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Photoperiod Affects Neuronal Nitric Oxide Synthase and Aggressive Behaviour in Male Siberian Hamsters (*Phodopus sungorus*)

J. C. Wen,*[a] A. K. Hotchkiss,* G. E. Demas† and R. J. Nelson*
*Departments of Psychology and Neuroscience, Ohio State University, Columbus, OH, USA.
†Department of Biology, Indiana University, Bloomington, IN, USA.

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Abstract

Many nontropical animals display physiological and behavioural changes in response to seasonal environmental cues including photoperiod (day length). Male Siberian hamsters (*Phodopus sungorus*) housed in short photoperiod undergo testicular regression accompanied by reduced circulating testosterone and decreased reproductive behaviour. By contrast to the majority of small mammals studied, aggressive behaviour is elevated in short-day Siberian hamsters when blood testosterone concentrations are not detectable. Because gonadal steroid hormones influence neuronal nitric oxide synthase (nNOS), and this enzyme has been implicated in aggressive behaviour, we hypothesized that nNOS expression would be decreased in short-day male Siberian hamsters and negatively correlated with the display of territorial aggression. Adult male Siberian hamsters were individually housed in either long (LD 16 : 8 h) or short (LD 8 : 16 h) photoperiods for 10 weeks. Hamsters were assigned to one of two categories by assessing testicular volume and plasma testosterone values: (i) photoperiodic responsive (i.e. regressed testes and low testosterone concentrations) or (ii) photoperiodic nonresponsive (i.e. testes size and circulating testosterone concentrations equivalent to hamsters maintained in long days). At week 10, aggression was assessed using a resident–intruder test. Latency to initial attack, frequency of attacks and duration of total attacks were recorded during a 10-min aggression trial. Brains were collected immediately after behavioural testing and stained for nNOS expression using immunohistochemistry. All short day-housed hamsters were significantly more aggressive than long-day animals, regardless of gonadal size or testosterone concentrations. Short-day animals, both reproductively responsive and nonresponsive morphs, also had significantly less nNOS-immunoreactive cells in the anterior and basolateral amygdaloid areas and paraventricular nuclei compared to long-day hamsters. Together, these results suggest that seasonal aggression in male Siberian hamsters is regulated by photoperiod, through mechanisms that are likely independent from gonadal steroid hormones.

Many nontropical animals display seasonal morphological, physiological and behavioural changes in response to environmental factors; one of the most salient seasonal cues is photoperiod, or day length (1, 2). Individuals of many mammalian species undergo marked seasonal adaptations when exposed to changes in day length under laboratory conditions. For example, rodents maintained in short, winter-like photoperiods (e.g. < 12 h of light/day) display decreased body mass, changes in pelage colour, gonadal regression (including reduction of gonadotropin and gonadal steroid hormone secretion, which in turn affects reproductive activity), changes in general locomotor activity and several other behavioural adjustments (3). These changes are primarily due to the seasonal changes in the pattern of pineal melatonin secretion, which is directly correlated to the prevailing photoperiod (4). Presumably, these morphological, physiological and behavioural responses to the seasons help individuals cope with the changing environment and are adaptive.

Siberian hamsters (*Phodopus sungorus*) show robust seasonal adjustments mediated by photoperiod. Hamsters

Correspondence to: Professor Randy J. Nelson, Department of Psychology, Ohio State University, Columbus, OH 43210, USA (e-mail: rnelson@osu.edu).
[a]Present address: Department of Psychology, University of Chicago, Chicago, IL 60637, USA.

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housed in short photoperiods (< 13 h light/day) for prolonged periods display coat colour changes from the summer black-brown to 'winter-white', and also reduce general locomotor activities and body mass, as well as gonadal size and function (5). As the gonads undergo regression, plasma concentrations of gonadal steroid hormones fall to undetectable levels (3). In most rodent species studied to date, reduction in circulating testosterone concentrations result in reduced mating and aggressive behaviours (6, 7). However, both Syrian (*Mesocricetus auratus*) and Siberian hamsters display increased aggressive behaviour in short photoperiods despite low circulating gonadal steroids (8–10).

