The influence of temporal spacing on time–place discrimination

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The conditions under which circadian and interval-timing mechanisms are used in time–place discrimination were investigated. Rats earned the first daily meal by pressing a lever beginning 3.5 h after the start of the session and a second daily meal by pressing another lever. The second meal started 3.5 or 7 h (Experiment 1) or 0.75 or 1.75 h (Experiment 2) after the start of the first meal, using independent groups. In Experiment 1, approximately half of the rats used an interval-timing mechanism, and the other half used a circadian mechanism. In Experiment 2, the rats timed two intervals, one from the start of the session until the first meal and the other from the first to the second meal. A circadian mechanism is relevant to timing intervals in the range of 1.75–3.5 h, and an interval-timing mechanism can be used to time intervals from 0.75–7 h.

Time–place discrimination refers to the ability to acquire resources (i.e., food, mates, and prey) that are available under specific temporal and spatial contingencies. A variety of mechanisms have been identified and tested in the study of time–place discrimination. Nontiming strategies include alternation between two locations (Pizzo & Crystal, 2004a), a win–stay, lose–shift strategy (Pizzo & Crystal, 2004b), and an ordinal representation of the sequence in which locations provide reward (Carr & Wilkie, 1997). Two timing strategies that can be used to solve time–place discrimination are the use of an interval-timing mechanism and an endogenous circadian oscillator.

Carr and Wilkie (1998) investigated the role of an interval-timing mechanism in time–place discrimination. Each of four levers provided reward at one of four different times. Reinforcement was available for three groups of rats at each of the four locations for 4, 6, or 8 min. For example, lever pressing for one group at the four locations was available for 6, 6, 6, and 6 min, respectively, in 24-min daily sessions. The rats restricted the majority of their responses to the correct lever at the appropriate time. An analysis of the response rate distribution at each lever showed that the variability was constant. This meant that the spread of the distribution was proportional to the

interval being timed, suggesting that the rats were timing successive intervals during the session rather than one interval equal to the session length. These results are consistent with the use of an interval-timing mechanism.

Evidence for time–place discrimination based on the use of a circadian mechanism was documented by Biebach, Gordijn, and Krebs (1989). The chamber in which the garden warblers, *Sylvia borin*, lived had a central corridor attached to four adjacent rooms. Each of these rooms provided momentary access to food for a different 3-h interval of a 12-h session, each day. The birds learned to restrict most visits to each location when it provided food. An open-hopper session, in which food could be obtained from each location throughout a 12-h session, revealed that the birds continued to restrict their visits to the correct rooms at their previously rewarded times. This manipulation ruled out the possibility that the birds were searching randomly among all locations. Further evidence for the use of a circadian mechanism was found when access to the adjacent rooms was blocked for different 4-h segments followed by access to all locations (Krebs & Biebach, 1989). The birds continued to visit the locations at the previously trained times of day. In another study, a phase shift of the light cycle was conducted in order to determine whether the birds were timing an interval with respect to the light onset or whether they were utilizing an endogenous mechanism to visit the correct food location. Although the time of light onset was advanced, the birds continued to visit the appropriate location at the correct time of day, lending additional support for the use of a circadian mechanism (Biebach, Falk, & Krebs, 1991).

Studies investigating time–place discrimination suggest some factors that influence the use of these two timing strategies. Previous experiments have shown that when the temporal spacing between successive meals is large (e.g., 3–7 h), a circadian mechanism is utilized (Biebach et al., 1991; Mistlberger, de Groot, Bossert, & Marchant,

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1996). In contrast, when the temporal spacing is small (e.g., 4–16 min), an interval-timing mechanism is used (Carr & Wilkie, 1998; Pizzo & Crystal, 2004b). However, these studies have used different training procedures, manipulations, and dependent variables; consequently, these studies have not identified the conditions under which interval-timing and circadian mechanisms are employed in time–place discrimination. It is necessary to vary the length of time between successive meals to identify the conditions under which interval-timing and circadian mechanisms are used.

In the present study, we investigated the use of four temporal spacings between meals in a time–place discrimination task. In Experiment 1, two conditions were selected in the range of a few hours, because this range is expected to implicate a circadian mechanism (Biebach et al., 1991; Mistlberger et al., 1996). In Experiment 2, two other conditions were selected at approximately an hour, because this may be a point of transition from circadian to interval-timing mechanisms (Aschoff, 1984, 1985, 1989, 1993, 1998; Aschoff & Daan, 1997).

EXPERIMENT 1

In Experiment 1, we investigated the role of a circadian mechanism in time–place discrimination using large temporal spacings between successive meals. A circadian mechanism has been implicated in time–place discriminations involving meals separated by 3.5 h and by 7 h (Biebach et al., 1991; Mistlberger, 1996). However, documenting the use of a circadian mechanism, in the absence of any evidence for a nontiming strategy, appears to be dependent on minor procedural details (Pizzo & Crystal, 2004a). Therefore, to identify the role of temporal spacing in constraining the use of circadian versus interval-timing mechanisms, it is necessary to document circadian and interval-timing mechanisms using the same experimental paradigm. Consequently, in Experiment 1, we used conditions (3.5- and 7-h separations between meals) thought to implicate a circadian mechanism. In Experiment 2, we placed the meals closer to one another, in order to identify the temporal limits of circadian and interval-timing mechanisms.

Food was available when a different lever in an operant chamber was pressed during each of the two meals in daily sessions that started at a constant time of day. The first meal was available when one of the levers was pressed 3.5 h after the start of the session. The duration of each meal was 1 h. There was no consequence for pressing the incorrect lever during the meal. After the first meal, there was a period of time in which neither lever provided reward. Next, food was available when the other lever was pressed during the second meal. The second meal started either 3.5 h or 7 h after the start of the first meal for independent groups of rats.

