 | 0.58 | -0.14 | 0.76 | -0.10 | 0.76 | -0.41 | 0.14 | |
| Number of Chases | -0.44 | 0.17 | 0.23 | 0.53 | 0.28 | 0.32 | 0.50 | 0.17 | 0.37 | 0.26 | -0.13 | 0.62 | |
| Chase Duration | -0.47 | 0.15 | 0.23 | 0.53 | 0.14 | 0.61 | 0.62 | ***0.08*** | 0.14 | 0.66 | -0.08 | 0.77 | |
| Latency to First Attack | 0.47 | 0.14 | -0.09 | 0.80 | -0.02 | 0.94 | 0.07 | 0.86 | -0.28 | 0.40 | 0.09 | 0.75 | |
| Aggression Frequency | -0.43 | 0.19 | 0.35 | 0.33 | 0.17 | 0.54 | 0.32 | 0.41 | 0.34 | 0.31 | -0.34 | 0.19 | |
| NTN Frequency | 0.73 | **0.01** | 0.07 | 0.84 | -0.22 | 0.43 | 0.48 | 0.19 | -0.52 | ***0.08*** | 0.23 | 0.39 | |
| NTN Duration | 0.64 | **0.04** | 0.39 | 0.26 | -0.15 | 0.59 | 0.52 | 0.15 | -0.58 | ***0.05*** | 0.18 | 0.51 | |
| AGI Frequency | 0.64 | **0.03** | -0.25 | 0.49 | -0.39 | 0.15 | -0.04 | 0.91 | -0.39 | 0.21 | 0.02 | 0.94 | |
| AGI Duration | 0.45 | 0.17 | 0.10 | 0.78 | -0.30 | 0.28 | 0.22 | 0.58 | -0.36 | 0.26 | 0.10 | 0.72 | |
| Investigation Frequency | 0.65 | **0.03** | -0.09 | 0.81 | -0.30 | 0.27 | 0.24 | 0.53 | -0.49 | 0.11 | 0.06 | 0.82 | |
| Investigation Duration | 0.55 | ***0.09*** | 0.39 | 0.26 | -0.26 | 0.35 | 0.32 | 0.41 | -0.49 | 0.11 | 0.12 | 0.66 | |
| PCInv | 0.60 | ***0.06*** | 0.09 | 0.81 | -0.32 | 0.25 | 0.25 | 0.52 | -0.49 | 0.11 | 0.09 | 0.74 | |
| Grooming Frequency | 0.15 | 0.66 | 0.32 | 0.37 | -0.54 | **0.04** | 0.24 | 0.54 | 0.00 | 1.00 | 0.35 | 0.19 | |
| Grooming Duration | 0.16 | 0.63 | 0.10 | 0.81 | -0.30 | 0.28 | 0.18 | 0.65 | -0.26 | 0.42 | 0.36 | 0.17 | |

Spearman’s rank correlations between adrenal *mt1* expression and aggressive, investigative, and self-grooming behaviors in long-day hamsters infused with the control (CON) lentivirus (LD CON), short-day hamsters infused with the CON lentivirus that were responsive to photoperiodic treatment (SD-R CON), SD hamsters infused with the CON lentivirus that were non-responsive to photoperiodic treatment (SD-NR CON), LD hamsters infused with the MT1 receptor-expressing (MT1) lentivirus (LD MT1), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON), and LD hamsters infused with the MT1 lentivirus and given timed melatonin injections (LD-M MT1). Correlation coefficients (ρ) and *P*-values (*P*) are shown for each analysis, which was performed within each treatment group (LD CON: *n* = 10-11, SD-R CON: *n* = 9-10, SD-NR CON: *n* = 13-15, LD MT1: *n* = 7-9, LD-M CON: *n* = 11-12, LD-M MT1: *n* = 14-16). *P*-values for significant correlations (*P* < 0.05) are shown in **bold**, and *P*-values for correlations that trended towards significance (*P* < 0.10) are shown in **bold** and *italics*. *Abbreviations: AGI, anogenital investigation; NTN, nose-to-nose investigation; PCInv, composite investigation score.*

**FIGURES**



**Figure S1. Long-day hamsters infused with the MT1 receptor-expressing lentivirus and given timed melatonin injections exhibited a reduction in circulating DHEA.** Serum dehydroepiandrosterone (DHEA) levels of long-day hamsters infused with the control (CON) lentivirus (LD CON; purple), short-day hamsters infused with the CON lentivirus that were responsive to photoperiodic treatment (SD-R CON; green), SD hamsters infused with the CON lentivirus that were non-responsive to photoperiodic treatment (SD-NR CON; yellow), LD hamsters infused with the MT1 receptor-expressing (MT1) lentivirus (LD MT1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT1 lentivirus and given timed melatonin injections (LD-M MT1; cyan). Box plot shows medians and interquartile ranges (LD CON: *n* = 11, SD-R CON: *n* = 8, SD-NR CON: *n* = 13, LD MT1: *n* = 8, LD-M CON: *n* = 10, LD-M MT1: *n* = 14), and boxes with different letters indicate a significant difference between treatment groups (*P* < 0.05, Kruskal-Wallis one-way ANOVAs on ranks with Dunn’s post-hoc tests for multiple comparisons). *Outliers excluded from statistical analysis (not shown): two SD-R CON hamsters, two SD-NR CON hamsters, two LD MT1 hamsters, two LD-M CON hamsters, and two LD-M MT1 hamsters.*