Another factor that has an important role in regulating aggression is neuronal nitric oxide synthase (nNOS or NOS-1; 11). nNOS produces the neurotransmitter, nitric oxide (NO), as a byproduct of the conversion of arginine into citrulline in the central and peripheral nervous systems (12, 13). Nitric oxide from neurones appears to be involved in regulating some aggressive behaviours. For example, male mice with targeted disruption of the nNOS gene (nNOS–/–) display sustained aggressive behaviour and persistent sexual behaviour (11, 14). Pharmacological treatment with 7-nitroindazole (7-NI), a selective nNOS inhibitor, also elevates aggression in wild-type mice, suggesting that the aggressive behaviour in nNOS–/– mice is caused by loss of NO from neuronal sources, rather than developmental abnormalities (15). Such aggressive behaviour in nNOS–/– mice is due, in part, to selective decrement in serotonin (5-HT) turnover and deficiency of serotonin receptors 5-HT_{1A} and 5-HT_{1B} function in brain regions regulating emotion (16). However, inappropriate aggressive behaviour has been observed only in male nNOS–/– mice; female nNOS–/– mice display little or no aggression and a decrease in maternal aggression (11, 17). Castration and testosterone replacement studies have shown that testosterone is necessary, but not sufficient, to provoke elevated aggressive behaviour in nNOS–/– mice (18). Furthermore, nNOS expression and enzymatic activities in the hypothalamus and amygdala are negatively correlated with blood testosterone concentrations (19). Together, these data for nNOS–/– mice are consistent with the well documented relationship between androgens and aggression (7).

Siberian hamsters, unlike inbred strains of mice and rats that are not reproductively responsive to photoperiod, provide a naturalistic model for the study of seasonal fluctuations in aggressive behaviours, as well as the neuroendocrine mechanisms underlying these behavioural changes. Although the photoperiodic effect on aggression in hamsters has been previously reported (8, 10), to our knowledge, no study has examined the role of photoperiod on neuronal nitric oxide synthase and aggression. An additional advantage to studying Siberian hamsters is the natural variation in reproductive responsiveness to photoperiod (i.e. within a population of individuals, a small subset of animals remains reproductively unresponsive to photoperiod changes) (20–22). The neuroendocrine mechanisms by which such individuals maintain reproductive function despite the inhibitory short-day signals vary among species. Individuals of some species (e.g. Siberian hamsters) differ in aspects of the circadian pacemaker, whereas individuals of other species (e.g. deer mice) display alterations in target site responsiveness to melatonin (22). Despite their potential value in testing the neuroendocrine regulation of behaviour, the behavioural characteristics of nonresponsive animals are often overlooked.

Thus, the goal of the present study was to examine how photoperiod may affect the nNOS pathway and its regulation of aggression in Siberian hamsters. We manipulated photoperiod, assessed aggressive behaviour using a resident–intruder model, and measured nNOS expression in the anterior (AA) and basolateral amygdaloid area (BLA), as well as the paraventricular nucleus (PVN). The basolateral amygdaloid area was chosen because this nucleus has been reported to mediate intermale aggression (23). The PVN was chosen because it contains a large number of vasopressinergic neurones and is also an integral part of the hypothalamic-pituitary-adrenal (HPA) neuroendocrine axis; both of these systems have been implicated in aggression in rodents (24). Finally, The AA was used as a control area because this brain region had not been previously associated with aggressive behaviours in Siberian hamsters (17). We hypothesized that, because Siberian hamsters display photoperiodic variation in aggression and because NO mediates aggression in mice, photoperiodic changes in aggression may be modulated by the nNOS pathway. Specifically, we predicted that aggressive behaviour in short-day Siberian hamsters would be negatively correlated with nNOS expression in select brain regions regulating aggression.

Materials and methods

Animals and housing

Adult (> 60 days of age) male Siberian hamsters (*P. sungorus*) were obtained from our laboratory breeding colony at The Ohio State University. Animals were weaned at 21 days of age and housed with same-sex siblings in polypropylene cages (28 × 17 × 12 cm) with a 16 : 8 h light/dark cycle (lights on 22.00 h EST). Ambient temperature of the room was 22 ± 2 °C and relative humidity was maintained at 50 ± 5%. Food (LabDiet 5001; PMI Nutrition, Brentwood, MO, USA) and water were provided *ad libitum*, and cotton nesting material was available in the cage throughout the experiment. Two weeks before the experiment, hamsters were housed individually in a colony room with a long photoperiod (LD 16 : 8 h). Additional hamsters used as nonaggressive intruders during the behavioural testing were group-housed siblings (three to five per cage), because group-housed hamsters are generally not aggressive (25). These animals were the same age as experimental animals and were housed in long-day conditions (LD 16 : 8 h). All animals were treated in accordance with the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Photoperiod manipulation

Siberian hamsters (n = 24) were randomly assigned into either short days (n = 14) (LD 8 : 16 h, lights on 06.00 hours EST) or the long-day control group (n = 10) (LD 16 : 8 h) for 10 weeks. Such light/dark cycle schedules are effective in inducing photoperiod responses in Siberian hamsters, and 8 weeks is sufficient to induce seasonal adaptations in this species (5, 26). Photoperiodic responsiveness was evaluated based on changes in body mass and estimated testicular size at week 10. The testicular size was estimated by measuring the length and width of the testes through the abdominal skin. Actual testis mass was also measured postmortem for confirmation. Short-day hamsters that did not show significant reduction of body mass and testicular size were categorized as nonresponders and separated from responders during the subsequent statistical analysis.

