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The effects of photoperiod and social environment on behavior and neurobiology in meadow voles (*Microtus pennsylvanicus*)

By

Kelley C. Power

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Annaliese Beery, Chair

Professor Lance Kriegsfeld

Professor Linda Wilbrecht

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Abstract
The effects of photoperiod and social environment on behavior and neurobiology
in meadow voles (Microtus pennsylvanicus)

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Professor Annaliese Beery, Chair

The extent to which a mammal interacts with others varies widely across species. Although these interactions are a critical part of mammalian life, the mechanisms underlying social interactions aren't well understood. While many studies of sociality rely on comparative studies, some species have flexible social structures, providing a distinct opportunity for study within a single species. One such species is the meadow vole (*Microtus pennsylvanicus*). In the wild, meadow voles undergo predictable shifts in social organization in response to environmental cues. During the summer months females primarily live alone, but interact with males for mating, and other females while defending their territory. In the winter however, they live communally in mixed-sex groups, with a shared territory. Altering photoperiod in lab settings recapitulates behavioral changes seen in the wild, largely demonstrated by changes in short-term dyadic interactions during behavioral tests. My dissertation examines how daylength affects group dynamics of free-moving voles over longer time scales, how daylength, social history, and their interaction affect behavior and physiology, and how daylength affects striatal dopamine.

In chapter 1, I used radiofrequency identification (RFID) technology to track groups of voles housed in a multichambered arena. I compared grouping dynamics, temperature & activity patterns, and social networks of female voles in long daylengths (LD) and short daylengths (SD) over extended time periods. Daylength affected grouping behavior, with winter-like SD voles spending more time in groups, and forming larger, evenly mixed groups than summer-like LD voles. SD voles had highly correlated ultradian rhythms in temperature and activity even across groups. These correlations were not present in LD voles.

Although daylength and social history co-vary in the wild, their individual and combined effects on behavior and physiology remain understudied. In chapter 2, I housed female meadow voles in one of four ways (LD solo, LD pair, SD solo, SD pair) to untangle the effects of these environmental cues. Daylength affected aggression with a stranger and anxiety-like behavior in the light dark box, while housing affected anxiety-like behaviors in the open field test and oxytocin levels. The interaction between daylength and social history uncovered a particularly interesting relationship. When social history conflicted with daylength (e.g. LD pair, SD solo), voles exhibited elevated corticosterone concentration, engaged in fewer affiliative behaviors, and were slower to approach an unfamiliar conspecific than their counterparts in socially aligned housing (e.g. LD solo, SD pair),

demonstrating the importance of considering ethologically relevant factors even in laboratory settings.

In chapter 3, I examined how daylength alters dopamine dynamics using near-infrared catecholamine nanosensors (nIRCats) to examine dopamine at the synaptic level in the striatum of LD and SD meadow voles in slice preparation. Daylength had a significant effect on dopamine dynamics. SD females had increased dopamine release and higher release site density than LD females. Further investigation using pharmacological and extracellular manipulations found that SD voles exhibit heightened responsiveness to dopamine-increasing interventions and resilience against dopamine-suppressing conditions, unlike LD voles. Within SD, there was no significant difference in dopamine release or number of releasing sites between sexes. These findings highlight a significant association between dopamine signaling and photoperiod-driven changes in social behavior and establish nIRCats as an effective tool for expanding our understanding of dopamine dynamics across non-model organisms.

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Introduction

Sociality, or the degree to which individuals of the same species associate with one another, is remarkably variable across the animal kingdom. All mammals must interact with one another in a reproductive context, but some spend a majority of their time alone, while others form a selective relationship with a specific individual (pair-bond), and still others live in complex societies with multiple relationship types between members. Comparative studies between solitary and social species offer important insights on mechanisms underlying sociality, however, studying a single species with a flexible social system provides a unique opportunity to examine sociality without the confounds of species-specific traits. Meadow voles are one such species with a flexible social system that changes seasonally.

During the summer breeding season, female meadow voles maintain and defend exclusive territories, while rearing their young alone (Madison, 1980; Webster & Brooks, 1981). As winter arrives, however, females lose their territoriality and individuals come together to reside in mixed-sex groups (Madison et al., 1984). Meadow voles remain active throughout the winter and rely on the thermoregulatory benefits that huddling with other group members provides. Like most mammals living in temperate climates, meadow voles use daylength (photoperiod) as the primary cue to time changes in behavior and physiology, as it is the most reliable (Paul et al., 2008; Prendergast et al., 2002). In the lab, daylength has also been used to recapitulate many of the seasonal changes seen in the wild (reviewed in Beery, 2019).

In chapter 1, I established a multi-chambered arena with radiofrequency identification (RFID) positional tracking to study groups of free-moving LD and SD voles. I compared group dynamics, temperature & activity patterns, and social networks of voles in the arena over the course of a week. My findings largely align with seasonal changes in meadow vole social behavior in the wild, providing support for the use of meadow voles as a model of environmentally driven variation in social behavior and extend our understanding of social behavior in group settings.

Most prior studies on meadow voles kept housing consistent across daylengths to establish the main effect of daylength. However, in the wild, social history co-varies with daylength and may also contribute to seasonal changes in social behavior. In chapter 2, I explored the individual and combined factors of social history and daylength to determine their effects. I housed female meadow voles in one of four ways (alone in LD in pairs in LD, alone in SD, and pairs in SD) to assess anxiety-like behaviors, social interactions with an unfamiliar conspecific, and hormonal profiles. Daylength, social history, and their interaction affected unique aspects of behavior and physiology. This chapter illustrates the importance of the alignment between daylength and expected social environment, and the consequences when the two are not aligned.

In chapter 3, I used near-infrared catecholamine nanosensors (nIRCats) to examine evoked dopamine in slice preparation for the first time in voles. These nanosensors provide high spatiotemporal resolution and can be used in non-model species, unlike traditional methods and tools used to study dopamine. I investigated differences in dopamine dynamics between daylength (LD, SD), social history (solo, pair, quad), and sexes (female and male), and found daylength to have the biggest overall impact. Together, these chapters highlight how combining non-traditional

animal models with innovative techniques and naturalistic environmental contexts provides a better understanding of sociality.

Chapter 1

A social switch: daylength drives meadow vole group dynamics in automatically tracked habitats

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ABSTRACT

Sociality has evolved on numerous occasions, with important fitness benefits and consequences for individuals in group-living species. Despite the prevalence of group living, it remains challenging to study the proximate factors that promote variation in this behavior. Meadow voles, *Microtus pennsylvanicus*, vary in social organization in response to seasonal cues in the wild, providing a remarkable opportunity to study mechanisms that support changing grouping behavior within a single species. In summer, female meadow voles are territorial and maintain exclusive home ranges, while in winter, they live in mixed-sex groups. Laboratory manipulations of photoperiod reproduce seasonal variation in social behavior in this species, demonstrated by altered short-term dyadic interactions during behavioral tests. To assess changes in group social dynamics, we implemented an automated tracking system using radiofrequency identification technology to monitor free-moving voles housed in long daylengths and short daylengths in multichambered habitats over extended intervals. We developed open-source software to model each animal's position within the habitat and to describe co-occupancy patterns. Photoperiod drove robust and pronounced changes in group social behavior, recapitulating natural seasonal transitions in the wild. Voles housed in short, winter-like days spent more time in groups, formed larger, more evenly mixed groups and shared a single home-chamber more often than long-day voles. Short-day voles were more active than long-day voles but rested in groups, thus cohabitation was strongly correlated with ultradian rhythms in activity. These metrics of group dynamics will allow more nuanced assessment of the effects of manipulations on social behavior in voles and other species, as both habitat and software are adaptable for use with other rodents.

1.1 Introduction

While all mammals interact prosocially to mate or rear young, in some cases the benefits of group living outweigh the fitness costs, leading to the evolution of complex social structures (Hamilton, 1971; Krause & Ruxton, 2002; Patterson & Ruckstuhl, 2013; Terrien et al., 2011). The resulting social groups can vary in size, stability, kinship and many other attributes, both within and between species (Hofmann et al., 2014). Features of the physical and social environment, including photoperiod, rainfall, temperature, food availability and early social experience, can also impact group formation and composition (Ahern et al., 2021; Lee & Beery, 2024; Perkeybile et al., 2013;

Prehn et al., 2019). When group dynamics vary predictably under different environmental conditions, it provides a rare opportunity to gain insight into the processes that covary with and may support sociality. To develop such a model, we measured free-living social behavior in small groups of meadow voles, a seasonally social species, under different daylength conditions.

The meadow vole, Microtus pennsylvanicus, is a small rodent distributed across eastern North America. Meadow voles are active year-round, and changes in season coincide with major changes to their social organization (Beery, 2019). During the summer breeding season, females are solitary and aggressive and maintain exclusive territories, which they defend from others (Madison, 1980; Webster & Brooks, 1981). Males are nomadic during the summer, travelling through multiple females' territories to breed (Madison, 1980; Webster & Brooks, 1981). As winter approaches, females' exclusive territories collapse, and voles cohabit in mixed-sex groups of up to 10 conspecifics throughout the nonreproductive season (Madison & McShea, 1987). Meadow voles are promiscuous breeders (Getz, 1972), allowing dissociation of the mechanisms underlying peer social relationships from those involved in pair bonds with mates (i.e. monogamy).

For high-latitude mammals, daylength is the most reliable and salient indicator of changing seasons and can be used in the laboratory to induce downstream changes in physiology and behavior (Paul et al., 2008; Prendergast et al., 2002). In the laboratory, meadow voles housed in long daylengths maintain reproductive function and behavioral phenotypes associated with the summer, while meadow voles housed in short daylengths undergo gonadal regression (Dark et al., 1983) and shift towards behavioral phenotypes associated with winter group living. In particular, female meadow voles housed in short daylengths exhibited greater interest in the odours of same-sex conspecifics (Ferkin & Zucker, 1991), and they spent more time in side-by-side contact during a 3 h partner preference test, than did females housed in long daylengths (Beery et al., 2008). While short-day voles prefer familiar over novel companions, they also exhibit a higher tolerance of strangers than do long-day voles (Lee et al., 2019). These foundational studies establish meadow voles as a model to study the effects of photoperiod on social behaviors, but none explicitly examined factors related to group formation or persistence.

Laboratory assessments of group social behaviors in rodents are uncommon. Early research on environmental enrichment compared rats housed in groups in free environments to those in standard or deprived laboratory housing (Bennett et al., 1964, Hebb, 1947). Years later, the “visible burrow system” (VBS) was developed, initially to address questions surrounding sexual behavior in groups (McClintock & Adler, 1978). Similar set-ups were then used to investigate social stress and hierarchy in rats (Blanchard & Blanchard, 1989; McEwen et al., 2015; Tamashiro et al., 2004). In mice, groups housed in large habitats that simulate above- and below-ground regions have been used to study neurobiological mechanisms associated with social dominance (So et al., 2015; Williamson et al., 2017). Such studies are more ethologically relevant than standard housing but often rely exclusively on time-intensive direct observations of behavior. Some studies have begun to get around this issue by incorporating automated tracking using radiofrequency identification (RFID) and computer vision (König et al., 2015; Levy et al., 2023; Puścian et al., 2016; Shemesh et al., 2013; Walcott & Ryabinin, 2021) in place of or in addition to observation.

Much more commonly, social behavior is assessed through laboratory observations of dyadic interactions. Standardized assessments such as the social interaction test (File & Seth, 2003) and the three-chamber sociability test (Crawley, 2004; Moy et al., 2004) investigate social interactions with an unfamiliar conspecific. Preference and motivation for a familiar (versus unfamiliar) conspecific can be assessed using partner preference tests and operant conditioning with social

stimuli as rewards (Beery, 2021; Beery et al., 2021; Williams et al., 1992). These tests yield important insights into specific aspects of social behavior in dyadic interactions, but they do not address the dynamics of social groups and longer timescales. In contrast, field assessments of social behavior often make use of automated movement tracking to develop a greater understanding of group dynamics (e.g. Böhm et al., 2008; Sabol et al., 2018; Smith et al., 2018). To improve the study of environmentally driven changes in group living in the laboratory, we employed RFID tracking in multichambered habitats housing groups of meadow voles.

Across multiple trials in an RFID habitat (Fig. 1.1), we asked whether groups of four unfamiliar meadow voles given free access to multiple housing chambers would display photoperiod-dependant variation in measures of group living. We also investigated the temporal dynamics of these outcomes over multiple days of cohousing. We developed open-source software, Voletron, to infer position and direction of movement from the raw RFID reads. Based on field descriptions of seasonal variation in meadow vole social organization, we predicted that short-day voles would spend more time in larger groups than long-day voles. This study demonstrates that more naturalistic and continuous assessment of cohabitation provides a valuable complement to standard behavioral testing for assessing group social dynamics and further establishes meadow voles as a model of predictable environmental change of group-living behavior.

1.2 Methods

Animal Subjects and Timeline

Meadow voles were bred in an in-house colony outbred with wild voles caught in Hampshire County, Massachusetts, U.S.A. to maintain genetic diversity. Voles were bred in long daylengths (14:10 h light: dark cycle, lights on 0300–1700 hours). Voles were housed in clear plastic cages (45 × 25 × 15 cm) with aspen bedding, nesting materials and an opaque plastic refuge tube. Ad libitum food (LabDiet Mouse Chow 5015) and water were available. Diet was supplemented two to three times/week with apple or carrot. Long-day voles additionally received leafy greens (lettuce or spinach) twice weekly.

All subjects in this study were female, as female meadow voles display a more dynamic change in social behavior between photoperiods in both the field (Edwards et al., 2019; Madison & McShea, 1987) and the laboratory (Beery et al., 2009a).

Voles were group-weaned with siblings on postnatal day 20. Within 1 week, voles were sexed and separated into experimental housing in long daylengths or short daylengths (LD 10:14 h, lights on 0700–1700 hours). The temperature in both short-day and long-day rooms was 21 ± 1 °C.

We tested 12 cohorts of four voles each: six cohorts were tested in short daylengths and six cohorts in long daylengths; all 48 voles completed at least 1 day of testing. Cohorts were maintained for up to 1 week and were stopped early if there were any technical or husbandry issues (e.g. PIT tag loss, power outage). Five cohorts housed in short daylengths (20 voles) and three cohorts in long daylengths (12 voles) completed a full week of testing. In a separate study, four short-day and four long-day solo-housed voles were monitored in their home cages for body temperature and activity.

Experimental Design

Voles were housed in their experimental daylength for at least 45 days from weaning before the

start of testing to allow transition to a short-day phenotype and were solo-housed from weaning or for at least 3 weeks before testing. Three to seven days prior to placement in the RFID habitat, we anaesthetized voles using isoflurane and subcutaneously injected PIT tags (APT12 FDX-B, Biomark, Boise, ID, U.S.A.) between the shoulder blades. After implantation, a unique pattern was shaved into the vole's fur for visual identification during testing. At the start of each trial, four adult voles (mean ± SE age = 84.5 ± 1.67 postnatal days) were united in the RFID habitat and remained there for up to 1 week. The habitat was not disturbed throughout testing but was visually inspected at least once daily for health checks. Observations of vole identity and position were collected during health checks to validate the RFID system's positional data for each vole.

RFID Habitat

Each RFID habitat was composed of a large central cage (~50 × 42 × 20 cm) with four smaller cages (~47 × 25 × 20 cm) attached by acrylic plastic tubes (Fig. 1.1 a). Each side chamber contained bedding, nesting material, a plastic hiding tube and ad libitum food and water. The large central chamber contained only bedding.

Each habitat was equipped with eight ring antennas, positioned at either end of each tube connecting different chambers (Fig. 1.1 b, c). Antennas were connected to the AniLoc4mice Socialbox (FBI-Science GmbH), which used a CAN2USB-interface for input to a computer running OLCUS (FBI-Science GmbH) for data acquisition and basic statistics. Raw data files were exported from OLCUS for further processing using Voletron.

RFID habitats were set up in adjoining rooms on different light cycles to enable simultaneous data acquisition from cohorts of long-day and short-day voles. Antenna cables were directed through a light-tight wall port to the acquisition computer.

Activity and Temperature Monitoring

We anaesthetized eight solo-housed voles (four short-day and four long-day) using isoflurane and injected them subcutaneously with thermal sensing PIT tags (BioTherm13 Thermal FDX-B, Biomark, Boise, ID, U.S.A.; LifeChip with Bio-Thermo Technology, Destron Fearing, DFW Airport, TX, U.S.A.). We added infrared motion sensors (Actimetrics/Lafayette Instruments, Lafayette, IN, U.S.A.) to the lid of their home cage and placed an RFID antenna beneath their cage. We monitored body temperature and activity for 1 week in 30 s intervals and processed the data using ClockLab (Actimetrics/Lafayette Instruments).

Ethical Note

All experimental procedures were conducted according to federal and institutional guidelines and protocols were approved by the Institutional Animal Care and Use Committees of Smith College (19R-AB-272) and UC Berkeley (2021-05-14304). PIT tagging was performed under anaesthesia by trained personnel, and voles were carefully monitored post implantation to ensure full recovery. Once in the arena, all voles were visually checked daily for signs of poor health, stress or injury. Had significant aggression occurred, we would have halted the experiment immediately and removed all voles from the arena (no occurrences). Aside from daily health checks, voles were left undisturbed for the duration of the experiment, with ad libitum access to food and water in each of the four side chambers.

RFID Read Processing (Voletron)

Raw RFID reads were processed for specific time ranges using Voletron version 1.0 (https://github.com/BeeryLab/voletron), developed for this study. Voletron imports a map of the antenna locations in each RFID habitat together with raw antenna reads to infer a vole's location over a period of time defined by the user.

The raw data obtained from the apparatus consists of a set of time-stamped events, each indicating that a given PIT tag was in proximity to a specific antenna. Antenna reads do not indicate the specific chamber location or direction of travel; thus, a main function of Voletron is to algorithmically assign each animal's location to the cage or tube that lies between the locations of antennas triggered at the beginning and end of that time window.

Voletron incorporates heuristics to resolve ambiguous situations. These situations include consecutive reads at a specific antenna (interpreted as stationary within a tube) and "skipped reads", when the antenna failed to detect a passing animal (in which case the location information is split across the two locations between recorded reads). Thus, given the raw data (antenna reads) and a map of the habitat, Voletron assembles a complete description of the locations of animals within the habitat over time. It can then derive metrics of interest, such as the total duration that specific animals were in the same chamber ("cohabitation"), the distribution of group sizes (number of animals in the same chamber) over time and so forth.

Statistical Analysis

Data were analysed in 24 h intervals, and additionally for one cohort in hourly intervals, over 7 days. Outputs from Voletron in each interval included the time each vole spent alone or in a pair, trio or quad, average group sizes across the test (including and excluding when voles were alone), cohabitation times for each complement of voles (regardless of other voles present), the time spent in each cage and a matrix of cohabitation durations between each pair of voles. Finally, we compared daily visual observations of vole locations to their antenna reads using the validation flag in Voletron.

We ran a repeated measures mixed effects model using daily time series data (days 1–7) for all animals completing 1 week of testing. Although all voles were individually housed prior to testing, some trials contained former littermates. Analysis of prior familiarity revealed no effect of littermate status on time spent in cohabitation between pairs of voles on day 1, so this factor was not included in further models. Model factors included the effect of daylength (between groups) and day of testing (repeated measure), with run nested within daylength as a random variable to account for cohort effects. Test day was significant in all analyses, so we made specific comparisons on day 1 and day 7 to assess whether daylength differences were present after 1 day of testing and, if so, whether these differences remained at the conclusion of testing.

We performed cross-correlations between time series of activity, body temperature and percentage of time solo at hourly intervals using one long-day and one short-day cohort in the RFID arena and solo-housed long-day and short-day voles in their home cages. We performed additional cross-correlations to investigate the relation between body temperature and home cage activity of long-day and short-day voles at 15 min intervals and to compare subcutaneous body temperature to previously published core body temperature readings at 15 min intervals. We used a 95% confidence interval as the reporting threshold for substantive correlations in cross-correlation analyses.

We constructed social networks as undirected, weighted networks, with each network representing

a particular trial in the habitat. For each network, nodes represented individual animals within the habitat, and weighted edges represented the amount of time two animals were present in the same location divided by the total duration of the analysis period. Social network diagrams were constructed for each trial on days 1 and 7 using the package 'iGraph' (version 1.3.0; Csárdi & Nepusz, 2006Csárdi & Nepusz, 2006) in R (version 4.1.2; R Core Team, 2021). We quantified the evenness of mixing by calculating the percentage of the coefficient of variation (CV) of edge weights for each group, with lower CVs indicating more even mixing among group members and higher CVs indicating more uneven relationships (for example, formation of consistent pairs within the group or exclusion of one group member). We computed one mean % CV for each cohort and ran t tests to compare differences between daylengths. We also analysed home cage preferences by cohort, as further described in the Results.

