

Olfactory Cues Accelerate Reentrainment following Phase Shifts and Entrain Free-Running Rhythms in Female *Octodon degus* (Rodentia)

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Abstract Social interactions between conspecifics is a type of nonphotic zeitgeber common to several species. In the diurnal rodent *Octodon degus*, social interactions enhance reentrainment after phase shifts and can act as a weak zeitgeber. Olfactory stimuli appear necessary for these effects since bullectomy eliminates socially enhanced reentrainment. In Experiment 1, the authors examined whether stimulation of the main olfactory system was sufficient to enhance reentrainment after 6-h phase advances and delays in the adult female *O. degus*. When test animals received conspecific odor cues during reentrainment, they entrained 39% faster after phase advances ($p < 0.05$) and 33% faster after phase delays ($p < 0.001$) than when they did not receive odor cues. Thus, olfactory cues from distant female donors were sufficient to enhance rates of entrainment in female *O. degus* and provided results equivalent to earlier studies with donors and shifters housed in the cages together. In Experiment 2, the authors examined whether discrete 3-h and 1-h daily pulses of airborne odors from a group of 5 entrained female degus would be sufficient to produce entrainment of wheel-running activity in adult female conspecifics. During the period of exposure to 3-h pulses, 50% (4/8) of the subjects temporarily entrained to a 24-h cycle, while 12.5% (1/8) of the subjects fully entrained. Exposure to 1-h pulses allowed 37.5% (3/8) of the subjects to temporarily entrain and 12.5% (1/8) of the subjects to fully entrain. Duration of entrained episodes was positively correlated with Ψ , daily onset of activity with respect to the timing of odor exposure (Pearson $r = 0.731$; $p < 0.05$), such that animals with the entraining odor pulse beginning during subjective day ($\Psi = 7.8$ h, CT 7.8 ± 1.4) had longer periods of entrainment (22.2 ± 5.6 days) than animals with the entraining pulse occurring during subjective night ($\Psi = -4.6$ h; CT 19.4 ± 0.9 ; 5.6 ± 0.9 days; $p < 0.001$). In addition, for each animal, the combined duration of all episodes of 24-h entrainment correlated with increased period length (τ) of free-running rhythms (Pearson $r = 0.733$; $p < 0.05$). Thus, daily discrete pulses of odors with durations of either 1 or 3 h from female conspecifics were sufficient to produce both temporary and full entrainment to a 24-h cycle in the majority of female *O. degus*, and the likelihood of long periods of entrainment correlated with long taus and coordination of the odor pulse with mid subjective day.

Key words circadian, nonphotic, activity, degu

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The common critical zeitgeber for nearly all species is the daily light-dark cycle (Bruce, 1960; Moore-Ede et al., 1982). Recent studies, however, have indicated that nonphotic periodic cues may also act as zeitgebers in a number of species. Social cues, or time of day information resulting from social interaction between conspecifics presented in a rhythmical fashion are one type of nonphotic zeitgeber. Social interactions can influence free-running circadian rhythms by coordinating individuals' rhythms with each other in such species as hamsters (*Mesocricetus auratus*) (Davis and Gorski, 1985), deer mice (*Peromyscus maniculatus*) (Crowley and Bovet, 1980), Wistar rats (*Rattus norvegicus*) (Takahashi et al., 1984), and marmosets (*Callithrix jacchus*) (Erkert and Schardt, 1991).

Periodic exposure to social cues may also entrain free-running rhythms in mammalian and avian species. For example, daily periods of song playback entrain some species of birds, including siskins (*Carduelis spinus*) (Gwinner, 1966), serins (*Serinus serinus*) (Gwinner, 1966), and house sparrows (*Passer domesticus*) (Menaker and Eskin, 1966; Reeb, 1989). Periodic exposure to conspecifics can also entrain circadian rhythms in some, but not all, hamsters (*Mesocricetus auratus*). When 9 free-running male hamsters were exposed to an entrained hamster placed daily for 1 h in the free-running animals' cage, 44% of the animals showed full entrainment and 22% showed probable partial entrainment (Mrosovsky, 1988). Continuous, rather than episodic, exposure to conspecifics, entrained by a light-dark cycle, also can entrain free-running rhythms in some species (*Mus musculus*, Halberg et al., 1954; *Hipposideros speoris*, Marimuthu et al., 1981).

Social cues may also function as zeitgebers for some humans. While previous studies on the effects of social interactions on entrainment rate in humans are problematic due to simultaneous light exposure (Aschoff et al., 1971; Klein and Wegmann, 1974), a recent study shows that melatonin and core body temperature rhythms of 9 out of 15 (60%) retinally blind or enucleated individuals were entrained to a 24-h day. The remaining subjects (40%) were free running. To rule out the possibility that light was operating as a zeitgeber, despite the absence of light-induced suppression of melatonin (negative melatonin suppression test), 2 of the 9 entrained subjects were exposed to a bright-light stimulus, which resulted in phase shifts in sighted individuals. This stimulus failed to produce a similar response in the blind individuals, indicating

that the entrainment of these individuals was probably due to the effects of nonphotic zeitgebers of unknown origin (Klerman et al., 1998). As with the golden hamsters (Mrosovsky, 1988), social cues can produce full entrainment of free-running rhythms in some, but not all, human subjects.

Exposure to social cues can also accelerate entrainment rates following phase shifts. For example, when male golden hamsters were exposed to estrous females for 3 h on the initial day of an 8-h phase advance, the wheel-running activity rhythms of these males reentrained to the new light-dark cycle significantly faster than when they were shifted alone (Honrado and Mrosovsky, 1989). Recent studies in our laboratory indicate that social cues also act as a functional zeitgeber in the diurnal species *Octodon degus*, a hystricomorph South American rodent (Woods and Boraker, 1975). Females separated by mesh barriers that prevented physical contact from unfamiliar conspecifics but allowed the animals to experience visual, auditory, and olfactory stimuli reentrained more quickly after phase advances (30%-42% faster) (Goel and Lee, 1995a) and phase delays (20%-35% faster) (Goel and Lee, 1995b).