Testosterone radioimmunoassay (RIA)

One day before behavioural testing, animals were lightly anaesthetized and blood samples (100 μl) were drawn from the retro-orbital sinus. The blood samples were centrifuged at 4 °C for 30 min at 2500 r.p.m and the supernatant was collected and stored at −74 °C until assayed. Testosterone concentrations were determined in duplicate in a single assay using an ^{125}I RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). All of the instructions provided by the manufacturer were followed. The lower limit of the assay was 0.05 ng/ml and the intra-assay coefficient of variation was < 10.0%.

Behavioural testing

Aggressive behaviour was assessed using a resident–intruder model (27). During the first 2 h after lights were extinguished, a group-housed nonaggressive intruder was introduced into the home cage of the experimental animal for 10 min, while agonistic behaviour was videotaped. A small patch of skin on the dorsal surface of intruder animals was shaved for purposes of identification. The bedding in the home cages remained unchanged for 10 days before testing to maintain the territorial defensiveness of the experimental animal. An observer who was uninformed about the experimental treatment groups scored for latency to first attack, total number of attacks and duration of attacks.

Immunohistochemistry

Immediately following the aggressive encounter, hamsters were deeply anaesthetized with an overdose of sodium pentobarbital. Hamsters were perfused with 0.9% saline, followed by 4% paraformaldehyde in phosphate-buffered saline (PBS) (0.1 M, pH = 7.4). Following the perfusion, the brains were collected, postfixed over night at 4 °C, placed in 20% glucose in PBS cryoprotectant at 4 °C for 2 days, and then frozen in crushed dry ice and stored in −74 °C until staining. Free floating sections were stained using the avidin–biotin peroxidase system. The brains were sliced into 40-μm sections using a cryostat, washed in anti-nNOS PBS (10 mM, pH = 7.4), incubated with 3% hydrogen peroxide, washed in PBS with 0.2% Triton X-100 (PBS-X), blocked with 5% normal goat serum in PBS-X, and incubated overnight at 4 °C with rabbit anti-nNOS (C-terminal) antibodies (10 : 1000) (ImmunoStar, Inc., Hudson, WI, USA). After washes in PBS-X, the sections were exposed to secondary antibodies (1 : 500) and avidin–biotin complex with VECTASTAIN Elite ABC Kit (Rabbit IgG) and visualized using VECTOR VIP Substrate Kit (VECTOR Laboratories, Burlingame, CA, USA). The sections were mounted on slides, coverslipped and sealed with Permount (Fisher Scientific, Pittsburgh, PA, USA).

Cell counting

Consecutive alternating frontal brain sections were taken from each animal starting from the merging point of the anterior commissure. Sections containing anterior amygdaloid area (AA), basolateral amygdaloid area (BLA) and paraventricular nucleus (PVN) were identified according to the mouse atlas by Franklin and Paxinos (28), and nNOS-immunoreactive (nNOS-ir) cells within these areas were counted at ×200 magnification under bright-field microscopy. Quantification of nNOS-immunoreactive cells was performed by an individual who was uninformed about experimental treatments; a random sample of cells were recounted by a second uniformed individual, and cell numbers varied by < 2%.

Statistical analysis

Differences between treatment means were analysed using separate one-way analysis of variance for behavioural and immunohistochemical data (StatView 5, SAS Institute, Cary, NC, USA). Post-hoc comparisons were conducted using Fisher's PLSD test. Correlations between nNOS-immunoreactive cell counts and behavioural data were also calculated. In all cases, mean differences and other results were considered statistically significant at $P < 0.05$.

Results

Of all the hamsters housed in the short-day condition, 50% (n = 7) were nonresponsive in terms of changes in body mass, testicular mass and plasma testosterone concentrations. Short-day responders weighed significantly less than short-day nonresponders ($P < 0.0001$) or long-day hamsters ($P < 0.0001$), whereas short-day nonresponders had similar body mass to long-day animals ($P > 0.05$) (Fig. 1A). Short-day responders also had smaller testes than nonresponders ($P < 0.0001$) or long-day hamsters ($P < 0.0001$); again, nonresponders had similar testicular masses to long-day animals ($P > 0.05$) (Fig. 1B). Short-day responders had lower plasma testosterone concentrations compared to nonresponders ($P < 0.05$) or long-day animals ($P < 0.05$); nonresponders had similar plasma testosterone concentrations compared to long-day animals ($P > 0.05$) (Fig. 1C).

