

Time-of-day discriminative learning in homing pigeons, *Columba livia*

CHERI A. BUDZYNSKI and VERNER P. BINGMAN
Bowling Green State University, Bowling Green, Ohio

Pigeons were trained on an operant procedure to discriminate between morning and afternoon when location did not vary (Experiment 1). The pigeons were placed on a fixed interval (FI) schedule in the morning and on a different FI schedule in the afternoon. Probe trials that occurred at the beginning of the training sessions were examined. The pigeons responded differently, depending on the time of day, reflecting the learning of a stable 24-h memory representation of the association between the FI schedules and the time of day. The pigeons from Experiment 1 were then clock shifted and tested twice, to determine whether they were relying on an endogenous circadian oscillator, an hourglass mechanism influenced by the photoperiod, or environmental noise to make the time-of-day discrimination (Experiment 2). The results of the second experiment indicated a circadian mechanism was most important for the observed time-of-day learning.

Beginning early this century, the ability of organisms to use temporal and spatial information to guide behavioral responses has been of experimental interest. Organisms are able to extract temporal and spatial information efficiently from the environment to exploit resources, while minimizing energy expenditure (Enright, 1970). The ability to associate a specific location with a specific time of day (24-h time-place associative learning) has been observed in many species. For example, honeybees, *Apis mellifera*, not only recognize different food locations, but also arrive at the different locations at appropriate times of day when food is available (Beling, 1929; Gould, 1987). Twenty-four-hour time-place associative learning has been documented in natural (European kestrels, *Falco tinnunculus*: Rijnsdorp, Daan, & Dijkstra, 1981), seminatural (garden warblers, *Sylvia borin*: Biebach, Falk, & Krebs, 1991; Biebach, Gordijn, & Krebs, 1989; Biebach, Krebs, & Falk, 1994; Falk, Biebach, & Krebs, 1992; fish, Pisces: Cyprinidae: Reeb, 1996), and operant (rats: Boulos & Logothetis, 1990; Mistlberger, de Groot, Bossert, & Marchant, 1996; pigeons, *Columba livia*: Saksida & Wilkie, 1994) settings.

A survey of the time-place associative learning literature indicates that animals readily learn to associate locations with time of day and use this information to coordinate behavior. However, it is unknown to what extent spatial information and time-of-day information are processed independently during time-place associative learning. Spa-

tial learning without a discriminative temporal component is a ubiquitous feature of animal learning. Obvious examples include water maze learning in rats (Morris, Garrud, Rawlins, & O'Keefe, 1982) and the navigational map of homing pigeons (Wallraff, 1991); both can be expressed irrespective of time of day. However, to the best of our knowledge, time-of-day learning (i.e., the ability to perform different learned behaviors at different times of day when location does not vary) has not been reported. Therefore, an interesting question in understanding the range of conditions in which learning can occur is whether time-of-day learning can take place when location does not vary.

EXPERIMENT 1 Time-of-Day Discrimination

In Experiment 1, we adopted a discrete-trial peak-interval procedure to determine whether the pigeons were discriminating between morning and afternoon sessions (Catania, 1970; Meck, 1988; Roberts, 1981). The discrete-trial peak-interval procedure involved sessions consisting of two types of trials. One type of trial consisted of food reinforcement after a fixed time interval. The other type of trial was nonreinforced and consisted of a relatively long interval, in comparison with the reinforced trials. The two types of trials were randomly intermixed within a session. On nonreinforced trials, an animal typically responds more vigorously around the time that food would be available during the reinforced trials (see Meck, 1988; Roberts, 1981). By training pigeons on one fixed time interval during the morning and on a different fixed time interval during the afternoon, we used the discrete-trial peak-interval procedure to measure time-of-day discriminative learning without varying location.

The discrete-trial peak-interval procedure has been previously employed to examine the accuracy, as well as the neural mechanisms, of short-interval timing (e.g., intervals

This study was supported in part by grants from the National Institutes of Health (R03-MH52315-02). We would like to acknowledge A. Kacelnik, H. Broadbent, and M. Bateson for their input into the experimental design. Sincere gratitude is expressed to Rosemary Strasser and Joyce Ehrlinger for lending their help in the data collection. Correspondence concerning this article should be addressed to C. A. Budzynski, Department of Psychology, Bowling Green State University, Bowling Green, OH 43403 (e-mail: cherib@bgnet.bgsu.edu).

of less than 1 min) in animals (Meck, 1988; Roberts, 1981). Our experiment, by contrast, was designed to examine time-of-day discriminative learning that was stable over a 24-h period. Therefore, our research question was very different from the questions posed in previous discrete-trial peak-interval procedure experiments and required a different type of data analysis (see the Method section). In short-interval timing experiments (Meck, 1988; Roberts, 1981), the first trials of a session were excluded, because the researchers were not interested in examining the animal's stable long-term memory. Second, the response patterns were generated by pooling a large number of trials (Meck, 1988; Roberts, 1981). By contrast, the initial nonreinforced trials were the critical trials needed to answer our research question. Only the response patterns from these initial trials, which effectively reflect stable learning over a 24-h period, were analyzed in our study.

Method

Subjects. The subjects were 10 homing pigeons, *Columba livia*, housed separately in a temperature-controlled room with a 14:10-h light:dark cycle (0600–2000). The birds were maintained at 85% of their free-feeding body weight and were provided with water and grit ad lib. They received food during training sessions; supplementary food was provided at random times during the day, to maintain their weight. The pigeons were trained 6 days per week.