Behavioral data demonstrated that both male and female entrained conspecifics (donors) housed with female phase-shifters spent more time scent marking and sniffing the cage and the barrier than did donors housed with animals entrained to the same light-dark cycle as the donors. In addition, all phase-shifters showed more investigatory behaviors compared with the donors (Goel and Lee, 1996). These data suggested the hypothesis that olfactory cues were the necessary component of these social cues in *O. degus*. Consistent with this hypothesis, bilateral olfactory bulbectomies in female degus prevented the socially enhanced increased rate of reentrainment while leaving responses to the light zeitgeber unaltered (Goel and Lee, 1997a).

The goals of this study were twofold: first, to determine if an isolated olfactory stimulus, uncontaminated by other social cues associated with the physical presence of another animal, could accelerate rates of reentrainment following phase shifts in female degus as described in earlier studies (Goel and Lee, 1995a, 1995b), and second, to determine if discrete pulses of this olfactory stimulus could entrain the free-running rhythms of female degus. Specifically, we first tested the hypothesis that continuous exposure to airborne olfactory cues from entrained female degus was suffi-

cient to accelerate reentrainment rates of wheel-running activity rhythms in female *degus* experiencing both 6-h phase advances and delays (Experiment 1). Based on the enhanced rates of reentrainment in female shifters produced by female donors in earlier studies (Goel and Lee, 1995a, 1995b), we predicted that continuous exposure to airborne olfactory cues from groups of donor females would produce accelerated rates of entrainment in the females experiencing 6-h phase shifts.

Second, we hypothesized that discrete daily pulses of airborne odors from a group of entrained female *degus* would be sufficient to produce entrainment of free-running wheel-running activity rhythms in adult female *degus* (Experiment 2). Previous data indicate that temporary or partial entrainment can occur with continuous exposure to female *O. degus*. Daily exposure to conspecifics for 23 h entrained locomotor activity rhythms of female *degus* for 5 to 12 days out of 30 tested in 5 of 6 (83%) *degus* (Goel and Lee, 1997b). Since exposure to a single donor female produced partial entrainment in free-running females, we predicted that concentrated olfactory cues from 5 donors would produce longer periods of entrainment, minimally, and possibly full entrainment in the females experiencing olfactory cues. Five *degus* were used as odor donors to represent typical *degus* group size in the field (Fulk, 1976).

MATERIALS AND METHODS

Subjects

Subjects were 16 mature female *O. degus* (1-3 years of age, with an average life span of 5-7 years) born in a laboratory colony at the University of Michigan ($n = 8$ in Experiment 1, and different $n = 8$ in Experiment 2). Prior to the start of this experiment, animals were housed in LD 12:12, lights-on at 0600 h (average light intensity of 250 lux), with constant room temperature ($18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) and humidity maintained at 50% to 60%. Animals were maintained on a diet of LabDiet rodent chow (#5001). Food and water were available *ad libitum*.

Housing

Subjects were individually housed in plastic cages ($42.5 \times 22 \times 19$ cm) fitted with Nalgene running wheels

(9 cm wide \times 34.5 cm in diameter) in ventilated, light-tight environmental chambers (average light intensity of 250 lux; temperature at $18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). Chambers are ventilated by a constantly running fan that draws air through the box while simultaneously providing a background white noise. Each environmental chamber contained either 1 or 2 individually housed subjects. Running-wheel activity data were collected, recorded, and stored in 10-min bins either by Dataquest III or Vitalview (Mini-mitter, Inc., SunRiver, OR, USA). Additional mature female *O. degus* (1-3 years of age) (donors) were housed in plastic cages (either $42.5 \times 22 \times 19$ cm or $42.5 \times 46 \times 19.5$ cm) in ventilated environmental chambers. Each of these environmental chambers contained one smaller cage with two siblings and one larger cage with three siblings. All cages were cleaned weekly.

Procedure

Experiment 1

At the start of this experiment, subjects (phase-shifters) were entrained to a 12:12 LD cycle for a minimum of 3 weeks. Each animal then experienced a 6-h phase advance of its LD cycle, with both the times of lights-on and lights-off occurring 6 h early. On the initial day of the phase advance, each environmental chamber containing a shifter was connected via 1.53 m of hose to a box containing 5 entrained donors. Prior to the start of the study, donors were entrained to the phase-advanced light cycle that phase-shifting subjects would experience. Air was pulled continuously from the donors' box to the shifters' box through the hose by two fans (one on each box) for a minimum of 3 weeks, or until all the shifters were reentrained. Goel and Lee (1996) previously found no correlation between any calls or noises animals make and entrainment. But in the event of such a possibility, the white noise from the two fans was sufficient to mask any sounds from *degus* in one chamber from reaching the other. Once the shifters were stably entrained to the phase-advanced light cycle, they experienced another 6-h phase advance with the hoses between the donors' and shifters' boxes disconnected. As before, animals were given a minimum of 3 weeks to reentrain to the new LD cycle.

A similar procedure was followed for the phase delays, with the exception that animals were shifted with and without olfactory cues using a counter-

balanced design. Animals entrained to a 12:12 LD cycle experienced a 6-h phase delay both with and without a connection from the chamber housing the donors to the chamber housing the shifters. Again, the connection between the boxes was first made on the day on which the animals were shifted, and the donor animals were entrained to the phase-delayed LD cycle prior to the subjects' shift. Animals were given a minimum of 3 weeks to entrain to the shifted LD cycle.