Long-day hamsters were significantly less aggressive and were slower to initiate the first attack than short-day

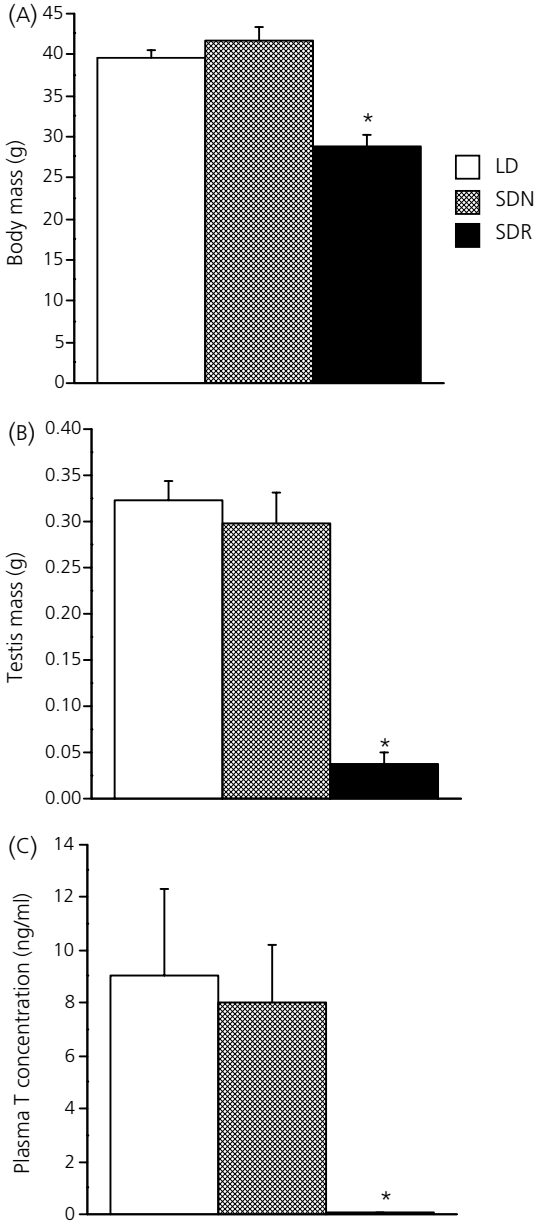

FIG. 1. Mean ± SEM body mass (A), testes mass (B) and plasma testosterone concentration (C) of adult male Siberian hamsters housed in either long-day (LD 16 : 8 h) or short-day (LD 8 : 16 h) conditions after 10 weeks. LD, Long-day; SDN, short-day nonresponders; SDR, short-day responders. Significant differences between pair-wise means are indicated by an asterisk (*$P < 0.05$).

responders ($P < 0.01$) or nonresponders ($P < 0.05$). Short-day responders and nonresponders were equally aggressive and required less time to initiate the first attack ($P > 0.05$) (Fig. 2A). Long-day hamsters attacked significantly less frequently than short-day responders ($P < 0.05$) or nonresponders ($P < 0.01$); whereas short-day responders and nonresponders did not differ in attack frequency ($P > 0.05$) (Fig. 2b). There was a marginal effect of photoperiod on duration of attacks [$F(2,20) = 3.39$, $P = 0.054$] (Fig. 2c).

Long-day hamsters had significantly more nNOS-ir cells in the AA than short-day responders ($P < 0.005$) or nonresponders ($P < 0.005$); short-day responders and nonresponders did not differ on this parameter ($P > 0.05$). Long-day hamsters had more nNOS-ir cells in the PVN than short-day nonresponders ($P < 0.05$) and a marginal difference to short-day responders ($P = 0.06$). There was no effect of photoperiod on nNOS-ir cell counts in the BLA [$F(2,20) = 2.809$, $P = 0.08$] (Fig. 3). However, when short-day responder and nonresponder groups are pooled together, long-day hamsters had significantly more nNOS-ir cells in both the PVN ($P < 0.05$) and BLA ($P < 0.05$). Latency to first attack significantly correlated with nNOS-ir cell counts in the AA ($r = 0.6$, $P < 0.005$), BLA ($R = 0.59$, $P < 0.005$) and PVN ($r = 0.47$, $P < 0.05$). In addition, there was a significant correlation between latency to first attack and total nNOS-ir cell counts in these areas ($r = 0.64$, $P < 0.005$) (Fig. 4).

