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Adrenal MT_1 melatonin receptor expression is linked with seasonal variation in social behavior in male Siberian hamsters

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ABSTRACT

Many animals exhibit pronounced changes in physiology and behavior on a seasonal basis, and these adaptations have evolved to promote survival and reproductive success. While the neuroendocrine pathways mediating seasonal reproduction are well-studied, far less is known about the mechanisms underlying seasonal changes in social behavior, particularly outside of the context of the breeding season. Our previous work suggests that seasonal changes in melatonin secretion are important in regulating aggression in Siberian hamsters (*Phodopus sungorus*); it is unclear, however, how melatonin acts via its receptors to modulate seasonal variation in social behavior. In this study, we infused a MT_1 melatonin receptor-expressing (MT_1) or control (CON) lentivirus into the adrenal glands of male Siberian hamsters. We then housed hamsters in long-day (LD) or short-day (SD) photoperiods, administered timed melatonin or control injections, and quantified aggressive and non-aggressive social behaviors (e.g., investigation, self-grooming) following 10 weeks of treatment. LD hamsters infused with the MT_1 lentivirus had significantly higher adrenal *mt1* expression than LD CON hamsters, as determined via quantitative PCR. While melatonin administration was necessary to induce SD-like reductions in body and relative reproductive mass, only LD hamsters infused with the MT_1 lentivirus displayed SD-like changes in social behavior, including increased aggression and decreased investigation and grooming. In addition, SD CON and LD hamsters infused with the MT_1 lentivirus exhibited similar relationships between adrenal *mt1* expression and aggressive behavior. Together, our findings suggest a role for adrenal MT_1 receptor signaling in regulating behavior, but not energetics or reproduction in seasonally breeding species.

1. Introduction

In order for individuals to be successful in their native environment, they must reproduce and survive. Because reproduction and survival both require a considerable investment of resources, natural selection has favored the evolution of seasonal adaptations that enable individuals to prioritize investing in the neuroendocrine mechanisms underlying reproduction and survival based on the time of year (Bronson and Heideman, 1994; reviewed in Stearns, 2000; Whittier and Crews, 1987). To mediate these energetic tradeoffs across the annual cycle, animals have evolved the ability to utilize cues in their environment to anticipate seasonal conditions and alter their physiology and behavior accordingly. Although a suite of environmental factors vary on a seasonal basis, photoperiod (i.e., day length) provides a reliable, 'noise-free' cue from which animals can coordinate these seasonal adaptations with the appropriate time of year (Goldman, 2001; Stevenson et al., 2017; reviewed in Walton et al., 2011). In mammals, the hormone melatonin serves as the biochemical cue from which organisms establish and maintain biological and circadian rhythms (Goldman, 2001; Stevenson et al., 2017; reviewed in Wood and Loudon, 2014). Photoperiod is translated from an environmental cue into a biochemical signal via a multisynaptic pathway, in which environmental light is perceived by retinal ganglion cells, processed in the hypothalamus, and transduced from a neural to an endocrine signal through the release of melatonin by the pineal gland. Because melatonin production is high at night and low during the day, the pattern and duration of melatonin secretion closely

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tracks changes in photoperiod throughout the year and, thus, conveys information about day length to the central nervous system and peripheral tissues that are sensitive to melatonin (reviewed in Bartness et al., 1993; Goldman, 2001).

Following its secretion into circulation, melatonin can exert its effects by binding to one of two subtypes of membrane-bound G protein-coupled receptors: the MT_1 melatonin receptor [also known as the Mel_{1a} receptor ($Mel_{1a}R$) in non-mammalian vertebrates and MTNR1A in humans] and the MT_2 melatonin receptor [also known as the Mel_{1b} receptor ($Mel_{1b}R$) in non-mammalian vertebrates and MTNR1B in humans; reviewed in Dubocovich and Markowska, 2005; reviewed in von Gall et al., 2002; reviewed in Witt-Enderby et al., 2003]. Of these subtypes, the MT_1 receptor is considered to be primarily responsible for photoperiodic signal transduction (reviewed in Reppert, 1997), since the MT_1 receptor is expressed in brain regions and endocrine tissues that are important sites for the biological and circadian actions of melatonin (e.g., the suprachiasmatic nucleus of the hypothalamus and pars tuberalis of the anterior pituitary gland; reviewed in Kennaway and Rowe, 1995; reviewed in Wood and Loudon, 2014), whereas the MT_2 receptor is largely absent from the hypothalamus and pituitary gland of mammals and is absent entirely from several species of seasonally breeding rodents, including Syrian hamsters (*Mesocricetus auratus*) and Siberian hamsters (*Phodopus sungorus*; reviewed in Reppert, 1997; Weaver et al., 1989; Weaver et al., 1996). In addition to the suprachiasmatic nucleus and pars tuberalis, the MT_1 receptor has been localized in several other brain regions and peripheral endocrine glands, including the midbrain, thalamus, hippocampus, cerebellum, gonads, and adrenal glands (reviewed in Dubocovich and Markowska, 2005; reviewed in von Gall et al., 2002; reviewed in Witt-Enderby et al., 2003). To date, the role of the MT_1 receptor in regulating seasonal changes in physiology and behavior has mostly been studied in the context of reproduction. Prior studies suggest that neural and peripheral MT_1 receptor signaling modulate seasonal reproduction by activating or suppressing the hypothalamic-pituitary-gonadal (HPG) axis in many seasonally breeding species (reviewed in Dawson et al., 2001; Stevenson et al., 2017; reviewed in Wood and Loudon, 2014). While the regulatory actions of the MT_1 receptor on seasonal reproduction have been well-studied, it is unclear whether MT_1 receptor signaling is also important in regulating seasonal changes in social behaviors that affect an organism's fitness, such as aggression.

Aggressive behavior is well-conserved across animal taxa and enables individuals to compete with conspecifics for access to limited resources in their environment, such as food, territories, and mates (Jalabert et al., 2018; Nelson, 2006). Consequently, many species show high levels of aggressive behavior during the breeding season, when acquiring a mate and actively defending a territory is critical to increasing an individual's chances of reproductive success. Indeed, much of the research conducted on the neuroendocrine processes underlying aggressive behavior has focused extensively on the role of gonadal steroids [e.g., testosterone (T) and estradiol (E_2)] in regulating breeding aggression (reviewed in Cunningham et al., 2012; reviewed in Soma, 2006). Some animals, however, show equivalent or higher levels of aggression during the non-breeding season despite gonadal regression, suggesting that these species face additional selective pressures that favored the evolution of alternative neuroendocrine mechanisms, which are independent of gonadal steroids, to regulate aggressive behavior year-round (reviewed in Munley et al., 2018; reviewed in Soma et al., 2015). Our previous work suggests that melatonin and the adrenal androgen dehydroepiandrosterone (DHEA) are important in regulating non-breeding aggression in Siberian hamsters, a solitary, seasonally breeding species in which individuals actively defend their territories year-round (Wynne-Edwards, 2003). We have shown that male and female hamsters housed in long-day (LD) photoperiods (characteristic of the breeding season) and given timed melatonin injections, which mimic the pattern of melatonin secretion exhibited by animals exposed to short-day (SD) photoperiods (characteristic of the non-breeding season), display higher levels of aggression than LD hamsters given control injections and show SD-like changes in baseline and aggression-induced circulating androgen and estrogen concentrations (Munley et al., 2020; Munley et al., 2021; Rendon et al., 2020; Rendon et al., 2015). Moreover, treating adrenal glands with melatonin *in vitro* elevates DHEA production in SD, but not LD females, while treating cultured ovaries with melatonin elevates DHEA production in LD, but not SD females (Rendon et al., 2015), suggesting that melatonin acts on the adrenal glands to elevate DHEA secretion and aggression during the non-breeding season. More recently, we found that timed melatonin administration and exposure to SDs reduces levels of DHEA, T, and E_2 in brain regions associated with aggressive behavior in male hamsters (Munley et al., 2021). Although our results suggest that melatonin regulates seasonal aggression by altering both peripheral and neural steroidogenesis, the role of the MT_1 receptor in mediating seasonal variation in social behavior (e.g., aggression) is unclear.

In the present study, we tested the hypothesis that melatonin acts via the adrenal glands to regulate seasonal changes in behavior, but not energetics or reproduction in Siberian hamsters. Because Siberian hamsters lack a functional MT_2 receptor (reviewed in Reppert, 1997; Weaver et al., 1989; Weaver et al., 1996), this species provides an excellent opportunity to characterize how melatonin receptors modulate seasonal variation in energetics, reproductive physiology, and social behavior. To assess how adrenal melatonin receptors affect these physiological and behavioral responses, we manipulated adrenal MT_1 receptor expression via lentiviral vector infusion, in which adult male hamsters were infused with either a MT_1 melatonin receptor-expressing (MT_1) or control (CON) lentivirus into the adrenal glands. We then housed these hamsters in LD or SD photoperiods, administered timed melatonin or control injections, and measured body and reproductive tissue mass and aggressive and non-aggressive social behaviors (e.g., investigation and self-grooming) following 10 weeks of treatment (Fig. 1). Moreover, we quantified serum DHEA levels to determine whether adrenal MT_1 receptors mediate seasonal changes in behavior by altering DHEA production. We predicted that adrenal MT_1 overexpression and timed melatonin administration will cause SD-like changes in circulating DHEA levels and social behavior, including increases in serum DHEA concentration and aggressive behavior and reductions in non-aggressive behaviors (i.e., investigative and self-grooming behavior), in male hamsters. In contrast, we hypothesized that energetic and reproductive responses to seasonal changes in

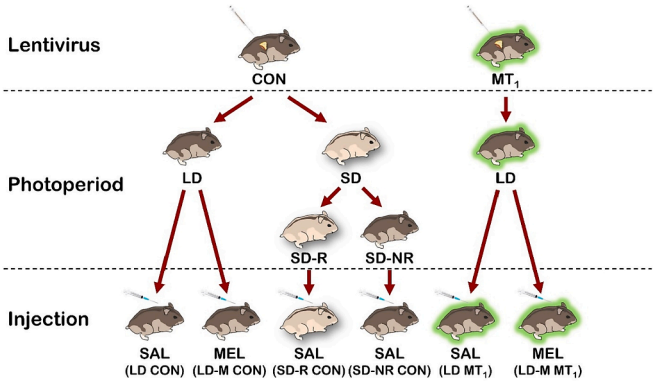

Fig. 1. Schematic of experimental design. Male hamsters were infused with either a MT_1 melatonin receptor-expressing (MT_1) or control (CON) lentivirus into the adrenal glands. Upon completion of surgery, hamsters were housed in either long-day (LD) or short-day (SD) photoperiods. Following 14 d of surgical recovery and post-operative monitoring, hamsters were administered timed subcutaneous injections of melatonin (MEL) or control (SAL) solution for a period of 10 weeks. At the end of the 10-week study, SD hamsters were classified as responsive or non-responsive to photoperiodic treatment (SD-R or SD-NR, respectively; see Section 2.5 for detailed description of *a priori* criteria used to classify seasonal phenotypes).

photoperiod occur independently of adrenal MT_1 receptor signaling. Thus, we expected that a SD-like circulating melatonin signal (i.e., timed melatonin injections or exposure to SDs), but not adrenal MT_1 overexpression, will induce gonadal regression and a reduction in body mass in male hamsters.

2. Materials and methods

2.1. Experimental animals

Adult male Siberian hamsters (*Phodopus sungorus*, >60 d of age) were reared and maintained in a breeding colony under long-day photoperiods (LDs; light:dark, 16 h:8 h; lights off at 1800 h Eastern Standard Time, EST) and group-housed at weaning (post-natal day 18) in polypropylene cages (28 × 17 × 12 cm). Sani-chip bedding (Teklad, laboratory grade; Envigo, Madison, WI, USA) was used in each cage, and hamsters were given *ad libitum* access to standard laboratory rodent chow (Teklad global 18% protein diet; Envigo, Madison, WI, USA) and tap water. Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee (BIA-CUC) at Indiana University (protocol #17-001 and 20-002).