Experiment 2

At the start of this experiment, subjects were entrained to a 12:12 LD cycle for a minimum of 3 weeks. Subjects were released into constant dark conditions (DD) for a minimum of 3 weeks. The τ of each subject's free-running rhythms was calculated, as described in the Data Analysis section for Experiment 2.

When the τ of each subject's free-running rhythms was stable (after a minimum of 3 weeks in DD), each environmental chamber containing a shifter was connected via 1.53 m of hose to a box containing 5 donors for 3 h daily. Prior to the start of the study, 5 donors housed in a separate environmental chamber were entrained to a 12:12 LD cycle (lights-on at 0600 h and lights-off at 1800 h). Preliminary data indicated that 5-h pulses of odors from 5 entrained female donors produced full entrainment in 1 animal, temporary or partial entrainment in 4 animals, and disrupted rhythms in the 3 other animals (Governale, unpublished). Thus, 3-h and 1-h pulses were chosen to examine whether a more discrete pulse would be equally, if not more, effective in producing entrainment. For the duration of each daily 3-h pulse, air was pulled continuously from the donors' box to the subject's box through the hose by two fans (one on each box). Each subject received pulses at the same clock time each day; 4 subjects received pulses from 0800 h to 1100 h, 3 subjects received pulses from 1130 h to 1430 h, and 1 subject received pulses from 1430 h to 1730 h. Pulses continued daily for 9 weeks.

Next, the pulses were shortened to a 1-h duration to determine if entrainment would be altered. The pulses began at the same times as for the 3-h pulses but ended 2 h earlier, with 4 subjects receiving pulses from 0800 h to 0900 h, 3 subjects receiving pulses from 1130 h to 1230 h, and 1 subject receiving pulses from 1430 h to 1530 h. Pulses for all subjects continued daily for 9 weeks. Finally, subjects were maintained in DD for an additional 2 weeks with no pulsing.

Control Treatments

Two procedures were carried out to check whether air pressure or other changes consistent with attaching the odor source chamber to the test chamber might alter rates of reentrainment or cause phase shifts in the absence of an odor source. Eight female degus were phase-advanced, as described in Experiment 1, while exposed to clean air which was passed through both chambers or while exposed to clean air passed only through their housing chamber. The rates of reentrainment did not differ between the two conditions. Another group of 8 female degus were released into DD and pulsed with clean air from the empty source chamber for 1 h at CT 4 or continuously exposed to clean air passing through the test chamber alone. A similar pulse of odors from 5 donor animals at CT 4 causes a significant phase advance of nearly 1 h (Jechura and Lee, unpublished data). When exposed to the pulse of air through the empty source chamber, the free-running rhythms were unaltered and were indistinguishable from the rhythms when there was no pulse. These data support the hypothesis that the results described below are the result of odor exposure and not other changes generated by connecting the chamber with the source odors to the chamber with the experimental animals.

Data Analysis

Experiment 1

Prior to and after each experimental change, Ψ was calculated for wheel-running activity using double-plotted actograms as well as daily histograms. Ψ was defined as the relationship between the time of lights-on and the time of the onset of morning activity and was used to determine the number of days necessary for stable entrainment. Activity onset was defined as 40 counts of activity/10 min for at least a 20-min period following a minimum hiatus of activity of 4 h (Goel and Lee, 1995a, 1995b). Thus, Ψ was calculated first for the entrained animal, using an average of the data from the 4 days prior to the shift. The animal was considered stably entrained after the phase shift when Ψ returned to and was maintained at the preshift value (± 10 min). Ψ was first approximated by analyzing the double-plotted actograms, and this analysis was checked using the corresponding daily histograms by an observer blind to the treatment con-

ditions. Two-tailed paired *t*-tests were used for statistical analysis, with each animal serving as its own control. Differences were considered significant if $p < 0.05$, and data are presented as means \pm SEM.

Levels of mean daily wheel running (number of revolutions per 10 min) were also examined for each animal at four different times during each shift using Dataquest III software to determine whether activity level varied either as a function of exposure to odor cues or as a function of time. Time 1 was the average of the mean daily activity on the 3 days prior to the shift, Time 2 was the mean daily activity on the initial day of the shift, Time 3 was the mean daily activity on the 2nd day of the shift, and Time 4 was the average of the mean daily activity on the 8th, 9th, and 10th days after the initial day of the shift. A two-way analysis of variance test with post hoc paired comparisons was used to examine differences in daily activity levels across time (repeated measures) during the shifts as well as between the groups exposed and the groups not exposed to olfactory cues. Differences were considered significant at $p < 0.05$.

Experiment 2

For each animal, levels of mean daily wheel running, maximum daily wheel running, and daily amplitude (mean daily activity subtracted from maximum daily activity) were calculated and then averaged for each of the five experimental conditions. For these three variables, a two-way analysis of variance test was used to examine differences in daily activity across time (repeated measures) between all experimental conditions.

When animals were entrained to the LD cycle, Ψ was calculated as described in the Data Analysis section for Experiment 1. For each animal, activity onset, activity offset (the point at which activity levels fell below the daily mean) (Labyak et al., 1997), and α (the difference between activity onset and offset) were calculated and then averaged for this experimental condition (Dataquest III or VitalView). When the animal was free running, τ was determined by cosine analysis, either with Dataquest III or Cosinor (Clopton and Klemfuss, 1993).

