TIME-PLACE LEARNING

by

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ABSTRACT

The ability to learn spatiotemporal characteristics of biologically significant events is advantageous for an animal and is known as *time-place learning* (TPL). Gallistel (1990) proposed an influential theory positing that whenever a biologically significant event occurred, a memory code was automatically formed, encoding the nature of the event, and the time and place in which it occurred. When the animal is later faced with a biological need it could consult these memory codes and determine when and where that need had been met in the past. This information could be used to guide current behaviour. Importantly, Gallistel theorized that the encoding of the spatiotemporal characteristics of an event into a tripartite code was an automatic process.

Despite the appealing power and simplicity of Gallistel's theory, I have provided arguments suggesting that it has serious limitations. Perhaps the most damaging evidence against this theory is the reluctance of rats to demonstrate daily TPL (i.e., events that vary in location depending on time of day). Widman, Gordon, and Timberlake (2000) argue that for TPL to occur the response cost for incorrect decisions must be high. While this hypothesis is unable to explain the inconsistencies in TPL, it does highlight the fact that animals do not automatically store time-place-event information as a tripartite code. If they did, it would not make sense for them to ignore such information in some tasks.

I have provided an alternative hypothesis that states that whenever a biologically significant event occurs two bipartite memory codes (time-event and place-event) are automatically formed. Only under some conditions, perhaps those with high response cost, do animals form tripartite codes. For this reason, rats often have difficulty learning a TPL task; although rats easily learn a place preference for those places that provide

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reinforcement (place-event), and easily learn a go/no-go discrimination (time-event). This thesis provides data from both the daily and interval TPL realms supporting the proposed theory of bipartite codes.

Although rats do not readily learn daily TPL tasks, they do demonstrate knowledge of interval TPL under a variety of conditions designed to enhance the ecological validity of the task. The properties of interval TPL are discussed.

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CO-AUTHORSHIP STATEMENT

I was the first author on all manuscripts presented in this thesis. In Dr. Wilkie's Comparative Cognition lab the first listed author is the senior author. I was involved in all stages of the research including, but not limited to, conception of the ideas, designing the experiments, conducting the studies, analyzing the data, and writing the manuscripts.

Research assistants and volunteers often helped with conducting the experiments, but always under my supervision. Mollie Bates and Vanja Petrovic worked on Chapters Two and Four respectively as part of Directed Studies projects and as such were given authorship on these manuscripts.

Donald Wilkie was also involved in all aspects of the research included in this dissertation. As well, he acted as PhD supervisor and is included as author on all manuscripts.

CHAPTER ONE: INTRODUCTION

It is sometimes said that the only constancy in life is change. Luckily that change is often predictable. The availability of biologically significant events such as food, mates, and predators generally varies systematically across both space and time. The ability to learn the spatiotemporal variability of these events would be advantageous because it would allow animals to maximally exploit resources. This ability has been demonstrated in a number of species and has come to be known as *time-place learning (TPL)*.

The importance of the spatiotemporal characteristics of biologically important events is reflected in Gallistel's (1990) theory of learning and memory. He theorized that whenever a biologically significant event occurs, a memory code is automatically formed that includes the nature of the event, as well as the time and place in which it occurred. When the animal is later faced with a biological need, it consults these time-place-event memory codes and determines when and where that need has been met in the past and uses that information to guide its current behaviour.

In Gallistel's theory, animals' temporal and spatial knowledge is of fundamental importance. The psychology of space and time has a long history. Until recently, the topics have been studied independently by different research groups. It is surprising that it took researchers so long to begin examining the conjunction of these two topics given that all events occur jointly in time *and* space. This dissertation focuses on animals' knowledge of temporal and spatial information.

Carr and Wilkie (1997a) distinguished among three timing systems: ordinal, phase, and interval. *Ordinal timing* allows an animal to predict the order in which events

occur within a time period. For example, breakfast, dinner, and supper occur in the same order each day, but the time of the day at which they occur may not be constant. It has been shown that rats sometimes learn the order in which different levers provide food within a day without learning the actual time of day that they do so (Carr & Wilkie, 1997b; 1999).

Phase timing allows an animal to predict events that are cyclic or occur with a fixed periodicity. This periodicity may be circadian, circalunar (tidal), or circannular. One of the first field studies of TPL observed oystercatchers, a type of seabird, who learned the timing of the tides (Daan & Koene, 1981). These birds flew from inland roosts to mussel beds that were exposed at low tide. Because of coastal irregularities, mussels were exposed at different times and spatial locations. The birds were able to learn the spatiotemporal variability of the mussels' availability as demonstrated by the ability to time their trips to the correct spatial locations, despite not being able to see the mussel beds from their roosts.

Interval timing allows an animal to predict an event that reliably occurs a fixed amount of time after some external event. For example, animals respond to a fixed interval (FI) schedule with a typical "scalloped" pattern of responding. Interval timers are thought to measure durations in the range of seconds to minutes.

It is important to realize that each of these timers rely on endogenous oscillators. This means that the temporal knowledge originates from within the animal, rather than relying on external indictors of time. The oscillators persist in running in the absence of external cues such as light (although the period may deviate slightly) or regularly scheduled meals. Animals may use endogenous oscillators to measure the duration of

time passage (interval timing) or they may consult the oscillator to determine the time of day, month, or year (phase timing).

Each of these three timers is specialized for measuring durations of varying lengths. In addition, these timers have unique characteristics. Ordinal timers are resetable and incorporate sequence information. Phase timers are self-sustaining, entrainable, and run continuously. Interval timers have stopwatch properties of start, stop, reset, and restart (Carr & Wilkie, 1997a). The existence of multiple timing systems may have evolved because the characteristics that make one timing system efficient are *functionally incompatible* with the efficient performance of the other system (see Sherry & Schacter, 1987 for a detailed discussion of this concept; see Wilkie, 1995 for a discussion of the different requirements of interval and phase timing).

This dissertation is written in a manuscript-based format rather than the traditional format. Each of Chapters Two through Seven are meant to be stand-alone manuscripts containing their own Introduction and Reference sections, while Chapters One and Eight are the General Introduction and Discussion of the entire dissertation. Some of the chapters have already been published and others are drafts that will be submitted in the near future. While this format has many advantages, it has two clear limitations. The first is that there must necessarily be a certain amount of repetition and for that I beg your forgiveness and patience. The second is that each chapter does not necessarily flow into the next. To help combat this particular limitation I will clearly lay out the objectives of each chapter in this Introductory chapter and attempt to tie the findings of each chapter together in the General Discussion (Chapter Eight).

This dissertation can be broken down into two parts. The first part (Chapters Two and Three) focuses on the reluctance of rats to learn *daily* time-place contingencies, how this may require revision of Gallistel's theory of learning and memory, and the formulation of an alternative theory to explain this reluctance. In no way is this meant to suggest that rats are incapable of daily TPL, only that they do not automatically store time-place-event information in a tripartite code as suggested by Gallistel. The second part (Chapters Four through Seven) examines the properties of *interval* TPL using what is assumed to be more ecologically valid designs. Each of these two parts will now be discussed in turn.

Daily Time-Place Learning

Timing Mechanisms

The majority of research on TPL has been done using a daily time-place task in which food is available at one spatial location at one time of day and at another spatially distinct location at another time of day. Biebach, Gordijn, and Krebs (1989) provided the first laboratory demonstration of daily TPL. In their experiment, garden warblers were tested for 12 h each day in a chamber that was divided into a central living area and four compartments. Each compartment contained a grain dispenser that provided grain on an intermittent schedule for a specific 3-h period each day. It was found that the birds entered the correct rooms at the correct times and anticipated when the rooms would provide food as demonstrated by their tendency to enter a room before it started providing food. Furthermore, on probe sessions in which no food was given, the birds continued to enter the rooms at the correct times. Anticipation and performance on test sessions suggest that the birds were using an endogenous timer to track the availability of

food. To verify that the warblers were using a circadian timer (rather than an interval timer), tests were conducted in which the light-dark schedule was changed so that the lights remained on for 24 hours a day. This change did not disrupt the birds' performance on the first day of the test. However, over continued days of testing, the periodicity dropped from 24 hours to approximately 23 hours (free running time). These results suggest that the timer used was a circadian timer that was entrained to the light-dark cycle (Biebach, Falk, & Krebs, 1991).

There is also evidence to suggest that, in addition to using a circadian timer in this task, the birds also use an ordinal timer (Carr & Wilkie, 1997b). Krebs and Biebach (1989) prevented warblers from entering any of the rooms for the first 3 h period. The warblers were then given free access to all of the rooms. If their behaviour was driven solely by a circadian timer, then the birds should have gone to Room 2; if, however, their behaviour was driven by an ordinal timer, they should have gone to Room 1. In fact, five of the nine warblers preferred Room 1, suggesting their behaviour was at least partially controlled by an ordinal timer.

Daily TPL has also been demonstrated in pigeons using an operant chamber that contained a key on each of its four walls (Saksida & Wilkie, 1994). The chamber was transparent to allow distal room cues to be available. Pigeons received two daily sessions: one in the morning and one in the afternoon. One key provided food in morning sessions, whereas another key provided food in afternoon sessions. The first few pecks never provided reinforcement and were recorded to see to which key the pigeons responded. Pigeons quickly learned to peck the correct key. To verify that the pigeons were timing, rather than using a non-temporal strategy such as alternation, skip session tests were

conducted in which either the morning or afternoon session on a probe day was omitted. If pigeons were alternating, they should respond on the incorrect lever following all skip sessions. If they were using an ordinal timer, they should respond on the correct lever following skip PM sessions, but not skip AM sessions. Following skipped sessions, the pigeons still responded on the correct lever, thus ruling out an alternation or ordinal timing strategy. Manipulations of the light-dark cycle, similar to those discussed in the Biebach et al. (1991) paper, suggested that the pigeons were using a light entrained circadian timer. Although not noticed by Saksida and Wilkie (1994), Carr and Wilkie's (1997a) re-analysis of these data found evidence that the pigeons, like the warblers, displayed some control by an ordinal timer.

In a similar study, Carr and Wilkie (1997b, 1999) observed TPL in rats. Rats were tested twice daily, once in the morning and once in the afternoon, in a chamber similar to that used for pigeons that had a lever mounted on each of its four walls. For each rat, one lever provided food during the morning sessions, whereas a different lever provided food during the afternoon sessions. Rats learned to press the appropriate lever depending on the time of day. Interestingly, skip session probe tests conducted after the rats had successfully learned the discrimination revealed that the rats were not using a circadian timer, but rather an ordinal timer; that is, they learned the order in which the places provided food during the day. In the afternoon, after a skipped morning session, the rats responded on the morning lever; however, in the morning of a day following a skipped afternoon session, the rats responded on the correct morning lever. The latter finding suggests that the ordinal timing system was reset by some (unknown) end of day or start of day cue. Another possibility is that the rats could not remember the afternoon

omission; however, this possibility is unlikely given the rat's well known spatial memory ability.

Other researchers have shown that rats sometimes use a circadian rather than an ordinal timer on a TPL task. Pizzo and Crystal (2002) successfully trained rats on a daily TPL task in which food was available at one of three locations depending on the time of day. There were two groups: Group AB-C received two sessions in the morning and one session in the afternoon, whereas Group A-BC received one session in the morning and two sessions in the afternoon. To determine whether a circadian or ordinal timer was in effect, the time of Session B was shifted either very late or very early. It was found that by shifting the time of Session B, performance dropped significantly contrary to what would be expected from ordinal timing. (Interval timing from light onset was ruled out by switching the rats to a continuous light schedule.)

Similarly, Mistlberger, de Groot, Bossert, and Marchant (1996) successfully trained rats to press a lever in one arm of a T-maze in morning sessions and a lever in the other arm in afternoon sessions. An alternation strategy was ruled out through skip sessions. Interestingly, rats with lesions of the suprachiasmatic nucleus (SCN; a brain area known to govern circadian rhythms) were still able to learn this task, suggesting that the rats were not using a light entrained circadian timer (see Mistlberger, Antle, & Kilduff, 2003; Mistlberger & Marchant, 1995 for discussion of food versus light entrained circadian timers). The authors suggested that the circadian timer used was probably food entrained. Using corticosterone levels, Lukoyanov, Pereira, Mesquita, and Andrade (2002), also found evidence to suggest the use of a food entrained timer in daily

TPL tasks. The research cited above suggests that during daily TPL tasks, rats make use of either an ordinal timer or a food-entrained circadian timer.

Difficulty Demonstrating Daily Time-Place Learning

Laboratory studies have shown daily TPL in honey bees (Wahl, 1932 as cited in Reebs, 1993), ants (Schatz, Beugnon, & Lachaud, 1994; Schatz, Lachaud, & Beugnon, 1999), fish (inangas) (Reebs, 1999), warblers (Biebach et al., 1989), pigeons (Saksida & Wilkie, 1994), and rats (Carr and Wilkie, 1997b; 1999). Interestingly, daily TPL is sometimes elusive. This is surprising given that Gallistel's (1990) theory posits that TPL is a low-level, automatic process that should be fairly easy to demonstrate in a variety of animals and circumstances. However, recent publications suggest that this may not be the case.

Reebs attempted to demonstrate daily TPL in fish for the last decade with only limited success. In 1993 he fed cichlid fish in different corners of their aquarium at different times of day. Although the fish learned the times at which food was given (as demonstrated by increased searching of the corners during the feeding times), they did not limit their searching to only the correct corner suggesting they did not learn which corner was associated with which time of day. Reebs speculated that this failure to learn the time-place association may have been due to the low response-cost ratio associated with not going to the correct place initially; that is, not much energy was expended, and there was no punishment for going to the incorrect location. At the correct time of day, the fish could swim around the aquarium until they found the food. Alternatively, the

failure to learn the time-place association may have been due to interference among the locations.

Reebs (1996) was able to demonstrate TPL in another fish, the golden shiner. However, the fish were only able to learn the spatiotemporal association when two places were involved. When the number of associations was increased to three, the fish no longer successfully anticipated the location of food. Similar findings were reported with another fish, the inanga; however, TPL was not shown with an aversive stimulus, namely simulated heron attacks (Reebs, 1999).

Boulos and Logothetis (1990) trained rats to press one lever during a 1 or 2 h time period and another lever located on the opposite side of the testing chamber during another 1 or 2 h time period. Only half of the rats showed pressing of the time appropriate lever on no-food test sessions. The remaining rats showed a preference for one lever over another. White and Timberlake (1990, unpublished data cited in Widman, Gordon, & Timberlake, 2000) were also unable to demonstrate TPL in rats using a paradigm similar to that of Boulos and Logothetis (1990). Furthermore, they could not demonstrate learning on a three arm radial maze in which each arm provided food at a particular time of day.

Means, Ginn, Arolfo, and Pence (2000b) trained rats to go to one arm of a T-maze in morning sessions and to the other arm in afternoon sessions for food with only limited success. Only 63% of rats were able to learn this task after significant training. Furthermore, with continued training, rats' performance actually decreased. In addition, when external time-related cues such as noises and feeding were minimized, the trials to

criterion increased. Skip session probes suggested that the rats likely used an ordinal timer.

In a later study, Means, Arolfo, Ginn, Pence, and Watson (2000a) again showed that rats have difficulty learning a spatiotemporal contingency in a T-maze. Neither making the two places more distinctive, nor making the two times farther apart during the day significantly increased rats' success rate on the task. Yet, rats were able to learn a time-of-day go/no-go discrimination. In this task, food was available on both arms of the T-maze in one session, but no food was available in the other session at a different time of day. Rats' latency to leave the start arm of the T-maze was significantly shorter on food sessions than on no-food sessions.

Widman, Gordon, and Timberlake (2000) discovered that, by increasing the response cost in TPL tasks, they increased the probability that the rats would learn the task. (While Widman et al. (2000) use the term response cost, an equally descriptive term would be effort.) In a simple radial arm maze similar to those described above (i.e., one arm baited in morning sessions, another arm baited in afternoon sessions), the rats did learn a general place preference (i.e., they learned which two arms were baited), but they did not learn when those places were baited. By switching to a vertical maze and increasing the height the rats had to climb for food (and thereby increasing the response cost), they increased the success rate for TPL. Two other studies have also provided evidence suggesting that an increase in response cost can result in an increase in acquisition of daily TPL. Lukoyanov et al. (2002) found that rats that were severely food-restricted (60% of ad libitum daily portion) were more likely to learn a daily TPL task conducted in the Morris water maze, than rats fed ad libitum. As Widman, Sermania, and

Genismore (2004) point out, the food-restricted rats were likely in a state of high metabolic need. Given that the rats were required to swim, it is reasonable to assume that there was a high response cost associated with incorrect responding. Widman et al. (2004) increased response cost in rats by putting weights on them and requiring them to find a platform in the Morris water maze. The weighted rats learned the TPL contingency, while the non-weighted rats did not (Widman, Sermania, & Genismore, 2004).

However, there are some obvious inconsistencies in the daily TPL literature that Widman et al.'s response cost theory cannot explain. Some researchers have found daily TPL in operant response tasks (Carr & Wilkie, 1997, 1999; Mistlberger, de Groot, Bossert, & Marchant, 1996; Pizzo & Crystal, 2002), whereas others using very similar paradigms (and similar reinforcement schedules) have failed to find it (Boulous & Logothetis, 1990; White & Timberlake, 1990). Although Widman et al. (2000) believe that response-cost ratios (the relative cost of making an error) are the critical determinant of whether an animal learns a TP task, it is not clear why this should be the case.

Objectives

Chapter Two contains a series of experiments showing that rats have difficulty learning daily TPL contingencies using a variety of tasks including a place preference task, an avoidance task (Morris water maze), radial arm maze and T-maze. Because our statistical tests are based on the null hypothesis, it is difficult to prove that an animal did not learn a task; however, converging evidence from several researchers using various manipulations and tasks allows for easier justification of this claim.

Chapter Two also contains a series of experiments designed to increase success rate. Methods included increasing the distinctiveness of the time, place and event portions of the time-place-event code. Whenever timing was demonstrated, skip session probes were conducted to determine the type of timer involved.

Animals are clearly capable of learning daily TPL tasks as discussed above. However, converging evidence from Chapter Two as well as that conducted by various researchers (e.g., Means et al., 2000a, b; Widman et al., 2000) show that rats do not readily encode a tripartite time-place-event code as proposed by Gallistel (1990). Chapter Three formulates an alternative theory stating that animals automatically encode two bipartite (time-event and place-event) codes when faced with biologically significant events.

Interval Time-Place Learning

Interval TPL differs from daily TPL in the length of duration being timed. In daily TPL the location of stimuli varies depending on the time of day (i.e., 24 h). Whereas, in interval TPL, the location of stimuli varies depending on how much time has passed since the onset of some stimulus. The duration of the timed event is usually in the seconds to hours range.

Much of the knowledge about the timer involved in interval timing comes from research employing the peak procedure. In this procedure, animals are first trained on a fixed interval (FI) task in which there is a signal indicating the start of a trial. Once the animal has learned this task, peak probe trials are introduced, in which the signal remains on for approximately twice as long as the interval being timed and no reinforcement is

given. When the response rate on these peak probe trials is plotted as a function of time elapsed since signal onset, a normal distribution with the peak response rate at the usual reinforcement time is obtained (e.g., S. Roberts, 1981). The width or spread of the response rate distribution is taken as a measure of error in the timing system. If the response rate distributions are acquired for many different FI durations, it can be seen that, as the duration of the FI timed increases, the error (spread) of the response distributions increases proportionally. Therefore, interval timing is said to obey Weber's Law, a property also known as scalar timing (e.g., Cheng & Roberts, 1991; Gibbon, 1991). Scalar timing is also evidenced in interval time-place learning. Carr and Wilkie (1998) trained three groups of rats on an interval time-place task in which the levers provided reinforcement for either 4-, 6-, or 8-min. As expected from the Scalar Timing Theory, the greater the duration, the greater the error.

Numerous theories have been developed to explain interval timing (e.g., Church & Broadbent, 1990; Gibbon, Church, & Meck, 1984; Killeen & Fetterman, 1988; Machado, 1997; Miall, 1989; Staddon, Higa, & Chelaru, 1999). The three most influential theories will be discussed in turn. The first is an information processing theory called *Scalar Expectancy Theory (SET*; Gibbon, Church, & Meck, 1984). This theory states that there is an internal clock consisting of three stages: clock, memory, and decision. The *clock stage* consists of a pacemaker that is thought to emit pulses with a certain periodicity. These pulses are then transmitted through a gate into an accumulator. The accumulator holds the total number of pulses received from the pacemaker until such time as it is reset. The *memory stage* consists of both working and reference memory. If for some reason the stimulus being timed is temporarily stopped, for example in the gap

procedure, the value in the accumulator can be stored in working memory. When the stimulus being timed is followed by a biologically significant event, such as food, the value in the accumulator is stored in reference memory and can later be used to guide behaviour. If the subject learns that the biologically significant event usually occurs after a set amount of time has passed, it can use its internal timer to tell it when to expect the event. For example, if a rat learns that it will be given a piece of food every 30 s, it can compare the value in its accumulator to the values it has stored in reference memory to determine when it will be reinforced again. This is the third stage of the internal clock, the *decision stage*. If the value in the accumulator is close enough to the value in reference memory by some predetermined decision rule, the rat will expect food.

In the *Behavioural Theory of Timing (BET)* the pacemaker is started upon signal onset and drives an animal through different "behavioural" states with each pulse of the pacemaker. Each of the states may be of different durations and the responses associated with the states may differ in terms of rate and intensity. Furthermore, the speed of the pacemaker increases with the rate of reinforcement. Therefore, short intervals will be timed more accurately than long intervals (scalar timing). The BET does not include clock, comparators, or decision components (Killeen & Fetterman, 1988).

Oscillator/Coincidence Detection Theories (e.g., Beat Frequency theory; Miall, 1989) do not have a pacemaker-accumulator component. Instead, there are a variety of fast oscillator periods (~5-15 HZ) that are initiated upon signal onset. The time code consists of those neurons that fired spikes at the criterion time. Matell and Meck (2000) argue that this theory is superior because it can be mapped onto a neural framework – namely the firing of cortical neurons can act as the oscillators.

Interval TPL differs from interval timing in two important ways. First, in the TPL task there is an association between time and place. Second, the duration of time being measured is much longer (minutes to hours) in the TPL task than in the interval timing (seconds) task. In the Biebach, et al. (1989) paper (discussed in the daily TPL section), warblers were trained to go to four different chambers which provided food in consecutive 3-h periods. These birds used a circadian timer to solve the task. This is not to say that an interval timer could not have been used – no one has yet determined what the upper and lower limits of the interval timer are. To ensure that animals are using an interval timer rather than a circadian timer, the animals are trained at different times of day so that the time of day information is irrelevant.

Wilkie and colleagues (Wilkie & Willson, 1992; Wilkie, Saksida, Samson, & Lee, 1994) tested pigeons in a modified version of the Biebach et al. (1989) task. Pigeons were placed in a transparent box that contained a key and a hopper on each of the four walls. Lights signalled the start of the session. Each key provided reinforcement on a variable interval schedule for 15 min. Only one key provided reinforcement at any given time, and the order in which the keys provided reinforcement remained constant. Three major findings were observed from the pigeon TPL experiments. First, the pigeons restricted the majority of their responding to the key that was providing reinforcement. Second, the pigeons *anticipated* the arrival of food on a key as shown by their tendency to start responding on a key immediately prior to when it started providing reinforcement. Third, when given probe tests in which a reinforced key stopped providing reinforcement partway through the active period, the pigeon continued to peck at the appropriate key. These three findings suggest that the pigeons were timing rather than using a non-timing

strategy. One such non-timing strategy is to simply discriminate when the keys are providing food. The pigeon would then peck on a key as long as it provided reinforcement (win-stay) and move to another key when it failed to give reinforcement (lose-shift).

Wilkie et al. (1994) went on to determine some characteristics of the timer in interval TPL. Through the use of probes in which the lights were turned off and no reinforcement was given and probes in which the pigeons were temporarily removed from the chamber partway through the session, it was determined that the interval timer used in this task has the properties of stop, restart, and reset.

Research has also been conducted into the properties of interval TPL in rats using a similar paradigm as in pigeons. Rats also restrict the majority of their responding to the correct lever and anticipate reinforcement on an upcoming lever. In addition, they anticipate depletion of reinforcement on a lever before it stops providing reinforcement (Carr & Wilkie, 1998). While anticipation indicates the use of a timing strategy, open hopper test (OHT) probe sessions confirm this. On OHT probes, all levers provide reinforcement on a variable ratio schedule for the complete duration of the session. Rats continue to move from lever to lever at approximately the correct times despite there being no contingencies in effect to necessitate their doing so (Carr, Tan, Thorpe, & Wilkie, 2001). It is worth noting that there is more variability in OHT sessions than in baseline sessions, suggesting that rats are able to discriminate when a lever stops providing reinforcement in baseline sessions and use this information to keep error from accumulating.