The time–place discrimination could be solved by interval-timing, circadian, or alternation mechanisms. It is possible that the rats could also be searching both locations randomly in order to obtain food. During train-

ing, these cues were confounded; subsequent tests were used to unconfound these mechanisms. Baseline performance was compared with a random model of behavior in order to rule out random search behavior. A meal was occasionally skipped (i.e., omission testing) to dissociate timing and alternation mechanisms. For example, Meal 1 was skipped, and an assessment at Meal 2 (i.e., a nonreinforced probe) was conducted. If a rat was responding on the basis of an alternation strategy, and the last location that provided reward was Location 2, then the rat should respond at the alternate location (i.e., the location that usually provides food during Meal 1). This strategy does not require information about when to respond at a particular location, only to search the alternate location with respect to where food was previously obtained. Because our dependent measure was the proportion of correct responses with respect to time of day, low performance on the probe would be consistent with an alternation strategy. In contrast, high performance would be consistent with circadian and interval-timing mechanisms, because both time of day and time with respect to the start of the session are associated with the location that usually provides food during Meal 2. Finally, a circadian mechanism and an interval-timing mechanism were dissociated by conducting a phase shift of the testing time (i.e., by starting the session 3.5 h prior to the time at which the second meal normally occurred). If an interval was being timed with respect to the start of the session, the rats should approach the location that usually provides reward during the first meal. However, if the rats are discriminating time of day, responses should be allocated to the location that usually provides reward during the second meal.

Method

Subjects. Male Sprague-Dawley rats ($N = 16$, *Rattus norvegicus*; Charles River) were used. Eight rats were randomly assigned to each of the 3.5- and 7-h conditions. The rats were 63 days old at the beginning of the experiment, and they were housed in constant darkness. Dim red lights were present in the colony and testing rooms at all times. The rats were individually housed in clear plastic cages lined with bedding (The Andersons, Bed-o'-cobs, Maumee, OH). The rats were given food pellets (Noyes Precision pellets, Improved Formula A/I; Research Diets, Inc., New Brunswick, NJ) consumed during testing sessions and a ration of 5001 Rodent Diet (LabDiet, Brentwood, MO), which was adjusted so that the total daily intake was 15 g. Water was continuously available in the home cage and during testing. The average weight of the rats at the start of the experiment was 275 g.

Apparatus. The rats were tested in 16 operant chambers ($30.5 \times 24.1 \times 29.2$ cm; Med Associates, Inc., ENV-007-VP, Georgia, VT), each equipped with two retractable levers (ENV-112CMX, 4.8×1.9 cm) that were 6.4 cm above the floor with 11.5 cm between the nearest edges of the levers. The floor of the chamber consisted of 19 stainless steel rods (4.8 mm diameter, 1.6 cm spacing between them) with a stainless steel waste tray located beneath it. A pellet dispenser (ENV-203IRX), capable of dispensing 45-mg pellets, was positioned outside the testing chamber and attached to a food trough (ENV-200R2M) located between the levers. Inside the food trough, there was a photobeam (ENV-254). A water bottle attached to the outside of the chamber positioned across from the food trough supplied water through a tube inserted into the chamber. Each box contained a speaker (ENV-224AM, 8.0×11.0 cm), a relay (ENV-

135M), and a houselight (ENV-227M). Each chamber was placed in a ventilated sound-attenuated cabinet (ENV-016M, $66.0 \times 35.6 \times 55.9$ cm). Two Celeron computers (366 and 500 MHz) running Med-PC for Windows (Version 4.0) located in a nearby room controlled experimental events and recorded the time of each event with 10-msec accuracy. Efforts to minimize the effect of cues correlated with time of day included masking noise provided by the ventilation fans, the use of sound-attenuated chambers, eliminating access to the testing room during testing sessions, placement of control equipment and computers in a room separated from the operant chambers, and use of a temperature and humidity controlled environment.

Procedure. The rats were placed in the chambers daily, 7 days per week. Pretraining consisted of a session in which food was paired with a click every 60 sec, for 30 min. This was followed by two sessions in which the levers were inserted, and each leverpress produced a pellet and a click; after 10 presses on the left lever, the left lever was retracted and the right lever was inserted. The levers continued to alternate until 60 pellets were earned or 60 min had elapsed. The rats were randomly assigned to the testing chambers at the start of the experiment.

After pretraining, the time-place contingencies were introduced. At the start of the session, the levers were inserted into the chamber. This was followed by a 3.5-h period in which pressing either lever had no effect. The first meal was available 3.5 h after the start of the session for 1 h. Following the first meal, neither lever provided food for a period of time. The second meal was available 3.5 or 7 h after the start of the first meal for two groups of rats: the 3.5-h group and the 7-h group. The second meal was also 1 h in length. The rats obtained the first and second meals by pressing different levers; left and right levers were counterbalanced across rats. The levers were retracted, and the session terminated at the end of the second meal.

The rats were required to satisfy a fixed ratio (FR) in order to receive their first pellet of food at the start of each meal. Initially, the requirement was an FR1; the ratio was doubled until FR32 or FR64 was reached, thereby increasing the response cost; a high response cost promotes time-place discrimination (Widman, Gordon, & Timberlake, 2000; Widman, Sermania, & Genismore, 2004). Research in our laboratory has suggested that rats show inconsistent performance when the response cost is too high. Some rats temporarily stopped responding when the FR was increased. The FR was decreased if responding stopped. Consequently, the highest attainable FR was used for each rat. The highest requirement was FR64 ($n = 9$) and FR32 ($n = 2$); 5 rats did not reach either of these criteria. In each meal, food was delivered on a variable interval (VI) schedule of reinforcement after the initial FR.

The VIs that were in effect during the meals were adjusted so that food consumption was above the threshold for circadian entrainment. Stephan (1997) has estimated that the threshold of caloric intake that is necessary to entrain a feeding-entrainable oscillator is approximately 25.2 kcal, which is approximately 7 g of food pellets. The rats earned 7–10 g of pellets per meal. This amount varied on a daily basis. If a rat earned less than 200 pellets during a meal, the VI was decreased. At the end of training, the VIs during the two meals ranged from 1 to 14 sec.

Reinforcement during each meal was available by the rats' pressing the right lever or the left lever. The assignment of left and right levers to Meals 1 and 2 was randomly determined for each rat; this assignment was used for each rat throughout the remainder of the study. The start of each session occurred at a constant time of day. We carefully controlled the time at which the start of daily testing occurred using a high-precision clock (Atomic Moon Phase Clock, Model 86715). This ensured that time of day was consistently associated with food availability.