When animals were under conditions of constant darkness and were exposed to odor cues, Ψ was defined as the relationship between the time of the onset of the odor cues and the time of the onset of the activity bout associated with lights turning on during the previous LD cycle (CT 0). Activity onset was

defined as 40 counts of activity/10 min for at least a 20-min period following a minimum hiatus of 4 h (Goel and Lee, 1995b). Ψ was approximated first by analyzing the double-plotted actograms, and this analysis was checked by using the corresponding daily histograms (Dataquest III or Vitalview). Following the convention used for Ψ between activity onset and light onset, activity starting prior to the odor pulse has a positive Ψ , while activity beginning after the odor pulse has a negative Ψ (see Figs. 1 and 2 for examples).

When an animal maintained Ψ at a stable value (± 30 min) for 3 or more consecutive days, τ for this episode was estimated by Fourier analysis (MESA), MESA analysis (MESA), and cosine analysis (Cosinor; Clopton and Klemfuss, 1993). An episode of temporary entrainment was defined as a period of time during which τ was between 23.9 and 24.1 h. Full or temporary entrainment was defined as continuous entrainment throughout the period of pulsing, once an animal had "locked on" to the cue (Figs. 1 and 2). Partial entrainment was defined as all other variable, discontinuous episodes of entrainment with τ approximating 24.0 h. For each animal, activity onset, activity offset (the point at which activity levels fell below the daily mean) (Labyak et al., 1997), and α (the difference between activity onset and offset) were calculated and then averaged for all episodes of full or temporary entrainment (Dataquest III or VitalView) for correlational analysis (Systat, v. 7.0). Finally, the end of all episodes of entrainment was examined to determine whether free-running rhythms began at the point of release by an observer blind to the experimental conditions.

For the episodes of full or temporary entrainment during exposure to 3-h and 1-h pulses, two-tailed paired *t*-tests were used to compare mean daily activity levels, maximum daily activity levels, amplitude, activity onset, α , Ψ , and duration (number of days) of the episodes. Episodes of full or temporary entrainment occurring during both pulsing periods were then pooled, and two-tailed paired *t*-tests were used to compare activity onset, α , mean activity levels, maximum activity levels, and amplitude between these pooled episodes and entrainment under the 12:12 LD cycle.

For all episodes of full or temporary entrainment, two-tailed Pearson correlation analysis was used to evaluate the correlation of Ψ of the episodes of entrainment with τ of free-running rhythms as well as the correlation of the duration of the episodes of



Figure 1. Double-plotted actogram of degu #1005 demonstrating long periods of entrainment by 3-h and 1-h odor pulses from entrained female conspecifics delivered through a 1.5 m air duct connecting the donors' housing chamber with that of the subject. The animal was initially housed in LD 12:12 (noted by open and closed bar at the top of the actogram) and was released into DD on the day noted on the right of the actogram. Three weeks later, she was exposed daily to 3-h pulses of odors at the time noted by shading. This period of exposure was later reduced to 1 h per day as noted on the figure. The period with no data is the result of failure in data collection equipment and is not included in analysis. During the 3-h pulse, $\Psi = 10.69$ h, and $\Psi = 9.34$ h during the 1-h daily pulses. Days with very intense activity every 18 to 20 days are periods of estrous (see Labyak and Lee, 1995, for details).

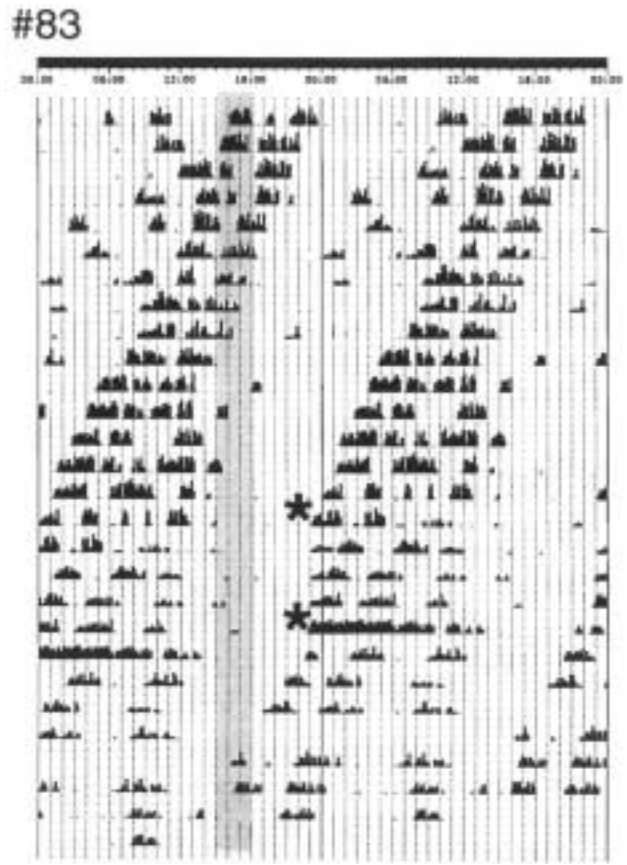


Figure 2. Double-plotted actogram of degu #83, housed in DD, demonstrating a short period of temporary entrainment (noted by two asterisks) in response to 3-h odor pulses from entrained conspecifics (noted by shaded area). During the period of entrainment, $\Psi = -7.27$ h. The intense activity on the last entrained day is likely a day of estrous.

entrainment with Ψ of the episodes of entrainment. For each animal, two-tailed Pearson correlation analysis was used to evaluate the correlation of the combined lengths of all episodes of full or temporary entrainment with τ of free-running rhythms. For all statistical tests, $p < 0.05$ is considered significant, and data are presented as means \pm SEM.

RESULTS

Experiment 1

Reentrainment Rate

Exposure to olfactory cues from 5 entrained donors significantly accelerated reentrainment following both the 6-h phase advances and the delays (Fig. 3).

