Plasticity in photoperiodic regulation of adrenal, but not testicular, function in Syrian hamsters

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ABSTRACT

Transfer from long days (LD) to short days (SD) increases aggressive behavior, but it suppresses the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes in male Syrian hamsters. The present study sought to determine whether social instability (group housing from days 1–70, single housing from days 71 to 84, and 10-min social encounters during the light or dark phase on days 82 and 83) could reverse SD-induced quiescence in the aggression-promoting HPA and HPG axes. Controls were housed in stable groups during LD or SD exposure. Euthanasia occurred on day 84 during the light or dark phase (unstable condition) and during the dark phase (stable condition). SD exposure in the unstable condition increased aggression during social pairings, and it elevated circulating corticosterone, cortisol, and adrenocorticotropic hormone (ACTH) concentrations, assessed by RIA, particularly during the dark phase. Although anterior pituitary pro-opiomelanocortin (POMC) immunoreactivity was unaltered by these experimental conditions, SD and the dark phase during social instability elevated POMC mRNA levels, assessed by solution hybridization assay. In socially stable controls, SD exposure increased aggression, assessed by bite marks, reduced cortisol and ACTH, but not corticosterone, secretion, and it reduced anterior pituitary POMC mRNA, but not immunoreactivity, levels. SD exposure in both conditions reduced testicular function, indicated by more than 77% reduction of testis mass. These results suggest that social instability, rather than aggression per se, reversed SD-induced suppression of HPA, but not HPG, function.

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1. Introduction

Transfer from long days (LD: at least 12.5 h of light per day) to short days (SD: less than 12.5 h of light per day) has been shown to increase aggressive behavior toward conspecific opponents in male and female Syrian hamsters [2,6,7,13]. Although corticosteroid signaling, particularly that of cortisol, is critical for the diurnal rhythm of aggression in LD-exposed hamsters [11,15,16], its role in SD-induced aggression is poorly understood. Given the importance of corticosteroid signaling in responses to metabolic and stressful stimuli, it is plausible that in SD-exposed hamsters environmental factors other than photoperiod influence adrenal and testicular functions. It is proposed here that social instability might serve as a stimulus to restore adrenal and testicular function during SD exposure, thereby increasing corticosteroid secretion in anticipation of the potential need for aggressiveness during future social conflicts.

In rats and mice, corticosteroid signaling depends on corticosterone synthesis, secretion, and metabolism. Like humans, however, hamsters synthesize and secrete cortisol in addition to corticosterone, and there is evidence that corticosterone and cortisol exert different, but overlapping, roles in hamsters [18,24]. For example, intact cortisol rhythmicity, but not that of corticosterone, is essential for the diurnal rhythmicity of aggression [15], and cortisol signaling appears to be a more important regulator of metabolic activity in hamsters [18,24]. By contrast, corticosterone and cortisol exert similarly important roles on the immune system, by promoting splenic hypertrophy and thymic involution [18,24]. Seasonal regulation of aggression, therefore, might depend on changes in one or both of these corticosteroids. Seasonal regulation of adrenal cortex function has not been reported extensively, but SD exposure has been shown to decrease cortisol secretion throughout its 24-h rhythm, and to abolish the 24-h rhythm of corticosterone secretion [18–20,22]. It is, therefore, plausible that reduction of cortisol secretion during SD exposure might alter social behavior. Alternatively, an elevated corticosterone-to-cortisol ratio during SD exposure might do so.

During SD exposure, male hamsters also exhibit testicular regression, which is well characterized by the suppression of steroidogenesis

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(e.g., androgen biosynthesis), spermatogenesis, and testis mass [8,18,20]. Testicular recrudescence occurs after approximately 20 weeks when hamsters become refractory to SD effects [23,25]. In addition to corticosteroids, gonadal steroids, including testosterone, promote aggressive behavior in hamsters [4,5]. Because SD-induced aggression occurs in stably housed hamsters exhibiting complete testicular regression [7], it is unlikely that gonadal hormones play a stimulatory role. If, however, social instability delays the onset, or reduces the magnitude, of testicular regression, a failure to suppress steroidogenesis fully might allow androgens to promote aggression.

2. Materials and methods

2.1. Experimental subjects

Forty-eight male Syrian golden hamsters (Mesocricetus auratus) of the Lake View Gorge strain (Lak:LVG(SYR)BR) were purchased from Charles River (Kingston, NY) at age 8 weeks. Subjects were acclimated in group housing (4/cage) and maintained on a long day (LD) light–dark schedule (14 h light/10 h darkness: lights on from 2200–1200 h) at 23 ± 3 °C. Rodent chow pellets and tap water were provided ad libitum. Procedures used were approved by the Institutional Animal Care and Use Committees.

2.2. Experimental design

After a two-week period of acclimation, half the subjects for both experiments were kept in LD, and the remaining halves were transferred to a short day (SD) light–dark schedule (10 h light/14 h darkness: lights on from 2400–1000 h) in a separate room (Fig. 1). Unfamiliar pairs were videotaped during social encounters either at the onset of the light or dark phase at ~400 or ~40 lux, respectively. Room illumination has been shown to have no significant affect on aggressive behavior between hamsters during the first few hours of the dark phase [16].