2.2. Lentiviral vector development

Primers were designed to amplify and purify a region of the Siberian hamster MT_1 melatonin receptor (a gift from Dr. David Weaver) and included the coding sequence of the *P. sungorus* MT_1 receptor, along with *BamHI* and *HindIII* restriction sites. The resulting polymerase chain reaction (PCR) products were then cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). Subsequently, the MT_1 receptor fragment was subcloned into the lentivirus transfer vector pLenti-GFP-Puro, fused to and downstream of the enhanced green fluorescent protein (EGFP) coding sequence. Expression of the fusion protein was verified through transient transfection in human embryonic kidney 293T (HEK293T) cells and Siberian hamster adrenal glands. Viral production was followed from procedures outlined in Tiscornia et al. (2006) with minor modifications (Jasnow et al., 2009a; Jasnow et al., 2009b). Briefly, active viral particles were produced by co-transfecting the lentiviral expression vector with packaging plasmids coding for pLP1, pLP2, and VsVG into HEK293T cells using calcium phosphate transfection procedures. The packaged, unconcentrated virus was collected over a period of 3 d post-transfection, and then was concentrated via ultracentrifugation and resuspended in sterile 1× Hanks' balanced salt solution. The resulting titer was assessed in HEK293T cells after serially diluting the concentrated virus (virus titers: 0.9×10^8–1.2×10^9 TU/mL).

2.3. Surgical procedures and photoperiodic manipulations

Prior to the start of photoperiodic manipulations, experimental animals ($n = 83$; see Section 2.1) were individually housed for a two-week acclimation period on a LD light cycle. Following acclimation, hamsters underwent bilateral surgeries for infusion of a control ($n = 48$; referred to hereafter as "CON" lentivirus) or a MT_1 melatonin receptor-expressing lentivirus ($n = 35$; referred to hereafter as "MT_1" lentivirus; Section 2.2; Fig. 1) into the adrenal glands. Before surgery, hamsters were administered meloxicam (1 mg/kg; Covetrus, Dublin, OH, USA) and a midazolam and butorphanol cocktail (0.4 mg/kg each; Covetrus, Dublin, OH, USA) subcutaneously as pre-operative analgesics. Hamsters were deeply anesthetized using isoflurane (Covetrus, Dublin, OH, USA) delivered via a SomnoSuite low-flow anesthesia system (Kent Scientific Corporation, Torrington, CT, USA). Bilateral incisions were made on the dorsum over the kidneys, and the adrenal glands were visualized using a Leica GZ6E stereo zoom microscope (Leica Microsystems, Wetzlar, Germany). A single 2 μL injection of the CON or MT_1 lentivirus was delivered to each adrenal gland using a Hamilton syringe (1700 series; Hamilton, Reno, NV, USA), and needles were left in place for 1–2 min before removal to facilitate diffusion of the lentivirus into the adrenal glands. Upon completion of surgery, buprenorphine (0.05 mg/kg; Covetrus, Dublin, OH, USA) was administered subcutaneously as a post-operative analgesic. Hamsters were then transferred to a room on a short-day (SD) light cycle (CON: $n = 25$; light:dark, 8 h:16 h; lights on at 1000 h EST) or were relocated to a new room on a LD light cycle (CON: $n = 23$, MT_1: $n = 35$; Fig. 1). Meloxicam (1 mg/kg) and buprenorphine (0.05 mg/kg) were administered every 24 h for 3 d post-surgery and as needed thereafter for pain management. Hamsters recovered from surgery and were monitored post-operatively for 14 d before the start of *in vivo* melatonin administration (Section 2.4).

2.4. In vivo melatonin administration

After surgical recovery, a subset of LD hamsters (LD-M; CON: $n = 12$, MT_1: $n = 22$) was administered timed subcutaneous injections of melatonin [15 μg/day (USP Reference Standard; Sigma-Aldrich, St. Louis, MO, USA) dissolved in 1:10 ethanol:saline solution], as described in prior studies (Munley et al., 2020; Munley et al., 2021; Stetson and Tay, 1983). All remaining hamsters in the study (CON: $n = 36$, MT_1: $n = 13$) received daily injections of a control (1:10 ethanol:saline) solution (Fig. 1). Injections were administered 2 h prior to lights out (1530–1630 h EST), which extended the LD pattern of endogenous melatonin secretion in LD-M hamsters to mimic that of SD hamsters (Stetson and Tay, 1983), for a period of 10 weeks. This manipulation allowed us to dissociate the effects of circulating melatonin on energetics, reproduction, and social behavior from those associated with adrenal MT_1 overexpression.

2.5. Seasonal phenotypes

Following 10 weeks of treatment with timed melatonin or control injections, seasonal phenotypes were determined based on *a priori* criteria that have been previously described for Siberian hamsters (Munley et al., 2021; Scotti et al., 2007). Body mass was measured weekly for the duration of the study, and paired testes were collected and weighed at the end of the study. Body and paired testes mass were the primary criteria used to classify hamsters as responsive or non-responsive to photoperiodic treatment, and each of the two variables used for classification were in agreement for all hamsters in the LD CON and SD CON groups. LD CON hamsters had functional testes (i.e., had a paired testes mass between 0.600 and 1.000 g) and showed no significant change in body mass (<6%). In contrast, SD CON hamsters that were responsive to photoperiodic treatment (SD-R CON; $n = 10$) had regressed testes (i.e., had a paired testes mass that was >2 standard deviations less than the mean paired testes mass of LD CON hamsters) and displayed a significant reduction in body mass (≥6%). LD MT_1 hamsters exhibited a similar phenotype to LD CON hamsters, whereas LD-M CON and LD-M MT_1 hamsters exhibited a phenotype that was intermediate to LD CON and SD-R CON hamsters. A subset of SD CON hamsters ($n = 15$, 60.0%) failed to respond to photoperiodic treatment and were classified as "non-responders" (SD-NR CON) using the same criteria described above for LD CON hamsters. Non-responsiveness to SDs, in which hamsters do not undergo gonadal regression or reduce body mass in response to SDs and generally respond physiologically and behaviorally like LD hamsters, has been previously documented in this species (Gorman and Zucker, 1997; Puchalski and Lynch, 1986; Rendon et al., 2017).

2.6. Behavioral testing

Social behavior was quantified within the first 3 h of the dark phase

(1830–2100 h EST) using a 5 min same-sex resident-intruder paradigm (Munley et al., 2021; Rendon et al., 2015). Staged male dyads were composed 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 not more than once per testing period (i.e., group of behavior trials conducted in a single day, which was approximately 2–3 h in duration and consisted of 2–4 trials). Hamsters used as intruders (n = 30) 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 aggressive (i.e., latency to first attack, number and duration of attacks and chases) and non-aggressive social behaviors (i.e., frequency and duration of nose-to-nose investigation, anogenital investigation, and self-grooming) were scored for each experimental animal by a single trained observer who was blind to the experimental conditions using Behavioral Observation Research Interactive Software (BORIS) version 7.9.8 (Friard and Gamba, 2016). Three hamsters (LD MT_1: $n = 1$, LD-M MT_1: $n = 1$, SD-NR CON: $n = 1$) did not display attacking behavior during the testing period and were assigned an attack latency of 300 s for the purpose of statistical analysis. Measures of aggression, investigation, and grooming were defined according to prior studies on same-sex social behavior in male Siberian hamsters (Munley et al., 2020; Scotti et al., 2015).

Principal component analyses (PCAs) were used to reduce aggression data (i.e., latency to first attack, number and duration of attacks and chases) to a composite 'aggression score' (PC_{Agg}) and to reduce investigation data (i.e., frequency and duration of nose-to-nose and anogenital investigation) to a composite 'investigation score' (PC_{Inv}). In addition, a composite 'social behavior score' (PC_{Soc}) was generated using all social behaviors that were measured during the study (i.e., aggression, investigation, and grooming). 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 4.0.3 (R Core Team, 2020; Revelle, 2019). The data that comprised each PCA had a Kaiser-Meyer-Olkin measure of sampling adequacy > 0.600 and a significant P-value ($P < 0.05$) for Bartlett's test of sphericity and, thus, were considered appropriate for factor analysis (Dziuban and Shirkey, 1974; Kaiser, 1974; PC_{Soc} – Kaiser-Meyer-Olkin measure of sampling adequacy = 0.730, Bartlett's test of sphericity: $\chi^2 = 664.315$, d.f. = 55, $P < 0.001$; PC_{Agg} – Kaiser-Meyer-Olkin measure of sampling adequacy = 0.710, Bartlett's test of sphericity: $\chi^2 = 290.157$, d.f. = 10, $P < 0.001$; PC_{Inv} – Kaiser-Meyer-Olkin measure of sampling adequacy = 0.690, Bartlett's test of sphericity: $\chi^2 = 277.826$, d.f. = 6, $P < 0.001$). PCAs were conducted using the *prcomp* function of the *stats* package (R Core Team, 2020), and data were standardized using Z-scores to account for differences in scaling between variables (Jolliffe and Cadima, 2016). The first principal component of each analysis explained a significant proportion of the total variance (PC_{Soc}: 43.3%, PC_{Agg}: 67.4%, PC_{Inv}: 79.8%), had a large eigenvalue (>3), and was strongly loaded by most of the variables included in a given analysis (i.e., loading values <−0.3 or >0.3; Table 1). Thus, PC_{Soc}, PC_{Agg}, and PC_{Inv} and were used to assess the effects of photoperiodic, lentiviral vector, and melatonin treatment on social behavior, aggression, and investigation, respectively.

2.7. Blood sampling and tissue collection

After behavioral testing (1840–2120 h EST), adrenal glands and reproductive tissues were collected from each experimental animal.

Table 1
Principal component loading values and eigenvalues for variables that were used to generate composite social behavior, aggression, and investigation scores via principal component analysis.

Variables	PC_{Soc}	Variables	PC_{Agg}	Variables	PC_{Inv}
Number of attacks	**0.371**	Number of attacks	**0.512**	NTN frequency	**0.526**
Attack duration	**0.304**	Attack duration	**0.436**	NTN duration	**0.474**
Number of chases	**0.327**	Number of chases	**0.501**	AGI frequency	**0.511**
Chase duration	**0.304**	Chase duration	**0.470**	AGI duration	**0.488**
Attack latency	−0.289	Attack latency	−0.278		
NTN frequency	**−0.350**				
NTN duration	**−0.302**				
AGI frequency	**−0.357**				
AGI duration	**−0.325**				
GR frequency	−0.170				
GR duration	−0.104				
Eigenvalues	4.758		3.370		3.192

Composite social behavior, aggression, and investigation scores (PC_{Soc}, PC_{Agg} and PC_{Inv}, respectively) were used to determine the effects of adrenal MT_1 receptor overexpression, timed melatonin injections, and photoperiod on social behavior in male hamsters. PC_{Soc} accounted for 43.3% of the total variance, PC_{Agg} accounted for 67.4% of the total variance, and PC_{Inv} accounted for 79.8% of the total variance. Bold values indicate variables that loaded strongly (<−0.3 or >0.3) onto a given principal component. *Abbreviations: AGI, anogenital investigation; GR, grooming; NTN, nose-to-nose investigation.*

Hamsters were euthanized with a lethal intraperitoneal injection (0.3 mL) of ketamine (150 mg/kg) and xylazine (30 mg/kg) cocktail in 0.9% saline within 5 min of behavioral testing. Adrenal glands were rapidly extracted using RNase-free tools (<5 min following euthanasia), cleaned of fat, weighed to the nearest mg, collected into sterile polypropylene microcentrifuge tubes, flash frozen on dry ice, and stored at −80 °C until quantitative polymerase chain reaction (qPCR) analysis. Paired testes and epididymal white adipose tissue (EWAT) were also removed, separated, and weighed individually and were used to calculate relative reproductive mass, which is reported as the sum of a hamster's reproductive tissue mass (i.e., paired testes mass and EWAT mass) divided by its body mass.

