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Vocalizations convey sex, seasonal phenotype, and aggression in a seasonal mammal

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HIGHLIGHTS

- We recorded vocalizations from hamsters of different sexes and photoperiods.
- Ultrasonic vocalization subtypes differentially reflect seasonal phenotype and sex.
- Broadband calls reflect seasonal phenotype and sex, and relate to aggression.
- USVs and BBCs are signals used during same-sex encounters of Siberian hamsters.

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ABSTRACT

Seasonal variation in social behavior is often accompanied by seasonal variation in communication. In mammals, how seasonal environmental cues influence aggressive vocalizations remains underexplored. Photoperiod is the primary cue coordinating seasonal responses in most temperate zone animals, including Siberian hamsters (*Phodopus sungorus*), a species that undergoes reproductive inhibition and increased aggression in winter. During same-sex aggressive encounters, hamsters emit both broadband calls (BBCs) and ultrasonic vocalizations (USVs) that indicate aggression and the vocalizer's sex, respectively; however, it is not known whether these rodents adjust specific elements of their vocal repertoire to reflect their photoperiod-induced seasonal phenotypes. To address this, we recorded vocalizations emitted during dyadic interactions between male or female pairs of hamsters housed in long or short photoperiods and measured serum testosterone levels. USV emission rate remained stable across photoperiods, but proportional use of USV subtypes varied in novel ways: 'jump' USVs were sensitive to seasonal phenotype, but not the vocalizer's sex, whereas 'plain' USVs were sensitive only to the sex of the vocalizer. BBC emission rate varied with seasonal phenotype; short-day non-reproductive hamsters produced more BBCs and demonstrated increased aggression compared with reproductive hamsters. Testosterone, however, was not related to vocalization rates. Collectively, these findings demonstrate that changes in the vocal repertoire of Siberian hamsters reflect sex, aggression, and seasonal phenotype, suggesting that both BBCs and USVs are important signals used during same-sex social encounters.

1. Introduction

Many temperate zone species demonstrate marked seasonal variation in social behavior, including gregariousness [1], aggression [2,3], and reproductive behaviors [4]. Coupled with variation in social behavior, animals modify the use of their communicative signals on a seasonal basis. Excellent species for studying seasonal variations in communication exist across multiple vertebrate classes. For example, the substantial body of work in multiple species of songbirds has provided a detailed picture of seasonal variation in vocal behavior, particularly for vocalizations related to courtship (e.g., [5–7]). This work illustrates that animals modify acoustic signals seasonally. Vocalizations are important determinants of mate acquisition for many species, and thus, many species that exhibit seasonality in reproduction also exhibit seasonal shifts in vocal behavior with animals exhibiting increased vocalization rates during the breeding season (e.g., coyotes, frogs, humpback whales, midshipman fish, red deer, sea lions [8–13]).

Whereas courtship-related vocalizations are imperative to an animal's fitness, aggressive vocalizations can also provide fitness benefits. For example, the soft song of song sparrows (*Melospiza melodia*) is a signal of "aggressive intent," which could allow signalers to avoid potential injuries incurred during an aggressive encounter [14]. Furthermore, the production of these calls varies on a seasonal basis, with

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proportionally more soft song being produced during the non-breeding season when territoriality dominates these sparrows' social behavioral repertoire [14]. Thus, seasonal changes in the proportional use of vocalization types may indicate differences in the information content being conveyed by animals across different seasonal contexts, or may indicate that animals have shifted between seasonal states. Here, we explicitly test the idea that vocalizations reflect shifts in seasonal phenotypes in a seasonal rodent by inducing seasonal shifts with changes in photoperiod (i.e., day length).

Photoperiod serves as the primary environmental cue used by most mammalian species to coordinate seasonally appropriate responses [15]. Temperate zone-inhabiting mammals undergo marked morphological, physiological and behavioral changes in response to changes in photoperiod. For example, animals maintained in short "winter-like" days (i.e., <12 h of light/day) undergo gonadal regression, decreases in sex steroids, and changes in critical social behaviors, such as aggression [16]. Therefore, by manipulating photoperiod within the laboratory we can gain insight into seasonal changes in vocal behavior and the relationship of this behavior to seasonal phenotypes of individuals.