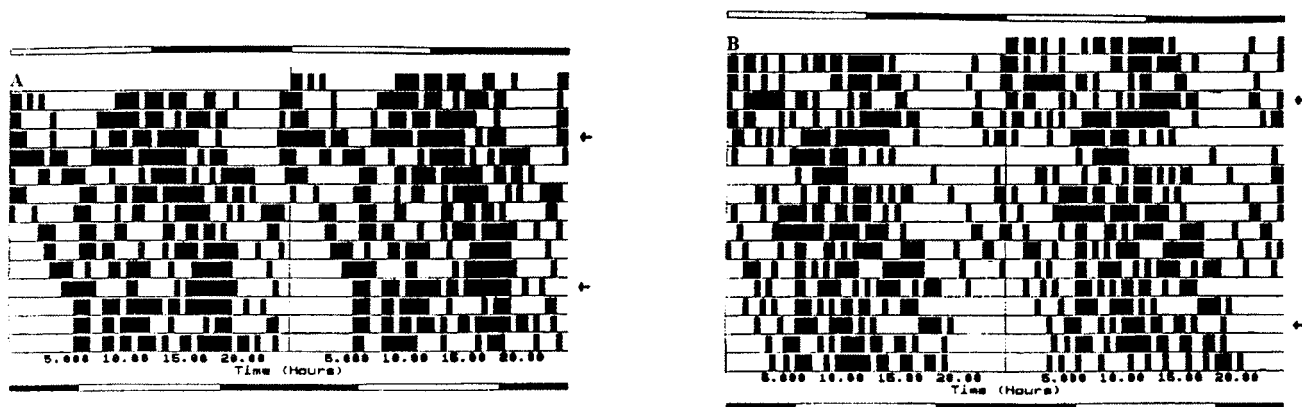


Figure 3. Example double-plotted actograms demonstrating reentrainment after a 6-h phase delay of the light cycle for 1 degu exposed to conspecific odors (A) or to photic changes only (B). The light cycle prior to the shift is shown above the actogram, and the new light cycle is shown below the actogram with open and closed bars. The arrows on the right side of the graph show the day of the phase shift and the day judged to be the end of the reentrainment period.

Animals entrained 38.9% faster, on average, following 6-h phase advances ($p < 0.05$) (Fig. 4A) and 32.7% faster, on average, following 6-h phase delays ($p < 0.001$) when exposed to odor cues than when not (Fig. 4B). As previously reported (Goel and Lee, 1995b), the rate of reentrainment with or without exposure to conspecifics or their odors during phase delays was unaffected by the order of exposure to the treatments when tested by repeated measures ANOVA.

Mean Daily Activity

Repeated measures ANOVA did not indicate significant differences in mean daily activity levels either across time or as a function of exposure to olfactory cues during the phase delays or advances.

Experiment 2

Entrainment

During the period of exposure to 3-h pulses, 50% (4/8) of the subjects temporarily entrained to a 24-h cycle (τ : 23.99 ± 0.02 ; duration of episode: 11.5 ± 4.7 days), while 12.5% (1/8) of the subjects fully entrained to a 24-h cycle (τ : 24.0; duration: 33 days). During the period of exposure to 1-h pulses, 37.5% (3/8) of the subjects temporarily entrained to a 24-h cycle (τ : 24.0 ± 0 ; duration: 15.0 ± 6.6 days), and 12.5% (1/8) of the subjects fully entrained to a 24-h cycle (τ : 24.0; duration: 41 days). Three of the 5 subjects that demonstrated entrainment to a 24-h cycle exhibited multiple episodes. Animals produced free-running activity

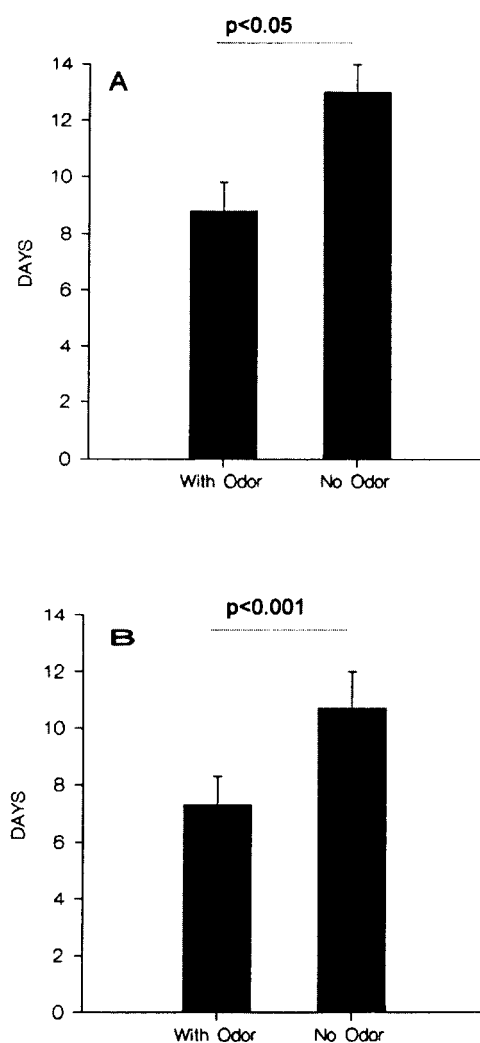


Figure 4. Summary data of rates of reentrainment (days) for degus exposed to conspecific entrained odors or to photic cues only during 6-h phase advances (A) and 6-h phase delays (B).

Table 1. Variables describing episodes of temporary or full entrainment with τ between 23.9 and 24.1 h.