2.8. qPCR

To confirm the efficacy of the MT_1 lentivirus in overexpressing the MT_1 melatonin receptor sequence, the relative mRNA expression of *mt1* (referred to here and in parts of the main text and Supplementary Material as a gene and not as a protein and, thus, abbreviated in italics with all lowercase letters) was measured in the adrenal glands of each experimental animal via qPCR. Total RNA was extracted from adrenal gland tissue using Maxwell® simplyRNA tissue kits and a Maxwell® Rapid Sample Concentrator instrument (Promega, Madison, WI, USA). RNA quality and quantity were determined using a Take3 micro-volume plate and an Epoch microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). Complementary DNA (cDNA) was reverse transcribed from 75 ng of total RNA using SuperScript™ III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) per the manufacturer's instructions.

The resulting cDNA was used to measure the mRNA expression of *mt1* and the reference genes 18s ribosomal RNA (*18srrna*) and glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) via qPCR. We chose to include two reference genes for normalization because the use of multiple reference genes has been found to significantly increase the reliability of relative mRNA expression quantification (Vandesompele et al., 2002). A custom primer set was designed for *mt1* using a Siberian hamster transcriptome sequence (GenBank accession number: U14110.1; Reppert et al., 1994; Weaver et al., 1996), and the primer sets

for *18srrna* and *gadph* have been previously published in Siberian hamsters (Banks et al., 2016; Bao et al., 2019; Supplementary Material, Table S1). All primers were validated using serial dilutions (replication efficiencies – *mt1*: 101.68%, *18srrna*: 99.77%, *gapdh*: 98.24%), and melt curves were used to confirm that reactions yielded a single product. qPCR reactions (10 μL) were run in triplicate alongside no template controls (NTCs) in a QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Waltham, WA, USA) using PerfeCTa SYBR Green SuperMix with low ROX (Quanta Biosciences, Gaithersburg, MD, USA). Each well contained 3 μL cDNA diluted 1:2 (or 3 μL nuclease-free water for NTCs), 0.5 μL forward and reverse primers (4 μM concentration for *mt1* primers, 10 μM concentration for *18srrna* and *gapdh* primers), 1 μL nuclease-free water, and 5 μL PerfeCTa SYBR Green SuperMix for a total volume of 10 μL. The following thermocycling conditions were used: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s. A final melting stage of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s was run to confirm single-product specificity of each reaction. cDNA samples from different treatment groups were counterbalanced across 5 MicroAmp™ optical 384-well reaction plates (Applied Biosystems, Foster City, CA, USA). Inter-plate coefficients of variability (CVs) were determined by comparing the average Ct values of the reference genes across plates, and intra-plate CVs were determined by calculating the average CV (generated from Ct values) of each gene within a given plate. The inter-assay variability was ≤11.03% (*18srrna*: 11.03%, *gapdh*: 6.94%), and the average intra-assay variability was ≤1.47% (*mt1*: 1.47 ± 0.07%, *18srrna*: 1.02 ± 0.11%, *gapdh*: 0.76 ± 0.10%).

QuantStudio™ Real-Time PCR software version 1.3 (Thermo Fisher Scientific, Waltham, WA, USA) was used to determine the relative mRNA expression of *mt1* via the comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method, in which the mRNA expression of a gene of interest is calculated as the fold-change in expression relative to a calibrator sample and normalized to the expression of one or more reference genes (Schmittgen and Livak, 2008). The geometric mean Ct of *18srrna* and *gapdh* was used as the reference cycle for calculating ΔCt for a given sample. In addition, the mean relative adrenal *mt1* expression of LD CON hamsters was used as a calibrator for determining ΔΔCt. For experimental animals that were infused with the MT_1 lentivirus, individuals that exhibited adrenal *mt1* expression > 2 standard deviations above the mean expression of LD CON hamsters were classified as showing adrenal MT_1 overexpression. Hamsters infused with the MT_1 lentivirus that did not exhibit adrenal MT_1 overexpression ($n = 9$, 25.7%) were excluded from the study.

2.9. Quantification of circulating DHEA levels

Serum DHEA concentrations were quantified using a commercially-available enzyme immunoassay kit (DHEA ELISA kit ADI-901-093; Enzo Life Sciences, Farmingdale, NY, USA; assay sensitivity = 2.90 pg/mL), which has been previously validated in male Siberian hamsters (Munley et al., 2020). This assay has some cross-reactivity with sulfated DHEA (DHEA-S, 30%); low cross-reactivity with androstenedione (0.73%), androsterone (0.29%), pregnenolone (0.28%), and T (0.10%); and negligible or undetectable cross-reactivity with other steroid hormones (<0.06%). Serum samples were run neat or diluted 1:2, 1:4, or 1:8 with assay buffer to ensure 20–80% binding on a 4-parameter logistic standard curve (Microplate Manager 6 version 6.2; Bio-Rad Laboratories, Hercules, CA, USA). Samples were run in duplicate according to the manufacturer's instructions, and all samples contained levels of DHEA that were above the limit of detection for the assay. Serum samples from different treatment groups were counterbalanced across 4 plates of the same kit lot number (lot number: 12142008). Samples with a CV greater than 10% and a maximum binding less than 20% or greater than 80% were re-analyzed. Inter-assay and intra-assay CVs were determined using a pooled serum sample from LD CON hamsters. The inter-assay variability was 10.77%, and the intra-assay variability was ≤7.01% (average: 4.64 ± 0.87%).

2.10. Statistical analyses

All data are presented as mean ± standard error of the mean (SEM). Statistical testing was performed using R version 4.0.3 (R Core Team, 2020), and statistical significance was attributed at $P < 0.05$ after controlling for false discovery rate (Verhoeven et al., 2005). 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 (see table and figure legends in the main text and Supplementary Material). For each variable, normality of linear model residuals was assessed with Shapiro-Wilk tests using the *shapiro.test* function of the *stats* package (R Core Team, 2020), and homogeneity of variances was assessed with Levene's tests using the *leveneTest* function of the *car* package (Fox and Weisberg, 2019). Data that did not exhibit a normal distribution were visualized with Cullen and Frey plots using the *descdist* function of the *fitdistrplus* package, and distribution fit was assessed using the *fitdist* function of the *fitdistrplus* package (Delignette-Muller and Dutang, 2015).

Because our experimental design is not in matrix form (Fig. 1), we used two-way analyses of variance (ANOVAs; for data that were normally distributed and exhibited homogeneity of variances) and generalized linear models (GLMs; for data that violated the assumptions of normality and/or homogeneity of variances) to probe for an interaction of lentiviral infusion and subcutaneous injection on adrenal *mt1* expression, body and organ mass, social behavior, and circulating DHEA levels in LD hamsters using the *lm* and *glm* functions of the *stats* package, respectively (R Core Team, 2020). There was no evidence of a significant interaction of lentiviral infusion and subcutaneous injection for any of these variables (interaction: $P \geq 0.253$ for all analyses); thus, we collapsed lentiviral infusion, photoperiod, and subcutaneous injection into a single factor, which allowed us to compare these measures between all six treatment groups. For data that were normally distributed and exhibited homogeneity of variances, one-way ANOVAs were conducted to examine the effects of adrenal MT_1 overexpression, photoperiodic treatment, and timed melatonin injections on body mass, reproductive tissue mass, and adrenal mass using the *lm* function of the *stats* package (R Core Team, 2020). For data that did not satisfy the assumptions of normality and/or homogeneity of variances, non-parametric Kruskal-Wallis one-way ANOVAs on ranks were conducted to examine the effects of adrenal MT_1 overexpression, photoperiodic treatment, and melatonin administration on adrenal *mt1* expression, social behavior, and circulating DHEA levels using the *kruskal.test* function of the *stats* package (R Core Team, 2020). If a statistical test reported a significant effect of treatment, pairwise comparisons were examined with Tukey's Honestly Significant Difference (HSD) post hoc tests (for one-way ANOVAs) or Dunn's post hoc tests for multiple comparisons (for Kruskal-Wallis one-way ANOVAs on ranks) using the *glht* function of the *multcomp* package (Hothorn et al., 2008) and the *dunn.test* function of the *dunn.test* package (Dinno, 2017), respectively. Effect sizes were calculated for all univariate analyses and their respective post hoc tests and are expressed as η^2 for one-way ANOVAs, transformed η^2 for Kruskal-Wallis one-way ANOVAs on ranks (Cohen, 1988; Rosenthal, 1994), Cohen's *d* for Tukey's HSD post hoc tests, and Hedge's *g* for Dunn's post hoc tests to account for unequal sample sizes between treatment groups (Ellis, 2010). Finally, to determine whether associations between adrenal *mt1* expression and social behavior differed across seasonal phenotypes and in response to adrenal MT_1 overexpression and timed melatonin injections, Spearman's rank correlations with a Holm-Bonferroni correction for multiple comparisons were conducted for each treatment group using the *corr.test* function of the *psych* package (Revelle, 2019).

3. Results

3.1. Hamsters infused with the MT_1 lentivirus exhibited higher adrenal *mt1* expression

Hamsters infused with the MT_1 receptor-expressing lentivirus had significantly higher *mt1* expression in the adrenal glands relative to hamsters infused with the CON lentivirus (Fig. 2). On average, adrenal *mt1* expression was 4.3-fold higher in LD MT_1 hamsters than in LD CON hamsters (Kruskal-Wallis one-way ANOVA on ranks: $H = 13.622$, d.f. = 5, $P = 0.018$, $\eta^2 = 0.127$; Dunn's post hoc test: $P = 0.013$, $g = 7.269$), whereas adrenal *mt1* expression was 0.9-fold higher in LD-M MT_1 hamsters than in LD-M CON hamsters ($P = 0.020$, $g = 2.768$). There was no difference in adrenal *mt1* expression, however, between hamsters infused with the CON lentivirus, regardless of photoperiodic or melatonin treatment ($P \geq 0.324$; $g \leq 2.221$; Fig. 2A). Furthermore, there was no effect of treatment on relative adrenal mass (one-way ANOVA: $F_{5,65} = 1.555$, $P = 0.185$, $\eta^2 = 0.107$; Fig. 2B), suggesting that neither lentiviral infusion surgeries nor timed melatonin injections adversely affected adrenal morphology.