1.3 Results

Daylength Impacted Group Occupancy

A repeated measures mixed effects model of the 7-day time series revealed a significant effect of daylength and day on time spent in groups, but no interaction between them (Fig. 1.2 b, Table 1.1). In the first 24 h in the habitat (day 1), individual short-day voles spent significantly more time in groups than did long-day voles (Fig. 1.2 a, Table 1.3.1). By day 7, both long-day and short-day voles spent more time cohabiting than on day 1, but short-day voles still spent significantly more time in groups than did long-day voles (Table 1.1).

Time spent in groups was concordant with differences in group size. Group size was affected by daylength, day and the interaction between daylength and day, both including and excluding time spent alone (model results in Table 1.1). By the end of day 1, short-day voles already cohabited in significantly larger groups than did long-day voles, both considering average group size across the recording interval (Fig. 1.1 c) and average group size only when cohabiting with at least one other animal (Table 1.1). These differences in group sizes between short-day and long-day voles remained on day 7 (Table 1.1).

Daylength Impacted Group Composition

Time series analysis indicated that both daylength and day had effects on time spent in trios and in quads (Table 1.2). Short-day voles spent significantly more time in groups of three (trios) on average than long-day voles did on day 1 and day 7 (Fig. 1.3 a, Table 1.2). Both long-day and short-day voles spent more time in trios on day 7 than on day 1, but this increase was only significant for short-day voles ($t19 = 5.956$, $P < 0.0001$; Fig. 1.3 c).

Similarly, short-day voles spent significantly more time in groups of four (quads) than long-day voles did on the first day in the habitat (Fig. 1.3 a, Table 1.2). On day 7, short-day voles still spent significantly more time in groups of four than did long-day voles (Table 1.2).

Cohabitation Changed over Time in Both Daylengths

Both time in groups and group size increased over the 7-day test interval (Fig. 1.3, Table 1.2). In short-day voles, this was reflected in an increased tendency to spend time in trios and quads (Fig. 1.3 c, Table 1.2), while long-day voles increased their time spent in pairs (day 1 versus day 7: t11

= 2.397, P = 0.029; Fig. 1.3 b).

Daylength Impacted Social Network Metrics

By day 7, cohorts of long-day voles appeared more likely to form consistent pairwise associations, while short-day cohorts formed evenly mixed groups with no preference for specific individuals (Fig. 1.4 a, b). Short-day voles had lower coefficients of variation between social network edge weights than did long-day voles on days 1 and 7 (Fig. 1.4 c), but this difference was only significant on day 7 (day 1: t10 = −1.592, P = 0.1425; day 7: t6 = −2.94, P = 0.0258). While there was no change in social network CV evident in short-day voles (t4 = 4.848, P = 0.3244) between day 1 and day 7, there was a tendency towards increased CV between days 1 and day 7 in long-day voles (t3 = 2.945, P = 0.0985). These results are consistent with less even mixing within long-day group cohorts.

Home Environment Preference

To assess whether cohorts of short-day and long-day voles preferred a common home cage within the habitat, we used a ranking system. We averaged the percentage of time individual voles within a cohort spent in each of the five chambers (four cages and central arena) to determine each chamber's rank. Chambers were then ranked in order of the cohort's preference, with rank 1 being the cohort's most preferred chamber (highest average time in chamber) and rank 5 being the cohort's least preferred chamber (lowest average time in chamber), producing a single ranking for each cohort. On day 1, short-day voles spent significantly more time in their cohort's highest ranked cage than did long-day voles (t38.33 = 2.64, P = 0.012; Fig. 1.5 a), indicating a greater tendency to establish a shared home cage. On day 7, this pattern remained (t23.75 = 2.90, P = 0.008). Time series analysis revealed a significant effect of daylength (F1,30 = 0.51, P = 0.0005), but no effect of day (F1,30 = 0.33, P = 0.26) and no interaction between daylength and day (F1,30 = 0.082, P = 0.908; Fig. 1.5 b). We also evaluated the stability of the home cage location (i.e. does the home cage location change over each day of testing, or does it remain stable once established?). Cohorts of short-day and long-day meadow voles changed locations of the shared home cage at similar rates (t3.07 = −1.42, P = 0.248), but short-day meadow vole cohorts spent more days in their overall most preferred cage over the duration of the test (t5.97 = 3.39, P = 0.0147).

Ultradian Variation in Social Behavior Related to Activity and Body Temperature

We further analysed one cohort each of short-day and long-day voles in hourly intervals to determine whether grouping dynamics varied throughout the day. As spending time in close proximity to other voles provides thermoregulatory benefits (Madison et al., 1984; Madison & McShea, 1987), we compared social grouping data from the RFID habitat to activity and body temperature data collected from four short-day and four long-day voles housed alone in their home cages to identify activity and temperature rhythms in the absence of huddling.

Short-day voles were overall more active than long-day voles (Fig. 1.6). Cross-correlation (CC) of these time series revealed a strong correlation between when short-day voles in the tracked habitat were alone and patterns of activity in solo housing (CC = 0.728 at lag 0; Fig. 1.6 a), indicating that endogenous ultradian patterns of activity likely drove time spent alone in short-day voles. In contrast, the correlation between these variables in long-day voles did not extend beyond the 95% confidence interval (CC = 0.329 at lag 0; Fig. 1.6 b). In both short-day and long-day voles, home

cage activity was correlated with body temperature, but this relationship was stronger in short-day voles (long-day: CC = 0.365 at lag 0; short-day: CC = 0.816 at lag 0; Supplementary Fig. S1.1). Finally, to validate the temperature data we collected with thermal PIT tags placed subcutaneously, we compared our temperature data to previously collected core body temperature data from solo-housed short-day meadow voles (Beery et al., 2008a) and found a strong correlation at lag 0 (CC = 0.841; Supplementary Fig. S1.2).

Automated RFID Tracking Reliably Captured Vole Location

Manually recorded observations of vole positions within the habitat matched the respective adjacent recording antennas 96% of the time (N = 135 observations). Specific vole locations (chambers and tubes) inferred by Voletron from the sequence of successive antenna reads matched recorded observations 85% of the time (N = 135).

1.4 Discussion

All prior studies of seasonal changes in social behavior in the laboratory have been limited to dyadic interactions in constrained behavioral tests. For the first time, we demonstrate that daylength alone is sufficient to drive changes in free-living social behavior in meadow voles, including time spent in groups, group size and social network characteristics. These findings recapitulate seasonal differences in space use and social organization that have been documented in the wild (Madison et al., 1984b; Madison & McShea, 1987) and provide a foundation for probing the mechanisms underlying life in social groups.

Short-day voles spent more time in groups and in larger group sizes than did long-day voles. Detailed analysis of group composition showed that short-day voles cohabited in groups of three and four more often than did long-day voles. Short-day groups were also more homogeneous than long-day groups, with no strong preference for specific individuals within their cohort. This aligns with prior studies of meadow voles housed in trios, which form selective but nonexclusive bonds with each known cagemate relative to a novel stranger (Beery et al., 2009a). It also complements previously described daylength differences in interactions with unfamiliar conspecifics, with short-day voles showing more prosocial interaction with strangers (Beery et al., 2008; Lee et al., 2019) and showing a preference for larger group sizes over even a known partner (Ondrasek et al., 2015a).

Social networks highlight connectedness among individuals, as well as the strength of specific pairwise relationships between group members (Croft et al., 2011; Farine & Whitehead, 2015; James et al., 2009; Silk, 2023). By the end of 1 week of cohousing between four initially unfamiliar companions, long-day meadow voles formed more subgroup relationships than did short-day voles, who cohabited more evenly with all group members. This is consistent with the capacity to form multiple, equivalent pairwise partner preferences in short-day meadow voles (Beery et al., 2009a).

With four side chambers within the habitat, voles could freely select whether to isolate or share a chamber with other voles. In both daylengths, voles engaged in more social interaction as the testing interval progressed. The 1-week period allowed voles to become familiar with each other over time, even though they all started as strangers. Long-day voles spent more than half the time alone at the start of the test but cohabited with at least one other vole ∼50% of the time by the end of the week. The extent of cohabitation in long-day voles indicates that photoperiod alone is not

sufficient to replicate all seasonal differences from the field. Uniform social history likely reduced the difference between daylengths. All voles, regardless of daylength, were solo-housed prior to testing; in the field, summer and winter voles have very different social life histories. Voles born in autumn often remain with their natal litter until mixed lineages condense (Madison & McShea, 1987); thus, voles in short or decreasing daylengths may never have experienced life alone. In contrast, voles born in early summer disperse to solitary burrows. We previously demonstrated that solitary housing reduces affiliative social interactions in short-day meadow voles (Lee et al., 2019) solo housing in the present study likely reduced social behavior in short-day voles, minimizing the difference in time voles spent in contact with others. Abiotic factors such as temperature and nutrient availability also play a role in the full complement of seasonal changes (Beery, 2019a; Cranford, 1983; Ondrasek et al., 2015a) and may contribute to further variation in social behavior across the seasons.

In addition to choosing whether to cohabitate with or isolate from their cohort, voles chose where to spend their time within the habitat. We found that short-day voles spent significantly more time in their cohort's most preferred cage than long-day voles did, both on the first day and across all days of testing. This suggests that short-day meadow voles established and maintained a shared home cage early in the experiment, mimicking the formation of stable nesting groups in winter.

There was striking concordance between the times of day short-day voles were active in their home cage and the times they were alone in the RFID habitat, suggesting that movement was the primary cause of separation from the group. Critically, activity and body temperature were collected in separate animals housed alone, so this ultradian rhythm in activity was independent of huddling. Spending time in a group, particularly while huddling, provides thermoregulatory benefits, which are crucial for voles' survival in the cold winter months (Gilbert et al., 2010). Long-day voles lacked such a relationship between activity and time alone. This is likely in part because long-day voles spent more time alone and were less active than short-day voles.

While multiple seasonal cues shape changes in physiology and social behavior, photoperiod alone was sufficient to drive pronounced differences in group size and social preferences, mirroring seasonal differences in field social behavior. These findings will enable the use of RFID habitat-based assessments of the impacts of manipulations of neural and endocrine mechanisms that may underlie changing social behavior, including gonadal steroids, oxytocin and vasopressin, corticotropin-releasing factor, other molecules and their receptors (Beery, 2019a; Beery et al., 2014a; Beery & Zucker, 2010).

Habitat Validation, Adaptation and Benefits

Behavioral tests in the laboratory, such as the partner preference test, social interaction test and three-chamber social choice test provide important empirical measures of social interaction that can be assessed following environmental and physiological manipulations (Beery, 2021; File & Seth, 2003; Williams et al., 1992; M. Yang et al., 2011) At the same time, these tests are limited, typically focusing on dyadic interactions over relatively brief periods. Outside of the laboratory, field studies of group dynamics provide critical insights into how natural and environmental interactions within and between groups shape both individual and collective social behavior (Farine & Whitehead, 2015; Silk, 2023). Field studies, however, are difficult to compare and manipulate and often rely on scan and focal sampling to infer group dynamics.

Recent years have seen an increasing focus on long-term monitoring of behavior in group settings

using RFID tracking combined with video and processed with machine learning tools, both in the field (Bonter & Bridge, 2011; Duret et al., 2022; Kays et al., 2015; Smith et al., 2018; Wilmers et al., 2015) and in the laboratory (e.g. Forkosh et al., 2019; Levy et al., 2023; Puścian et al., 2016). Although lacking in assessment of specific behavioral interactions, the present RFID system provides continuous information about each individual's position, even when voles are obscured by housing and refuge materials (e.g. cage lids, food and hiding tunnels) or when lights are off, with an easily processed data set size. Future implementations could benefit from coupling movement data with detailed characterizations of behavior from video data in a subset of the trial duration.

RFID monitoring, used with Voletron to track group co-occupancy, is easily adaptable for use with other group-living species in a more ethologically relevant laboratory setting. The habitat set-up can be flexibly expanded or condensed to support different chamber numbers and configurations. The monitoring system can be used with any number of animals that can travel through tubes monitored by ring antennas. This system complements standardized behavioral testing by providing a means to assess continuous, free-living social behavior, especially in group settings, under controlled conditions that can be manipulated and replicated.

1.5 Chapter 1 Figures and Tables

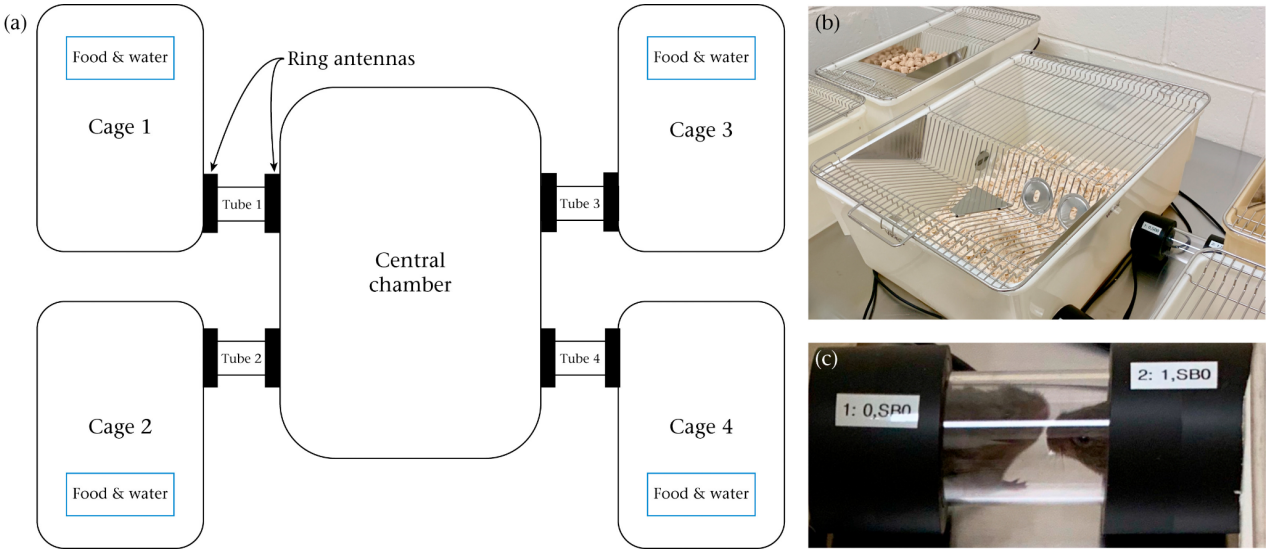


Figure 1.1 *Radiofrequency identification (RFID) habitat.* (a) Schematic and (b) photograph of the RFID-tracked habitat. (c) Two voles interact within a tube in the habitat.

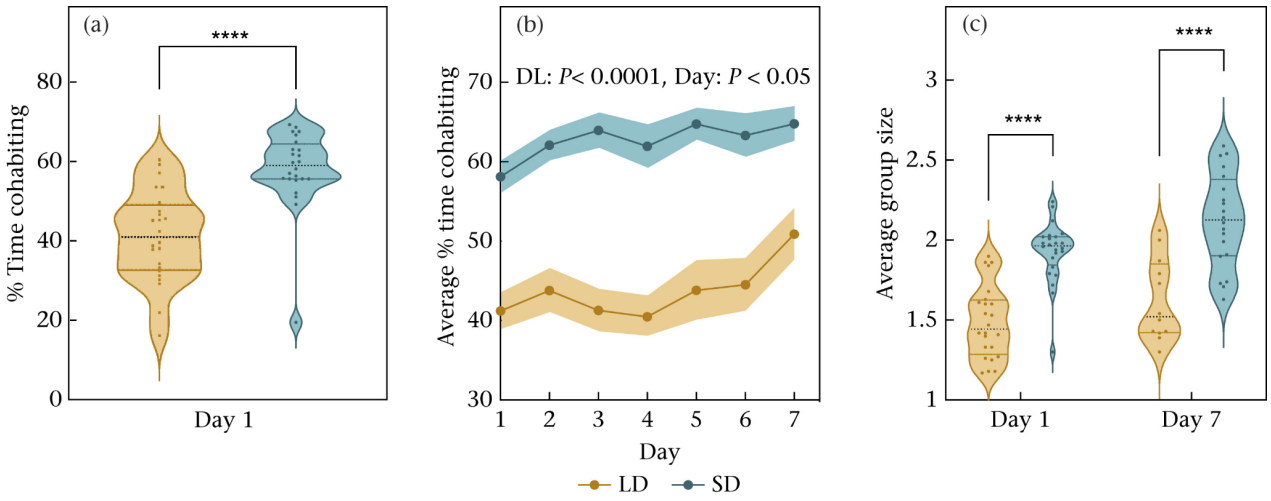


Figure 1.2 *Cohabitation and group metrics. SD voles spent more time cohabiting and cohabited in larger groups*. (a) Percentage of time cohabiting with at least one other vole on day 1 for short daylengths and long daylengths. (b) Average percentage of time cohabitating over the course of the experiment for short-day (SD) and long-day (LD) voles. Shaded areas represent ±1 SE. (c) Average group size for short-day and long-day voles on day 1 and day 7. DL: daylength. ****P < 0.0001.

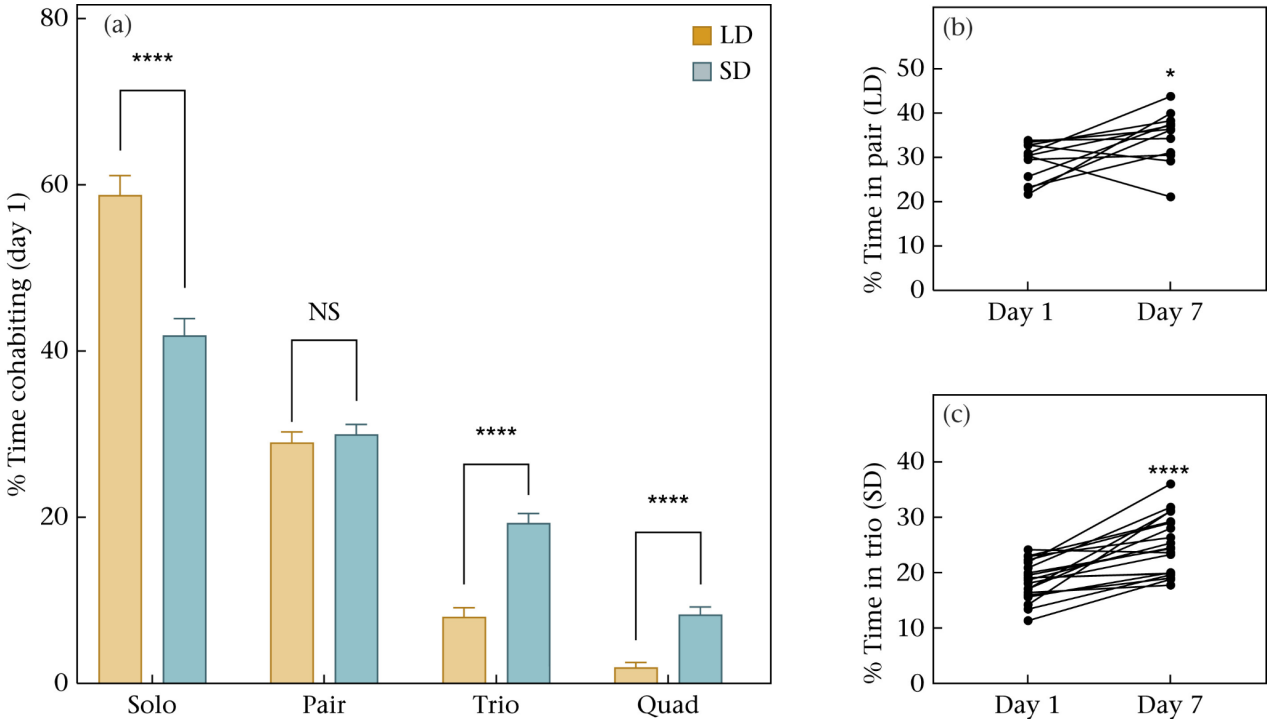


Figure 1.3 *Percentage of time cohabiting in groups of different sizes.* (a) Percentage of time long-day (LD) and short-day (SD) voles spent alone, in a pair, trio or quad on day 1 in the habitat. (b) Percentage of time long-day voles spent in pairs from day 1 to day 7. (c) Percentage of time short-day voles spent in trios from day 1 to day 7. $*P < 0.05$; $****P < 0.0001$.

