AN ORDINAL TIMING SYSTEM

by

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We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March 1996

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ABSTRACT

In this series of Experiments we examined the behaviour of laboratory rats in a daily time-place learning task. The rats received two daily sessions (one at 09:30, and a second at 15:30) in a large, clear, test chamber. A lever was mounted on each of the four chamber walls. Each rat could work for food on one lever during 09:30 sessions and on a different lever during 15:30 sessions.

Over the course of Experiment 1 the rats clearly learned which lever would provide food during 09:30 and 15:30 sessions. In Experiment 2, we examined the affect skipping 09:30 and 15:30 sessions had on the rats' time-place behaviour. In the 09:30 sessions which followed a skipped 15:30 session the rats continued to expect food at their 09:30 levers. However, in the 15:30 sessions which followed a skipped 09:30 session the rats incorrectly expected food at their 09:30 levers. These results suggest that; (1) receiving a 09:30 session, and not the passage of time, was necessary for the rats to anticipate the location of food in 15:30 sessions, and (2) receiving a 15:30 session was not necessary for the rats to anticipate the location of food during 09:30 sessions.

These results suggest that the rats' learned to press one lever during their first session of each day and to then press a second lever during their second session of each day. We called this a *daily route strategy*. As the rats' time-place behaviour was not disrupted when 15:30 sessions were skipped, some event other than 15:30 sessions must have been capable of resetting the rats route each day.

In Experiment 3 we determined where the rats expected food during probe sessions at 11:45, 13:00, and 14:15. At 11:45 the rats mainly pressed their 15:30 levers. This is also consistent with the use of a daily route strategy. However, at 11:45 the rats pressed their 15:30

levers relatively less, and they pressed the two levers which never provided food relatively more, than they did during baseline 15:30 sessions. This effect was also evident in the probe sessions at 13:00 and 14:15, but it's magnitude decreased the closer the probe session was in time to 15:30. This result suggests that a second timing system had weak, but detectable, control over the rats' time-place behaviour.

In Experiments 4a&b we demonstrated that the rats did not solely rely on the daily transitions of the colony light-dark cycle to reset their route each day. Additionally, in Experiment 4c we demonstrated that the rats did not solely rely on the occurrence of either the transitions of the colony light-dark cycle or a 15:30 session to reset their daily routes. Later, Experiment 5 showed that the occurrence of a 15:30 session was not even sufficient for the rats to reset their daily routes. We suggest that the rats reset their daily routes when a food-entrained circadian phase timer attained some fixed phase angle each day.

We also propose that the daily route employed by the rats in the present time-place learning task is an exemplar of *ordinal timing* -- the knowledge of the order of a set of events within a period of time. We contrast the temporal information provided by ordinal timing with that provided by the more well known phase and interval timing. We then suggest that ordinal, phase, and interval timing provide animals with representations of time at the ordinal, interval, and ratio levels of measurement respectively. Finally, we suggest that animals posses these three timing systems because each system is specifically adapted to enable animals to anticipate a specific type of spatiotemporal regularity.

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Introduction

Many animals have prey, mates, or predators whose locations in *space* vary predictively over *time*. Animals that were able to exploit moving resources or predators efficiently would have attained a fitness advantage over conspecifics who lacked this ability. Consequently, I suspect that many animals posses cognitive mechanisms that enable them to abstract, anticipate, and exploit, biologically important events that display spatiotemporal regularity. To examine this idea, laboratory and field work has been conducted to examine if, and how, animals track food availability as it varies in space over time. This ability is called *time-place learning* (for a review of this concept see Wilkie (1995)). This thesis examines time-place learning in the laboratory rat. The long-term goal of this research is to develop a model system for the investigation of the timing systems that control time-place behaviour.

There is a growing body of field evidence of time-place learning in birds. Rijnsdorp, Daan, and Dijkstra (1981) studied kestrels' daily hunting activity. These birds prey mainly on voles whose surface foraging trips display a pronounced, roughly 2 hr, ultradian rhythm. Rijnsdorp et al. found that kestrels restricted their hunting flights to the times-of-day when vole surface activity was at its highest. They also found that when a kestrel was successful hunting in an area, it tended to return and hunt in that area 24 hr later. Daan and Koene (1981) studied oystercatchers' foraging flights from their inland roosts to nearby tidal mudflats. Although these birds could not see the flats from their roosts, they flew to the flats just before the mussels were exposed by low tide. This observation suggests that the oystercatchers tracked the tidal cycle. Other work has demonstrated time-place learning in invertebrates. For example, Wahl (1932, as cited in Gallistel, 1990a) trained bees to visit one feeding station from 09:00 to 10:30 and to visit a second station from 15:30 to 17:00.

Recently Wilkie, Carr, Siegenthaler, Lenger, Liu, and Kwok (in press) found that a variety of generalists foragers including pigeons, gulls, and crows flocked in anticipation of a reoccurring mid-day increase in the amount of food discarded by humans at two outdoor locations. This finding suggests that the ability to track the location of food over time is not restricted to specialist foragers. Taken together, these field studies suggest that time-place learning may play an important role in the foraging activities of a wide variety of animals.

Timing in Laboratory Animals

A great deal of laboratory work has examined timing in animals. This work suggests that animals posses at least two different classes of endogenous timing systems: *interval timers* and *circadian phase timers*. These two timing systems provide animals with *two different types* of temporal information (Church, 1984; Aschoff, 1989; Gallistel, 1990a; Wilkie, 1995).

Interval Timers

Much work has demonstrated that rats and pigeons posses a remarkably precise ability to anticipate events which tend to occur a fixed amount of time *after some external event*. For a review of this literature see Gallistel (1990a, chapter 9). The classic example of this form of timing is the temporal control exhibited by animals responding on fixed interval schedules ranging in length from a few seconds to many hours (Ferster & Skinner, 1957). The formal properties of this form of timing are well described by Scalar Timing Theory (Gibbon, 1991). In brief, when estimating temporal intervals of different lengths animals tend to time a stable, animal-unique, proportion too fast or too slow, and the standard deviation of their timing estimates is a fixed proportion of the length of the interval being timed.

Information processing and connectionist models of interval timing have been developed (Church & Brodbent, 1990). In the information processing model, the passage of time is

captured by the accumulation of pulses emitted by a pacemaker. In the connectionist model, the time of the beginning and end of an interval is represented by recording the status of a bank of endogenous oscillators of ranging periodicities. The length of the interval is then calculated by subtracting the time at the end of the interval from the time at which the interval began.

Significant external cues control the operation of interval timers as they stop, reset, and restart timing (Roberts & Holder, 1984; Holder & Roberts, 1985). These characteristics give interval timers stopwatch-like properties (Wilkie, Saksida, Samson & Lee, 1994).

Circadian Phase-Timers

Other work suggests that animals posses one or more circadian (24 hr) timing systems. For example, a wide variety of animals use a circadian timing system to anticipate daily meals (Mistleberger, 1994), and to control their daily sleep-awake cycle (Binkley, 1990). Other work has shown that bees (Gould, 1980), desert ants, (Wehner & Lanfranconi, 1981) and homing pigeons, (Keeton, 1969; Foa & Saviozzi, 1990) use a circadian clock to perform sun-compass navigation. A third line of work has shown that regardless of the initial time of training, a rat's tendency to display previous learning is maximal 12, 24 and 36 hr post-training (Wansley & Holloway, 1975; Holloway & Wansley, 1973). Many workers have suggested that the circadian timing inherent in these behaviours is provided by the phase angle of circadian biological rhythms or their associated pacemakers (Aschoff, 1989; Crystal, unpublished manuscript; Daan & Koene, 1981; Enright, 1970; Gallistel, 1990a; Stephan & Kovacevic, 1978; and Mistleberger, 1994). This type of timing is therefore referred to as *circadian phase-timing* (Church, 1984; Gallistel, 1990a).

In both invertebrates and vertebrates a wide variety of physiological and behavioural variables vary with a 24-hr periodicity (for reviews see Aschoff, 1989; Mistleberger & Rusak,

1994; Hall, 1995; Jacklet, 1985; Turek, 1985). Certain exogenous cues (Zietgebers) with a roughly circadian periodicity adjust, or entrain, endogenous oscillators within the circadian timing system so that each oscillator has the same periodicity as it's entraining cue. Two major Zietgebers are the light-dark cycle (LD cycle) and periods of food availability. Each of these Zeitgebers appears to entrain a separate circadian pacemaker (Rosenwasser & Adler, 1986; Meijer & Rietveld, 1989; Mistleberger & Rusak, 1994; Mistleberger, 1994). Circadian oscillations (e.g., the sleep-awake cycle) always attain the same phase relationship with their entraining cue. This ensures that a given phase angle of an endogenous oscillator always coincides with the same phase angle of it's exogenous entraining oscillator. When a Zietgeber is removed (e.g., an animal is held in constant light (LL)), endogenous oscillators persist but often attain a periodicity slightly different from 24 hr. This self-sustaining rhythmicity is taken to reflect the inherent periodicity of the circadian pacemaker(s) driving the oscillatory system. When in this state, an oscillator is said to be in free-run. If a free-running oscillator has periodicity less than 24 hr it will reach a certain phase angle progressively earlier each day. Conversely, if a free-running oscillator has a periodicity greater than 24 hr it will reach a certain phase angle progressively later each day. If a Zietgeber is phase advanced (occurs earlier each day), or phase delayed (occurs later each day), the oscillator(s) it controls drift in the direction of the phase shift and re-entrain to the Zietgeber. See Binkley (1990) for a more detailed treatment of entrainment.

Although the mechanisms envisioned vary depending on the type of circadian phase-timing (see Gallistel, 1990a; Mistleberger & Marchant, 1995), all phase timing models propose that the circadian temporal information inherent in circadian phase-timing is provided by the phase angle of an entrained endogenous circadian oscillator, or it's associated pacemaker.

Laboratory Demonstrations of Daily Time-Place Learning

Laboratory time-place learning tasks examine if, and how, animals exploit a food source that varies in space over time. In *daily time-place learning* tasks the location of food depends on the time-of-day. For example, animals might be required to exploit a food source that is available at one location at 09:30 and at a second location at 15:30.

Daily time-place learning has been extensively studied in birds. Biebach, Gordijn and Krebs (1989) studied daily time-place learning in garden warblers. The birds lived in a large apparatus which consisted of a central area and four surrounding rooms. Lighting was provided on a 12:12 LD cycle. Food was available in each of the four surrounding rooms for one 3 hr interval during the 12 hr light period. Food was available in the rooms in the same order every day. For example, each day a given bird might be able to obtain food in Room 1 from 0600 to 0900, in Room 2 from 09:00 to 12:00, in Room 3 from 12:00 to 15:00, and in Room 4 from 15:00 to 18:00. The garden warblers quickly learned to enter the correct room at the correct time-of-day. The birds maintained this pattern of room entries on a test day when food was made available in all the rooms throughout the entire day. This result suggests that their room entries were controlled by an endogenous timing mechanism.

The warblers' pattern of room entries also persisted the day after they were switched to a LL cycle and food was made available in all four rooms throughout the entire day (Biebach, Falk & Krebs, 1991). Over the next few days the warblers' pattern of room entries attained a periodicity of 23 hr. This caused the warblers to enter each room progressively earlier each day. This result strongly suggests that the warblers' room entries were phase locked to a circadian phase timer that was going into free-run.

In another experiment Biebach et al. (1991) phase advanced light onset by 6 hr (i.e., lights came on 6 hr early and went off 6 hr early). This manipulation caused a partial shift (mean of 2.6 hr) in the birds' pattern of room entries on the next day. Over the next few days their pattern of room entries gradually advanced by 6 hr. This result suggests that the warblers' time-place behaviour was controlled by a circadian phase timer re-entraining to the new LD cycle.

Saksida and Wilkie (1994) investigated daily time-place learning in pigeons. They adopted a different procedure than Biebach et al. (1989). Pigeons were transported to a large, clear, square testing chamber twice per day; once between 09:00 and 10:00 and at second time between 15:30 and 16:30. The birds were well orientated in space as a number of salient distal spatial cues were visible from within the chamber. A pecking key was mounted on each of the four chamber walls. Pecks to one key produced grain in morning sessions and pecks to a different key produced grain during afternoon sessions. Pecks to the other two keys were recorded but had no consequences. Each session began with a brief period of variable length during which key pecks were recorded but no food was available. Anticipatory key pecks during this period were used to infer where the pigeons *expected* food. The pigeons learned to peck the correct key in morning and afternoon sessions. When either morning or afternoon sessions were skipped, the birds still anticipated the location of food during the following session. This rules out a strategy based solely on alternating between the two keys which provided food and suggests instead that the pigeons were using some form of endogenous timing mechanism.

Saksida and Wilkie performed various lighting manipulations similar to those employed by Biebach et al. (1991). The results of these manipulations suggested that their pigeons also used a circadian phase timer to track the location of food over the course of a day. For

example, placing the birds in LL initially had no effect on their performance. However, over the next few days their ability to anticipate the location of food in the morning and afternoon sessions gradually declined. Similarly, a 6-hr phase advance of the colony LD cycle caused a small decline in their performance on the first day. Accuracy then declined over the next six sessions.

Despite the clear evidence of daily time-place learning in birds there is little published evidence of this behaviour in mammals. This is surprising as most of my knowledge of the formal properties, and neurobiology, of circadian rhythms is based on the study of laboratory rodents. Daan, Leiwakabessy, Overkamp, and Gerkema (1994) outlined some unpublished work using house mice (C57B1). They used a procedure similar to that of Biebach et al. (1989). Mice lived in a central cage connected to four outer cages. Each of the outer cages contained a running wheel. Wheel revolutions in each room only produced food during one 3-hr interval of the light period. Food was available in the cages in the same order every day. The mice learned to enter each room at the correct time-of-day. When their lighting was switched from LD to LL, and food was made available in all the rooms throughout the entire day, the mice's pattern of room entries initially conformed to the usual pattern of food availability. Their room entries then gradually fell out of phase over the next 5 days. This result strongly suggests that their room visits were controlled by a circadian phase timer that was going into free-run.

Boulos and Logothetis (1990) examined time-place learning in the laboratory rat. The rats lived in circular light-tight chambers. Food was provided at one lever at one time-of-day and at a second lever at a second time-of-day. Intact rats and rats with ablated suprachiasmatic nuclei (SCNX) were tested in LL and LD. Fewer than half of the rats correctly anticipated the location of both daily meals. The SCNX rats acquired the task to varying degrees, but the intact

animals housed in a stable LD cycle performed the task best. As the SCN is the site of the LD-entrained pacemaker in the rat (Meijer & Rietveld, 1989), daily time-place learning in the rat may be primarily controlled by a food-entrainable circadian phase timer. However, as Boulos and Logothetis did not include any tests to prove that their rats were not using an alternation strategy to track the location of food it is impossible to be sure that their rats were using a circadian phase-timer.

Mistleberger (1994) describes an unpublished investigation of daily time-place learning in rats. He trained intact and SCNX rats to bar press for food at two locations in an open arena. One lever provided food in morning sessions and a second lever provided food in afternoon sessions. Again less than half of the rats discriminated between the morning and afternoon sessions.

In summary, there is clear evidence suggesting that pigeons and garden warblers can acquire a daily time-place learning task. Additionally, these birds appear to use a circadian phase-timing system to track the location of food over the course of a day. There is weaker but suggestive evidence for this learning ability in mammals. The present series of experiments was conducted to further examine daily time-place learning in the rat. In these studies I used a procedure similar to that employed by Saksida and Wilkie (1994). In Experiment 1 I found that rats can track the location of food over the course of two daily sessions. I then conducted a series of experiments to characterize the mechanism controlling the rats' time-place behaviour.

Experiment 1: Acquisition Training and the First Baseline Period

Method

Subjects

Four male hooded Long Evans rats obtained from Charles River, Quebec served as subjects. The rats were experimentally naive, approximately 90 days old at the beginning of the experiment, and weighed between 400 and 420 g. The rats were maintained at a minimum of 90% of their free-feeding weight (adjusted for age) and received free access to water except during the experimental sessions. The rats were fed 45 mg Noyes reward pellets during experimental sessions and standard rat chow during post-session feedings.