Apparatus. An operant chamber was used for the training procedures. The chamber measured 42 × 36 × 40 cm. One wall was equipped with three white pecking keys (left/right key: 2 cm; middle key: 3 cm, in diameter), which could be back-illuminated (44 lux), and a food hopper. The left key was illuminated to initiate each trial of a session, and the center key was illuminated during the discriminative phase of a trial. The chamber was dimly lit (88 lux) and ventilated by a fan. White noise was used to mask environmental sounds.

Procedures. Each pigeon was initially shaped to peck at a single illuminated key for mixed-grain reinforcement. Subsequently, a pigeon was placed into the operant chamber 5 min before the initiation of a training session. After the 5-min habituation period, the key to the left of the center key was illuminated. The first peck to the left key was followed by a 3–5 sec variable interval. After the 3–5 sec variable interval, the first trial was initiated by the illumination of the center key. All the subsequent trials followed the same sequence of left-key trial initiation, center-key illumination, and then a variable intertrial interval of 10–35 sec. Sessions consisted of 50 training trials.

As was noted above, the training sessions were modeled after the discrete-trial peak-interval procedure (see Roberts, 1981). During training sessions, the birds received reinforced fixed interval (FI) training trials and nonreinforced probe trials. Reinforced FI training trials consisted of the illumination of the center key for a standard FI of either 5 or 25 sec (see below). In the probe trials, the center key was illuminated for 70 sec, after which the light was extinguished and no mixed-grain reinforcement was provided. The FI training trials and the probe trials were randomly alternated, with the condition that there be no more than 3 consecutive probe trials. Initially, all 50 trials in a session were reinforced FI training trials (100% reinforcement). The percentage of reinforced training trials was successively dropped. After 28 days, 50% of the trials were FI training trials; by the 9th week, 30% of the trials were FI training trials.

On each day of training, the pigeons received a morning only session, an afternoon only session, or both morning and afternoon sessions. Each week, the pigeons received 2 days with morning only sessions, 2 days with afternoon only sessions, and 2 days with both morning and afternoon sessions, with the condition that they not receive more than two of the same type of session consecutively. On

each day of training, the same experimenter handled the pigeons during the morning and afternoon sessions. Each session lasted about 1 ½ h. The pigeons received training for 10 weeks (80 sessions).

During morning sessions, the pigeons were trained between 0800 and 1230 (each individual was trained at the same time in the morning throughout training). Four pigeons received reinforced training trials at a FI of 5 sec in the morning. The other 6 received reinforced training trials at a FI of 25 sec in the morning. On reinforced training trials, the first peck at the illuminated key after the FI provided mixed grain for 2–3 sec. The afternoon sessions for each bird occurred 6 h after the morning sessions (1400–1830). Pigeons on a FI of 5 sec in the morning were placed on a FI of 25 sec in the afternoon. Pigeons that were on a FI of 25 sec in the morning were on a FI of 5 sec in the afternoon. All other variables within the afternoon sessions were identical to those described for the morning sessions.

Data analysis. A computer (8088-based IBM compatible), interfaced to the operant chamber, recorded the number of pecks per trial in 1-sec bins. After a pigeon completed the 10 weeks of training, the last 15 probe trials (for both the morning and the afternoon sessions) that occurred *before any reinforced training trials within a session* were examined. Thus, 30 probe trials were analyzed per bird. The probe trials during each session were randomly generated by the computer program. Therefore, the probe trials examined for any 2 birds were not necessarily taken from the same sessions or the same days. Likewise, the probe trials may have been taken from days in which the pigeons received only a morning session, only an afternoon session, or both sessions.

Each analyzed probe trial occurred before any reinforced training trial. Therefore, the analyzed probe trials were independent of within-session learning. Within a session, the task was fairly easy, and pigeons quickly learned to increase their rate of responding just before reinforcement in the typical scalloped fashion (data not shown). However, the within-session learning did not indicate whether memory for the time-of-day discrimination was stable over a 24-h period. Therefore, the initial nonreinforced probe trials were critical and were the only trials used in the data analysis.

The pigeons in this study were counterbalanced, so that some received a 5-sec FI session in the morning and others received a 25-sec FI session in the morning. Therefore, to aggregate the response patterns of the pigeons, the data were analyzed with respect to the session type (i.e., 5-sec FI session or 25-sec FI session) rather than the time of day (morning or afternoon). Group response rate curves were calculated for the 5-sec and 25-sec FI sessions. First, a response rate curve was generated for each pigeon. As was noted previously, the number of pecks per trial were recorded in 1-sec bins. A median number of pecks per trial was computed for each 1-sec bin (i.e., all 70 bins) across the 15 probe trials of the 5-sec FI sessions and across the 15 probe trials of the 25-sec FI sessions for each pigeon. Medians were used to minimize the influence of outliers. The medians for each 1-sec bin were then normalized as percentages of the maximum number of pecks for each 1-sec bin. The response rate patterns of the individual birds were then combined: A group or pooled median for each 1-sec bin was calculated from the median response rate of the individual birds at each 1-sec bin for both the 5-sec FI sessions and the 25-sec FI sessions. The group response rate curves generated from the group median bins for the 5-sec FI and the 25-sec FI sessions were plotted, and a third-order polynomial line was then fit to the data for each type of training session.

To provide a quantitative assessment of the response pattern and to determine whether any apparent differences in the response rate curves could be statistically verified, a two-way analysis of variance (ANOVA) was calculated. For an individual bird, the total number of pecks for each trial was calculated for (1) bins 3–7 and (2) bins 23–27. We examined bins 3–7 and bins 23–27 because these bins provided a relative measure of the pigeons' performance during the probe trials at the times when reinforcement would have occurred. The sums were then normalized as percentages as a function of the

maximum number of pecks recorded over any five consecutive bins within the trial. For an individual bird, medians were computed for the normalized percentages of bins 3–7 and bins 23–27 from the 15 probe trials of the 5-sec FI sessions and the 15 afternoon probe trials of the 25-sec FI sessions. The two-way ANOVA was then computed from the individual birds' median percentages of bins 3–7 and bins 23–27. The two-way ANOVA was implemented to determine whether there were any statistical differences in the pecking response between session type (5- or 25-sec FI session) and bin type (bins 3–7 or bins 23–27).