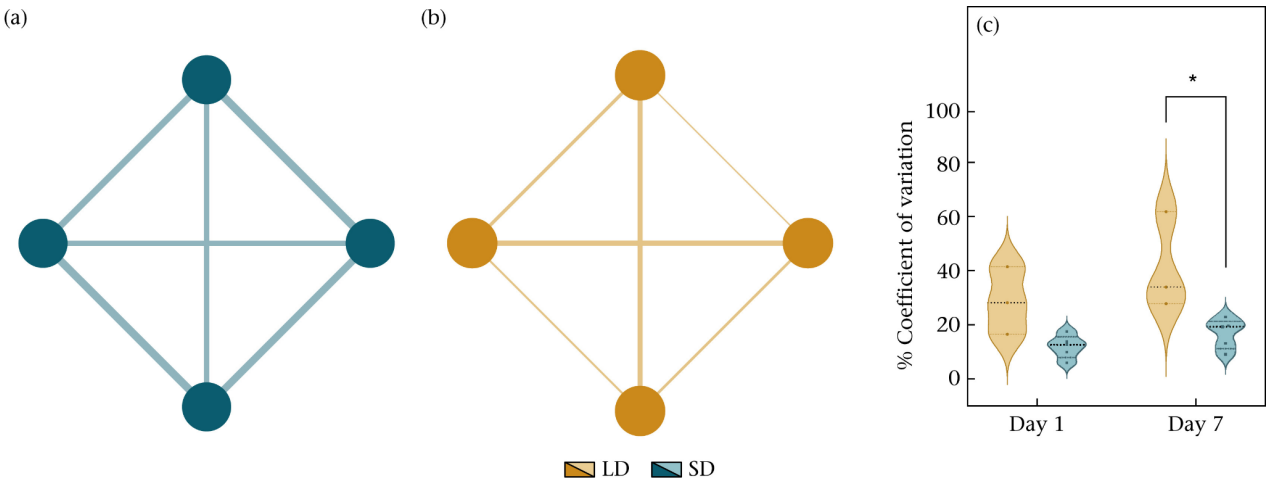


Figure 1.4. *Sample social networks from a single cohort on day 7 in (a) short-day (SD) and (b) long-day (LD) habitats.* Each node represents an individual vole, while edge weights represent the time pairs of voles spent in the same chamber. (c) Percentage of coefficient of variation for short-day and long-day cohorts on day 1 and day 7. $*P < 0.05$.

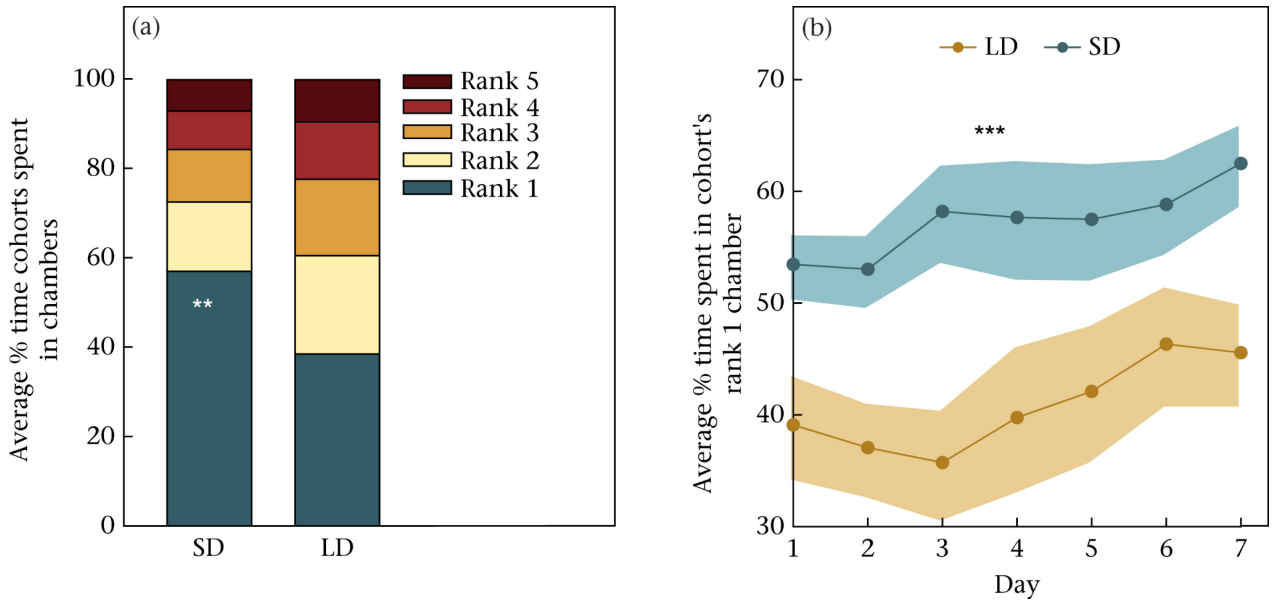


Figure 1.5 *Home cage preferences.* (a) Average percentage of time short-day (SD) and long-day (LD) voles spent in the chambers of each cohort's preference (rank 1–5) on day 1. (b) Time series of average percentage of time short-day and long-day voles spent in their cohort's most preferred cage over the duration of the experiment. ***P < 0.001.

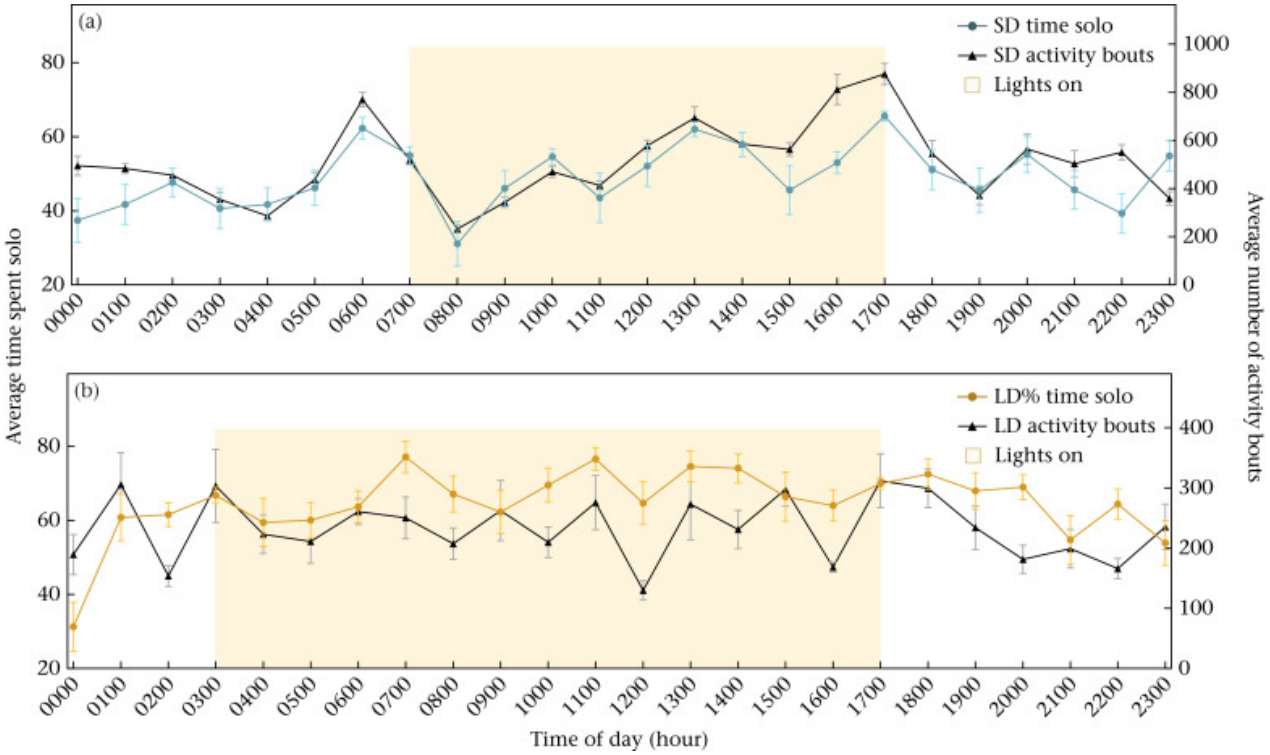


Figure 1.6 *Hourly analysis of time spent solo and average number of activity bouts.* Percentage of time (a) short-day (SD) and (b) long-day (LD) voles spent alone at each hour (four voles measured for 7 days). Also shown are activity data (black symbols) collected in the home cage of short-day and long-day solo-housed voles.

Grouping metrics

Variable/day	Short daylengths (day 1, $N = 24$; day 7, $N = 20$)	Long daylengths (day 1, $N = 24$; day 7, $N = 12$)	Daylength effects	Time series analysis (model effects for day*daylength)
% Time cohabiting				**DL**: $F_{1,24} = 5.16$, $P < 0.0001$
Day 1	58.13 ± 2.04	41.21 ± 2.34	$t_{46} = 5.45$, ***P* < 0.0001**	**Day**: $F_{6,19} = 3.05$, $P < 0.029$
Day 7	64.79 ± 2.19	50.88 ± 3.27	$t_{30} = 3.67$, ***P* = 0.0009**	DL*day: $F_{6,19} = 1.42$, $P = 0.2588$
Average group size				**DL**: $F_{1,24} = 13.77$, $P < 0.0001$
Day 1	1.93 ± 0.04	1.49 ± 0.05	$t_{46} = 7.26$, ***P* < 0.0001**	**Day**: $F_{6,19} = 2.13$ $P = 0.0006$
Day 7	2.13 ± 0.07	1.62 ± 0.07	$t_{30} = 4.87$, ***P* < 0.0001**	**DL*day**: $F_{6,19} = 1.35$, $P = 0.0069$
Average group size when cohabiting				**DL**: $F_{1,24} = 93.07$, $P < 0.0001$ **Day**: $F_{6,19} = 12.47$, $P < 0.0001$
Day 1	2.62 ± 0.03	2.27 ± 0.04	$t_{46} = 7.71$, ***P* < 0.0001**	**DL*day**: $F_{6,19} = 7.11$, $P < 0.0001$
Day 7	2.74 ± 0.06	2.30 ± 0.06	$t_{30} = 5.20$, ***P* < 0.0001**	

Table 1.1 *Average time spent in a group and average group sizes across the recording interval and excluding time spent alone are reported on day 1 and day 7 in short daylengths and long daylengths with comparisons across daylengths at these time points.* Outcomes of a repeated measures mixed effects model using the daily time series are reported for the effects of daylength, DL (short daylengths, long daylengths), day (1–7) and their interaction, with cohort accounted for as a random variable (see Statistical Analysis). Means are listed ± SE. Bolded results are statistically significant.

Group composition

Variable/day	Short daylengths (day 1, $N = 24$; day 7, $N = 20$)	Long daylengths (day 1, $N = 24$; day 7, $N = 12$)	Daylength effects	Time series analysis (model effects for day*daylength)
% Time in quad				**DL**: $F_{1,24} = 9.56 \times 10^{14}$, $P < 0.0001$
Day 1	8.27 ± 0.97	1.92 ± 0.65	$t_{46} = 5.45$, ***P* < 0.0001**	**Day**: $F_{6,19} = 9.63 \times 10^{12}$, $P < 0.0001$
Day 7	11.94 ± 1.88	2.05 ± 0.53	$t_{30} = 5.07$, ***P* < 0.0001**	**DL*day**: $F_{6,19} = 2.19 \times 10^{12}$, $P < 0.0001$
% Time in trio				**DL**: $F_{1,24} = 9.63$, $P < 0.0001$
Day 1	19.30 ± 1.17	8.04 ± 1.08	$t_{46} = 7.08$, ***P* < 0.0001**	**Day**: $F_{6,19} = 2.97$, $P < 0.0001$
Day 7	24.95 ± 1.18	12.04 ± 2.84	$t_{30} = 4.85$, ***P* < 0.0001**	DL*day: $F_{6,19} = 0.59$, $P = 0.142$
% Time in pair				DL: $F_{1,24} = 0.0009$, $P = 0.89$
Day 1	29.99 ± 1.19	29.02 ± 1.27	$t_{46} = 0.56$, $P = 0.5784$	Day: $F_{6,19} = 0.64$, $P = 0.11$
Day 7	27.27 ± 1.63	34.64 ± 1.71	$t_{30} = -2.96$, ***P* = 0.0059**	**DL*day**: $F_{6,19} = 1.73$, $P = 0.0019$
% Time alone				**DL**: $F_{1,24} = 3.74$, $P < 0.0001$
Day 1	41.87 ± 2.04	58.79 ± 2.34	$t_{46} = -5.45$, ***P* < 0.0001**	**Day**: $F_{6,19} = 1.05$, $P = 0.020$
Day 7	35.21 ± 2.19	49.12 ± 3.27	$t_{30} = -3.67$, ***P* = 0.0009**	DL*day: $F_{6,19} = 0.43$, $P = 0.28$

Table 1.2 *Time spent alone, in pairs, trios and quads on day 1 and day 7 in short daylengths and long daylengths with daylength comparisons.* Model effects for repeated measures mixed effects models on daily time series are also reported for the effects of daylength (DL), day and their interaction, with cohort accounted for as a random variable (see Statistical Analysis). Means are listed ±SE. Bolded results are statistically significant.

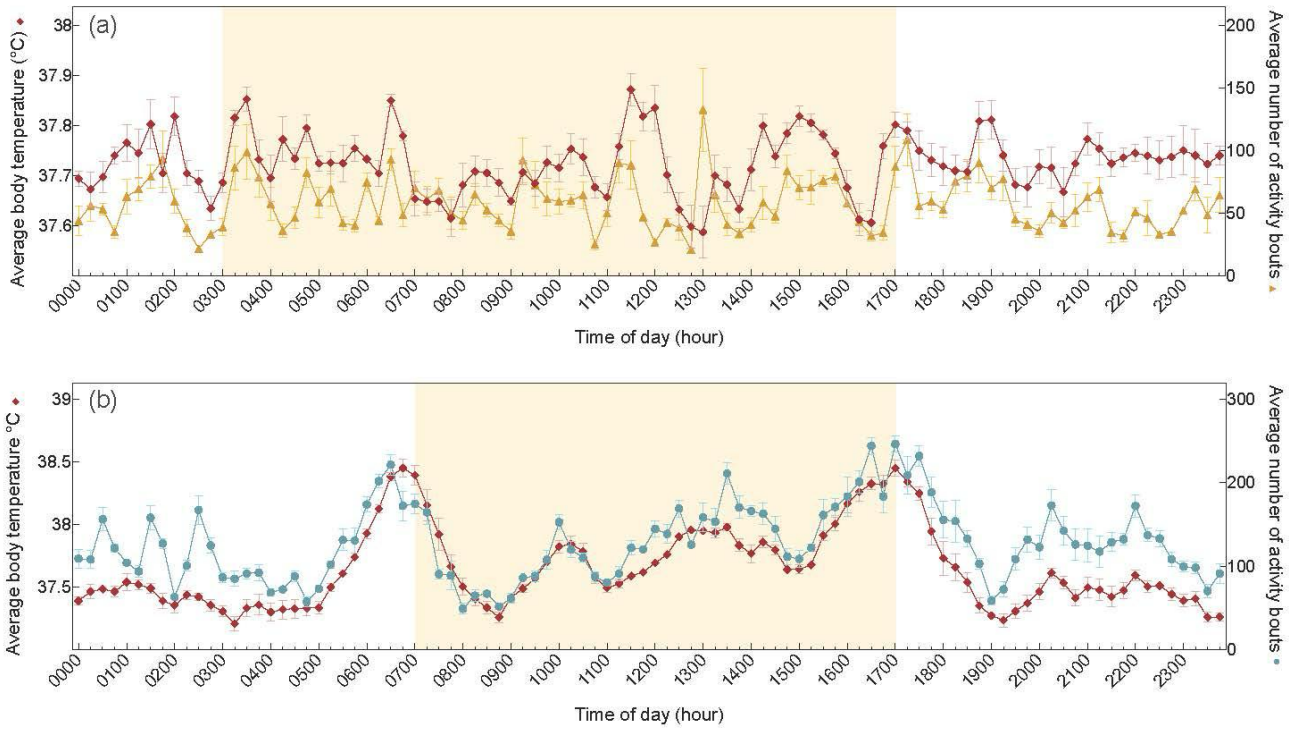


Figure S1.1 *Analysis of body temperature and number of activity bouts in 15 min intervals of solo-housed voles in their home cage.* Number of activity bouts for (a) long-day (yellow symbols) and (b) short-day (blue symbols) voles averaged across four voles/daylength over 7 days. Activity is co-plotted with average body temperature (red symbols) collected from the same voles. Yellow shading denotes lights on. A significant correlation between activity and temperature was present in both long-day and short-day voles, but was stronger in short-day voles (81.6%) than in long-day voles (36.5%) at lag 0.

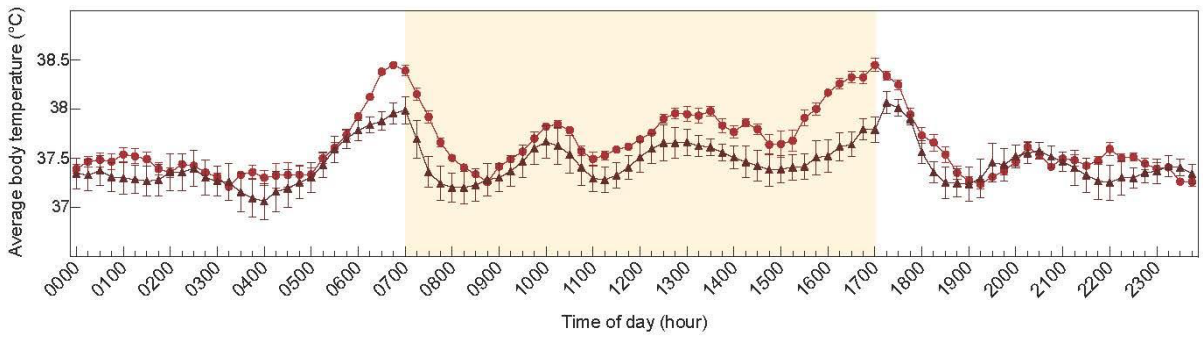


Figure S1.2. *Consistency of core and subcutaneous measures of body temperature in short-day solo-housed voles in their home cage from distinct data sources.* Red dots display body temperature data taken in the present study using subcutaneously implanted BioTherm transponders (averaged across four voles over 7 days). Core body temperature measured using iButton implants (maroon triangles) was recorded in a distinct set of short-day solo-housed females in Beery et al. (2008). Yellow shading denotes lights on. A strong correlation (84.1%) existed between the two data sources measured 15 years apart with different devices and placements, highlighting the robustness of this temperature rhythm.

Chapter 2

Daylength and social history impact behavior and physiology in meadow voles

ABSTRACT

While group living is common throughout the animal kingdom, uncovering the mechanisms that drive social behavior remains difficult. The meadow vole (*Microtus pennslyvanicus*) offers an ideal model to study these mechanisms as individuals shift their social structure in response to seasonal cues. During the summer breeding season, females live alone, defending exclusive territories while rearing their young. In the winter however, they live communally in mixed-sex groups. Manipulating daylength alone in laboratory settings mimics many of the behavioral and physiological shifts that occur in the wild. However, social history naturally co-varies with season, and is likely to also impact social behavior and physiology.

To untangle the effects of social history, daylength, and their interaction on social behavior in meadow voles, we housed voles in long, summer-like, or short, winter-like daylengths alone or in same-sex pairs. We assessed how housing and daylength differentially affected social interactions, anxiety-like behaviors, and circulating hormones. Daylength impacted anxiety-like behavior in the light dark box and aggression during a social interaction, with short day voles spending more time in the light portion of the light dark box, and having more bouts of aggression than long day voles. Social history impacted behavior in the open field test and oxytocin concentration. Solo-housed voles spent more time in the center of the open field test and had higher oxytocin concentration than pair-housed voles. Voles reared in "seasonally unaligned" conditions–where social history did not match daylength–had higher levels of corticosterone, engaged in less affiliative behaviors, and had lower latency to interact with a stranger than those housed in "seasonally aligned" housing. Together these findings indicate that daylength and social history shape distinct aspects of organismal responses to the environment, while interacting to shape social behavior.

2.1 Introduction

Sociality, or group living, is an integral part of mammalian life. The degree to which an animal is social varies widely. Some species only interact with others to mate and provide some maternal care, while others live in large cooperative groups with multiple complex relationships. Despite it's importance, proximate mechanisms underlying sociality, particularly those outside of a reproductive context, are poorly understood. Meadow voles (*Microtus pennsylvanicus*) provide an excellent model with which to study social behavior within a single species, as they experience a seasonal shift in sociality, driven largely by daylength. We investigated daylength, social environment, and their interaction to determine their effects on behavior and circulating hormone levels in meadow voles.