The rats lived individually in opaque plastic cages lined with Sani-Cell bedding. They were periodically given a variety of paper products to build nests. Unless stated otherwise, colony light onset was at 07:30 and light offset was at 19:30 producing a 12:12 LD cycle. The subjects received two daily enrichment sessions during which they could interact socially, explore, and manipulate objects. Daily enrichment is a standard component of my animal care program. Throughout this series of experiments the animals were cared for in strict accordance with Canadian Council on Animal Care guidelines.

Apparatus

The rats were tested in a large Plexiglas chamber (length = 40 cm, width = 40 cm, height = 42 cm). The testing chamber was located on a bench in a small (length = 3 m, width = 2 m), well lit testing room. From within the testing box the subjects could see a variety of distal spatial cues including a PC and monitor, a door and door frame, and various geometric shapes cut from construction paper that were mounted on the walls of the testing room.

A lever was mounted in the middle of each of the four walls of the testing chamber. Each lever was placed 10 cm above the chamber floor. A brass food cup was mounted adjacent to each lever. Each lever operated a switch which recorded lever presses. Four reward pellet hoppers were mounted on the top of the test chamber. When operated, the hoppers dispensed 45 mg Noyes reward pellets into the food cup mounted adjacent to their associated lever. A small covered cue light was mounted above each lever. Data collection and equipment control was carried out by a Turbo C++ program running on a nearby networked PC.

The enrichment chamber measured 90 cm (length) by 63 cm (width) by 175 cm (height), and had three interconnected levels. The enrichment chamber framework was made of painted wood and the sides and floors were made of wire mesh or sheet metal. All solid surfaces were lined with sani-cell bedding and various play objects were placed on the floors. Water was freely available in the enrichment chamber.

Procedure

Initially the subjects were exposed individually to the testing chamber. During these 30 min sessions every lever press resulted in the delivery of a reward pellet into the adjacent food cup. After six of these sessions all the rats were steadily pressing all four levers for food. Reward pellets were then provided on a variable ratio (VR) schedule. The VR was gradually increased until all the subjects were pressing steadily on a variable ratio 15 (VR15).

Acquisition training then began. The rats were transported as a group from the colony room to the enrichment chamber at 09:30 and 15:30, 5 to 7 days per week. The rats were then taken one at a time, and in a quasi-random order, from the enrichment chamber to the testing chamber.

In the testing chamber each rat could press for food on one lever during 09:30 sessions (his 09:30 lever) and on a second, different, lever during 15:30 sessions (his 15:30 lever). This was counterbalanced across the rats. A session began when a rat pressed any lever. This turned on the four cue lights. The onset of the cue lights was followed by a period of variable length (range 4 to 40 sec) during which lever presses were recorded but no reward pellets were given. The rats' bar presses during this initial nonrewarded period served as the dependent variable. I reasoned that these responses could be used to infer where the rats *expected* food to be available that session. This expectation was expressed in the form of a mean percent of all responses score for each of the three types of levers: (1) correct lever in that session (MPCorr), (2) correct lever in the alternate daily session (MPAlt), or (3) the incorrect levers which never provided food (MPInc). This score was calculated for each type of lever by dividing the number of responses to the lever(s) by the total number of responses to all the levers and then multiplying this ratio by 100. These three scores were calculated for each rat for every session.

Every initial nonrewarded period was followed by a 10 min rewarded period during which the designated lever provided reward pellets on a VR15. When each rat finished a session he was transported back to the enrichment box. After all the rats were tested they were returned to their home cages where they received a post-session feeding. The rats received their post-session feedings an average of 20 min (range 2 to 38 min) after the end of their experimental sessions. One or 2 days per week were rest days. On rest days the rats remained in their home cages and were fed when they usually received their standard post-session feedings.

Acquisition Training and the First Baseline Period took place over 14 weeks. During this time three changes were made to the general procedure. First, during the initial 6 weeks of training there was a positive correlation between the length of the initial nonrewarded period and

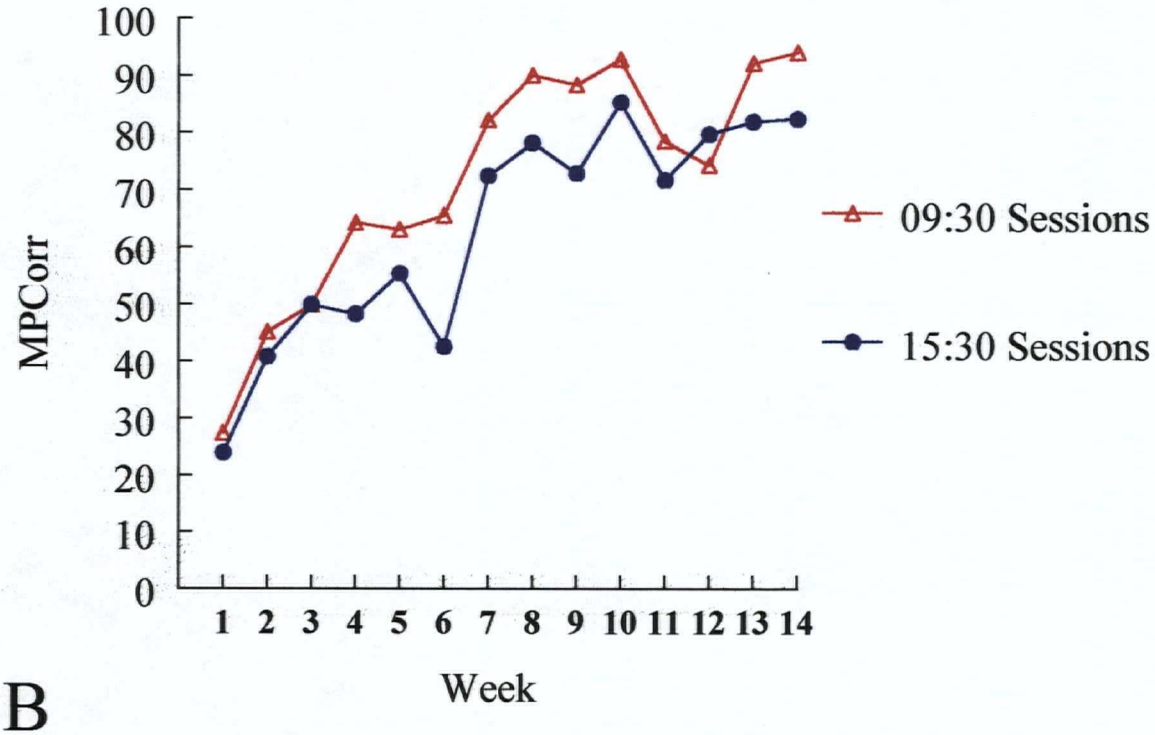
the rats' MPCorr scores. This observation suggested that the rats were patrolling the test chamber (and pressing many levers) before they started to press on the lever that they expected would provide food. This was not surprising as rats have demonstrated similar patrolling behaviour in other contexts (Cowan, 1977; Wilkie, Mumby, Needham, & Smeele, 1992). I attempted to discard these patrolling responses by adding a 10 s time-out period after the initial lever press which started each session. This time-out period was added at the beginning of Week 7. Responses during this time-out period were not recorded and did not produce food. The usual nonrewarded and rewarded periods followed the time-out period. Secondly, on Week 11 the enrichment chamber was changed to a smaller Plexiglas box which measured 62 cm (length) by 55 cm (width) by 25 cm (height). The new box had a wire mesh lid and was lined with sani-cell bedding. The new enrichment box contained many play objects and again water was freely available. Finally, during Week 12 the rats were handled by novel experimenters. From Week 13 onwards the original experimenter handled the rats.

Results

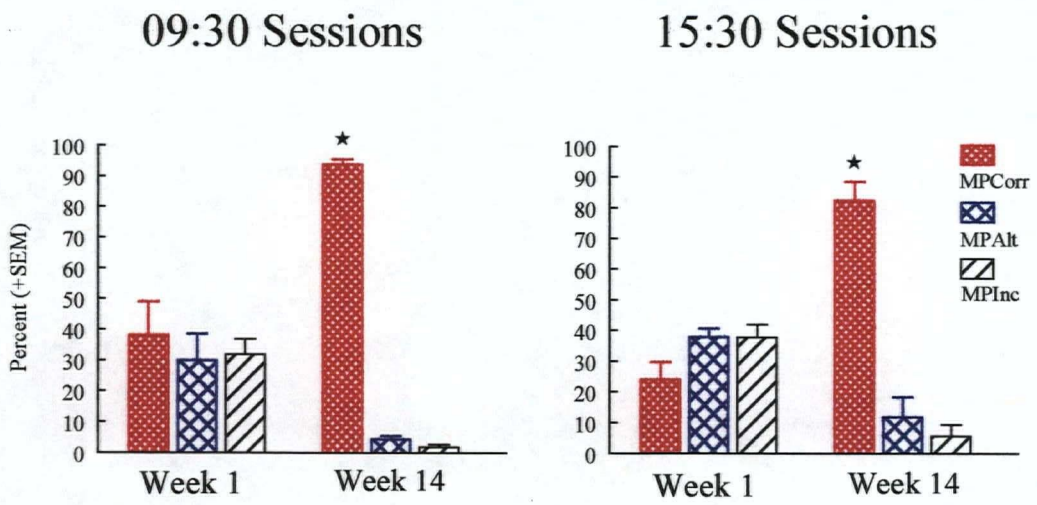
Figure 1A presents the rats' overall MPCorr scores (in 7-day blocks) during the 09:30 and 15:30 sessions over the acquisition and first baseline periods. During Week 1 the rats' MPCorr in the 09:30 and 15:30 sessions was roughly 25%. This indicates that initially the rats could not anticipate the future location of food in 09:30 or 15:30 sessions. However, over the following weeks the rats clearly learned the location of food in both 09:30 and 15:30 sessions. The rats' weekly MPCorr passed 50% correct in 09:30 sessions during Week 4. A MPCorr of 50% represents chance performance if the rats only learned which two levers provided food. After Week 4, the rats' 09:30 MPCorr exceeded their 15:30 MPCorr by roughly 10% during 9 of the following 10 weeks. By Week 7 the rats were clearly anticipating the location of food in

Figure 1. A) The rats' weekly overall 09:30 and 15:30 MPCorr scores over the 14 week acquisition and first baseline period. B) The rats' mean 09:30 and 15:30 MPCorr scores during week 1 and week 14. Asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.

A


MPCorr
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Week
09:30 Sessions
15:30 Sessions

B

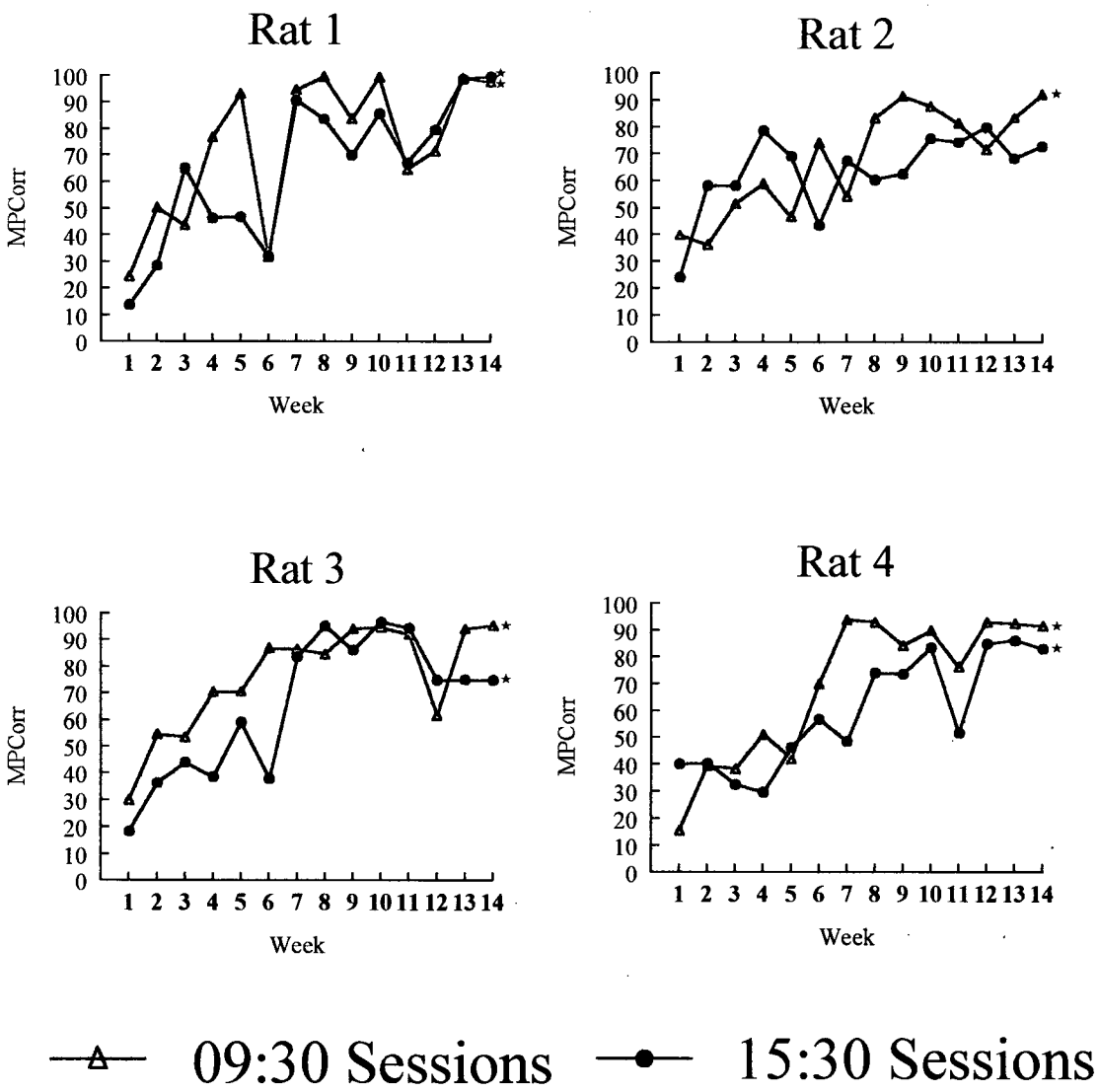

09:30 Sessions
15:30 Sessions
Percent (+SEM)
100
90
80
70
60
50
40
30
20
10
0
Week 1
Week 14
MPCorr
MPAlt
MPInc

09:30 and 15:30 sessions and during Week 8 their 09:30 and 15:30 MPCorr appeared to asymptote at roughly 90% and 80% respectively. The increase in the rats' 09:30 and 15:30 MPCorr on Week 7 is likely due to the addition of the 10 sec time-out period. The transient dip in the rats' 09:30 and 15:30 MPCorr during Weeks 11 and 12 may be the result of the change in their enrichment environment and handling.

These observations were confirmed by the results of a repeated-measures ANOVA on the weekly overall MPCorr scores. Session time (09:30 or 15:30) and training (Week 1 to Week 14) served as repeated-measures. This analysis revealed a significant effect of training, $F_{(13,39)} = 16.14$, $p<.001$, a significant effect of the time of a session, $F_{(1,3)} = 33.72$, $p < .02$, and a non-significant training by time of session interaction, $F_{(13, 39)} = <1$, $p<.77$. Figure 1B presents the rats' MPCorr, MPAlt, and MPInc scores for 09:30 and 15:30 sessions during Weeks 1 and 14. During Week 1, the rats clearly could not anticipate where food would be available in 09:30 and 15:30 sessions. In contrast, during Week 14 the rats' mean MPCorr scores in 09:30 and 15:30 sessions were 94.12 (*SEM* = 1.44) and 82.48 (*SEM* = 6.08) respectively. During Week 14, the rats' MPCorr was significantly greater than a conservative estimate (50%) of chance performance in both 09:30 and 15:30 sessions, (one sample t-test for 09:30 sessions, $t_{(3)} = 30.63$, $p<.001$, and for 15:30 sessions $t_{(3)} = 5.34$, $p<.014$).