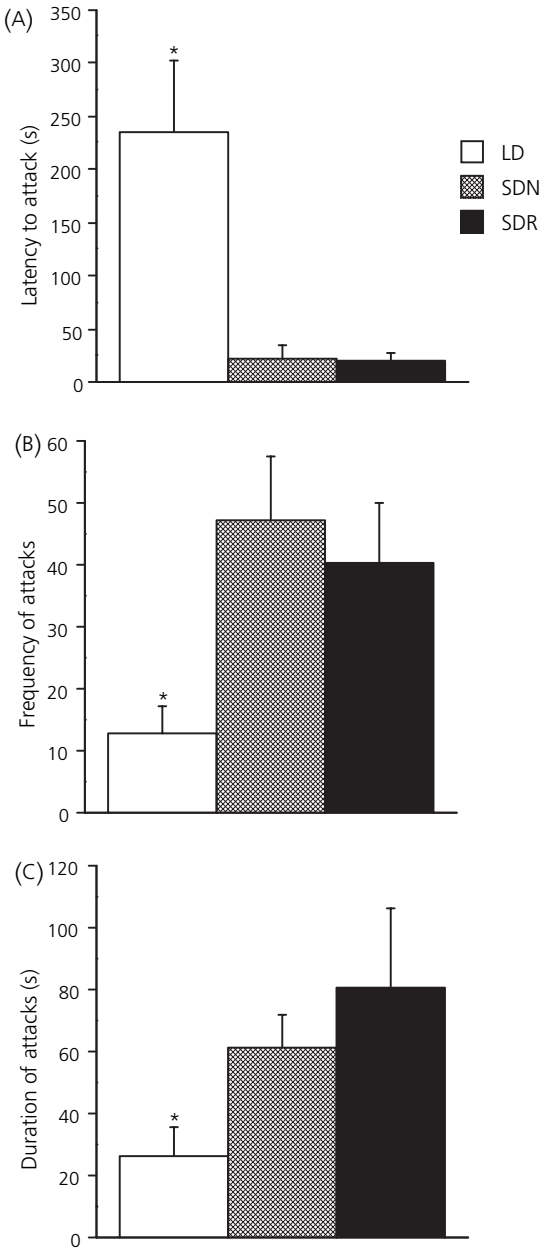

FIG. 2. Mean ± SEM latency to initial attack (A), frequency of attacks (B) and total duration of attacks (C) of adult male Siberian hamsters housed in either LD 16 : 8 h or LD 8 : 16 h after 10 weeks tested in resident-intruder model. LD, Long-day; SDN, short-day nonresponders; SDR, short-day responders. Significant differences between pair-wise means are indicated by an asterisk (*$P < 0.05$).

Discussion

The results of the present study support the hypothesis that photoperiod affects nNOS expression, thereby suggesting a

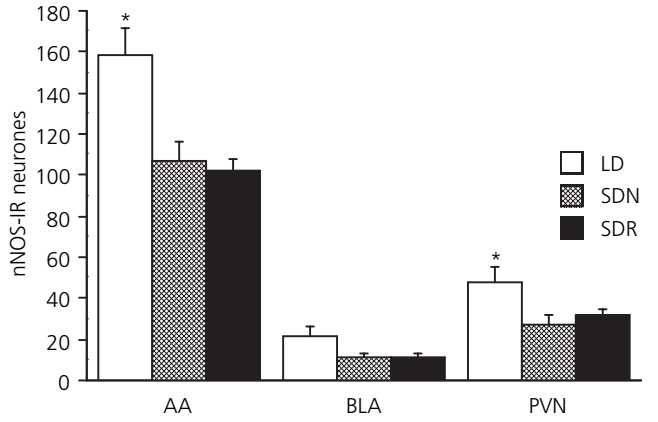

FIG. 3. Mean ± SEM number of neuronal nitric oxide synthase (nNOS)-immunoreactive cells in anterior amygdaloid area (AA), basolateral amygdaloid area (BLA) and paraventricular nucleus (PVN) of adult male Siberian hamsters housed in either long-day (LD 16 : 8 h) or short-day (LD 8 : 16 h) conditions after 10 weeks. LD, Long-day; SDN, short-day nonresponders; SDR, short-day responders. Significant differences between pairwise means are indicated by an asterisk (*$P < 0.05$).