The rats were tested until 75% of the responses given during the completion of the FR were at the correct lever ($M = 114$ sessions). Upon achieving this criterion, meals were occasionally manipulated to identify the mechanism used to solve the time-place discrimina-

tion. First, omission testing was implemented by withholding food during a scheduled meal. Following each type of omission (i.e., omitted Meal 1 or omitted Meal 2), an unrewarded probe was conducted during the next regularly scheduled meal. All responses were recorded, and no food was available during these probes. First and second meals were omitted on three occasions. The occurrence of an omission test was randomized within a seven-meal block, with the restriction that omitted meals were separated by at least four meals in addition to the unrewarded probe at the next meal. When the amount of food earned in an experimental session was reduced by an omitted meal, supplemental feeding after the session was adjusted to maintain total daily intake at 15 g.

After omission testing was completed for all the subjects, the rats underwent phase shift testing. Since this manipulation occurred at different times according to the group assignment of the rats (3.5 or 7 h), subsets of rats were tested on different days. Additional days of retraining were conducted before implementing the phase shift for some rats if performance had declined. This test placed the two meal times in conflict with one another. For example, in the 7-h training condition, the first meal was available at 2000 h, and the second meal was at 0300 h. The test involved placing the rats in the chambers 3.5 h prior to their second meal (i.e., the session started at 2330 h). A nonrewarded probe occurred at the time when the second meal usually occurred. The phase shift test started at 2000 h for the 3.5-h group. During the phase-shift probe, all responses were recorded, and no food was available.

Data analysis. The data consisted of the number of lever presses at each location during the initial FR prior to the start of the meal (i.e., prior to receiving any food at each meal). A ratio describing proportion correct was calculated using these data (correct leverpresses divided by the sum of all lever presses prior to earning the first pellet). The other data presented are the distribution of responses during the first 3.5 h of the session.

The proportion can be described in terms of different hypotheses. All mechanisms (e.g., interval-timing, circadian, and alternation strategies) were confounded during baseline conditions. For example, performance of 80% during baseline denotes that 80% of the responses were correct with respect to each hypothesis. However, timing predictions (i.e., circadian and interval-timing hypotheses) are dissociated from the prediction of an alternation strategy during omission testing. Therefore, performance may be characterized as low with respect to one prediction and high with respect to another. For example, performance of 80% with respect to a timing hypothesis on omission probes corresponds to 20% correct with respect to an alternation strategy. The data are presented in the figures below as proportions correct with respect to timing hypotheses in meal omission tests and with respect to interval timing in phase-shift tests, using a single measure to prevent presentation of redundant information. However, for the purpose of statistical tests, the raw data for each subject were transformed accordingly to evaluate each hypothesis. Throughout this article, the criterion for statistical significance was $p < .05$.

Five subjects were excluded from the analyses. One subject showed a large bias toward one lever. One subject showed variable performance and was deemed not suitable to undergo the manipulations. Three subjects displayed high performance at Meal 1 ($\bar{x} = .84$, $SE = .07$) and Meal 2 ($\bar{x} = .77$, $SE = .07$) only after they were tested for 168 sessions; these rats were not included because they did not reach high performance until nearly the end of the experiment. One subject in the 7-h condition was not tested on the phase-shift probe because performance declined after omission testing.

Results and Discussion

Temporal anticipation of the first meal. Figure 1 shows the response rate at each lever as a function of time since the start of the session during the first 3.5 h from the

Figure 1. Response rate at the first rewarded location (correct lever) and second rewarded location (incorrect lever) plotted as a function of time since the start of the session. The top and bottom panels represent the 3.5-h group and the 7-h group, respectively. Note that the first meal started 3.5 h after the start of the session.

last 15 sessions of training (baseline). The top and bottom panels show the data from the 3.5- and 7-h groups, respectively. Response rate at both levers was low at the start of the session but gradually increased to reach a maximum just prior to the first meal. The data suggest that the rats anticipated the start of the first meal but did not discriminate its location during the initial 3.5 h of the session. We confirmed these observations by subjecting the data shown in each panel of Figure 1 to an ANOVA using lever (correct and incorrect) and time as repeated measures. This analysis was restricted to the first meal, because any discrimination made at the second meal could have been based on receiving reward during the first meal. The analysis for the 3.5-h group revealed no effect of lever [$F(1,5) < 1, p > .05$] but did show a significant effect of time [$F(41,205) = 9.08, p < .001$]. The interaction was not significant [$F(41,205) < 1, p > .05$]. The same conclusions were reached for the 7-h group [no effect of lever, $F(1,4) < 1, p > .05$; an effect of time, $F(41,164) = 2.08, p < .01$; and no interaction, $F(41,164) = 1.05, p > .05$].

Spatial discrimination. The proportion of correct responses (with respect to time of day) was calculated dur-

ing the completion of the terminal FR value at the start of each meal. The proportion correct was calculated using the last 15 sessions of acquisition. Average performance for the two groups was .80 and .85 for Meals 1 and 2, respectively. Mean performance for the groups is illustrated by the filled bars in Figure 2 (labeled "Baseline" in the figure).

Comparison of baseline with chance performance.

In order to determine whether the rats preferred the correct lever at the start of the meals, it is necessary to compare baseline performance with the level expected by chance. Chance performance is the result of an independent, random selection of levers ($p = .5$). Performance during the first meal was significantly different from chance for both the 7-h group and the 3.5-h group [$t(4) = 6.8, p < .01$; $t(5) = 7.6, p < .001$, respectively]. Performance during the second meal was also significantly different from chance for the 7- and 3.5-h groups [$t(4) = 17.51, p < .001$; $t(5) = 17.71, p < .001$]. This suggests that the rats discriminated spatial locations. Although there was no evidence for spatial discrimination in the anticipatory responses, there was evidence for spatial discrimination

Figure 2. Proportion of responses at the correct lever with respect to time of day during baseline training and omission probes. The top and bottom panels show performance for the 3.5-h group and the 7-h group, respectively. The dashed line represents chance performance; error bars represent SE.

in the proportion correct during the FR (i.e., immediately prior to earning the first pellet of the meals). These data suggest that the rats did not demonstrate their knowledge of the correct spatial location until it was precisely the time to do so; the FR was completed in 3.8 min, on average ($SE = 0.6$). The relatively fast completion of the FR indicates that there was an increase in response rate at 3.5 h. This response rate during the completion of the FR was 39.3 responses/min ($SE = 3.9$), which was statistically higher than the rate before the start of the meal [$t(10) = -9.3, p < .001$].