The rats' individual acquisition curves are presented in Figure 2. Initially every rat performed at near chance (25%) levels. During training all the rats learned the location of food in both 09:30 and 15:30 sessions. With the exception of Rat 2 in 15:30 sessions, during Week 14 each rat's overall MPCorr score was significantly greater than a conservative (50%) estimate of chance performance in both 09:30 and 15:30 sessions, (one sample t-tests, all p's $<.05$).

Figure 2. Weekly overall 09:30 and 15:30 MPCorr scores over the 14 week acquisition and first baseline period for each rat. Asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.


Rat 1
Rat 2
Rat 3
Rat 4
MPCorr
Week
09:30 Sessions
15:30 Sessions

Consistent with their overall performance, each rat appeared to anticipate the location of food best in the 09:30 sessions.

Discussion and Four Possible Underlying Mechanisms

Experiment 1 clearly demonstrated that the rats could learn the location of food in 09:30 and 15:30 sessions. I initially reasoned that the rats could have employed at least four different mechanisms to discriminate between the two daily sessions.

A circadian phase-timer mechanism

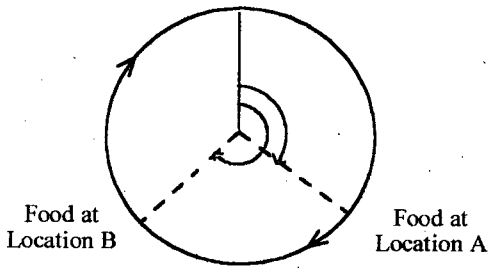
The rats could have used a circadian phase-timer to anticipate the location of food in 09:30 and 15:30 sessions (i.e., food is at location A when the circadian phase-timer is at phase angle A, and food is at location B when the circadian phase-timer is at phase angle B). A schematic representation of this strategy is presented in Figure 3A.

An interval-timer mechanism

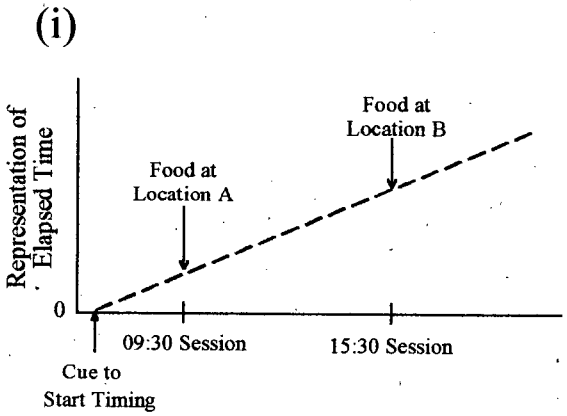
The rats could have used an interval timer started by some salient event that occurred reliably at the same time each day to anticipate the location of food in 09:30 and 15:30 sessions. The onset of the colony lights each day could serve this function (i.e., food is at location A 2 hr after light onset and food is at location B 8 hr after light onset). A schematic representation of this strategy is presented in Figure 3B(i). Alternatively, as the amount of time between sessions is not equal, the rats could have used an interval timer started by some event in each session to discriminate between the 09:30 and 15:30 sessions (i.e., 12 hr precede food at location A, whereas 6 hr precede food at location B). A schematic representation of this strategy is presented in Figure 3B(ii).

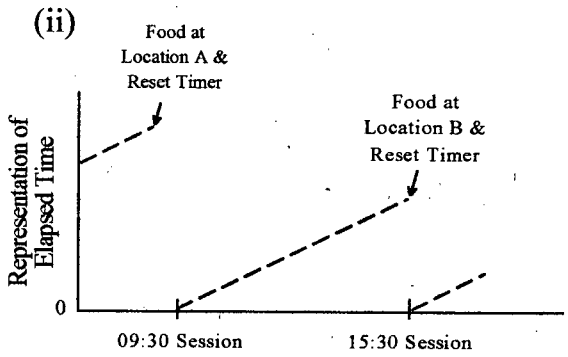
Figure 3. Schematic representations of the four mechanisms that I suspected rats could use to perform the daily time-place task.

A. Circadian Phase-Timer



B. Interval-Timer Strategies





C. Alternation Strategy

Food at Location A → Food at Location B → Food at Location A ...

D. Exogenous Cue Strategy

$S^{D}_{09:30}$ ⟶ Food at Location A

$S^{D}_{15:30}$ ⟶ Food at Location B

An alternation mechanism

Rats readily employ an alternation strategy in T-maze tasks and can perform well even when arm choices are separated by many hours (Livesey & Livesey, 1981). Therefore, the rats could have anticipated the location of food during 09:30 and 15:30 sessions by learning alternate between the two levers which provided food (i.e., Location A then Location B then Location A etc.). A schematic representation of this strategy is presented in Figure 3C.

A contextual cue mechanism .

The rats could have learned to press their 09:30 levers in the presence of one set of contextual cues and learned to press their 15:30 levers in the presence of another set of contextual cues (i.e., $S^{D}_{09:30}$ -> food is at Location A, $S^{D}_{15:30}$ -> food is at Location B). These cues could include general colony noise, laboratory temperature, or daily colony maintenance. A schematic representation of this strategy is presented in Figure 3D.

Experiment 2: Skipped 09:30 and 15:30 Sessions

To determine whether the rats were using one of the above four mechanisms to track the location of food over the course of a day, I first examined what effect skipping 09:30 and 15:30 test sessions had on their time-place behaviour.

Predictions made by the four Possible Mechanisms

In the circadian phase-timing based mechanism the rats' time-place behaviour is controlled by the phase angle of an endogenous 24 hr clock. Therefore, a rat using a phase timer to track the location of food should not be affected by a skipped 09:30 or 15:30 session.

I described two possible interval-timing mechanisms. These mechanisms can be differentiated on the basis of the event which starts timing. In the first mechanism, the timer is

started each day by some reoccurring event (such as light onset), and in the second mechanism the timer is started by some event in the test sessions. A rat using the first type of interval-timing mechanism should not be affected by a skipped 09:30 or 15:30 session as the cue which starts the timer each day would presumably not be disrupted. A rat using the second type of interval-timing mechanism should be impaired when either 09:30 or 15:30 sessions are skipped as the cue which starts and stops timing (an event during experimental sessions) did not occur.

In the alternation based mechanism the location at which food is expected is based solely on the last location where food was obtained. Therefore, a rat using an alternation based strategy would be dramatically effected if either a 09:30 or 15:30 session was skipped. If a 09:30 session was skipped, the rat would expect food at his 09:30 lever at the beginning of the following 15:30 session, and if a 15:30 session was skipped, the rat would expect food at his 15:30 lever at the beginning of the following 09:30 session.

The contextual cue mechanism relies solely on the presence of some different exogenous stimulus or stimuli during 09:30 and 15:30 sessions. Therefore, if the rats were using an exogenous cue mechanism, simply skipping 09:30 or 15:30 sessions would have no effect on their ability to anticipate the location of food during the next session.

Method

Subjects & Apparatus

The subjects and apparatus were the same as those used in Experiment 1.

Procedure

During baseline days the rats were handled exactly as they were during Experiment 1. However, on every fourth or fifth day a 09:30 or 15:30 session was skipped. The type of session skipped (09:30 or 15:30) was determined according to a quasi-random schedule. When

a session was skipped the rats were transported to the enrichment chamber at the usual time. They remained in the enrichment chamber for 45 min, and then they were transported back to their home cages. In their home cages they received their usual post-session meal plus additional food to compensate for their missed experimental session. This protocol ensured that on skip-session days the rats received the same amount of food at roughly the same times-of-day as they did during baseline sessions. Experiment 2 lasted 40 days during which the rats received 32 baseline days, four skipped 09:30 sessions, and four skipped 15:30 sessions.

Results

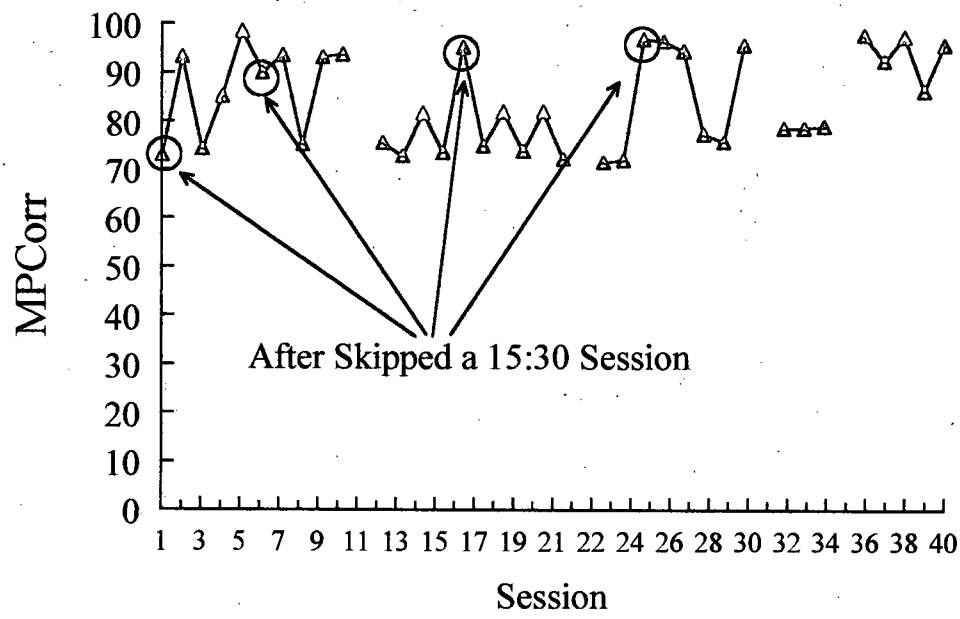
The rats' daily overall MPCorr scores in the 09:30 and 15:30 sessions of Experiment 2 are presented in Figure 4. During the baseline 09:30 sessions the rats' MPCorr was 82.59 (*SEM* = 5.11), and during the baseline 15:30 sessions their MPCorr was 83.17 (*SEM* = 3.53). Therefore, during Experiment 2 there was no evidence of the rats' previous tendency to perform best in the 09:30 sessions.

Inspection of Figure 4 reveals that skipping a 15:30 session had little impact on the rats' MPCorr in the following 09:30 session. On the other hand, skipping a 09:30 session caused a dramatic decrease in the rats' MPCorr during the following 15:30 session. In fact, the four lowest overall MPCorr scores during Experiment 2 were obtained in the 15:30 sessions following a skipped 09:30 session.

The rats' overall MPCorr, MPAlt, and MPInc scores in the 09:30 and 15:30 sessions immediately before a skipped session, in the sessions immediately after a skipped session, and in the second session after a skipped session are presented in Figure 5. Using this method of data presentation, it is again evident that skipping a 15:30 session had no effect on the rat's MPCorr in the following two 09:30 sessions. In all three types of 09:30 sessions, the rats' MPCorr

Figure 4. The rats' daily overall 09:30 and 15:30 MPCorr scores during Experiment 2. Missing data points correspond to skipped sessions.

9:30 SESSIONS



15:30 SESSIONS

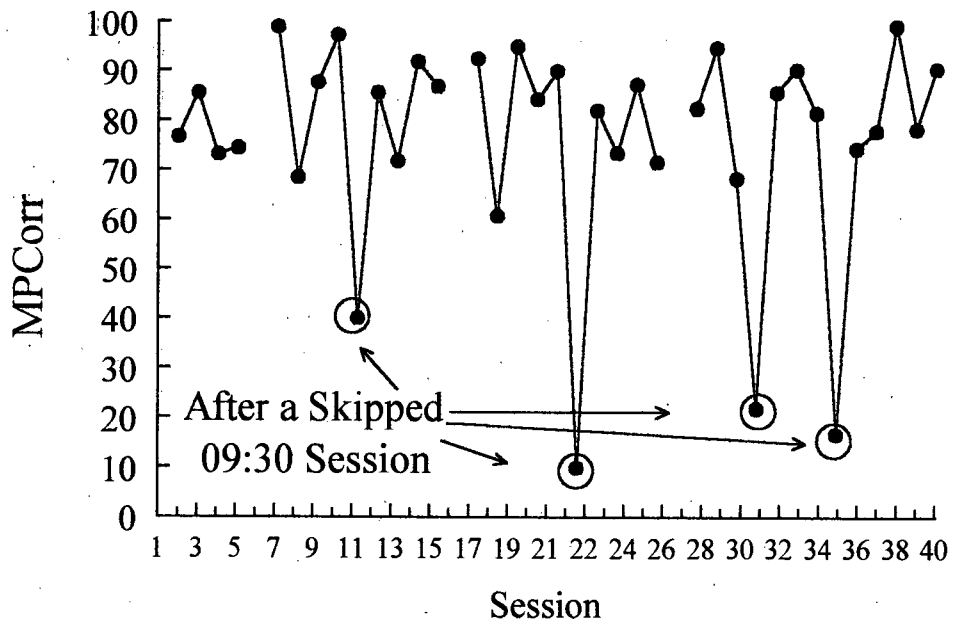
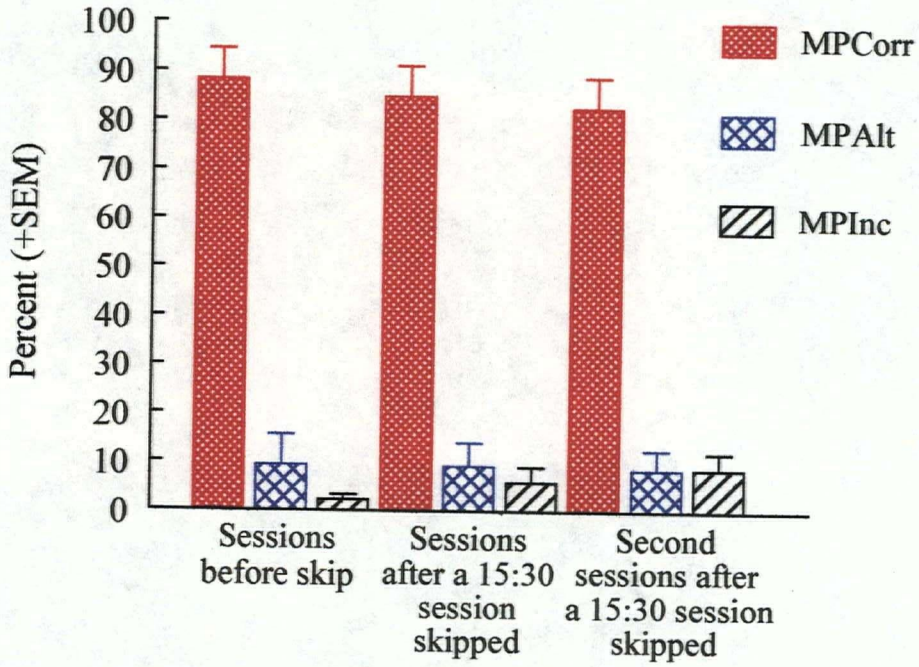
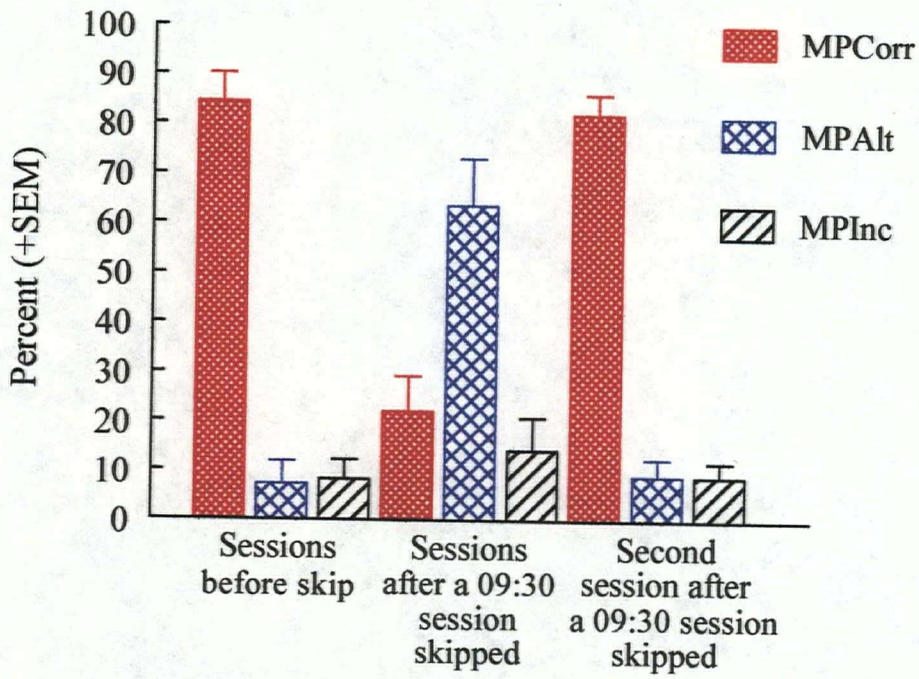


Figure 5. The rats' overall 09:30 and 15:30 MPCorr scores (+ SEM) in Experiment 2 during the sessions immediately before the skipped sessions, during the sessions immediately after skipped sessions, and during the second sessions after skipped sessions.