Siberian hamsters (*Phodopus sungorus*) are an excellent rodent species with which to examine how the vocalizations produced during male–male or female–female social encounters are influenced by the pair's sex, seasonal phenotype, and aggression. First, both male and female Siberian hamsters exhibit gonadal regression and display *increased* territorial aggression when housed in short "winter-like" days compared with hamsters housed in long "summer-like" days [2,3]. Most vertebrate species display aggression only when reproductively active, making it difficult to dissociate the individual effects of reproductive physiology, reproductive behavior, and aggression (reviewed in: [17]). Siberian hamsters, in contrast, have elevated levels of aggression when gonads are regressed. Whereas most short-day hamsters inhibit reproductive physiology (i.e., "short-day responders"), a subset of hamsters are unresponsive to short photoperiods (i.e., "short-day non-responders") and are physiologically indistinguishable from long-day hamsters. They maintain functional reproductive physiology, brown/gray pelage, and long-day-like body mass, food intake, and thermoregulation ([18,19]; reviewed in: [20]). Thus, Siberian hamsters exhibit distinct seasonal phenotypes: a "summer" morph (long-day hamsters and short-day non-responders) and a "winter" morph (short-day responders). These natural fluctuations in seasonal phenotypes make it possible to dissociate between the relative contributions of the physiological response to photoperiod and the photoperiod cue itself to seasonal variation in vocalizations.

We have recently shown that Siberian hamsters housed in long days vocalize during same-sex aggressive encounters, emitting two vocalization classes: high frequency (>20 kHz), narrowband ultrasonic vocalizations (USVs) and lower frequency, broadband calls (BBCs) [21]. Specifically, we found that the proportional use of different vocalization types is dependent on whether the same-sex pair of hamsters is male or female. Further, BBCs, but not USVs, are related to aggression during same-sex encounters [21], demonstrating that Siberian hamster vocalizations are sensitive to social context. It is important to note that all the animals in this study were in long-day, "summer" condition; whether production of these vocalizations varies in response to changes in seasonal phenotypes, and whether environmental cues such as photoperiod play a key role in coordinating changes in vocal repertoire, remains unknown.

In the present study, we investigated relationships among seasonal phenotypes, aggression, and vocal production, as well as the effects of these factors on specific aspects of the vocal repertoire. We also examined the potential role of photoperiodic variation in the gonadal steroid testosterone (T) in regulating vocal production. We predicted that changes in vocal behavior would reflect photoperiodic changes in aggression, such that non-reproductive hamsters, which display more aggression, would produce more BBCs and would use proportionally more 'rattle' BBCs, which are more closely related to aggression in breeding-condition Siberian hamsters [21]. Because USVs are not related to aggression, we predicted no changes in vocalization rate for USVs across photoperiods [21]. Lastly, because T is inversely related to aggression in males of this species [2], we predicted that T would be inversely related to BBCs. By testing these predictions, we can associate known photoperiodic changes in seasonal phenotypes and aggressive behavior with changes in the composition of the vocal repertoire of a seasonal rodent, and begin to address the physiological correlates of communication in a seasonal context.

2. Materials and methods

2.1. Animal housing and photoperiodic treatment

Adult (>60 days of age) hamsters were reared in a breeding colony at Indiana University, Bloomington. Hamsters were bred and maintained under long days (light:dark, 16:8 h) and group-housed at weaning (postnatal day 18). Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. Hamsters were given ad libitum access to tap water and laboratory rodent chow (Lab Diet 5001, PMI Nutrition). All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University..

Resident hamsters were individually housed (females: $n = 40$; males: $n = 40$) and intruder hamsters were pair-housed (females; $n = 20$; males, $n = 20$) in the colony room for a one-week acclimation period. Subsequently, a random subset of hamsters was transferred to a room on a short-day light cycle (light:dark, 8:16 h), and the remaining hamsters were relocated to a new room on the same long-day light cycle as the colony room. All hamsters remained in their respective photoperiods for ten weeks.

2.2. Determination of seasonal phenotypes

Photoperiodic-induced changes in physiology and morphology indicative of seasonal phenotypes were determined based on *apriori* criteria previously established for Siberian hamsters [2,3]. Following collection of behavioral data, animals were given a lethal dose of a ketamine/xylazine cocktail, necropsies were performed, and reproductive tissues were collected to confirm functional reproductive physiology. Hamsters were deemed reproductively competent if they had functional reproductive tissue weights (i.e., paired testes mass of >0.25 g for males or the combined mass of ovaries, uterine horns, and parametrial white adipose tissue >0.1 g for females), displayed no significant changes in body mass (<10%), and maintained a brown/gray coat color (long-days; LD; females: $n = 14$; males: $n = 14$). Estrous cycles were monitored via vaginal cytology [3] to confirm cycling in reproductive females. In contrast, hamsters were deemed reproductively incompetent if they had regressed reproductive tissue masses, lost >10% of their body mass, and had a "winter" white pelage (short-day responder; SD-R; females: $n = 10$; males: $n = 15$); non-reproductive females did not demonstrate estrous cycling. As previously documented, a subset of short-day hamsters failed to respond reproductively to the short-day photoperiod treatment and remained reproductively competent (short-day non-responder, SD-NR; females: $n = 16$; males: $n = 11$) [18,19]. After examining reproductive mass, two pairs of females and two pairs of males were excluded because they were determined to be of different seasonal phenotypes.

2.3. Aggressive and vocal behavior recording and analysis

Dyadic interactions were staged between a resident hamster and a same-sex intruder hamster of the same photoperiodic and seasonal