For species living in temperate climates, one of the most salient environmental cues is the duration of the light phase, or daylength (photoperiod). Unlike food availability or temperature, which can vary unpredictably, daylength is a consistent, reliable cue and is used to synchronize behavior and physiology with the season (Paul et al., 2008; Prendergast et al., 2002). Meadow voles use daylength as a primary cue to time changes in reproduction, physiology, and social behavior (Dark & Zucker, 1983). Female meadow voles live alone in the summer months, only permitting males in their territories for short intervals to breed(Madison, 1980; Webster & Brooks, 1981). As the days shorten and winter arrives, females shift to living in mixed-sex groups of up to 10 conspecifics (Madison & McShea, 1987). Initially, these groups are composed of a female and her last litter of undispersed offspring, but due to high predation, by the end of the season the groups are composed of unrelated voles (Madison & McShea, 1987). Consequentially, females born at the beginning of the breeding season disperse and live alone until the end of the fall, while those born at the end of the breeding season don't reside alone until the following spring. While breeding females do cohabit with their young before dispersal, they do not cohabit with other adults for extended periods of time during the breeding season. This nuance in social history may be an important part of preparing voles for the transition in social behavior.

In the lab, altering daylength is sufficient to induce many of the changes in physiology and behavior associated with the season in the wild. Meadow voles living in long, summer-like daylengths spend more time alone, are less affiliative towards others, and maintain reproductive capacity (Beery et al., 2008; Lee et al., 2019; Power et al., 2025). Females in long day lengths (LD) have higher total corticosterone (CORT) (Anacker, Reitz, et al., 2016), and higher CRFR1 binding in the hippocampus than do their counterparts in short day lengths (SD)(Beery et al., 2014a). In short, winter-like daylengths, meadow voles lose reproductive capacity (Dark et al., 1983) but spend more time in groups (Ondrasek et al., 2015a; Power et al., 2025), huddle more with known conspecifics, and are more tolerant of strangers (Beery et al., 2008, 2009a; Lee et al., 2019). SD voles also have increased oxytocin receptor density in multiple regions (Beery & Zucker, 2010; Parker et al., 2001), increased CRFR2 binding in the hippocampus (Beery et al., 2014a), and increased dopamine release (Mun et al., 2026). Many of these neurobiological changes are known to influence social behavior in voles and other species.

While most of the prior laboratory research in meadow voles has focused on the effects of daylength, there are other factors that can alter behavior. One of these factors is social history (housing). In SD, housing voles alone (against their natural social housing history in the wild), results in less pro-social contact and fewer bouts of social investigation than SD voles housed in pairs (Lee et al., 2019) SD solo-housed voles also differ in aggressive behaviors-they have fewer instances of lateral attack/threat than SD pair-housed voles (Lee et al., 2019), though there is not a significant difference in overall aggression score. In a study examining effects of isolation on anxiety behavior and corticosterone, male meadow voles had no differences in anxiety behavior or corticosterone concentration due to isolation, suggesting isolation isn't a stressor like it is in many species (Stowe et al., 2005). Isolation in LD female meadow voles has yet to be examined. These findings suggest that social history plays an important role in meadow vole social behavior in addition to daylength.

Despite research on the effects of daylength and social history independently, few studies address how these environmental cues interact to shape behavior and physiology. In the wild, meadow voles need to integrate multiple cues to shape season-appropriate behavior. To understand how meadow voles shift their social behavior seasonally, we need to understand the roles these cues and their

interaction play. We altered both daylength (SD, LD) and social history (solo, pair) to disentangle the effects of daylength, social history, and their interaction on anxiety, social behaviors, and hormone concentration in female meadow voles.

2.2 Methods

Animal Subjects

Meadow voles were bred locally in LD (14 hour light:10 hour dark, lights on 03:00-17:00) and housed in clear plastic cages (45 x 25 x 15 cm) with aspen bedding, nesting materials, and an opaque plastic refuge tube. Temperature was maintained at 20 +/- 4 °C. *Ad libitum* food (LabDiet Mouse Chow 5015) and water were available. Diet was supplemented twice a week with apple, carrot, or sunflower seeds. LD voles additionally received leafy greens (lettuce or spinach) weekly. All procedures adhered to federal and institutional guidelines and were approved by the University of California, Berkeley Animal Care and Use Committee.

Voles were group weaned with siblings between postnatal day 19-20. Within one week, female voles were sexed and separated into experimental housing in LD or SD (SD 10 hour light: 14 hour dark, 07:00-17:00). When applicable, cage mates were same-sex, age-matched conspecifics. Males were not used as they do not exhibit as distinct of a shift from solitary to social living.

Experimental Overview

To assess the effects of daylength, social history, and their interaction on anxiety behaviors, social behaviors, and circulating hormones, voles were housed in one of the following ways— alone in LD, in pairs in LD, alone in SD, and in pairs in SD, between PND 70-90, all focal voles underwent an open field test, light dark box test, and a social interaction test on consecutive days. Within two minutes of the end of the social interaction test, focal voles were sacrificed, and blood was collected.

Behavioral Tests

Open Field Test (OFT)

Voles were placed at the edge of the outermost circle of the OFT; a large circular open arena (56 cm diameter) and filmed for 10 minutes. Experimenters left the testing room during the test. We assessed behaviors including latency to center, time spent in the center, and total distance travelled using a custom script (OpenField1.3.pl, AKB) and Ethovision (Noldus, Leesburg, VA, USA). One SD pair animal was excluded from all tests due to video error. Two videos were not able to be properly tracked in Ethovision and were excluded only from distance travelled metrics.

Light Dark Box (LDB)

Voles were placed int the dark portion of the LDB, a two chambered apparatus with a light chamber and dark chamber (49 × 20.5 × 19.5 cm) and filmed for 10 mins. Experimenters left the testing room during the test. We assessed behaviors including latency to enter the light and time spent in the light using a custom script (LightDarkBox1.6.pl, AKB). Two animals (LD solo and LD pair) were excluded due to video error.

Social Interaction (SIT)

The focal vole was placed into a neutral cage (45 x 25 x 15 cm) with a same-sex, daylength- and social history-matched stranger, and filmed for 10 minutes. The stranger was partially shaved (2cm x 2cm patch) for identification during these tests. Experimenters were present in the room to monitor aggressive bouts during the duration of the test. Tests were stopped early if three significant bouts of aggression occurred (two occurrences). We used BORIS (https://www.boris.un ito.it) to assess the following behaviors: aggression, huddling, allogrooming, and social investigation.

Corticosterone Quantification

Immediately following the SI test, voles were anesthetized using isoflurane, and trunk blood was collected, spun down at 4500 rpm for 20 minutes, and serum was stored at -80°C. A Corticosterone ELISA was conducted (Enzo Life Sciences, Lot 44KE180). Samples were diluted 1 part sample : 40 parts assay buffer, then processed following the procedure from Enzo. For total CORT measurements steroid displacement reagent (SDR) was added to the diluted samples prior to plating. For free CORT measurements, SDR was omitted from the samples. The plate was read at 405 nm with an Absorbance 96 plate reader (Byonoy, GmbH). One sample did not meet our pre-determined criteria of > 15% coefficient of variability between replicates and was excluded.

Oxytocin Quantification

An oxytocin ELISA (500440; Cayman Chemical Batch 0817512) was conducted at a dilution of 1 part sample: 3 parts assay buffer following kit protocols. The plate was read at 405 nm with an Absorbance 96 plate reader (Byonoy, GmbH). All samples met our pre-determined criteria of > 15% coefficient of variability between replicates and were included.

Data Analyses

All statistical analyses were performed using JMP version 20 and figures were constructed in GraphPad Prism version 11. All behavioral tests were analyzed with a GLMM, with post-hoc t-tests for planned pairwise comparisons of interest. Pearson's correlation coefficient was calculated between a number of variables.

2.3 Results

Social history affected behavior in the Open Field Test

In the OFT, there was a main effect of social history (housing), where solo-housed voles spent more time in the center of the arena than pair-housed voles (Fig. 2.1A) (GLMM: Housing *prob>f*=0.0054, $F_{1,46}$ = 8.53; Daylength *prob>f*=0.7574, $F_{1,46}$ = 0.7574; Daylength*housing *prob>f*=0.440, $F_{1,46}$ = 0.4396*).* Solo-housed voles also had lower latency to enter the center of the test regardless of daylength (Fig. 2.1B) (GLMM: Housing *prob>f* = 0.0018, $F_{1,46}$ = 10.979; Daylength *prob>f*=0.714, $F_{1,46}$ = 0.136; Daylength* housing *prob>f*=0.677, $F_{1,46}$ = 0.177). Overall distance travelled did not differ by social history, daylength, or their interaction. (GLMM: Housing *prob>f*=0.483, $F_{1,44}$ =0.501; Daylength *prob>f*=0.420, $F_{1,44}$ = 0.663; Daylength*housing *prob>f*=0.713*,* $F_{1,44}$ = 0.137).

Daylength affected behavior in the Light Dark Box

In the light dark box, there was a main effect of daylength for time spent in the light portion of the box. SD voles spent more time fully in the light than LD voles (Fig. 2.1C) (GLMM: Daylength prob>f = 0.0017, F1,45 = 11.162; Housing prob>f=0.0926, F1,45 = 2.954; Daylength* housing prob>f=0.531, F1,45= 0.398). There was a main effect of daylength, social history, and a trend towards the interaction between the two for latency to leave the dark portion of the box (Fig. 2.1D) (GLMM: DL prob>f = 0.0356, F1,45= 4.709; housing prob>f = 0.0248, F1,45= 5.395; Daylength* housing prob>f = 0.0584, F1,45= 3.769). Latency was ultimately driven by LD pair-housed voles who had the longest latency to leave the box. There was a significant difference between LD pair and LD solo (Student's t-test: p=0.0045), and between LD pair and SD pair (Fig. 2.1D) (Student's t-test: p=0.0053).

Interaction between daylength and social history affected affiliative behavior

In the social interaction test, there was a main effect of the interaction between daylength and social history on huddling duration (GLMM: Daylength*housing prob>f =0.0281, F1,42= 5.171; Daylength prob>f =0.4137, F1,42= 0.681; Housing prob>f =0.3093, F1,42= 1.059) (Fig. 2.2A) and allogrooming duration (GLMM: Daylength*housing prob>f =0.0159, F1,42= 6.316; Daylength prob>f =0.243 F1,45= 1.400; Housing prob>f =0.172, F1,42= 1.929). SD pair-housed voles huddled the most (LD pair vs SD pair (Student's t-test: p=0.0268), SD pair vs SD solo (p=0.0215)). LD solo-housed voles spent the most time allogrooming (LD solo vs LD pair (Student's t-test: p=0.0099). Huddling and allogrooming are both considered affiliative behaviors and are combined in Figure 2.2B (GLMM: DL*Housing prob>f =0.0132, F1,42=6.699; Daylength prob>f = 0.668, F1,42=0.187; Housing prob>f = 0.577, F1,42= 0.315). For both affiliative behaviors, voles housed in "seasonally aligned" housing (where season matches social history in the wild-LD solo and SD pair) are more affiliative towards a stranger than those in "seasonally unaligned" housing.

Daylength affected number of aggressive bouts

SD voles had more aggressive bouts than LD voles (Fig. 2.2A) (GLMM [Poisson]: Daylength prob>f =0.0001; Housing prob>f =0.4081, Daylength*housing DL prob>f =0.159) (Fig. 2.2B). SD solo-housed voles had the highest number of aggressive bouts within SD (Student's t-test: p=0.0288), and SD pair-housed voles had more aggressive bouts than LD pair-housed voles (Student's t-test: p=0.0050). There was no significant effect of daylength, housing, or their interaction on the overall duration of aggression, however (GLMM: Daylength prob>f =0.0963, F1,42= 2.929; Housing prob>f =0.248 F1,42= 1.372, Daylength*housing DL prob>f =0.250, F1,42= 1.363).

Interaction between daylength and social history affected latency to social interaction

Latency to first social interaction, regardless of type of interaction, was also affected by the interaction between daylength and social history (GLMM: Daylength*housing prob>f =0.0140, F1,42= 5.676; Daylength prob>f =0.639, F1,42= 0.154; Housing prob>f =0.585, F1,42= 0.334) (Fig. 2.2C). Voles housed in "seasonally aligned" housing interact more quickly than those in "seasonally unaligned" housing (Student's t-test: LD pair vs SD pair p=0.300; SD solo vs SD pair p=0.0296).

Interaction between daylength and social history affected free CORT

There was a main effect of the interaction between daylength*housing on free CORT following a social interaction with a stranger (Fig. 2.3A) (Daylength*housing prob>f = 0.0086, F1,24= 8.180; Daylength prob>f =0.6880, F1,24= 0.688; Housing prob>f =0.8035, F1,24= 0.063). LD pair had higher free CORT than LD solo (Student's t-test: p=0.0519) and SD pair (Student's t-test: p=0.0372). SD solo had higher free CORT than SD pair (Student's t-test: p=0.0560). Voles housed in "seasonally aligned" housing had lower free CORT than those housed in "seasonally unaligned" housing (Figure 2.3D) (Student's t-test: p=0.0063).

Social history and the interaction between daylength and social history affected total CORT

Total CORT following a social interaction with a stranger was impacted by housing (GLMM: prob>f = 0.0001, F1,23= 22.572) and daylength* housing (GLMM: prob>f = 0.0305, F1,23= 5.317). Daylength was not a main effect (GLMM: prob>f =0.3907, F1,23= 0.765 (Fig. 2.3B). LD pair-housed voles had the highest total CORT overall (Student's t-test: LD pair vs LD solo p <0.0001; LD pair vs SD pair p =0.0436). Voles housed in "seasonally aligned" housing had nearly significantly lower total CORT than "seasonally unaligned" voles (Fig. 2.3E) (Student's t-test: p= 0.0526).

Social history affected oxytocin

Social history affected OT following a social interaction test (GLMM: Housing prob>f = 0.0004, F1,31= 15.995; Daylength prob>f =0.8485, F1,31= 0.037; Daylength*housing prob>f =0.3808, F1,31= 0.790) (Fig. 2.3C). Solo-housed voles had higher OT than pair-housed within each daylength. There was no difference in OT between voles housed in "seasonally aligned" and those in "seasonally unaligned" housing (Fig. 2.3F) (Student's t-test: p= 0.4030).

Correlations across hormones and behavior

Since our data were all gathered in the same animals, we examined pairwise correlations between behaviors and hormones. Across all data, total corticosterone was positively correlated with free corticosterone (Fig. 2.4A), latency to exit in the light dark box (Fig. 2.4C), and latency to reach the center of the open field test (SI Fig. S2.1). Total corticosterone was negatively correlated with oxytocin (Fig 2.4B). We also found positive correlations between the amount of time a vole was aggressive during the social interaction test and overall social investigation (Fig. 2.4D), oxytocin (Fig. 2.4E), and the amount of time spent in the light in the light dark box (Fig. 2.4F)

2.4 Discussion

Our findings indicate that social behaviors, anxiety-like behaviors, and circulating hormones in female meadow voles are not driven by a single environmental factor. Instead, daylength, social history, and their interaction mediate distinct aspects of physiology and behavior.

Anxiety-like Behaviors

Notably, the results from our two behavioral assays typically referred to as measuring anxiety like behavior are different. The open field test showed a main effect of social history, while the light dark box had a main effect of daylength. Although both assays are both widely used to measure anxiety-like behaviors, a number of studies suggest these two tests are measuring different facets of anxiety (e.g. Çevik et al., 2025; Snyder et al., 2021). The open field test measures the conflict between exploration and the avoidance of wide-open spaces where predation is more likely. We found solo-housed voles spent more time in the center of an open field test and entered the center of the test arena more quickly than pair-housed voles, regardless of daylength. Typically, animals that spend more time in the center of the OFT and show lower latency to reach the center are interpreted as having lower anxiety, but sometimes this is interpreted as higher risk-taking behavior, or simply increased exploration. Our results also reveal another facet of the OFT, since this is the first time pair-housed voles are tested alone. It is possible that the stress of being separated from their partner for a short period of time and placed into a novel environment led to lower time in the center for pair-housed voles. Solo-housed voles, who are used to being alone, did not have this additional stressor, which may have led to them spending more time in the center.

Instead of measuring aversion to wide open spaces, the LDB measures aversion to brightly lit areas. There is still an element of the LDB that measures exploration, though the LDB arena is much closer in size to the voles' home cage than the OFT. Commonly, animals that spend more time in the light are considered less anxious, which can also be interpreted as having higher risk-taking behavior. In the LDB, we found a main effect of daylength. SD voles spent more time in the light portion of the box than LD voles. Daylength and social history had a main effect on latency to exit the box, with SD voles exiting more quickly than LD, and solo-housed exiting more quickly than pairs. Latency was particularly driven by LD pairs who took the longest to leave the box. These results suggest that SD voles are less anxious than LD, and particularly LD pairs are the most anxious. The results between the pair-housed LD and SD voles are consistent with findings from a previous study in meadow voles (Ossenkopp et al., 2005), where LD pairs avoided the light more than SD pairs.
To further support that the LDB and OFT are indeed measuring different aspects of anxiety, there was not a significant correlation between time in the light in the LDB and time in the center in the OFT across all groups, nor in latency to enter the center or the light. Together, our LDB and OFT findings highlight the benefits of using multiple behavioral assays to get a clearer picture of an individual’s and group’s behavior.

Social Interaction

Interestingly, voles housed in “seasonally aligned” housing conditions (LD solo and SD pair) showed the lowest latency to interact with each other and had the highest durations of affiliative behavior. In SD pairs, affiliation was primarily driven by huddling behavior, while in LD solos, by allogrooming. In the winter, huddling is necessary for survival due to its thermoregulatory benefits (Gilbert et al., 2010). It is likely then, that LD solo voles don’t huddle as much since the thermoregulatory benefit huddling provides is not critical for survival.

Previous studies in the lab suggest that LD voles engage in less affiliative behavior than their SD counterparts (Beery et al., 2008; Lee et al., 2019), although these studies used pair-housed voles in both daylengths. Because LD voles are typically solitary in the wild, pair housing in the lab may act as a chronic stressor. Given that stress disrupts same-sex bond formation and reduces affiliation in SD meadow voles (Anacker, Reitz, et al., 2016), a parallel stress mechanism may drive the lack

of affiliative behavior seen in LD pair-housed voles. Conversely, the absence of cohabitation stress in LD solo-housed voles may enable them to engage in more affiliative behaviors when exposed to a peer.

In a social interaction test, SD solo-housed had the highest instances of aggressive bouts, but aggression duration wasn't different between groups. Similarly, in another lab based study, SD meadow voles had higher frequency of attacks than LD voles (Lee et al., 2019). As Lee et al., suggested, it is possible that SD voles are overall more likely to engage with any conspecific, including aggressive bouts, and over time, these behaviors become more affiliative and lead to cohabitation (2019).

Across all voles, aggression was positively correlated with time in the center in the OFT, time in the light in the LDB, and time investigating during the SIT. In order to manage their response to environmental stress, individuals within a population display consistent correlated behaviors that fall along a continuum from proactive to reactive (reviewed in Laskowski et al., 2022)). In voles, increased aggression, investigation, time in the center, and time in the light constitute a more bold or proactive phenotype. Our results suggest although daylength and social environment contribute to group level effects, some aspects of behavior remain consistent within an individual vole and determine how it responds to its environment.

Circulating Hormones Following SIT

Consistent with many of our behavioral findings in the SIT, daylength and social history interact to drive corticosterone differences between groups. Total corticosterone measures the entire pool of circulating corticosterone (both bound and unbound), while free corticosterone measures the small percentage unbound to carrier protein that is biologically active. The LD pair-housed voles had the highest total corticosterone. Free corticosterone was highest in voles in "seasonally unaligned" housing, which suggests that a mismatch between daylength and expected social environment leads to increased HPA axis activity and is a stressor, which ultimately impacts social interactions.