9:30 SESSIONS



15:30 SESSIONS



remained high while their MPAlt and MPInc remained very low. In contrast, in the 15:30 sessions following a skipped 09:30 session, the rats' MPAlt increased and their MPCorr decreased. In other words, in the 15:30 sessions which followed a skipped 09:30 session, the rats displayed a greatly increased preference for their 09:30 levers and a greatly reduced preference for their 15:30 levers. The effect of skipping a 09:30 session was not permanent as the rats' MPCorr returned to 80-90% during the second 15:30 session after a skipped 09:30 session.

These observations were confirmed statistically with a repeated-measures ANOVA on the rats' MPCorr scores. The time of a session (09:30 or 15:30), the type of session (session before a skipped session, session immediately after a skipped session, or the second session after a skipped session), and skip replication (1st, 2nd, 3rd, or 4th) served as repeated-measures. This analysis revealed a significant effect of the time of a session, $F_{(1,3)} = 118.45$, $p<.003$, a significant effect of the type of session, $F_{(2,6)} = 9.44$, $p<.015$, and a non-significant effect of skip replication, $F_{(3,9)} <1$, n/s. The only significant interaction found was that between the time of a session and the type of session, $F_{(2,6)} = 25.66$, $p<.002$. This interaction confirms that the effect of skipping a session depended on time of the skipped session.

The rats' overall MPAlt in the 15:30 sessions following a skipped 09:30 session was significantly higher than their overall MPAlt in the preceding and following 15:30 sessions (paired t-tests versus the preceding 15:30 session, $t_{(3)} = 4.79$ $p <.018$, and versus the following 15:30 session, $t_{(3)} = 4.73$, $p<.019$). Their overall MPCorr in the 15:30 sessions following a skipped 09:30 session was also significantly lower than their overall MPCorr in the preceding and following 15:30 sessions (paired t-tests versus preceding the 15:30 session, $t_{(3)} = 6.93$. $p <.007$, and following 15:30 session, $t_{(3)} = 6.77$, $p<.008$).

Discussion

Experiment 2 found that when a 15:30 session was skipped, the rats were still able to anticipate the location of food in the following 09:30 session. However, when a 09:30 session was skipped, the rats incorrectly expected food at their 09:30 lever during the following 15:30 session.

This outcome is not consistent with the operation of any of my four proposed mechanisms. As the rats were impaired when 09:30 sessions were skipped but unimpaired when 15:30 sessions were skipped, I can rule out control by a simple alternation strategy. The usual contextual cues were present during the 15:30 sessions which followed the skipped 09:30 sessions, so primary control by contextual cues can also be ruled out. This leaves the endogenous timing mechanisms. Primary control by a circadian phase-timer can be ruled out because when 09:30 sessions were skipped, the rats incorrectly expected food at their 09:30 levers during the following 15:30 session. Additionally, the rats did not primarily use either of the two proposed interval timing strategies. First, I can rule out control by an interval timer started by an external cue (such as light onset) as the rats were impaired in the 15:30 sessions which followed a skipped 09:30 session. I can also rule out control by an interval timer started by some event during each session as the rats were impaired when 09:30 sessions were skipped, but unimpaired when 15:30 sessions were skipped.

At this point the mechanism employed by the rats was best characterized as a *daily route strategy*: They learned to lever press for food at one location during their first session of each day, and to lever press at a second location during their second session of each day (i.e., Location A then Location B, *each day*). It is the daily nature of this strategy that differentiates it from an alternation based strategy. This daily route strategy is similar to Krebs and Biebach's

(1989) notion of a fixed route as the rats had to receive a 09:30 session to be able to anticipate the location of food in the following 15:30 session.

Second Baseline Period

After completing Experiment 2, the rats received two weeks of baseline training. Figure 6A presents the rats' overall MPCorr scores for the 09:30 and 15:30 sessions during the two week baseline period. The rats continued to anticipate the location of food in both the 09:30 and 15:30 sessions and again there was no difference between their performance in the 09:30 and 15:30 sessions. Their overall MPCorr was 85.0 (*SEM* = 4.97) in the 09:30 sessions and 85.57 (*SEM* = 4.30) in the 15:30 sessions. The rats' overall MPCorr score was significantly greater than a conservative estimate (50%) of chance performance in both the 09:30 and 15:30 sessions (one sample t-test for 09:30 sessions, $t(_3) = 11.85$, $p < .002$, and for 15:30 sessions $t(_3) = 6.88$, $p < .007$).

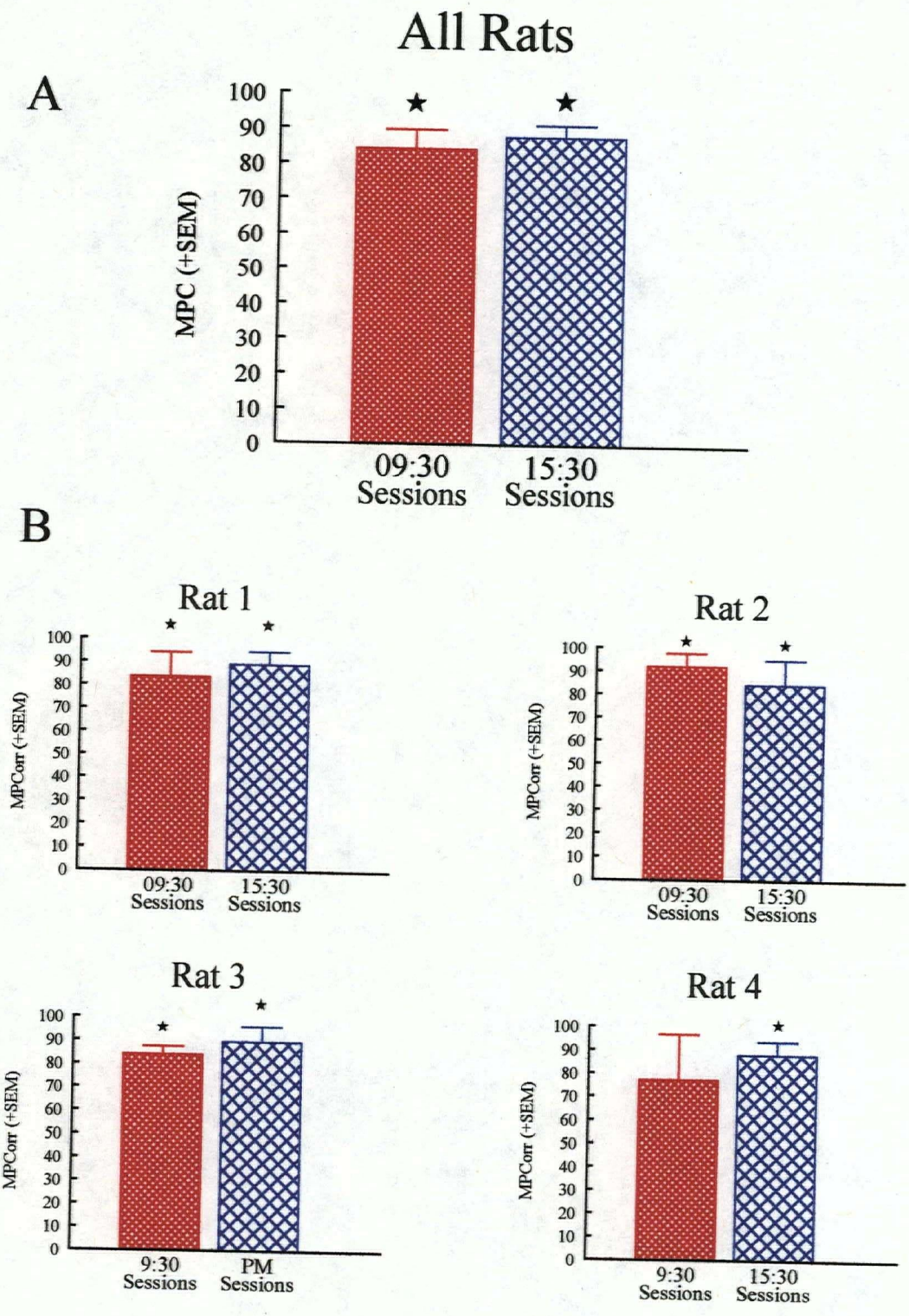
Each rat's overall MPCorr for the 09:30 and 15:30 baseline sessions is presented in Figure 6B. With the exception of Rat 4 in 09:30 sessions, every rat clearly continued to anticipate the location of food in 09:30 and 15:30 sessions, and this was confirmed statistically with one-sample t-tests against a conservative (50%) estimate of chance, (all p's $< .05$).

Experiment 3: Interpolated Probe Sessions at 11:45, 13:00, & 14:15

Experiment 2 suggested that the rats were primarily using a daily route strategy to track the location of food over the course of a day. This strategy entailed lever pressing at one location during their first session of each day and lever pressing at a second location during their second session of each day.

As the baseline 09:30 sessions and the 15:30 sessions which followed a skipped 09:30 session were both the rats' first session of the day, they should have been treated identically if

Figure 6. A) The rats' overall 09:30 and 15:30 MPCorr scores (+ SEM) during the Second Baseline Period. B) Each rat's mean 09:30 and 15:30 MPCorr scores during the Second Baseline Period. For A and B, asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.


All Rats
A
MPC (+SEM)
100
90
80
70
60
50
40
30
20
10
0
09:30 Sessions
15:30 Sessions
B
Rat 1
MPCorr (+SEM)
09:30 Sessions
15:30 Sessions
Rat 2
MPCorr (+SEM)
09:30 Sessions
15:30 Sessions
Rat 3
MPCorr (+SEM)
9:30 Sessions
PM Sessions
Rat 4
MPCorr (+SEM)
9:30 Sessions
15:30 Sessions

the rats were *solely* using a daily route strategy to track the location of food. This was clearly not the case. In the 15:30 sessions which followed a skipped 09:30 session the rats did not favor their 09:30 levers as strongly as they preferred their 09:30 levers in baseline 09:30 sessions (63.6 (*SEM* =9.7) versus 82.59 (*SEM* =5.11)). Or from the other point of view, during the 15:30 sessions which followed a skipped 09:30 session, the rats pressed their 15:30 levers relatively more than they usually pressed their 15:30 levers in baseline 09:30 sessions (22.2. (*SEM* =7.13) versus 7.4 (*SEM* =4.6)). It appears that the rats were not completely tricked when 09:30 sessions were skipped.

The ordinal position of these two types of sessions was the same (first in the day), but they differed in the time-of-day at which they occurred (09:30 versus 15:30). It therefore seemed reasonable to propose that the rats' expectation of the future location of food was based on two types of information; (1) a daily route strategy which had primary control over the rats' time-place behaviour, and a (2) secondary timing mechanism which had relatively weak control over the rats' time-place behaviour.

In Experiment 3 I looked for further evidence of behavioural control by a secondary timing mechanism. I did this by measuring where the rats' expected food 1.15 hr, 2.30 hr, and 3.45 hr after the end of the 09:30 session. The 09:30 sessions typically ended at 10:30. If the rats' expectation of where food would be available was determined solely by a daily route strategy, they should prefer their 15:30 levers equally 1.15, 2.30, 3.45, and 5 hr after a 09:30 session. If, however, the rats' expectation of where food would be available was partially determined by the status of an endogenous timing system, their preferences for their 15:30 levers should display a temporal gradient: They should prefer their 15:30 levers least at 11:45 (when the daily route strategy and timing mechanism predict food at the 15:30 and 09:30 locations

respectively) and their preference for their 15:30 levers should then increase the closer the probe session is in time to 15:30 (when the daily route strategy and timing mechanism both predict food at the 15:30 location).

Method

Subjects and Apparatus

The subjects and apparatus were the same as those used in Experiments 1 and 2.

Procedure

Over the course of the next 24 days the rats received 15 baseline days and nine probe days. Baseline and probe days were presented in three 8-day blocks. Each 8-day block contained a probe session at each of the probe times (11:45, 13:00, and 14:15) and 5 baseline days. The sessions were presented in a quasi-random order, and probe days were always separated by at least one baseline day. On probe and baseline days the rats received their 09:30 and 15:30 test sessions and post-session meals as per usual. On probe days the rats also received a single brief probe session at 11:45, 13:00, or 14:15. During the probe sessions the rats were transported to the enrichment and testing chambers as per usual. When a rat was placed in the testing chamber his first lever press turned on the cue light above each of the levers. The onset of the cue lights was followed by a 40 s period during which lever presses were recorded but no food was provided. At the end of this nonrewarded period the cue lights were extinguished and the rat was taken back to the enrichment chamber. After all the rats were tested they were returned to their home cages. No food was given during the probe sessions.

The proportion of the rats' responses that were on their 09:30 (MP09:30), 15:30 (MP15:30), and incorrect levers (MPInc), in the nonrewarded period at the beginning of every session served as the dependent variable. These scores were calculated for each type of lever by

dividing the number of responses to the lever(s) by the total number of responses to all the levers and then multiplying this ratio by 100. These three scores were calculated for each rat for every session.

Results

Figure 7 displays the total number of lever presses recorded during the probe sessions in each of the three 8-day blocks of sessions. As the length of the probe sessions was held constant at 40 s, the rats' rate of lever pressing clearly decreased over the three blocks of probe trials. This effect was examined with a repeated measures ANOVA on the total number of responses recorded for each rat during each of the three blocks of probe sessions. The block of probe sessions (one through three) served as the repeated measure. This analysis failed to confirm a main effect of probe block, ($F_{(2,6)} = 2.99$, $p<.13$). Along with this marked, although not statistically significant, decrease in responding the rats' lever choices became erratic over the course of the second and third block of probe sessions. As a result the rats did not display a consistent preference for any type of lever at any probe time during the second and third block of probe sessions. Consequentially, the results of the second and third block of probe sessions were not included in the following analysis.

Figure 8 presents the rats' overall MP09:30, MP15:30, and MPInc scores during the 24 baseline 09:30 sessions, 15 baseline 15:30 sessions, and the first probe session at each probe time (11:45, 13:00, and 14:15). There were 24 baseline 09:30 sessions and only 15 baseline 15:30 sessions because the nine 15:30 sessions which followed an interpolated probe session were not treated as baseline sessions. During the baseline 09:30 sessions the rats continued to mainly press their 09:30 levers. During the first probe at 11:45, the rats mainly pressed their 15:30 levers. However, during this probe session the rats pressed their 15:30 levers

Figure 7. The total number of responses recorded during the probe sessions in each of the three 8-day blocks of sessions in Experiment 3.