There are two possible timing strategies that the rats could use in this task. First, they could time from the start of the session and move when the timer reaches three consecutive criterion values (time session strategy). Secondly, they could time each lever separately (time location strategy). If the rats use a time session strategy we would expect the error (spread) to increase across levers, whereas if they use a time location strategy we would not expect the error to increase. It was found that the error did not increase across levers suggesting that the rats timed each individual lever (Carr & Wilkie, 1998). It is not known however what starts the timer (although a likely possibility is the first reinforced response on a lever).

Objectives

The second purpose of this PhD thesis is to further elucidate the properties of interval TPL in the rat using more ecologically relevant procedures. By doing this the ecological validity of interval TPL is increased. Ecological validity is the extent to which the behaviours observed in the artificial laboratory can be generalized to natural settings. This is particularly important in the interval TPL domain given that, to date there have been no field studies examining interval TPL. (There have been field studies examining daily TPL learning however. Wilkie, Carr, Siegenthaler, Lenger, Liu, and Kwok (1996) found that scavenging birds were able to learn the spatiotemporal variability of food.) This is not to say that we would not expect to find evidence of interval TPL in the natural environment, only that it has not been explicitly studied yet.

In the typical interval TPL design some attempts are made to create ecological validity. Rats are reinforced in the interval TPL task on an intermittent schedule to mimic

the fact that in the natural environment animals do not receive reinforcement after every response. As well, animals have access to a variety of spatial cues in the natural environment, therefore rats are given access to large distal cues in the laboratory. Chapters Four through Seven challenge the limits of interval TPL using more ecologically valid designs and procedures.

First rats were given a distraction during the interval TPL task as might happen when a foraging animal must chase away a competitor (Chapter Four). Secondly, a group of rats was trained on an interval TPL task in which the levers provided food reinforcement for varying durations. In addition to discovering if rats were capable of learning this task, the errors made on each lever were analyzed to determine if they conformed to Weber's Law (Chapter Five). Thirdly, a group of rats was trained on a task in which there were eight "places" rather than the usual three or four, and each place provided food more than once during the session (Chapter Six). Finally, two groups of rats were trained with differing reinforcement contingencies for each lever. Both the capability of rats to learn the task and the effect of reinforcement rates on amount of anticipation were of interest (Chapter Seven).

Note on Methodology

Throughout my dissertation, whenever possible, the small-N design advocated by Sidman (1960) is used. In traditional research designs, the behaviour of many subjects is averaged. One of the rationales behind large-N designs is to control for variability. Sidman argues that a better approach is to reduce the variability by controlling extraneous confounding variables. In my case, I controlled for variability by training subjects until

their performance reaches a steady state. This often entails a large number of training days. At this point, manipulations are introduced and their effect on behaviour is determined. In addition, the effects that I am looking for are usually so pronounced that sophisticated statistical tests are not necessary. A final advantage of small-N designs is an ethical one – a reduction in the number of animals used in research.

Note on Terminology

Throughout this dissertation the term *biologically significant event* is used. There are many possible interpretations of this term. In the human domain, one might mean something that induces an episodic memory or a "flashbulb" memory. In the learning domain, one might mean something that invokes one-trial learning such as taste avoidance. For the purposes of this dissertation, I define biologically significant events as events that increase the biological fitness of an individual. These events would include, but are not limited to, food, predators, and mates.

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CHAPTER TWO: RATS HAVE TROUBLE ASSOCIATING ALL THREE PARTS OF THE TIME-PLACE-EVENT MEMORY CODE[1]

We live in a world that is always changing, sometimes predictably and sometimes not. The availability of food, mates, predators, and other biologically significant stimuli varies across both space and time. If these stimuli vary predictably, it is advantageous for animals to learn this spatiotemporal variability, so that they might maximally exploit these resources. The ability to learn spatiotemporal variability has become known as *time-place learning.*

In *The Organization of Learning*, Gallistel (1990) put forth a theory that stated that whenever a biologically significant event occurs, a memory code is made that includes the nature of the event, and the time and place in which it occurred. When the animal is later faced with a biological need, it can consult these memory codes and determine when and where that need has been met in the past, and use that information to guide its current behaviour.

Carr and Wilkie (1997) distinguished between two different types of time-place learning on the basis of the length of the time periods involved. On *interval time-place tasks* the temporal intervals extend for several seconds or several minutes. On *phase time-place tasks* the temporal intervals extend over a day or longer intervals. (There exists an extensive literature on interval time-place learning (e.g., Carr, Tan, Thorpe, & Wilkie, 2001; Thorpe & Wilkie, 2002; Wilkie, Saksida, Sampson, & Lee, 1994; Wilkie & Willson, 1992), unfortunately it is beyond the scope of the present paper. The current article will examine only circadian or daily time-place learning.)

Phase time-place learning has been demonstrated in several species in both field and laboratory studies. In one of the first field studies, Daan and Koene (1981) observed foraging oystercatchers. These birds flew from inland roosts, over dykes, to mussel beds that were exposed at low tide. Because of coastal irregularities, mussels were exposed at different times at different spatial locations. The birds were able to learn the spatiotemporal variability of the mussels availability as demonstrated by the ability to time their trips to the correct spatial locations, despite not being able to see the mussel beds from their roosts.

Biebach, Gordijn, and Krebs (1989) provided the first laboratory demonstration of time-place learning. In their experiment, garden warblers were tested for 12 hr each day in a chamber which was divided into a central living area and four compartments. Each compartment contained a grain dispenser which provided grain for a specific 3 hr period every day. Other laboratory studies have shown similar time-place learning in honey bees (Wahl, 1932 as cited in Reebs, 1993), pigeons (Saksida & Wilkie, 1994) and fish (inangas) (Reebs, 1999).

Carr and Wilkie (1997) have also observed time-place learning in rats. In their experiment, rats were tested twice daily, once in the morning and once in the afternoon, in a chamber which had a lever mounted on each of its four walls. For each rat, one lever provided food during the morning session, while a different lever provided food during the afternoon session. Rats learned to press the appropriate lever depending on the time of day. Interestingly, probe tests conducted after the rats had successfully learned the discrimination revealed that the rats were not using a circadian clock but rather engaged

in ordinal timing. That is, they learned the order in which the places provided food during any given day.

From Gallistel's (1990) theory we are led to believe that time-place learning is an almost automatic process and that it should be fairly easy to demonstrate in a variety of animals and circumstances. However, a few recent publications suggest that this may not be the case.

Reebs has investigated time-place learning in fish for the last decade. In 1993 he published a paper in which cichlid fish were fed in different corners of their aquarium at different times of the day. While the fish learned the time at which food was given (as demonstrated by increased searching of the corners during the feeding times), they did not learn which corner was associated with which time of day (that is, they did not limit their searching to only that corner). Reebs speculated that a few different factors could explain this lack of learning. First, perhaps this failure to learn the time-place association was due to the low response-cost ratio associated with not going to the correct place initially. That is, not much energy was expended and there was no punishment for not going to the correct location. As long as the fish knew the correct time of day, they could swim around the aquarium until they found the food. Second, the failure to learn the time-place association may have been due to the interference in memory from learning various associations. That is, maybe they learn that it is in Corner A, but then the next time it is in Corner B, then Corner C, etc. These different associations between places and food could interfere with one another.

In 1996, Reebs was able to demonstrate time-place learning in another fish, the golden shiner. However, the fish were only able to learn the time-place association when

two places were involved. When the number of time-place associations was increased to three the fish no longer successfully completed the task. In 1999, he replicated this ability to associate two places with different times of day for a food reward in another fish, the inanga. However, he was unable to show time-place learning for an aversive stimuli, namely simulated heron attacks.

Means, Arolfo, Ginn, Pence, and Watson (2000) showed that rats have difficulty learning a time-place association in a T-maze. Rats were trained that one arm of the T-maze provided food in the morning session while the opposite side provided food in the afternoon session. Neither making the two places more distinctive nor making the two times farther apart during the day significantly increased their success rate on the task. Rats were able to learn a time-of-day go/no-go discrimination, however. In this task, food was available on both arms of the T-maze in one session, but no food was available in the other session at a different time of day. Rats' latency to leave the start arm of the T-maze was significantly less on food sessions than on non-food sessions.

Widman, Gordon, and Timberlake (2000) discovered that by increasing the response cost in time-place tasks they increased the probability that the rats would learn the task. In a simple radial arm maze similar to those described above (i.e., one arm baited in the morning sessions, another arm baited in the afternoon sessions), the rats did learn a general place preference (i.e., they learned which two arms were baited) but they did not learn when those places were baited. By switching to a vertical maze and increasing the height the rats had to climb for food (and thereby increasing the response cost), they increased the success rate for time-place learning. Widman et al. also point out that this seems to be a contradiction of what would be expected from Gallistel's theory.

That is, rats do not seem to automatically store and/or retrieve time-place-event information from their memory store.

The present research sought to replicate previous studies showing that rats have difficulty learning daily time-place tasks. It is difficult to prove that an animal did not learn a task because our statistical tests are based on the null hypothesis. However, if we have converging evidence from several researchers using various manipulations and tasks we can more easily justify this claim. We attempted to show time-place learning using a variety of tasks including a place preference task, an avoidance task, namely the Morris water maze, radial arm maze and t-maze. Finally, we sought to understand why the rats might have difficulty with these tasks.

Experiment 1: Time-of-Day Place Preference Task

All previous time-place tasks have involved the subject exploring various places and finding food in one location at one time of day and in another location at another time of day. The first experiment attempts to demonstrate time-place learning in a task in which the subject does not get to explore all locations at all times. Here the subject is not given the opportunity to learn that sometimes the places are not associated with food – the rat is only ever in a place when there is food. Previous studies have shown that when rats receive reinforcement in an environment and are later given the opportunity to spend time in that environment or in another, rats prefer the environment in which they had received reinforcement. This is known as place preference learning (see Calcagnetti & Schechter, 1992; Fleming, Korsmit, & Deller, 1994; Spyraki, Fibiger, & Phillips, 1982). The present experiment is a slight modification of the traditional place preference task.

Rats were given two exposures to the place preference box – one in the morning and one in the afternoon. One side of the box was associated with food in the morning while the opposite side was associated with food in the afternoon. During the training, the rats had no choice in which side of the box they were placed. Later rats were given free access to both sides in a morning and an afternoon test session and the time spent in each side was recorded.

Method

Subjects

Three male Long Evans rats were obtained from Charles River (St. Constant, Quebec). All rats were approximately 1 year old and experimentally naïve when testing began. They were individually housed in large, opaque plastic cages which contained Carefresh (Absorption Corp., Bellingham, WA) bedding and paper products that were available to build nests. The colony room was maintained on a 12 hr light-dark cycle, with light onset at 0730 and offset at 1930. Rats were behaviourally enriched approximately two to three times per week. During enrichment, rats were placed in a chamber containing plastic toys (e.g., tubes, ladders, boxes) for 20 min. Rats were fed 20 g of standard rat diet (PMI Feeds, Inc., St. Louis, MO) each day during the experiment and had free access to water at all times. Rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

Materials and Apparatus

The place preference box (Figure 2.1) was made of clear Plexiglas. The box measured 61 cm by 52 cm, with a height of 41 cm. Inside the box consisted of a black
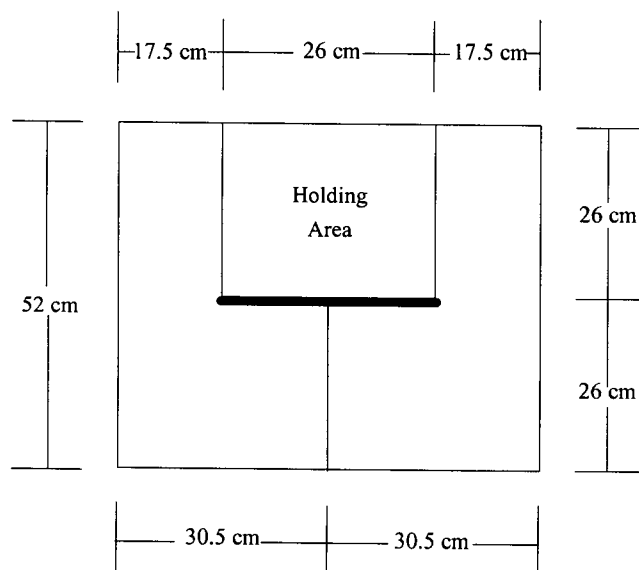
Figure 2.1. Place preference box used in Experiment 1. The dark line on the holding area represents the door that can be removed to allow the rat access between the two sides on test sessions.

Plexiglas partition, which split the box into two sides, creating the two places from which the rat could choose. The black partition was 26 cm long and 41 cm high. A rat holding area was located in the back of the box, with black Plexiglas walls. The holding area measured 26 cm by 26 cm, and was situated equidistant from either exterior side wall. The black partition connected to the holding area. There were two openings in the holding area (one on either side of the partition) through which the rat could gain access to either side of the box. In order to control such access, the front wall of the holding area slid up and out, acting as a door controlled by the experimenter. All interior and exterior Plexiglas walls measured 5 mm thick. The bottom of the testing area was lined with Bed O'Cobbs bedding.

Procedure

Rats were given two daily sessions, one in the morning (between 0930 – 1100) and one in the afternoon (between 1430 – 1600). Each rat was assigned a morning side and an afternoon side. This was quasi counterbalanced across rats. During each session the rats were placed into their corresponding side where there was 10 g of standard rat food. They were then given 20 min in which to eat the food. On those rare occasions in which the rat did not finish the food in 20 min, the remaining food was put into their home cage immediately following the session. During these daily sessions the door was closed so that rats did not have access to the other side of the box. After 40 sessions of exposure to these conditions, rats were given a morning and an afternoon test session.

Test sessions were conducted at the same time of day as previous exposure training. During test sessions the rats were placed into the holding area. No food was

present. The door was lifted and rats were able to move freely from one side of the box to the other. Time spent in each side was recorded.

Because the rats did not display a preference for either side, a once daily condition was added in which one side of the box was made more distinctive from the other by lining it with white opaque paper and the same rats were given only one trial per day in which they received 10 g of food. (The other 10 g was given at the end of the day between 1630 and 1730.) The time of this one daily session varied across days. Rats were exposed to this once daily condition for 15 sessions before they were given a test session that was identical to the ones given before.

Following the once daily sessions the rats were put back onto the previous twice daily condition. (The added distinctiveness to one side of the box remained.) Again, another set of two test sessions was given and time in each side was recorded. These tests sessions were conducted after the rats had received 17 sessions back on the twice daily protocol.

Results

Figure 2.2 shows the time spent in each side for all three conditions. As can clearly be seen in the left hand panel, the rats developed a preference for the side that was associated with food in the once daily condition ($t(2) = 20.05, p = .002$). However, the rats did not develop a preference for the side associated with food only at a certain time-of-day ($t(2) = .86, p = .48$ for the first test and $t(2) = .21, p = .85$ for the second test).
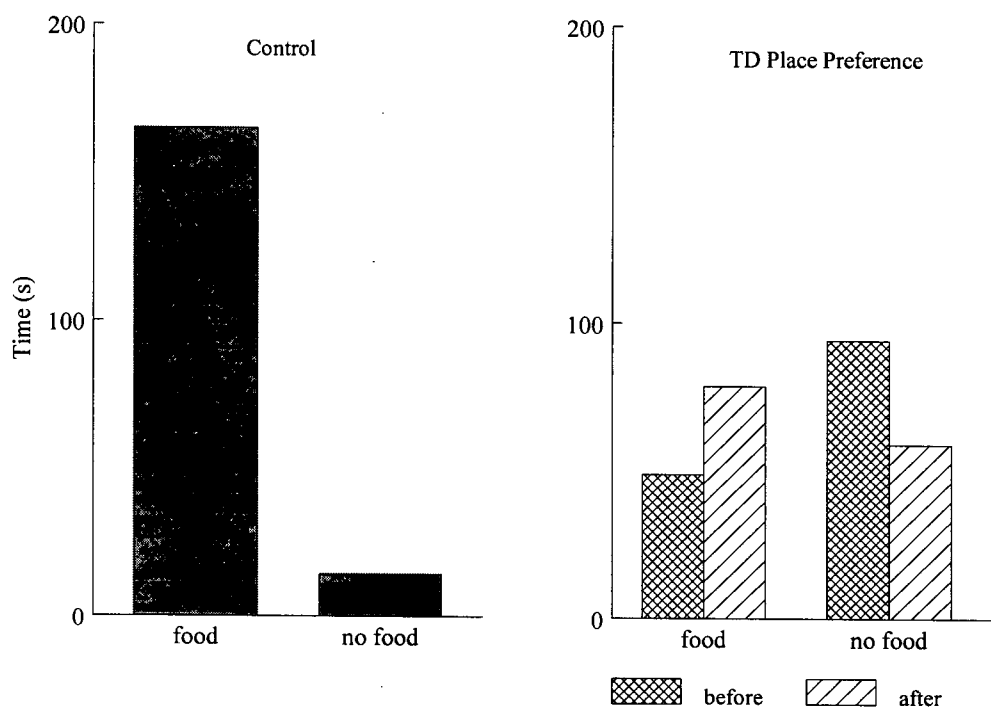
Figure 2.2. Time (s) spent in each side of the place preference box during test sessions for Experiment 1. Food refers to the side that is normally associated with food at that time of day while no food refers to the side that is not associated with food. The left panel shows the data for the once daily test sessions while the right panel shows the data for the time-of-day associated condition. The time-of-day associated condition has data for two tests: one done before the once daily test and one done after.

Discussion

In test sessions, the rats did not prefer the side that was associated with food at the time of testing. This was true even when the distinctiveness of the two sides was increased. Rats had a preference for the side that was associated with food only if there was just one session per day. It is possible that the rats did not attend to the time-of-day in this paradigm because it was not necessary for their success. That is, they were always given food regardless of their behaviour. The experiments that follow all demand that the rat make a choice (with the possible exception of Experiment 5).

Experiment 2: Aversive Time-Place Task (Morris Water Maze)

All previous studies of time-place learning in the rat have used food reward as the motivator. Only one study has used a non-food motivator – the study of simulated heron attacks on the inanga (Reebs, 1999). In this study, shoals of five inangas (*Galaxias maculates*) were kept in a large tank which had an opaque partition to divide the tank into halves. A small space was left open between the far edge of the partition and the wall so that the fish could move from one side of the tank to the other. Twice a day (once in the morning and once in the afternoon) a wooden model of a white-faced heron's bill (a natural predator of the inanga) was used to strike the water in the tank several times. In the morning, the strikes would be administered from one side of the tank (e.g., the right side) and in the afternoon the strikes would be administered from the opposite side of the tank (e.g., the left side). Because inangas had previously displayed time-place learning based on food (Reebs, 1999), it was hypothesized that they would also demonstrate time-place associations based on predation risk by avoiding the appropriate side of the tank at

the appropriate time of day. Interestingly, the inangas did not show a preference for the "safe" side of the tank at the time just prior to the heron strikes. The fish were afraid of the heron once they saw it (i.e., they quickly moved to the other side of the pool), but they did not anticipate the heron strike by moving to the other side of the pool before the heron appeared. In light of this study, the following question can be raised: Would other species, such as rats, exhibit time-place learning in response to an adverse situation, or is food the primary motivation for this phenomenon?

Method

Subjects

Five male Long Evans rats were obtained from Charles River (St. Constant, Quebec). All rats were 5 months old and experimentally naïve when testing began. Housing and enriching were the same in all experiments. In this and all following experiments, rats were fed standard rat chow to maintain their weight at approximately 90% of free-feeding weight (adjusted for age). Feeding occurred 30 – 60 min after the end of the testing day (i.e., following the afternoon session). We therefore cannot determine if any observed circadian timing was the result of food or light entrainment (Mistlberger & Marchant, 1995).

Materials and Apparatus

The maze was a circular pool with a diameter of 193 cm. The water was filled to a depth of 70 cm and was rendered opaque by the addition of powdered nontoxic white paint (Washable Dry Temp, Palmer Paint Products, Troy, MI). The platform was a

square piece of mesh (17 x 8.5 cm) placed on a cylindrical jar and was located 3 cm below the surface of the water. There were several distal visual cues on the walls surrounding the maze (e.g. posters, a lamp), the locations of which remained unchanged throughout the experiment. A stopwatch was used to time the amount of time spent on the platform during the latent learning phase training. A computer tracking system was used to measure the amount of time spent in each quadrant (HVS Image, Hampton, UK) during the active learning phase training and test sessions. The testing room contained an opaque screen which separated the pool from the tracking equipment.

Procedure

Each rat was randomly assigned a morning platform location and a different afternoon platform location. Possible platform locations were north-west quadrant, north-east quadrant, south-west quadrant and south-east quadrant. Platforms were placed in the center of the appropriate quadrant. For the first phase of this experiment (Phase 1), rats were trained using a latent learning technique (for a detailed description of latent spatial learning see Keith & McVety, 1988). During a morning training session, rats were placed on a platform located at their morning position for 60 s. The experimenter remained behind an opaque screen during this time and removed the rat after 60 s had elapsed. Rats that fell off the platform were guided back to the platform by the experimenter if they did not immediately climb back on. Each rat performed five trials per training session. Afternoon training sessions were conducted in the same fashion with the exception that rats were placed on the platform located at their afternoon position. Training was done twice daily, with morning sessions occurring between 0900-1030 and

afternoon sessions between 1400-1530. After two weeks of training, test sessions were conducted (one in the morning and one in the afternoon) in which the rats were placed in the water at random locations around the edge of the pool and time spent in each quadrant was recorded. There were no platforms in the pool during test sessions and rats were removed after 60 s. Rats performed two trials during test sessions.

Because rats failed to learn their platform locations after latent learning training, an active learning technique was employed (Phase 2). During active learning training sessions, rats were placed in the water at random locations around the edge of the pool and were required to swim to the platform. If rats did not find the platform within 60 s, they were guided to it by the experimenter. Rats were left on the platform for 10 s before they were removed. Active learning training was otherwise identical to latent learning training. After 2 weeks of active learning training, test sessions were conducted (one in the morning and one in the afternoon) which were identical to the previous test sessions.