Unlike glucocorticoids, which were affected by the interaction between daylength and social history, the oxytocin system showed a main effect of social history. Solo-housed voles in both daylengths exhibited significantly higher OT concentrations following the SIT than their pair-housed counterparts. Increased oxytocin in solitary rodents is a widespread phenomenon. Prairie voles have increased circulating OT following an extended period of isolation (Grippo, Gerena, et al., 2007), and isolated mice and voles have increased OT cell counts in the paraventricular nucleus (Grippo, Lamb, et al., 2007; Musardo et al., 2022). Elevated OT during isolation may be an important part of maintaining social drive to interact with conspecifics (Chang et al., 2025; D. Liu et al., 2025). OT, while well known for its affiliative effects, can also promote aggression and other antisocial behaviors (Beery, 2015). It's therefore possible that the endogenous increase in OT lead to an increase in affiliation as seen by allogrooming in LD solo-housed voles, but an increase in aggression in SD solo-housed voles, driven in part by seasonal changes in OT receptors (Beery & Zucker, 2010; Parker et al., 2001).

Summary

Our data show that behavior and physiology in female meadow voles rely on the interaction between multiple environmental factors. Decoupling daylength from housing reveals that an environmental

mismatch leads to elevates HPA activity acting as a stressor, whereas aligning both daylength and housing lead to increases in affiliation and decreases in corticosterone. Daylength and housing both impacted behavior and physiology independently also. Main effects of daylength were evident in the light dark box and bouts of aggression. Housing affected circulating oxytocin and open field test metrics. Standard housing in lab-based studies can introduce a number of confounds when the animal's natural history is not accounted for and should be carefully considered.

2.5 Chapter 2 Figures

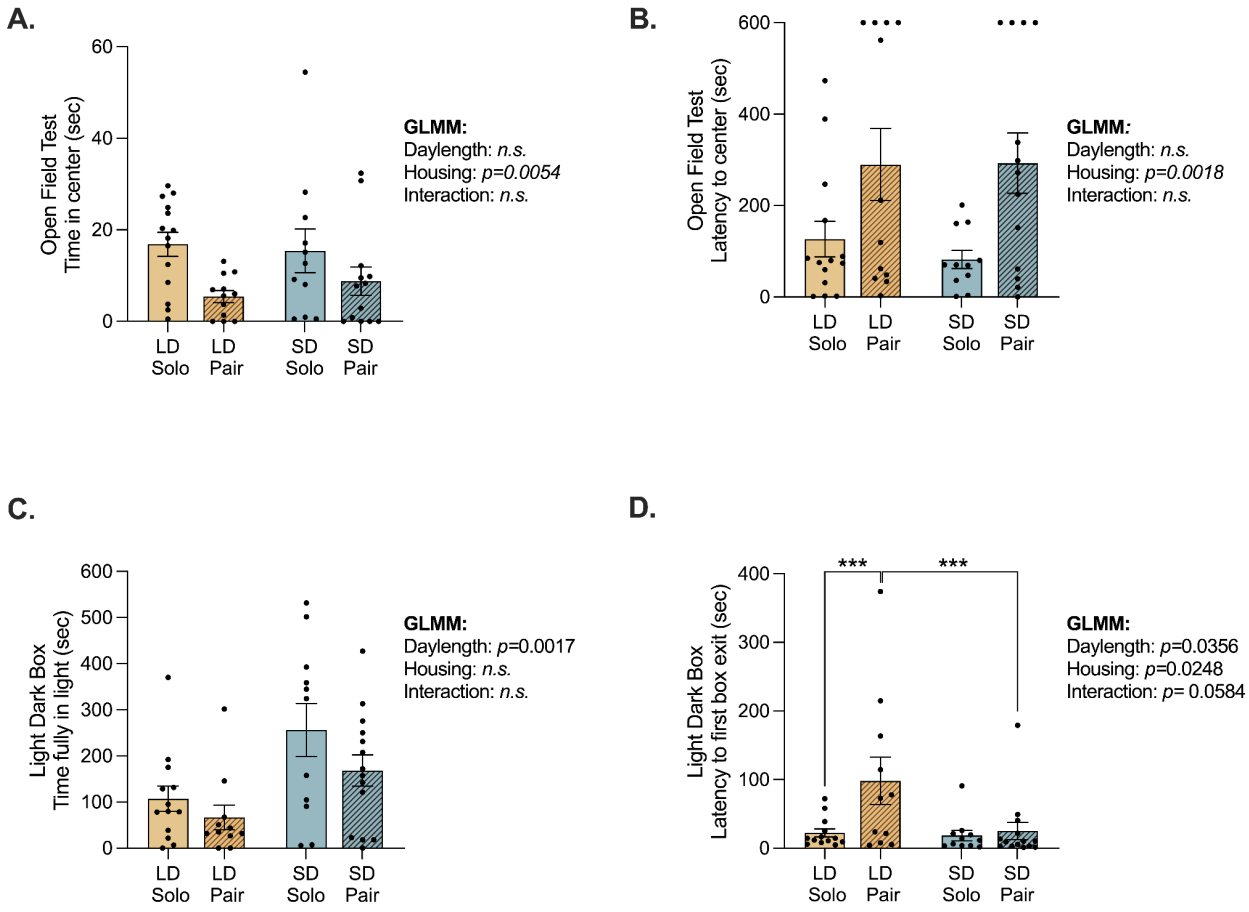


Figure 2.1. *Behavior in Open Field Test and Light Dark Box.* **A.** Solo-housed voles in both daylengths spend more time in the center of an Open Field Test than pair-housed voles. (LD solo n=14; LD pair n=12; SD solo n=11; SD pair n=13) **B.** Pair-housed voles are slower to enter the center of the test than solo-housed voles (LD solo n=13; LD pair n=11; SD solo n=11; SD pair n=14) **C.** SD voles spend more time in the light portion of a Light/Dark Box than LD. **D.** LD pair-housed voles are the slowest to leave the Light Dark Box. Post-hoc pairwise comparisons shown when interaction effect was significant. Mean and SEM shown. *** =<0.001.

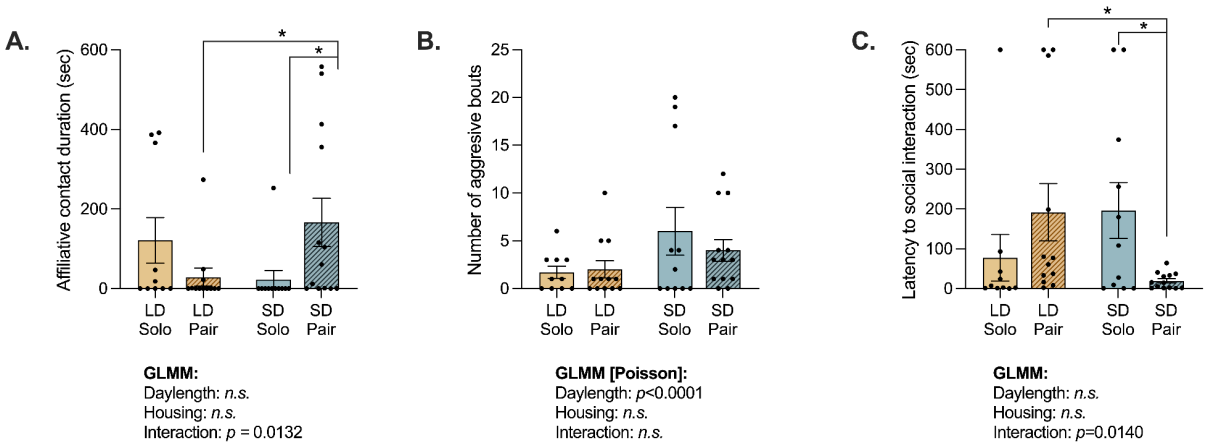


Figure 2.2. *Behavior in a social Interaction Test* **A.** SD voles have more bouts of aggression than LD voles. SD solo-housed voles are the most aggressive. (LD solo n=10; LD pair n=12; SD solo n=11; SD pair n=13) **B.** Voles in "seasonally aligned" housing (i.e. LD solo and SD pair) spent more time in affiliative contact with a stranger than voles in "seasonally unaligned" (i.e. LD pair and SD solo). **C.** Voles housed in "seasonally aligned" housing also interact more quickly with a stranger than "seasonally unaligned" voles. Mean and SEM shown. *= <.05, ***=<0.001.

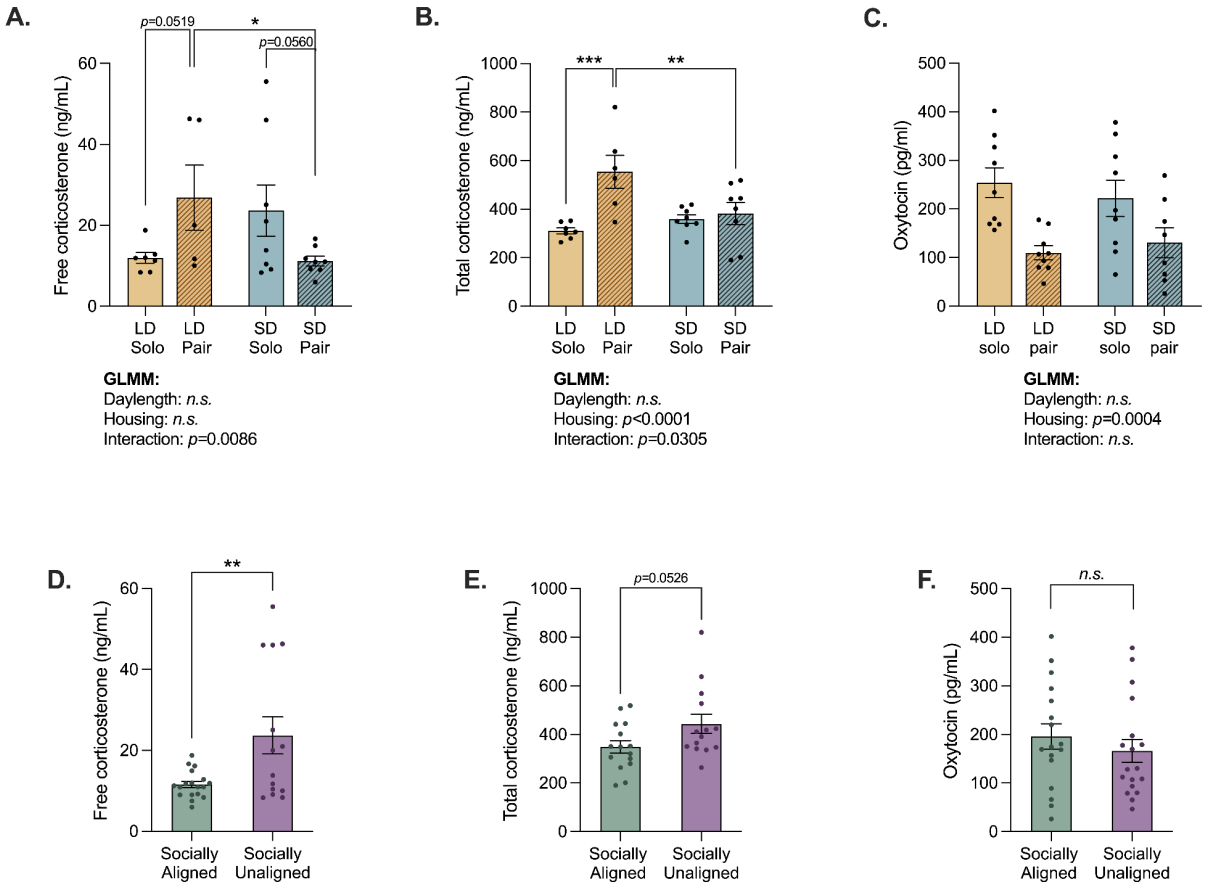


Figure 2.3. *Plasma hormones following a social interaction with a stranger.* **A.** Voles in "seasonally unaligned" housing conditions have higher free corticosterone than voles in "seasonally aligned" housing conditions. There was a significant effect of daylength*housing (LD solo n=7; LD pair n=5; SD solo n=8; SD pair n=8) **B.** LD pair-housed voles have the highest levels of total corticosterone. There were significant effects of housing and daylength* housing. (LD solo n=7; LD pair n=6; SD solo n=8; SD pair n=8) **C.** Solo-housed voles have higher oxytocin in both daylengths than pair-housed voles. There was a significant effect of housing, but not daylength or daylength*housing (LD solo n=9; LD pair n=9; SD solo n=9; SD pair n=8). Free corticosterone (**D.**), total corticosterone (**E.**), and oxytocin (**F.**) data replotted by "social alignment" status. Mean and SEM shown. * = <0.05, ** = <0.01, *** = <0.001

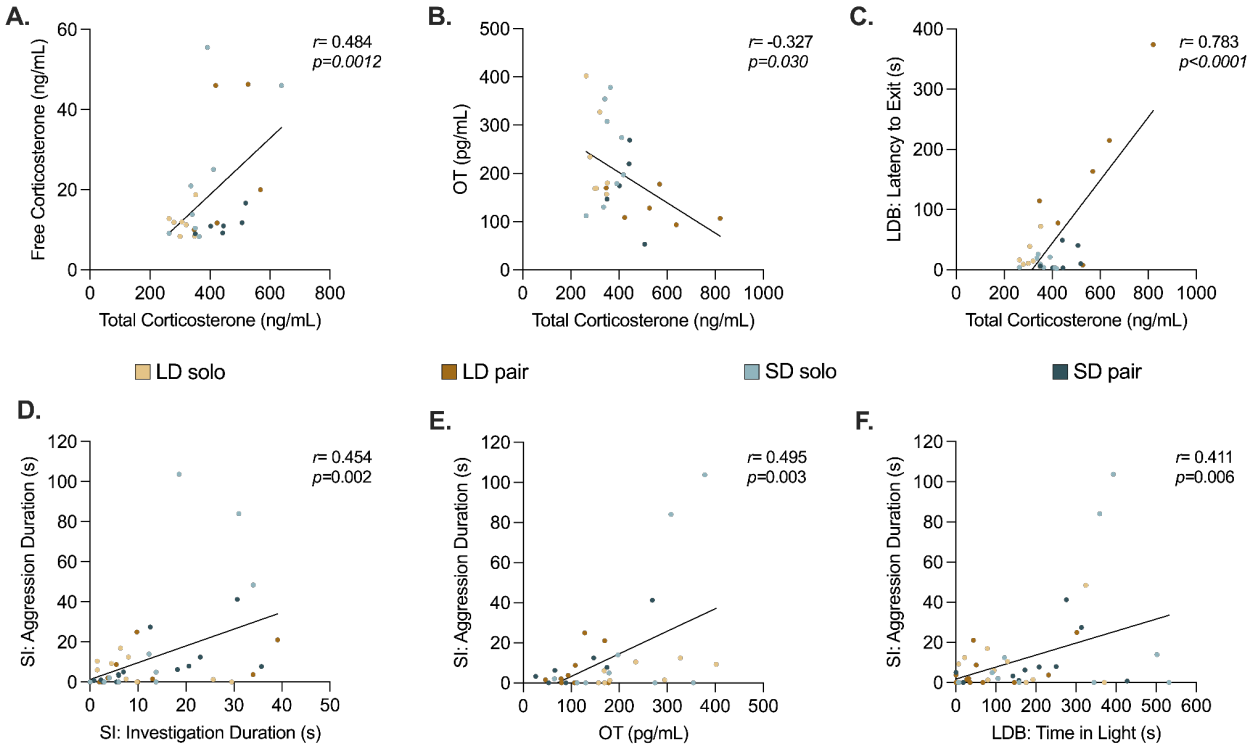
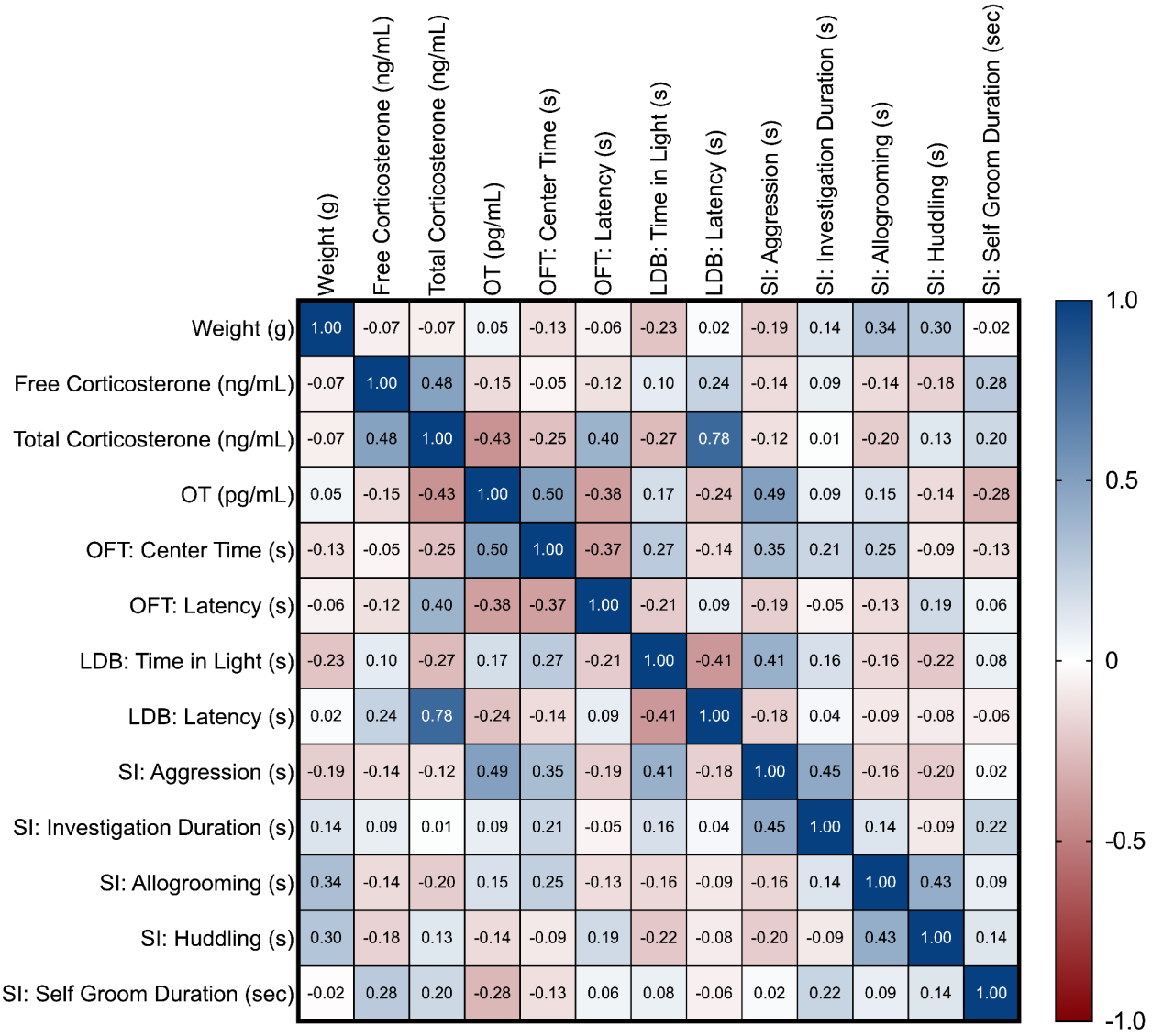


Figure 2.4. *Correlations between variables across all groups* **A.** Total corticosterone and free corticosterone were positively correlated **B.** Total corticosterone was negatively correlated with oxytocin **C.** Total corticosterone was positively correlated with the amount of time it took for voles to exit the LDB. The amount of time voles were aggressive during the social interaction test was positively correlated with **D.** investigation duration during the social interaction test, **E.** oxytocin, and **F.** the amount of time they spent in the light portion of the LDB. LD solo-housed voles are colored in light yellow; LD pair-housed voles are colored in dark yellow; SD solo-housed voles are colored in light blue; and SD pair-housed voles are colored in dark blue for all figures.



SI Figure S2.1. *Correlations between data.* Pearson's r values for each pairwise correlation. Blue color indicates a positive correlation between variables while red indicates a negative correlation. Darker colors indicate stronger correlations, while lighter shades represent weak/no correlation.

Chapter 3

Neurochemical imaging reveals changes in dopamine dynamics with photoperiod in a seasonally social vole species

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* J.M and K.C.P. contributed equally to this work

ABSTRACT

Studying dopamine signaling in nonmodel organisms is crucial for understanding the broad range of behaviors not represented in traditional model systems. However, exploring new species is often hindered by a scarcity of tools suitable for nongenetic models. In this work, we introduce near-infrared catecholamine nanosensors (nIRCats) to investigate dopamine dynamics in meadow voles, a rodent species that exhibits distinct changes in social behavior and neurobiology across photoperiods. We observe increased dopamine release and release site density in voles housed in short photoperiods (which induce a social phenotype), suggesting adaptations linked to environmental changes. Moreover, pharmacological and extracellular manipulations demonstrate that short photoperiod/social voles exhibit heightened responsiveness to dopamine-increasing interventions and resilience against suppressive conditions. These findings highlight a significant association between dopamine signaling and photoperiod-driven changes in social behavior and establish nIRCats as an effective tool for expanding our understanding of dopamine dynamics across nonmodel organisms.