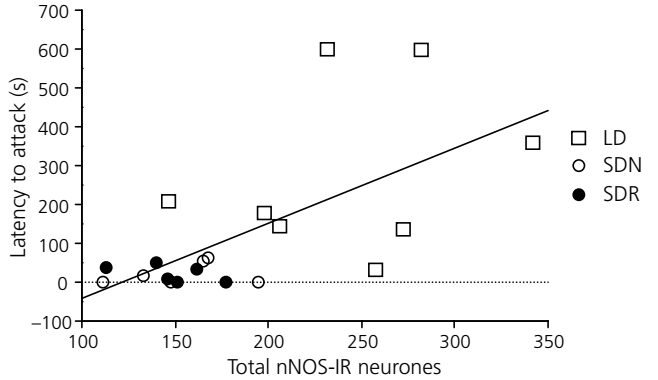

FIG. 4. Linear regression of latency to initial attack and total number of neuronal nitric oxide synthase (nNOS)-immunoreactive cells in AA, BLA and PVN of adult male Siberian hamsters housed in either LD 16 : 8 h or LD 8 : 16 h after 10 weeks. LD, Long-day; SDN, short-day nonresponders; SDR, short-day responders. $r = 0.64$, $P < 0.005$.

potential involvement of nNOS in short-day aggression in Siberian hamsters. Short-day hamsters were more aggressive and had fewer nNOS-ir cells in the anterior and basolateral amygdaloid areas and paraventricular nucleus compared to long-day animals. These results confirm and extend previous studies reporting that short photoperiod increases aggression in hamsters (8–10). The reduction of nNOS expression in the BLA and PVN in the short photoperiod suggests the potential involvement of nNOS in photoperiod-induced aggression. The reduction of nNOS expression in the AA, a control region not previously associated with aggression, although not examined in this species in such a context, at least suggests that short photoperiods in general decrease nNOS expression in these areas. This general reduction of nNOS expression in short photoperiod also correlates with increased aggressive behaviour, which is consistent with previous findings that nNOS negatively regulates aggressive behaviour (15). However, there were no differences in aggressive behaviour or nNOS expression between short-day reproductively responsive and nonresponsive animals, which suggests that photoperiodic differences in nNOS expression are independent of gonadal steroid hormones, and likely to be a direct result of short photoperiod operating through melatonin-evoked changes. Together, these results support the hypothesis that photoperiod affects nNOS expression, which may be related to the short-day evoked aggression previously reported in Siberian hamsters.

The evidence suggesting that testosterone regulates aggressive behaviour is pervasive in behavioural endocrinology. Thus, it is notable that short-day aggression in hamsters is independent from testosterone, and possibly regulated directly by photoperiod via melatonin. However, several studies suggest that aggression can be induced with short-term melatonin injections mimicking short photoperiods in house mice (*Mus musculus*) (29), Syrian hamsters (*Mesocricetus auratus*) (30), and Siberian hamsters (31). These studies also suggest the involvement of adrenal hormones in melatonin-induced aggression through the HPA axis. Melatonin-induced aggression can be attenuated by adrenalectomy in Siberian hamsters (31), which strongly suggests the involvement of the HPA axis and adrenal hormones in short-day aggression. One of the adrenal androgens, dehydroepiandrosterone (DHEA), has been proposed to play an important role in territorial aggression during the nonbreeding season in song birds (*Melospiza melodia morphna*) (32) and spotted antbirds (*Hylophylax n. naevioides*) (33). These birds display territorial aggression throughout the nonbreeding season, during which they secrete virtually no gonadal steroids; DHEA concentrations are positively correlated with aggression in birds, and may serve as a source of androgen (33). DHEA can be metabolized into testosterone or aromatized into oestradiol in the brain, and therefore is potentially capable of activating aggressive behaviour. More recently, it has been demonstrated that DHEA can stimulate nitric oxide release from endothelial cells via cell-surface receptors (34), suggesting a potentially important role for this hormone in the regulation of aggression by NO. DHEA concentrations are unaffected by castration in Syrian hamsters (35), which suggest that DHEA is a potential source of androgen for gonadally regressed short-day hamsters. Collectively, these data suggest a potential mechanism to explain short-day aggression in Siberian hamsters through DHEA.

The literature generally supports the hypothesis that nNOS is regulated by steroid hormones (36). Typically, testosterone down-regulates nNOS through androgen receptors (37), whereas oestradiol up-regulates nNOS through oestrogen receptors (38). However, the lack of difference in nNOS expression between short-day responders and nonresponders, which had low and high testosterone concentrations, respectively, again supports a photoperiodic effect that is independent of gonadal steroids. One previous study has suggested that androgenic effects in the medial amygdaloid area are photoperiod-dependent in Siberian hamsters (39), and nNOS expression in the anterior and basolateral amygdaloid areas and paraventricular nucleus may be regulated through similar mechanisms. Photoperiod may affect nNOS expression in the limbic system directly or potentially through the HPA axis. Adrenalectomy blocks the reduction of nNOS activity in the paraventricular nucleus in rats under food deprivation (40), suggesting a regulatory function of adrenal steroids (i.e. glucocorticoids, DHEA) on nNOS. Short-day Siberian hamsters have higher plasma glucocorticoids concentrations than long-day hamsters (41). Considered together, these data suggest a potential pathway regulating nNOS expression that is independent of gonadal steroid hormones.