Results

Figure 2.3A shows the mean percent of time spent in the correct quadrant, the opposite quadrant, and the other two quadrants during the morning and afternoon test sessions of the latent learning phase. The correct quadrant is defined as the quadrant where the platform is located during training sessions, the opposite quadrant is defined as the quadrant where the platform is located during the opposite daily training session (e.g. the afternoon quadrant if the testing is occurring in the morning), and the other two quadrants are defined as quadrants which never contain platforms during either daily
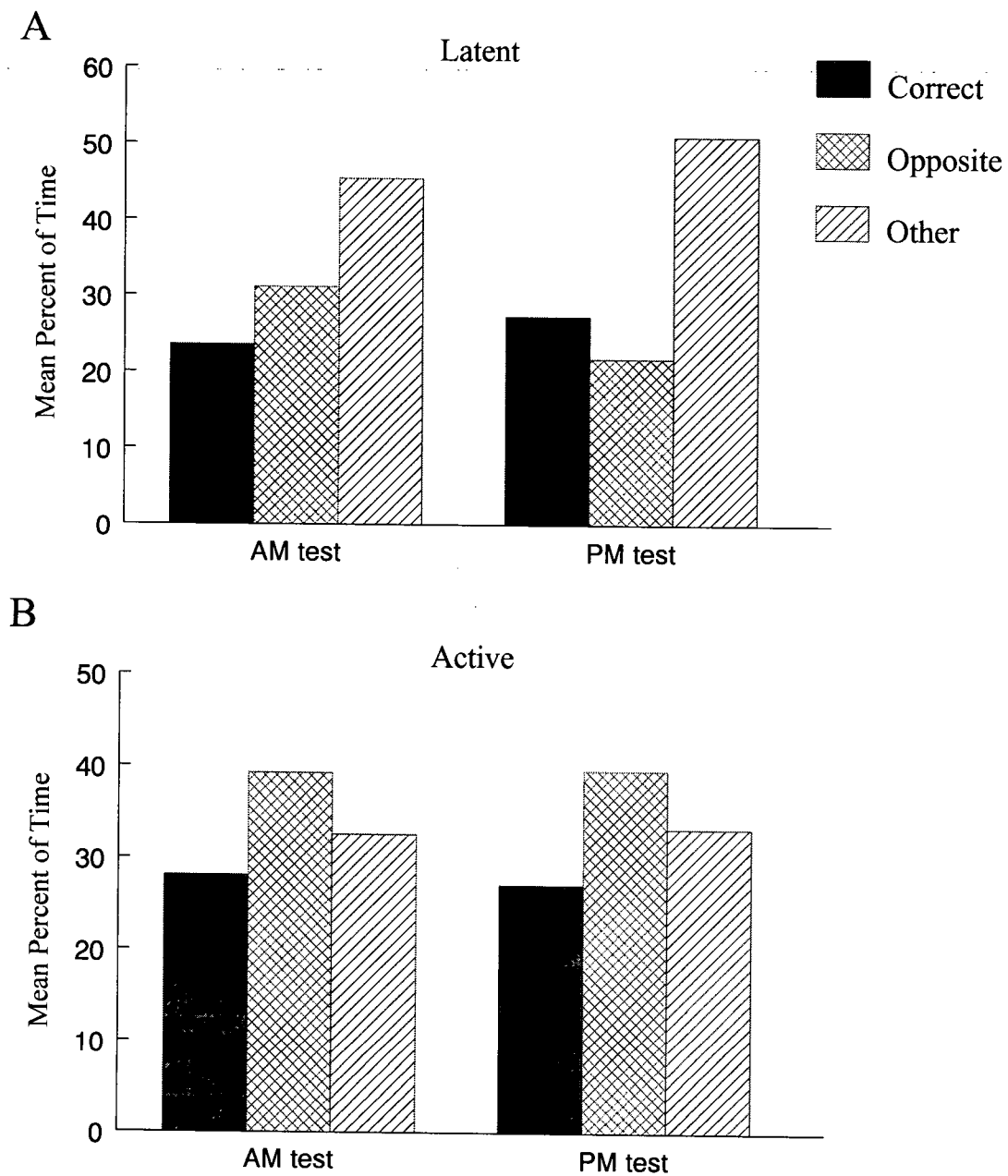
Figure 2.3. Mean percentage of time spent in the correct, opposite, and other quadrants during morning and afternoon test sessions of the latent (A) and active (B) learning phases of Experiment 2.

session. Two-tailed paired t-tests revealed that rats spent significantly less time in the correct quadrant compared to the other three quadrants ($t(4)= 7.99, p= .001$). In addition, the correct and opposite quadrants were compared to determine if the rats had learned a time-of-day discrimination. It was found that the rats did not spend significantly more time in the correct quadrant compared to the opposite quadrant ($t(4)=0.55, p=.61$). The combined time spent in the correct and opposite quadrants was analyzed to determine if the rats had learned a general place discrimination (i.e., which two quadrants provided food). No significant differences existed between the combined amount of time spent in the correct and opposite quadrants compared to the combined time spent in the other two quadrants ($t(4)= 1.01, p= .37$).

Figure 2.3B shows the mean percentage of time spent in the correct quadrant, the opposite quadrant, and the other two quadrants during the morning and afternoon test sessions of the active swimming phase. Rats spent significantly less time in the correct quadrant compared to the other three quadrants ($t(4)= 4.76, p= .009$). Again, rats did not spend significantly more time in the correct quadrant compared to the opposite quadrant ($t(4)=1.74, p=.16$). They did however spend significantly more time in the combined correct and opposite quadrants compared to combined time spent in the other two quadrants ($t(4)= 3.68, p= .02$).

Discussion

During both the latent and active learning phases of the water maze experiment, the rats spent significantly less time during test sessions in the quadrant that typically had the platform at the time of day in which they were being tested. This could suggest that the rats were avoiding the location that held the platform. This explanation can be ruled

out however, by noting that the rats did not spend significantly more or less time in the correct location compared to the opposite location. This suggests that the rats could not distinguish between the two locations based on time of day. That is, the rats learned a place-event association, but neither a time-event nor a time-place-event association.

During the latent learning phase, the rats did not learn a general place preference for the locations that normally had a platform associated with them at some point in time. By the end of the active learning phase they did have a general place preference for the locations that had platforms over those locations that never had platforms.

The fact that the rats learned the quadrants in which the platforms were located, but not the times in which they were in that quadrant, is in line with the results of Widman et al (2000). This is further evidence showing that rats have difficulty learning a daily time-place task and that this difficulty extends to aversive tasks as well as food related tasks.

Experiment 3: Radial Arm Task

There are two possible explanations as to why the rats in Experiment 2 did not learn the task. First, it is possible that the rats simply cannot learn a time-place association. Second, it is also possible that the rats could learn the time-place association but only as it relates to a food reward. That is, maybe time-place learning is specific to food motivated tasks, which is line with the work by Reebs (1999) in which inangas learned a time-place association for food reward but not simulated heron attacks. If it is the case that animals can learn a time-place association only for food rewarded tasks, Gallistel's theory should be modified/restricted. To determine which of these two

possibilities was more likely, the same rats were trained in an appetitive task. If the rats could demonstrate learning in the appetitive task, then the reason for the lack of learning in the water maze task was probably due to the aversive nature of the task. If however, the rats do not learn the appetitive task then it is further support for the idea that rats have difficulty learning to associate time and place with any event.

Methods

Subjects

The same rats used in Experiment 2 were also used in Experiment 3. Feeding was also consistent across the two experiments.

Materials and Apparatus

The maze was a white, wooden, eight-arm radial arm maze with a diameter of 190 cm, arm length of 58 cm and an arm width of 9 cm. The maze was placed inside the emptied pool used in Experiment 2 and was raised 37 cm off the ground. Ninety-four mg Noyes A/I Pellets (P.J. Noyes Company, Inc., Lancaster, NH) were used as rewards and were placed in depressed food cups located at the ends of the arms.

Procedure

Each rat was assigned a morning and an afternoon arm which was positioned at the center of the same quadrant as their morning platform and their afternoon platform, respectively. During morning sessions, two food pellets were placed in the depression at the end of the rats' morning arm. Rats were then placed in the center of the maze and

their arm choices were recorded. Rats were allowed to make multiple choices, but were removed as soon as they either ate the food, or chose their afternoon arm. Each rat performed five trials per session. Afternoon sessions were the same as morning sessions with the exception that the food pellets were placed at the end of the rats' afternoon arm. Testing was conducted at the same times as in Experiment 2 and continued for 35 days. During analysis, only the first choice were considered.

Results

During analysis, only the first trial of each session was considered for each rat, because all subsequent trials were confounded by the fact that the rats only needed to remember where to go based on where they found the reinforcements in the initial trial of that session.

Figure 2.4A shows the mean percent choices to the correct and opposite arms in blocks of 10 trials (5 morning, 5 afternoon). The correct arm is defined as the arm which contains food, the opposite arm is defined as the arm which contains food during the opposite daily session, and the other arms are defined as arms which never contain food. As can clearly be seen the rats did not appear to have learned the task.

Figure 2.4B shows the mean percent of correct arm choices, opposite arm choices and other arm choices during the last 5 trials of the morning and afternoon test sessions. The rats clearly did not learn the time-place discrimination task in the radial arm maze, and therefore statistical analyses were not conducted. To determine whether the rats had learned a general preference for the two arms that were correct in the AM and PM sessions, a one-sample t-test comparing the amount of time spent in the combined correct
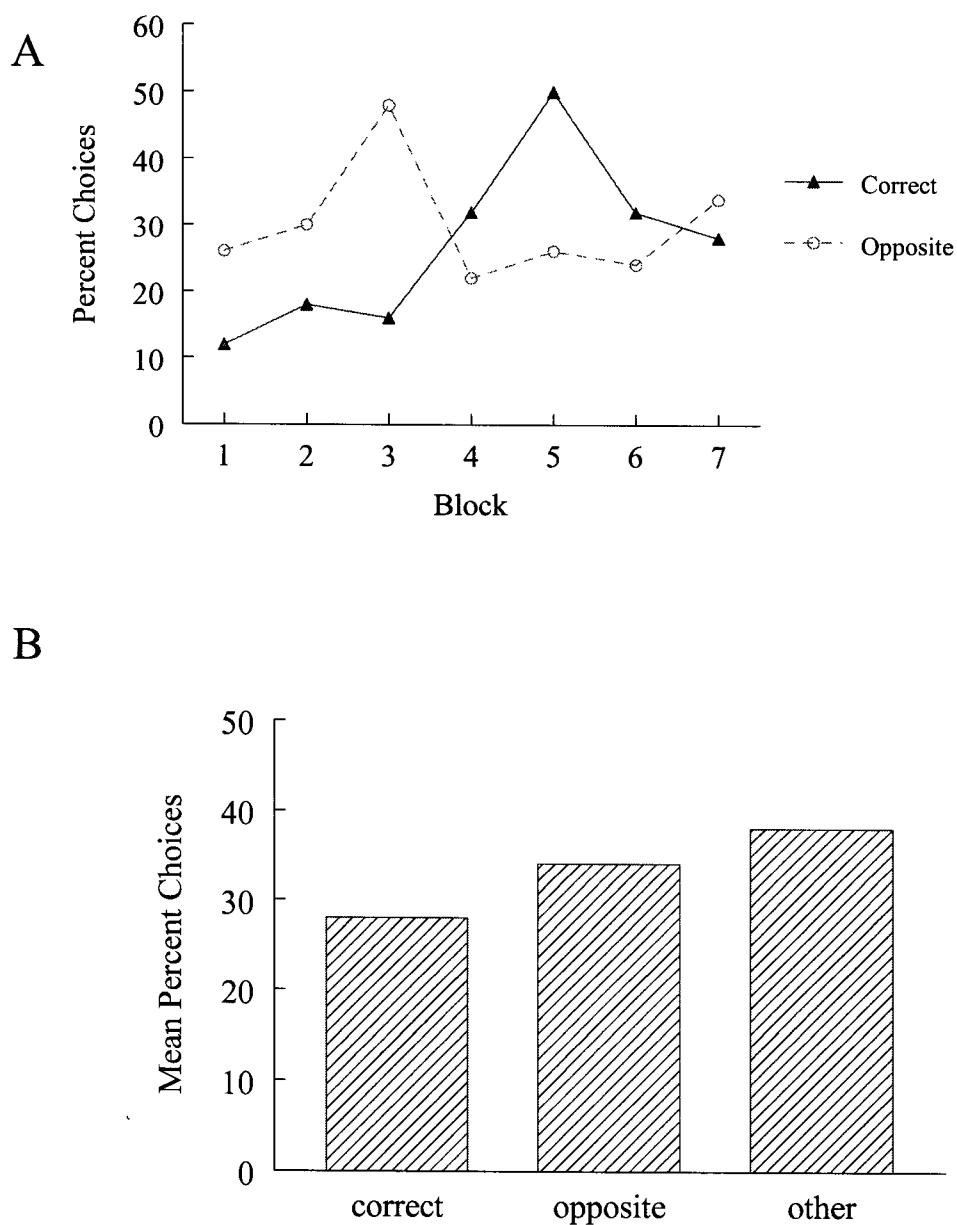
Figure 2.4. A. Percent choices on correct arm (i.e., the arm that is baited at that time of day) and opposite arm (i.e., the arm that is baited at the other time of day) for the 7 blocks that rats were trained during Experiment 3. Each block consists of 5 morning trials and 5 afternoon trials (total 10 trials). Only the first trial of each session is considered. B. Mean percent choices on the correct, opposite, and other arms during last 10 trials of the morning and afternoon sessions of Experiment 3.

and opposite arms with chance (i.e., 25%) was conducted. It was found that there was a trending preference for those arms ($t(4) = 2.656, p = .057$). With increased sample size this finding would probably reach significance. It is still clear however, that the rats did not learn a time-place association.

Discussion

Again, the rats did not demonstrate time-place learning as would be demonstrated by the rats choosing the correct arm at the correct time of day. As in the last experiment, rats had a tendency to choose the arms that were correct at some point in the day over those that were never correct. This suggests that they did learn the locations in which food could be found but could not make the association as to when those places were correct (i.e., learned a place-event association but not a time-place-event association).

Experiment 4: Lighting Condition as a Discriminative Cue for Place

A finding that is consistent across Experiment 2 and 3, as well as the experiment by Widman et al. (2000) is that rats do learn which two places are associated with food, as shown by their tendency to prefer those locations over other locations. Since they know the locations why do they not connect them to the time-of-day? There are at *least* three possible explanations. First, maybe the rats have learned that there are two arms that give food on a partial reinforcement schedule (i.e., 50%), but they do not "realize" that there is a discriminative cue (e.g., time-of-day) that could increase their success rate. Second, they may be unable to use a discriminative cue to guide their behaviour. Third, it is possible that they can use a discriminative cue, but that time-of-day is not salient

enough. The purpose of the present experiment was to determine whether using a more salient discriminative stimulus that indicates which place is being reinforced, would enable rats to maximize their success by restricting their choices to the correct arm. The discriminative stimuli were two levels of room illumination.

Method

Subjects

Five male Long Evans rats were obtained from Charles River (St. Constant, Quebec). The rats were experimentally naïve and were tested at 75 days old. Housing, feeding, and enriching were the same as in previous experiments.

Materials and Apparatus

The radial arm maze was the same one used in Experiment 3. However, rather than being placed inside the pool it was put on legs (62 cm from the floor) in another room. The experiment took place in a room with several visual cues, including a poster, door, counter, and a filing cabinet. A radio provided background noise. During the "light" sessions, standard fluorescent overhead lighting was used and during the "dark" sessions, a lamp containing a 60-watt light bulb illuminated one corner of the room.

Procedure

The eight arms of the radial arm maze were divided equally into food arms, where the rats would find reinforcements, and release arms, where they would be placed on the maze. The arms perpendicular to the walls of the room were assigned as release arms

and the arms at diagonals to the walls of the room were assigned as food arms. Each rat was assigned two food arms (out of the potential four food arms), one for morning sessions and one for afternoon sessions. The release arms were chosen pseudo-randomly at the start of each session, such that a release arm was not used more than twice in a session. Three 92 mg food pellets were placed at the end of each rat's assigned food arm during each trial.

Two sessions were run each day, one in the morning (0830-0930 for the first month, then due to a change in scheduling, the morning session was run between 0900-1000) and one in the afternoon (1430-1530), with five trials per session. Three of the rats were ran with the room brightly lit during the morning session and with the room dimly lit in the afternoon session. The remaining two rats were run with the room dimly lit in the morning and the room brightly lit in the afternoon. During a pre-experimental training period, the rats were placed on the maze in the random release arm and were given 120 s in which to find the reinforcements. The rats were removed from the maze once they found the food, and if they did not find it in 120 s, the rats were "assisted" by drawing the rat to the correct food arm by tapping that arm. The pre-experimental training sessions ended and the test sessions began once the rats were proficient at searching the arms until they found food. During each trial, the rats were placed in the release arm and were removed from the maze upon finding the food in the food arm. No time restrictions were given. The experimenter recorded each arm that the rat entered until the food was found. After each trial, the maze was rotated zero, 45, or 90 degrees in either a clockwise or counterclockwise direction. If a rat urinated or defecated on the maze, the maze was wiped clean with water. The experiment lasted for 85 morning and 85 afternoon sessions

and was run 5 days per week. A skip AM probe test, where the rats were only tested in the afternoon for one day, was used to check for the use of an alternation strategy by the rats.

Once all rats were clearly demonstrating learning of the task, the light discriminative cue was removed and rats were tested in the same way as above for a further 35 sessions. Rats were randomly assigned to be tested in either all light or all dark This was to determine if they could now use the knowledge that discriminative cues can be used to maximize efficiency and then use time-of-day as a discriminative cue.

Results

Figure 2.5 shows the average percent of correct arms chosen for all five subjects during the morning and afternoon sessions. Results are shown in blocks of 5 trials. Repeated-measures ANOVA tests revealed that, during the morning sessions, subjects showed significant improvement overall throughout the experiment ($F(13,52) = 4.78, p < .001$). A two-tailed paired-samples t-test showed a significant difference between the first five trials and the last five trials ($t(4) = -14.70, p < .001$) and a two-tailed one-sample t-test revealed that, during the last five trials, subjects performed significantly above 50% ($t(4) = 8.573, p = .001$). Similar results were found for the afternoon sessions. A repeated-measures ANOVA revealed an overall significant improvement over the span of the experiment during afternoon sessions ($F(13,52) = 7.13, p < .001$); a two-tailed paired samples t-test showed a significant improvement between just the first five trials and the last five trials ($t(4) = -6.52, p = .003$); and a two-tailed one-sample t-test revealed that performance during the last five trials was significantly above fifty-percent ($t(4) = 7.76, p$
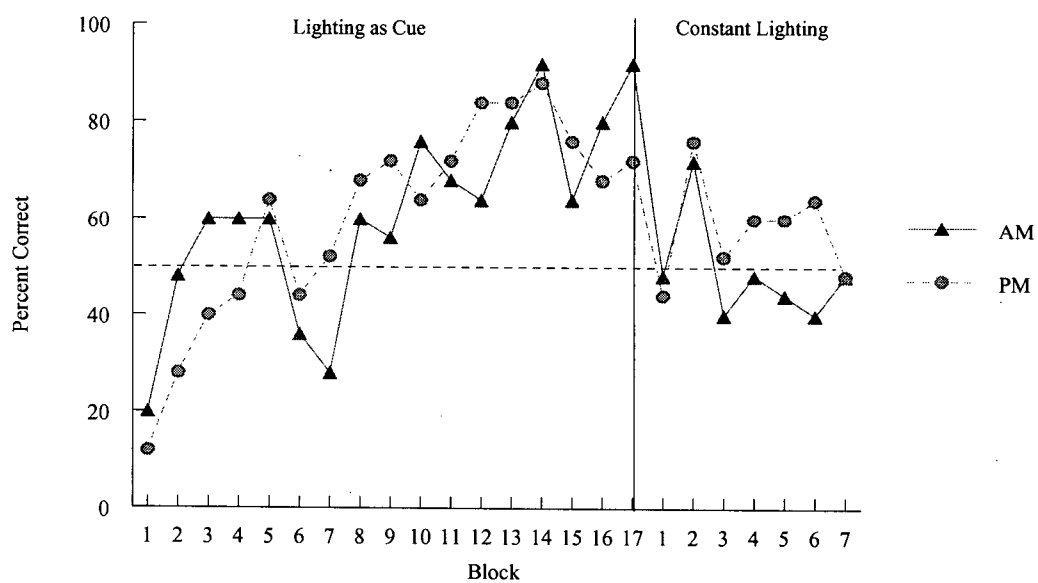
Figure 2.5. Mean percent correct arms chosen for rats during the morning and afternoon sessions during Experiment 4. Results are shown in blocks of 5 trials. The left side of the graph shows the data when lighting was used as a discriminative cue. The right side of the graph shows the data when the lighting was kept constant.

= .001).

Results from the skip-AM probe test revealed that four out of five rats chose the correct arm. The probability of such an event occurring by chance is .156 if we assume that the rats were choosing between two arms or .004 if we assume that the rats were choosing amongst all eight arms.

The results of the switch from lighting as a discriminative cue to constant lighting condition is shown in the right side of Figure 2.5. As can clearly be seen the percent correct dropped significantly when the lighting cue was removed. This was confirmed by a two-tailed paired t-test using the average of the morning and afternoon data for the last block of the Lighting as Cue condition and the first block of the Constant Lighting condition ($t(4) = 9, p = .001$). At the end of training on the constant lighting condition (70 trials) performance still had not increased above 50% ($t(4) = 0.23, p = .83$).

Discussion

Rats could successfully learn to go to two different places at different times of day only if there was a salient discriminative cue telling them which place was correct. Once this discriminative cue was removed performance fell back to chance levels. This could suggest one of two things: First, maybe rats cannot tell the difference between the two times of day (perhaps because they are too close together in time). Second, maybe they do know the time-of-day, but are unable (or reluctant) to combine it with place information to guide behaviour.

It is interesting to note that there is an obvious nontemporal solution to this task, namely alternation. That is, the rats could have learned on Session 1 go to Place 1, on Session 2 go to Place 2, on Session 3 go to Place 1, and so on. Yet, the results of the

skipped session as well as the failure to master the task after the lighting is made constant show that the rats do not use this solution.

Experiment 5: Go/No-Go Task

Experiment 4 raised the question of whether the rats were unsuccessful at the time-place tasks because they were unable to discriminate the two times of day. To verify that this was not the case we replicated the study by Means, Arolfo, Ginn, Perce, and Watson (2000) discussed in the Introduction. Rats were trained on a t-maze such that food was available in both arms during one daily session, but not available in either arm during the other session. Means et al. (2000) showed that rats' latency to leave the start arm was significantly shorter at the times in which food was available indicating that they did learn when food was available. Unfortunately, they did not do probes to determine whether the rats were using circadian or ordinal timing. Carr and Wilkie (1997) found evidence that rats could learn to associate two levers with different times of day for food reinforcement. However, when skipped probes were conducted in which rats were either not run in the morning or in the afternoon session, it was discovered that the rats were not using circadian timing. Instead, rats appeared to use an ordinal timer. It is possible that the rats in the Means et al (2000) paper were using circadian or ordinal timing or even alternation (a nontemporal strategy) to solve the task. The present experiment had probe sessions to determine which strategy the rats were using.

Method

Subjects

Seven male Long Evans rats were obtained from Charles River (St. Constant, Quebec). All rats weighed approximately 450g and were 1-2 years of age when tested. Again, housing feeding, and enriching were identical to all previous experiments.

Materials and Apparatus

The apparatus was an open, elevated, white wooden T-maze which was raised 70 cm above the floor. The stem and choice arms were 15 cm wide and 61 cm long. A circular 2 cm diameter food well was located at the end of each choice arm. Food wells were 0.5 cm deep. The maze was placed in a normally lighted room with several distal visual cues present including a chair, boxes, cupboards and two counters. Ninety-four milligram Noyes A/I Pellets (P.J. Noyes Company, Inc., Lancaster, NH) were used to bait the choice arms. A stopwatch was used to time start-arm-exit latencies.

Procedure

All rats were trained twice daily, once in the morning and once in the afternoon. Morning sessions occurred between 0930-1100, while afternoon sessions occurred between 1430-1600. Each rat was randomly assigned to either an AM group or a PM group. For AM rats, both choice arms of the T-maze were always baited during the morning session and never baited during the afternoon session. For PM rats, both arms were always baited during the afternoon session and never baited during the morning session. Each choice arm was baited with two food pellets. During each session, rats were

placed at the far end (i.e., the end not connected to the choice arms) of the start-arm. Once the rats' hind legs exited the start arm, a barrier was placed at the entrance of the start arm to prevent re-entry. If the choice arms were baited, rats were removed once they had eaten all four food pellets. If the choice arms were not baited, rats were removed after 20 s had elapsed. Rats were timed from the moment they were placed on the maze until their hind legs left the start-arm. This was considered the start-arm-exit latency. Rats that did not exit the start arm after 2 min were removed from the maze. Each rat performed three trials per training session. Training sessions occurred 5 days a week for 8 months (with the exception of rats #3 and #6 who were only trained for 2 months because they learned the task quicker than the other rats).

Upon successfully learning the task, probe tests were administered. A maximum of one probe test was conducted per week. These were intermixed with baseline sessions. On probe days, either the morning or the afternoon session was skipped. The procedure was identical to non-probe days with the exception that choice arms were not baited for either group and only one trial was performed. Five probe sessions were performed in which the morning session was skipped, and five probe sessions were performed in which the afternoon session was skipped.

If rats were using an alternation strategy, it was expected that they would behave as though it were morning following a skipped morning session, and would behave as though it were afternoon following a skipped afternoon session. If rats were using circadian timing, it was expected that their performance would be unaffected by skipped sessions. If rats were using ordinal timing, it was expected that they would behave as

though it were morning following a skipped morning session, and would also behave as though it were morning following a skipped afternoon session.

Results

Figure 2.6A shows the mean start-arm-exit latencies for the morning and afternoon sessions. Only baseline data from the last 10 days are used. As in Experiment 3 only the first trial of each session was considered for each rat. Rats 1, 2, 3 and 4 are AM group rats, while rats 5, 6, and 7 are PM group rats. Two-tailed paired t-tests revealed that start-arm-exit latencies were significantly faster on food trials than on non-food trials ($t(6) = 9.65, p < .001$). It is also worth noting that in the last 10 days none of the rats had to be removed from the maze because 120 s had elapsed on food trials. However, on non-food trials, rats had to be removed 16% of the time for this reason. For the purposes of data analysis this was scored as 120s.