Results and Discussion

Nine of the 10 pigeons were included in the data analysis. One pigeon was excluded because it did not consistently work during the morning session. The aggregate response rate curves for the 5- and 25-sec training sessions are presented in Figure 1. Inspection of the response rate curves indicates that the pigeons were able to use time-of-day information to discriminate between the two FI schedules. The pigeons displayed a high rate of pecking early during the 5-sec FI session trials, with a rapid decline as the trials progressed. In contrast, the pigeons displayed a relatively sustained rate of pecking throughout the 25-sec FI session trials. The data summarized in the response rate curves of Figure 1 reflect learning across sessions that was mediated by a stable (at least 24-h) memory representation of the time-of-day/FI association.

The two-way ANOVA yielded a statistically significant interaction [$F(1,32) = 4.24, p < .05$]. The results of the statistical analysis are consistent with the differences in the response rate curves of Figure 1. The pigeons displayed a higher rate of pecking during the 3–7 sec bins and a low

rate of pecking during the 23–27 sec bins during the 5-sec FI sessions. In contrast, the pigeons displayed a high rate of pecking during the 3–7 sec bins and sustained pecking during the 23–27 sec bins during the 25-sec FI sessions.

The results of the aggregate response rate curves and the two-way ANOVA indicate that time-of-day learning can occur when location does not vary. Our results are consistent with previous reports that animals can use time-of-day information to find a food reward (Biebach et al., 1991; Biebach et al., 1989; Biebach et al., 1994; Boulos & Logothetis, 1990; Falk et al., 1992; Mistlberger et al., 1996; Reeb, 1996; Rijnsdorp et al., 1981; Saksida & Wilkie, 1994). Time-of-day learning in birds has been easily demonstrated when time-of-day information was associated with a particular spatial location (see Biebach et al., 1989; Saksida & Wilkie, 1994). In contrast, there was a notable amount of variability in our experimental results when location did not vary. Homing pigeons live in environments where integrating spatial and temporal information is essential (e.g., sun compass orientation; see Schmidt-Koenig, Ganzhorn, & Ranvaud, 1991). Therefore, pigeons may not readily learn time-of-day associations when spatial information does not vary. Nonetheless, the results indicate that time-of-day information, to some extent, is processed independent of locality variation.

**EXPERIMENT 2
Circadian Clock or Hourglass?**

The internal timing mechanisms that allow animals to use time-of-day information have been of great interest for

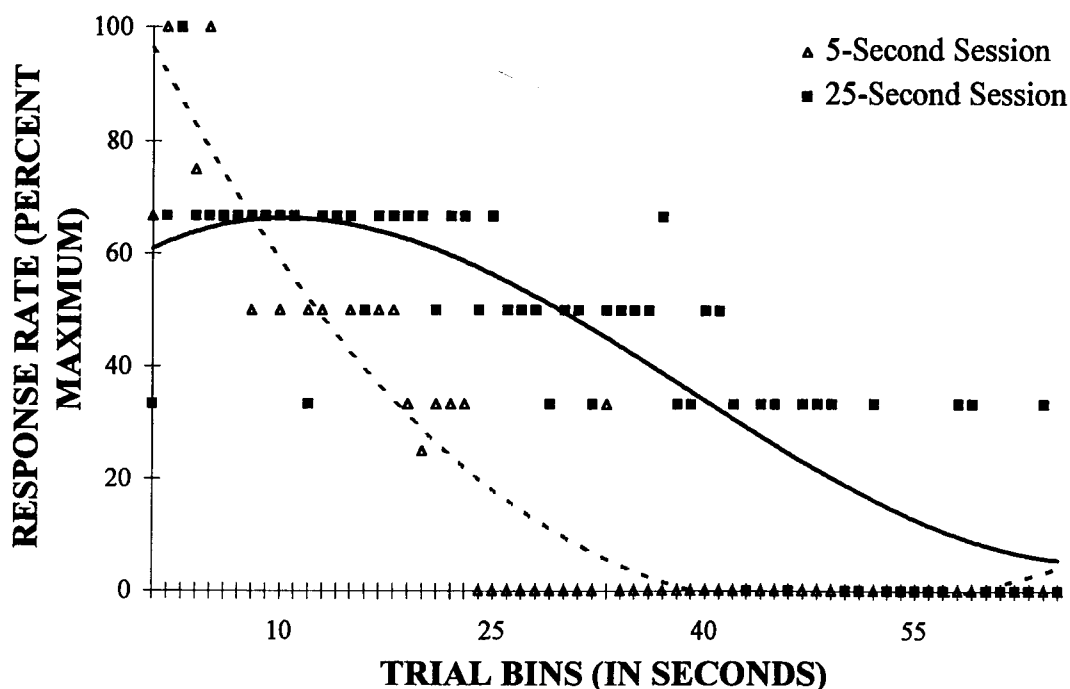


Figure 1. Aggregate response rate curves for the 5-sec fixed interval (FI) session (open data points and dashed line) and the 25-sec FI session (filled data points and solid line) probe trials. Data are only shown to 65 sec.

researchers involved in animal behavior and learning. Two hypotheses have emerged as possible models for the 24-h internal timing mechanism: an hourglass and a circadian timer. Theoretically, animals can use either mechanism to make time-of-day discriminations. However, time-place associative learning is usually regulated by a circadian mechanism (Biebach et al., 1991; Saksida & Wilkie, 1994).