The gonadal steroid-independent aggression in short days suggests that such aggression is probably unrelated to reproductive behaviour. Competition for mates is not likely to be an important selective pressure outside of the breeding season for Siberian hamsters, given that they are not monogamous. However, competing for scarce food and other resources in winter is critical for survival, and more dominant animals may better secure the necessary resources to survive and therefore ensure future reproductive success. Regulation through the HPA axis might also mean that short-day aggression is related to winter stressors, and may reflect the environmental selection pressures confronting individuals of this species in their tundra habitat. Regardless of the adaptive significance of such short-day mediated aggression, the current data suggest a photoperiodic effect on nNOS expression in anterior and basolateral amygdaloid areas and the paraventricular nucleus. Importantly, nNOS expression correlates with seasonal aggression, and this relationship is likely to be independent of gonadal steroid hormones. Collectively, these results suggest that seasonal changes in agonistic behaviour are regulated, at least in part, by changes in neuronal nitric oxide with specific brain regions in Siberian hamsters, and possibly other seasonally breeding species as well.

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References

1 Goldman BD. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *J Biol Rhythms* 2001; **16:** 283–301.
2 Prendergast BJ, Mosinger B Jr, Kolattukudy PE, Nelson RJ. Hypothalamic gene expression in reproductively photoresponsive and photorefractory Siberian hamsters. *Proc Natl Acad Sci USA* 2002; **99:** 16291–16296.
3 Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res* 1993; **15:** 161–190.
4 Elliot JA, Goldman BD. Seasonal reproduction: Photoperiodism and biological clocks. In: Adler N, Adler T, eds. *Neuroendocrinology of Reproduction: Physiology and Behavior*. New York, NY: Plenum Press, 1981: 377–423.
5 Hoffmann K. The critical photoperiod in the Djungarian hamster *Phodopus sungorus*. In: Aschoff J, Daan S, Groon G, ed. *Vertebrate Circadian Systems*. Berlin: Springer, 1982: 297–304.
6 Knol BW, Egberink-Alink ST. Androgens, progestagens and agonistic behaviour: a review. *Vet Q* 1989; **11:** 94–101.
7 Rubinow DR, Schmidt PJ. Androgens, brain, and behaviour. *Am J Psychiatry* 1996; **153:** 974–984.
8 Garrett JW, Campbell CS. Changes in social behavior of the male golden hamster accompanying photoperiodic changes in reproduction. *Horm Behav* 1980; **14:** 303–319.
9 Badura LL, Nunez AA. Photoperiodic modulation of sexual and aggressive behavior in female golden hamsters (*Mesocricetus auratus*): role of the pineal gland. *Horm Behav* 1989; **23:** 27–42.
10 Jasnow AM, Huhman KL, Bartness TJ, Demas GE. Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). *Horm Behav* 2000; **38:** 102–110.
11 Nelson RJ, Demas GE, Huang P, Fishman MC, Dawson VL, Dawson TM, Snyder SH. Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* 1995; **378:** 383–386.
12 Eliasson MJ, Blackshaw S, Schell MJ, Snyder SH. Neuronal nitric oxide synthase alternatively spliced forms: prominent functional localizations in the brain. *Proc Natl Acad Sci USA* 1997; **94:** 3396–3401.
13 Nelson RJ, Chiavegatto S. Molecular basis of aggression. *Trends Neurosci* 2001; **24:** 713–719.
14 Nelson RJ, Kriegsfeld LJ, Dawson VL, Dawson TM. Effects of nitric oxide on neuroendocrine function and behavior. *Front Neuroendocrinol* 1997; **18:** 463–491.
15 Demas GE, Eliasson MJ, Dawson TM, Dawson VL, Kriegsfeld LJ, Nelson RJ, Snyder SH. Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. *Mol Med* 1997; **3:** 610–616.
16 Chiavegatto S, Dawson VL, Mamounas LA, Koliatsos VE, Dawson TM, Nelson RJ. Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. *Proc Natl Acad Sci USA* 2001; **98:** 1277–1281.
17 Gammie SC, Nelson RJ. Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. *J Neurosci* 1999; **19:** 8027–8035.