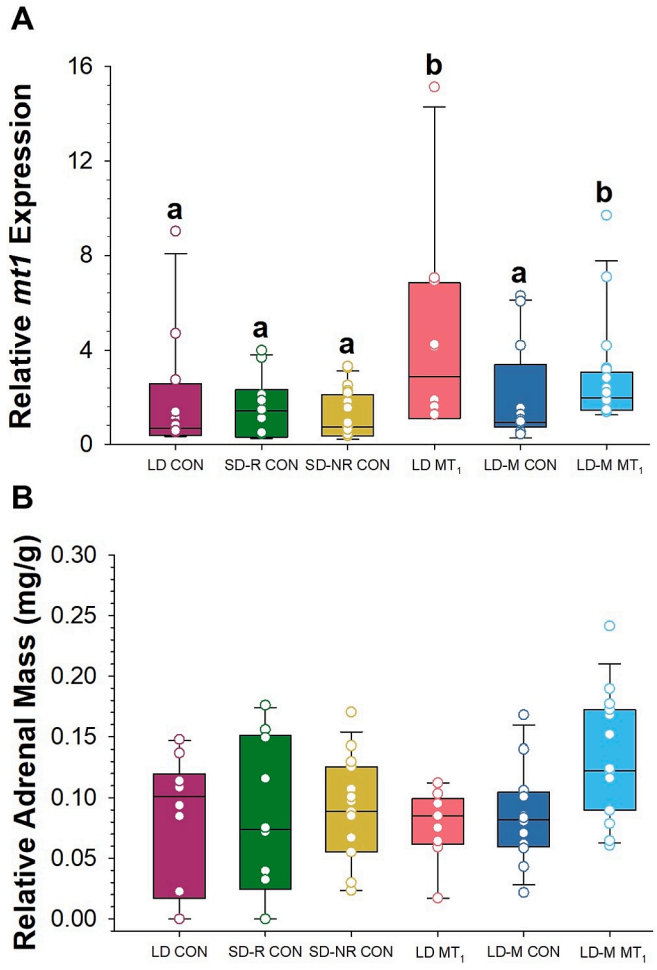

Fig. 2. Hamsters infused with the MT_1 receptor-expressing lentivirus had higher adrenal *mt1* expression relative to hamsters infused with the control lentivirus. (A) Adrenal *mt1* expression and (B) relative adrenal mass of long-day hamsters infused with the control (CON) lentivirus (LD CON; purple), 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 MT_1 receptor-expressing (MT_1) lentivirus (LD MT_1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT_1 lentivirus and given timed melatonin injections (LD-M MT_1; cyan). Box plots show medians and interquartile ranges (LD CON: $n = 11$, SD-R CON: $n = 10$, SD-NR CON: $n = 15$, LD MT_1: $n = 10$, LD-M CON: $n = 12$, LD-M MT_1: $n = 16$), and boxes with different letters indicate a significant difference between treatment groups ($P < 0.05$; adrenal *mt1* expression: Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests for multiple comparisons, relative adrenal mass: one-way ANOVA with Tukey's HSD post hoc tests). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Timed melatonin injections induced seasonally appropriate changes in energetics and reproduction

Melatonin administration and exposure to SDs produced characteristic changes in body mass and reproductive physiology (Fig. 3). SD-R CON and LD-M hamsters showed significant reductions in percent change in body mass (one-way ANOVA: $F_{5,67} = 24.49$, $P < 0.001$, $\eta^2 = 0.646$) and relative reproductive mass (one-way ANOVA: $F_{5,68} = 25.12$, $P < 0.001$, $\eta^2 = 0.649$) relative to LD CON, SD-NR CON, and LD MT_1 hamsters, regardless of lentiviral infusion. These decreases in percent change in body mass and relative reproductive mass, however, were more pronounced in SD-R CON hamsters than in LD-M hamsters (percent change in body mass: $P < 0.001$, $d \leq -5.040$; relative reproductive mass: $P < 0.001$, $d \leq -6.384$). In contrast, there was little evidence that adrenal MT_1 overexpression enhanced or diminished the effects of SDs and timed melatonin injections on energetics and reproduction. LD MT_1 and LD-M MT_1 hamsters showed no significant difference in percent change in body mass or relative reproductive mass compared to LD CON and LD-M CON hamsters, respectively (percent change in body mass – LD MT_1 vs. LD CON: $P = 0.981$, $d = -1.253$; LD-M MT_1 vs. LD-M CON: $P = 0.867$, $d = -1.820$; relative reproductive mass – LD MT_1 vs. LD CON: $P = 0.961$, $d = -2.384$; LD-M MT_1 vs. LD-M CON: $P = 0.801$, $d = -1.854$; Fig. 3A–B).

3.3. Adrenal MT_1 overexpression and exposure to SDs produced similar changes in social behavior

Hamsters infused with the MT_1 lentivirus and SD-R CON hamsters exhibited similar changes in social behavior (Fig. 4). PC_{Soc} was strongly loaded by measures of aggressive behavior (i.e., number and duration of attacks and chases) in the positive direction and by measures of investigative behavior (i.e., frequency and duration of nose-to-nose and anogenital investigation) in the negative direction (Table 1). Thus, a positive PC_{Soc} value indicates that a hamster displays higher levels of aggression relative to investigation, whereas a negative PC_{Soc} value suggests that a hamster shows higher levels of investigation relative to aggression. SD-R CON hamsters had a significantly higher PC_{Soc} than LD CON, SD-NR CON, LD-M CON, and LD-M MT_1 hamsters (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 9.367$, d.f. = 5, $P \leq 0.038$, $g \geq 3.945$). In addition, LD MT_1 hamsters had a significantly higher PC_{Soc} than LD-M CON hamsters ($P = 0.032$, $g = 2.795$), and LD-M MT_1 hamsters showed a trend towards an increase in PC_{Soc} relative to LD-M CON hamsters ($P = 0.096$, $g = 1.456$). Conversely, there was no significant difference in PC_{Soc} between LD CON, SD-NR CON, and LD-M CON hamsters ($P \geq 0.110$, $g \leq 1.758$; Fig. 4). Together, these data suggest that hamsters which exhibit adrenal MT_1 overexpression or are responsive to SD photoperiods display higher levels of aggressive behavior relative to investigative behavior.

3.4. Adrenal MT_1 overexpression and SD photoperiods increased aggressive behavior

Hamsters that were infused with the MT_1 lentivirus or were responsive to SDs displayed higher levels of aggression than CON hamsters (Fig. 5; Supplementary Material, Table S2). SD-R CON and LD-M MT_1 hamsters had a significantly shorter attack latency than LD CON and LD MT_1 hamsters (Kruskal-Wallis one-way ANOVA on ranks: $H =$

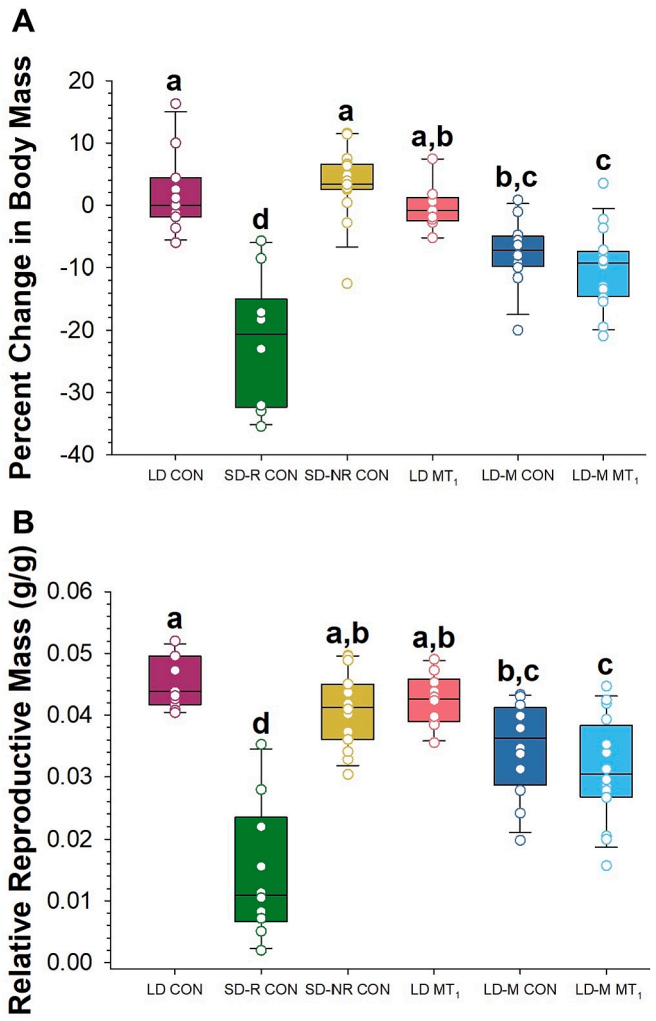

Fig. 3. Treatment with short-day photoperiods and timed melatonin injections, but not the MT_1 receptor-expressing lentivirus, reduced body and reproductive tissue mass. (A) Percent change in body mass and (B) relative reproductive mass of long-day hamsters infused with the control (CON) lentivirus (LD 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 MT_1 receptor-expressing (MT_1) lentivirus (LD MT_1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT_1 lentivirus and given timed melatonin injections (LD-M MT_1; cyan). Box plots show medians and interquartile ranges (LD CON: n = 10–11, SD-R CON: n = 10, SD-NR CON: n = 15, LD MT_1: n = 9–10, LD-M CON: n = 12, LD-M MT_1: n = 15–16), and boxes with different letters indicate a significant difference between treatment groups ($P < 0.05$, one-way ANOVAs with Tukey's HSD post hoc tests). *Outlier excluded from statistical analysis (not shown): one LD MT_1 hamster for percent change in body mass.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

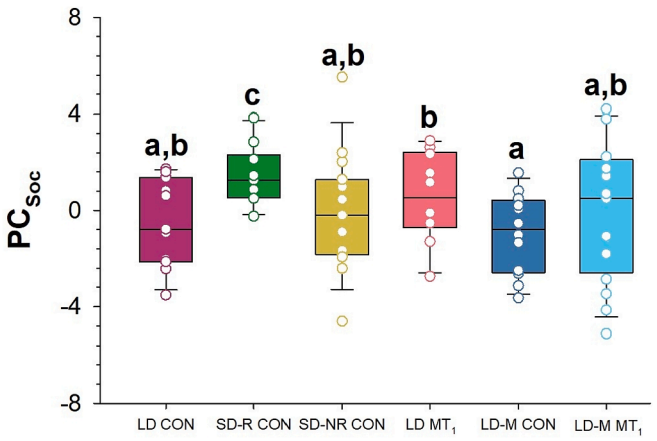

Fig. 4. Hamsters infused with the MT_1 receptor-expressing lentivirus and hamsters that were responsive to short-day photoperiods showed similar changes in social behavior. Composite social behavior score (PC_{Soc}) of long-day hamsters infused with the control (CON) lentivirus (LD CON; purple), short-day 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 MT_1 receptor-expressing (MT_1) lentivirus (LD MT_1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT_1 lentivirus and given timed melatonin injections (LD-M MT_1; cyan). Box plot shows medians and interquartile ranges (LD CON: n = 11, SD-R CON: n = 10, SD-NR CON: n = 15, LD MT_1: n = 10, LD-M CON: n = 12, LD-M MT_1: n = 16), and boxes with different letters indicate a significant difference between treatment groups ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests for multiple comparisons). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

13.206, d.f. = 5, $P = 0.022$, $\eta^2 = 0.130$; Fig. 5B). SD-R CON hamsters also trended towards increases in number of attacks (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc test: $H = 4.776$, d.f. = 5, $P = 0.095$, $g = 2.374$; Fig. 5A) and PC_{Agg} (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc test: $H = 4.704$, d.f. = 5, $P = 0.098$, $g = 2.234$; Fig. 5E) compared to LD CON hamsters. Furthermore, LD-M MT_1 hamsters exhibited a significant increase in number of chases (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc test: $H = 3.123$, d.f. = 5, $P = 0.046$, $g = 3.525$; Fig. 5C) and trended towards increases in aggression frequency (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc test: $H = 3.699$, d.f. = 5, $P = 0.071$, $g = 3.428$; Supplementary Material, Table S2), aggression duration (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc test: $H = 4.121$, d.f. = 5, $P = 0.051$, $g = 2.866$; Fig. 5D), and PC_{Agg} ($P = 0.091$, $g = 3.047$; Fig. 5E) relative to LD-M CON hamsters. While there was some evidence that LD MT_1 hamsters were more aggressive than LD CON hamsters (e.g., number of attacks and chases, aggression frequency and duration, PC_{Agg}), none of these trends reached significance ($P \geq 0.212$, $g \leq 1.082$). There was also no significant difference in aggression between LD CON, SD-NR CON, and LD-M CON hamsters, with the exception of latency to first attack (latency to first attack – SD-NR CON vs. LD CON: $P = 0.049$, $g = -2.440$; all other aggressive behaviors: $P \geq 0.102$, $g \leq 1.720$; Fig. 5; Supplementary Material, Table S2).