Figure 2.6B shows mean start-arm-exit latencies for morning and afternoon sessions, as well as mean start-arm-exit latencies for skip PM sessions (sessions that occurred in the morning following a skipped afternoon session) and skip AM sessions (sessions that occurred in the afternoon following a skipped morning session). During skip PM sessions, Rat 1's start-arm-exit latencies appear to be most similar to its baseline afternoon latencies, while its skip AM start-arm-exit latencies are most similar to its baseline morning latencies. This suggests that Rat 1 was using an alternation strategy. During both skip PM and skip AM sessions, Rats 2 and 4's start-arm-exit latencies are most similar to their baseline morning latencies. This suggests that Rats 2 and 4 were
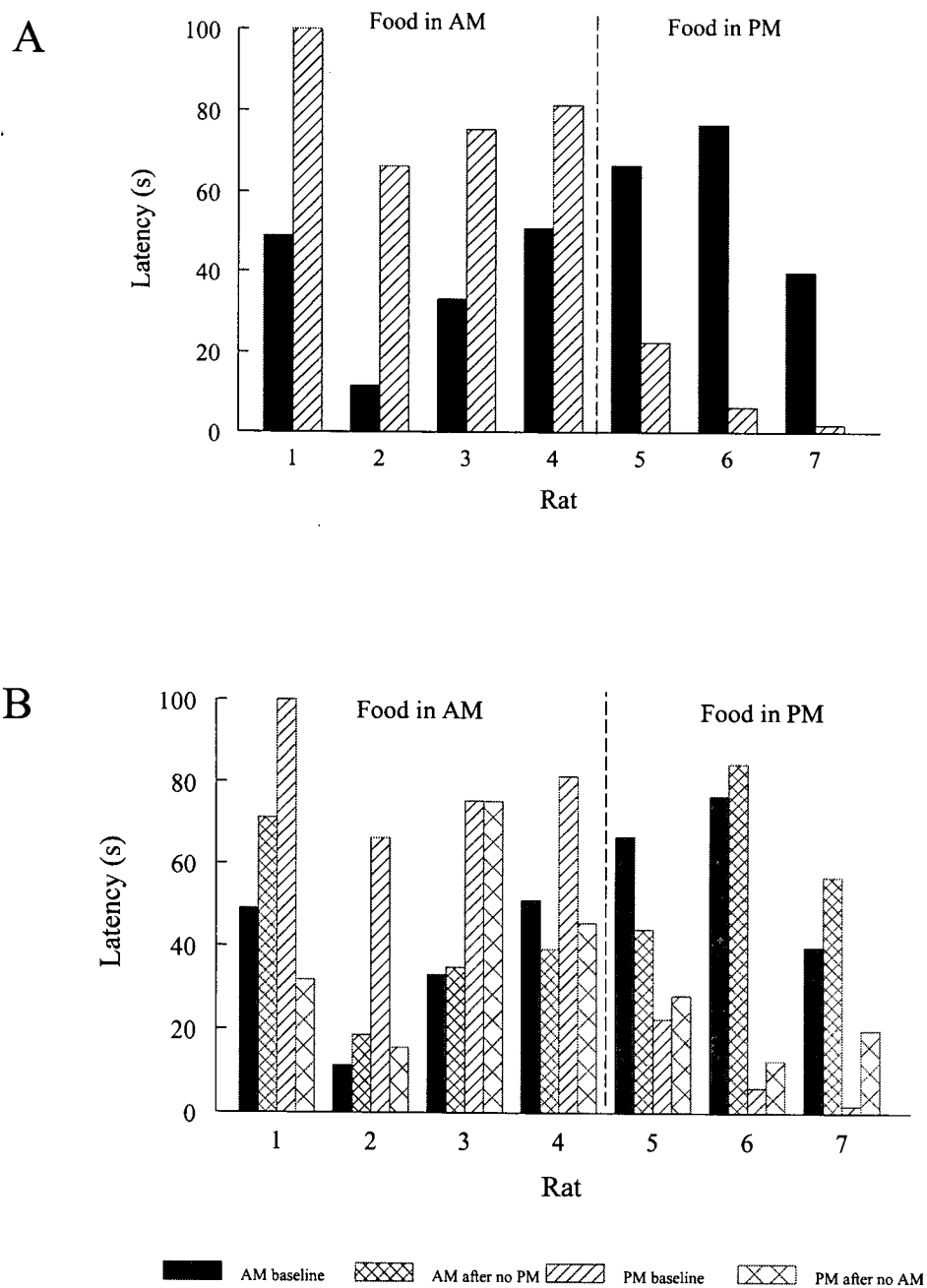
Figure 2.6. The start-arm exit latencies (s) for rats in Experiment 5 on 10 morning and 10 afternoon baseline sessions (A) and on Skip AM/PM test sessions (B). The left panels show rats that were reinforced in the morning, while the right panels show rats that were reinforced in the afternoon.

using an ordinal timing strategy. Rats 3, 5, 6 and 7, have skip PM session start-arm-exit latencies that are most similar to baseline morning latencies, and have skip AM session latencies that are most similar to baseline afternoon latencies. This suggests that Rats 3,5, 6 and 7 are using circadian timing to complete this task.

Discussion

Rats latency to leave the start arm was significantly shorter on trials in which food was presented than on trials in which no food was presented indicating that the rats had learned the time-of-day discrimination. The fact that the rats are able to tell the difference between morning and afternoon sessions in the go/no-go task argues against the idea in Experiment 4 that the rats could not use time-of-day as a discriminative cue because they were unable to discriminate time-of-day. Failure to use it as a cue indicates that that are unwilling or unable to use it in a time-place discrimination task.

Probe tests were conducted after the rats had learned the task and showed that the rats could and did use a variety of methods to tell the time of day. These included circadian and ordinal timing and alternation. An important finding was that at least some of the rats were able to make a circadian discrimination. Carr and Wilkie (1999) tried to encourage rats to use a circadian timer rather than an ordinal timer by training rats with morning session only days, afternoon session only days, and both session days. With this procedure rats could only solve the task by using a circadian timer. They found no evidence that the rats could in fact use a circadian timer. Thus the finding that rats in this task could use it is an important contribution. Means, Ginn, Arolfo, and Pence (2000) also found some evidence suggesting that rats could be using circadian timing in a time-

place discrimination task. However, the rats performance on both baseline and probe sessions was quite variable and the percent correct was barely above chance levels.

General Discussion

The results of the present series of experiments demonstrate that rats have difficulty associating time-of-day with a specific location in a daily time-pace task. This finding is in line with previous research by Widman et al. (2000) and Means et al. (2000). A variety of paradigms have been examined including the semi-aversive water maze, and the food rewarded place preference task, radial arm maze and T-maze. This is not to say that rats are never able to demonstrate daily time-place learning. Both Carr and Wilkie (1997) and Widman et al. (2000) have demonstrated this ability. The fact remains however that time-place learning is not always easy to demonstrate in the rat. Widman et al. (2000) argue that the key factor in a rat being able to learn these types of tasks is that the response cost has to be high. This is in disagreement with Gallistel's (1990) theory, however which suggests that the formation of time-place-event associations is automatic.

Gallistel posits that whenever a biologically significant event occurs a memory code is made which stores the time and place information as well as the nature of the event. Whenever the animal faces a biological need it consults this memory store to see when and where the need has been met in the past. There are numerous training sessions in all of the above paradigms that would allow the association between the three parts of the code to be strengthened.

As demonstrated in Experiments 4 and 5, rats have no difficulty learning tasks in which only two parts of the tripartite code are required. That is, if either the time-of-day

requirements or the differing places are removed from the task the rats are quite successful. This would suggest that perhaps two separate codes are being stored: One that records the time at which the event occurred and another which records the place at which the event occurred. In the typical time-place task, when the rat consults its memory it has a roughly equal probability of getting the time-event code or the place-event code, which means that it can be successful only 50% of the time (which is what we find). In the go/no-go experiments, there is no place-event code, only a time-event code so the rat can be successful. Likewise, in the discriminative cue experiment there is no time-event code competing with the place-event code so the animal can be more successful.

In low response cost situations, rats code only time-event or place-event information. However, during high response cost situations, rats appear to code event-time-place information. In future research it will be interesting to attempt to determine how response effort determines the structure of the memories encoded on daily time-place tasks.

It is worth noting that McDonald, Hong, Ray, and Ralph (2002) have also begun to call into question the existence of a tripartite memory code (event-time-place). These researchers trained and tested rats at either the same time of day or at different times of day. Again they used a number of different paradigms and procedures. They found that testing rats at a different time of day from the training time did not decrease their performance. If there was a tripartite memory code and one part of that code (time) was altered it would be expected that performance would be decreased (i.e., show a generalization decrement). It is also worth pointing out that the researchers have an article in preparation that shows that hamsters are adversely affected by a change in time-

of-day from training to testing sessions using almost identical procedures. This calls into question whether different species are more likely to code all three aspects (event-time-place). Saksida and Wilkie (1994) have also shown that pigeons readily acquire a daily time-place discrimination. In future research it will also be important to determine why some species (e.g., pigeons, Saksida & Wilkie, 1994) encode event-time-place information under low response cost situations.

The current study is also important in that it clearly demonstrates that at least some rats are capable of consulting a circadian clock. Means et al. (2000) did not conduct probe tests, so their results were unclear as to the mechanisms that the rats used to make time-of-day discriminations. Thus it appears that the present data are among the first to demonstrate that rats can use a circadian timer in a time-of-day discrimination.

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CHAPTER THREE: RATS LEARN A LOW RESPONSE-COST DAILY TIME PLACE LEARNING TASK IN WHICH THERE ARE DIFFERENTIAL AMOUNTS OF FOOD[2]

In *The Organization of Learning*, Gallistel (1990) theorized that whenever a biologically significant event occurs, a memory code is made that includes the nature of the event, and the time and place in which it occurred. The animal can consult these memory codes to determine when and where current needs have been met in the past, and use that information to guide its current behaviour.

Many of the biologically significant events that an animal encounters have some degree of spatiotemporal regularity. For example, the availability of food, mates, and predators varies predictably across both space and time. The ability to learn the spatiotemporal variability of stimuli is known as *Time-Place Learning (TPL)*.

The current article will focus on one particular type of TPL: daily TPL (For a discussion of other types of TPL see Thorpe & Wilkie, in press). Biebach, Gordijn, and Krebs (1989) provided the first laboratory demonstration of daily TPL. In their experiment, garden warblers were tested for 12 hr each day in a chamber which was divided into a central living area and four surrounding compartments. Each compartment contained a grain dispenser which provided grain intermittently for a specific 3 hr period every day. The warblers learned to visit the compartments when they provided grain. Other laboratory studies have shown similar time-place learning in honey bees (Wahl, 1932 as cited in Reebs, 1993), pigeons (Saksida & Wilkie, 1994) and fish (inangas) (Reebs, 1999).

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[2] A version of this chapter will be submitted for publication. Thorpe, C. M., & Wilkie, D. M. (in prep). Rats Learn A Low Response-Cost Daily Time Place Learning Task In Which There Are Differential Amounts Of Food.

There has been some recent debate about the ability of rats to display daily TPL. Thorpe, Bates, and Wilkie (2003) attempted to demonstrate daily time-place learning using a variety of tasks including a place preference task, a semi-aversive water maze task, a radial arm maze task, and a T-maze task. They found that rats learned the locations in which food (or platform in case of water maze task) was presented, as shown by their tendency to prefer those locations over others that had never been associated with food (or platform). However, the rats did not associate those places with the time of day they provided food (or platform).

The Thorpe et al. (2003) paper adds to the growing list of papers that have either failed to find daily TPL or did so only with minimum success. Boulos and Logothetis (1990) trained rats to press one of two levers depending on the time of day. Only half of the rats learned the task. The remaining rats showed a preference for one lever over another. White and Timberlake (1990, unpublished data cited in Widman, Gordon, & Timberlake, 2000) were also unable to demonstrate TPL in rats using a similar paradigm to that of Boulos and Logothetis (1990). Furthermore, they could not demonstrate learning on a three arm radial maze in which each arm provided food at a particular time of day.

Means, Ginn, Arolfo, and Pence (2000b) were able to train rats to go to one arm of a T-maze in morning sessions and to the other arm in afternoon sessions for food. However, only 63% of rats were able to learn this task after significant training. Furthermore, with continued training, rats' performance actually decreased. In addition, when external time-related cues such as noises and feeding were minimized, the trials to criterion increased.

In a later study, Means, Arolfo, Ginn, Pence, and Watson (2000a) again showed that rats have difficulty learning a TP association in a T-maze. Neither making the two places more distinctive, nor making the two times farther apart during the day significantly increased rats' success rate on the task.

Widman, Gordon, and Timberlake (2000) discovered that, by increasing the response cost (i.e., effort) in TP tasks, they increased the probability that the rats would learn the task. In a simple radial arm maze similar to those described above (i.e., one arm baited in morning sessions, another arm baited in afternoon sessions), the rats did learn a general place preference (i.e., they learned which two arms were baited), but they did not learn when those places were baited. By switching to a vertical maze and increasing the height the rats had to climb for food (and thereby increasing the response cost), they increased the success rate for TPL.

There are some obvious inconsistencies in the daily TPL literature. Some researchers have found daily TPL in operant response tasks (Carr & Wilkie, 1997, 1999; Mistlberger, de Groot, Bossert, & Marchant,1996; Pizzo & Crystal, 2002), whereas others using very similar paradigms (and similar reinforcement schedules) have failed to find it (Boulous & Logothetis, 1990; Thorpe et al., 2003; White & Timberlake, 1990). Although Widman et al. (2000) believe that response-cost ratios (the relative cost of making an error) are the critical determinant of whether an animal learns a TP task, it is not clear from a theoretical perspective why this should be the case. Nor does the response cost theory account for the many inconsistencies in the research findings.

It is also not clear whether the rats that are successfully learning these daily TPL tasks are using circadian timing. Carr and Wilkie (1997a) identified three timing systems;

interval, ordinal, and phase. *Interval timing* allows an animal to anticipate the occurrence of an event that occurs a fixed amount of time after some external event. Interval timing is used in the measurement of durations ranging from milliseconds to hours and thus is not applicable to the daily TPL tasks. *Ordinal timing* allows an animal to predict the order in which events occur within a particular time frame. For example, Carr and Wilkie (1997) found that when rats trained on a daily TPL task were given Skip AM probe tests, they responded in the afternoon as if it were morning. When they were given Skip PM sessions, the rats went to the correct location the following morning. These skip probes suggest that the rats learned the order in which locations provided food within a given day, but that their timers were reset sometime following the afternoon session. The third timing system is *phase timing*. The most often studied example of phase timing is circadian timing. If a circadian timer is used, then following either Skip AM or Skip PM sessions, the animals should go to the correct location. In addition to these timing systems it is important to rule out the use of the non-timing, alternation strategy. If rats are relying on an alternation strategy then following both Skip AM and Skip PM sessions, the rats should respond incorrectly.

Both Carr & Wilkie (1997b) and Means et al. (2000b), using skip session probes, have concluded that their rats were likely to have been engaged in ordinal timing. Other than these two instances, researchers have not used Skip sessions to determine which type of timer is used.

The aim of the present study was to demonstrate daily TPL in a task with low response cost. Rats were trained in a standard daily TPL task that differed in one respect: the reward also differed depending on the time of day. For example, Place A provided a

large amount of food at Time 1, while Place B provided a smaller amount of food at Time 2. It is hypothesized that we will see daily TPL in this task for two reasons. First, the present task is likely to be more ecologically valid. That is, in the animal's natural environment the qualities of the food are likely to change depending on the spatiotemporal characteristics. Second, if memory is organized in a time-place-event fashion as hypothesized by Gallistel (1990), then in the past paradigms there has been a great amount of repetition in the memory codes. By making the morning and afternoon event more distinctive (i.e., differing the amounts of food), we may decrease the probability of interference and therefore increase the learning success rate.

Method

Subjects and Apparatus

Six male Long Evans rats were obtained from Charles River (St. Constant, Quebec). At the start of the experiment the rats were approximately 100 days old. They were maintained at approximately 90% of free-feeding weight, adjusted for age. Rats received standard rat diet (PMI Feeds Inc., Richmond, IN) at the end of test days and on non-test days to maintain their weight. During test sessions rats received Froot Loops (Kellogg Company, Battle Creek, MI). Rats had free access to water except during test sessions.

Rats were individually housed in large opaque, plastic cages lined with Aspen chips (Nepco, Warrensburg, NY) bedding. Rats were given paper products weekly with which to build nests. Rats received four to six sessions per week of group behavioural enrichment which consisted of being placed in a chamber containing various tubes, ladders, toys, and other rats for 20 min per session. The colony room was maintained on a

12 h light-dark cycle, with light onset at 0730 and offset at 1930. Throughout the duration of the experiment, the rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

The apparatus was an open, elevated, white wooden T-maze which was raised 70 cm above the floor. The stem and choice arms were 15 cm wide and 61 cm long. A circular 2 cm diameter food well was located at the end of each choice arm. Food wells were 0.5 cm deep. The maze was placed in a normally lighted room with several distal visual cues present including a chair, boxes, cupboards and two counters.

Procedure

Rats were trained twice daily, once in the morning (0930-1100) and once in the afternoon (1530-1700). Rats were assigned to one of two groups: AM (those who received a full Froot Loop in the morning and 1/5 of a Froot Loop in the afternoon) and PM (those who received a full Froot Loop in the afternoon and 1/5 of a Froot Loop in the morning). The location of the food differed depending on the time of day. For example, Rat 1 received a full Froot Loop in the left arm in morning sessions and 1/5 of a Froot Loop in the right arm in afternoon sessions. The location of the AM and PM food was quasi-counterbalanced across rats.

Each rat received three trials in each session. Each trial was separated by approximately 8-10 min. For each trial the rat was placed at the far end (i.e., the end not connected to the choice arms) of the start-arm. They were timed from the moment they were placed on the maze until their hind legs left the start-arm. This was considered the start-arm-exit latency. Rats that did not exit the start arm after 2 min were removed from

the maze. If rats went to the incorrect arm they were allowed to go the correct arm to receive the food reward. Training occurred for 5-6 days per week and continued for 13 months in total.

Upon successfully learning the task, probe tests were administered to determine the timing strategy that the rats engaged in. A maximum of one probe test was conducted per week. Probes were only administered if during the last 5 days the latencies and choices indicated that they had learned the task. Probes were intermixed with baseline sessions. On probe days, either the morning (Skip AM) or the afternoon (Skip PM) session was skipped. The procedure was identical to non-probe days with the exception that choice arms were not baited for either group and only one trial per session was performed. Each rat received 4-8 Skip AM and 1-5 Skip PM probes.

If rats were using an alternation strategy, it was expected that they would behave as though it were morning following a skipped morning session, and would behave as though it were afternoon following a skipped afternoon session. If rats were using circadian timing, it was expected that their performance would be unaffected by skipped sessions. If rats were using ordinal timing, it was expected that they would behave as though it were morning following a skipped morning session, and would also behave as though it were morning following a skipped afternoon session. The outcomes are shown in Figure 3.1.

Results

Figure 3.2 shows the average start-arm-exit latencies and percent correct arm choices for the first trial of each session for the 10 days prior to the start of probe sessions. Two-tailed paired t-tests revealed that start-arm-exit latencies were significantly

	PM (after Skip AM)	AM (after Skip PM)
Alternation	AM	PM
Ordinal	AM	AM
Circadian	PM	AM

Figure 3.1. Behaviour of rats on Skip AM and Skip PM sessions if they are using alternation, or ordinal or circadian timing. For example, if the rat was using an ordinal timer, it would be expect to behave as though it were the morning following both Skip AM and Skip PM probe sessions.
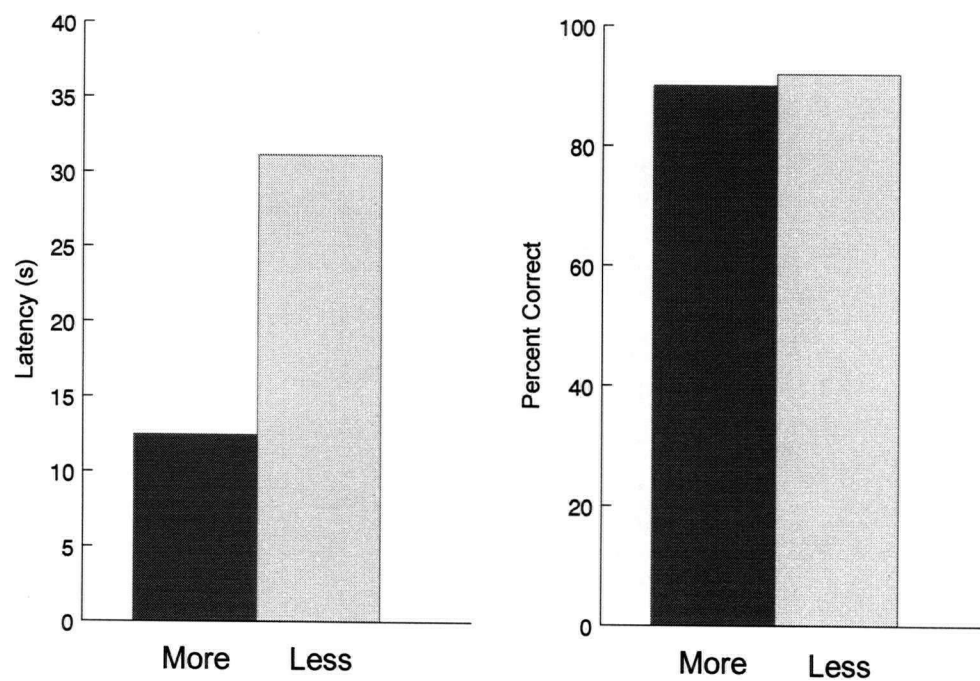
Figure 3.2. Mean latency (s) and percent correct scores on the last 10 days of training before probes sessions started.

faster on trials with more food than on trials with less food ($t(5)=3.74, p=.013$). A one-sample t-test, with scores pooled across sessions, revealed that the rats chose the correct arm significantly greater than chance ($t(5)=13.59, p<.001$).

Figure 3.3 shows mean start-arm-exit latencies for AM and PM sessions, as well as mean start-arm-exit latencies for skip PM sessions (sessions that occurred in the morning following a skipped afternoon session) and skip AM sessions (sessions that occurred in the afternoon following a skipped morning session). These scores, unlike percent correct, can be used to ascertain the timing strategies employed by the rats. The mean start-arm-exit latencies following skip sessions suggest that Rats 1 and 3 are using an ordinal timer (latencies following both types of skip sessions are similar to baseline morning latencies), while the mean start-arm-exit latencies for Rats, 2, 4, 5, and 6 following skip sessions suggest they are using a circadian timer (latencies following both types of skip sessions are consistent with the actual time of day).

Discussion

Rats' latencies to leave the start arm were significantly shorter on trials in which more food was presented than on trials in which less food was presented indicating that the rats had learned the time-of-day discrimination. The rats also went to the correct location at the correct time of day as indicated by the high percent correct scores. Thus, the rats learned the daily TPL task.