3.1 Introduction

Significant breakthroughs in neuroscience have emerged from the study of diverse species from the barn owl to the sea slug, reinforcing August Krogh's famous declaration: "for many problems there is an animal on which it can be most conveniently studied" (Krebs, 1975; Krogh, 1929). Studies of diverse organisms also enable comparisons across species, improving our understanding of shared mechanisms and of species-specific adaptations (Hale, 2019; Laurent, 2020; Taborsky et al., 2015; Yartsev, 2017). Nowhere is this more evident than in the study of social behavior, which can be highly variable between even closely related species (Hofmann et al., 2014b; Lee & Beery, 2019; Phelps et al., 2010). Although the study of diverse species is crucial, it is accompanied by methodological challenges, as many tools and techniques available in model organisms are genetically based and challenging to apply outside of the model organisms for which they have been developed. The development of nongenetically encoded neurochemical sensors that can be applied in wide-ranging species promises to advance research on behaviors exhibited only by "weird," nontraditional species. Here, we validate and apply synthetic catecholamine sensors to investigate photoperiod-driven changes in dopamine release, reuptake, and diffusion in

the striatum of meadow voles (Microtus pennsylvanicus), a nonmodel organism that offers a rare opportunity to study pathways underlying seasonal changes in social dynamics. Using our tool, we find previously uncharacterized dopamine signaling differences in voles under short photoperiods in which they exhibit increased social interactions, indicating a potential role for changing dopamine in mediating this shift.

Dopamine, a neuromodulatory catecholamine most often associated with reward and pleasure, also plays important roles in regulating social behavior. Striatal dopamine in particular is elevated after mating, facilitates social bonding following mating in some species, and is involved in social reward processing in a number of species, including humans (Báez-Mendoza & Schultz, 2013; Bhanji & Delgado, 2014). However, dopamine release and modulatory dynamics at the synaptic level that may support alterations in social behaviors remain largely unknown. Many current methods used to examine dopamine dynamics are limited by spatial and/or temporal resolution. Microdialysis, although capable of measuring extracellular dopamine with high specificity, has poor spatial and temporal resolution. Fast-scan cyclic voltammetry, while able to quantify extracellular dopamine at high temporal resolution, is not capable of visualizing dopamine in high spatial resolution. Genetically encoded dopamine-based sensors have overcome many of these challenges and are capable of imaging at high temporal resolution but are often limited to use in model organisms such as mice and rats and have yet to achieve spatial resolutions enabling single release site quantification. We recently developed near-infrared catecholamine nanosensors (nIRCats) that are capable of imaging dopamine with high spatiotemporal resolution (Beyene et al., 2019; S. J. Yang et al., 2021). nIRCats enable direct visualization of dopamine volume transmission by labeling the brain's extracellular space with release site resolution, and because of their synthetic nature, they can be readily used in diverse, nonmodel organisms.

Meadow voles provide a unique opportunity to study mechanisms supporting social behavior because they exhibit predictable transitions in grouping behavior within a single species in response to environmental cues (Beery, 2019b). Meadow voles are seasonally social: Females are solitary and territorial in summer months but come together in mixed-sex groups in the winter (Madison, 1980; Webster & Brooks, 1981). In the wild, photoperiod is the primary cue many mammals use to determine the season (Paul et al., 2008; Prendergast et al., 2002) and is sufficient in lab settings to induce changes in behavioral and physiological phenotypes associated with each season, including social behaviors (Fig. 3.1 A). In long photoperiods (LP; 14 h light:10 h dark), female meadow voles interact less with and are less affiliative toward conspecifics than their counterparts housed in social, short photoperiods (SP; 10 h light:14 h dark). In contrast, short photoperiod voles form selective partner preferences for peer companions, and prefer to cohabit in groups (Beery et al., 2008; Power et al., 2025). Many neurobiological pathways change in concert with the transition between photoperiods and social phenotypes, including changes in multiple neuropeptide signaling pathways (Anacker, Christensen, et al., 2016; Beery et al., 2014b; Beery & Zucker, 2010). In prairie voles, a closely related monogamous vole species, dopamine signaling is integral to the formation and maintenance of social bonds with mate partners (Aragona et al., 2006; Y. Liu & Wang, 2003; Pierce et al., 2024; Resendez et al., n.d.; Wang et al., 1999). Photoperiodic changes in dopamine signaling have been documented in mice and chipmunks (Deats et al., 2015; Goda et al., 2015; Jameson et al., 2023) and may contribute to changes in behavior. In meadow voles, changes in dopamine signaling across photoperiods associated with different grouping behavior have never been examined.

In the present study, we validated the use of nIRCats in meadow voles to investigate whether

striatal dopamine release dynamics differed between voles housed in conditions that promote social and nonsocial behavioral phenotypes. We found that in short photoperiods, voles exhibited greater dopamine release than long photoperiod voles, both across matched and photoperiod-typical housing. We also investigated the effects of sex and regional variability within the striatum on dopamine dynamics. Finally, we modulated dopaminergic signaling using sulpiride (D2 receptor antagonist), quinpirole (D2 receptor agonist), and extracellular Ca^{2+} to explore how voles with different social phenotypes respond differently to these key regulators of dopamine signaling. In socially housed, short photoperiod voles, dopamine release was more strongly enhanced in response to dopamine-increasing manipulations. In contrast, in nonsocial, long photoperiod voles, dopamine release was more strongly suppressed in response to dopamine-decreasing manipulations. These data provide evidence for increased striatal dopamine in short photoperiods versus long, nonsocial photoperiods and establish nIRCats as a valuable tool to measure dopamine dynamics at the synaptic level in nonmodel organisms.

3.2 Results

We sought to understand how dopamine signaling might be affected by photoperiodic changes in meadow voles, as day length is the primary cue regulating a major transition in their social behavior. To investigate how photoperiod impacts dopamine signaling, we synthesized dopamine-sensitive optical probes, nIRCats, following established protocols, and used these probes to label the extracellular space of brain slices prepared from adult voles exposed to the above-mentioned photoperiods with custom near-infrared microscopy (Beyene et al., 2019; S. J. Yang et al., 2021). We hypothesized that nIRCats would enable us to image changes in dopamine signaling through the brain extracellular space induced by different photoperiod exposures. We further hypothesized that there may be subtle changes in dopamine signaling, depending on whether neuronal synapses are stimulated with a mild stimulus to release predominately their readily releasable pool of dopamine, or whether synapses are promoted to release intracellular dopamine reserves with a strong stimulus to release most intracellular dopamine.

One key advantage of nIRCats is the ability to image dopamine release and reuptake with high spatiotemporal resolution. Labeled brain slices were analyzed using a custom Python code (Ouassil, 2022), which identified brain regions where dopamine fluorescence changes exceeded twice the SD of baseline fluctuations upon stimulation. These dopamine "hotspots" were designated as regions of interest (ROIs), which we term dopamine release sites. Recent studies identified such release sites as tyrosine hydroxylase positive axonal varicosities colocalized with the presynaptic protein Bassoon (Bulumulla et al., n.d.; Elizarova et al., 2022). Thus, the number of release sites in our labeled slices may closely correspond to the number of single synaptic dopamine release sites. Because nIRCats exhibit high sensitivity and a low detection limit (Beyene et al., 2018), they can effectively respond to dopamine release when it is at or above the sensor's nanomolar sensitivity (Elizarova et al., 2022).

The degree of fluorescence modulation in each release site was quantified as $\Delta F/F_0$, defined as the fluorescence change ($F-F_0$) divided by baseline fluorescence (F_0), where peak $\Delta F/F_0$ corresponds to the amount of dopamine released from each release site. Therefore, the total amount of released dopamine is a function of both the number of release sites and the peak $\Delta F/F_0$ in each release site, and we used these two parameters to characterize dopamine release. Multiple measurements were

performed for each brain slice to calculate the slice-averaged number of release sites and peak $\Delta F/F_0$ (Fig. 3.1*B*).

We first imaged dopamine release prompted by a single-pulse electrical stimulation applied under two different stimulation strength conditions to induce varying levels of dopamine release by adjusting the stimulation current and the distance between the imaging field of view and the electrode (Fig. 3.1*C*). When a stimulation current greater than 0.3 mA was applied with the electrode positioned close (5 μm) to the field of view, the number of release sites became saturated and did not change with further increases in current beyond 0.3 mA (Fig. 3.1*D*). This strong stimulation condition enabled activation of the maximum number of dopamine release sites, without saturating the $\Delta F/F_0$ within individual sites or depleting the readily releasable pool of dopamine. We referred to this condition as "MAX," as it represents the maximum observable dopamine release in a field of view upon stimulation and likely represents a supraphysiological level of dopamine release. In contrast, when the electrode was positioned 80 μm away from the imaging field of view, changes in stimulation current significantly affected the amount of dopamine release (Fig. 3.1*D*). [$\Delta F/F_0$: $p = 0.024$ (0.1 mA & 0.3 mA), $P = 0.011$ (0.1 mA & 0.3 mA), $P = 0.037$ (0.3 mA & 0.5 mA) Release site: $p = 0.047$ (0.1 mA & 0.3 mA), $P = 0.026$ (0.1 mA & 0.3 mA, $P = 0.297$ (0.3 mA & 0.5 mA)]. The enhancement of dopamine release reflected both the activation of additional release sites and elevated $\Delta F/F_0$ within individual sites, with the latter contributing more substantially. In this "non-MAX" condition, only a portion of vesicles are released from the readily releasable dopamine pool (Fig. 3.1*C*). We chose 0.3 mA with the stimulator placed 80 μm away from the field of view as a representative non-MAX condition to investigate nonsaturated dopamine release.

We primarily focused on female meadow voles housed in short or long photoperiods, as females undergo a more pronounced transition in social behavior induced by season or photoperiod than do males. We first assessed the effect of photoperiod alone, using matched housing across short and long photoperiods. Because social grouping and photoperiod normally shift in tandem for females (i.e., in the summer females live alone, but in winter they live in groups), we next assessed voles in "naturalistic housing" (alone in long photoperiods, or in a group of four in short photoperiods). Finally, since both females and males huddle in mixed sex groups during the winter, we also investigated the effect of sex in groups housed in short photoperiods for both MAX and Non-MAX conditions.

Max Stimulation Effects

We applied MAX stimulation to the dorsomedial striatum (DMS) of short photoperiod and long photoperiod voles. We chose to investigate the dorsal striatum due to its dense dopaminergic innervation and the absence of norepinephrine-releasing inputs, which the catecholamine sensors can also detect (Berridge & Waterhouse, 2003).

We first investigated the influence of daylength on dopamine release. In this cohort, voles in both long and short photoperiods were pair-housed, with only the photoperiod varied to precisely assess its effect on dopamine release under MAX stimulation protocols. MAX stimulation evoked an instantaneous increase in nIRCat fluorescence in both photoperiods. This fluorescence response was reversible, with fluorescence levels returning to baseline after 15 s through a combination of dopamine reuptake and diffusion away from the release site (Fig. 3.2 *A* and *B*).

The peak $\Delta F/F_0$ was then calculated for each release site, rather than the entire field of view, to better quantify the relative amount of dopamine released as the release site density across experimental groups. As shown in Fig. 3.2*C*, pair-housed voles in short photoperiods had significantly larger peak $\Delta F/F_0$, indicating more dopamine release than pair-housed voles in long photoperiods ($P = 0.0036$, n = 8 voles in LP, 7 voles in SP). There was not a significant difference between the number of release sites in long and short photoperiods ($P = 0.26$, n = 8 voles in LP, 7 voles in SP).

Then, to assess whether the effect of photoperiod on dopamine release persisted in more naturalistic housing, we assessed voles in long, nonsocial photoperiods housed alone, and voles in short, social photoperiods housed in groups of four. These groups reflect the combined photoperiod and social environment change that female voles would experience in the wild and self-select in the lab. Social voles in short photoperiods (social/SP) had a larger peak of $\Delta F/F_0$ than solitary voles in long photoperiods ($P = 0.05$, n = 7 voles in LP, 6 voles in SP) (Fig. 3.2*D*). Social/SP voles also had a higher number of release sites than solitary/long photoperiod voles ($P = 0.0068$, n = 7 voles in LP, 6 voles in SP) (Fig. 3.2*D*). Data from both studies were then compared. No significant effect of housing was detected within LP (pair versus or solo, ROI: $P = 0.80$; $\Delta F/F_0$: $P = 0.75$) or within SP (pair versus group, ROI: $P = 0.97$; $\Delta F/F_0$: $P = 0.16$). Data were thus pooled by day length for visualization (Fig. 3.2*A*) and subsequent analysis. In the pooled dataset, voles in short photoperiods had a significantly higher $\Delta F/F_0$ ($P = 0.001$) and significantly more release sites ($P = 0.017$) than voles in long photoperiods. Social housing may act as a stressor for long photoperiod voles. Therefore, as changes in dopamine release were evident in both housing paradigms (matched housing across day lengths and naturalistic housing), we standardized on naturalistic housing to better emulate seasonal environmental changes for all future experiments.

Non-MAX Stimulation Effects

We next applied Non-MAX stimulation to the DMS of a new cohort of voles in both long and short photoperiods in naturalistic housing (alone in long photoperiods and in a group of four in short photoperiods). Non-MAX stimulation resulted in an increase in dopamine release in both short and long photoperiods, with much less dopamine release observed in both cohorts compared to the MAX stimulation condition, as shown in Fig. 3.2 *E–G*. Under non-MAX stimulation conditions, nIRCats again showed clear reversibility, with their fluorescence returning to baseline as dopamine is reuptaken and diffuses away from the release site within 10 s of stimulation.

Under these non-MAX stimulation conditions, group-housed voles in short photoperiods showed a trend toward increased dopamine release per site compared to solitary long photoperiod voles, although the difference was not statistically significant ($P = 0.10$; n = 12 brain slices in LP, n = 10 brain slices in SP) (Fig. 3.2*G*). As for release sites, voles in short photoperiods showed a significantly larger number of release sites (310 ± 29, n = 10 brain slices) than those in long photoperiods (150 ± 31, n = 12 brain slices) ($P = 0.0008$) (Fig. 3.H). Taken together, our data show that under mild stimulation conditions, group housed voles in short, social photoperiods exhibited more activated dopamine release sites than solitary voles in long photoperiods, though release sites effluxed similar quantities of dopamine per site between photoperiods.

Finally, it is known that voles of both sexes housed in a short photoperiod form selective

preferences for known (versus unknown) same-sex conspecifics and huddle in groups (Beery et al., 2009b; Lee & Beery, 2019). To identify whether sex differences in dopamine release exist, we assessed male and female meadow voles housed in groups of four in short photoperiods. Because of their similar social behavior in this photoperiod, we hypothesized that group-housed males and females would not show significant differences in dopamine release. Upon applying MAX and non-MAX electrical stimulation in the DMS, indeed neither $\Delta F/F_0$ nor the number of release sites differed significantly between male and female voles in short photoperiods.

Regional Variability within the Dorsal Striatum

Having observed differences in dopamine signaling as a function of stimulation strength, we next studied whether there were subregional differences in dopamine signaling within the dorsal striatum of group housed voles in short photoperiods. The dorsal striatum can be divided into two regions-the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS). While both subregions are implicated in motor control, they serve distinct behavioral functions. Dopamine in the DMS plays a critical role in goal-directed behaviors and behavioral flexibility, while dopamine in the DLS is responsible for habitual behaviors and navigation (Devan et al., 2011; Vandaele et al., 2019). To this end, dopamine was electrically evoked in these two regions within the same brain slices under MAX condition. Under MAX stimulation, we found no significant difference in peak $\Delta F/F_0$ in release sites between the DMS and DLS. However, the DMS had significantly more release sites than the DLS. (*SI* Fig. S3.1)

Effects of D2 Autoreceptor Manipulation

Having examined differences in dopamine release dynamics across sexes, housing conditions, photoperiods, and striatal subregions, we next investigated how dopamine D2 autoreceptor manipulation affects evoked dopamine release in short and long photoperiods. D2 autoreceptors play a crucial role in regulating dopamine signaling by suppressing dopamine release and synthesis when necessary, maintaining homeostasis in the brain's dopamine system. These receptors are primarily located on the presynaptic terminals of dopaminergic neurons (Fig. 3.1*C*). When dopamine is released into the synaptic cleft, it can bind to these autoreceptors, which then send a feedback signal to the neuron to reduce further dopamine release and synthesis. Due to their critical function, D2 autoreceptors have been widely studied in the context of dopamine-related psychiatric diseases such as Parkinson's disease and schizophrenia (Ford, 2014; Seeman, 2013).

It has previously been demonstrated that nIRCats are compatible with dopamine pharmacology, including the use of sulpiride (a D2 autoreceptor antagonist) and quinpirole (a D2 autoreceptor agonist) (Beyene et al., 2019). Based on our above data showing differences in dopamine release site density and amount as a function of photoperiod, we hypothesized that solitary and social voles may have differing levels of dopamine receptor sensitivity to pharmacological intervention. We first examined the effects of sulpiride, a D2 autoreceptor antagonist, on electrically evoked dopamine release in voles housed alone in long photoperiods and in groups in short photoperiods. After imaging dopamine release in artificial cerebrospinal fluid (ACSF), 5 μM of sulpiride was bath-applied for 30 min and electrically evoked dopamine release was then imaged in the same field of view. For this experiment, non-MAX stimulation was used exclusively, as overstimulation could override any effects of the applied pharmacological agents.

We found that a 5 μM concentration of sulpiride significantly increased dopamine release in the

DMS, as shown in Fig. 3.3 *A* and *B*. $\Delta F/F_0$ per release site in voles in long photoperiods increased by 74%, from a peak $\Delta F/F_0$ of 0.031 ± 0.002 presulpiride to 0.054 ± 0.006 postsulpiride (n = 5 brain slices), while in short photoperiods, the peak $\Delta F/F_0$ increased by 92%, from 0.037 ± 0.005 presulpiride to 0.072 ± 0.012 postsulpiride (n = 5 brain slices) (Fig. 3.3*C*). These results confirm that antagonism of presynaptic D2 autoreceptors using sulpiride effectively increases the amount of dopamine released from each dopamine release site.

While the application of 5 μM sulpiride increased the amount of dopamine released per release site in both photoperiods, sulpiride's effects were less evident in the number of release sites, which were not significantly different from pretreatment in either long or short photoperiods (Fig. 3.3*D*). Interestingly, after 5 μM sulpiride treatment, most release sites in short photoperiod voles were activated, with the number of release sites approaching that seen during MAX stimulation conditions (452 ± 9, n = 13 brain slices) (Fig. 3.2*D*). However, even with 5 μM sulpiride, most dopamine release sites in long photoperiod voles remained deactivated, and the number of release sites fell short compared to those measured with MAX stimulation conditions (392 ± 20, n = 15 brain slices).

Next, we investigated how quinpirole, a D2 autoreceptor agonist, affects electrically evoked dopamine release in the DMS of solitary voles in long photoperiods and group-housed voles in short photoperiods. Dopamine release upon non-MAX electrical stimulation was measured in the same field of view before and after bath application of 5 μM quinpirole. Surprisingly, although quinpirole substantially reduced integrated fluorescence across the field of view, the amount of dopamine released from each release site decreased only marginally in voles in long and short photoperiods (Fig. 3.3 *E*–*G*). The $\Delta F/F_0$ measured from release sites changed from 0.028 ± 0.004 and 0.036 ± 0.007 prequinpirole to 0.024 ± 0.004 and 0.026 ± 0.002 postquinpirole for long (n = 7 brain slices) and short (n = 6 brain slices) photoperiods, respectively. Instead, the major source of the clear decrease in dopamine response was the reduction in the number of dopamine release sites. The number of release sites decreased in short photoperiod voles by 55%, from 307 ± 48 prequinpirole to 137 ± 49 postquinpirole (n = 6 brain slices), while the release site number in long photoperiod voles was reduced by 88%, from 165 ± 50 prequinpirole to 20 ± 5 postquinpirole (n = 7 brain slices). Although voles in short photoperiods retained nearly half of their release sites after quinpirole application, dopamine release sites in long photoperiod voles were mostly deactivated, suggesting that these solitary voles in long photoperiods may have a higher sensitivity to D2 autoreceptor agonism relative to their social counterparts in short photoperiods. This could potentially be due to altered D2 autoreceptor expression or autoreceptor functional responsiveness. We did not see a statistically significant difference between photoperiods in D2 receptor mRNA expression, although this pilot dataset of n = 4 animals is insufficient to conclude whether differences in pharmacological sensitivities are due to changes in autoreceptor expression or downstream differences in sensitivity.