3.5. *SD-R CON and LD MT_1 hamsters displayed decreases in investigative and self-grooming behaviors*

Adrenal MT_1 overexpression and SDs caused reductions in investigation and self-grooming (Fig. 6; Supplementary Material, Table S2). SD-R CON and LD MT_1 hamsters exhibited a significant decrease in nose-to-nose investigation frequency compared to LD CON hamsters (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 9.153$, d.f. = 5, $P \leq 0.048$, $g \leq -2.356$; Fig. 6A). Moreover, SD-R CON hamsters showed significant reductions in anogenital investigation frequency (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 8.469$, d.f. = 5, $P \leq 0.014$, $g \leq -3.797$; Fig. 6B), anogenital investigation duration (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 7.784$, d.f. = 5, $P \leq 0.023$, $g \leq -3.447$; Fig. 6C), investigation frequency (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 9.721$, d.f. = 5, $P \leq 0.012$, $g \leq -4.360$), investigation duration (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 6.031$, d.f. = 5, $P \leq 0.045$, $g \leq -3.293$;

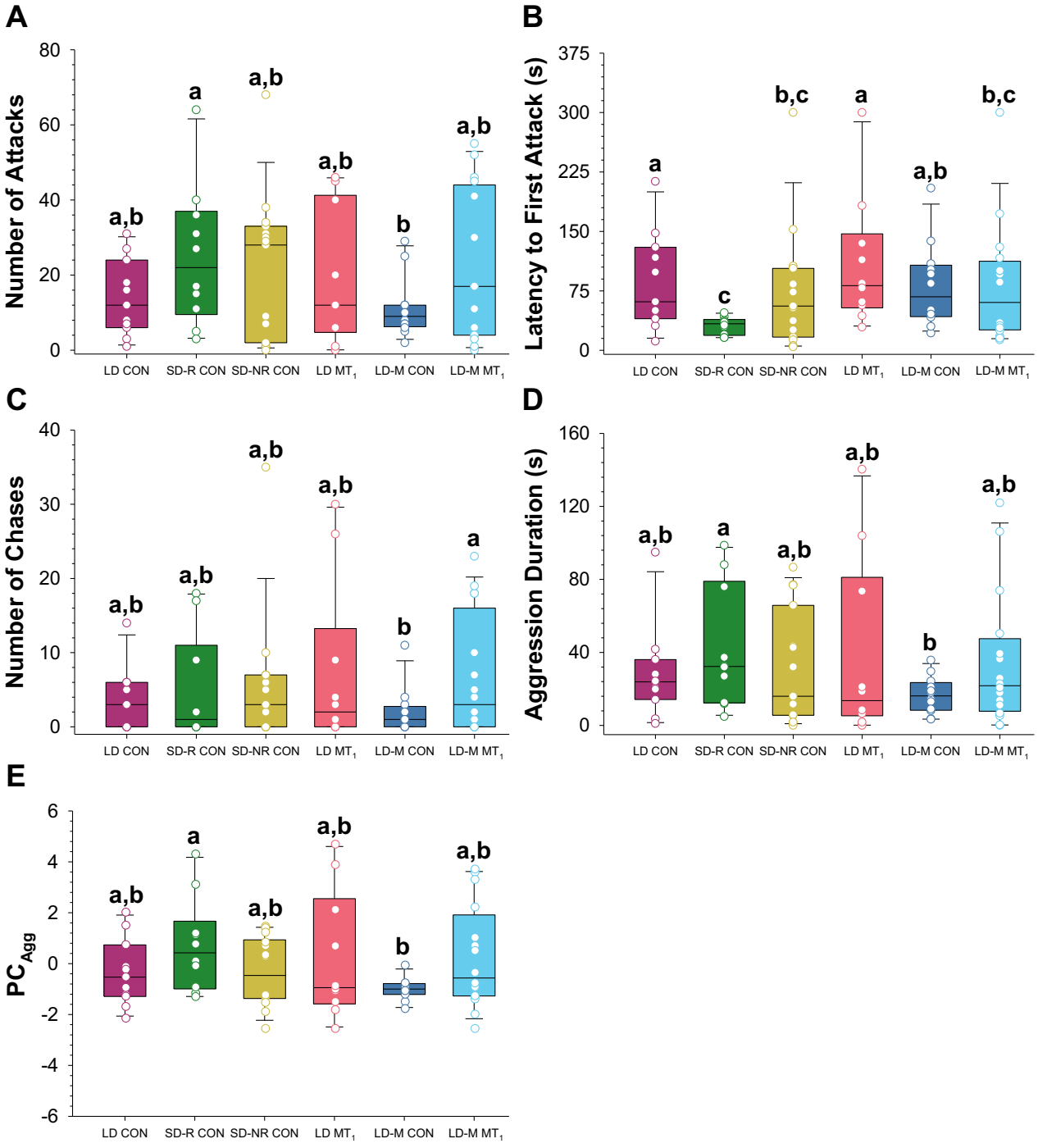

Fig. 5. Adrenal MT_1 receptor overexpression and short-day photoperiods increased aggressive behavior. (A) Number of attacks, (B) latency to first attack, (C) number of chases, (D) aggression duration, and (E) composite aggression score (PC_{Agg}) of long-day hamsters infused with the control (CON) lentivirus (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 MT_1 receptor-expressing (MT_1) lentivirus (LD MT_1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT_1 lentivirus and given timed melatonin injections (LD-M MT_1; cyan). Box plots show medians and interquartile ranges (LD CON: $n = 11$, SD-R CON: $n = 10$, SD-NR CON: $n = 13$–15, LD MT_1: $n = 10$, LD-M CON: $n = 11$–12, LD-M MT_1: $n = 14$–16), 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): one LD-M CON hamster, two LD-M MT_1 hamsters, and two SD-NR CON hamsters for latency to first attack; one LD-M CON hamster for number of chases; and one LD-M CON hamster and one SD-NR CON hamster for PC_{Agg}.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Supplementary Material, Table S2), and PC_{Inv} (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 7.953$, d.f. $= 5$, $P \leq 0.020$, $g \leq -3.387$; Fig. 6D) relative to LD CON, SD-NR CON, LD-M CON, and LD-M MT_1 hamsters. LD MT_1 hamsters also trended towards a decrease in investigation frequency relative to LD CON hamsters ($P = 0.082$, $g = -2.266$; Supplementary Material, Table S2). Conversely, there was no significant difference in investigative behavior between LD CON, SD-NR CON, LD-M CON, and LD-M MT_1 hamsters ($P \geq 0.229$, $g \leq$

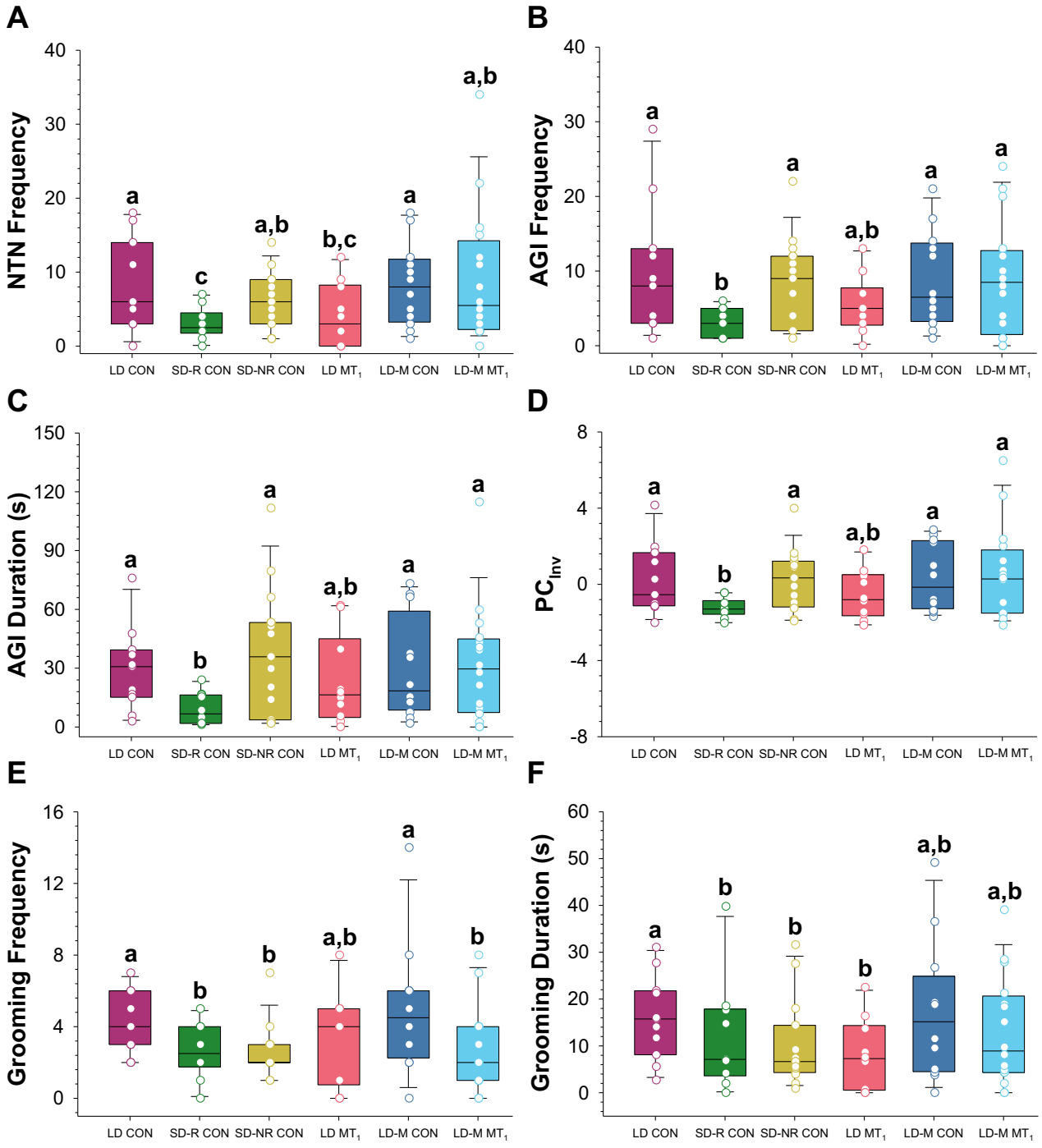

Fig. 6. Long-day hamsters infused with the MT_1 receptor-expressing lentivirus and hamsters that were responsive to short-day photoperiods displayed decreased investigative and self-grooming behaviors. (A) Nose-to-nose investigation (NTN) frequency, (B) anogenital investigation (AGI) frequency, (C) AGI duration, (D) composite investigation score (PC_{Inv}), (E) grooming frequency, and (F) grooming duration 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 MT_1 receptor-expressing (MT_1) lentivirus (LD MT_1; pink), LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON; blue), and LD hamsters infused with the MT_1 lentivirus and given timed melatonin injections (LD-M MT_1; cyan). Box plots show medians and interquartile ranges (LD CON: $n = 11$, SD-R CON: $n = 9$–10, SD-NR CON: $n = 15$, LD MT_1: $n = 10$, LD-M CON: $n = 11$–12, LD-M MT_1: $n = 16$), 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): one LD-M CON hamster for grooming frequency and one SD-R CON hamster for grooming duration.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1.686; Fig. 6A–D; Supplementary Material, Table S2). With respect to grooming behavior, SD-R CON and LD-M MT_1 hamsters had a significantly lower grooming frequency than LD CON and LD-M CON hamsters (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 8.170$, d.f. $= 5$, $P \leq 0.049$, $g \leq -2.344$; Fig. 6E), and SD-R CON and LD MT_1 hamsters had a significantly shorter grooming duration than LD CON hamsters (Kruskal-Wallis one-way ANOVA on ranks with Dunn's post hoc tests: $H = 5.985$, d.f. $= 5$, $P \leq 0.047$, $g \leq -2.918$; Fig. 6F). Interestingly, SD-NR CON hamsters also showed significant reductions in grooming frequency and duration relative to LD CON hamsters (grooming frequency: $P = 0.023$, $g = -3.426$; grooming duration: $P = 0.041$, $g = -2.405$). There was no significant difference in grooming behavior, however, between LD CON and LD-M CON hamsters ($P \geq 0.340$, $g \leq 0.279$; Fig. 6E–F).