When rats have been trained on similar daily TPL T-maze tasks they are either unable to learn the task (Thorpe et al., 2003; Widman et al., 2000) or learn it only moderately after substantial training (Means et al., 2000a; Means et al, 2000b) Widman et al. (2000) argue that rats learn a daily TPL task when the response cost is high. The
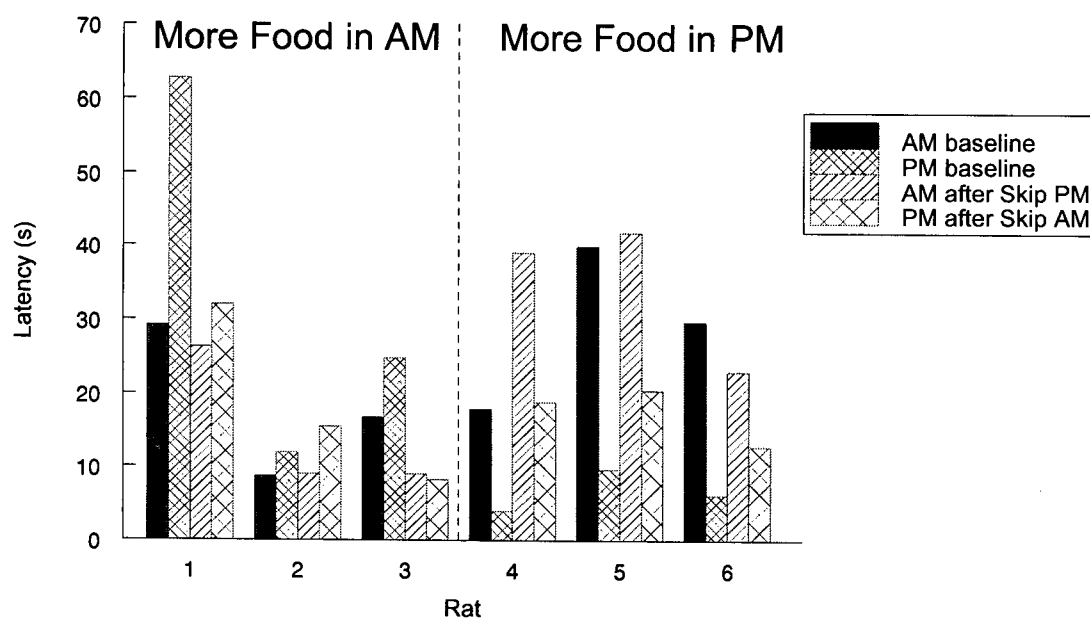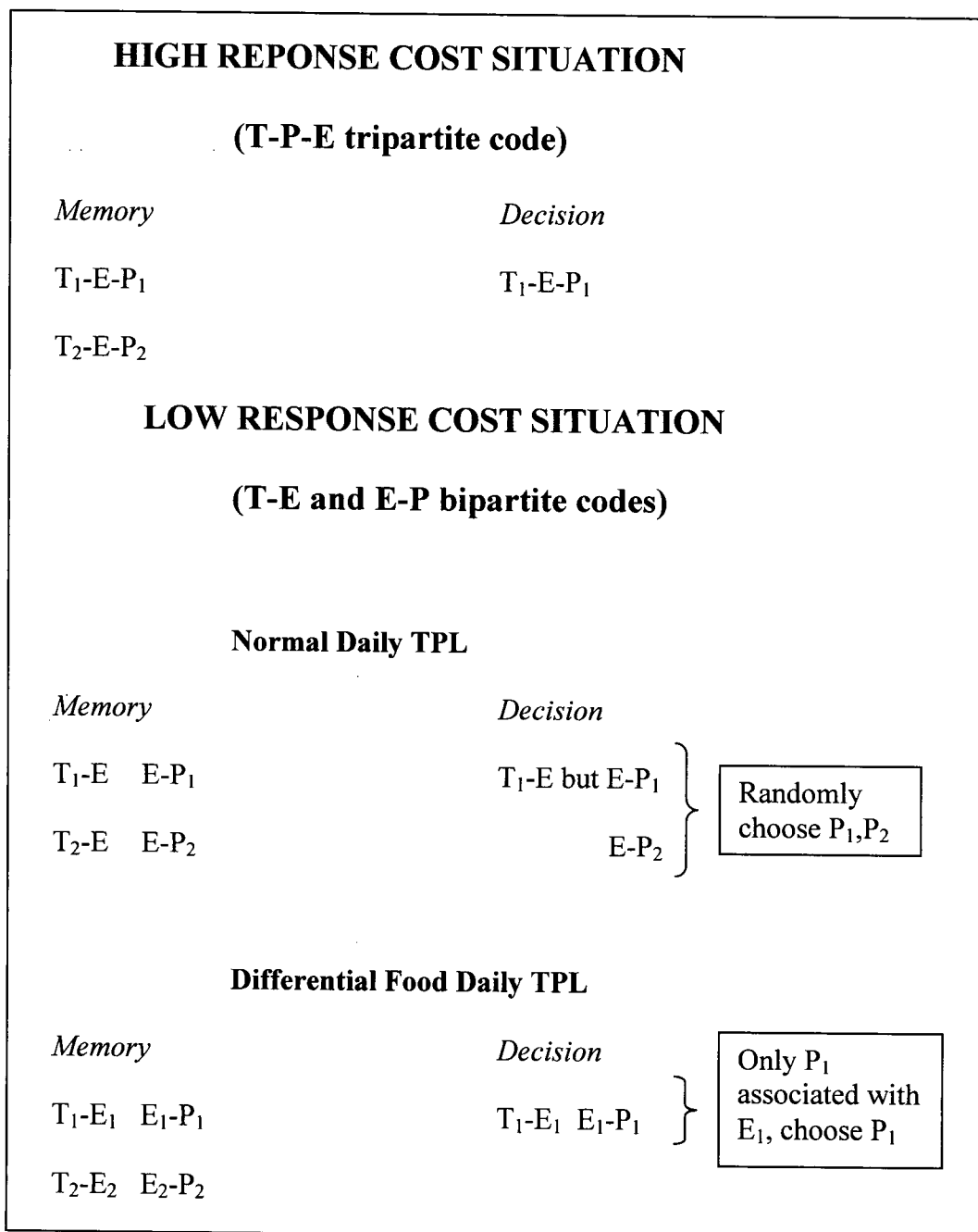
Figure 3.3. Individual latency (s) data for each rat on baseline and probe sessions. Rats 1, 2, and 3 received the larger amount of food in morning sessions. Rats 4, 5, and 6 received the larger amount of food in afternoon sessions.

current study is a demonstration of an instance in which rats learned a daily TPL task in which the response cost was low.

It would appear that in high response cost situations a tripartite code in made that encodes the time, place, and event (Widman et al., 2000). Gallistel (1990) posits that the formation of these tripartite codes is automatic. However, in low response cost situations it does not appear that rats automatically store or retrieve time-place-event information (Thorpe et al., 2003; Widman et al., 2000).

In low response cost situations however, it is possible that two bipartite codes are made: time-event and place-event. Figure 3.4 shows a schematic representation of the possible codes. In the normal daily TPL situation, $time_1$, $place_1$, $time_2$, and $place_2$ are all associated with the same event (food). When the rat is faced with a certain time (e.g., time1) he has a 50% chance of picking the correct place (because both places are associated with the event). This is why in low response cost situations, rats learn a general place preference for the locations that provide food and go to the correct location at chance levels. In the differential food condition described in this paper, there is no confusion due to there being only one event. In the example in which $time_1$ contained $event_1$ in $location_1$, $event_1$ is only paired with $place_1$. In go/no-go experiments (Means et al., 2000a; Thorpe et al, 2003) in which there is no place-event code (i.e., the presence or absence of food depend on the time of day, but there is no spatial variability), only a time-event code exists therefore the rat is successful. Likewise, in the discriminative cue experiment (Thorpe et al., 2003) in which there is no time-event code, only place-event codes exist so the animal can be successful.

HIGH REPONSE COST SITUATION

(T-P-E tripartite code)

Memory   *Decision*

T_1-E-P_1 T_1-E-P_1

T_2-E-P_2

LOW RESPONSE COST SITUATION

(T-E and E-P bipartite codes)

Normal Daily TPL

Memory   *Decision*

T_1-E   E-P_1 T_1-E but E-P_1 Randomly
                                                          choose P_1,P_2
T_2-E   E-P_2 E-P_2

Differential Food Daily TPL

Memory   *Decision*   Only P_1
                                                 associated with
T_1-E_1 E_1-P_1 T_1-E_1 E_1-P_1 E_1, choose P_1

T_2-E_2 E_2-P_2

Figure 3.4. Theorized memory codes and decision processes involved in high and low response cost daily TPL situations. The low response cost situations are further divided into those with the same event (i.e., equal amounts of food) and those with different events (i.e., different amounts of food). T=Time, P=Place, E=Event. The hypothesized memory codes are shown on the left side. The decision process that would occur at T_1 are shown on the right side.

Further evidence against the existence of a time-place-event tripartite memory code comes from the work of McDonald, Hong, Ray, and Ralph (2002). These researchers trained and tested rats at either the same time of day or at different times of day using a number of different paradigms and procedures. They found that testing rats at a different time of day from the training time did not decrease the rats' performance. If there was a tripartite memory code and one part of that code (time) was altered it would be expected that performance would be decreased.

While it has now been shown that rats are capable of learning a low response cost daily TPL task, there still remains the question of what type of timer is being used. The latency data for the skip session probes showed four of the six rats were using a circadian timer, while the other two rats appeared to be using an ordinal timer. Interestingly, all of the rats that received the most food in the afternoon used a circadian timer. In the group receiving the most food in the morning, two used an ordinal timer and one used a circadian timer. Similar findings were found in the Thorpe et al. (2003) go/no-go time of day procedure. Food was available in only the morning or afternoon sessions. If food was available it was in both arms of the T-maze, the rats' latency was significantly shorter on food trials than on no food trials indicating that they learned the temporal variability of food. Furthermore, when skip sessions were conducted it was found that all three of the rats in the afternoon group used a circadian timer. In the morning group, two rats used an ordinal timer, one used a circadian timer, and one used a non-timing alternation strategy. It would thus suggest that a circadian timer is more likely to be used when the afternoon session is associated with more food than in the morning session. When circadian timers

are used, evidence suggests that the timer is a food-entrained, rather than a light-entrained

oscillator (Lukoyanov, Pereira, Mesquita, & Andrade, 2002; Mistlberger et al., 1996).

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CHAPTER FOUR: HOW RATS PROCESS SPATIOTEMPORAL INFORMATION IN THE FACE OF DISTRACTION[3]

Many animals appear to use spatiotemporal information to guide their foraging behaviour. Gallistel (1990) has posited that whenever a biologically significant event occurs, a code is stored in memory consisting of the nature of the event, and the time and spatial location at which it occurred. A striking example of an animal using spatiotemporal information to guide their foraging behaviour was described by Daan and Koene (1981). In their field study they observed oystercatchers flying from their inland roosts to mussel beds located in tidal mudflats located some distance from the roosts. The timing of these foraging trips was critical. Flying too early or too late would not result in foraging success. Despite the fact that low tide depends upon both circadian and circalunar cycles the oystercatchers were able to arrive at the mussel beds at the "correct" times.

Another interesting field study of birds' foraging behaviour was reported by Davies and Houston (1981). They studied the pied wagtail, a passerine bird that defends a feeding territory in the winter. These birds feed on dead insects that wash up on river banks. The birds forage for 90% of daylight hours and must eat an insect every 4 s in order to achieve an energy balance. The defended territory averages about 600 m of river bank. Territory owners regularly circuit their territory, a strategy that avoids revisiting recently visited (and depleted) sites. Several circuits are made each day.

An interesting phenomenon happens when another bird intrudes into a defended territory. The territory owner breaks off feeding and chases the intruder from the

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[3] A version of this chapter has been published. Thorpe, C. M., Petrovic, V., & Wilkie, D. M. (2002). How rats process spatiotemporal information in the face of distraction. *Behavioural Processes, 58*, 79 – 90.

territory, sometimes for distances of 500 m. Interestingly, after the intruder has been evicted the owner returns to the place on the river bank at which it was feeding before chasing the intruder. There are two possible explanations for how this behaviour occurs. First, the wagtails may continue to process spatiotemporal information about food availability during territory defence. The second possibility is that the wagtail does not keep track of the time that has passed while it was defending its property and simply remembers the spatial location it was at before the interruption and returns to that same spatial location. It is impossible to determine from this field study which of these two strategies the wagtails are using. The present laboratory research investigates how animals process spatial and temporal information in the face of distraction.

Rats were trained on an interval time-place task (Carr & Wilkie, 1997) in which four levers provide food, in succession, for equal periods of time. Thus Lever 1 provided food in the first period, Lever 2 in the second period, and so forth. Sessions began with the illumination of each of the four lamps located above each lever. All lamps remained on until the end of the session. After training on the task the rats were given probe sessions in which a piece of highly preferred food – a piece of cheese - was dropped into the test chamber at selected times. Post-cheese behaviour was observed. Several outcomes of the cheese distraction were theoretically possible: 1) the cheese could "disrupt" processing of the spatiotemporal sequence so that the rats would restart the sequence at a random location; 2) the cheese could "reset" processing of the spatiotemporal sequence so that the rats would start the time-place sequence at the beginning (i.e., on Lever 1); 3) the cheese could have no effect on processing of the spatiotemporal sequence so that the rats would continue the sequence at the "correct"

time-place location; 4) the cheese could "stop" processing of the spatiotemporal sequence for the duration of the cheese consumption so that the rats would be "late" when restarting.

Timing on the interval time-place task seems to make use of an internal clock. Numerous studies have examined the properties of the internal clock, including the properties of reset, stop, and restart. For example, Roberts and Church (1978) and Roberts (1981) trained rats on fixed interval schedules (FI-60 and 40 s respectively). They then introduced short breaks during probe trials. It was found that these breaks caused a rightward shift in the peak time of approximately the length of the break, suggesting that the rats stopped timing during the break and then added the time pre-break to the time post- break. Cabeza de Vaca, Brown, and Hemmes (1994) have suggested that during breaks the clock stops and time accumulated during the break is gradually lost during the break. Therefore, the rightward shift would be somewhat less than would be expected if the clock had simply stopped and restarted.

There are at least two major differences between these studies and the present study. First, the durations in the present study are much longer than those in the abovementioned studies. Secondly and perhaps more importantly, the abovementioned studies used discrete reinforcers at a rate of one per trial. In the present study there are numerous reinforcers available throughout the entire rewarded period.

Matell and Meck (1999) used multiple reinforcers per trial in their study examining the effect of intertrial reinforcements on clock functioning. They found that reinforcement within a trial caused the clock to reset. Again, this study examined intervals much shorter than those used in the present study.

Wilkie, Saksida, Samson, and Lee (1994) performed a manipulation that examined how pigeons reacted to a distraction. Pigeons were trained on a time-place task in which each of four illuminated pecking keys produced grain for a fixed amount of time. In probe sessions, all keys were turned off for a period of time (i.e., a time-out). When the keys were re-illuminated the birds behaved as if their internal clock had been stopped and restarted. Given this result one might expect that when cheese is used as a distraction in the present study, the rats will stop and restart their internal clock. However, such an expectation must be tempered by the fact that striking differences have been observed in rat and pigeon timing processes. For example, while Saksida and Wilkie (1994) found that pigeons could use a circadian timer to make a time-of-day discrimination, Carr and Wilkie (1997) reported that rats made the time-of-day discrimination on the basis of an ordinal timing system rather than by consulting circadian clock. Thus it was important to see how rats reacted to a distraction manipulation.

Method

Subjects and Apparatus

Five experimentally naïve, male, Long-Evans rats were obtained from Charles River (St. Constant, Quebec). At the start of training rats were approximately 80 days of age. They were maintained at 90% of their free-feeding weight (adjusted for age) on Purina Rat Chow diet (PMI Feeds, Inc., St. Louis, MO). During test sessions rats received 45 mg Noyes A/I Pellets (P. J. Noyes Company, Inc., Lancaster, NH). Tap water was available ad libitum. Initially rats were group-housed in plastic cages (dimensions: 40 x 30 x 20 cm), lined with Bed o' Cobs bedding (Andersons, Maumee, OH). They were handled every day for a week, after which they began

behavioural enrichment at least twice weekly. Enrichment consisted of being placed in a chamber containing various tubes, ladders, and toys with two other rats. Upon commencement of training sessions (one month after arrival to colony), the animals were moved to single-housing, plastic bins (dimensions: 46 x 26 x 20 cm), also lined with Bed o' Cobs bedding. Each week rats were given paper products to build nests. The colony was maintained on a 12 hr light-dark cycle with lights on at 07:30 and off at 19:30, and at a temperature of $21.0 \pm 1.0°C$. Throughout the duration of the experiment, the rats were maintained in strict accordance with the Canadian Council on Animal Care (CCAC) Guidelines.

All subjects were individually tested in a Plexiglas chamber (dimensions: 40 x 40 x 40 cm). The chamber was situated on a tabletop in a small, well-lit testing room. The chamber was transparent, permitting the rat to view various distal room cues. The door to the testing room was left slightly ajar so that the rats would be habituated to hallway traffic and to permit the experimenter to drop cheese into the test chamber. The testing room also contained a radio that provided masking sounds.

The floor of the chamber was covered with 2 cm of Bed o' Cobs bedding. A lever (dimensions: 1.2 x 3.4 cm) was mounted in the centre of each of the four walls of the chamber 4 cm above the floor. Four pellet hoppers (Scientific Prototype Mfg. Corp., New York, NY, Model No. D700), located on top of the chamber, delivered 45-mg Noyes A/I Precision food pellets (P.J. Noyes Company, Inc. Lancaster, NH) through a wall opening into a brass food cup, positioned to the left of the lever. In addition, a small cue light (28V DC) was mounted above each lever.

A C++ program running on a nearby networked PC carried out data collection and equipment control.

Procedure

Rats were initially exposed in groups of three to the testing chamber. During this initial phase all four of the levers provided food reinforcement on a continuous reinforcement (CRF) schedule. Once all the rats were consistently lever pressing they began individual training. During individual training the rats were gradually shifted from a CRF schedule to a variable ratio (VR) 16 schedule.

Baseline Sessions: Once rats were consistently lever pressing on the VR-16 schedule, training on the interval time-place task began. Rats received a single session 5 days per week. The order in which the rats were trained varied randomly across days. Sessions began between 1 and 5 hrs after colony light onset. Rats were removed from the colony rooms in their home cages and placed into a waiting room near the testing room where they remained until all the rats were finished their sessions and were then returned to the colony room.

At the start of each session, rats were placed into the testing chamber and a 2-min non-reinforcement period began. Responses had no consequences during this time. The purpose of this non-reinforcement period was to allow the rats sufficient time to patrol and inspect the chamber and food sites before testing began. After this 2-min non-reinforced period all four cue lights came on and stayed on for the entire session. (For a more detailed discussion of this non-reinforced period see Wilkie, Willson, & Carr, 1999).

Sessions were 20-min long and were divided into four equal length quarters. During each quarter only one lever provided food according to a VR-16 schedule. The four levers provided food in the same order for all rats in all sessions. The order in which the levers provided food was in a monotonic counter clockwise direction. The timing of each lever press and reward delivery was recorded to a 1-s accuracy on a remote server computer. A data file was generated in which

the time at which each response occurred was recorded (time-stamp), in addition to which lever was pressed, and whether the response resulted in the delivery of a food pellet.

At the end of the session, rats were transported back to their home cages in the holding room. When all rats were finished testing they were returned as a group to the colony room. Rats were fed at the end of the work day (approximately 5:00 p.m.) the remaining amount of their daily food ration to maintain them at approximately 90% of their body weight. Rats received 131 baseline sessions prior to the start of the cheese sessions.

Open Hopper Tests: In addition to baseline sessions rats also received six "open hopper test" (OHT) sessions interspersed between the baseline sessions. During OHTs all levers provided food at all times during the 20-min session according to the VR-16 schedule. OHTs were administered to provide further evidence that the rats were in fact engaged in timing rather than some other strategy. One alternative strategy could be to discriminate when a lever is providing food and when it is not. This would be somewhat difficult (but not impossible) because the levers provided food on a VR 16. According to this strategy the rats could simply press one lever until it fails to give them food and then search for the next lever providing food. During OHTs it is usually the case that the animals switch to the next lever at approximately the correct time even though there are no contingencies in effect that necessitate them to do so. (For further discussion of OHTs see Carr, Tan, Thorpe, & Wilkie, 2001).

Cheese Sessions: Rats received nine cheese tests which were conducted weekly. Cheese tests were identical to baseline sessions with the exception that at some point during the session a piece of cheese (approximately 5 g) was dropped into the chamber by the experimenter. For the first three cheese tests, the cheese was dropped in 6 min into the session. During cheese tests four

to six the cheese was dropped in 11 min into the session, and during cheese tests seven to nine the cheese was dropped in 9 min and 55 s into the session. The experimenter observed the behaviour from the hallway and recorded the time the cheese was dropped in, when the rat started and finished eating the cheese, and which lever the rat pressed after it finished eating the cheese.

Results

Baseline Sessions: Results are reported for the last 20 baseline sessions. We computed each rat's mean response rate on each of the four levers during each of the 40 30-s recording bins. Any differences in overall response rates across the levers were removed by normalizing each rat's mean response rate distribution for each lever. This normalization was accomplished by expressing each rat's mean response rate on a given lever during each bin as a percentage of the rat's maximum response rate per bin on that lever. This yielded normalized mean response rates that ranged from 0 to 100.

An overall response rate distribution was then computed for each lever. This was accomplished by finding the average for each bin across animals. This was again normalized so that a maximum of 100 existed for each lever. The normalized overall response rate distributions are presented in Figure 4.1. As can be seen in this graph the rats restricted the majority of their responding to the quarter in which the lever provided food.

Figure 4.2 shows the average normalized response rate collapsed across levers. This was accomplished by replotting the normalized response rate distributions in Figure
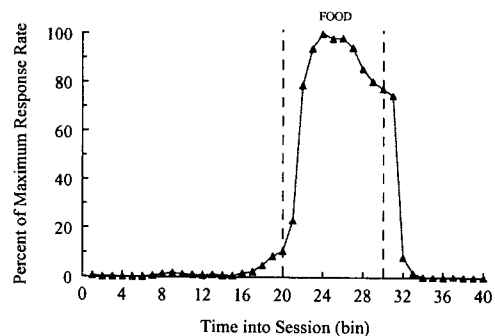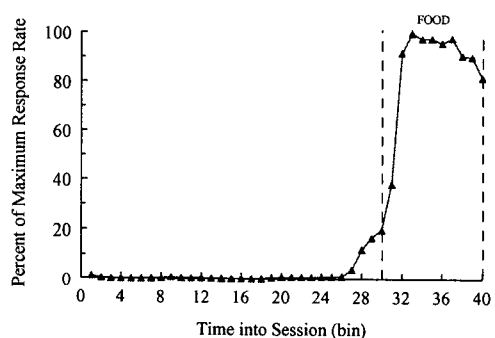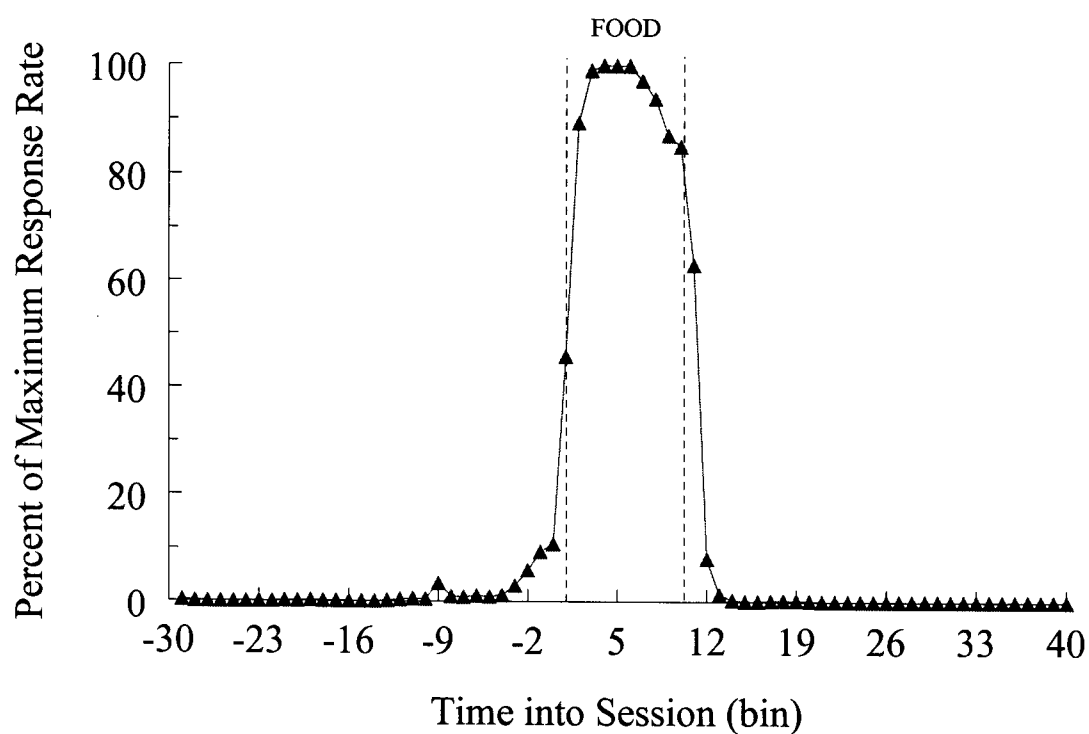
Lever 1
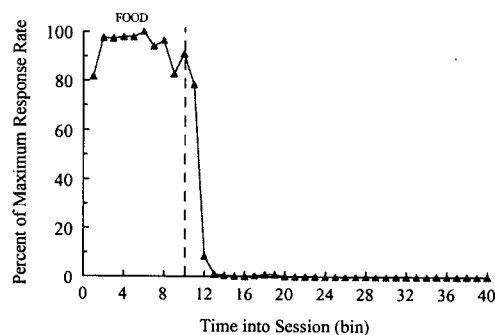
Lever 2
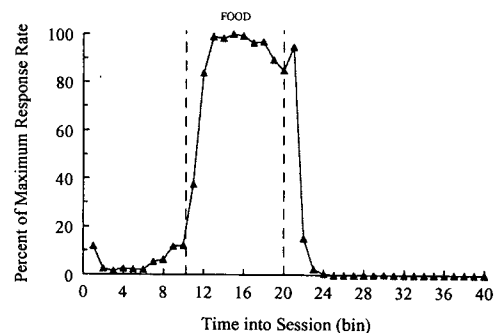
Lever 3

Lever 4

Figure 4.1. The normalized overall response rate distribution for the rats on Levers 1, 2, 3, and 4 during the 32 baseline sessions that were interspersed between the cheese test probes. Responses on each lever were reinforced during the period bounded by the vertical dashed lines.
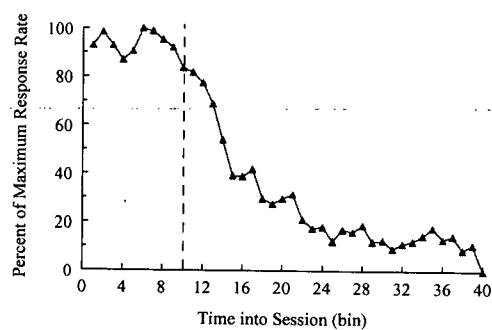
Figure 4.2. The normalized overall response rate distributions collapsed across levers for the 32 baseline sessions that were interspersed between the cheese test probes. Responses on each lever were reinforced during the period bounded by the vertical dashed lines.