A circadian mechanism involves the entrainment of internal, endogenous circadian rhythms to an environmental stimulus. Endogenous circadian oscillators, when entrained, enable organisms to use temporal information to synchronize activity with members of their species and more effectively exploit the environment (Aschoff, 1989). The most powerful environmental entrainment stimulus (zeitgeber) is the naturally occurring 24-h light:dark cycle. If one removes the zeitgeber (e.g., the photoperiod), an endogenous circadian mechanism continues to function with a slightly altered period length. Furthermore, if the zeitgeber is experimentally altered (e.g., changing the photoperiod), endogenous rhythms can readjust or re-entrain to the altered photoperiod.

Environmental stimuli regulate an hourglass mechanism differently. Environmental cues such as daylight or darkness activate the hourglass. An hourglass timer requires a starting cue and a cue to "turn it over" to measure a new period. An hourglass timer measures only continuous, single time periods. Without the environmental activation cues, an hourglass timer does not function (Biebach et al., 1991; Wilkie, Saksida, Samson, & Lee, 1994). Furthermore, if the environmental activation stimulus is experimentally altered, an hourglass timer immediately adjusts to the alterations.

It is important to note that the photoperiod is the environmental stimulus most often manipulated when trying to discriminate between the two mechanisms. However, any environmental stimulus with characteristics of constancy and strength may influence both mechanisms. For example, environmental noise can entrain circadian rhythms (Moore-Ede, Sulzman, & Fuller, 1982) and may serve to "turn over" an hourglass mechanism. Therefore, it is always important to consider environmental stimuli other than the photoperiod when examining the mechanism of time-of-day learning in animals.

Under a natural photoperiod, it is impossible to determine whether a circadian mechanism or an hourglass mechanism is regulating time-of-day learning. Therefore, an experimental manipulation is necessary to determine what mechanism is being used. The experimental manipulation employed in this study involved phase shifting or clock shifting the pigeons. Clock shifting has been reliably used in past experiments to discriminate between the timing mechanisms (Biebach et al., 1991). Clock shifting is also a valuable cue for determining whether an animal is relying on environmental stimuli other than the photoperiod to determine the time of day. Distinct predictions can be made about the behavior of the birds, depending on the mechanism employed during the time-of-day learning task (see Figure 2).

Considering past research on time-of-day discriminative learning (Biebach et al., 1991; Boulos & Logothetis, 1990; Mistlberger et al., 1996; Saksida & Wilkie, 1994), we hypothesized that the pigeons used a circadian timer during the time-of-day discrimination task of Experiment 1. There were two alternative hypotheses: (1) The pigeons relied on an hourglass mechanism influenced by the photoperiod, or (2) the pigeons relied on environmental noise to learn the time-of-day discrimination.

Method

Subjects. The 9 pigeons that completed Experiment 1 were used in Experiment 2.

Apparatus. The same apparatus as that described in Experiment 1 was used.

Procedure. The pigeons were moved to an adjacent room after 9 weeks of training (described in Experiment 1). Training continued for 6 days under the normal photoperiod (0600–2000), to ensure that their behavior was not influenced by the move. Training sessions before the move were compared with training sessions after the move; no changes were noted for any of the birds. After 6 days in the new room, the pigeons were subjected to a clock-shift manipulation. Six of the pigeons were subjected to a fast shift, in which lights were turned on at 2400 and were turned off at 1400. The remaining 3 pigeons were subjected to a slow shift, in which lights were turned on at 1200 and were turned off at 0200.

The day immediately following the shifted photoperiod, the fast-shifted pigeons were tested with five probe trials between 0800 and 1230 (each bird was tested at the real time of its morning training). The slow-shifted pigeons were tested with five probe trials between 1400 and 1830 (each bird was tested at the real time of its afternoon training). This first clock-shift test (Test 1) was critical for discriminating between a circadian timing mechanism and an hourglass mechanism (Figure 2).

The pigeons were tested again 7 days after they were continually maintained in the shifted photoperiod. The fast-shifted pigeons were tested with five probe trials between 0800 and 1230 (each bird was tested at the real time of its morning training). The slow-shifted pigeons were tested with five probe trials between 1400 and 1830 (each bird was tested at the real time of its afternoon training). This second clock-shift test (Test 2) was critical for discriminating between a circadian timing mechanism and a training cue of environmental noise (Figure 2).

The reader is referred to Figure 2 for the behavioral differences predicted for a circadian timer, an hourglass timer, and environmental noise. We predicted that the pigeons would rely on a circadian timing mechanism. If true, the fast-shifted birds' response pattern in Test 1 should resemble their morning training response pattern. Likewise, the slow-shifted birds' response pattern in Test 1 should resemble their afternoon training response pattern. This behavior was predicted because an endogenous rhythm would not have re-entrained to the altered light:dark cycle after only 1 day (for a 6-h clock shift, it takes about 6 days for the circadian rhythms to re-entrain; Schmidt-Koenig, 1960). Thus, the birds' rhythms would still be approximately in phase with the natural light:dark cycle and not in phase with the shifted light:dark cycle. An hourglass mechanism would cause an immediate shift in behavioral response after a phase shift. Therefore, the fast-shifted birds' response pattern in Test 1 should resemble their afternoon training response pattern if they were relying on an hourglass mechanism. Likewise, the slow-shifted birds' response pattern in Test 1 should resemble their morning training response pattern.