2.2.1. Social instability experiment

Hamsters exhibit a diurnal rhythm of aggressive behavior that coincides with peak (dark phase onset) and nadir (light phase onset) corticosteroid secretion [16], and intact corticosteroid signaling is an essential stimulus for this behavior rhythm [15]. This experiment was designed to determine whether social instability could restore HPA function during SD exposure, in conjunction with the rhythmicity of aggressive behavior. Subjects were grouped housed (4/cage) and exposed to LD ($n = 16$) or SD ($n = 16$) from days 1 to 70, and singly housed from days 71 to 84. On day 82, each subject was allowed a single 10-min social interaction with an unfamiliar subject in a homotypic (LD vs. LD or SD vs. SD) pair. On day 83, each subject was allowed another 10-min social interaction in a heterotypic (LD vs. SD) pair. On day 84, subjects were euthanized ~22 h after the termination of the second social encounter. Thus, there were four treatment groups ($n = 8$ per treatment): LD/Light; SD/Light; LD/Dark; and SD/Dark (Fig. 1).

2.2.2. Social stability experiment

SD exposure in socially stable conditions disrupts the diurnal rhythmicity of corticosterone (B) secretion, and it suppresses cortisol (F) secretion, but not its diurnal rhythmicity, in male hamsters [18,20,21]. This experiment was designed to illustrate that SD exposure in socially stable hamsters suppresses HPA and HPG functions, while stimulating aggressive behavior. Each subject was housed with a familiar cage mate, and exposed to either LD ($n = 4$ pairs) or SD ($n = 4$ pairs), without additional treatments, between days 1 and 84. On day 84, subjects were euthanized in the hour after the onset of the dark phase. Thus, there were two exper-

imental treatment groups ($n = 8$ per treatment): LD/Dark; and SD/Dark (Fig. 1).

2.3. Aggressive behavior

For socially unstable subjects, 10-min social encounters in a neutral cage were assessed, essentially as described by the author [17]. Each testing pair was matched by body mass (±10%). Behaviors were scored by trained observers who were blind to the treatments. Aggressive behavior was scored as climbing onto, standing over, chasing or initiation of biting, striking, or kicking. Defensive behavior was scored as crouching, fleeing, vocalizing, active avoidance, or counterattacking. Social dominance and subordination of each pairing were determined primarily by chasing and fleeing, respectively. This assessment was supported by determining the ratio of aggressive-to-defensive behavior. Social behavior was scored as approaching or sniffing. Non-social behavior was scored as self-grooming or rearing. Each subject in an encounter was scored independently. For socially stable subjects, aggressiveness was estimated post mortem by bite mark scores, determined by visual inspection of abdominal skin, and by visual inspection and palpation of dorsal skin. Each animal was given a score for four categories: 1 (0–10 marks); 2 (10–20 marks); 3 (20–30 marks); and 4 (30–40 marks). Scores from each category were totaled for each animal. The analysis of territorial aggression has face validity, as social defeat generates among mammals, including humans (bullying and workplace harassment), common consequences for endocrine disturbances and emotional behavior. Two scorers were trained by the author, using practice videotapes, to obtain scores within ten percent of each other, and average scores were used.

2.4. Tissue collection

Subjects were decapitated at the onset of the light or dark phase of the respective LD and SD schedules. Trunk blood was collected in tubes with or without EDTA (Becton–Dickinson, Franklin Lakes, NJ) on ice, and plasma (for ACTH) or serum (for corticosteroids), respectively, was separated at 3000g and 4 °C for 10 min and stored at −20 °C. Adrenal glands, spleens, and testes were excised and weighed. Anterior pituitaries were excised and stored at −80 °C.

2.5. Radioimmunoassays

Serum corticosterone was measured using 195-B3 rabbit corticosterone antiserum. Serum was diluted in phosphate buffer (pH 7.5) with 0.25% BSA, and incubated at 70 °C for 30 min. Diluted serum was incubated with 50 µl of 195-B3 antiserum (1:12,800 final dilution), raised at the Molecular & Behavioral Neuroscience Institute, and 8000–10,000 cpm of [^{3}H]corticosterone in RIA buffer at 4 °C for 16 h. Bound radioactivity was precipitated with 0.5 ml of 1% Norit A charcoal and 0.1% dextran in phosphate buffer (pH 7.5) on ice for 10 min and centrifugation at 3000g and 4 °C for 8 min. Supernatants were mixed with 6 ml of scintillation fluid and radioactivity was counted for 2 min in a beta counter. Intra-assay variation: 10%. Limit of detection: 0.8 ng/ml. Serum corticosterone and cortisol were measured using Coat-a-Count RIA kits (Diagnostic Products, Los Angeles, CA). Intra-assay variations were 8%, and 5%, respectively. Limits of detection were 6 and 2 ng/ml, respectively. Duplicate samples were assayed according to manufacturers' instructions and in single assays to eliminate inter-assay variation. Serum cortisol was measured using a Coat-a-Count RIA kit (Diagnostic Products, Los Angeles, CA), as described [19]. Intra-assay variation: 5%. Limit of detection: <0.5 ng/ml.

Plasma ACTH was measured using rabbit antiserum against human ACTH provided by Dr. Morano (Molecular & Behavioral Neuroscience Institute). Plasma was diluted and incubated with 50 mM