Difference between anticipation and proportion correct during the FR. Discrimination at the start of the meal (FR performance, Figure 2) but not earlier in the session (response rate performance, Figure 1) could be based on either of two hypotheses. According to the *noise hypothesis*, the rats were able to hear the food being dispensed in nearby boxes. Consequently, the majority of the rats would allocate a high percentage of responses to the correct lever during the FR; earlier in the session (i.e., before the meal was scheduled to start), noises from the pellet dispensers would not be available. According to the *timing hypothesis*, the rats had accurate estimates of the expected start time of the meal; the preference for the correct location was expressed at the start of the meal but not earlier in the session.

There are three lines of evidence that conflict with the noise hypothesis. First, sound levels were measured using

a digital sound level meter (No. 33-2055, RadioShack, Fort Worth, TX) inside one of the operant chambers while noise was created within adjacent chambers. The noise used for these tests was larger than the noise that occurred in the sessions (i.e., simultaneously dispensing food in all operant chambers other than the one in which the sound level was measured). Sound levels were also measured when food was not dispensed in any chamber. Note that each chamber was shut and enclosed within a sound-attenuated cabinet. There was no change in measured sound levels in these tests. This suggests that any noise created in one box was not likely to have been detected inside another box. Second, the performance of the first rat to complete the FR requirement each day was high ($\bar{x} = .85$; $SE = .09$). High performance by the first rat to complete the FR on each day occurred before any food pellets were dispensed. Third, performance during omission testing (as discussed in the next section) remained high during unrewarded probes. These data were collected in the absence of noises associated with food. In conclusion, these three independent lines of evidence are in conflict with the noise hypothesis; taken together, they lend support to the timing hypothesis. The rats appeared to have a precise estimate of time at which the meal was scheduled to occur. Prior to the meal, the rats did not express their knowledge about the correct location (Figure 1); at the start of the meal (but before any food had been delivered), the rats searched at the correct location (Figure 2).

Omission testing. Performance immediately prior to probes was not disrupted by previous manipulations [$t(10) = 1.29, p > .05$]. If the rats were using an alternation strategy, performance should have been low following an omitted meal when data were presented in terms of a time-of-day hypothesis. However, performance should have remained high if the rats were using a circadian or interval-timing mechanism. Figure 2 shows proportion correct with respect to time of day during baseline and omission probes for the 3.5- and 7-h groups (top and bottom panels). High performance was observed on the probes during both meals. A three-way ANOVA using condition (baseline and probe), meal (first and second), and group (3.5 and 7 h) was conducted on these data. The effects of conditions, meals, and groups were not significant [$F_s(1,9) < 1, p_s > .05$]. None of the interactions were significant [$F_s(1,9) < 1, p_s > .05$]. These results suggest that omitting a meal had no effect on the rats' performance. These results are consistent with the use of a timing strategy. The observed null effects were due to the presentation of the data with respect to time of day; this limitation was offset by transforming the data to measure performance with respect to an alternation hypothesis.

The omission probe data were transformed to express performance with respect to an alternation strategy and were subjected to a three-way ANOVA using the same conditions as in the analysis described above. This test revealed a significant difference between conditions [$F(1,9) = 127.0, p < .001$]. There was no difference between meals or groups [$F_s(1,9) < 1, p_s > .05$]. None of the interactions were significant [$F_s(1,9) < 1, p_s > .05$].

This analysis provides statistical support for the conclusion that performance in the omission tests was reliably different than behavior predicted by an alternation mechanism. The data are consistent with behavior based on an interval-timing or circadian mechanism.

Phase shift. Phase-shift performance is presented in terms of an interval-timing hypothesis. The average proportions correct during the phase shift probe for the 7- and 3.5-h conditions were .60 and .59, respectively. High performance on the phase shift would indicate that the rats pressed the lever associated with the first meal and is consistent with the use of interval timing. In contrast, low performance on the phase shift would indicate that the rats pressed the lever associated with the second meal and is consistent with discriminating time of day. The observed intermediate performance (i.e., between the predictions of interval-timing and circadian mechanisms) does not correspond to the predictions of either mechanism. Intermediate performance could indicate that individual rats approached each lever without expressing a preference. Alternatively, intermediate performance in the group average would also occur if half the rats performed according to circadian and half performed according to interval-timing mechanisms. In order to evaluate this possibility, we examined the performance of individual rats. Examination of individual subjects' performance revealed that 6 had high proportions, whereas 4 had low proportions. Since intermediate performance in the average did not reflect the individuals' performance, the subjects were subdivided into two groups: those that performed above .5 and those that performed below .5. Figure 3 shows the baseline and phase-shift performances of each of these groups in the 3.5- and 7-h conditions (top and bottom panels). Performance was compared with its prior baseline performance in terms of each mechanism for the data averaged across 3.5- and 7-h conditions. This means that each subject's phase-shift performance was expressed with respect to a circadian mechanism (to evaluate support for an interval mechanism) and with respect to an interval-timing mechanism (to evaluate support for a circadian mechanism) and was compared with performance during baseline. The group with performance below .5 on the probe showed low performance with respect to interval timing. This group's performance was significantly different from performance expected by an interval-timing hypothesis [$t(3) = 15.2, p < .05$]. In order to evaluate whether this group's performance differed from the prediction of a circadian mechanism, it was necessary to transform the data as described above. There was no difference between probe performance and the time-of-day hypothesis [$t(3) = -0.63, p > .05$]. Similar analyses were conducted on the performance of the group with performance above .5 on the probe. This group's performance was high when expressed in terms of interval timing. Probe performance was not significantly different from performance expected by the interval-timing hypothesis [$t(5) = -0.31, p > .05$]. Next, accuracy was expressed in terms of a circadian mechanism by transforming the data; performance was significantly different from perfor-

Figure 3. Proportion of photobeam breaks at the correct food trough during baseline training and omission probes for the groups that performed below 0.5 (circadian) and the groups that performed above 0.5 (interval) on the phase shift probe. The top and bottom panels represent the 3.5-h group and the 7-h group, respectively. The dashed line represents chance performance; error bars represent SE.

mance expected by the circadian hypothesis [$t(5) = -10.4, p < .05$]. The results were the same when the identical tests were conducted separately on the subgroups of the 3.5- and 7-h conditions. These results suggest that the group with performance above .5 was based on an interval-timing mechanism, and the group with performance below .5 was based on a circadian mechanism. Figure 4 shows the average response rate during the first 3.5 h for each subgroup described above; response rates were averaged across correct and incorrect levers. Response rate increased prior to the meal for both groups. This increase appears to have occurred closer to the meal in the circadian subgroup relative to that in the interval-timing subgroup. However, this difference was not confirmed by an ANOVA [$F(35,315) = 1.3, p > .05$].