After 7 days in the manipulated photoperiod, the birds' endogenous circadian rhythms would have re-entrained to the altered light:

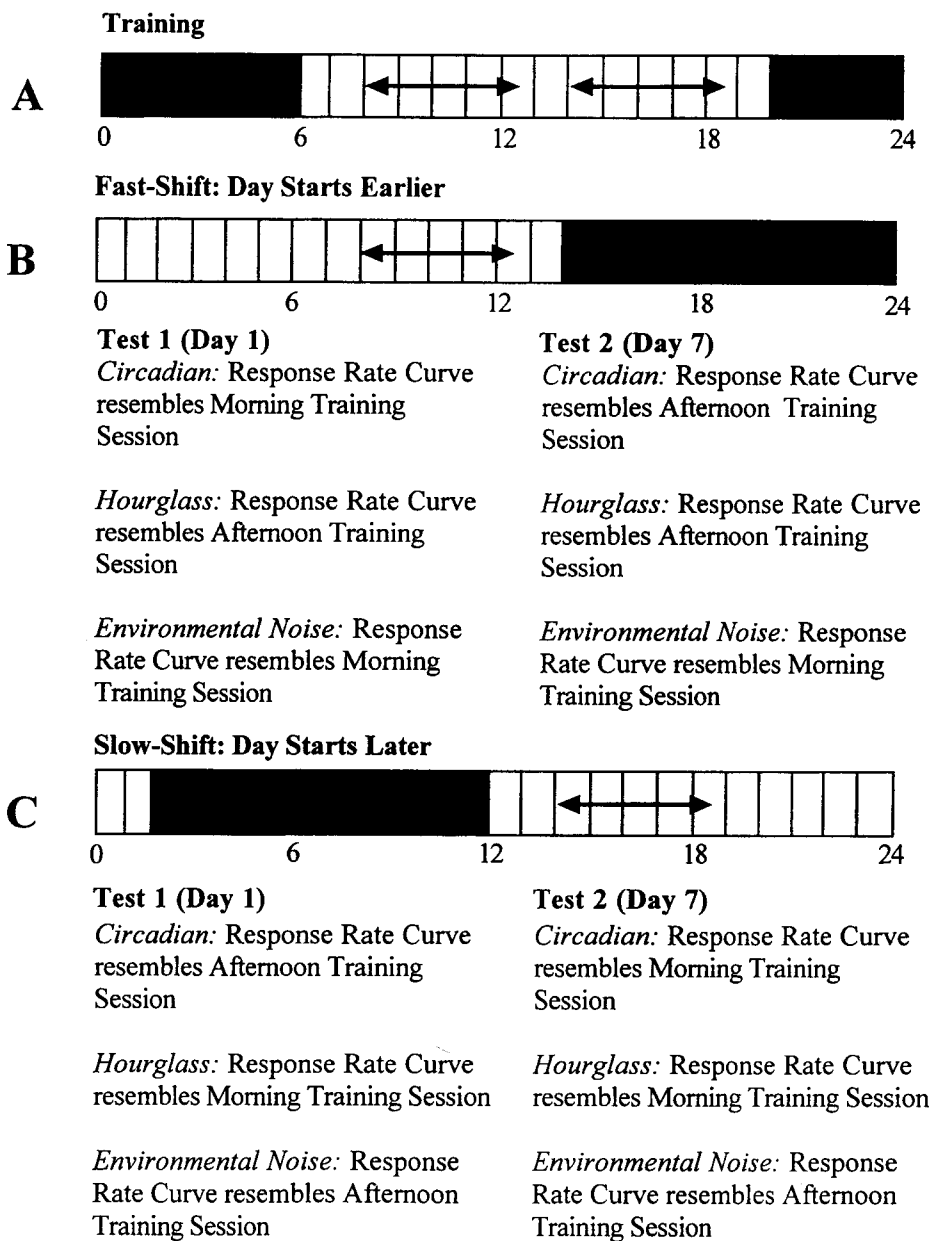


Figure 2. A schematic representation of the photoperiod conditions during (A) training, (B) the fast-shift manipulation, and (C) the slow-shift manipulation. Arrows indicate time of training/testing. Behavioral predictions based on the circadian hypothesis, the hourglass hypothesis, and the environmental noise hypothesis following the clock shift are included. 0 = 12:00 a.m.

dark cycle, leading to a change in the response pattern with respect to real time. If they were relying on a circadian mechanism, the fast-shifted pigeons' response pattern in Test 2 should now resemble their afternoon training response pattern. Likewise, the slow-shifted birds' response pattern in Test 2 should now resemble their morning training response pattern. No response difference between Test 1 and Test 2 would be expected if the time-of-day discrimination was based on environmental noise.

Data analysis. To examine the group response patterns of the pigeons during the clock shift, it was necessary to divide the birds into

two groups (Group 1 and Group 2) on the basis of our hypothesis. For both groups, the data were analyzed for Test 1 and Test 2. Group 1 ($n = 4$) was predicted to display a response pattern similar to the 25-sec FI session response pattern during Test 1 and a response pattern similar to the 5-sec FI session response pattern during Test 2, if the pigeons were relying on a circadian timer. Group 2 ($n = 5$) was predicted to display a response pattern similar to the 5-sec FI session response pattern during Test 1 and a response pattern similar to the 25-sec FI session response pattern during Test 2, if the pigeons were relying on a circadian timer. Both groups consisted of

birds that were fast shifted or slow shifted; likewise, both groups consisted of birds that were trained at a 5-sec FI in the morning and a 25-sec FI in the morning.

The same procedures were used as those described in Experiment 1 to generate the response rate curves for both groups. Likewise, a two-way ANOVA was calculated as was described in Experiment 1, to statistically compare the response rate curves. However, analyses were only computed on the 5 probe trials in each test session, in contrast to the 15 probe trials taken from numerous morning and afternoon sessions for the analyses of Experiment 1.