Based on our measurements, we identified several characteristics of how D2 autoreceptors are differentially manipulated by dopamine receptor pharmacology within and between photoperiods. Although both drugs affect the number of dopamine release sites and the amount of dopamine released from each site to some extent, they operate through different mechanisms. Specifically, sulpiride is more effective at increasing the amount of dopamine released from each site, while quinpirole is better at deactivating release sites in both photoperiod-housing conditions. In addition, we found that voles in short, social photoperiods respond more sensitively to sulpiride,

which increases dopamine, but are less responsive to quinpirole, which decreases dopamine.

Extracellular Ca^{2+} Effects

Finally, we investigated how extracellular Ca^{2+} affects electrically evoked dopamine release in naturalistically housed voles in long and short photoperiods. The release of neurotransmitters such as dopamine from presynaptic dopaminergic neurons is highly dependent on the influx of Ca^{2+} through voltage-gated calcium channels (Fig. 3.1*C*). Consequently, the amount of dopamine released is influenced by the concentration of extracellular Ca^{2+}. We hypothesized that voles in long, nonsocial photoperiods, and short, social photoperiods might differentially respond to varying concentrations of extracellular Ca^{2+}, as suggested by our prior data showing differing sensitivities of each photoperiod to MAX versus non MAX stimulation conditions.

For this experiment, acute brain slices from voles housed alone in long photoperiods, and in groups in short photoperiods, were prepared and incubated in Ca^{2+}-deficient ACSF buffer before imaging. This environment ensures that prior Ca^{2+} exposure does not influence dopamine release upon stimulation. Electrically evoked dopamine was imaged under non-MAX stimulation conditions in the Ca^{2+}-deficient buffer. After this initial imaging, Ca^{2+} was added to final concentrations of 2 mM and 5 mM, and dopamine release was imaged in the same field of view. In 0 mM Ca^{2+} buffer, we observed that dopamine release was highly suppressed in both conditions, as expected (Fig. 3.4). Solitary voles in long photoperiods exhibited a negligible amount of dopamine release, with a peak $\Delta F/F_0$ of 0.009 ± 0.004 and an average of 8 ± 3 release sites (n = 6 brain slices). Compared to dopamine release in regular ACSF buffer (Fig. 3.2 *E–H*), most dopamine release sites were deactivated (a 94% reduction in the number of release sites) in the absence of Ca^{2+}, and the few active sites released significantly smaller amounts of dopamine relative to standard 2 mM Ca^{2+} buffer conditions in long photoperiods. Interestingly, voles in short photoperiods still maintained some of their release sites even in a Ca^{2+}-deficient environment, with a peak $\Delta F/F_0$ of 0.016 ± 0.007 and an average of 68 ± 30 release sites (n = 5 brain slices). These observations suggest that social voles in short photoperiods exhibit higher resistance to Ca^{2+} deficiency in maintaining dopamine release, even in the absence of extracellular Ca^{2+}.

Next, a 2 mM Ca^{2+} buffer was supplied for 30 min, and upon stimulation, dopamine release became clearly observable, confirming that extracellular Ca^{2+} plays a critical role in dopamine release, as expected (Fig. 3.4*B*). Under these 2 mM Ca^{2+} extracellular calcium conditions, voles in short photoperiods exhibited both a higher peak $\Delta F/F_0$ (0.029 ± 0.003 for short photoperiods, n = 5 brain slices, and 0.022 ± 0.002 for long photoperiods, n = 6 brain slices) and a greater number of release sites compared to long photoperiod voles (231 ± 36 for short photoperiods, n = 5 brain slices, and 124 ± 27 for long photoperiods, n = 6 brain slices) (Fig. 3.4 *C* and *D*). It should be noted that when imaging was performed in regular ACSF without prior exposure to a 0 mM Ca^{2+} environment, the difference in peak $\Delta F/F_0$ between long and short photoperiods was insignificant (Fig. 3.2 *G* and *H*). These results indicate that voles in short, social photoperiod recover more quickly from Ca^{2+}-deficient conditions than their counterparts in long photoperiods. However, neither photoperiod showed full recovery of dopamine release to the levels observed when measured without prior exposure to a 0 mM Ca^{2+} environment. Next, a 5 mM Ca^{2+} buffer was supplied for another 30 min, which further increased both the peak $\Delta F/F_0$ and the number of release sites upon electrical stimulation. Short photoperiod voles exhibited a peak $\Delta F/F_0$ of 0.056 ± 0.006 and 429 ± 22 release sites (n = 5 brain slices). Interestingly, 5 mM of extracellular Ca^{2+} fully activated available

dopamine release sites, whereby the observed number of release sites was comparable to that measured under MAX stimulation conditions (Fig. 3.2*D*). Although at 5 mM Ca^{2+} voles in long photoperiods also exhibited increases in peak $\Delta F/F_0$ (0.036 ± 0.005) and the number of release sites (222 ± 48, n = 6 brain slices) relative to lower Ca^{2+} conditions, the release site numbers remained well below values observed under MAX stimulation (Fig. 3.2*D*).

In summary, based on all observations from varying extracellular Ca^{2+} concentrations, we confirmed that Ca^{2+} plays a significant role in gating dopamine release, with increasing Ca^{2+} levels leading to higher peak $\Delta F/F_0$ and a greater number of release sites. Our findings are consistent with previous studies showing that elevated extracellular calcium concentrations can increase the likelihood of neurotransmitter vesicle release, expand the size of the readily releasable pool, and shift inactive boutons into more active states (Leitz & Kavalali, 2011; Thanawala & Regehr, 2013). Similar to what we observed with D2 autoreceptor drugs, voles housed in short, social photoperiods showed lower dopamine suppression under zero Ca^{2+} concentrations, which reduces dopamine release, and demonstrated faster enhancement in dopamine release as Ca^{2+} concentrations increased.

This pattern, together with their higher release site density, indicates that photoperiod may affect both the availability of release machinery and its functional coupling with presynaptic Ca^{2+} channels across different Ca^{2+} conditions. As a result, dopamine signaling that remains stable at low Ca^{2+} levels while being strongly amplified at high Ca^{2+} levels may allow voles in short photoperiods to maintain reliable baseline transmission and produce robust surges of dopamine during heightened activity. This broader dynamic range may enable their dopaminergic system to flexibly scale with the demands of social interactions, providing a physiological basis for greater adaptability and plasticity in social behavior. In contrast, voles in long, nonsocial photoperiods display a smaller number of release sites which may translate to a narrower Ca^{2+}-dependent range of dopamine release, leading to more constrained dopaminergic signaling and reduced capacity for modulation of social motivation and reward processing.

3.3 Discussion

Nontraditional species enable the study of multiple behaviors not found in model organisms. For example, meadow voles undergo a transition in social behavior in response to photoperiod that allows us to examine how changes in the brain contribute to changes in social behavior (and vice-versa). Studying nonmodel organisms comes with trade-offs, however, many of the tools available in model organisms are genetically based and may not work across nonmodel species. In this study, we use nIRCats to explore dopamine dynamics in meadow voles, between photoperiods linked to group size. We observed striking differences in synaptic-scale striatal dopamine signaling between voles housed in long and short photoperiods, both in matched and naturalistic (photoperiod-typical) group sizes.

Across a wide variety of experimental conditions including stimulation profiles, Ca^{2+} concentrations, agonist and antagonist applications, evoked dopamine was altered in a consistent manner. Photoperiod alone significantly influenced the amount of dopamine released per site with a nonsignificant trend toward increased release sites, while voles in naturalistic housing

(alone in LP and social in SP) had significant effects on dopamine release per site and number of release sites. There were no detectable housing differences and pooled analysis revealed a greater number of dopamine release sites and higher dopamine release per site in voles housed in short photoperiods in the MAX condition. Having explicitly tested effects of both photoperiod alone, and photoperiod in the context of naturalistic housing (with solitary long photoperiod voles housed alone, and social short period voles housed in groups), all further experiments were conducted using naturalistic housing. Under mild (non-MAX) stimulation, voles in short photoperiods again activated more release sites than those in long photoperiods, but there was no difference in the amount of dopamine each site released. Additionally, we observed that voles in short photoperiods demonstrated heightened resistance to conditions that reduce dopamine release, such as quinpirole treatment or lack of extracellular calcium. In the wild, winter groups are essential for survival and thermoregulation for meadow voles (Madison, 1980; Madison & McShea, 1987; Ondrasek et al., 2015b). It is possible that group living may be supported not only by elevated dopamine but also resistance to reduced dopamine, ensuring voles remain in a group throughout the winter. To the same end, voles in short photoperiods showed greater sensitivity to agents that increase dopamine release, such as sulpiride and 5 mM extracellular calcium, further supporting the role of dopamine in regulating social behaviors and the possibility of dopamine synthesis upregulation or differences in autoreceptor sensitivity induced by photoperiod.

While social behavior changes markedly across photoperiods, this change is part of a larger suite of physiological and behavioral traits that shift as animals prepare for the transition to a winter environment. In winter and short photoperiods, voles are reproductively quiescent, weigh less, decrease food intake (Dark et al., 1983), and are more active than those housed in long photoperiods (Power et al., 2025). Increased dopamine levels in short, winter-like photoperiods may support this increase in locomotion and may also contribute to changes in motivational states related to reproduction, feeding, and other behavioral drives.

Interestingly, many rodent studies of the interactions between dopamine and photoperiod find evidence of *lower* dopamine in short photoperiods relative to long photoperiods (Deats et al., 2015; Goda et al., 2015; Jameson et al., 2023), opposite the findings in this study. Humans, however, show increased striatal dopamine during the fall and winter (Eisenberg et al., 2010), in line with findings in the present study, and there is some evidence in rodents that photoperiodic effects on dopamine vary between species and brain regions (Goda et al., 2015), further highlighting species-specific variation. Furthermore, social contexts can also impact dopamine. Historically, studies of social isolation on dopamine have shown an association between isolation and increased dopamine response (Hall et al., 1998; Karkhanis et al., 2019; Yorgason et al., 2016). In meadow voles, isolation is not a stressor for voles in long, nonsocial photoperiods as it is in many other rodent species (Curtis et al., 2003; Stowe et al., 2005), whereas paired housing may act as a stressor in long, but not short photoperiods.We tested voles in both matched and naturalistic housing conditions and found no differences by housing. conditions; it is possible that testing voles housed alone in short photoperiods when they would not normally be alone would lead to similar changes in dopamine response.

The nIRCats used in this study were used in slice preparation, which is most suitable for detecting stable alterations in release-site scale dopamine signaling, e.g., across different environmental conditions such as photoperiod. Our results herein compare differences in the number of release sites, and the amount of dopamine released per release site, where some conditions induce changes

in one but not both metrics. These results highlight the importance of release site scale dopamine imaging to measure nuanced differences likely unobservable using bulk measurement approaches. As we look toward the future of nongenomically encoded sensor use, future studies will benefit from nIRCats or other sensors that are capable of imaging nonmodel organisms in vivo to measure context-dependent dopamine signaling. Additionally, since dopamine can act as a neuromodulator and alter the activity of other neuropeptides like oxytocin and cortisol that are known to vary seasonally, sensors that can measure multiple molecules simultaneously would be beneficial to further elucidate these relationships.

The present findings not only validate the use of nIRCats in voles but also highlight the importance of dopamine in modulating changes in social behavior due to photoperiod in meadow voles. This work paves the way for future studies of dopamine signaling in nontraditional organisms and demonstrates the potential of nIRCats for advancing our understanding of neurotransmitter dynamics across species.

3.4 Methods

Animal Subjects

Meadow voles were bred locally at UC Berkeley in long photoperiods (14 h light: 10 h dark). Within 1 wk of weaning, voles were separated into experimental housing and either remained in long photoperiods or were moved to short photoperiods (10 h light: 14 h dark). For the initial experiments using the MAX stimulation protocols, female meadow voles were housed in one of four groups: housed alone in long photoperiods, housed in a pair in long photoperiods, housed in a pair in short photoperiods, or housed in a group of four in short photoperiods. When housed socially, cagemates were same-sex, age-matched conspecifics. For all other conditions, male and female meadow voles were housed in same-sex, age matched groups of four in short photoperiods, and females were housed alone in long photoperiods. Voles were all adults at the time of the experiment. All animal procedures were approved by the University of California Berkeley Animal Care and Use Committee.

Materials

HiPco SWNTs were purchased from NanoIntegris (batch #27 to 104, diameter: 0.8 to 1.2 nm, length: 400 to 700 nm). $(GT)_6$ oligonucleotides purified with standard desalting were purchased from Integrated DNA Technologies. Quinpirole and sulpiride were purchased from Tocris Bioscience. All other agents were purchased from Millipore Sigma.

Sensor Synthesis and In Vitro Characterization

Near-infrared catecholamine sensors were synthesized using previously reported protocols. Briefly, SWNTs and $(GT)_6$ (2 mg each) were bath-sonicated (Branson Ultrasonic 1800) for 3 min in 1 mL of 0.1 M NaCl, followed by probe tip-sonication for 10 min (Cole-Parmer Ultrasonic Processor, 3-mm diameter tip, 5 W power) in an ice bath. To remove nondispered SWNTs, the resulting suspension was centrifuged at 16,000 g for 60 min (Eppendorf 5418), and the supernatant containing stable $(GT)_6$-SWNT was collected and used. The absorbance of the $(GT)_6$-SWNT was measured at 632 nm (NanoDrop One, Thermo Scientific) to calculate the SWNT concentration of the suspension. [extinction coefficient $\varepsilon = 0.036\ (mg/L)^{-1}\ cm^{-1}$] The $(GT)_6$-

SWNT suspension was diluted to 5 μg mL^{-1} and 200 μg mL^{-1} for in vitro characterization and ex vivo slice experiments, respectively.

For in vitro characterization, fluorescence measurements were performed using a 20× objective on an inverted Zeiss microscope (Axio Observer D1) with a SCT 320 spectrometer (Princeton Instruments) and a liquid nitrogen-cooled InGaAs linear array detector (PyLoN-IR, Princeton Instruments). Aliquots of 99 μL of $(GT)_6$-SWNT were placed in each well of a 384-well plate. The fluorescence spectra of $(GT)_6$-SWNT were obtained before and after the addition of 1 μL of dopamine using a custom-built spectrometer and microscope. A 721 nm laser (Opto Engine LLC) was used to excite the nanosensor suspensions. nIRCats exhibit a 24-fold increase in fluorescence upon exposure to dopamine, with high selectivity against various neurochemicals in vitro (Beyene et al., 2018).

Brain Slice Preparation and Sensor Labeling

Brain slices were prepared using established protocols(Beyene et al., 2019; Piekarski et al., 2017; S. J. Yang et al., 2021). Briefly, meadow voles were anesthetized under isoflurane and then intraperitoneally injected with a combination of ketamine (10 mg mL^{-1}) and xylazine (5 mg mL^{-1}) in saline (1 mL). While anesthetized, transcardial perfusion was performed with ice-cold cutting buffer (119 mM NaCl, 26.2 mM $NaHCO_3$, 2.5 mM KCl, 1 mM NaH_2PO_4, 3.5 mM $MgCl_2$, 10 mM glucose, and 0 mM $CaCl_2$), followed by rapid brain dissection over the same buffer. The brain was mounted onto a vibratome (Leica VT 1000S) and coronally sliced into 300-μm thick sections. Sections containing the dorsal striatum were incubated for 30 min at room temperature in oxygen-saturated ACSF buffer (119 mM NaCl, 26.2 mM $NaHCO_3$, 2.5 mM KCl, 1 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 10 mM glucose, 2 mM $CaCl_2$). The slices were transferred to a small volume incubation chamber (Scientific Systems Design Inc., AutoMate Scientific) containing 5 mL of oxygen-saturated ACSF. nIRCat nanosensors were added to the incubation chamber (final concentration of 200 μg mL^{-1}),and slices were incubated for 15 min. Then, the slices were rinsed with ACSF buffer to remove excess nonlocalized sensors.

Dopamine Imaging in Acute Brain Slices

Slice imaging was performed using established protocols and a custom-built upright epifluorescent microscope (Olympus, Sutter Instruments) mounted onto a motorized stage (Beyene et al., 2019; S. J. Yang et al., 2021). A 785 nm laser (Opto Engine LLC) was used to excite nanosensors, which was expanded to a final diameter of ~1 cm using a Keplerian beam expander with two plano-convex lenses (f = 25 and 75 mm; AR coating B, Thorlabs) The beam was passed through a fluorescence filter cube [excitation: 800 nm shortpass (FESH0800), dichroic: 900 longpass (DMLP990R), and emission: 900 longpass (FELH0900); Thorlabs] to a 60× Apo objective (numerical aperture, 1.0; working distance, 2.8 mm; water dipping; high nIR transmission; Nikon CFI Apo 60XW nIR). Emission photons collected from the sample were passed through the filter cube, were focused onto a two-dimensional InGaAs array detector [500 to 600 nm: 40% quantum efficiency (QE); 1,000 to 1,500 nm: >85% QE; Ninox 640, Raptor Photonics] and were recorded using the Micro-Manager Open Source Microscopy Software.

A bipolar stimulation electrode (Platinum/Iridium Standard Tip, MicroProbes for Life Science) was positioned in the dorsal striatum using a 4× objective lens. A total of 600 frames were captured in the nIR using a 60× objective lens at eight frames per second, with electrical

stimulation applied after 200 frames of baseline. For the saturated condition, the electrode was placed directly next to the field of view, and a single-pulse 0.5 mA stimulation was applied for 1 ms. For the nonsaturated condition, the electrode was placed 80 µm away from the field of view, and a single-pulse 0.3 mA stimulation was applied for 1 ms. nIRCats demonstrated fluorescence modulation in response to evoked dopamine release with high spatial (µm) and temporal (ms) resolution.

For drug experiments, imaging was initially conducted in plain ACSF. Then, either quinpirole or sulpiride (5 µM) was added to the imaging chamber through ACSF perfusion. The brain slice was incubated with either drug for 30 min before imaging continued in the same field of view. For calcium experiments, initial incubation occurred in ACSF without calcium, then calcium was later applied in concentrations of 2 mM and 5 mM. Brain slices were exposed to new calcium concentrations for 30 min before imaging.

Image Processing and Data Analysis

Image files were processed using a custom Python application (https://github.com/NicholasOuassil/NanoImgPro). A field of view was divided into 25 × 25 pixel grids, and those with fluorescence modulation more than two times the SD of baseline fluctuations were identified as ROIs, which we term release sites. Recent studies identified such release sites as tyrosine hydroxylase-positive axonal varicosities colocalized with the presynaptic protein Bassoon (Bulumulla et al., n.d.; Elizarova et al., 2022). Thus, the number of release sites may closely correspond to the number of single synaptic dopamine release sites. $\Delta F/F_0$ of each release site was calculated as $(F\ F_{o0})/F_{o0}$, where F_{o0} is the average intensity of baseline fluorescence and F is the dynamic fluorescence intensity. $\Delta F/F_{o0}$ was averaged over release sites to draw an average $\Delta F/F_{o0}$ in release sites as a function of time. The maximum $\Delta F/F_o$ of each release site was identified and averaged to calculate the average peak $\Delta F/F_0$ in release sites. The total amount of released dopamine is a function of both the number of release sites and the peak $\Delta F/F_{o0}$ in each release site, and we use these two parameters to describe electrically evoked dopamine release.

RT-qPCR

Six punches (2 mm in diameter) were collected from dorsomedial striatum per each animal, which were treated as one sample. RNA was extracted from pooled punches using the TRI reagent method following the manufacturer's instructions. To remove any contaminating DNA from samples, samples were treated with the TURBO DNA-free Kit from Invitrogen following the "rigorous" DNAse protocol. RNA was then converted to cDNA using the iScript cDNA synthesis kit (Bio-rad). RT-qPCR was run for each target in triplicate using 5 ng of cDNA per well and SYBR Green Universal master mix (Applied Biosystems). Primers for each target are listed below (Table 1). Primers for each target gene were designed using the NCBI primer design tool, and primers for the housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (HPRT), were taken from Vitale et al., 2023, after confirming complementation with the *M. pennsylvanicus* genome. Melt curves were used to confirm amplification of only a single product per target. To control for DNA contamination, a no reverse transcriptase control (NRTC) was run for each sample—no amplification was observed in any of these samples, indicating successful DNA removal. For analysis, dCq values for each target were calculated by taking the difference between the raw cycle count (Cq) of the target and Cq of the housekeeping gene. Fold change was calculated as 2^(−dCq). ddCq analysis was not conducted as we sought to simply

directly compare mRNA expression across 2 d lengths and there was no “untreated” control for further normalization.