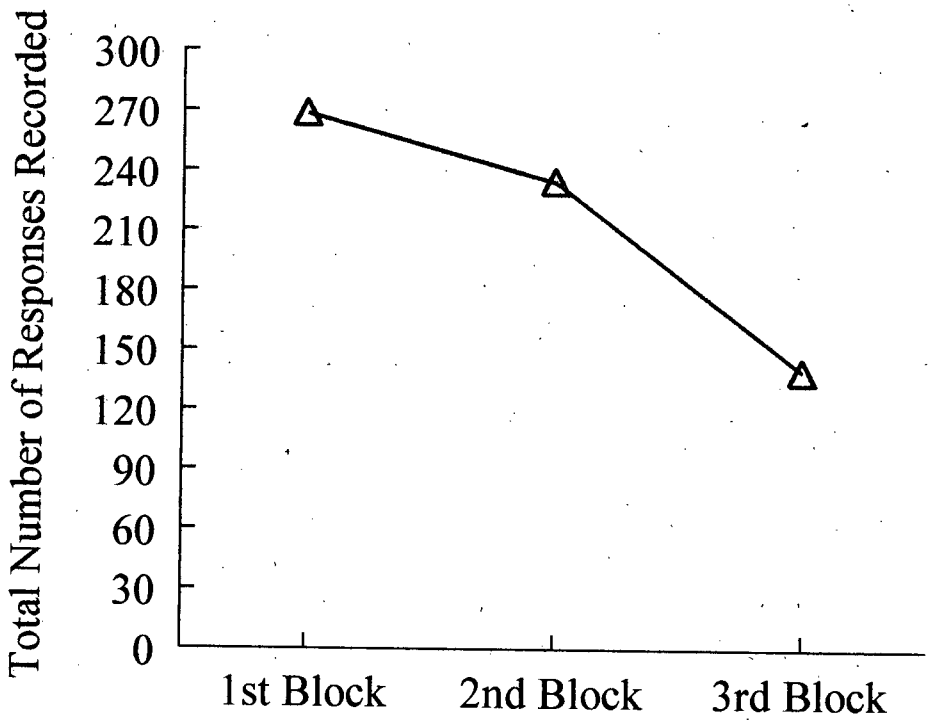
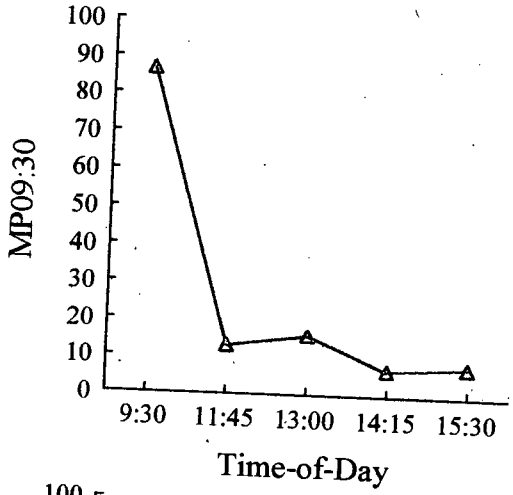
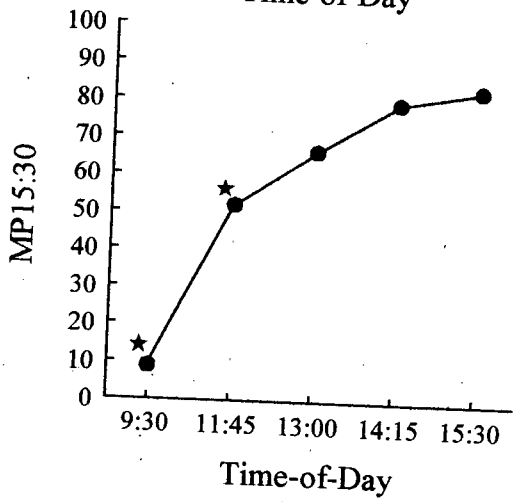
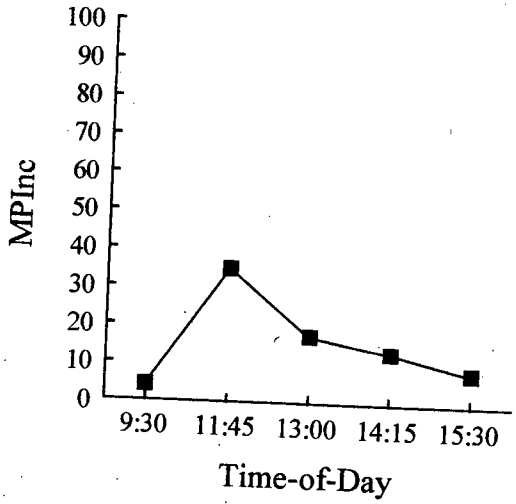

Total Number of Responses Recorded
300
270
240
210
180
150
120
90
60
30
0
1st Block
2nd Block
3rd Block

Figure 8. The rats' overall MP09:30, MP15:30, and MPInc scores during the first block of probe sessions in Experiment 3. Asterisks indicate the corresponding mean igreater than a conservative (50%) estimate of chance performance, $p<.05$.


MP09:30
100
90
80
70
60
50
40
30
20
10
0
9:30
11:45
13:00
14:15
15:30
Time-of-Day


MP15:30
100
90
80
70
60
50
40
30
20
10
0
9:30
11:45
13:00
14:15
15:30
Time-of-Day


MPInc
100
90
80
70
60
50
40
30
20
10
0
9:30
11:45
13:00
14:15
15:30
Time-of-Day

proportionately less (52.0 (*SEM* = 13.5) versus 83.3 (*SEM* = 4.89)), and their incorrect levers proportionately more (34.8 (*SEM* = 8.9) versus 8.5 (*SEM* = 1.5)) than they did during baseline 15:30 sessions. This decreased preference for their 15:30 levers and increased preference for their incorrect levers was also evident in the first probe sessions at 13:00 and 14:15. However, the size of this effect decreased the closer the probe session was in time to 15:30. During the baseline 15:30 sessions the rats continued to mainly press their 15:30 levers.

These observations were confirmed statistically by the results of a repeated measures ANOVA on the rats' MP15:30 scores. The time of a session (09:30, 11:45, 13:00, 14:15 and 15:30) served as the repeated measure. This analysis revealed a significant effect of the time of a session, ($F_{(4,12)} = 7.27$, $p<.004$). One-tailed paired t-tests confirmed that the rats responded proportionately less on their 15:30 levers during 09:30 and 11:45 sessions than they did during baseline 15:30 sessions, ($t_{(3)} = 11.32$, $p<.001$, and $t_{(3)} = 2.46$, $p<.05$. respectively).

Discussion

Once the rats received food at their 09:30 levers they did not expect food to be available at that location for the rest of that day, even during the interpolated probe session at 11:45. This constitutes further support for the daily route strategy. However, during the first 11:45 probe session the rats did not prefer their 15:30 levers as strongly as they did during the baseline 15:30 sessions. Instead, they displayed an increased preference for their incorrect levers. The rats' preference for their 15:30 levers then increased, and their preference for their incorrect levers then decreased, as the time of the probe session approached 15:30. This outcome is consistent with my suggestion that the rats' expectation of where food would be available was determined, at least in part, by the status of an endogenous timing mechanism. When the output of this timing mechanism differed from that of the daily route, as was the case in probe sessions

at 11:45, the rats displayed a reduced preference for their 15:30 levers and an increased preference for their incorrect levers. This increased preference for levers which had not provided food for over 175 testing days is interesting, and may reflect exploratory sampling.

During the second and third block of probe tests the rats lever pressed less frequently and they did not display a clear preference for any type of lever at any probe time. As there was no change in the rats' behaviour during baseline 09:30 and 15:30 sessions during this time, it appears that the rats learned to discriminate between probe sessions and baseline sessions. The only difference between probe sessions and the initial unrewarded period of baseline 09:30 and 15:30 sessions was the time-of-day at which they occurred. Perhaps then, the rats learned to detect probe sessions on the basis of their unusual time-of-occurrence, and consequently they did not expect food to be available at any location during my second and third block of probe sessions. If this is the case, it is remarkable how quickly this discrimination was acquired.

Experiment 4a: Constant Light

At this point it is useful to characterize in greater detail what I knew about the daily route strategy employed by the rats. I knew that the rats began each day expecting food to be available at their 09:30 levers (Experiments 1, 2, and 3). Then, after the rats received food at their 09:30 levers, they expected that food would be available *next* at their 15:30 levers (Experiments 1, 2 and 3). This shift in lever preference occurred as early in the day as 11:45. If the rats did not receive a 09:30 session (but did receive their morning post-session meal), they continued to expect that food would be available next at their 09:30 levers until at least 15:30 (Experiment 3). This suggests that receiving food at their 09:30 levers, and not the passage of time, was *necessary and sufficient* for the rats to expect food at their 15:30 levers during 15:30

sessions. However, when 15:30 sessions were skipped, the rats correctly expected that food would be available at their 09:30 levers during the following 09:30 session. This means that receiving food at their 15:30 levers was *not necessary* for the rats to expect food at their 09:30 levers during 09:30 sessions. I therefore suspected that some other event was capable of "resetting" the rats daily route each day.

This reset function could conceivably be triggered either endogenously or exogenously. If the reset was triggered exogenously the external trigger stimulus would likely be the most salient, temporally reliable stimulus available to the rats. I reasoned that the daily onset and offset of the colony lights was the best candidate. Alternatively, if this daily reset was triggered endogenously, the internal trigger would likely be provided by a circadian phase-timer. If this were the case, the rats' time-place behaviour would not be immediately effected by the manipulation of any exogenous cue.

To test whether the rats' daily sequence was exogenously reset by the onset or offset of the colony lights each day, I determined whether housing the rats in constant light (LL) disrupted their ability to discriminate between 09:30 and 15:30 sessions. If placing the animals in LL *immediately* caused them to expect food at their 15:30 levers during the first 09:30 session after the switch to LL, I would have strong evidence that the daily colony LD cycle served as the *necessary* daily reset stimulus. Also, if the rats were immediately impaired when they were switched to LL whilst continuing to receive 15:30 sessions, I would also know that receiving a 15:30 session was not *sufficient* to make the rats reset their daily routes.

Method

Subjects and Apparatus

The subjects and apparatus were the same as those used in Experiments 1, 2 and 3.

Procedure

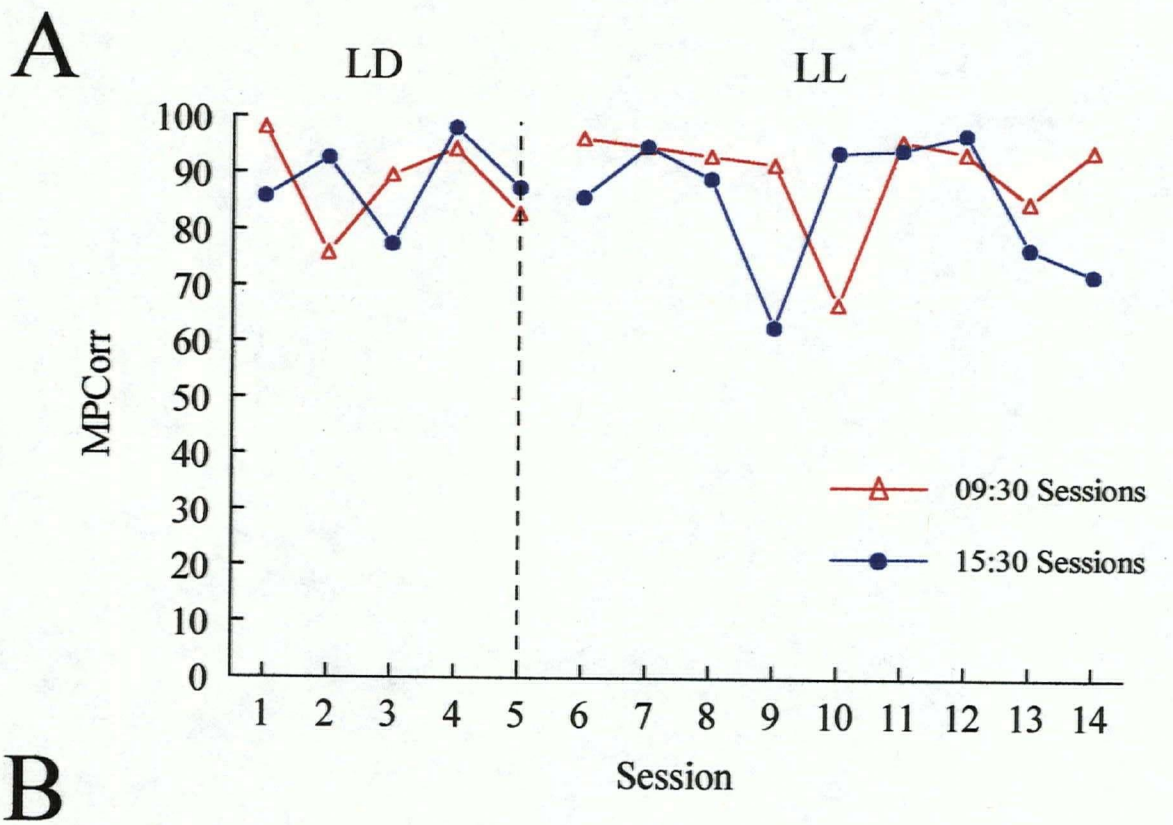
The rats received 5 days of LD sessions. During this time, the rats received their usual 09:30 and 15:30 test sessions and post-session meals. The colony was lit according to the usual 12:12 LD, 07:30 onset schedule. After the 15:30 test session on Day 5 the colony lighting was switched to LL for the next 9 days. During the LL period the rats received their usual 09:30 and 15:30 test sessions and post-session meals.

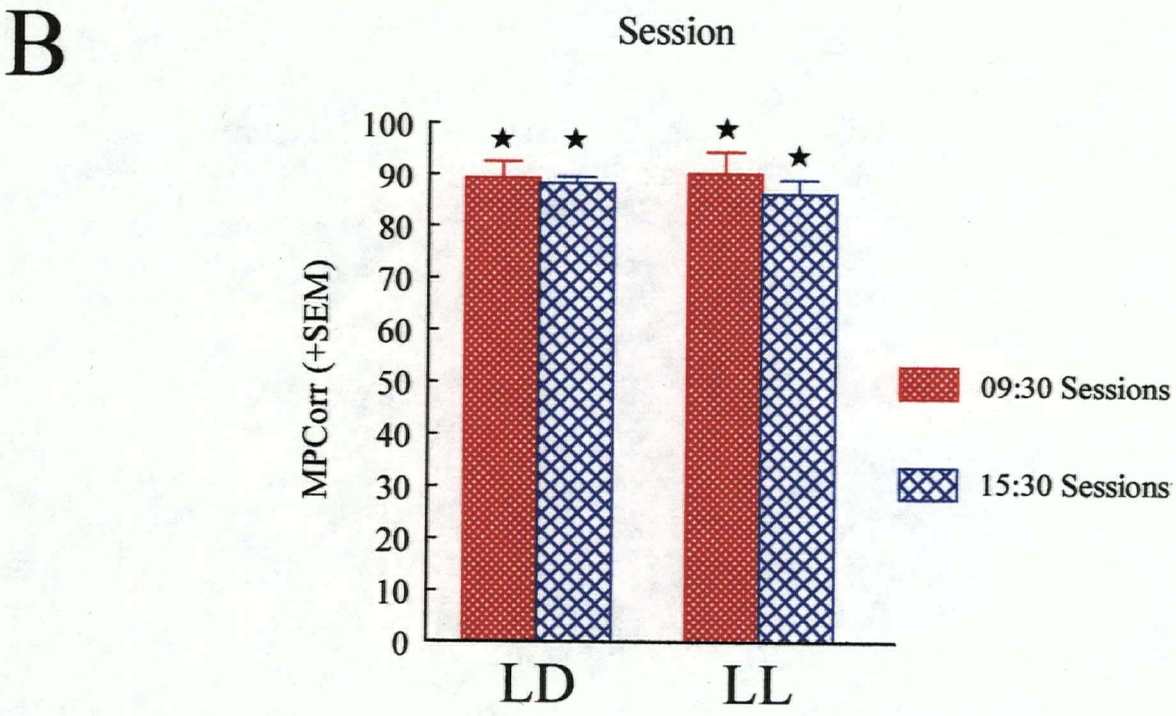
Results

Figure 9a presents the rats' daily overall 09:30 and 15:30 MPCorr scores during the 5-day LD period and 9-day LL period. During the LD period the rats continued to anticipate the location of food in both 09:30 and 15:30 sessions and again their MPCorr was roughly equal during 09:30 and 15:30 sessions. Their overall MPCorr was 89.4 ($SEM = 3.27$) in 09:30 sessions and 88.4 ($SEM = 1.32$) in the 15:30 sessions. The rats' overall MPCorr score during the LD period was significantly greater than a conservative estimate (50%) of chance performance in both the 09:30 and 15:30 sessions (one sample t-test for 09:30 sessions , $t(_3) = 12.07$, $p < .001$, and for 15:30 sessions $t(_3) = 29.16$, $p < .001$).