Animal ID	1- or 3-h Pulsing	Duration of Episode (days)	τ of Episode	Free Running at End of Episode?	Mean Daily Activity	Maximum Daily Activity	Amplitude	Time of Activity Onset	α (hours)	Ψ	τ of Free-Running Rhythms
1005	3	5	24.0	Yes	70.79	426.00	355.21	0.72	18.42	-10.78	23.87
1005	3	4	24.0	No	62.04	438.25	376.21	6.40	16.42	-5.10	—
1005	3	33*	24.03	Yes	103.32	660.44	557.12	22.19	15.55	10.69	—
1005	1	41*	24.0	Yes	82.28	595.08	512.80	20.84	14.51	9.34	—
808	1	4	24.0	Yes	76.50	414.67	338.17	6.89	15.61	-1.61	23.25
83	3	4	23.92	Yes	109.96	627.00	517.04	1.23	15.36	-7.27	23.17
83	1	9	24.0	Yes	116.22	742.17	625.95	16.97	13.54	8.47	—
860	3	16	24.0	Yes	54.59	418.53	363.94	10.51	12.53	2.01	23.75
860	1	9	24.0	Yes	26.37	390.88	364.51	5.22	11.60	-3.28	—
860	1	12	24.0	Yes	30.59	382.64	352.05	17.00	12.30	8.50	—
847	3	7	24.0	Yes	98.62	475.67	377.05	5.62	18.58	-5.88	23.17

*These episodes of entrainment were continuous over the switch from 3-h to 1-h pulses and can be combined into a single episode.

rhythms at the end of all of these episodes from the time of activity onset, except for one (Tables 1 and 3), indicating that entrainment, not masking, had occurred. One animal never entrained to the odor pulses (Fig. 5).

In addition, during the 3-h pulses, 50% (4/8) of the subjects experienced partial entrainment with a τ close to 24 h (τ : 24.28 ± 0.02 ; duration: 4.5 ± 0.7 days), and 12.5% (1/8) of the subjects experienced similar partial entrainment (τ : 24.29; duration: 4.0 days) during exposure to the 1-h pulses. One of 5 subjects that demonstrated temporary entrainment with a τ close to 24 h exhibited multiple episodes. Animals produced free-running activity rhythms from the point of activity onset at the end of all of these episodes (Tables 2 and 3).

None of the two-tailed paired *t*-tests comparing mean daily activity, maximum daily activity, amplitude, activity onset, α , Ψ , or duration of entrainment, between episodes of full or temporary entrainment during exposure to 3-h and 1-h odor pulses, reached significance. However, α during episodes of entrainment with 3-h pulses (16.14 ± 2.25 h) was significantly longer than with 1-h pulses (13.51 ± 1.62 h; $df = 8,9$; $p = 0.05$; Table 1).

Mean Daily Activity, Maximum Daily Activity, and Amplitude

Repeated measures ANOVA did not indicate significant differences in amplitude or maximum daily activity levels between conditions. Of the two-tailed paired *t*-tests comparing variables between the combined episodes of full or temporary entrainment from

both 3-h and 1-h pulses (mean daily activity: 75.57 ± 30.45 revolutions/10 min) and the initial entrainment under a 12:12 LD cycle (mean daily activity: 107.62 ± 35.03 revolutions/10 min), only differences in mean daily activity level approached significance ($df = 13,9$; $p = 0.06$).

Correlations between Ψ , τ , and Duration of Episodes

For each separate episode of full or temporary entrainment, two-tailed Pearson correlation analysis was used to evaluate the correlation of entrained Ψ with τ of free-running rhythms and the correlation of the duration of the episodes of entrainment with Ψ . Duration of entrained episodes was positively correlated with the phase angle of entrainment (Pearson $r = 0.731$; $p = 0.011$). In addition, as expected, the combined durations of all episodes of entrainment increased as the period (τ) of free-running rhythms increased (Pearson $r = 0.733$; $p = 0.039$). However, there was no significant correlation between τ of free-running rhythms and Ψ of entrained episodes (Pearson $r = 0.194$; $p = 0.567$).

Circadian Phase of Odor Pulse during Entrained Episodes

Each episode of entrainment occurred such that the odor pulse either occurred during subjective day or late during subjective night, except for one episode (#1005, $\Psi = 10.8$ with $\alpha = 18.4$ h), which is ambiguous and was not included in this analysis. The onset of the odor pulse occurred at CT 7.8 ± 1.4 for episodes with



Figure 5. Double-plotted actogram of degu #808, housed in DD while exposed to daily 3-h periods of odor exposure. This animal never demonstrated entrainment or changes in τ until the last 50 days (not shown) when the activity pattern became arrhythmic. Estrous periods can be recognized by the increased activity at regular intervals.

positive phase angles of activity onset ($n = 5$; Table 1 and Fig. 1). The alpha of 13.7 ± 0.5 h and duration of entrained episodes of 22.2 ± 5.6 days was significantly different ($p < 0.05$ and 0.001 , respectively) from the episodes that occurred with a negative Ψ (CT 19.4 ± 0.9 ; $n = 5$; $\alpha = 15.5 \pm 1.0$ h; duration: 5.6 ± 0.9 days; Table 1 and Fig. 2). Tau and the circadian time of the odor pulses were uncorrelated, and entrainment during both subjective day and subjective night occurred in 3 out of 5 entraining animals.

Table 2. Variables describing episodes of partial entrainment with τ between 24.1 and 24.4 h.

Animal ID	3-h or 1-h Pulsing	Duration of Episode (days)	τ	Free Running at End of Episode?
895	3	3	24.34	Yes
144	3	6	24.24	Yes
83	1	5	24.24	Yes
83	3	4	24.29	Yes
808	3	4	24.29	Yes

Arrhythmia

During exposure to the odor pulses, the 1 animal of the 8 (12.5%) that did not demonstrate any kind of entrainment showed arrhythmia of wheel-running activity for a period of 50 days.