18 Kriegsfeld LJ, Dawson TM, Dawson VL, Nelson RJ, Snyder SH. Aggressive behavior in male mice lacking the gene for neuronal nitric oxide synthase requires testosterone. *Brain Res* 1997; **769:** 66–70.
19 Singh R, Pervin S, Shryne J, Gorski R, Chaudhuri G. Castration increases and androgens decrease nitric oxide synthase activity in the brain: physiologic implications. *Proc Natl Acad Sci USA* 1999; **97:** 3672–3677.
20 Hoffmann K. Effects of short photoperiods on puberty, growth and molt in the Djungarian hamsters (*Phodopus sungorus*). *J Reprod Fertil* 1978; **54:** 29–35.
21 Hoffmann K. Photoperiod, pineal, melatonin and reproduction in hamsters. *Prog Brain Res* 1979; **52:** 397–415.
22 Prendergast BJ, Kriegsfeld LJ, Nelson RJ. Photoperiodic polyphenisms in rodents. Neuroendocrine mechanisms, costs, and functions. *Q Rev Biol* 2001; **76:** 293–325.
23 McGregor A, Herbert J. Differential effects of excitotoxic basolateral and corticomedial lesions of the amygdala on the behavioural and endocrine responses to either sexual or aggression-promoting stimuli in the male rat. *Brain Res* 1992; **574:** 9–20.
24 Delville Y, De Vries GJ, Ferris CF. Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain Behav Evol* 2000; **55:** 53–76.
25 Brain PF. Effects of isolation/grouping on endocrine and fighting in male and female golden hamsters (*Mesocricetus auratus*). *Behav Biol* 1972; **7:** 349–357.
26 Prendergast BJ, Flynn AK, Zucker I. Triggering of neuroendrocrine refractoriness to short-day patterns of melatonin in Siberian hamsters. *J Neuroendocrinol* 2000; **12:** 303–310.
27 Crawley JN. *What's Wrong with My Mouse: Behavioral Phenotyping of Transgenic and Knockout Mice*. New York, NY: Wiley-Liss, 2000.
28 Franklin KBJ, Paxinos G. *The Mouse Brain: in Stereotaxic Coordinates*. San Diego, CA: Academic Press, 1997.
29 Paterson AT, Vickers C. Melatonin and the adrenal cortex: Relationship to territorial aggression in mice. *Physiol Behav* 1981; **27:** 983–987.
30 Jasnow AM, Huhman KL, Bartness TJ, Demas GE. Short-days and exogenous melatonin increase aggression of male Syrian hamsters (*Mesocricetus auratus*). *Horm Behav* 2002; **42:** 13–20.
31 Demas GE, Polacek KM, Durazzo A, Jasnow AM. Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*) 2004; **46:** 582–591.
32 Soma KK, Wingfield JC. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. *Gen Comp Endocrinol* 2001; **123:** 144–155.
33 Hau M, Stoddard ST, Soma KK. Territorial aggression and hormones during the non-breeding season in a tropical bird. *Horm Behav* 2003; **45:** 40–49.
34 Liu D, Dillon JS. Dehydroepiandrosterone stimulates nitric oxide release in vascular endothelial cell: evidence for a cell surface receptor. *Steroids* 2004; **69:** 279–289.
35 Pieper DR, Lobocki CA. Characterization of serum dehydroepiandrosterone secretion in golden hamsters. *Proc Soc Exp Biol Med* 2000; **224:** 278–284.
36 Scordalakes EM, Shetty SJ, Rissman EF. Roles of estrogen receptor α and androgen receptor in the regulation of neuronal nitric oxide synthase. *J Comp Neurol* 2002; **453:** 336–344.
37 Hull EMJ, Lorrain DS, Matuszewich L. Testosterone, preoptic dopamine, and copulation in male rats. *Brain Res Bull* 1997; **44:** 327–333.
38 Warembourg M, Leroy D, Jolivet A. Nitric oxide synthase in the guinea pig preoptic area and hypothalamus: distribution, effect of estrogen, and colocalization with progesterone receptor. *J Comp Neurol* 1999; **407:** 207–227.
39 Cooke BM, Hegstrom CD, Breedlove SM. Photoperiod-dependent response to androgen in the medial amygdala of the Siberian hamster, *Phodopus sungorus*. *J Biol Rhythms* 2002; **17:** 147–154.
40 Jahng JW, Lee JH, Kim GT, Kim YM, Houpt TA, Kim DG. Adrenalectomy abolishes fasting-induced down-regulation of NADPH-diaphorase in the rat paraventricular nucleus. *Yonsei Med J* 2004; **45:** 123–128.
41 Bilbo SD, Dhabhar FS, Viswanathan K, Saul A, Yellon SM, Nelson RJ. Short day lengths augment stress-induced leukocyte trafficking and stress-induced enhancement of skin immune function. *Proc Natl Acad Sci USA* 2002; **99:** 4067–4072.