During the LL period, the rats continued to anticipate the location of food in both 09:30 and 15:30 sessions. During the first 3 days of the LL period the rats appeared to be completely unaffected by the lighting manipulation. Over the next few sessions, their performance appeared to become more variable, but at no point over the entire LL period did the rats' MPCorr during 09:30 or 15:30 sessions drop below 60%. During the LL period their overall MPCorr was 90.4

Figure 9. A) The rats' daily overall 09:30 and 15:30 MPCorr scores during the 5-day LD period and during the 9-day LL period in Experiment 4a. B) The rats' overall 09:30 and 15:30 MPCorr scores (+ SEM) during the LD and LL periods in Experiment 4a. Asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.


A
LD
LL
MPCorr
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Session
09:30 Sessions
15:30 Sessions


B
MPCorr (+SEM)
100
90
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70
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50
40
30
20
10
0
LD
LL
09:30 Sessions
15:30 Sessions

(*SEM* = 4.16) in 09:30 sessions and 86.4 (*SEM* = 2.82) in 15:30 sessions. The rats' overall MPCorr score during the LL period was also significantly greater than a conservative estimate (50%) of chance performance in both 09:30 and 15:30 sessions, (one sample t-test for 09:30 sessions , $t(3) = 9.7, p < .003$, and for 15:30 sessions $t(3) = 12.92, p < .002$).

Therefore, changing the colony lighting to a LL schedule did not appear to cause an immediate change in the rats' ability to anticipate the location of food in either 09:30 or 15:30 sessions. This is clearly seen in Figure 9B. This impression was supported by the results of a repeated-measures ANOVA. The time of a session (09:30 or 15:30) and the lighting condition (LL or LD) served as repeated-measures. This analysis revealed that there was no main effect of the time of a session, ($F(1, 3) = 2.52, p < .22$), nor was there a main effect of lighting condition, ($F(1,3) < 1, p < .91$). Although the rats' 15:30 MPCorr was slightly depressed in LL, the time of a session and the lighting condition did not interact significantly, ($F(1,3) < 1, p < .58$).

Discussion

The rats appeared to be largely unaffected by the change to a LL lighting schedule as there was no difference between their mean 09:30 or 15:30 MPCorr scores in the LD and LL periods. However, the rats MPCorr scores did appear to become more variable from the third day onwards in the LL period. Additionally, their 15:30 MPCorr decreased slightly in LL.

When viewed together the results of Experiments 2 and 3 suggest that the co-occurrence of *both* the daily LD cycle *and* a 15:30 test session was not necessary for the rats to expect food at their 09:30 levers during 09:30 sessions. But was the occurrence of *either* sufficient, and the occurrence of at least one of them *necessary*, for the rats to reset their daily routes? I addressed this possibility in Experiment 4c.

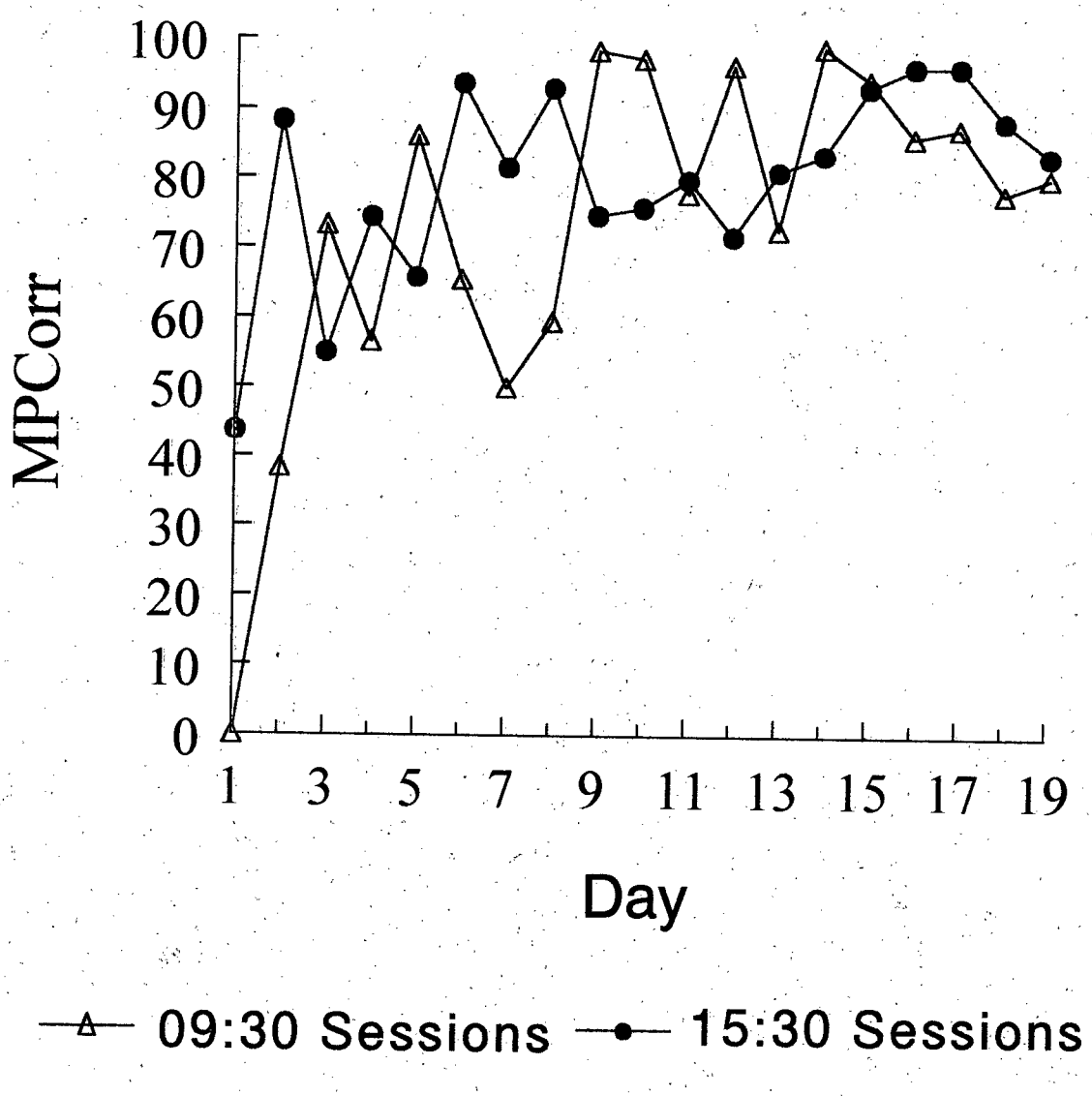
Free-Food and Reacquisition Period

Due to a vacation the rats did not receive their daily test sessions for the 2-week period following the completion of Experiment 4a. During this time the rats remained in their home cages and they received free access to food and water. At the end of the two week free-food period, the rats were returned to their original feeding schedule and they received 19 days of reaquisition and baseline training.

Figure 10 presents the rats' daily overall 09:30 and 15:30 MPCorr scores during the reacquisition period. The rats reacquired the task very quickly and by day 9 their overall MPCorr scores in the 9:30 and 15:30 sessions appeared to have asymptoted. This training effect was confirmed by the results of a repeated-measures ANOVA in which training days (1 through 19) and the time of session (09:30 or 15:30) served as a repeated-measures. This analysis revealed no main effect of the time of a session, ($F_{(1,3)} = 1.63$, $p<.30$) and a main effect of training, ($F_{(18,54)} = 2.74$, $p<.003$). The time of a session and training did not interact, ($F_{(18,54)} = 1.10$, $p<.39$).

During the last 11 days of the reacquisition period, the rats' overall MPCorr was 88.0 ($SEM = 2.90$) in 09:30 sessions and 84.1 ($SEM = 2.58$) in 15:30 sessions. During this time, the rats' overall MPCorr scores was significantly greater than a conservative estimate (50%) of chance performance in both 09:30 and 15:30 sessions, (one sample t-test for 09:30 sessions, $t_{(3)} = 13.1$, $p< .001$, and for 15:30 sessions $t_{(3)} = 13.21$, $p<.001$).

Figure 10. The rats' daily overall 09:30 and 15:30 MPCorr scores during the reacquistion period.


MPCorr
100
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30
20
10
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1
3
5
7
9
11
13
15
17
19
Day
09:30 Sessions
15:30 Sessions

Experiment 4b: Replication of the Constant Light Manipulation

In Experiment 4a I found that switching the rats' lighting from a LD to a LL schedule did not immediately effect their overall ability to anticipate the location of food in 09:30 and 15:30 test sessions. However in LL; (1) the rats' MPCorr scores seemed to become more variable, and (2) their 15:30 MPCorr was slightly depressed. To determine whether these are reliable phenomena I re-examined the effect LL had on the rats' time-place behaviour.

Method

Subjects and Apparatus

The subjects and apparatus were the same as those used in Experiments 1, 2, 3, and 4a..

Procedure

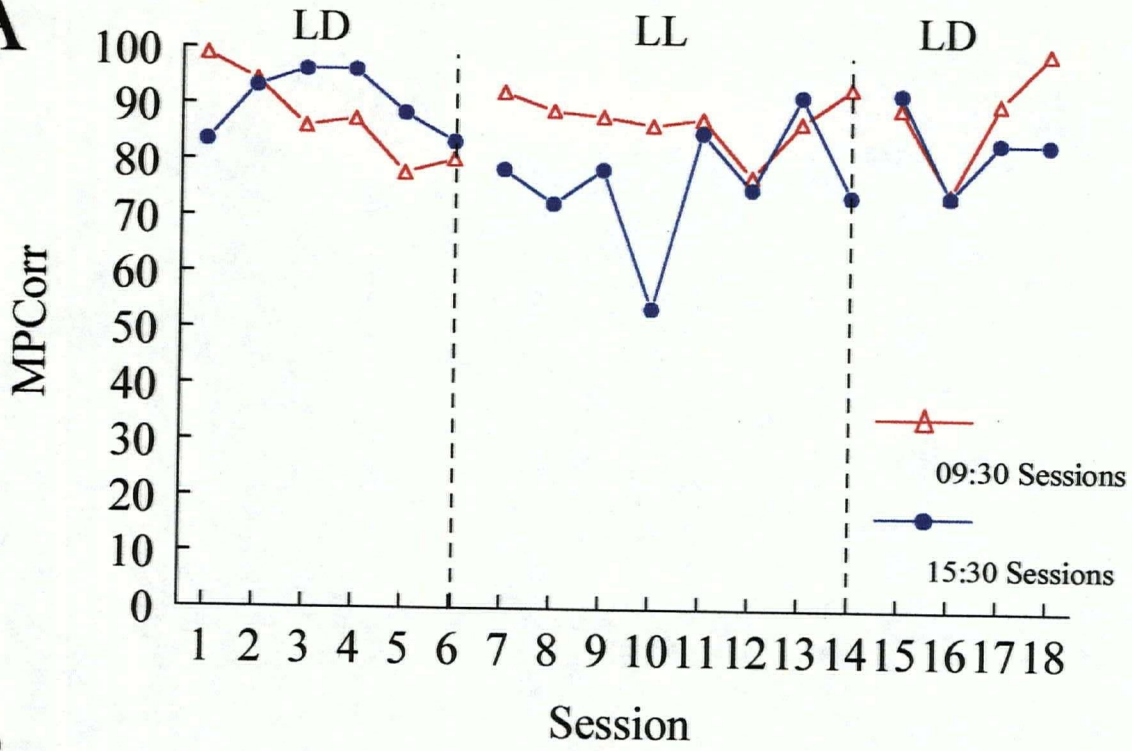
After the reacquisition training the rats were switched to a LL schedule. The rats were then held in LL for 8 days. During the LL period the rats received their 09:30 and 15:30 test sessions and post-session meals as per usual. I then returned the colony to the original 12:12, 07:30 onset, LD cycle for a further 4 days.

Results

Figure 11A presents the rats' daily overall 09:30 and 15:30 MPCorr scores for the last 6 days of the reaquisition period, the 8-day LL period, and for the first four days after the rats were returned to LD. During the LL period, the rats continued to anticipate the location of food in both 09:30 and 15:30 sessions, but again their MPCorr was depressed during 15:30 sessions (see Figure 11B). As in Experiment 4a the rats appeared to be largely unaffected by the change to LL for the first 3 days. Then their performance became more variable over the next few sessions. During the LL period their overall MPCorr was 87.83 (*SEM* = 5.47) in 09:30 sessions and 76.2 (*SEM* = 11.36) in 15:30 sessions. The rats' overall MPCorr score during the LL

Figure 11. A) The rats' overall 09:30 and 15:30 MPCorr scores during the 6-day first LD period, during the 8-day day LL period, and during the 4-day second LD period. B) The rats' overall 09:30 and 15:30 MPCorr scores (+ SEM) during the first LD, LL, and second LD periods in Experiment 4b. For A and B, asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.

A


LD
LL
LD
MPCorr
100
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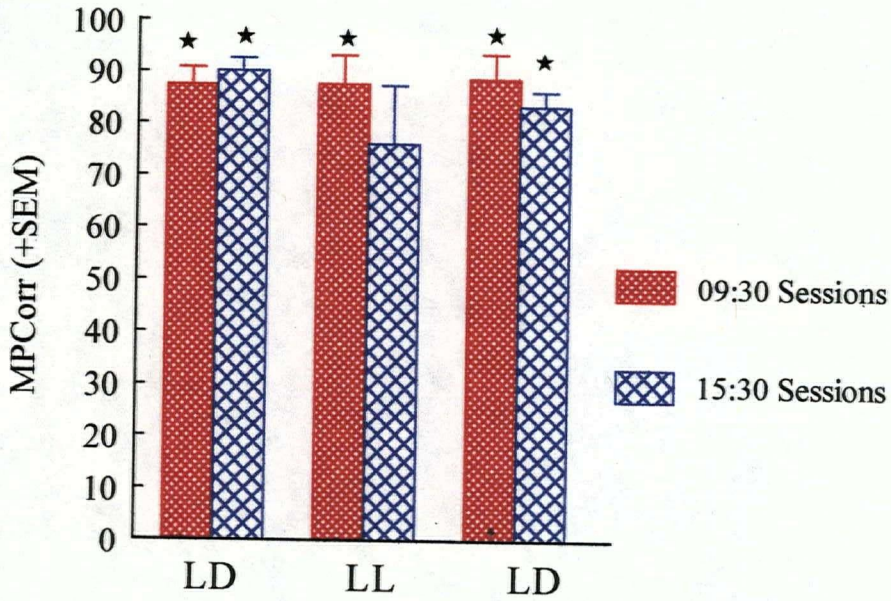
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Session
09:30 Sessions
15:30 Sessions

B


MPCorr (+SEM)
100
90
80
70
60
50
40
30
20
10
0
LD
LL
LD
09:30 Sessions
15:30 Sessions

period was again significantly greater than a conservative estimate (50%) of chance performance in 09:30sessions but not in 15:30 sessions, (one sample t-test for 09:30 sessions , $t(_3)= 6.91$, $p<$ .007, and for 15:30 sessions $t(_3) = 2.3$, $p<.11$). When the rats were returned to LD they continued to anticipate the location of food in 09:30 and 15:30 sessions, and their MPCorr during 15:30 sessions returned to near normal levels. Their mean MPCorr in the second LD period was 88.9 (*SEM* = 4.7) in 09:30 sessions, and 83.6 (*SEM* = 2.95) in 15:30 sessions. Both of these scores represent performance significantly greater than a conservative (50%) estimate of chance performance, ($t(_3) = 8.11$, $p<.005$, and $t(_3) = 11.38$, $p<.002$ for the 09:30 and 15:30 sessions respectively).