3.6. LD hamsters treated with the MT_1 lentivirus and melatonin showed a decrease in circulating DHEA

LD hamsters infused with the MT_1 lentivirus and administered timed melatonin injections exhibited a reduction in circulating DHEA levels (Supplementary Material, Fig. S1). Specifically, LD-M MT_1 hamsters had a lower serum DHEA concentration than LD CON, LD MT_1, and LD-M CON hamsters (Kruskal-Wallis one way ANOVA on ranks: $H = 11.053$, d.f. $= 5$, $P = 0.050$, $\eta^2 = 0.104$; Dunn's post hoc tests: $P \leq 0.013$, $g \leq -1.963$) and showed a trend towards a decrease in serum DHEA relative to SD-NR hamsters ($P = 0.075$, $g = -1.192$). There was no significant difference in serum DHEA levels, however, between LD CON, SD-R CON, SD-NR CON, LD MT_1, and LD-M CON hamsters ($P \geq 0.100$, $g \leq 1.999$).

3.7. Adrenal MT_1 overexpression, timed melatonin injections, and SDs produced similar relationships between adrenal mt1 expression and social behavior

LD and SD hamsters showed distinct associations between adrenal *mt1* expression and PC_{Soc}, and this relationship was affected by both adrenal MT_1 overexpression and melatonin administration (Fig. 7). Adrenal *mt1* expression was negatively correlated with PC_{Soc} in LD CON and LD-M MT_1 hamsters (LD CON – Spearman's rank correlation: $\rho = -0.77$, $n = 11$, $P < 0.01$; LD-M MT_1 – Spearman's rank correlation: $\rho = -0.34$, $n = 16$, $P = 0.20$; Fig. 7A), whereas adrenal *mt1* expression was positively correlated with PC_{Soc} in SD-R CON, SD-NR CON, LD MT_1, and LD-M CON hamsters (SD-R CON – Spearman's rank correlation: $\rho = 0.25$, $n = 10$, $P = 0.49$; SD-NR CON – Spearman's rank correlation: $\rho = 0.29$, $n = 15$, $P = 0.29$; LD MT_1 – Spearman's rank correlation: $\rho = 0.28$, $n = 9$, $P = 0.46$; LD-M CON – Spearman's rank correlation: $\rho = 0.48$, $n = 12$, $P = 0.12$; Fig. 7B). Collectively, these results suggest that adrenal MT_1 overexpression, timed melatonin injections, and SDs produce a positive association between adrenal *mt1* expression and behavior, in which hamsters with higher adrenal *mt1* expression display higher levels of aggressive behavior relative to investigative behavior.

3.8. SD-R CON and LD MT_1 hamsters showed similar associations between adrenal mt1 expression and aggression

Hamsters that were infused with the MT_1 lentivirus or were responsive to SDs showed similar relationships between adrenal *mt1* expression and aggressive behavior (Fig. 8; Supplementary Material, Table S3). Specifically, SD-R CON and LD MT_1 hamsters displayed positive associations between adrenal *mt1* expression and number of attacks (SD-R CON – Spearman's rank correlation: $\rho = 0.35$, $n = 10$, $P = 0.33$; LD MT_1 – Spearman's rank correlation: $\rho = 0.37$, $n = 9$, $P = 0.33$; Fig. 8B), aggression frequency (SD-R CON – Spearman's rank correlation: $\rho = 0.35$, $n = 10$, $P = 0.33$; LD MT_1 – Spearman's rank correlation: $\rho = 0.32$, $n = 9$, $P = 0.41$; Supplementary Material, Table S3), aggression duration (SD-R CON – Spearman's rank correlation: $\rho = 0.48$, $n = 10$, $P = 0.16$; LD MT_1 – Spearman's rank correlation: $\rho = 0.30$, $n = 9$, $P = 0.43$; Fig. 8D), and PC_{Agg} (SD-R CON – Spearman's rank correlation: $\rho = 0.25$, $n = 10$, $P = 0.49$; LD MT_1 – Spearman's rank correlation: $\rho = 0.32$, $n = 9$, $P = 0.41$; Fig. 8F). In addition, SD-R CON hamsters exhibited a positive correlation between adrenal *mt1* expression and attack duration (Spearman's rank correlation: $\rho = 0.59$, $n = 10$, $P = 0.07$), and LD MT_1 hamsters exhibited positive correlations between adrenal *mt1* expression and chasing behavior (adrenal *mt1* expression and number of chases – Spearman's rank correlation: $\rho = 0.50$, $n = 9$, $P = 0.17$; adrenal *mt1* expression and chase duration – Spearman's rank correlation: $\rho = 0.62$, $n = 9$, $P = 0.08$; Supplementary Material, Table S3). There was also some evidence of a positive association between adrenal *mt1* expression and aggression in LD-M CON hamsters, although these correlations were

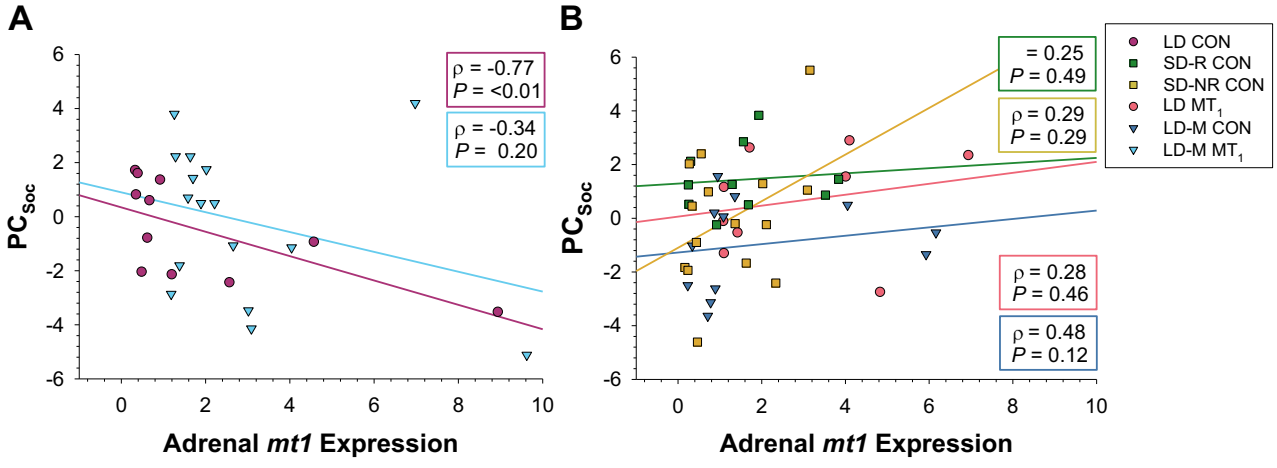

Fig. 7. Long-day hamsters infused with the MT_1 receptor-expressing lentivirus or given timed melatonin injections and hamsters exposed to short-day photoperiods showed similar relationships between adrenal *mt1* expression and social behavior. (A) Adrenal *mt1* expression was negatively correlated with composite social behavior score (PC_{Soc}) in long-day hamsters infused with the control (CON) lentivirus (LD CON; purple circles) and LD hamsters infused with the MT_1 receptor-expressing (MT_1) lentivirus and given timed melatonin injections (LD-M MT_1; cyan triangles). (B) Adrenal *mt1* expression was positively correlated with PC_{Soc} in short-day hamsters infused with the CON lentivirus that were responsive to photoperiodic treatment (SD-R CON; green squares), SD hamsters infused with the CON lentivirus that were non-responsive to photoperiodic treatment (SD-NR CON; yellow squares), LD hamsters infused with the MT_1 lentivirus (LD MT_1; pink circles), and LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON, blue triangles). Regression lines were generated from Spearman's rank correlations within treatment groups (LD CON: $n = 11$, SD-R CON: $n = 10$, SD-NR CON: $n = 15$, LD MT_1: $n = 9$, LD-M CON: $n = 12$, LD-M MT_1: $n = 16$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