Implications. In this experiment, we evaluated the mechanisms used in time-place discrimination using two large spacings between meals (i.e., 3.5 and 7 h). Response rate increased as a function of time prior to the start of the first meal. Performance during the first 3.5 h of the session did not reveal discrimination of spatial locations. However, performance during the initial FR suggests that the rats discriminated spatial locations at the start of the

Figure 4. Average response rate across both levers for the phase shift subgroups plotted as a function of time since the start of the session.

meal, prior to the delivery of the first pellet. The observation that performance in spatial discrimination changed immediately prior to the meals suggests that the timing mechanism is highly accurate. The variability of response rate functions has been widely used as a direct measure of temporal precision of short-interval timing. The results of the present experiment suggest that variability in response rate functions (Figure 1) may underestimate temporal precision (i.e., a two-alternative choice at the immediate but unsignaled start of the meal is a more sensitive measure than choice performance earlier in the interval; Figure 2).

Omission testing was conducted to determine whether the rats were using an alternation or a timing mechanism. The high performance during omission testing implies that the rats were not using an alternation strategy. Subsequently, a phase shift was conducted in order to determine whether the rats were utilizing an interval-timing mechanism or a circadian mechanism. Approximately half of the subjects utilized an interval-timing mechanism, and the other half used a circadian mechanism.

Experiment 1 provides information concerning the conditions under which interval-timing and circadian mechanisms are used in time-place discrimination. Circadian timing is associated with timing durations that are long, typically daily events separated by a few hours. The present experiment illustrates that a circadian mechanism can be used to make time-place discriminations that are separated by 3.5 and 7 h using an operant paradigm. In contrast, interval timing is associated with timing intervals in the seconds-to-minutes range. The results of Experiment 1 show that an interval-timing mechanism can be used to time 3.5 and 7 h in time-place discrimination.

Circadian and interval-timing cues were confounded during training because the handling cues and the time of testing remained constant. The task did not require that the rats use one mechanism or the other. In order to determine

which mechanism the rats were using, it was necessary to unconfound the available cues. As an alternative approach, training could be conducted in such a way that only one cue was available. For example, if sessions were started at randomly determined times during the day, while meals were fixed at the same time each day, this would make time of day the only reliable cue. Time-place discrimination in such a procedure could be solved only with the use of a circadian mechanism. Another possibility would be to start testing at randomly determined times during the day but to keep the meal fixed with respect to the start of the session. In such a procedure, the time-place discrimination could be solved only with the use of an interval-timing mechanism. Finally, under continuous testing, the only cue available would be the food obtained during the meals. This procedure would omit any handling cue that the rats may use. The results of Experiment 1 suggest that when both mechanisms are available, rats are equally likely to use circadian and interval-timing mechanisms. In Experiment 2, we investigated the temporal resolution of circadian and interval-timing mechanisms.

EXPERIMENT 2

The relatively long temporal spacings of the meals in Experiment 1 were chosen because previous time-place studies have provided evidence of circadian timing with similar conditions. Experiment 1 showed that circadian mechanisms could be used to time such intervals (i.e., 3.5 and 7 h). This is in agreement with other studies that have documented the use of circadian timing in time-place discrimination (Biebach et al., 1991; Mistlberger et al., 1996). It is possible that circadian mechanisms can be used to discriminate between events that are separated by smaller spacings, although the temporal limit of circadian discrimination is not known. Interval timing was also implicated in Experiment 1. Although interval timing is

often associated with timing intervals in the seconds-to-minutes range, Experiment 1 implicated interval timing of 3.5 and 7 h. It is likely that smaller intervals can be timed with the use of an interval-timing mechanism. These results set the stage for examining shorter intervals between meals, in order to identify the temporal constraints of circadian and/or interval-timing mechanisms in time-place discrimination.

In Experiment 2, we investigated temporal spacings above and below 1 h. Our reason for selecting these conditions relates to a study by Aschoff (1985), in which humans were housed voluntarily in temporal isolation and were asked to make judgments about time. The subjects lived underground and did not receive any time cues. They were required to signal when 1 h had passed. They were also required to time shorter intervals, in the range of 10–120 sec. The results of Aschoff's study revealed that there was a positive correlation between subjects' perception of 1 h and the length of their wake/sleep cycle. However, there was no correlation between their circadian cycle and the timing of shorter intervals. These results suggest that the timing of 1 h, but not shorter intervals, may be relevant to a circadian system. Evidence in support of circadian timing in the range of 1 h would challenge conventionally held views concerning timing with the use of this mechanism.

Interval timing has been associated with timing short intervals (e.g., seconds and minutes). This type of timing mechanism has a low level of precision (Gibbon, Fairhurst, & Goldberg, 1997), suggesting that it would be difficult to use for timing large durations. Evidence to support the use of an interval-timing mechanism around 1 h would provide further information regarding the range in which this mechanism can be used to time intervals.

Meals were available as in Experiment 1, except that the onsets of the meals were separated by 45 and 105 min in independent groups of rats. After baseline performance was established, a meal was occasionally skipped. Finally, a phase shift of the testing time was conducted.

Method

Subjects. Male Sprague-Dawley rats ($N = 30$, *Rattus norvegicus*; Charles River) were used in this experiment. Fifteen rats were randomly assigned to each of the 45- and 105-min groups. The rats were 63 days old at the beginning of the experiments. They were housed under the conditions described in Experiment 1. Each rat weighed approximately 251 g at the start of the experiment.

Apparatus. The equipment was the same as described in Experiment 1.

Procedure. The procedure was identical to the one used in Experiment 1, except as follows. The length of time between the onset of the first meal and the onset of the second meal was 45 min for half the rats, and it was 105 min for remaining rats. It was necessary to provide 30-min meals due to the close proximity of the two meals. Close proximity of the meals to one another might satiate the rats and reduce motivation to earn food during the second meal. Therefore, a maximum of 156 pellets (7 g) per meal was delivered. This amount is equal to the estimated threshold needed for entrainment of a feeding-entrainable circadian oscillator (Stephan, 1997). If a rat persistently earned less than 156 pellets because of a low response rate, the magnitude of the reward was adjusted (i.e., 2 pellets instead of 1 pellet). White noise (70 dB) was present throughout the session.