Data, Materials, and Software Availability

A custom image processing Python application is available online (https://github.com/NicholasOuassil/NanoImgPro). Other data are included in the article and/or *SI Appendix*.

3.5 Chapter 3 Figures and Tables

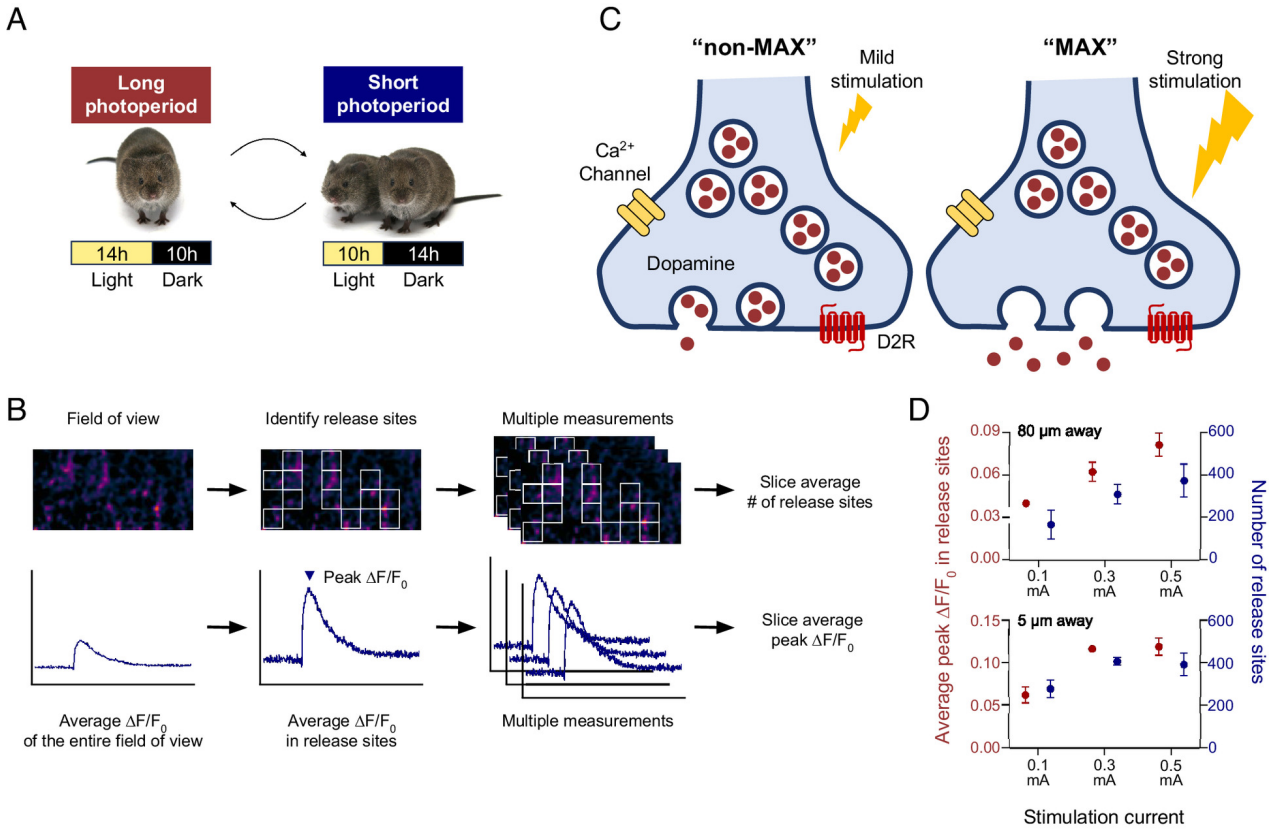


Figure 3.1. *Schematic of experimental design. (*A) In lab settings, altering photoperiod is sufficient to induce changes in behavior and physiology in meadow voles, with long photoperiods associated with solitary living and short photoperiods associated with group formation. (B) Schematic of data analysis methods used to examine the average number of release sites and the average peak ΔF/F0 per release site in a brain slice. The number of release sites and the peak ΔF/F0 correlate with the putative number of dopamine-releasing synaptic sites and the amount of dopamine released from each site, respectively. (C) Schematic of dopaminergic neurons under mild (Left, "non-MAX") and strong (Right, "MAX") electrical stimulation. More dopamine is released from the readily releasable dopamine pool with stronger electrical stimulation. (D) Change in average peak ΔF/F0 (red, Left y-axis) and the number of release sites (blue, Right y-axis) as a function of current, with the stimulator positioned at 80 µm (Top) and 5 µm (Bottom) from the field of view. (n = 3 measurements) Stimulation with 0.3 µA/80 µm was designated as the non-Max condition, while stimulation with 0.5 µA/5 µm was designated as the MAX condition.

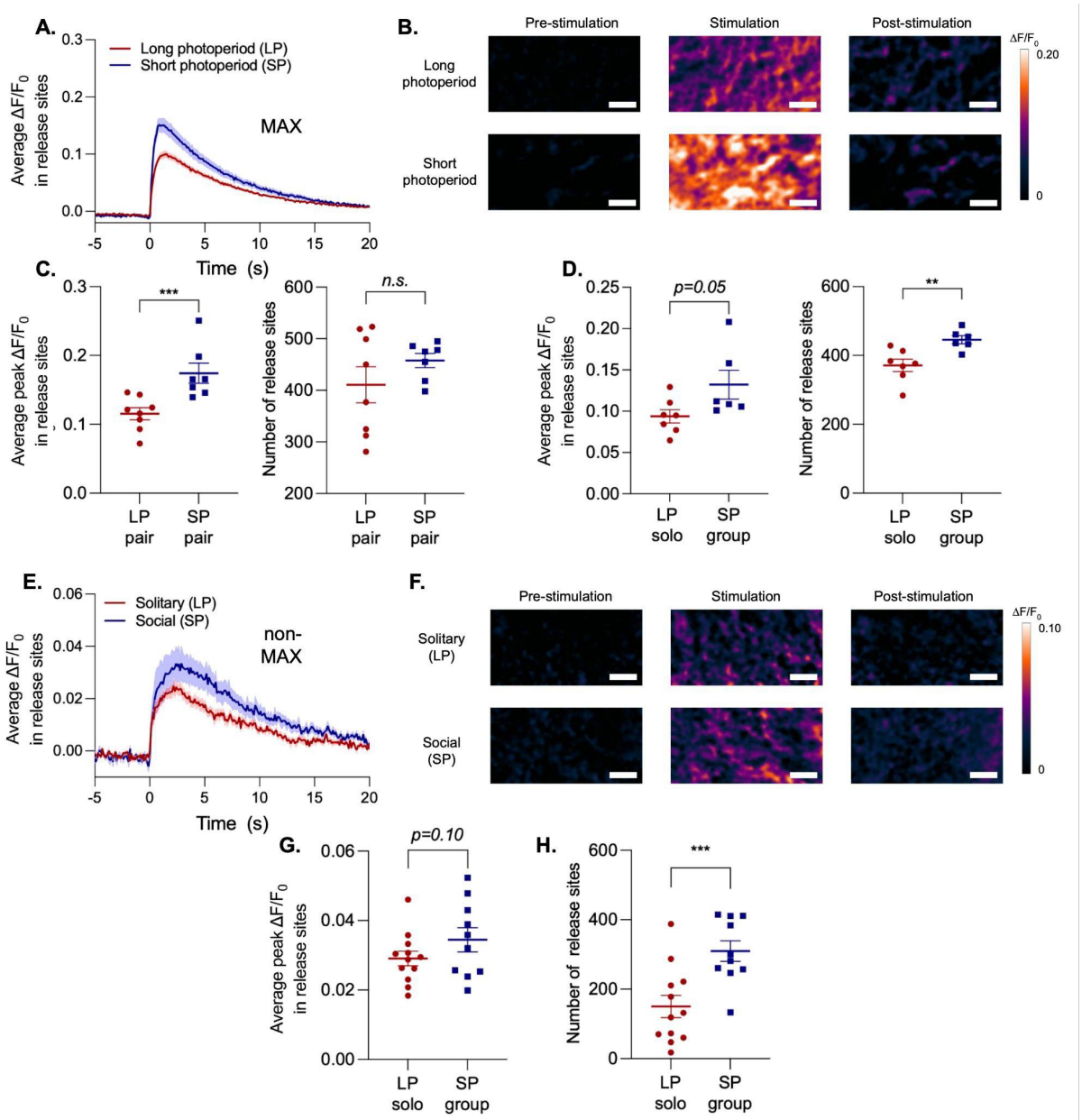


Figure 3.2 *Comparison of dopamine release dynamics in long photoperiods and short photoperiods*. In slice, imaging electrically evoked dopamine in the DMS of voles housed in long photoperiods (LP, in red) and short photoperiods (SP, in blue) following a 0.5 mA electrical stimulation applied adjacent to the field of view (MAX, A–D) and a 0.3 mA electrical stimulation applied 80 µm away from the field of view (non-MAX, E–G). (A) Average $\Delta F/F_0$ in release sites time trace (solid line) with the SE (shadow). (B) $\Delta F/F_0$ response images within the same field of view, (C) average peak $\Delta F/F_0$ in each release site, and number of release sites in the MAX condition for pair-housed voles in both photoperiods. (n = 8 animals in LP, n = 7 animals in SP). (D) Average peak $\Delta F/F_0$ in each release site, and number of release sites in the MAX condition for solo-housed voles in LP and group housed voles in SP. (n = 7 animals in LP, n = 6 animals in SP). (E) Average $\Delta F/F_0$ in release sites time trace (solid line) with the SE (shadow). (F) $\Delta F/F_0$ response images within the same field of view, (G) average peak $\Delta F/F_0$ in each release site, and (H) number of release sites in the non-MAX condition. (n = 12 brain slices from 12 animals in long photoperiods housed alone, n = 11 brain slices from 11 animals in short photoperiods housed in groups) "Prestimulation," "Stimulation," and "Poststimulation" represent nanosensor fluorescence before, immediately following, and after electrical stimulation, respectively. (Scale bar: 10 µm.), n.s.: not significant, *P < 0.05, **P < 0.01, ***P < 0.005.

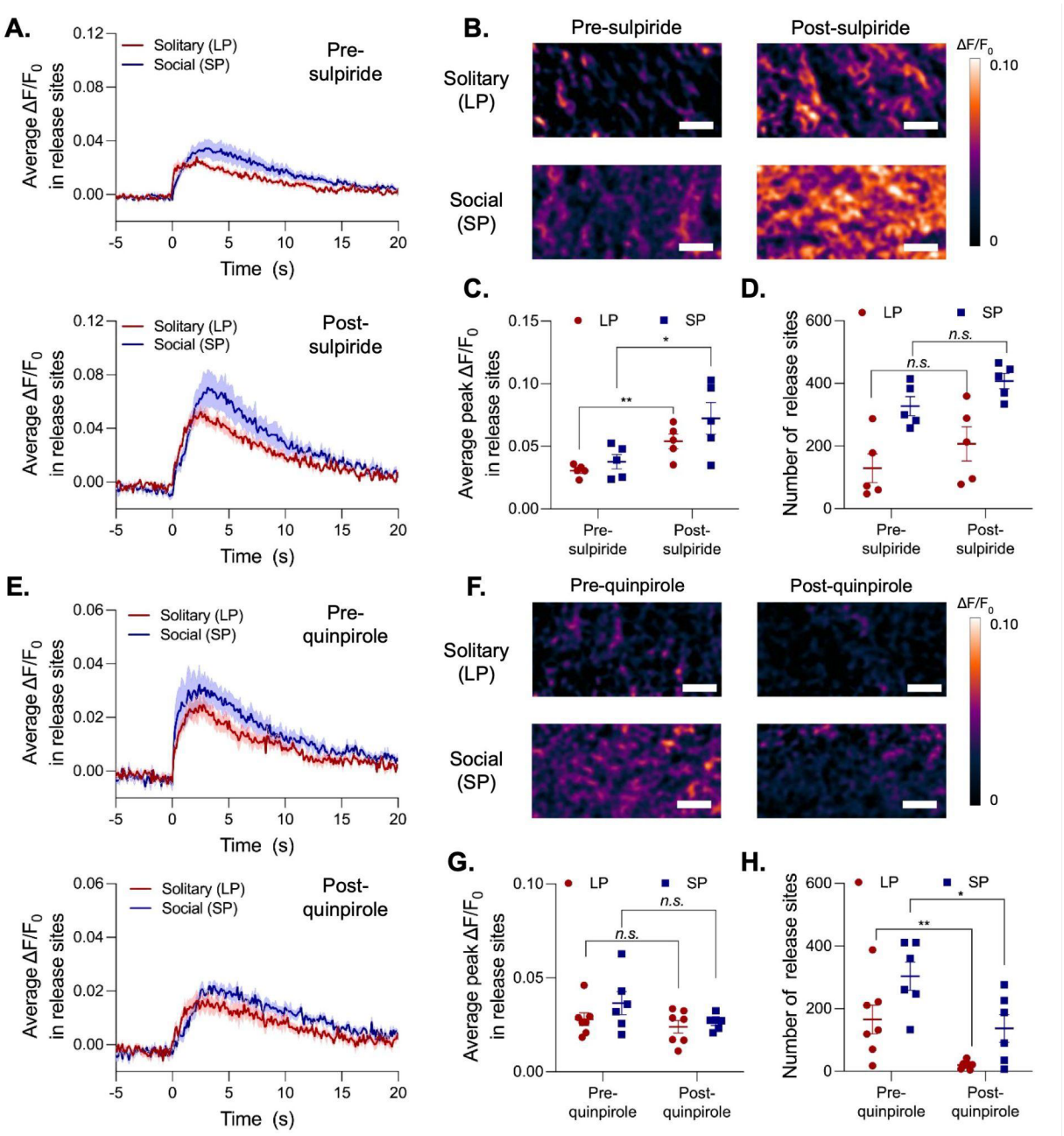


Figure 3.3. *Effects of D2 autoreceptor manipulation.* In slice, effects of D2-autoreceptor antagonist (sulpiride, A–D) and agonist (quinpirole, E–H) on electrically evoked dopamine release of voles housed alone in long photoperiods (LP; in red); and housed in groups in short photoperiods (SP; in blue). (A) Average $\Delta F/F_0$ in each release site's time trace (solid line) with the SE (shadow), (B) $\Delta F/F_0$ response images, (C) average peak $\Delta F/F_0$, and (D) number of release site following a 0.3 mA single-pulse electrical stimulation in the DMS of voles in solitary and social conditions before and after 5 µM sulpiride treatment. (n = 5 brain slices from 5 animals for LP voles, n = 5 brain slices from five animals for SP voles) (E) Average $\Delta F/F_0$ in each release site's time trace (solid line) with the SE (shadow), (F) $\Delta F/F_0$ response images, (G) average peak $\Delta F/F_0$ and (H) number of release sites following a 0.3 mA single-pulse electrical stimulation in the DMS of voles in social and solitary conditions before and after 5 µM quinpirole treatment. (n = 7 brain slices from seven animals for solitary LP voles, n = 6 brain slices from six animals for social SP voles) (Scale bar: 10 µm.), n.s.: not significant, *P < 0.05, **P < 0.01, ***P < 0.005.

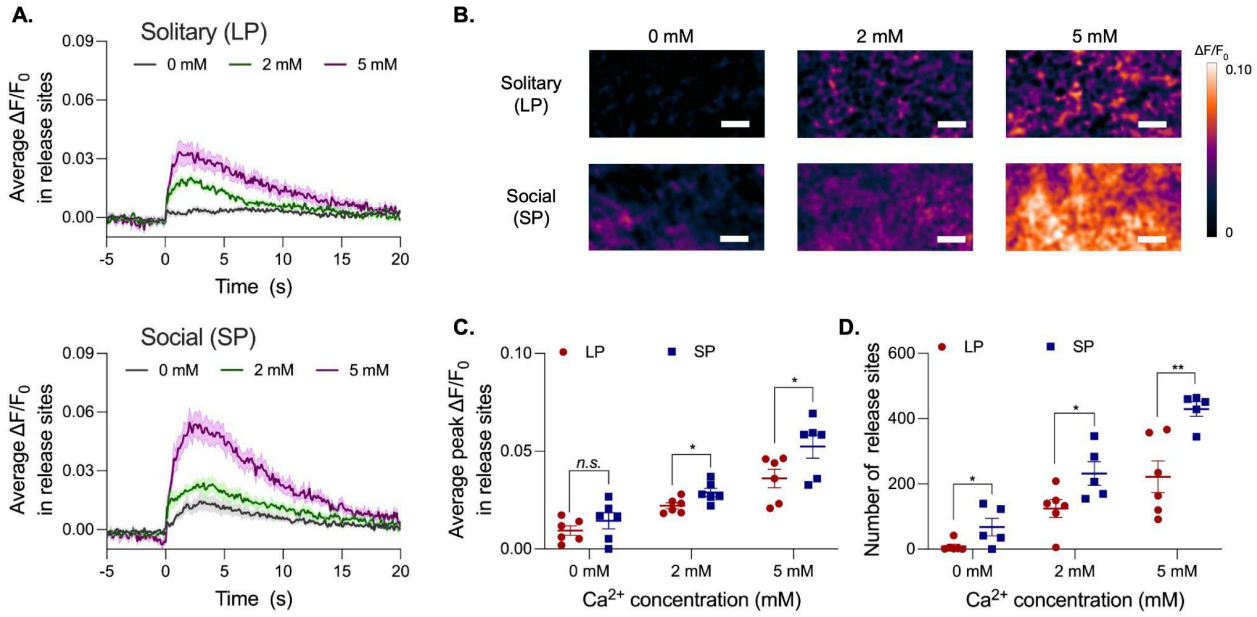


Figure 3.4. *Effects of extracellular Ca^{2+}* In slice, effects of extracellular Ca^{2+} on electrically evoked dopamine release of solitary voles in long photoperiods (LP; in red) and social voles in short photoperiods (SP, in blue). (A) Average $\Delta F/F_0$ in each release site's time trace (solid line) with the SE (shadow), (B) $\Delta F/F_0$ response images, (C) average peak $\Delta F/F_0$ and (D) number of release sites following a 0.3 mA single-pulse electrical stimulation in the DMS of voles in long and short photoperiods with various concentrations of Ca^{2+} (n = 6 brain slices from six animals for solitary LP voles, n = 5 brain slices from five animals in social SP voles) (Scale bar: 10 μm.), n.s.: not significant, *P < 0.05, **P < 0.01.

Target	Forward primer	Reverse primer
HPRT	CCC AGC GTC GTG ATT AGT GA	TCG AGC CAG TCT TTC AGT CC
DRD1	AGA AAC AAA TCC GGC GCA TC	TTC CAC GGG GTT GCC ATT AC
DRD2	CAA GCC AGA GAA GAA TGG GC	TAA GGG AGG TCC GGG TTT TG

Table 3.1. *Primers used for qPCR*

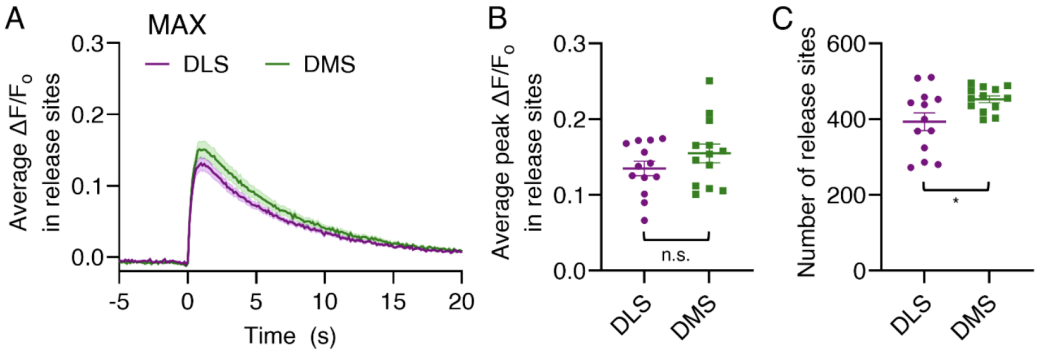


Figure S3.1. *Regional striatal differences. In slice, imaging electrically-evoked dopamine in the DLS (purple) and the DMS (green) of female voles housed in groups in short photoperiods following a 0.5 mA electrical stimulation applied adjacent to the field of view. (A) average ΔF/F₀ in release site time traces (solid line) with standard error (shadow), (B) average peak ΔF/F₀ in release sites, and (C) number of release sites under 'MAX' stimulation conditions. (n.s. not significant, *p<0.05)*

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