4.1 around the ten recording bins during which each lever provided food. The average response rate for each bin was then calculated by averaging across the levers that contributed to that bin. That is since Lever 1 and Lever 4 do not contribute to the first and last portions, the averages were only found across three levers in those portions.

From this figure we can see two things. First, the rats tended to start pressing on a lever just prior to when the lever starts providing food (on average three to four bins before). This tendency to start responding on a lever before it starts providing food is known as *anticipation*. Second, the rats decreased the amount of responding on a lever just prior to when the lever stopped providing food. This is known as *anticipation of depletion*.

Open Hopper Test Sessions: The results of the six OHTs were analyzed in the same way as the baseline sessions. The normalized overall response rate distributions for the OHTs are presented in Figure 4.3. Note that the rats moved from one lever to the next throughout the session, rather than staying on one lever for the entire session. This indicates that the rats were timing rather than relying on a win-stay/lose-shift strategy. However, it is also important to note that there was more variability in the OHTs than in the baseline sessions. This observation suggests that there is a built-in error checker in the baseline interval time-place task. For example, the rat may move to the next lever in the sequence if it have not received reinforcement in 30 s. This cannot be the only strategy the rats are using because if it were they would continue to stay on the same lever throughout the OHTs. Therefore, the rats seem to be using a combination of two strategies: timing and discrimination of boundaries between reward and non-reward.
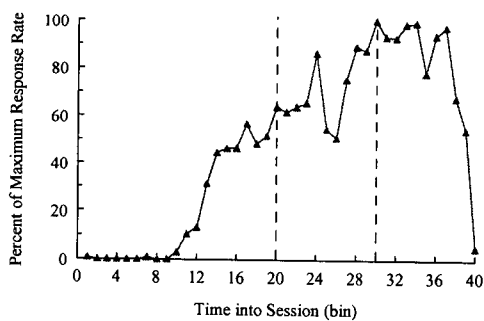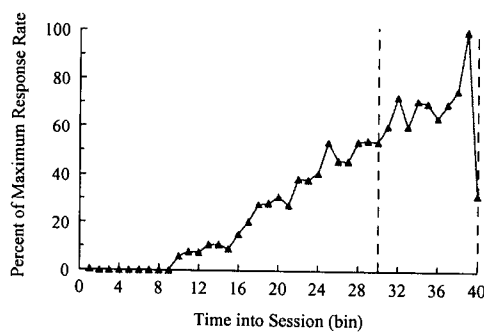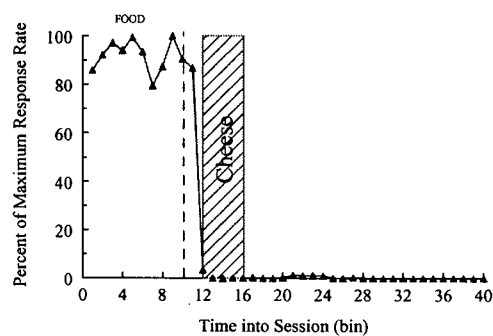
Lever 1
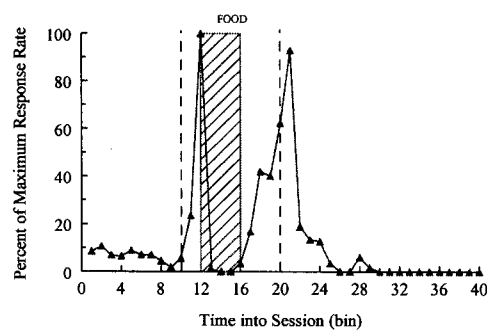
Lever 2
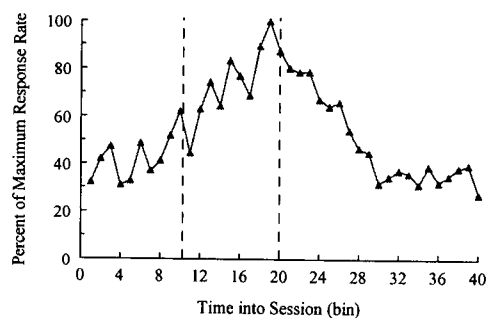
Lever 3

Lever 4

Figure 4.3. The normalized overall response rate distributions on Levers 1, 2, 3, and 4 during the 6 open hopper test sessions. Vertical dashed lines represent the period in which the responses on the lever normally provide food.
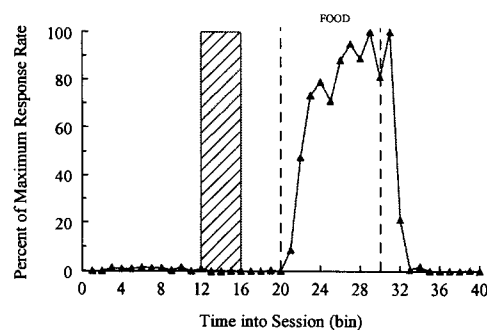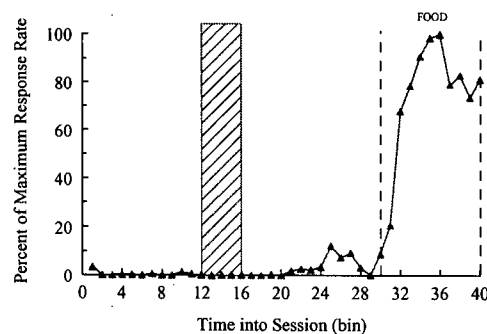
Lever 1

Lever 2

Lever 3

Lever 4

Figure 4.4. The normalized overall response rate distributions on Levers 1, 2, 3, and 4 for the probe sessions in which the cheese was presented at 6-min into the session. Responses on each lever were reinforced during the period bounded by the vertical dashed lines. The hatched bar represents the average time taken by the rats to eat the piece of cheese.
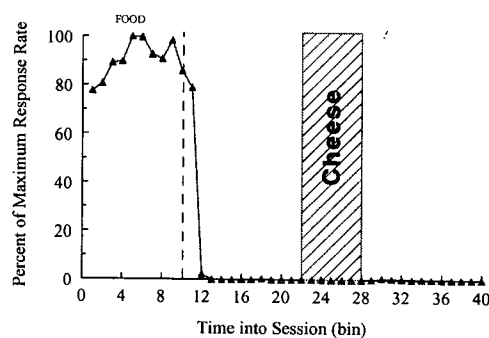
Lever 1
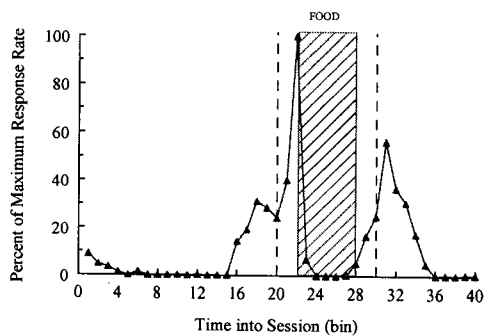
Lever 2
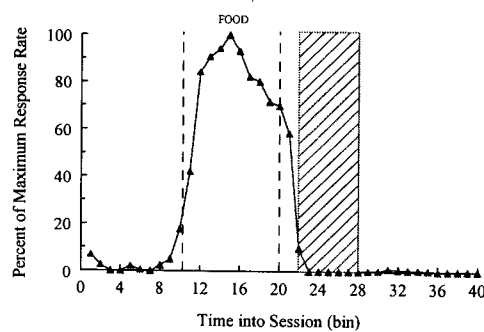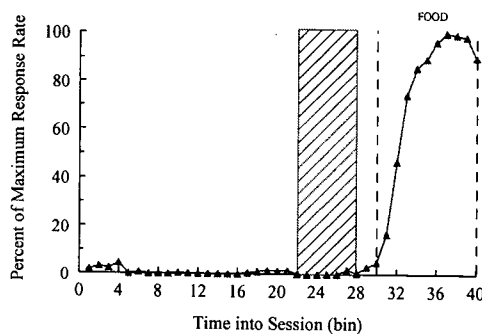

Lever 3

Lever 4

Figure 4.5. The normalized overall response rate distributions on Levers 1, 2, 3, and 4 for the probe sessions in which the cheese was presented at 11-min into the session. Responses on each lever were reinforced during the period bounded by the vertical dashed lines. The hatched bar represents the average time taken by the rats to eat the piece of cheese.

Lever 1

Lever 2

Lever 3

Lever 4

Figure 4.6. The normalized overall response rate distributions on Levers 1, 2, 3, and 4 for the probe sessions in which the cheese was presented at 9-min and 55 s into the session. Responses on each lever were reinforced during the period bounded by the vertical dashed lines. The hatched bar represents the average time taken by the rats to eat the piece of cheese.
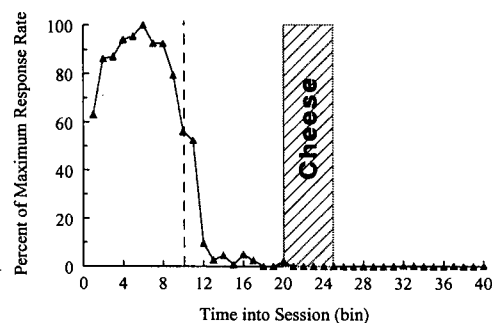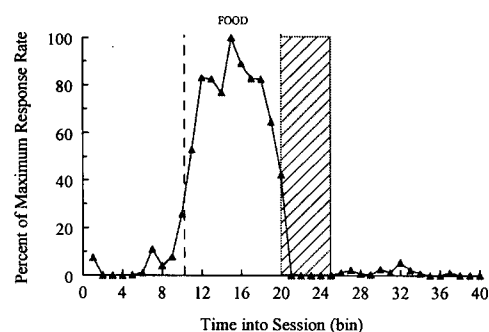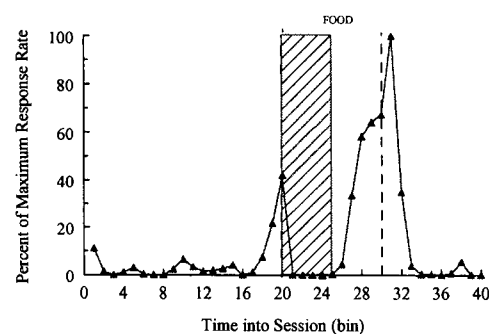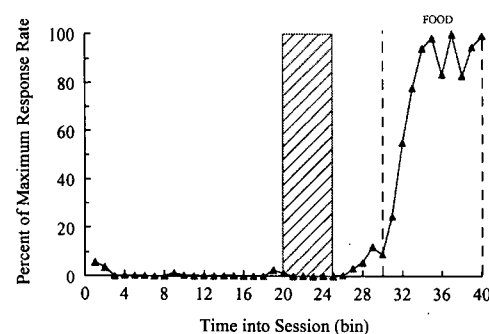
Cheese Tests: Figures were made for each of the cheese tests. The normalized overall response rate distributions for the three cheese tests conducted at 6 min, 11 min, and 9 min and 55 sec are presented in Figures 4.4, 4.5, and 4.6 respectively. One rat was not included in the calculations for the 6-min cheese test because it failed to stop pressing the levers after the cheese had been presented (i.e., it did not eat the cheese until long after it was presented).

Note the lack of anticipation on the lever following the presentation of the cheese. That is, in the 6 min cheese test there is no anticipation on Lever 3. Likewise in the 11 min cheese tests there is no anticipation on Lever 4. This lack of anticipation is most clearly seen in Figure 4.7 which shows the response curves for the lever immediately following the presentation of cheese (i.e., Lever 1 for 6:00 cheese test; Lever 4 for 11:00 and 9:55 cheese tests). This is superimposed over the baseline response curves for the same lever. Note that while the cheese tests that occur at 9 min and 55 sec do result in a slight amount of anticipation on Lever 4, it is noticeably less than during the baseline sessions.

To examine this lack of anticipation in more detail, anticipation was quantified as the area under the curve for the 5 bins immediately prior to that lever providing food. Figure 4.8 shows the area under the curve for both the cheese and baseline sessions on the lever immediately following the presentation of cheese. This is shown for all three of the cheese tests. A difference score was calculated between the area under the curve on baseline and cheese test sessions. A two-tailed t-test comparing this difference score to zero was conducted. The area under the curve on cheese test sessions was significantly

Lever 3: Baseline and 6:00 Cheese Test
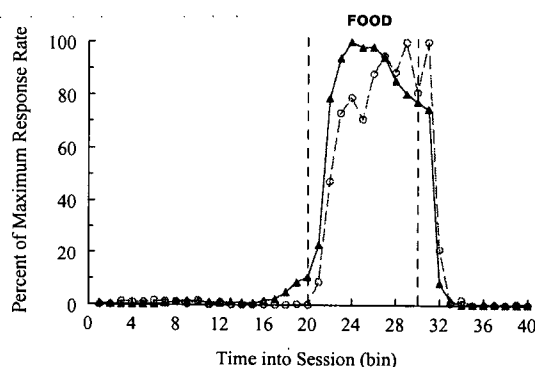
Lever 4: Baseline and 11:00 Cheese Test
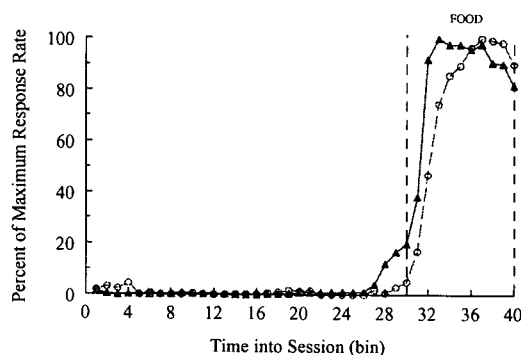
Lever 4: Baseline and 9:55 Cheese Test
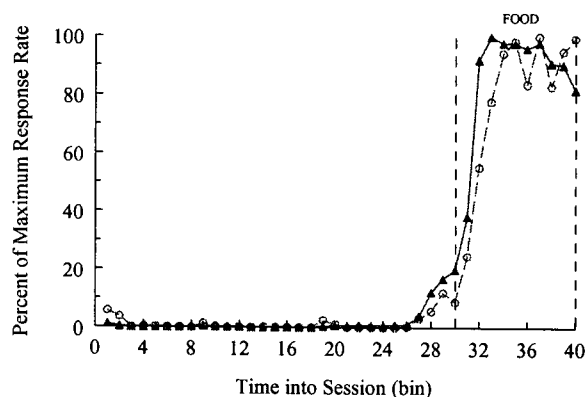
Figure 4.7. The overall response rate curves for the lever immediately following the presentation of cheese (open circles) and the overall response rate curves for the same lever on baseline sessions (closed triangles). The top panel shows the probe and baseline response rate curves for Lever 3 when cheese was presented at 6:00. The middle and bottom panels show probe and baseline response rate curves for Lever 4 on 11:00 and 9:55 cheese tests respectively.
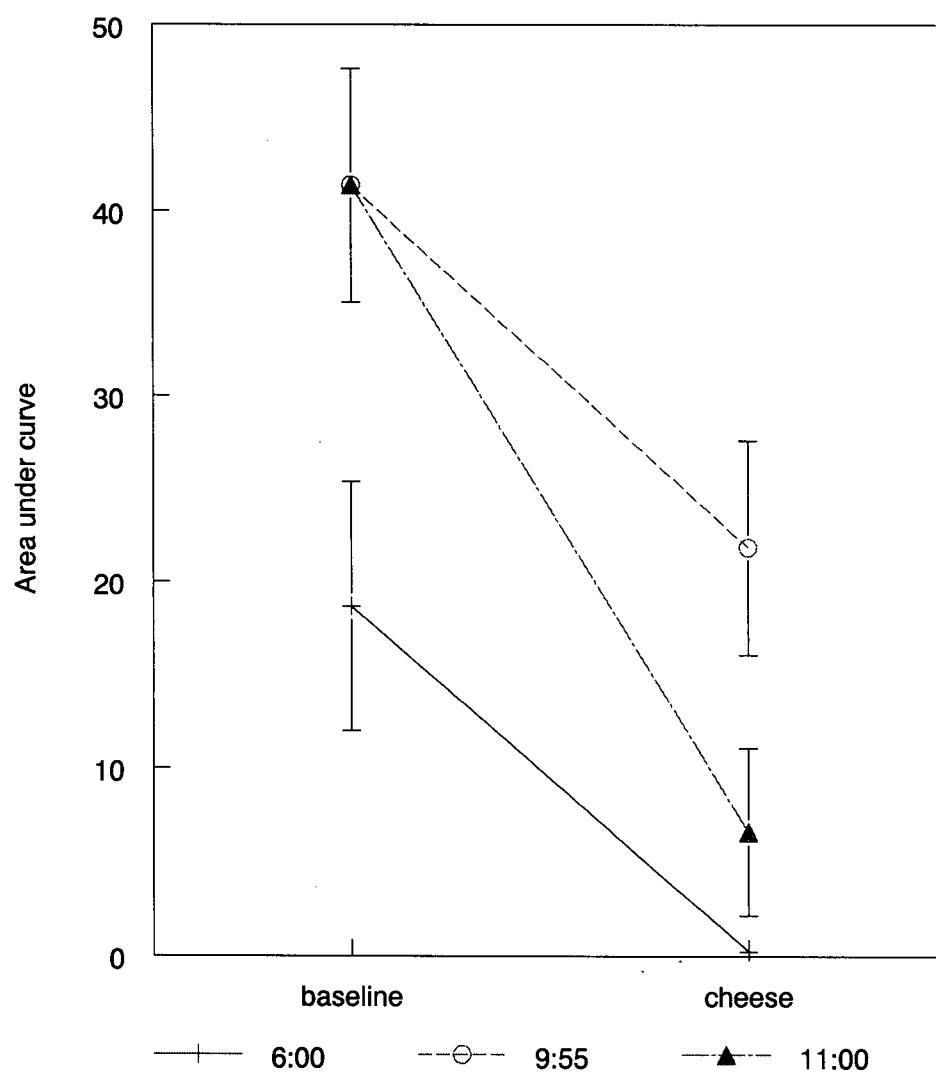
Figure 4.8. Area under the curve for the 5 bins immediately prior the lever next in sequence after cheese presentation. Shown for both baseline and cheese sessions.
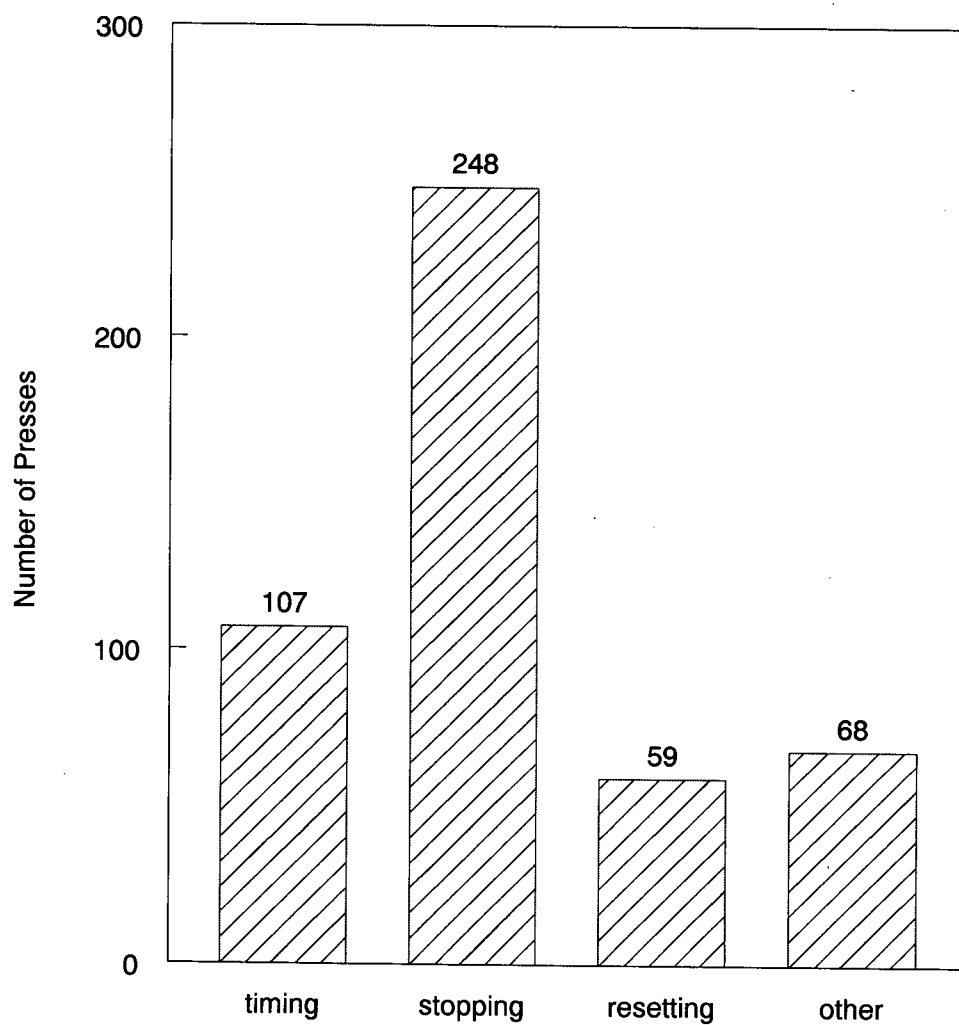
Figure 4.9. Number of presses on each lever corresponding to the four strategies for the period following the presentation of the cheese and prior to the first reinforced response after the cheese for the two probe sessions in which cheese had been presented at 6 and 11-min into the session.

smaller than on baseline sessions ($t(4) = 4.822$, $p = .009$), indicating that the rats did not anticipate the next lever providing food when it was preceded by cheese.

To further support the theory that the distraction leads the rats to stop timing during the presentation of cheese, the responses the rats made after they finished eating the cheese and prior to the first reinforcement after the cheese was examined. Based on the figures above it is apparent that the rats are not resetting their clocks and returning to the first lever in the sequence after the introduction of the cheese. There were two strategies the rats could have used if they were not resetting. First, they could have continued to time when the cheese was presented (i.e., a *timing* strategy). Alternately, they could have stopped timing when the cheese was presented (i.e., a *stopping* strategy). Only the data from the cheese tests at 6 and 11 min were examined because the 9:55 min data are more difficult to interpret. The data from the 6 and 11 min sessions are combined. (Again on three of the sessions Rat 1 did not respond after the cheese was dropped in so its data were omitted.) For 19 of the 27 trials in which cheese was presented, it was impossible to determine whether a timing or stopping strategy was used. This is because either strategy would lead to the same response. This occurs in sessions in which the rat finished eating in the same quarter in which the cheese was presented.

On the remaining eight trials timing and stopping strategies resulted in conflicting responses. This occurs in sessions in which the rat finished eating in a different quarter than the cheese had been presented (e.g., in the 6-min cheese tests if the rat did not finish eating the cheese until 11 min into the session). If the rat was relying on a timing strategy it should respond on the lever that is appropriate with respect to the time into the session. If the rat was relying on a stopping strategy however, it should respond on the lever that it

was responding on when the cheese was presented. Again the response the rat made between the time it had finished eating and the first reinforcement was examined. These results are illustrated in Figure 4.9. In these eight trials, 482 responses were recorded. Of these, 248 were consistent with the stopping strategy, whereas only 107 were consistent with the timing strategy.