Omission testing was conducted after the rats showed high baseline performance. Probes were randomized within a 6-day block, with the restriction that probes were separated by at least 4 days. The rats underwent phase-shift testing after a minimum of 5 days of additional training following the omission tests. During phase-shift testing, the rats in the 45- and 105-min groups were started 45 and 105 min later than their usual training start time, respectively.

Data analysis. The data were collected in the same manner as described in Experiment 1. However, the data presentation was different. Instead of calculating the proportion of correct responses during the FR at the start of the meals (as in Experiment 1), we calculated a proportion of correct responses during a 5-min window prior to the start of the meals. The change in dependent variables was necessary because 3 subjects exhibited low response rates; these subjects did not reach the terminal FR values of 32 or 64. Accuracy based on an FR consisting of 16 or fewer responses is highly variable. In order to include these subjects in the analysis, it was necessary to measure performance over a larger time window. Therefore, performance was assessed using the last 5 min before each meal. The average proportions correct based on the FR for the subjects that attained a high FR for Meal 1 ($M = .75$; $SE = .02$) and Meal 2 ($M = .74$; $SE = .03$) were comparable to the proportions correct for all subjects based on the 5-min window prior to Meal 1 ($M = .74$; $SE = .02$) and Meal 2 ($M = .77$; $SE = .02$).

Fourteen subjects acquired the task (i.e., .70 or higher on both levers). Three subjects were excluded due to mechanical failures in the testing equipment. Three subjects showed a large bias toward one lever. Ten subjects were excluded from the study because they displayed low performance on the task. One subject from the 45-min condition and 2 subjects from the 105-min condition were not tested on the phase-shift probe due to poor performance at the end of the experiment. Although some of the attrition of the subjects was due to technical difficulties, it appears that the task was difficult for the rats.

Results and Discussion

Temporal anticipation of the first meal. Figure 5 shows the response rate at each lever as a function of time since the start of the session during the first 3.5 h from the last 15 sessions of baseline. The top and bottom panels display data from the 45- and 105-min groups, respectively. Response rate at both levers was low at the start of the session but increased to a maximum just prior to the meal. The response rate at both levers was approximately the same until about two thirds into the interval. The response rate was larger at the correct lever prior to the meal. ANOVAs were conducted on the data shown in Figure 5 using lever (correct and incorrect) and time as repeated measures. The analysis for the 45-min group revealed an effect of lever [$F(1,7) = 23.3$, $p < .01$], an effect of time [$F(41,287) = 16.5$, $p < .001$], and a significant interaction [$F(41,287) = 15.0$, $p < .001$]. The same conclusions were reached for the 105-min group [an effect of lever, $F(1,5) = 47.5$, $p < .01$; an effect of time, $F(41,205) = 44.4$, $p < .001$; and a significant interaction, $F(41,205) = 29.1$, $p < .001$]. The significant interaction reflects the increase in response rate at the correct lever, relative to that at the incorrect lever. Figure 5 suggests that the rats anticipated the start of the meal and correctly discriminated its location.

Spatial discrimination. The proportion of correct responses (with respect to time of day) was calculated using the responses in the 5 min prior to the start of the meal. Acquisition proceeded until high performance was observed in this measure. The proportion correct was calculated

Figure 5. Response rate at the first rewarded location (correct lever) and second rewarded location (incorrect lever) plotted as a function of time since the start of the session. The top and bottom panels represent the 45-min group and the 105-min group, respectively. Note that the first meal started 3.5 h after the start of the session.

using the data from the last 15 sessions of acquisition. Average performances of the two groups were .74 and .77 for Meals 1 and 2, respectively. Mean performance for both groups is illustrated by the filled bars in Figure 6 (labeled “Baseline” in the figure).

Comparison of baseline with chance performance. Baseline performance was compared with the level expected by chance ($p = .5$). Performance during the first meal was significantly different from chance for both 45- and 105-min groups [$t(7) = 12.6, p < .01$; $t(5) = 8.4, p < .05$]. Performance during the second meal was also significantly different from chance for the 45- and 105-min groups [$t(7) = 9.4, p < .05$; $t(5) = 7.5, p < .05$]. This suggests that the rats discriminated the spatial locations.

Omission testing. The use of an alternation strategy should produce low performance following an omitted session when data are presented in terms of a time-of-day hypothesis. However, performance should remain high if the rats were using either a circadian or an interval-timing mechanism. Figure 6 shows performance in terms of time of day during baseline and omission probes for the 45- and 105-min groups. Performance during the probe at the first meal was high and comparable to baseline performance. In contrast, performance during the probe at the second meal was lower than baseline. A three-way ANOVA using condition (baseline and probe), meal (first and second), and

group (45 and 105 min) revealed no difference between groups [$F(1,12) = 0.15, p > .05$] but did reveal a significant difference between meals [$F(1,12) = 25.3, p < .001$] and conditions [$F(1,12) = 13.3, p < .01$]. The interaction of meal and condition was significant [$F(1,12) = 69.3, p < .001$]. The interaction of meal and condition reflects the fact that the difference between probe and baseline performance during the second meal was larger than in the first meal. None of the other interactions were significant [$F_s(1,12) < 1, p_s > .05$]. Because the three-way interaction was not significant, the data from both groups were pooled. The t tests revealed that there was no difference between probe and baseline performance at Meal 1 [$t(13) = -0.56, p > .05$]. However, there was a significant difference between probe and baseline performance during Meal 2 [$t(13) = 6.5, p < .05$]. Probe performance was significantly different from chance for Meal 1 but not for Meal 2 [$t(13) = 6.9, p < .05$; $t(13) = -2.3, p > .05$]. Moreover, for Meals 1 and 2, the probe performance was significantly different from alternation and ordinal predictions [$t(13) = -13.7, p < .01$; $t(13) = -7.4, p < .05$].