It appeared that I had again found that changing the colony lighting to a LL schedule did not cause a dramatic change in the rats' ability to anticipate the location of food in either 09:30 or 15:30 sessions. This impression was confirmed by the results of a repeated measures ANOVA. The time of a session (09:30 or 15:30) and the lighting condition (LD1, LL, or LD2) served as repeated measures. This analysis revealed that there was no main effect of the time of a session ($F(_{1,3}) = 4.48$, $p< .125$), nor was there a main effect of lighting condition, ($F(_{2,6}) = <1$, $p<.53$). Additionally, the session time and the lighting condition did not interact, ($F(_{2,6}) = 1.13$, $p<.38$).

Discussion

The rats' 09:30 MPCorr did not immediately decline when they were held in LL. Therefore, I again found that the rats' daily route was not necessarily reset by the daily transitions of the LD cycle. However, I again found that housing the rats in LL led to small decrease in their 15:30 MPCorr. Although this effect failed to meet statistical significance in Experiment 4a, it appears to be a reliable phenomena.

I have suggested that the rats' time-place behaviour was partially controlled by a secondary timing system. It is unlikely that this secondary timing system was a light-entrained phase timer for three reasons. First, housing the rats in LL for 9 days did not lead to a significant disruption of their time-place behaviour. Clearly if the rats' time-place behaviour was controlled by a light-entrained phase timer, placing the oscillator in free-run should cause a detectable disruption in the rats' time-place behaviour which increased as the LL period progressed. This was clearly not the case. Secondly, if this secondary timing system was a light-entrained phase timer it is not clear why the rats were more affected in 15:30 than 09:30 sessions when they were switched to LL. Thirdly, previous work has suggested that rats can acquire a daily-time place task with ablated SCN (Boulos & Logothetis, 1990; Mistleberger, 1994). This suggests that rats may use a food-entrained phase timing system in daily time-place learning tasks. Perhaps then, the rats' secondary timing system was a food-entrained phase timing system. As the food and light-entrained pacemakers appear to be weakly coupled in the rat (Mistleberger, 1994), the small decrease in the rats' 15:30 MPCorr could then be due to inference from a free-running light-entrained oscillator. Further work is clearly needed to address this issue.

Experiment 4c: Constant Light And Skipped 15:30 Sessions

To test whether the occurrence of *either* the daily transitions of the LD cycle *or* a 15:30 test session was necessary for the rats to reset their daily routes, I examined the effect skipping 15:30 sessions while in LL had on the rats' time-place behaviour. If while housed in LL, the rats pressed their 15:30 levers during the 09:30 sessions which followed a skipped 15:30 session, I would have strong evidence that the rats relied *solely* on the occurrence of either a 15:30 session

or the daily transitions of the LD cycle to reset their routes each day. If the rats' were not impaired when 15:30 sessions were skipped in LL, I would know some other event was capable of reseting the rats' route each day.

Method

Subjects and Apparatus

The subjects and apparatus were the same as those used in Experiments 1, 2, 3, 4a and 4b.

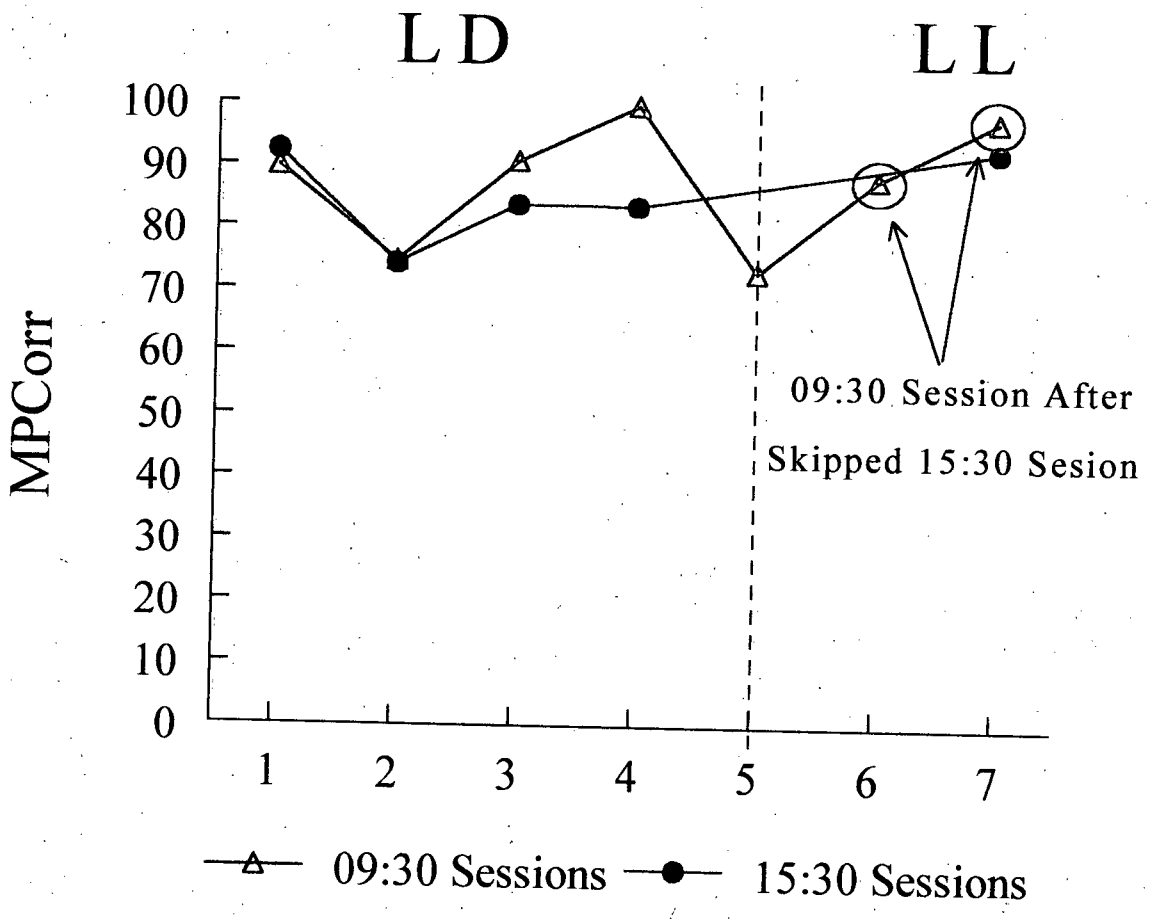
Procedure

The second LD period in Experiment 4b served as the four-day baseline period in Experiment 4c. On the fifth day the rats skipped their 15:30 sessions and they were held in LL that night. The next day the rats received their usual 09:30 test session, skipped their 15:30 session, and they were held in LL again that night. The following day, the rats received their 09:30 and 15:30 test sessions and their lighting returned to the usual 12:12 LD cycle. When the rats skipped 15:30 sessions they were treated as they were on probe days in Experiment 3.

Results

Figure 12 presents the rats' overall 09:30 and 15:30 MPCorr scores during the last four days of the second LD period in Experiment 4b and during the 2 days during which they were held in LL and skipped their 15:30 sessions. During the LD period the rats' overall 09:30 MPCorr was 85.8 (*SEM* = 5.1), and in the 09:30 sessions after the rats skipped their 15:30 sessions and were held in LL their overall MPCorr was 93.2 (*SEM* = 4.7). The rats' MPCorr scores in these two types of 09:30 sessions did not differ significantly, ($t_{(3)} = 1.15$, $p<.34$). Without a daily LD cycle *and without* a 15:30 session, the rats were clearly still able to anticipate the location of food in 09:30 sessions.

Figure 12. The rats' daily overall 09:30 and 15:30 MPCorr scores during the 4-day baseline period, and during the two days when they were held in LL and skipped their 15:30 sessions.


L D
L L
MPCorr
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
09:30 Session After
Skipped 15:30 Sesion
09:30 Sessions
15:30 Sessions

Discussion

The rats were clearly able to anticipate the location of food in 09:30 sessions when they skipped their previous 15:30 session *and* did not receive their daily 12 hr period of darkness. Clearly the occurrence of either of these two events was not necessary for the rats to reset their routes each day. As I outlined above, the rats' daily route could conceivably have been reset endogenously or exogenously. As I found that the two major exogenous temporally reliable cues available to the rats did not serve as necessary reset triggers, at least two potential remaining reset mechanisms exist. Firstly, their daily route could have been reset each day by another unknown contextual cue. Alternatively, their daily route could have been reset by an endogenous circadian phase-timer. I took care to remove any temporally reliable exogenous cues other than light onset, test sessions, and post-session meals. Additionally, the rats received test sessions 5 or 7 days per week over a period of more than half a year. Laboratory conditions varied tremendously over the course of each week and over the course of this series of experiments. Therefore, I believe it is unlikely that the rats daily route could be reset by some unknown temporally reliable contextual cue. Consequently, I suspect that the rats' daily route was reset when a circadian phase-timer reached a set phase angle each day. As the rats' time-place behaviour was not dramatically impaired when they were housed in LL for 9 days, this reset function was probably performed each day by a food-entrained phase timer.

Experiment 5: Interpolated Probe Sessions At 19:00

The results of Experiments 4a-c suggest that the rats *did not rely* on 15:30 test sessions or the daily transitions of the LD cycle to reset their daily route: when either, or both, of these events did not occur the rats still expected that food would be available at their 09:30 levers in

09:30 sessions. This suggests that some other mechanism was capable of resetting the rats' routes each day. I have suggested that this process may involve a food-entrained phase timer. But was the occurrence of a 15:30 session *sufficient* for the rats to reset their daily route? To address this question I examined where the rats expected food at 19:00 in two types of probe sessions. During the first type of probe session the rats received their 09:30 and 15:30 sessions, post-session meals, and a probe session at 19:00. During the second type of probe session the rats recieved their 09:30 sessions, *skipped* their 15:30 sessions, received both post-session meals, and a probe session at 19:00. I reasoned that as long as the rats' daily route was not reset by the mechanism that I detected in Experiments 2 and 4a-c before 19:00 each day, they would mainly press their 15:30 levers during the probe sessions at 19:00 which followed a skipped 15:30 session. However, I envisioned two possible outcomes in the probe sessions at 19:00 which did follow a 15:30 session. If the occurrence of a 15:30 session was sufficient for the rats to reset their daily routes they would prefer their 09:30 levers at 19:00. Alternatively, if the occurrence of a 15:30 session was not sufficient for the rats to reset their daily routes they would prefer their 15:30 levers at 19:00.

Method

Subjects and Apparatus

The subjects and apparatus were the same as those used in Experiments 1, 2, 3, 4a, 4b, and 4c.

Procedure

Experiment 5 lasted 14 days. Throughout this period the rats were held in their usual 12:12, 07:30 onset, LD cycle. Over this 14 day period the rats received 10 baseline days, and 4 probe days. On baseline days the rats received their 09:30 and 15:30 sessions and post-sessional

meals as per usual. On two of the probe days the rats received their 09:30 and 15:30 sessions and two post-session meals, plus probe sessions at 19:00. On the other two probe days the rats received their 09:30 sessions, skipped their 15:30 sessions, received their two usual post-session meals, and then probe sessions at 19:00. When 15:30 sessions were skipped the rats were treated as they were on probe days in Experiment 2. During the probe sessions at 19:00 the rats were treated as they were in probe sessions in Experiment 3. The rats' MP09:30, MP15:30, and MPInc scores during the initial unrewarded period of each session served as the dependent variable.

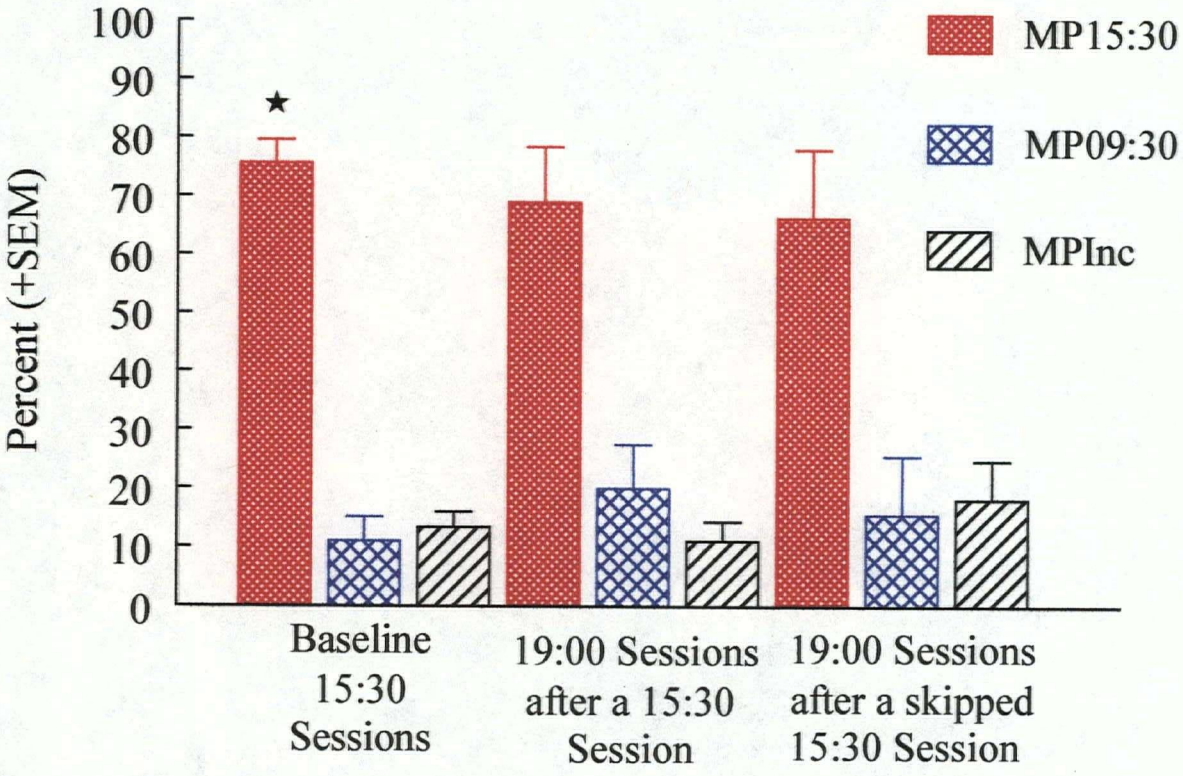
Results

Figure 13 presents the rats' MP09:30, MP15:30, and MPInc scores in the baseline 15:30 sessions and in both types of probe sessions at 19:00. The rats' overall MP15:30 was 75.7 (*SEM* = 3.9) in baseline 15:30 sessions, and this is greater than a conservative (50%) estimate of chance performance, ($t(_3)$ = 6.7, p<.008). Therefore, the rats clearly continued to anticipate the location of food in the baseline 15:30 sessions during Experiment 5.

The rats' overall MP15:30 score in the probe sessions at 19:00 which followed a 15:30 session was 66.4 (*SEM* = 10.1), and their overall MP15:30 score in the probe sessions at 19:00 which did not follow a 15:30 session was 69.1 (*SEM* = 6.6). Therefore, (1) in both types of probe sessions at 19:00, the rats preferred their 15:30 levers, and (2) the rats' overall MP15:30 scores in both types of 19:00 sessions were slightly lower than their overall MP15:30 score in baseline 15:30 sessions.

It therefore appeared that the rats' preference for their 15:30 levers at 19:00 did not depend on whether they received a 15:30 session that day. To examine this impression, I used a paired t-test to compare the rats' MP15:30 scores during each type of probe session. The rats'

Figure 13 The rats' overall MP09:30, MP15:30, and MPINC scores in the baseline 09:30 and 15:30 sessions, in the probe sessions at 19:00, and in the probe sessions at 19:00 after the rats skipped their 15:30 sessions. Asterisks indicate the corresponding mean is greater than a conservative (50%) estimate of chance performance, $p<.05$.