Discussion

Rats were able to learn the interval time-place task as demonstrated by their tendency to restrict the majority of their responses to the lever that provided food. Open hopper tests were conducted in which all levers provided food on a variable ratio schedule for the entire session. Despite the lack of contingency in effect requiring them to move from lever to lever the rats continued to do so. This suggests that the rats are in fact using a timing strategy to tell them when to move from lever to lever. However, it is important to note that a timing strategy is not the only strategy they are using. If it was the only guiding force in their behaviour, one would expect their performance on OHTs to be the same as on baseline sessions. This was not the case – on OHTs the response curves had increased variability as compared to baseline sessions. This indicates that while timing may be their dominant strategy they also use a built-in error checker. The most likely error checker in this task is to move from a lever once it stops providing reinforcement. It is unknown what criterion the rats are using to determine when they should move to the next lever, however one could speculate that the time since last reinforcement would be a good rule.

When rats were presented with a highly preferred food, they were able to keep an accurate record of the spatial location of the correct lever. That is, in most instances they

returned to the lever that had been providing food prior to the presentation of the cheese. This suggests that their spatial knowledge was intact. This was not the case for temporal knowledge. The rats' internal clock appeared to stop during cheese consumption. When they began pressing after cheese consumption their behaviour was consequently "late". The usual anticipation effect was absent after the cheese presentation. A comparison of the response curves following cheese presentation and on baseline sessions shows a lack of anticipation following distraction. This lack of anticipation suggests that the rat was not timing during cheese consumption. One might argue that if they had in fact stopped their internal clock during cheese consumption and then restarted it upon completion of consumption, their response curves should be shifted to the right by an amount proportional to the time they were eating the cheese. This is not the case however. This is due to the built-in error checker that was discussed in relation to the OHTs – once the rats did not receive reinforcement on a lever for some criterion length of time they shifted to the next lever. It is also possible that the rats stopped and reset their internal clock during cheese consumption as would be predicted by the Matell and Meck (1999) data. This could only occur if there was a decoupling of spatial and temporal information and the rat simply returned to the spatial location it was at prior to distraction. If this is the case we would also expect to see a rightward shift in the response curve. The magnitude of this shift would again be limited by the built-in error checker. Therefore, it is impossible in the present study to determine if the rats reset or restarted following distraction. It is known however, that they did stop.

Overall the pattern of results support a timing process that resembles the common stopwatch which has the properties of start, stop, restart, and reset. The results provided

further evidence against an hourglass type of timing described by Daan and Koene (1981). Hourglass timers have only two properties – start and finish. They cannot stop and restart. Wilkie et al. (1994) made similar arguments about pigeons' time-place behaviour.

The pattern of results observed in the present experiment have implications for the nature of representations formed on time-place tasks. One possible representation is that both spatial and temporal information are integrated in a single entity. Another possibility is that temporal and spatial information are represented in two entities that are processed in parallel. The present experiment supports the latter possibility.

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CHAPTER FIVE: UNEQUAL INTERVAL TIME-PLACE LEARNING[4]

Although there are probably many timing systems used by animals engaged in learning about spatiotemporal regularities in their environments, Carr and Wilkie (1997) distinguished among three. The first is a circadian timer that permits animals to make time of day discriminations. This mechanism in the one thought to permit Saksida and Wilkie's (1994) pigeons to correctly peck one key location in a morning test and to correctly peck a different key location during an afternoon test session. Saksida and Wilkie ruled out a simple response alternation strategy ("peck Key1 then peck Key 2") by skipping occasional morning and afternoon sessions. Evidence of the circadian nature of the timing was provided by photoperiod manipulations.

When Carr and Wilkie (1997) attempted to replicate Saksida and Wilkie's pigeon data with rats they discovered a second type of timing, which they described as ordinal timing. Although the rats successfully learned to press one lever location in the morning and a second lever location in the afternoon, their discrimination was disrupted by skipping sessions. More specifically, although skipping an afternoon session had little effect on the next morning's performance, skipping a morning session had a large effect: In the afternoon session the rats responded primarily on the lever location that was correct for the morning session. Rather than consulting a circadian clock as Saksida and Wilkie's pigeons appeared to do, Carr and Wilkie's rats appeared to learn a rule such as "press lever location 1 first, then press lever location 2, each day". Carr and Wilkie (1999) and Carr, Tan and Wilkie (1999) further demonstrated this ordinal timing process in rats.

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[4] A version of this chapter has been published. Thorpe, C. M., & Wilkie, D. M. (2002). Unequal interval time-place learning. *Behavioural Processes, 58,* 157 – 166.

The third timing system is called interval timing and is thought to be used for timing intervals in the range of seconds, minutes, and possibly a few hours. This process displays many of the characteristics of the common stopwatch (see Carr & Wilkie, 1997, for example), that is, it displays properties of start, stop, reset, and restart. Carr, Tan, Thorpe and Wilkie (2001) placed rats on a time-place learning task with temporal parameters in the interval timer range. More specifically the task was arranged such that food was intermittently available on each of four levers for 4 min. That is, Lever 1 provided food for 4 min, then Lever 2 provided food for 4 min, and so forth.

In all previous demonstrations of interval time-place learning in the rat, each food location provided food for an equal amount of time. Can rats learn an interval time place task when each lever location provides food for different durations? This question is interesting from an ecological viewpoint. One would assume that in the naturally occurring environment food in different patch locations would be available for varying lengths of time. The present experiment was designed to determine if rats are able to learn an unequal interval time-place task.

In the present experiment rats were trained on an interval time place task in which Lever 1 intermittently produced food for 6 min, then Lever 2 produced food for 4 min. Lever 3 and 4 provided food for 2 and 8 min, respectively. The rats also received "open hopper" tests interspersed in baseline sessions. During baseline sessions all levers provided food on the VR schedule for the entire session, that is there was no contingency in effect to necessitate their switching levers. These tests were conducted to provide further evidence that the animals were in fact timing rather than relying on some other strategy. One alternative strategy would be a win-stay/lose-shift strategy. That is, the

animal could continue to press on a lever until it stopped providing reinforcement, at which point it would shift to the next lever in the sequence. This would be somewhat difficult (but not impossible) because the levers provide food on a VR16. During OHTs, it is usually the case that the animals switch to the next lever at approximately the correct time even though there are no contingencies in effect to necessitate them doing so. (Carr, Tan, Thorpe, & Wilkie, 2001.)

While the main purpose of the present experiment was to determine if rats were capable of demonstrating time-place learning on an unequal interval task, a second issue was also addressed. Namely, the data from this experiment were examined to see if they conformed to Scalar Timing Theory/Weber's Law (Gibbon, 1991). Scalar Timing Theory is a specific instance of Weber's Law. Weber's Law states that as the size of a stimulus increases, the amount needed to be added to that stimulus for an observer to notice a difference, increases proportionally. A common example was that as humans judged the weights of objects, their error in judgement increased as the weights of the objects increased. Scalar Timing Theory applies the same idea to the temporal domain. It basically states that the longer the duration being timed, the greater the error in that timing (Gibbon, 1991). Data from a previous experiment, in which different groups of rats received training on different intervals in an equal interval time-place task, showed that Scalar Expectancy Theory/Weber's Law did apply (Carr & Wilkie, 1998). We wanted to determine if Scalar Expectancy Theory/Weber's Law also holds for the within subjects design used in the present experiment.

Method

Subjects and Apparatus

The subjects were four experimentally naive, male Long Evans hooded rats obtained from Charles River (St. Constant, Quebec). At the start of training the rats were approximately 85 days old. Rats were weighed once per week and the amount of food given was determined by allowing the rats to gain 5-10 g per week until they reached a maximum weight of approximately 450 g. If the rats did not eat food reinforcement in the experimental chamber when given to them their daily food ration was decreased. The rats received 45 mg Noyes A/I pellets (P. J. Noyes Company, Inc., Lancaster, NH) during test sessions. At the end of test days, and on non-test days, they received standard rat diet (PMI Feeds, Inc., St. Louis, MO). Rats had free access to water except during test sessions.

Rats were housed individually in opaque, plastic cages (46 x 26 x 20 cm) lined with Bed o' Cobs (Andersons, Maumee, OH) bedding. Each week the rats were given paper products with which to build nests. The colony in which the rats lived was maintained on a 12 h light-dark cycle, with light onset at 0730 and offset at 1930. Twice weekly, the rats received 20-min sessions of behavioural enrichment. Enrichment consisted of placing the rats in a large clear Plexiglas chamber containing various tubes, ladders, and toys. All four rats were placed in the chamber at the same time. Throughout the duration of the experiment, the rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

During experimental sessions the rats were tested in a transparent Plexiglas chamber (31 x 46 x 46 cm). The chamber was located on a bench (90 cm height) in a small, well lit room (157 x 206 x 273 cm). Several distal room cues were visible from the chamber (e.g., posters, door, etc.). The floor of the chamber was covered with 2 cm of Bed o' Cobs (Andersons, Maumee, OH) bedding. Centred on each of the four walls, 4 cm above the floor was a lever (Scientific Prototype Mfg. Corp., New York, NY, Model No. E2103). A brass food cup was located next to the lever. Lever presses were recorded by the closure of a micro-switch mounted on each lever. Four pellet hoppers (Scientific Prototype Mfg. Corp., New York, NY, Model No. D700) were mounted on top of the chamber. When operated, the hoppers dispensed one 45 mg Noyes A/I reward pellets into the food cups next to the levers. A small cue light (28 V DC) was mounted above each lever. A C++ program running on a nearby networked HP 286 PC carried out data collection and equipment control.

Procedure

Initially all four rats were placed into the testing chamber. During this phase all levers provided food reinforcement on a continuous reinforcement (CRF) schedule. The experimenter did not shape pressing, rather one rat would accidentally press a lever and obtain reinforcement and through observation all the rats began pressing. This usually required four to five days of group training. Once all the rats were consistently lever pressing they began individual training. During individual training rats were gradually shifted from a CRF to a variable ratio (VR) 16 schedule (i.e., on average every 16th response on a lever was reinforced).

When rats were consistently pressing on a VR16, they began training on the interval time-place task. Rats were placed into the testing chamber and a 2-min non-reinforced period began. Responses had no consequences during this time. The purpose of this non-reinforced period was to allow the rats sufficient time to patrol and inspect the chamber before testing began. Previous research has shown that initial responses may be due to causes other than forgetting or non-learning (for example the rats may simply be patrolling or investigating the area). To reduce the likelihood of intrusion of these errors Wilkie, Willson and Carr (1999) introduced a short non-reinforced period prior to the start of the experiment. After the non-reinforced period the cue lights came on and the session proper began.

Test sessions were 20 min long and were divided into four periods. During each period only one lever provided food according to a VR16. The four levers provided food in the same order for all rats on all sessions. The lever on the East wall provided reinforcement for the first period, the lever in the North wall provided food for the second period, the lever on the West wall for the third period, and the lever on the South wall for the fourth period.. The periods were 6, 4, 2, and 8 min in duration, respectively. The timing of each lever press and reward delivery was recorded to a 1-s accuracy on a remote server computer. A data file was generated in which the time at which each response occurred was recorded (time-stamp), in addition to which lever was pressed, and whether the response resulted in the delivery of a food pellet.

At the end of the session, rats were transported back to their home cages. At approximately 1600 the rats were fed the remaining amount of their daily food ration. Rats received a single daily session 5 days per week. The order in which the rats were

tested varied randomly across days. Sessions began between 1 and 4 hrs after colony light onset. Rats received 229 baseline sessions. Such a large number of baseline sessions were conducted because the task is relatively difficult to learn and because with such a small sample size we wanted to insure that there was as little variability as possible.
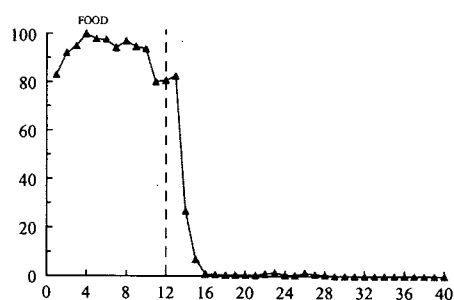
In addition to these baseline sessions rats also received 15 open hopper test (OHT) sessions. The OHTs were only begun once the rats were performing consistently during the baseline sessions, that is, once the performance appeared stable upon visual inspection. These sessions were interspersed among the baseline sessions in a quasi-random fashion. During OHTs all the levers provided food for the entire 20-min session on a VR16. The rats could therefore press any lever throughout the session and continue to receive food.

Results

Can Rats Perform an Unequal Interval Time-Place Task?

Results are reported for the first and last 20 baseline sessions. We computed each rat's response rate on each of the four levers during each of the 40 30-s recording bins that comprised the 20-min sessions. Any differences in overall response rates across the levers were removed by normalizing each rat's response rate distribution for each lever. This was accomplished by expressing each rat's mean response rate on a given lever during each bin as a percentage of the rat's maximum response rate per bin on that lever. This yielded normalized mean response rates that ranged from 0 to 100.

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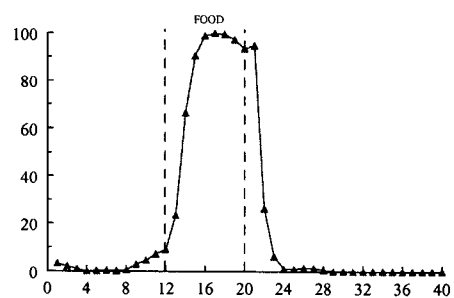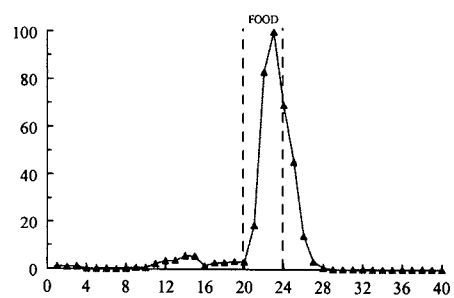
Figure 5.1. The normalized overall response rate distributions on the last 20 baseline sessions. Responses on each lever were reinforced during the period bounded by the vertical dashed lines.
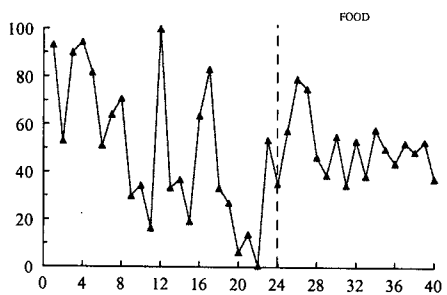
Figure 5.2. The normalized overall response rate distributions on the first 20 baseline sessions. Responses on each lever were reinforced during the period bounded by the vertical dashed lines.

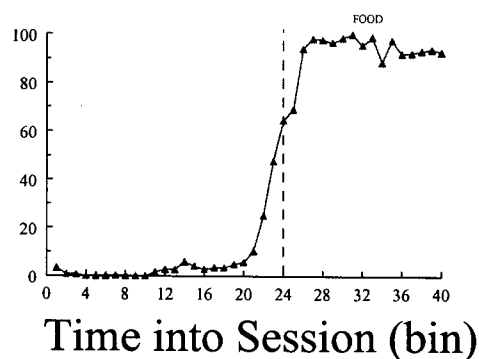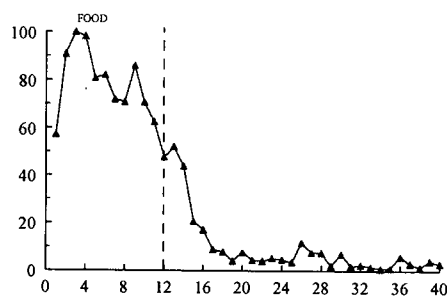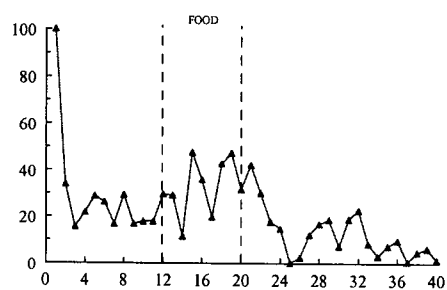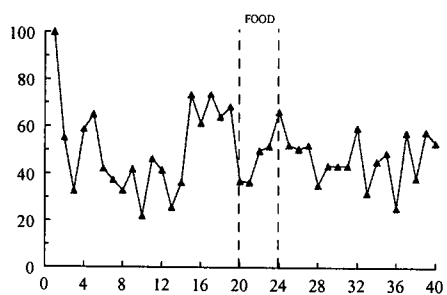An overall response rate distribution was then computed for each lever. This was accomplished by finding the average for each bin across animals. This was again normalized so that a maximum of 100 existed for each lever. The normalized response rate distributions for each of the 40 30-s bins are presented in Figure 5.1 for the last 20 baseline sessions and in Figure 5.2 for the first 20 baseline sessions.

A few points about the distributions are worth noting. First, the rats restricted the majority of their responding on all four levers to the period in which the levers provided food. Second, the rats began responding on a lever before that lever started to provide food, indicating they were able to predict the food availability. Both of these trends are noticeable on all levers, with the possible exception of the decrease in responding on Lever 4. Also, the increase in responding before the lever provided food could not be seen on Lever 1 nor could the decrease in responding before a lever stopped providing food on Lever 4 because these tails were not present.

The overall response rate distributions for the 15 OHTs are presented in Figure 5.3. As would be expected if the animals were engaging in a timing process, the rats' peak rate of responding is in the appropriate period. An exception exists for Lever 3. However, on this lever the second highest peak rate of responding was during the appropriate period of time in which that lever normally provided food.

The results of both the baseline and OHT sessions indicate that the rats are capable of performing an interval time-place task in which the periods the levers provide food vary in length. Further comment on this ability will follow in the Discussion.
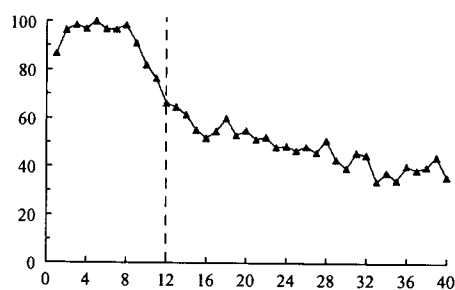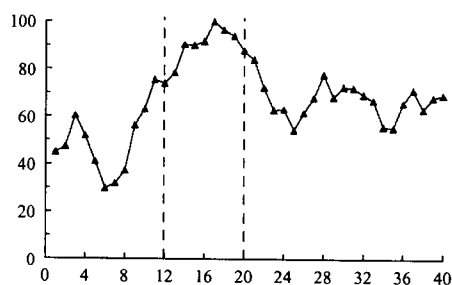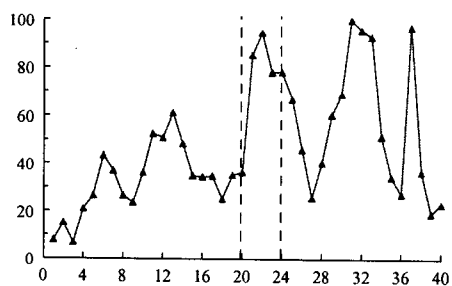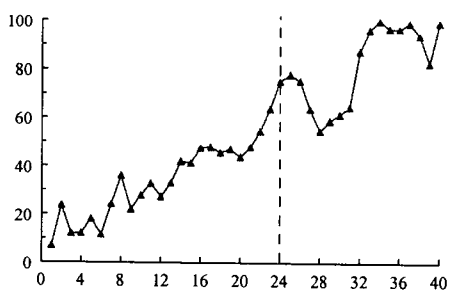
Figure 5.3. The normalized overall response rate distributions for the 15 OHT sessions. Responses on each lever were normally reinforced on baseline sessions during the period bounded by the vertical dashed lines.

Does Rats' Behaviour On The Unequal Interval Time-Place Task Follow Scalar Expectancy Theory/Weber's Law?

According to Gibbon (1991) there are two hallmark features of Scalar Timing: superposition and constant coefficient of variation.

1. Superposition. According to Gibbon (1991), if Scalar Expectancy Theory holds, response rate curves should superimpose when both axes are normalized, i.e., when relative response rate is plotted on the y-axis and relative time is plotted on the x-axis. To see if our results demonstrated such an effect the normalized response rate curves shown in Figure 5.1 were transposed so that the reinforced periods for each lever were equal. This was accomplished by stretching out the data points so that the reinforced period was 48 bins wide. This number of bins was chosen because it was the lowest common multiple of 4, 8, 12, and 16 (i.e., the number of bins reinforced on each lever). This means that for Lever 1, which provides food for 12 bins, there is only one data point for every 4 bins on the transposed graph. For Lever 2, which provides food for 8 bins, there is only one data point for every 6 bins on the transposed graph. For Lever 3, which provides food for 4 bins, there is only one data point for every 12 bins on the transposed graph. For Lever 4, which provided food for the 16 bins, there is only one data point for every 3 bins on the transposed graph. This transposed graph is shown in Figure 5.4. An alternative method of analyzing these data would be to re-bin the original data in time bins proportional to the corresponding interval. This method was decided against because we felt that it would create a bias in that the 2-min interval would not be averaged whereas the other intervals would receive varying amounts of averaging. This averaging would results in decreased variability and therefore the results and interpretations would
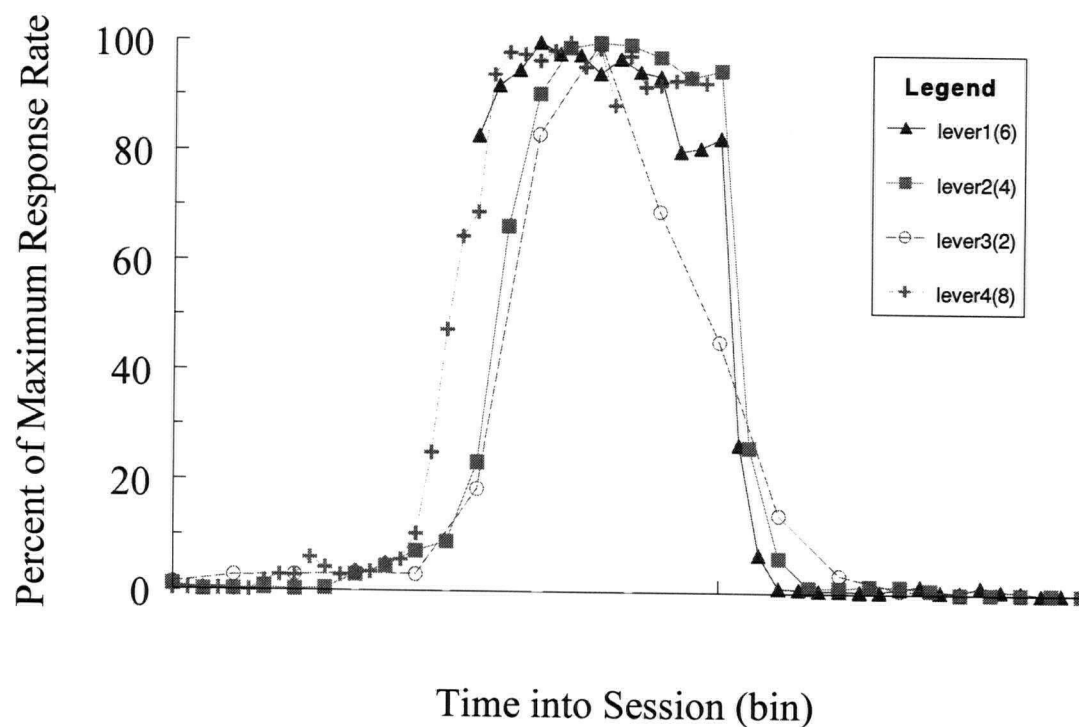
Figure 5.4. Transposed graph of Figure 5.1. The normalized overall response rate distribution curves in Figure 5.1 were "stretched" so that the reinforced period for each lever was equal. The number in brackets indicates the length of time in mins that the lever provided food.
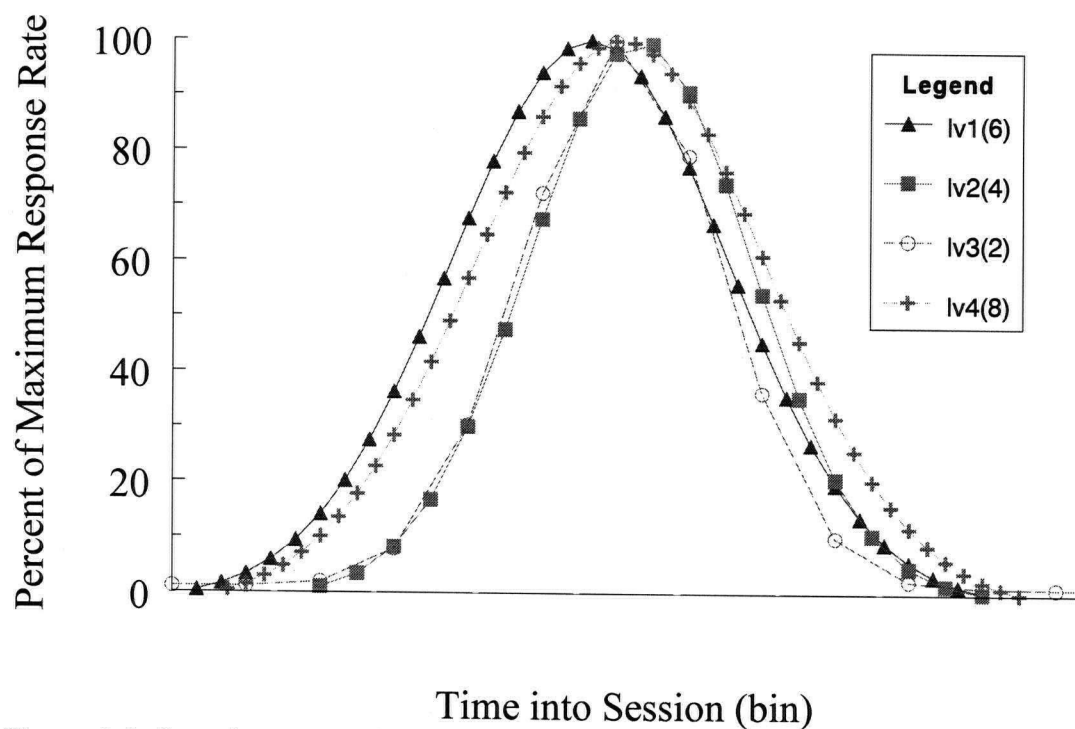
Figure 5.5. Gaussian curves fitted to the transposed distributions in Figure 5.3. The number in brackets indicates the length of time in mins that the lever provided food.

be based on our procedure rather than the data. Gaussian curves were then fitted to these data as is commonly the case (for example, Roberts, 1981) using the SlideWrite 3.0 graphing program (Advanced Graphics Software, Inc., Carlsbad CA). This program utilizes the Levenberg-Marquardt algorithm, which returns the coefficients which minimize the sum of the squared deviations. This fitting program also extrapolated the curves for Lever 1 and Lever 4. These fitted curves of the transposed data are shown in Figure 5.5. As can clearly be seen by visual inspection the curves for each of the four levers do not superimpose. To confirm this fact quantitatively the spread of each curve was calculated. Spread is defined as the width of the distribution at 50% of maximum response rate. If the curves superimposed the spreads should be equal. The spreads differed, a fact that was confirmed statistically. A repeated measures analysis of variance showed that the probability of the spreads differing as much as they do simply as a result of chance was low ($F(3,9) = 15.115, p = .001$). This lack of superposition is an apparent violation of Scalar Expectancy Theory/Weber's Law.