The data suggest that the rats were not alternating with respect to food obtained during the meals; alternation predicts low proportions on both omission tests. The data suggest that a circadian mechanism was not used at both meals; a circadian mechanism predicts high proportions on both omission tests. The data also suggest that the rats were not

Figure 6. Proportion of photobeam breaks at the correct lever during baseline training and omission probes. The top and bottom figures show performance for the 45-min group and the 105-min group, respectively. The dashed line represents chance performance; error bars represent SE.

timing a single interval with respect to the start of the session to get to each meal; timing a single interval predicts high proportions on both omission tests. Finally, the data were also not consistent with an ordinal timing hypothesis; an ordinal-timing mechanism predicts baseline level performance for Meal 1 and below-chance performance for Meal 2. These results were consistent with behavior based on an interval-timing strategy that times two intervals: one from the start of the session until Meal 1 and another interval from the first meal to the second meal.

Phase shift. Proportion correct during the phase shift is presented in terms of an interval-timing hypothesis. High performance on the phase shift would indicate that the rats pressed the lever associated with the first meal and is inconsistent with the use of interval timing. In contrast, low performance on the phase shift would indicate that the rats pressed the lever associated with the second meal and is consistent with discriminating time of day. Figure 7 shows the baseline and phase-shift performance. These data are presented as proportion correct in terms of an interval-timing hypothesis. A 2×2 ANOVA comparing condition (baseline vs. phase-shift probe) and group (45 vs. 105 min) was conducted on these data. This analysis revealed no significant differences between group [$F(1,9) = 2.2, p > .05$] or condition [$F(1,9) < 1, p > .05$]. There was no interaction between condition and group [$F(1,9) < 1, p > .05$]. These results suggest that performance during the probe was not different from the prediction of an interval-timing

mechanism. In order to contrast the data with the alternative hypothesis, it was necessary to transform the data from each rat in terms of a circadian timing hypothesis. A 2×2 ANOVA comparing condition and group was conducted on this transformed data. This revealed no significant difference between groups [$F(1,9) < 1, p > .05$]. However, there was a significant difference between conditions [$F(1,9) = 44.5, p < .001$]. There was no interaction between group and condition [$F(1,9) = 2.2, p > .05$]. These results suggest that probe performance was significantly different from what is predicted by a circadian mechanism. The data suggest that the rats used an interval-timing mechanism to obtain food during the first meal.

Implications. In this experiment, we evaluated the mechanisms used in time–place discrimination using two small spacings between meals (i.e., 45 and 105 min). The analysis of response rate during the first 3.5 h of the session suggests that the rats were timing the interval from the start of the session until the first meal (Figure 5). In order to determine whether the rats were using an alternation or a timing mechanism, omission testing was conducted following acquisition. Performance was high after the second meal was skipped. However, performance was disrupted when the first meal was skipped. The results of both of these probes imply that a circadian mechanism was not used to solve the time–place discrimination. It also suggests that the rats were not timing one interval with respect to the start of the session in order to get to both meals. These data suggest that the rats were timing two intervals: The first interval was timed with respect to the start of the session, and the second interval was timed with respect to the first meal. A subsequent phase shift provided further evidence that the rats were using an interval-timing mechanism in order to get food during the first meal. The rats utilized an interval-timing mechanism to get food at both of the meals by timing two intervals.

Experiment 2 provides information regarding the conditions under which circadian and interval-timing mechanisms are used in time–place discrimination. The data suggest that a circadian mechanism apparently cannot be used to make time–place discriminations that are separated by 45 and 105 min using the present method.

GENERAL DISCUSSION

Experiments 1 and 2 provide information about the conditions under which circadian mechanisms are used in time–place discrimination. The experiments suggest that a circadian mechanism is used under a wide range of conditions. Although a circadian mechanism has traditionally been used to describe events that are separated by a day, this mechanism can also be used to distinguish daily events that are separated by a relatively small amount of time. Experiment 1 suggests that circadian timing can be used to time several hours. This is consistent with evidence documented in other studies implicating a circadian mechanism for similar intervals (Biebach et al., 1991; Mistlberger et al., 1996). However, the conditions

Figure 7. Proportion of photobeam breaks at the correct food trough during baseline training and phase shift probe for the 45-min group (top panel) and the 105-min group (bottom panel). The dashed line represents chance performance; error bars represent SE.

tested in Experiment 2 (i.e., 45 and 105 min) suggest that a circadian mechanism is not used to time approximately 1 h. This is in contrast to reports by Aschoff (1984, 1985, 1989, 1993, 1998; Aschoff & Daan, 1997), which have suggested that a circadian mechanism is relevant to timing 1 h. The resolution of a circadian timing mechanism based on the present work is between 1.75 and 3.5 h.

Experiments 1 and 2 also provide information about the conditions under which interval-timing mechanisms are used in time-place discrimination. An interval-timing mechanism is implicated in timing intervals in the range of seconds to minutes (e.g., Gibbon, 1991). However, the present study has documented the use of this mechanism on a larger scale. Interval timing was implicated in all of the experimental conditions in Experiments 1 and 2. This implies that an interval-timing mechanism can be used to time intervals from 0.75 to 7 h. This raises the question, what is the limitation of interval timing? A fundamental characteristic of an interval timer is that the variability of its estimate increases in proportion to the to-be-timed interval (Gibbon, 1991)—that is, as the interval between meals increases, the time-place discrimination is less likely to be solved with the use of an interval-timing mechanism. Because a circadian mechanism is more precise, the closer the interval is to 24 h, the more likely a circadian mechanism is used to time the interval. The point of transition from an interval-timing mechanism to a circadian mechanism is between 7 and 24 h. This would suggest that an interval-timing system has a broader training range than previously proposed by Gibbon et al. (1997).