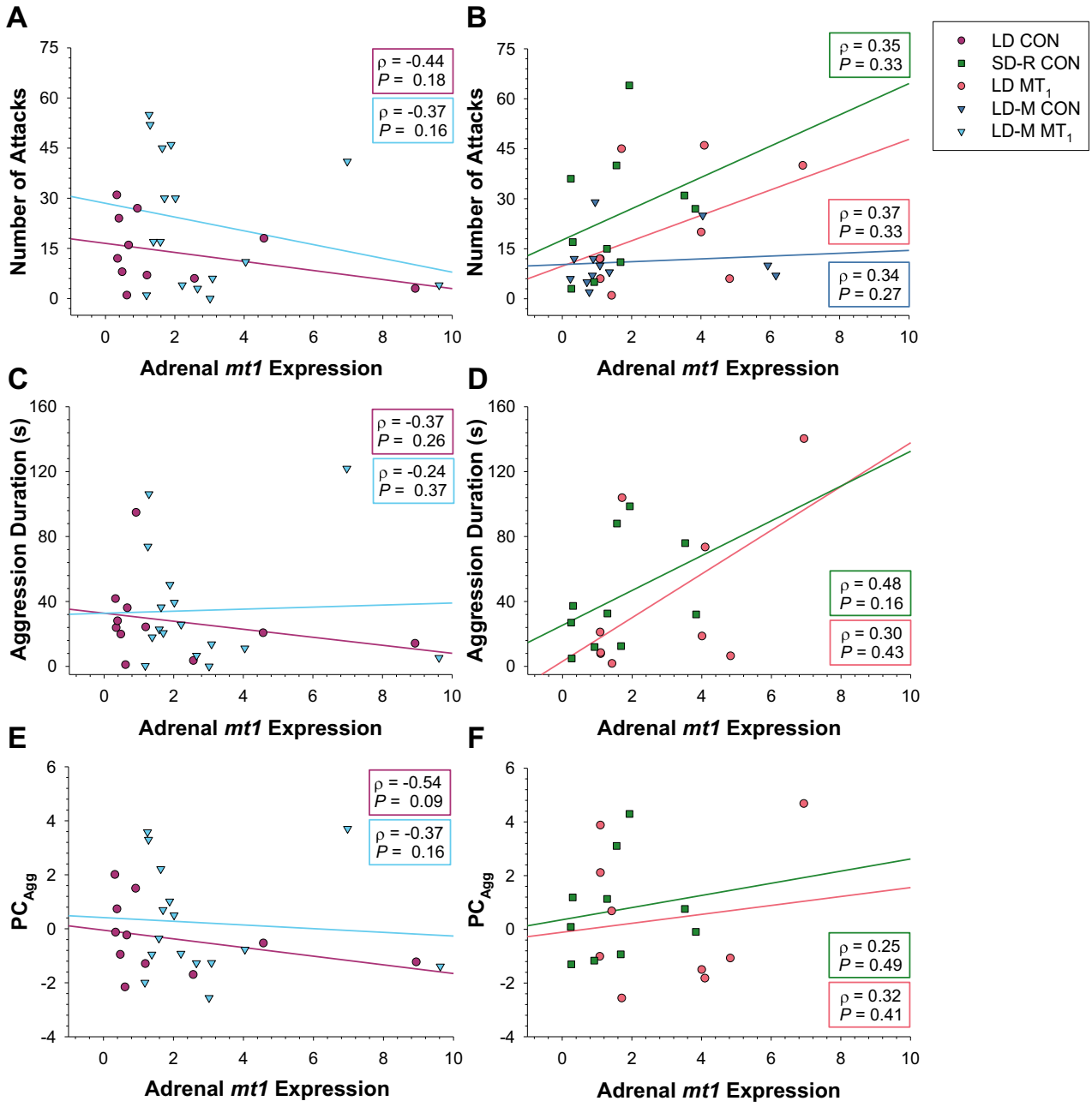

Fig. 8. Long-day hamsters infused with the MT$_1$ receptor-expressing lentivirus and hamsters that were responsive to short-day photoperiods showed similar associations between adrenal *mt1* expression and aggression. (A) Adrenal *mt1* expression was negatively correlated with number of attacks in long-day hamsters infused with the control (CON) lentivirus (LD CON; purple circles) and LD hamsters infused with the MT$_1$ receptor-expressing (MT$_1$) lentivirus and given timed melatonin injections (LD-M MT$_1$; cyan triangles). (B) Adrenal *mt1* expression was positively correlated with number of attacks in short-day hamsters infused with the CON lentivirus that were responsive to photoperiodic treatment (SD-R CON; green squares), LD hamsters infused with the MT$_1$ lentivirus (LD MT$_1$; pink circles), and LD hamsters infused with the CON lentivirus and given timed melatonin injections (LD-M CON, blue triangles). (C) Adrenal *mt1* expression was negatively correlated with aggression duration in LD CON and LD-M MT$_1$ hamsters. (D) Adrenal *mt1* expression was positively correlated with aggression duration in SD-R CON and LD MT$_1$ hamsters. (E) Adrenal *mt1* expression was negatively correlated with composite aggression score (PC$_{Agg}$) in LD CON and LD-M MT$_1$ hamsters. (F) Adrenal *mt1* expression was positively correlated with PC$_{Agg}$ in SD-R CON and LD MT$_1$ hamsters. Regression lines were generated from Spearman's rank correlations within treatment groups (LD CON: $n = 11$, SD-R CON: $n = 10$, LD MT$_1$: $n = 9$, LD-M CON: $n = 12$, LD-M MT$_1$: $n = 16$). *Note: SD-NR CON hamsters showed no relationship between adrenal mt1 expression and number of attacks, and LD-M CON and SD-NR CON hamsters showed no relationships between adrenal mt1 expression and aggression duration nor adrenal mt1 expression and PC$_{Agg}$. These relationships are not depicted in this figure.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

generally not as pronounced nor as consistent across measures of aggressive behavior as those observed for SD-R CON and LD MT$_1$ hamsters (adrenal *mt1* expression and attacking behavior – Spearman's rank correlations: $\rho \leq |0.34|$, $n = 11$–12, $P \geq 0.27$; adrenal *mt1* expression and chasing behavior – Spearman's rank correlations: $\rho \leq |0.37|$, $n = 11$–12, $P \geq 0.26$; Fig. 8B; Supplementary Material, Table S3).

Conversely, LD CON and LD-M MT$_1$ hamsters showed negative associations between adrenal *mt1* expression and aggressive behavior, including number of attacks (LD CON – Spearman's rank correlation: $\rho = -0.44$, $n = 11$, $P = 0.18$; LD-M MT$_1$ – Spearman's rank correlation: $\rho =$

−0.37, $n = 16$, $P = 0.16$; Fig. 8A), attack duration (LD CON – Spearman's rank correlation: $\rho = -0.43$, $n = 10$, $P = 0.21$; LD-M MT_1 – Spearman's rank correlation: $\rho = -0.41$, $n = 14$, $P = 0.14$), aggression frequency (LD CON – Spearman's rank correlation: $\rho = -0.43$, $n = 11$, $P = 0.19$; LD-M MT_1 – Spearman's rank correlation: $\rho = -0.34$, $n = 16$, $P = 0.19$; Supplementary Material, Table S3), aggression duration (LD CON – Spearman's rank correlation: $\rho = -0.37$, $n = 11$, $P = 0.26$; LD-M MT_1 – Spearman's rank correlation: $\rho = -0.24$, $n = 16$, $P = 0.37$; Fig. 8C), and PC_{Agg} (LD CON – Spearman's rank correlation: $\rho = -0.54$, $n = 11$, $P = 0.09$; LD-M MT_1 – Spearman's rank correlation: $\rho = -0.24$, $n = 16$, $P = 0.37$; Fig. 8E). In addition, LD CON hamsters displayed negative relationships between adrenal *mt1* expression and chasing behavior (adrenal *mt1* expression and number of chases – Spearman's rank correlation: $\rho = -0.44$, $n = 11$, $P = 0.17$; adrenal *mt1* expression and chase duration – Spearman's rank correlation: $\rho = -0.47$, $n = 11$, $P = 0.15$) and a positive relationship between adrenal *mt1* expression and latency to first attack (Spearman's rank correlation: $\rho = 0.47$, $n = 11$, $P = 0.14$; Supplementary Material, Table S3). There was little evidence of a relationship between adrenal *mt1* expression and aggression, however, in SD-NR CON hamsters (all correlations: $\rho \leq |0.28|$, $n = 13$–15, $P \geq 0.32$; Fig. 8; Supplementary Material, Table S3).

3.9. Timed melatonin injections and LDs produced distinct correlations between adrenal mt1 expression and investigation, but not self-grooming

Hamsters treated with melatonin or LD photoperiods showed contrasting relationships between adrenal *mt1* expression and investigative behavior (Supplementary Material, Table S3). LD CON hamsters displayed positive associations between adrenal *mt1* expression and nose-to-nose investigation (adrenal *mt1* expression and NTN frequency – Spearman's rank correlation: $\rho = 0.73$, $n = 11$, $P = 0.01$; adrenal *mt1* expression and NTN duration – Spearman's rank correlation: $\rho = 0.64$, $n = 11$, $P = 0.04$), anogenital investigation (adrenal *mt1* expression and AGI frequency – Spearman's rank correlation: $\rho = 0.64$, $n = 11$, $P = 0.03$; adrenal *mt1* expression and AGI duration – Spearman's rank correlation: $\rho = 0.45$, $n = 11$, $P = 0.17$), investigation frequency and duration (adrenal *mt1* expression and investigation frequency – Spearman's rank correlation: $\rho = 0.65$, $n = 11$, $P = 0.03$; adrenal *mt1* expression and investigation duration – Spearman's rank correlation: $\rho = 0.55$, $n = 11$, $P = 0.09$), and PC_{Inv} (Spearman's rank correlation: $\rho = 0.60$, $n = 11$, $P = 0.06$). Conversely, LD-M CON hamsters exhibited negative associations between adrenal *mt1* expression and nose-to-nose investigation (adrenal *mt1* expression and NTN frequency – Spearman's rank correlation: $\rho = -0.52$, $n = 12$, $P = 0.08$; adrenal *mt1* expression and NTN duration – Spearman's rank correlation: $\rho = -0.58$, $n = 12$, $P = 0.05$), anogenital investigation (adrenal *mt1* expression and AGI frequency – Spearman's rank correlation: $\rho = -0.39$, $n = 12$, $P = 0.21$; adrenal *mt1* expression and AGI duration – Spearman's rank correlation: $\rho = -0.36$, $n = 12$, $P = 0.26$), investigation frequency and duration (adrenal *mt1* expression and investigation frequency – Spearman's rank correlation: $\rho = -0.49$, $n = 12$, $P = 0.11$; adrenal *mt1* expression and investigation duration – Spearman's rank correlation: $\rho = -0.49$, $n = 12$, $P = 0.11$), and PC_{Inv} (Spearman's rank correlation: $\rho = -0.49$, $n = 12$, $P = 0.11$). There was also some evidence of a negative relationship between adrenal *mt1* expression and investigation in SD-NR CON hamsters, although this relationship was not as pronounced as that observed in LD-M CON hamsters (all correlations: $\rho \leq |\text{-}0.39|$, $n = 15$, $P \geq 0.15$). Interestingly, SD-R CON and LD MT_1 hamsters showed a positive relationship between adrenal *mt1* expression and nose-to-nose investigation (SD-R CON – adrenal *mt1* expression and NTN duration: $\rho = 0.39$, $n = 10$, $P = 0.26$; LD MT_1 – adrenal *mt1* expression and NTN frequency: $\rho = 0.48$, $n = 9$, $P = 0.26$; LD MT_1 – adrenal *mt1* expression and NTN duration: $\rho = 0.52$, $n = 9$, $P = 0.15$), but adrenal *mt1* expression was not correlated with any other measures of investigative behavior in these hamsters (all other correlations: $\rho \leq |0.32|$, $n = 9$–10, $P \geq 0.41$). There was little evidence of an association between adrenal *mt1* expression and investigation, however, in LD-M MT_1 hamsters (all correlations: $\rho \leq |0.23|$, $n = 15$, $P \geq 0.39$).

Conversely, there were few significant correlations between adrenal *mt1* expression and self-grooming (Supplementary Material, Table S3). SD-NR CON hamsters showed a negative relationship between adrenal *mt1* expression and grooming (adrenal *mt1* expression and grooming frequency – Spearman's rank correlation: $\rho = -0.54$, $n = 15$, $P = 0.04$; adrenal *mt1* expression and grooming duration – Spearman's rank correlation: $\rho = -0.30$, $n = 15$, $P = 0.28$), whereas LD-M MT_1 hamsters showed a positive relationship between adrenal *mt1* expression and grooming (adrenal *mt1* expression and grooming frequency – Spearman's rank correlation: $\rho = 0.35$, $n = 16$, $P = 0.19$; adrenal *mt1* expression and grooming duration – Spearman's rank correlation: $\rho = 0.36$, $n = 16$, $P = 0.17$). In contrast, there was little evidence of an association between adrenal *mt1* expression and grooming in LD CON, SD-R CON, LD MT_1, and LD-M CON hamsters (all correlations: $\rho \leq |0.32|$, $n = 9$–12, $P \geq 0.37$).

4. Discussion

Here, we used a molecular genetic approach to assess the role of adrenal MT_1 receptors in modulating seasonal variation in energetic, reproductive, and behavioral responses in male Siberian hamsters. We showed that adrenal MT_1 overexpression produces SD-like changes in behavior, including increased aggression and decreased investigation and self-grooming. Conversely, timed melatonin administration, but not adrenal MT_1 overexpression, was necessary to induce characteristic reductions in body and reproductive tissue mass, suggesting that adrenal MT_1 receptors mediate seasonal variation in behavior, but not energetics or reproduction. Finally, we determined that male hamsters exhibit distinct relationships between adrenal *mt1* expression and social behavior across seasonal phenotypes, but that LD MT_1 and SD-R CON hamsters display similar associations between adrenal *mt1* expression and behavior, including aggression. To our knowledge, this study is the first to suggest a role for adrenal MT_1 receptor signaling in regulating seasonal changes in behavior.

4.1. Melatonin administration, but not adrenal MT_1 receptor overexpression induces characteristic changes in energetics and reproduction

As predicted, we found that a SD-like circulating melatonin signal, but not adrenal MT_1 overexpression, is necessary to induce gonadal regression and decrease body mass in male hamsters. Interestingly, although both LD-M and SD-R hamsters exhibited reductions in body and reproductive tissue mass, these changes were more pronounced in SD-R hamsters than in LD-M hamsters. These results are consistent with our previous work, in which we showed that long-term timed melatonin administration decreases body and reproductive tissue mass in LD male and female Siberian hamsters, but that SD-R hamsters tend to exhibit more marked reductions in these measures than LD-M hamsters (Munley et al., 2020; Munley et al., 2021; Rendon et al., 2020). Because the present study was 10 weeks in duration and prior studies suggest that seasonal changes in energetics and reproductive physiology take 10–12 weeks to fully emerge in this species (Drazen et al., 2001; Jasnow et al., 2000), these differences in the magnitude of gonadal regression and body mass reduction between LD-M and SD-R hamsters could suggest that MT_1 receptors in tissues other than the adrenal glands may be important in modulating energetic and reproductive responses to changes in photoperiod.