DISCUSSION

In adult female *O. degus*, odor cues alone are sufficient to both enhance rates of reentrainment following 6-h phase advances and delays and produce entrainment in 75% of animals (5 out of 8) in constant darkness. These results are similar to those of previous studies, demonstrating that social cues from female conspecifics housed next to adult female degus could accelerate reentrainment to phase advances (Goel and Lee, 1995a) and delays (Goel and Lee, 1995b). The results are also similar to studies showing that daily exposure to entrained female conspecifics produced entrainment in 5 out of 6 (83%) female degus (Goel and Lee, 1997b). In addition, these results add to a burgeoning literature on the effects of social cues on both reentrainment rates following phase shifts (Honrado and Mrosovsky, 1989) and the entrainment of free-running rhythms (Klerman et al., 1998; Menaker and Eskin, 1966; Mrosovsky, 1988) in a variety of mammalian and avian species.

Experiment 1 demonstrated that airborne olfactory cues were sufficient to accelerate reentrainment rates in female *O. degus* following both 6-h phase advances and delays. The average enhancement for the advances (38.9%) and the delays (32.7%) was comparable to the enhancement found in the earlier studies in which social cues accelerated the rate of reentrainment of the activity rhythms of female phase shifters placed in a cage with female donors by an average of 31.0% following 6-h phase advances (Goel

Table 3. Variables describing entrainment during exposure to 3-h odor pulses, 1-h odor pulses, and two conditions combined.

	3-h Pulses		1-h Pulses		Combined	
	τ : 23.9-24.1	24.1-24.4	23.9-24.1	24.1-24.4	23.9-24.1	24.1-24.4
Animals showing temporary or partial entrainment	50% (4/8)	50% (4/8)	37.5% (3/8)	12.5% (1/8)	62.5% (5/8)	50% (4/8)
Animals showing full entrainment	12.5% (1/8)	0%	12.5% (1/8)	0%	12.5% (1/8)	0%
Episodes of temporary or partial entrainment	55.6% (5/9)	80% (4/5)	44.4% (4/9)	20% (1/5)	N/A	N/A
Episodes of full entrainment	50% (1/2)	0%	50% (1/2)	0%	N/A	N/A
τ of episode	23.99 \pm 0.02	24.28 \pm 0.02	24.0 \pm 0	24.29	24.00 \pm 0.83	24.28 \pm 0.02
Length of episode (days)	11.5 \pm 4.7	4.5 \pm 0.7	15.0 \pm 6.6	4.0	13.09 \pm 3.8	4.4 \pm 0.5
Ψ of episode	2.72 \pm 7.79	-4.28 \pm 6.18	N/A	N/A	N/A	N/A

and Lee, 1995a) and by an average of 42.9% following 6-h phase delays (Goel and Lee, 1995b). The lack of difference in mean daily activity between the groups exposed to donor odor cues and those not exposed is also comparable to the results from previous studies utilizing both phase advances (Goel and Lee, 1995a) and phase delays (Goel and Lee, 1995b). In contrast to these earlier studies, however, we did not find a significant increase in mean daily activity during the shift when compared with the preshift baseline data. Thus, the presence of another animal may be necessary in degus for this effect, which also occurs in golden hamsters exposed daily to a conspecific for 1 h following phase shifts (Mrosovsky, 1988). Thus, it seems that the phase-shifting effect of the odors in degus does not require enhanced activity levels.

As demonstrated in Experiment 2, 1-h and 3-h pulses of airborne olfactory cues were sufficient to produce short- and long-term entrainment of free-running rhythms in adult female degus. These discrete daily pulses of odors were more effective in producing long periods of entrainment of free-running rhythms than was the presence of an entrained conspecific in a previous study that found that the presence of an entrained female on the other side of a mesh barrier produced only temporary short duration entrainment of the free-running rhythms of 5 out of 6 (83.3%) adult female degus and full entrainment in none. In this study, we find 1-h and 3-h pulses of odor cues produced temporary entrainment in 5 out of 8 (62.5%) adult female degus and full entrainment in 1 out of 8 (12.5%). Odor pulses were more effective in producing longer durations of entrained periods (mean 22.2 \pm 5.6 days at CT 7.8) than were the continuous social cues from a donor in the cage, which produced periods of entrainment for an average of 7.2 \pm 1.2 days. The odor pulses may be more effective than

the presence of a conspecific in producing long periods of entrainment as a result of the discreteness of the pulses, the concentrated odors from 5 donors, or a combination of these two variables. Both methods produced similar rates of arrhythmia in subjects, with odor pulses producing arrhythmia in 1 out of 8 (12.5%) animals and the presence of a conspecific producing arrhythmia in 1 out of 6 (16.7%) animals (Goel and Lee, 1997b). Whether the odors produce entrainment or arrhythmia may be related to intraspecific variation in sensory capacity, salience of/attention to the odor, or sensitivity of the circadian system to nonphotic zeitgebers.

In Experiment 2, the difference in mean daily running-wheel activity between the combined episodes of entrainment from the two pulsing conditions and the initial entrainment under a 12:12 LD cycle approached significance ($df = 13, 9; p = 0.06$). This drop in mean daily activity level during the pulsing conditions may be explained by the interaction of exposure to constant darkness and conspecific odors, and it is consistent with other data in degus comparing mean activity levels under conditions of LD 12:12 and DD (Labyak and Lee, 1995). In addition, α was significantly longer ($df = 8, 9; p = 0.05$) in episodes of entrainment during the 3-h pulses (16.14 \pm 2.25 h) than during 1-h pulses (13.51 \pm 1.62 h).