Percent (+SEM)
100
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★
MP15:30
MP09:30
MPInc
Baseline 15:30 Sessions
19:00 Sessions after a 15:30 Session
19:00 Sessions after a skipped 15:30 Session

preference for their 15:30 levers clearly did not differ in the two types of probes sessions, (t ($_3$) = .7, p<.88).

Discussion

During both types of probe sessions at 19:00 the rats expected that food would be available at their 15:30 levers. Clearly receiving a 15:30 session was not sufficient for the rats to reset their daily routes. This suggests that the daily reset mechanism that I detected in Experiments 2 and 4a-c was a necessary component of the rats' daily route strategy.

Although the rats clearly preferred their 15:30 levers at 19:00, their overall MP15:30 scores during both type of 19:00 probe sessions were slightly lower than their overall MP15:30 score during baseline 15:30 sessions. As the 19:00 probe sessions occurred 3.5 hr later in the day than 15:30 sessions, this reduction in the rats' preference for their 15:30 levers at 19:00 may reflect the operation of the secondary timing mechanism that I detected in Experiments 2 and 3.

GENERAL DISCUSSION

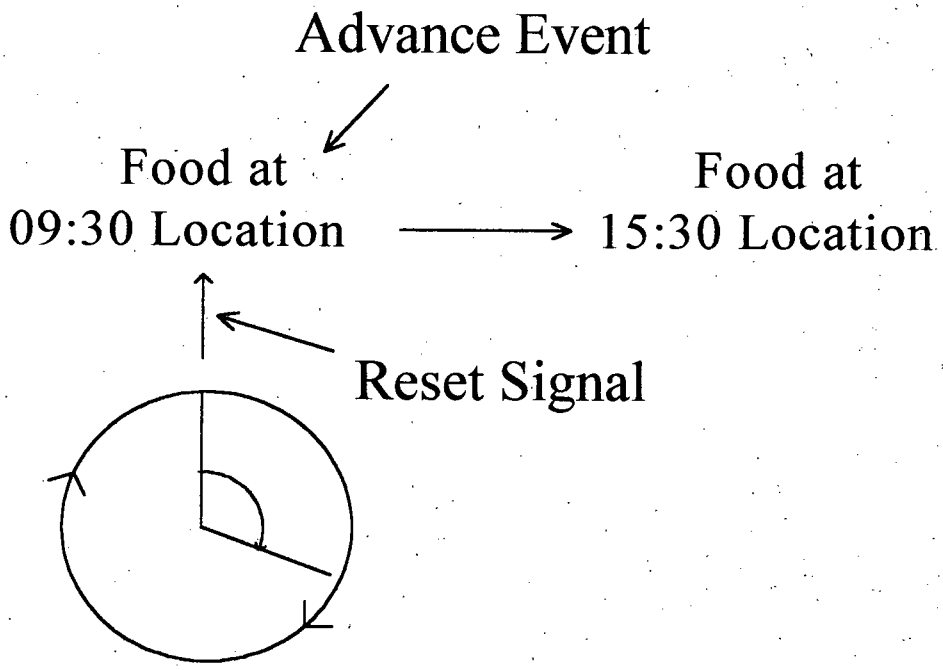
This series of experiments examined if, and how, rats track the location of a food source as it varies in space over the course of a day. Food was available at one location during morning sessions (2 hr after light onset), and at a second location during afternoon sessions (8 hr after light onset). The rats clearly learned the location of food in both morning and afternoon sessions. The rats primarily employed a daily route strategy to track the location of food. This strategy entailed lever pressing at one location during the first session of each day, and lever pressing at a second location during the second session of each day. This strategy displayed the following properties. Receiving a morning test session, and not the passage of time, was necessary and sufficient for the rats to expect food at their afternoon location during afternoon sessions. However, the rats did not use afternoon sessions to reset their routes each day. Nor did they use the daily transitions of the LD cycle to reset their routes each day. The rats' daily route may have been reset by some contextual cue other than the LD cycle. But as I outlined above, I suspect that this mechanism is unlikely. It is more likely that the rats reset their routes when a food-entrained circadian phase-timer reached a set phase angle each day. A schematic representation of this mechanism is presented in Figure 14.

Ordinal Timing

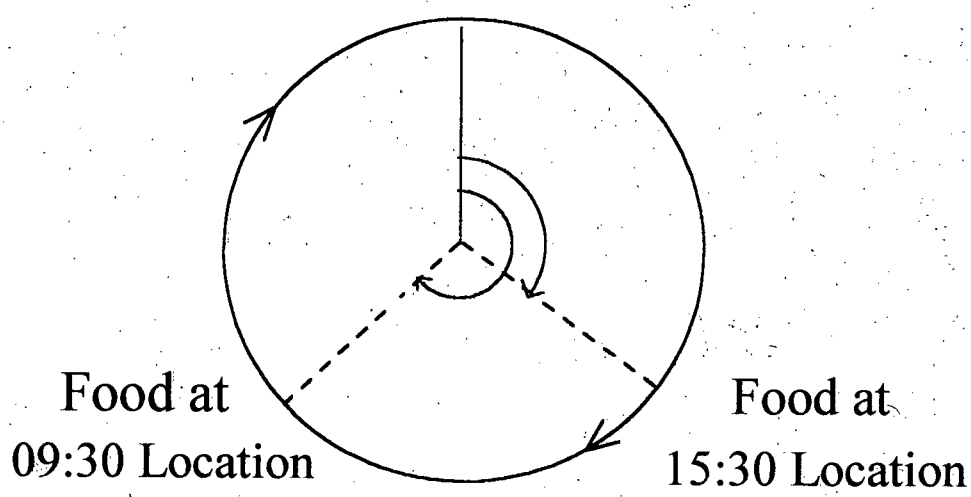
I propose that the daily route employed by these rats can be thought of as an exemplar of *ordinal timing*, or the *knowledge of the order of a set of events within a given period of time*. Admittedly, ordinal timing does not provide an animal with complex temporal knowledge. However, this ability does allow an animal to anticipate and exploit events which occur reliably in time. In this regard, ordinal timing is not different than phase or interval timing as they all tell an animal *when* something will occur. In other words interval, phase and ordinal timing all solve

Figure 14 A) A Schematic representation of the ordinal timing mechanism that rats employed to track the location of food in the present daily time-place task. B) A schematic representation of the secondary, food-entrained phase timer, the rats used to track the location of food in the present daily time-place learning task.

A


Advance Event
Food at
09:30 Location
Food at
15:30 Location
Reset Signal

B


Food at
09:30 Location
Food at
15:30 Location

the same basic problem. What does differ across ordinal, phase, and interval timing is the richness, or complexity, of the representation of time that they each provide an animal. I have found Gallistel's (1990a;1990b) computational-representational approach very useful when characterizing these types of timing. Accordingly, when I say a cognitive system represents temporal information, I mean that there is a formal correspondence between the operations of the timing system and the temporal properties of the resource it is tracking. Because of this formal correspondence between the operations of the representing and represented systems, or *functional isomorphism*, an animal is able to perform combinatorial operations within each timing system to generate the temporal information necessary for the animal to anticipate and exploit temporally graded resources. Furthermore, by determining the combinatorial operations which may be validly employed within each system I can, (1) characterize the temporal information provided by ordinal, phase, and interval timing, and (2) ascertain the relative richness of the representation of time provided by these three types of timing.

Levels of Timing

The most impoverished representation of time is captured by the proposed ordinal timing system. With an ordinal timing system an animal can only learn the *order* of events within a fixed period of time. This period of time can range from a day, as in the present series of experiments, to a few minutes, as in Terrace's simultaneous chaining paradigm (Terrace, 1993). Within this timing system the only valid combinatorial operations are equals (=), and earlier than (<) or later (>) than. Consequently, the ordinal timing system provides an animal with a representation of time which is at the ordinal level of measurement (Stevens, 1951).

Circadian phase-timing provides an animal with a more complex representation of time than an ordinal timer. With a circadian phase-timer an animal can learn the order of events

within a fixed period of time (the periodicity of the oscillator), and the phase angle of events from an arbitrary zero point of the oscillator. An arbitrary zero point is necessary as a circadian phase-timer does not have a true zero point. Consequentially, equal differences between the phase angles of events correspond to equal differences between their time-of-occurrence. However, it is impossible to use phase information to determine that some event is some proportion later or earlier *in time* than another event. For example, consider three events A, B, and C, which occur at phase angles of 60^0, 120^0, and 180^0 respectively from some arbitrary point in an oscillator. With this information I know that the amount of time between A and B is equal to the amount of time between B and C. However, as the phase angles of A, B, and C are based on an arbitrary zero point, is impossible to say that event C occurs three times later in time than event A. Because of these properties, equals (=), earlier than (<) and later than (>), and addition (+) and subtraction (-), are all valid combinatorial operations within this timing system. Consequentially, circadian phase-timing provides an animal with a representation of time which is at the interval level of measurement (Stevens, 1951).

An interval timer provides an animal with the most complex representation of time. With an interval timer an animal can the time of occurrence of an event from any defined point in time. Timing from any point in time is possible with an interval timer as it has a true zero point. Within this timing system equals (=), earlier than (<) and later than (>), addition (+) and subtraction (-), and multiplication (x) and division (/) are all valid combinatorial operations. Interval timing is therefore at the ratio level of measurement (Stevens, 1951).

Furthermore, I suggest that when an animal encounters a temporally graded biologically important event, it anticipates and exploits the resource using the timing system (or set of systems) best suited to representing, and therefore predicting, the event. For example, if the

occurrence of an event is best predicted by the amount of time that has passed since another event, an animal will use an interval timer to anticipate and exploit the event. If the occurrence of an event is best predicted by the time-of-day at which it occurs, an animal will use a circadian phase timer to anticipate and exploit the event. And finally, if the occurrence of an event is best predicted by its ordinal position in a recurring sequence of events, an animal will use an ordinal timer to anticipate and exploit the event. Finally, in situations where two or more timing systems (or sets of systems) are equally predictive, the least computationally complex timing system gains primary behavioral control.

How does this model account for the present data and the previous work on daily time-place learning in rodents and birds? Previous investigations of daily time-place learning with garden warblers and pigeons found that these birds used a phase timer to track the location of food in a daily time-place task (Biebach & Krebs, 1989; Saksida & Wilkie, 1994). There is also some evidence that rodents also use a phase timer in daily time-place learning tasks (Boulos & Logothetis, 1990; Daan et al. 1994; Mistleberger, 1994). However, I found that rats used a daily route, and not a phase timer, in a daily time-place task. This difference is unlikely solely due to task differences as the design employed in this study is almost identical to that employed by Saksida and Wilkie (1994). It is certainly possible that rats used a daily route in the present study out of necessity as they do not posses the robust circadian phase-timing ability demonstrated by birds. However, I believe the more likely, and intriguing, explanation is that rats are capable of phase timing but they used a ordinal timer in the present task as it is specifically designed to abstract the order of events within a period of time -- the essential temporal characteristic of food availability in the present time-place learning task.

To test whether rats are capable of using a phase timer to track a spatiotemporal regularity, I are currently examining how rats acquire a modified daily time-place task. In the new task, rats receive three types of training days; morning session only days, afternoon session only days, and morning and afternoon session days. This task is not solvable with an ordinal timer (e.g., daily route) but it is solvable with a phase or interval timer. Therefore, if the rats learn to anticipate the location of food in morning and afternoon sessions, and if their time-place behaviour displays the properties of a phase timer (i.e., self-sustaining, and phase shifts in response to Zeitgeber shifts), I will know that rats do posses a phase timing system which they can use to anticipate daily spatiotemporal regularities in food availability.

Other work in this lab supports my suggestion that when tracking a resource over time, animals use the timing system that best abstracts the temporal nature of the resource. This work used the *periodic time-place learning task*. In this task a subject receives test sessions in a 4 lever (or key) box at any time-of day. At the beginning of each session food is available at one location for a certain period of time. Food is then available at a second location for another period of time and so on throughout the course of the test session. Typically in these experiments each of the four levers (or keys) have produced food in succession. This time-place task is not best performed with a circadian phase timer as test sessions are short in length and occur at various times-of-the-day. Additionally, the use of only an ordinal timer (e.g., a fixed route) would not be the most efficient strategy in this time-place task as it would not allow an animal to anticipate the transition points when food moves from one location to another. Clearly, the periodic time-place learning task is best solved with an interval timer.

I have examined periodic time-place learning in pigeons and rats in sessions ranging in length from a few seconds to 60 min (Wilkie et al. 1994; Carr & Wilkie, in prep). Both pigeons

and rats were able to track the location of food throughout these test sessions. Their behaviour appeared to be controlled by an interval timer as they were able to anticipate the transition points when food moved from one location to another. Additionally, the subjects were able to stop and restart timing (Wilkie et al. 1994), and their timing performance displayed the scalar property across sessions of different lengths (Carr & Wilkie, in prep). Together, these results strongly suggest that rats and pigeons do use an interval timer to track the location of food in the periodic time-place task. Again, it appears that when animals are tracking the location of food in space over time, they employ the timing system which provides them with the best predictive ability.

While the rats' time-place behaviour in the present experiments was primarily controlled by an ordinal timer, I found that a second timing system had partial behavioural control. I suggested that this system was a food-entrained phase timer. The concurrent use of more than one timing system is also consistent with my multiple timing system model. In the present task, the location of food could be predicted by the ordinal, phase, and interval timing systems. However, the rats primarily used the ordinal system as it was the best predictor of the location of food. The other timing systems were also able track the location of food, but as their predictive ability was relatively lower, their behavioural control was correspondingly weak.

As I outlined above, previous work with pigeons (Saksida & Wilkie, 1994) and garden warblers (Krebs & Biebach, 1989) suggested that these birds primarily used a phase timer to track the location of food in a daily time-place task. However, both pigeons and garden warblers behaved like their time-place behaviour was *partially* controlled by a daily-route strategy. For example, when the pigeons did not receive their morning session, they displayed an increased tendency to peck their morning keys during the afternoon sessions which followed

a skipped morning session (mean increase = 25%, range 16% to 41% increase). This suggests that the pigeons were partially controlled by a daily route strategy similar to the strategy primarily employed by the rats in the present study. The garden warblers displayed a similar effect. In one experiment the warblers were prevented from entering any rooms during the first 3 hr period of a day. During this period the first room in their daily sequence usually provided food. After the locked period, all the rooms were unlocked and the warblers could then receive food in any room. At this time-of-day food was normally only available in the second room yet five of the nine birds clearly preferred the first room. This results suggests that the warblers' time-place behaviour was also partially controlled by a daily route. Consequentially I are left with the impression that behavioural control by more than one timing system is the norm rather than the exception.

Among others, Sutherland and Dyck (1984) have proposed that rats posses multiple spatial navigation mechanisms. These include praxis (response based), taxis (local cue based) and map based spatial navigation mechanisms. They also suggested that when faced with a spatial problem, rats use the spatial strategy which best fits the characteristics of the problem; when a task can only be solved using a praxis strategy that is the strategy rats adopt, whereas if the task can only be solved using a mapping strategy, that is the strategy rats adopt. Additionally, the representation of space employed in the spatial strategies described by Sutherland and Dyck range from minimal (in the praxis strategy) to very complex (in the map strategy).

The correspondence between my work in the temporal domain and Sutherland and Dyck's work in the spatial domain is striking and unlikely coincidental. It appears that spatial and temporal cognition in animals are not the products of a single general process mechanism,

nor are they the product of general process spatial and temporal mechanisms. Instead, animals posses a battery of different problem solving mechanisms within the temporal and spatial domains. All the mechanisms within each domain perform a similar function (e.g., they allow an animal to anticipate a temporal regularity or to navigate in space), but each mechanism is adapted to solve a particular *type* of problem. For example, interval timers are designed to time the amount of time between events, whereas ordinal timers are designed to abstract the order of events within a period of time. Perhaps these dedicated mechanisms are the products of environmental selection pressures on animals to solve a variety of *specific* problems over the course of evolution (Cosmides & Tooby, 1994). Consistent with this analysis, Sherry and Schacter (1987) have suggested that I should expect animals to posses multiple dedicated systems within each general problem domain as a single system which solves one problem well is, because of its' specialized nature, poorly suited to solving different types of problems.

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