Results and Discussion

The response rate curves for Test 1 and Test 2 are presented in Figure 3 (Group 1) and Figure 4 (Group 2). For Group 1, the response rate curve of Test 1 was characteristic of the 25-sec FI session response pattern; the rate of pecking was relatively sustained over the probe trials. The pecking response recorded during Test 2 was characteristic of the 5-sec FI session response pattern; the pecking response was higher earlier in the probe trials and declined quickly as the trials progressed. The response rate curves during the two clock-shift sessions were characteristic of a response pattern that would be expected if the pigeons were relying on a circadian timer.

For Group 2, the response rate curve of Test 1 was characteristic of the 5-sec FI session response pattern; the pecking response was higher earlier in the probe trials and

declined quickly as the trials progressed. The decline in the rate of pecking, however, was less steep during Test 1 (see Figure 4). The pecking response recorded during Test 2 resembled the 25-sec FI session response pattern; the rate of pecking stayed at a higher level over the probe trials. Despite the slower decline in the rate of pecking during Test 1, the response rate curves during the two clock-shift sessions generally resembled the response pattern that would be expected if the pigeons were relying on a circadian timer.

The two-way ANOVA for Group 1 yielded a statistically significant interaction [$F(1,16) = 29.15, p < .05$]. The two-way ANOVA for Group 2 did not yield a significant interaction or a significant main effect. The statistical analysis for Group 1 was consistent with the qualitative analysis presented in the response rate curves of Figure 3. Therefore, the response pattern of Group 1 notably matched the response pattern that would be expected if the pigeons were relying on a circadian mechanism (compare Figures 1 and 3). Although the qualitative analysis of the response rate curves for Group 2 was consistent with the circadian hypothesis, the slower decline in the rate of pecking during Test 1 and the statistical analysis precludes rejection of the hourglass hypothesis. The environmental noise hypothesis can be excluded, because both groups displayed a shift in the response pattern during

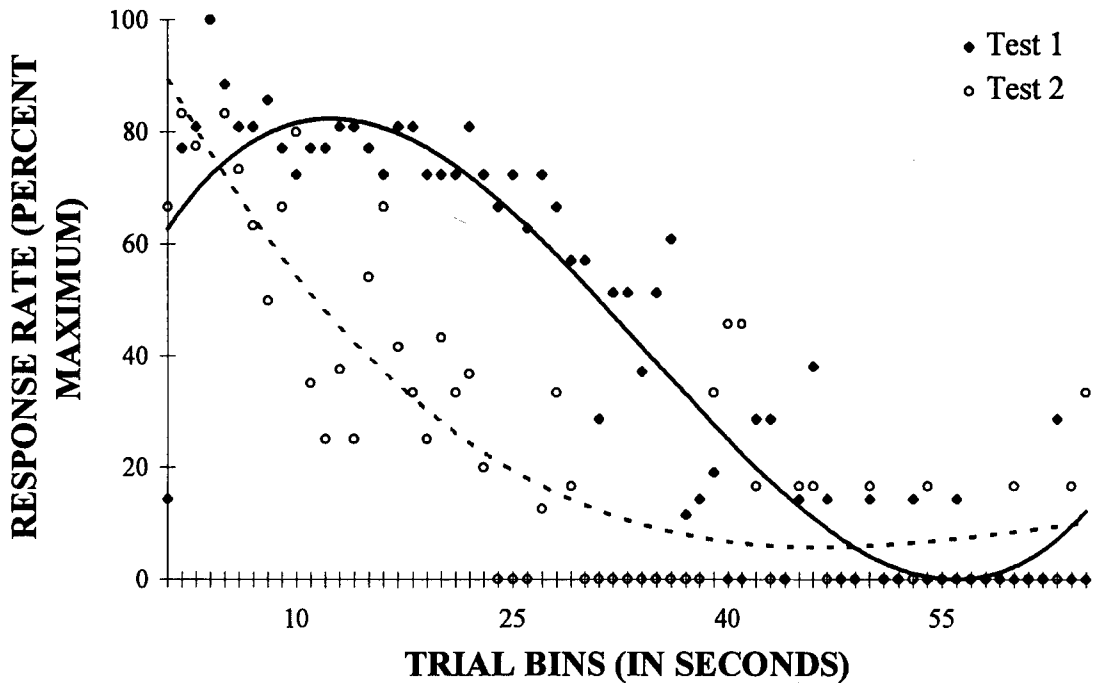


Figure 3. Aggregate response rate curves for Test 1 (day 1 after clock shift) and Test 2 (day 7 after clock shift) for Group 1 ($n = 4$). Group 1 was predicted to display a response pattern similar to the 25-sec fixed interval (FI) training sessions during Test 1 (filled data points and solid line) and a response pattern similar to the 5-sec FI training sessions during Test 2 (open data points and dashed line), if the pigeons were relying on a circadian timer. Data are only shown to 65 sec.