When the durations of all episodes of entrainment were combined for each animal, they were found to be positively correlated with τ (Pearson $r = 0.733; p < 0.05$). Thus, in this experiment, an animal with a τ close to 24.0 h was likely to show a larger total number of days of entrainment than an animal with a τ less similar to 24.0 h. Previous studies with nonphotic cues have shown that full entrainment can be difficult if the animal's τ falls far from 24 h (Menaker and Eskin, 1966; Hut et al., 1999). Thus, probably because social

cues are a weak zeitgeber (at least as we have presented them), it appears easier for a female degu to entrain to a 24-h cycle of odor pulses if the initial τ is very close to 24 h. Alternatively, nonphotic PRCs typically allow smaller phase delays than phase advances, and stable entrainment to a 24.0-h period for τ less than 23.75 h may not be possible (see Hut et al., 1999, for review).

The duration of entrained episodes was positively correlated with Ψ (Pearson $r = 0.731$; $p < 0.05$). Thus, an episode of entrainment with a more positive Ψ (activity onset occurring before the start of the pulse) was likely to have a greater length than an episode with an activity onset occurring after the start of the pulse. This resulted in two typical patterns of entrainment, animals with the odor pulses in subjective day at CT 7.8 and animals with the odor pulse in subjective night at CT 19.4. The mid to late subjective day effectiveness of the nonphotic odor zeitgeber is consistent with data from other species with other zeitgebers (see Hut et al., 1999, for review). It is particularly surprising that the latter allowed entrainment since it fell during mid subjective night when the animals are inactive and typically asleep (see Fig. 2; Goel and Lee, 1996; Kas and Edgar, 1999). This provides further indirect evidence that entrainment, at least for short periods, does not require that the nonphotic odor zeitgeber induce activity in the degu. In addition, 2 of the 8 animals showed disruption when the period of odor exposure coincided with daily activity onset, and 1 animal demonstrated true arrhythmia for a period of 50 days after a similar juxtaposition of odor exposure and activity onset. The construction of a PRC for conspecific odor pulses will shed further light on the effects of odors as a nonphotic zeitgeber on the free-running rhythms in the female degu.

An interesting characteristic of studies involving nonphotic cues is the species-specific nature of responsiveness to these cues; namely, certain nonphotic cues can act as zeitgebers in some species but not others. For example, while auditory cues can entrain the circadian rhythms of marmosets (Erkert and Schardt, 1991) and several avian species (Gwinner, 1966; Menaker and Eskin, 1966), similar procedures have failed to entrain squirrel monkeys (Sulzman et al., 1977) as well as rats (Vilaplana et al., 1995). Furthermore, while temperature cycles are effective entrainers in a number of species such as the palm squirrel (Rajaratnam and Redman, 1998), they have failed to entrain free-running rhythms in hamsters (Bruce, 1960) and flying squirrels (DeCoursey,

1960). Likewise, whereas social cues from the presence of a conspecific can entrain the free-running rhythms of bats (Marimuthu et al., 1981), mice (Halberg et al., 1954), and golden hamsters (Mrosovsky, 1988), social cues are ineffective entrainers in squirrel monkeys (Sulzman et al., 1977). This species-specificity lends credence to the hypothesis that species-specific ecology may determine the relative potency of a particular zeitgeber for a species; namely, the environment may determine which nonphotic cues critically influence survival, and thus a species may develop adaptations to allow these cues to influence the circadian system (Krebs and Davies, 1993). One would predict that in other highly social, fossorial rodent species, as in *O. degus*, social and olfactory cues could also function as zeitgebers.

Since the odor cue used in this study was airborne and traveled a relatively long distance of approximately 1.5 m through a hose, it is likely that the main olfactory system was the primary sensory system involved in producing the enhanced rate of entrainment rather than the vomeronasal organ (VNO), which is thought to primarily detect nonvolatile substances very near the animal (Meredith, 1983). In previous studies conducted with *O. degus* using the presence of a conspecific, it was impossible to determine which sensory system or combination of systems, whether auditory, visual, main olfactory, or accessory olfactory, was primarily liable for the enhanced rates of entrainment following phase shifts; moreover, since bulbectomies ablated both the main and accessory olfactory bulbs, it was not possible to determine what part of the lesions was responsible for the lack of response of bilaterally bulbectomized females to social cues (Goel and Lee, 1997a). With this experimental paradigm, however, it appears that the main olfactory system is sufficient to produce enhanced rates of entrainment comparable to those found in the studies with paired entrained odor donors and phase-shifters. Thus, the main olfactory system was probably the primary sensory system at work in the earlier studies using the presence of a live animal. Because the effects of long-distance air-borne odors on the VNO has not been often studied, we cannot completely exclude the possibility of its involvement as well. For example, particles may have traveled through the hoses to be detected by the VNO and thus activate the accessory olfactory system.

In conclusion, isolated continuous olfactory cues from entrained female donors were sufficient to accelerate reentrainment following 6-h phase shifts, and

discrete daily exposure to entrained conspecific odors entrain free-running rhythms in female *O. degus*. Whether these effects are unique to *O. degus* or shared by additional rodent or mammalian species remains to be seen. Regardless, their discovery adds to the growing body of knowledge of nonphotic cues and their role as zeitgebers for avian and mammalian species. While there seems to be a high degree of variation in which species responds to which nonphotic cue, and to what extent individuals within a species respond, responsiveness to nonphotic cues in general appears broadly applicable to most mammalian species. Further work is necessary to determine, among other things, the ecological significance of these nonphotic cues for different species as well as the neuroanatomical systems underlying the responses to these cues.

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