While our findings suggest that these physiological processes occur independently of adrenal MT_1 receptor signaling, previous work has shown that neural MT_1 receptors regulate seasonal changes in adiposity and reproductive physiology (Maywood and Hastings, 1995; Stevenson et al., 2017; reviewed in Walton et al., 2011; reviewed in Wood and Loudon, 2014). Pharmacological enhancement of MT_1 receptor

signaling via the agonist ramelteon and long-term melatonin administration to several forebrain sites (i.e., suprachiasmatic nucleus, dorsomedial hypothalamus, subzona incerta, and nucleus reuniens) induces gonadal regression and decreases body mass in LD male Siberian hamsters (Leitner and Bartness, 2010; Prendergast, 2010), suggesting that several melatonin-sensitive brain regions may mediate seasonal changes in energetics and reproduction. There is also evidence that neural MT_1 receptor abundance varies seasonally and in response to melatonin treatment in some species. SD photoperiods reduce MT_1 receptor density in the suprachiasmatic nucleus of male Syrian hamsters, and melatonin administration decreases MT_1 receptor abundance in the suprachiasmatic nucleus of LD male Syrian and Siberian hamsters (Schuster et al., 2001). Thus, it is likely that shifts in MT_1 receptor abundance and/or sensitivity in the brain, in addition to photoperiod-driven changes in circulating melatonin, are necessary to mediate the effects of SDs on energetics and reproduction. Our future studies will assess the role of neural MT_1 receptors in regulating seasonal changes in body mass and reproductive physiology in Siberian hamsters, specifically by examining the effects of hypothalamic MT_1 overexpression on these mechanisms using lentiviral vector infusions.

4.2. Adrenal MT_1 receptors modulate seasonal variation in social behavior

In the present study, we determined that adrenal MT_1 overexpression causes SD-like changes in social behavior, including increased aggression and decreased investigation and self-grooming. Specifically, we found that LD-M MT_1 hamsters exhibited an increase in chasing behavior and a decrease in grooming frequency relative to LD-M CON hamsters, and we showed that LD MT_1 hamsters displayed significant reductions in nose-to-nose investigation frequency and grooming duration relative to LD CON hamsters. Unexpectedly, similar changes in behavior were not observed in LD-M CON hamsters. Because we have previously shown that timed melatonin administration increases aggression in LD male and female hamsters (Munley et al., 2020; Munley et al., 2021; Rendon et al., 2020; Rendon et al., 2015), these results may be a consequence of lentiviral infusion and/or surgical procedures. Although we observed no differences in relative adrenal mass between treatment groups, it is possible that this procedure may have adversely affected neuroendocrine processes that mediate social behavior, such as adrenal androgen or glucocorticoid secretion. Despite these differences between the effects of adrenal MT_1 overexpression and timed melatonin injections on behavior, we found that LD-M CON hamsters generally exhibited positive relationships between adrenal *mt1* expression and social behavior (e.g., aggression), as observed in LD MT_1 and SD-R CON hamsters, whereas LD CON hamsters exhibited negative relationships between adrenal *mt1* expression, PC_{Soc}, and aggressive behavior. Moreover, LD-M CON hamsters showed negative associations between adrenal *mt1* expression and investigation, while LD CON hamsters showed positive associations between adrenal *mt1* expression and investigation. Collectively, these results suggest that both experimentally-induced and naturally-occurring variation in adrenal *mt1* receptor expression is related to social behavior in male hamsters and that this relationship is dependent on both circulating melatonin and adrenal MT_1 receptors.

Notably, we also found evidence that LD MT_1 and LD-M MT_1 hamsters exhibit differences in their neuroendocrine and behavioral responses to treatment. More specifically, LD-M MT_1 hamsters had lower circulating DHEA levels than all other treatment groups in our study and exhibited negative correlations between adrenal *mt1* expression and social behavior (e.g., aggression), which are similar to the relationships observed in LD CON hamsters, but contrast with the positive correlations between adrenal *mt1* expression and behavior that were observed in SD-R CON and LD MT_1 hamsters. Prior studies have shown that high (i.e., supraphysiological) concentrations of melatonin and prolonged melatonin exposure can cause MT_1 receptor desensitization, including an increase in MT_1 receptor density and decreased affinity of melatonin for the MT_1 receptor, in mammalian cell culture (Gerdin et al., 2004; Kokkola et al., 2007; reviewed in Liu et al., 2016; MacKenzie et al., 2002; reviewed in Witt-Enderby et al., 2003). Although LD-M MT_1 hamsters in our study were exposed to physiological concentrations of melatonin, it is possible that adrenal MT_1 overexpression induced by lentiviral vector infusion, in combination with the SD-like pattern of melatonin secretion produced by timed melatonin administration, resulted in MT_1 receptor desensitization in the adrenal glands and, ultimately, affected DHEA production and social behavior. Our findings and those of previous work suggest that the relationship between circulating melatonin and MT_1 receptor signaling is complex, and further investigation is necessary to determine how seasonal changes in melatonin secretion affect MT_1 receptor abundance and sensitivity in endocrine tissues and whether these processes alter behavior.

While our study is the first to examine the role of adrenal MT_1 receptors in modulating seasonal variation in social behavior, prior studies suggest that neural MT_1 receptors regulate behavior in both seasonally and non-seasonally breeding rodents. Male and female MT_1 receptor knockout C57BL/6 and C3H/HeN house mice (*Mus musculus*) display deficits in several behaviors compared to wild-type mice, including affective behaviors (i.e., anxiety- and depressive-like behaviors; Adamah-Biassi et al., 2014a; Comai et al., 2015; Liu et al., 2017; Weil et al., 2006), reward behavior (Clough et al., 2018; Clough et al., 2014; Comai et al., 2015), locomotion (Adamah-Biassi et al., 2014a; Pfeffer et al., 2017), and social interactions (Liu et al., 2017). Moreover, the MT_1/MT_2 receptor agonist ramelteon suppresses food intake in male Siberian hamsters (Prendergast, 2010), and melatonin-induced increases in depressive-like and aggressive behavior are blocked by the MT_1/MT_2 receptor antagonist luzindole in house mice (Dubocovich et al., 1990) and male California mice (*Peromyscus californicus*), respectively (Laredo et al., 2014). Taken together, these results and those of the present study suggest that both peripheral and neural MT_1 receptor signaling may be important in modulating social behavior. Additional studies are needed to distinguish the roles of peripheral and central MT_1 receptors in regulating behavior and to assess whether the relative importance of these signaling pathways differs across seasonal phenotypes.

4.3. Potential neuroendocrine mechanisms mediating the effects of adrenal MT_1 receptor signaling on behavior

Although our results suggest that adrenal MT_1 receptors regulate seasonal changes in social behavior, the neuroendocrine processes underlying this relationship are unclear. We have previously shown that male and female hamsters exhibiting a SD-like melatonin signal, either via timed melatonin administration or exposure to SDs, have higher levels of serum DHEA than LD hamsters and that circulating DHEA levels following a social challenge are positively associated with aggressive behavior (Munley et al., 2020; Rendon et al., 2020; Rendon et al., 2015). Furthermore, we have demonstrated that melatonin increases DHEA production in cultured adrenal glands from SD, but not LD female hamsters, whereas melatonin increases DHEA production in cultured ovaries from LD, but not SD females (Rendon et al., 2015), suggesting that melatonin acts directly on the adrenal glands to elevate DHEA secretion and aggression during SDs. In this study, however, we found little evidence that adrenal MT_1 overexpression alters DHEA production, since LD MT_1 hamsters showed no significant difference in circulating DHEA concentration relative to LD CON hamsters. Thus, our findings suggest that circulating melatonin, but not adrenal MT_1 receptor expression, is important in increasing adrenal DHEA secretion during the non-breeding season.

Because adrenal MT_1 overexpression does not influence DHEA production, it is likely that adrenal MT_1 receptors mediate seasonal changes in behavior via signaling mechanisms with neural substrates. Indeed, MT_1 receptors have been characterized in both the brain and peripheral endocrine tissues, including the hypothalamus (e.g., suprachiasmatic

nucleus and paraventricular nucleus; Lacoste et al., 2015; Weaver et al., 1989; Wu et al., 2006), midbrain (Green et al., 2015; Lacoste et al., 2015), pars tuberalis (Adamah-Biassi et al., 2014b; Williams et al., 1997), and gonads (Frungieri et al., 2005; McGuire et al., 2011). Notably, MT_1 receptors have been localized to each of the tissues that comprise the hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine circuit that is important in facilitating increased non-breeding aggression in some seasonally breeding species, including Siberian hamsters (reviewed in Munley et al., 2018). Furthermore, several of the brain regions in which MT_1 receptors are present have connections with nodes of the social behavior network, a collection of midbrain, hypothalamic, and basal forebrain nuclei that have been implicated in the regulation of social behavior in vertebrates (Goodson, 2005; O'Connell and Hofmann, 2011), including aggression (Delville et al., 2000; Fuxjager et al., 2010; reviewed by Nelson and Trainor, 2007). In further support of this hypothesis, we recently showed that timed melatonin administration and exposure to SDs decreases concentrations of DHEA, T, and E_2 in the anterior hypothalamus and periaqueductal gray, two brain regions associated with aggression (Munley et al., 2021), suggesting that seasonal changes in steroid hormone synthesis in the brain are dependent, at least in part, on melatonin signaling mechanisms. Our future research will examine how these melatonin-dependent changes in neurosteroids are regulated by MT_1 receptors.

5. Conclusions

In the current study, we showed that lentiviral-mediated overexpression of the MT_1 melatonin receptor in the adrenal glands results in SD-like changes in behavior, but not body or reproductive tissue mass in male Siberian hamsters. Specifically, we found that hamsters that were infused with the MT_1 lentivirus or were responsive to SD photoperiods exhibited an increase in aggressive behavior and a decrease in investigative and self-grooming behavior, suggesting a role for adrenal MT_1 receptor signaling in mediating seasonal changes in social behavior, but not energetics or reproduction. Additional studies are needed to characterize the neuroendocrine processes by which adrenal MT_1 receptors modulate social behavior and to assess whether the actions of adrenal MT_1 receptors on behavior occur via direct or indirect mechanisms. Collectively, these findings enhance our understanding of how melatonin, a biochemical cue that is essential in enabling animals to coordinate physiological and behavioral responses with the appropriate time of year, acts via the MT_1 receptor to regulate behavior in seasonally breeding species. More broadly, our study emphasizes how natural selection has acted, and will continue to act, on the neuroendocrine mechanisms that promote organismal fitness in a season-specific manner.

CRediT authorship contribution statement

K.M.M., A.M.J., and G.E.D. designed the experiments. S.D. and A.M.J. developed the lentiviral vectors. K.M.M. performed surgical procedures, administered photoperiodic treatment and timed melatonin injections, staged behavioral interactions, performed necropsies, collected blood and tissue samples, determined reproductive phenotypes, scored behavioral videos, performed qPCR, ran hormone assays, and conducted statistical analyses. K.M.M. and G.E.D. interpreted the data and drafted the manuscript, with editorial contributions from S.D. and A.M.J.

Declaration of competing interest

The authors have no competing interests to disclose, financial or otherwise.

Data availability

Data for this study are available in Mendeley Data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2021.105099.

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