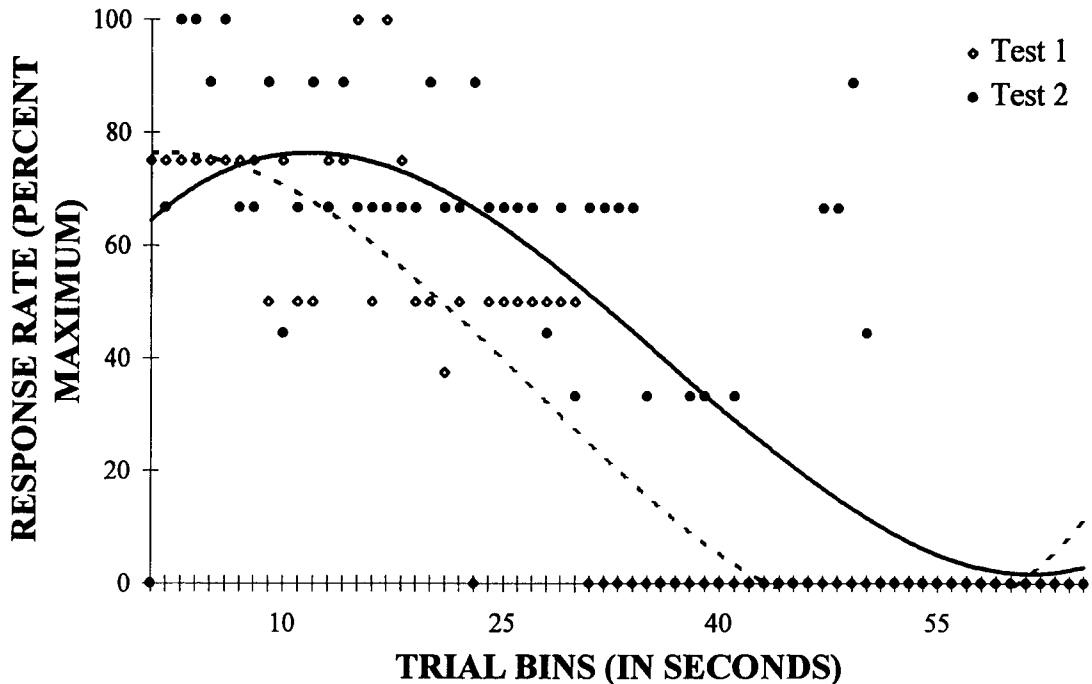


Figure 4. Response rate curves for Test 1 (day 1 after clock shift) and Test 2 (day 7 after clock shift) for Group 2 ($n = 5$). Group 2 was predicted to display a response pattern similar to the 5-sec fixed interval (FI) training sessions during Test 1 (open data points and dashed line) and a response pattern similar to the 25-sec FI training sessions during Test 2 (filled data points and solid line), if the pigeons were relying on a circadian timer. Data are only shown to 65 sec.

Test 2 (if they were relying on environmental noise as a training cue, there would not have been a shift in the response pattern).

In general, the results of the clock-shift experiment indicate that the pigeons were primarily relying on a circadian timing mechanism to make the time-of-day discrimination. This is consistent with the previous literature on time-place associative learning. However, the lack of a significant interaction for Group 2 leaves open the possibility that a small number of birds relied on an hourglass mechanism.

GENERAL DISCUSSION

The results of Experiment 1 provide insight into the central question of this study: Can pigeons discriminate between two times of day, using two FI schedules, when location does not vary? In Experiment 1, the qualitative and statistical analyses indicate that the pigeons were able to associate two FI schedules with two times of day. To the best of our knowledge, this is the first experiment with pigeons to report a learned association, stable over at least 24 h, between time of day and a response that did not depend on discriminating between alternative spatial responses or locations.

It was interesting to examine the timing mechanism employed by pigeons, once it was established that they could make time-of-day associations with two FI sched-

ules. The results of Group 1 were consistent with previous work on time-place associative learning: The pigeons relied on a circadian timing mechanism when making an association between time of day and a rewarding stimulus. Although the response rate curves of Group 2 were characteristic of the response that would be expected if they were relying on a circadian mechanism, the quantitative analysis was not significant. Therefore, the hourglass hypothesis could not be excluded.

Although the data from Experiment 2 indicate that birds generally rely on an endogenous circadian rhythm entrained to the photoperiod to perform time-of-day discriminations, we do not wish to suggest that birds necessarily rely on some perceived sense of time similar to an actual clock to perform the discrimination. For example, it may be that the time-of-day discrimination was directly controlled by some physiological state (e.g., body temperature) that cycled across the day and was regulated by an endogenous circadian timer. Therefore, the data do not permit us to identify the proximate stimuli that served as cues for the time-of-day learning. The clock-shift data, however, indicate that the time-of-day cues were either directly or indirectly controlled by an endogenous circadian rhythm.

Why do organisms rely on a circadian timing mechanism during time-of-day learning? From an evolutionary perspective, a circadian timing mechanism is particularly advantageous over an hourglass mechanism (Enright,

1970). For example, if one examines the work of Rijnsdorp et al. (1981) on the flight-hunt behavior of European kestrels, the evolutionary advantage of a circadian timer is clear. It has been documented that kestrels will revisit a site at the same time of day if that particular site has been rewarding in the past. If kestrels were to use an hourglass mechanism to make this association, changes in environmental conditions could alter the functioning of an hourglass. The most likely environmental stimulus used by the kestrels to derive information about time is the light:dark cycle. Therefore, on mornings in which daylight is delayed because of cloud coverage, an hourglass may be delayed because the environmental cue (daylight) that "turns over" the hourglass is delayed. Thus, the kestrels would have difficulty visiting previously rewarding sites at the appropriate time of day. If the kestrels were to rely on a circadian mechanism, variations in ambient light conditions would not influence time-place associative hunting. The kestrels would still be able to visit the appropriate sites at a particular time of day, because a circadian timer would function normally within a variable environment.

We designed Experiment 1 so that pigeons could not discriminate between the two FI schedules by using different spatial stimuli. Only time of day could be used as a discriminative cue. However, we cannot exclude the possibility that the spatial stimuli associated with the operant chamber were important in at least motivating the behavior of the animals. Therefore, at this level, the spatial stimuli of the operant chamber may have served as cues.