The differences observed in the anticipation functions between Experiments 1 and 2 are noteworthy. In Experiment 1, anticipation functions document that the response rates at the correct and incorrect levers did not diverge during the first 3.5 h of the session (see Figure 1). However, at the start of the meal, the rats expressed a preference for the temporally correct location before the first pellet was delivered. In contrast, response rate at the correct lever was higher than at the incorrect lever for more than 1 h before the first meal in Experiment 2 (see Figure 5). In summary, the rats in Experiment 1 demonstrated a rapid change in behavior at the precise time at which food was scheduled to be available; there was no evidence for such precision in Experiment 2. The differential precision in anticipating the first meal may be explained by the use of a circadian mechanism in Experiment 1 and an interval-timing mechanism in Experiment 2. The rationale for this proposal is outlined below. The temporal precision is high for circadian timing relative to interval timing (Gibbon et al., 1997). During training, both circadian and interval cues were available to solve the time-place discrimination. The phase shift in Experiment 1 suggests that the rats were equally likely to use interval-timing or circadian mechanisms when these cues were dissociated. The phase shift does not identify a subset of "circadian" rats that are missing an "interval" ability, in contrast to a subset of "interval" rats. The phase shift suggests that rats may use both mechanisms when both are available, and when given a forced choice to use one or the other, an equal number used circadian and

interval-timing mechanisms. Consequently, the use of a circadian mechanism, a highly precise timing mechanism, may have rendered interval timing irrelevant in baseline training. According to this proposal, the lack of divergence in the anticipatory functions in Figure 1 occurred because the rats were not using an interval-timing mechanism. The observation that the rats expressed knowledge of the correct location at precisely the right time (i.e., at the start of the meal) suggests that the rats used a circadian mechanism. Therefore, given the availability of a high-precision circadian mechanism, the rats did not use the less precise interval-timing mechanism during the 3.5-h interval before the first meal. In contrast, in Experiment 2, the rats were apparently unable to use a circadian mechanism when the meals were placed in close proximity to each other. The use of a circadian mechanism in Experiment 1 but not in Experiment 2 suggests that any remaining, uncontrolled stimuli that are correlated with time of day were not responsible for the observed circadian performance in Experiment 1 because these cues, presumably, would have been present in both experiments.

The results of Experiment 1 raise the question, why did the rats in Experiment 2 not withhold their spatial preference until the start of the meal as in Experiment 1 (note that the rats began to display a spatial preference a little more than 1 h before the meal in Experiment 2)? The results of Experiment 2 provide no evidence to support the use of a circadian mechanism when meals were close together. This suggests that the close spacing of meals in Experiment 2 rendered a circadian mechanism inoperative (i.e., beyond the temporal resolution of a circadian mechanism). This is the reason there was not a rapid transition from a low rate to a high rate as in Experiment 1. In the absence of a more precise circadian mechanism, the rats relied on the less precise interval-timing mechanism, according to this proposal. The low-precision interval-timing mechanism did not support the rapid change in behavior in Experiment 2; consequently, the rats in Experiment 2 began to express a preference for the correct location more than 1 h before the start of the meal. Note that the interval between the start of the session and the first meal was constant (3.5 h) for all groups in Experiments 1 and 2. Therefore, the temporal placement of the second meal, near or far, from the first meal determined the mechanism used to anticipate the first meal and the precision of this anticipation.

Since food takes time to consume (60 and 30 min in Experiments 1 and 2, respectively), there are constraints inherent in the procedure for assessing the temporal limits of circadian and interval-timing mechanisms. For example, it was necessary to separate the meals in time in order for the rats to consume the amount of food needed for entrainment. It is possible that if meals were placed closer together than they were in the present study, rats would not be able to consume enough food to entrain a circadian oscillator. This concern is unlikely to limit the conclusions of the present work, because precautions were taken to ensure that each rat obtained a minimum of 7 g per meal in Experiments 1 and 2.

One question that remains to be answered relates to how the temporal and spatial contingencies in these tasks are acquired. The training involved in Experiments 1 and 2 was extensive. The rats had considerable training ($M = 153$ sessions) before they exhibited a high level of performance on the task. During training, we observed that temporal discrimination was expressed prior to spatial discrimination (i.e., response rates increasing as a function of time before the first meal without a high proportion correct early in training; data not shown). Similar evidence has been documented in other time–place studies. Thorpe, Bates, and Wilkie (2003) showed evidence that rats acquired either a spatial or a temporal discrimination in a battery of time–place tasks using a T-maze, a radial-arm maze, a place preference box, and a Morris water maze. They found no evidence that rats discriminate time and place within the same task. Their results and those of Experiments 1 and 2 are contrary to a theory proposed by Gallistel (1990) regarding what is encoded into memory when significant biological events occur. Gallistel proposed that when an event occurs, animals store in memory the nature of the event, when the event occurred, and where the event took place. The animal consults this memory at a later time to determine which events are predicted by other stimuli (i.e., Pavlovian conditioning). To illustrate the use of this memory code, consider the contingencies under which food was available for the rats in Experiment 2. When food was available during the first meal, the rats encoded that “food” was available on the “left lever” at a particular “time of day.” All that the rats needed to do in the future to obtain food was to remember these three components. Because time and place are automatically encoded into memory with each experience, rats should learn when and where these events occur at the same rate. The results of Experiments 1 and 2 suggest that the rats had knowledge of the temporal component of the task prior to exhibiting any knowledge of the spatial component of the task. This result was not predicted by Gallistel’s proposal.

Thorpe et al. (2003) argued that separate event codes are used to encode time and place in time–place discrimination. This proposal is consistent with the observation that their rats learned either the temporal or the spatial components (but not both) of their task. This proposal is also consistent with the observation that the rats in Experiments 1 and 2 acquired the temporal discrimination prior to learning the spatial discrimination. Thorpe et al. also suggested that the reason that their animals did not acquire both components in time–place discrimination relates to the low response cost in their paradigms. Responses cost (i.e., making a choice effortful) promotes time–place discrimination (Widman et al., 2000). Thorpe et al. proposed that a high response cost is necessary to encode both time and place. The rats in Experiments 1 and 2 of the present study were required to press the correct lever 32–64 times at the start of the meal to obtain reward. Thus, a relatively high response cost was implemented in the task. The results of Experiments 1 and 2 suggest that extensive

experience is needed to acquire these tasks in addition to a high response cost. Thorpe et al. trained rats for different lengths of time in a variety of tasks. Tasks that required a time and a place component were trained with as little as 24 sessions and as many as 85 sessions. They showed evidence that rats were discriminating time of day in a go–no-go procedure. However, this result was obtained by training the rats for 160 sessions. This is comparable to the number of sessions of training that the rats had in Experiments 1 and 2. It should be noted that their go–no-go procedure was conducted in a T-maze in which spatial discrimination was not necessary. In their procedure, reward could be obtained from both locations at the rewarded time of day when it was available and from neither location when it was not available. In conclusion, the information from these studies suggests that rats encode the spatial and temporal components of the task independently.

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