Second, although the most powerful zeitgeber is the light:dark phases of an organism's environment, other cues can entrain circadian oscillators (Aschoff, 1989; Moore-Ede et al., 1982). Thus, it is desirable to test behavior in a closed economy (e.g., Biebach et al., 1991). In a closed economy, one can reduce the possibility that the pigeons are using entrainment cues other than the photoperiod (e.g., handling or social cues). The pigeons in the present study needed to be housed separately from the testing apparatus, because of resources. Therefore, interference from other entrainment cues was possible. However, similar testing procedures involving an apparatus separate from the housing chambers have been used in other time-place association studies (Saksida & Wilkie, 1994), and interference with entrainment to the light:dark cycle was not found. Therefore, the influence of other entrainment cues appears generally unimportant.

A third limitation of this study is that no biological rhythms were recorded. Therefore, there was no measurement of the internal state of the pigeons to compare with their behavioral responses. This is critical for interpreting the clock-shift manipulation. Although the behavioral data indirectly indicated the internal state of the pigeons, biological rhythm data would have provided an objective measurement. This objective measurement would have permitted us to correlate the behavioral responses of the pigeons on the 2 clock-shift test days with the internal state of the animals.

This study was the first to examine time-of-day learning in pigeons when location did not vary. The data should motivate researchers to recognize the ecological and evo-

lutionary importance of time-of-day information as a phenomenon independent of space. Furthermore, experimenters should begin to identify brain structures and mechanisms that control time-of-day learning.

REFERENCES

- ASCHOFF, J. (1989). Temporal orientation: Circadian clocks in animals and humans. *Animal Behaviour*, **37**, 881-896.
- BELING, I. (1929). Über das Zeitgedächtnis der Bienen [On bees' memory for time]. *Zeitschrift für Vergleichende Physiologie*, **9**, 259-338.
- BIEBACH, H., FALK, H., & KREBS, J. R. (1991). The effect of constant light and phase-shifts on a learned time-place association in garden warblers (*Sylvia borin*): Hourglass or circadian clock? *Journal of Biological Rhythms*, **6**, 353-365.
- BIEBACH, H., GORDIIN, M., & KREBS, J. R. (1989). Time-and-place learning by garden warblers, *Sylvia borin*. *Animal Behaviour*, **37**, 353-360.
- BIEBACH, H., KREBS, J. R., & FALK, H. (1994). Time-place learning, food availability and the exploitation of patches in garden warblers, *Sylvia borin*. *Animal Behaviour*, **48**, 273-284.
- BOULOS, Z., & LOGOTHETIS, D. E. (1990). Rats anticipate and discriminate between two daily feeding times. *Physiology & Behavior*, **48**, 523-529.
- CATANIA, A. C. (1970). Reinforcement schedules and psychophysical judgments: A study of some temporal properties of behavior. In W. N. Schoenfeld (Ed.), *The theory of reinforcement schedules* (pp. 1-42). New York: Appleton-Century-Crofts.
- ENRIGHT, J. T. (1970). Ecological aspects of endogenous rhythmicity. *Annual Review of Ecology and Systematics*, **1**, 221-238.
- FALK, H., BIEBACH, H., & KREBS, J. R. (1992). Learning a time-place pattern of food availability: A comparison between an insectivorous and granivorous weaver species (*Ploceus bicolor* and *Euplectes hordeaceus*). *Behavioral Ecology & Sociobiology*, **31**, 9-15.
- GOULD, J. L. (1987). Honey bees store learned flower landing behavior according to time of day. *Animal Behaviour*, **35**, 1579-1581.
- MECK, W. H. (1988). Hippocampal function is required for feedback control of an internal clock's criterion. *Behavioral Neuroscience*, **102**, 54-60.
- MISTLBERGER, R. E., DE GROOT, M. H. M., BOSSERT, J. M., & MARCHANT, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research*, **739**, 12-18.
- MOORE-EDE, M. C., SULZMAN, F. M., & FULLER, C. A. (1982). *The clocks that time us: Physiology of the circadian timing system*. Cambridge, MA: Harvard University Press.
- MORRIS, R. G. M., GARRUD, P., RAWLINS, J., & O'KEEFE, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**, 681-683.
- REBS, S. G. (1996). Time-place learning in golden shiners (Pisces: Cyprinidae). *Behavioural Processes*, **36**, 253-262.
- RUNSDORP, A., DAAN, S., & DIJKSTRA, C. (1981). Hunting in the kestrel, *Falco tinnunculus*, and the adaptive significance of daily habits. *Oecologia*, **50**, 391-406.
- ROBERTS, S. (1981). Isolation of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes*, **7**, 242-268.
- SAKSIDA, L. M., & WILKIE, D. M. (1994). Time-of-day discrimination by pigeons, *Columba livia*. *Animal Learning & Behavior*, **22**, 143-154.
- SCHMIDT-KOENIG, K. (1960). Internal clocks and homing. *Cold Spring Harbor Symposia on Quantitative Biology*, **25**, 389-393.
- SCHMIDT-KOENIG, K., GANZHORN, J. U., & RANVAUD, R. (1991). The sun compass. In P. Berthold (Ed.), *Orientation in birds* (pp. 1-15). Basel: Birkhäuser.
- WALLRAFF, H. G. (1991). Conceptual approaches to avian navigation systems. In P. Berthold (Ed.), *Orientation in birds* (pp. 128-165). Basel: Birkhäuser.
- WILKIE, D. M., SAKSIDA, L. M., SAMSON, P., & LEE, A. (1994). Properties of time-place learning by pigeons, *Columba livia*. *Behavioral Processes*, **31**, 39-56.