2. Constant Coefficient of Variation. The coefficient of variation is defined as the ratio of the standard deviation to the mean of the response distribution. That is,

$$C.V. = \sigma/\mu.$$

According to Gibbon (1991) this ratio should be constant if the Scalar Expectancy Theory holds. We computed the coefficient of variation using all 40 data points for each lever. Figure 5.6 shows these coefficients of variation. As can be seen from this figure this line is not flat and therefore is also a violation of Scalar Expectancy Theory/Weber's Law. This was confirmed in a linear regression analysis. The probability that the negative slope (-1.01) arose solely through chance factors is only .003 ($F(1,2) = 307.45$).
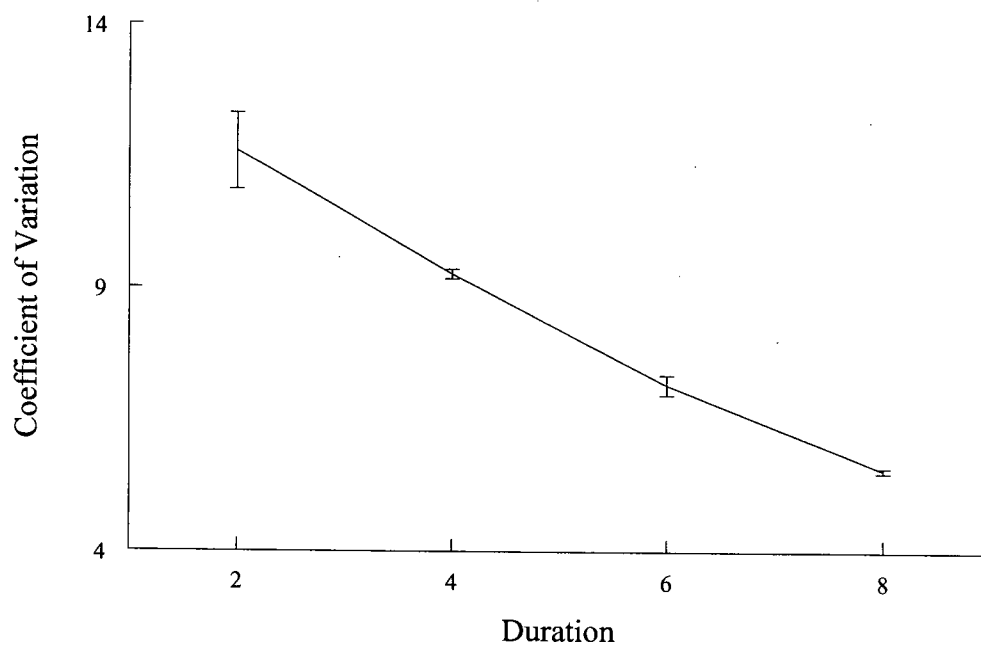
Figure 5.6. Coefficients of variation for the different levers (plotted on the x-axis as the interval length of the period in which the lever provided food). Vertical lines represent standard errors of the mean (SEM).

Discussion

On baseline sessions rats restricted the majority of their responding on a lever to the period in which that lever provided food. They also tended to start pressing on a lever before it started providing food (*anticipation*) indicating that they were able to predict food availability. They also began to decrease responding on a lever before it stopped providing food, indicating they were able to predict the end of food availability. This tendency to decrease responding on a lever before it stopped providing food is called *anticipation of depletion* (Thorpe, Petrovic, & Wilkie, 2002).

There are at least three lines of evidence suggesting that rats are using a timing strategy on the present unequal interval time-place task. First, rats anticipate the arrival and depletion of food. Second, rats performance on the final 20 baseline sessions is much improved from the first 20 baseline sessions. If the rats were relying solely on a win-stay/lose-shift strategy, one would expect their performance on the first 20 sessions to be closer to their performance on the last 20 baseline sessions. This conclusion must be tempered by the fact that the improved performance could be due solely to the rats becoming more proficient at discriminating the different variable ratio schedules. The third and most conclusive evidence showing that the rats are timing comes from the OHT sessions. As would be expected if the rats were timing, the rats' peak rate of responding occurred in the appropriate period, with the possible exception of Lever 3 (see discussion below). However, it is also important to note that there was more variability in the OHTs than in the baseline sessions. This observation suggests that there is a built-in error checker in the baseline interval time-place task. For example, the rat may move to the next lever in the sequence if it has not received reinforcement in 30 s. This cannot be the

only strategy the rats are using because if it were they would continue to stay on the same lever throughout the OHTs. Therefore, the rats seem to be using a combination of two strategies: Timing and discrimination of boundaries between reward and non-reward.

The fact that there is no anticipation on Lever 3 and the noisier behaviour on Lever 3 during the OHT suggests that the rats may not have been timing that lever. One possible explanation for why the rats did not time Lever 3 concerns the sequence of the durations. We have already determined that there is error in the timing system and that there is greater error for greater durations. Lever 2 provided food for 4-min and Lever 4 provided food for 8-min. It is possible that the error within these two levers "overshadowed" the timing of Lever 3. If the short duration had instead been at the beginning of the session the rats may have timed it. Even if we were to ignore the data from Lever 3 because the rats were not timing it, the data from the other three levers would lead to the same conclusions regarding the inadequacy of Weber's Law to explain the data.

The results of the baseline and OHT sessions confirm that rats are able to learn an unequal interval time-place task. However, their behaviour on this task indicates that it does not obey Weber's Law, as defined by Gibbon (1991). There are two important implications of this research. In this section we will discuss these two topics.

1. Implications for Foraging Behaviour

Rats were clearly capable of learning an interval time-place task in which each of the four levers provided food for different durations. The rats restricted the majority of their responding on a lever to the period in which that lever provided food. Furthermore, they were able to anticipate the availability of food and its depletion. The ability of the

rats to time these unequal intervals was further demonstrated by their tendency to correctly shift their responding from one lever to the next throughout the session.

Finding that rats are able to perform the unequal interval time-place task is important for two different reasons. First, all previous research on interval time-place tasks has used equal intervals. Finding that rats are able to perform the unequal variant of the task is an important extension of the generality of time-place learning. Although our results are clear in demonstrating that rats can perform the unequal task, our results do not indicate *how* rats did the task. One possibility is that the rats used a single 20-min timer, and switched from one lever to the next on the basis of different duration criteria stored in memory. Another possibility is that the rats engaged four separate timers, one for each of the four levers. For example, expiration of the first timer could serve as a cue for starting the second timer and so forth. Another possibility is that extinction of reinforcement could cause the first timer to stop and the second to start. Gallistel (1990) has suggested that animals possess multiple timers. From this perspective the latter possibility might be a more likely scenario.

The second reason why finding that rats can perform the unequal task is important concerns external validity. Time-place tasks are generally thought of as laboratory models of a problem faced by foraging animals, namely having information about the spatiotemporal distribution of resources such as food. Although it may be possible that a foraging animal might exploit a resource for the same amount of time in each of several spatial locations, a more plausible scenario is that a resource would be available for different periods of time in different locations. For example, Daan and Koene (1981) found that oystercatchers forage on mussel beds that are exposed at low tides. If the slope

of the shoreline varied from place to place, the duration that each mussel bed is exposed will also vary.

2. Implications for Scalar Timing / Weber's Law

The rats' behaviour on this task was not in agreement with Scalar Expectancy Theory/Weber's Law. The response rate curves did not superimpose and the coefficient of variation was not constant. This is not the only reported instance in which constant coefficients of variation have not been found.

Zeiler and Powell (1994) trained pigeons on FIs ranging in duration from 7.5 to 480 s. They found that the coefficients of variation of the pause durations increased as the durations increased. Weber's Law cannot explain this finding.

In a recent review of the timing literature Grondin (2001) argues that Weber's Law does not hold in situations where there is a sequence of intervals to be timed or in situations where there is extensive training. Both of these were the case in the present experiment and may explain why Weber's Law did not apply here. Therefore, it is not that surprising that the behaviour in this task did not support Weber's Law.

Crystal (2001) also argues that Weber's Law does not hold in all cases. He trained rats to discriminate different durations of noise. He calculated Weber fractions for the different durations. A Weber fraction is k in the formula

$$\Delta I = kI$$

where I is the intensity or magnitude of a stimulus, and ΔI is the change needed in I for a difference to be noticeable. According to Weber's Law, k should be a constant for all durations. However, Crystal found that k varied for different durations.

Machado and colleagues (Machado & Guilhardi, 2000; Machado & Keen, 1999) have also reported instances in which Scalar Expectancy Theory was unable to explain their data. These researchers are arguing that their learning to time model is better able to explain those instances in which Scalar Expectancy Theory is violated.

In summary, there is a growing body of evidence in the timing literature in which Weber's Law does not hold.

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CHAPTER SIX: RATS' PERFORMANCE ON INTERVAL TIME-PLACE TASKS: INCREASING SEQUENCE COMPLEXITY[5]

The ability to learn the spatiotemporal variability of biologically significant stimuli is known as time-place learning (TPL). This ability would presumably confer an advantage to a species by allowing it to plan the most efficient way to obtain access to stimuli such as food, mates, and predators. Some researchers have suggested that time-place information is so important to an animal that it likely forms the basis of how memories are encoded. Namely, Gallistel (1990) posits that whenever a biologically significant event occurs, a memory code is made that includes that time, place, and nature of the event. When the animal is later faced with a biological need it can consult these memory codes and determine when and where that need has been met in the past.

There are at least two types of TPL: daily and interval. In daily TPL, the location of some stimuli (usually food) is dependent on the time of day. For example, Biebach, Gordijn, and Krebs (1989) trained garden warblers to go to one of four rooms depending on the time of day for access to a food reward. The warblers learned the task. This ability has also been demonstrated in honey bees (Wahl, 1932 as cited in Reebs, 1993), ants (Schatz, Beugnon, & Lachaud, 1994; Schatz, Lachaud, & Beugnon, 1999), pigeons (Saksida & Wilkie, 1994), fish (Reebs, 1999), and rats (Widman, Gordon, & Timberlake, 2000; but see Thorpe, Bates, & Wilkie, 2003).

The second type of TPL is interval and it forms the basis of the current study. In interval TPL, the location of a stimulus depends on the amount of time that has passed since the onset of some other stimulus. In the first published paper on interval TPL,

Wilkie and Willson (1992) trained pigeons in a transparent operant chamber that contained a key and food hopper on each of its four walls. Responses on the keys were reinforced according to a time-place contingency. The first keylight provided food on a variable interval schedule for 15 min, followed by the second keylight for the next 15 min, followed by the third and fourth keylights also for 15 min. Only one key provided reinforcement at any given time and the order in which the keys provided reinforcement remained constant from session to session. The pigeons were able to successfully learn the time-place contingency. Similar studies have also been conducted using rats (e.g., Carr & Wilkie, 1998; Thorpe, Floresco, Carr, & Wilkie, 2002). A number of similarities exist between rats and pigeons trained on these tasks. First, both restrict the majority of their responding to the key or lever that is currently providing food. Second, both have a tendency to anticipate the occurrence of food as shown by their tendency to start responding on the key or lever before it starts providing food. Third, both have a tendency to anticipate depletion of reinforcement as shown by their tendency to decrease responding on the key or lever just prior to when it actually stops providing food. These three things strongly suggest that the animal is in fact timing the spatiotemporal movement of the reinforced key or lever. More concrete evidence for this claim comes from probe trials (known as "open hopper" trials, OHTs). In these probe trials, there are no time-place contingencies in effect. That is, all levers provide reinforcement for the entire session. OHTs are administered to provide further evidence that the rats are timing rather than relying on some other strategy such as discrimination of when a lever is and is not providing food. This would be somewhat difficult (but not impossible) because the levers provide food on a variable ratio schedule. According to this strategy the rats could

simply press one lever until it fails to give them food and then search for the next lever providing food. During OHTs animals continue to switch to the next lever at approximately the correct time despite there being no contingencies in effect to necessitate them doing so. (For further discussion of OHTs see Carr, Tan, Thorpe, & Wilkie, 2001).

In previous interval TPL tasks two things were always consistent. First, the duration the levers provided food was constant throughout the entire session. For example, all levers provided food for 4 min. Second, there were a maximum of four places. Recently, Thorpe and Wilkie (2002) manipulated the first of these by training rats on an interval TPL task in which each period was of differing duration. More specifically, the first lever provided food for 6-min, the second for 4-min, the third for 2-min, and the fourth for 8-min. Two interesting findings emerged from this study: First, the rats were able to learn this task as shown by the presence of anticipation on baseline sessions, as well as their tendency to move from lever to lever on OHTs. Second, the rats' behaviour on this task was not in agreement with Scalar Timing/Weber's Law which, as applied to timing, predicts that as the duration being timed increases, the error in timing increases proportionally. According to Gibbon (1991), there are two hallmark features of Scalar Timing: superposition and constant coefficient of variation. According to the rule of superposition, the response rate curves should superimpose when both axes are normalized. The coefficient of variation is defined as the ratio of the standard deviation to the mean of the response distribution and this ratio needs to be constant for Scalar Timing to hold. Neither of these conditions were meet in the Thorpe & Wilkie (2002) study. That

is, the response rate curves did not superimpose and the coefficient of variation was not constant.

The purpose of the present study was to determine how rats would respond to an interval TPL task in which there was an increase in the number of places. To date, the question of learning about more than 3 or 4 places has not been addressed. In nature, some animals seem to learn about only a small number of places. For example, Sibly and McCleery (1983) observed that herring gulls typically visited a grassy field in the morning and a garbage dump in the afternoon. But, on the other hand, animals such as humming birds visit a large number of places, staying as long as it takes to deplete the nectar in the flowers.

There are two possible ways of increasing the number of the places: increase the physical number of locations (e.g., have eight levers) or require the rats to visit the same place more than once (i.e., use a revisiting strategy). The current study increased the number of places by using the latter strategy. Four rats were trained on an interval TPL task in which each lever provided food for 3-min on a variable ratio 15 (VR15) schedule at two different times. The order in which the levers provided food is as follows: 1, 2, 4, 3, 2, 3, 1, 4.

Method

Subjects and Apparatus

The subjects were four experimentally naïve male Long Evans rats acquired from Charles River (St. Constant, Quebec). At the beginning of the experiment they were approximately 100 days old. The rats were maintained at approximately 90% of their free-feeding weight, adjusted for age. To maintain their weight, rats received standard rat

diet (PMI Feeds Inc., Richmond, IN) at the end of test days and on non-test days. During test sessions rats received 45 mg Noyes A/I Pellets (P. J. Noyes Company, Inc., Lancaster, NH). Rats had free access to water except during test sessions.

Rats were housed individually in large opaque, plastic cages lined with Bed o' Cobs (Andersons, Maumee, OH) bedding. Each week rats were given paper products to build nests. Rats received four to six sessions per week of group behavioural enrichment which consisted of being placed in a chamber containing various tubes, ladders, toys, and other rats for 20 min per session. The colony room was maintained on a 12 h light-dark cycle, with light onset at 0730 and offset at 1930. Throughout the duration of the experiment, the rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

Rats were tested in a Plexiglas chamber (40 x 40 x 40 cm). The chamber was located on a tabletop in a small, well lit room. The chamber was transparent, permitting the rat to view various distal room cues. The floor of the chamber was covered with 2 cm of Bed o' Cobs (Andersons, Maumee, OH) bedding. Centred on each of the four walls, 4 cm from the floor was a lever. A brass food cup was located next to the lever. Lever presses were recorded by the closure of a microswitch mounted on each lever. Four pellet hoppers (Scientific Prototype Mfg. Corp., New York, NY, Model No. D700) were mounted on top of the chamber. When operated, the hoppers dispensed 45 mg Noyes A/I reward pellets into the food cups next to the levers. A small cue light (28 V DC) was mounted above each lever.

Procedure

Rats were initially exposed in groups of three or four to the testing chamber. During this exposure phase, all four of the levers provided food reinforcement on a continuous reinforcement (CRF) schedule. Once all rats were consistently lever pressing they began individual training. During individual training rats were gradually shifted from the CRF schedule to a variable ratio (VR) 15 schedule (i.e., on average every 15th response is reinforced).

When rats were consistently pressing on the VR 15 schedule they began training on the TPL task. Rats received two sessions 5 to 6 days per week. By the completion of the experiment rats had received approximately 350 sessions. The order in which the rats were tested varied randomly across days. Sessions began between 1 and 5 hrs after colony light onset. Rats were placed into the testing chamber and a 1-min non-reinforcement period began. Responses had no consequences during this time. The purpose of this non-reinforcement period was to allow rats sufficient time to patrol and inspect the chamber and food sites before testing began. After this 1-min non-reinforced period the cue lights came on and the session proper began. (For a more detailed discussion of this non-reinforced period see Wilkie, Willson, & Carr, 1999.)

Test sessions were 24-min long and were divided into eight equal length periods. During each period only one lever provided reward pellets according to a VR 15 schedule. The levers provided food in the same order for all rats in all sessions. The levers were successively numbered one through four and the order in which they provided reinforcement was 1, 2, 4, 3, 2, 3, 1, 4. (The order was chosen in a semi-random way such that one lever did not follow another lever on two occasions and such that the

same lever was not reinforced for two consecutive periods.) The timing of each lever press and reward delivery was recorded to 1-s accuracy on a remote server computer. A data file was generated in which the time at which each response occurred was recorded, in addition to which lever was pressed, and whether the response resulted in the delivery of a food pellet.

In addition to baseline sessions rats also received three OHT sessions interspersed between the baseline sessions. During OHTs all levers provided food at all times during the 24-min session according to a VR 15 schedule. As discussed in the Introduction, the purpose of the OHTs was to provide further evidence that the rats were timing.

Results

Results are reported for the last 40 baseline sessions. The 24-min session was first broken down into 48 30-s recording bins. The total number of responses that each rat made during the 40 sessions in each of these bins was then calculated for each of the four levers. These individual response distributions were then averaged across all four rats. Figure 6.1 plots the average response distributions with each period graphed separately.

From these figures at least three factors suggest that the rats were timing. First, the rats restricted the majority of responding on a lever to the periods in which it provided food. Second, the rats tended to start pressing on a lever just prior to when the lever started providing food (*anticipation*; with the possible exception of P6-L3). Third, the rats decreased the amount of responding on a lever just prior to when the lever stopped providing food (*anticipation of depletion*).

To provide additional evidence that the rats were timing the results of the three OHTs were analyzed. These data were analyzed in the same manner as the baseline data.
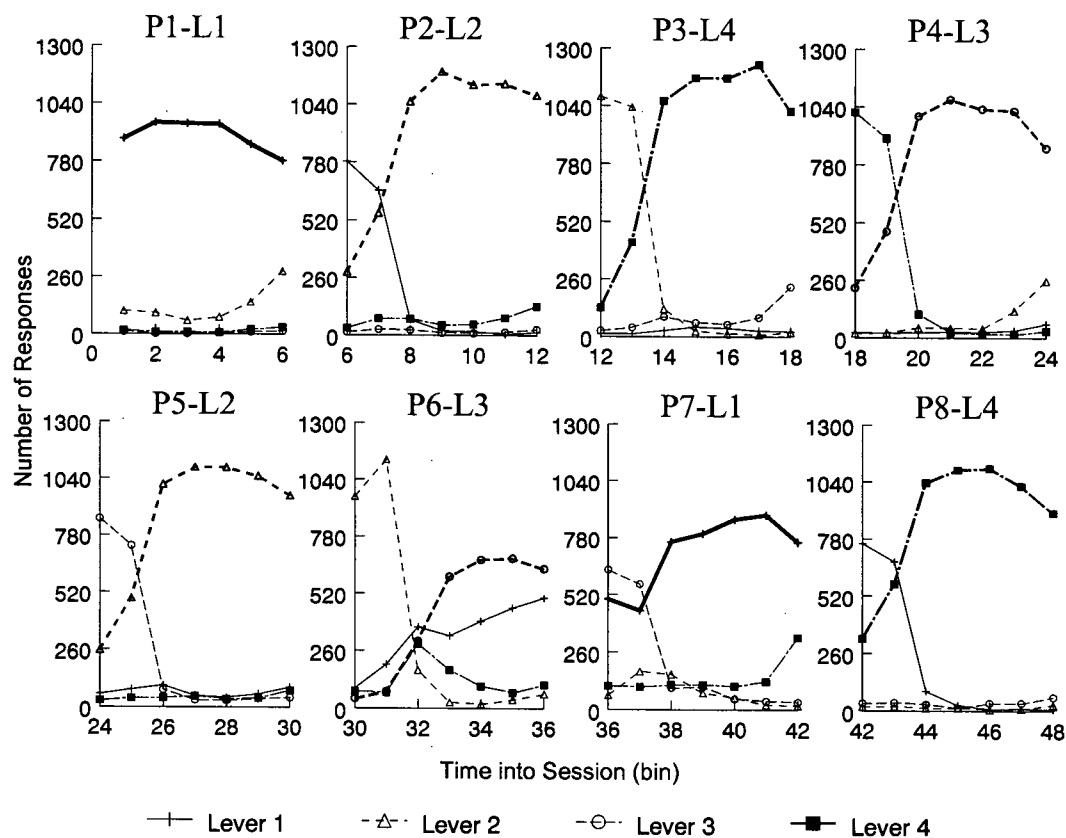
Figure 6.1. The average response rate distributions on Levers 1, 2, 3, and 4 during the 40 baseline sessions. Each period is represented in a separate graph. The labels along the top of the graph denote the period and lever that provided food (e.g., P3-L4 represents Lever 4 provided food during Period 3). The lever that is currently providing food is bolded.
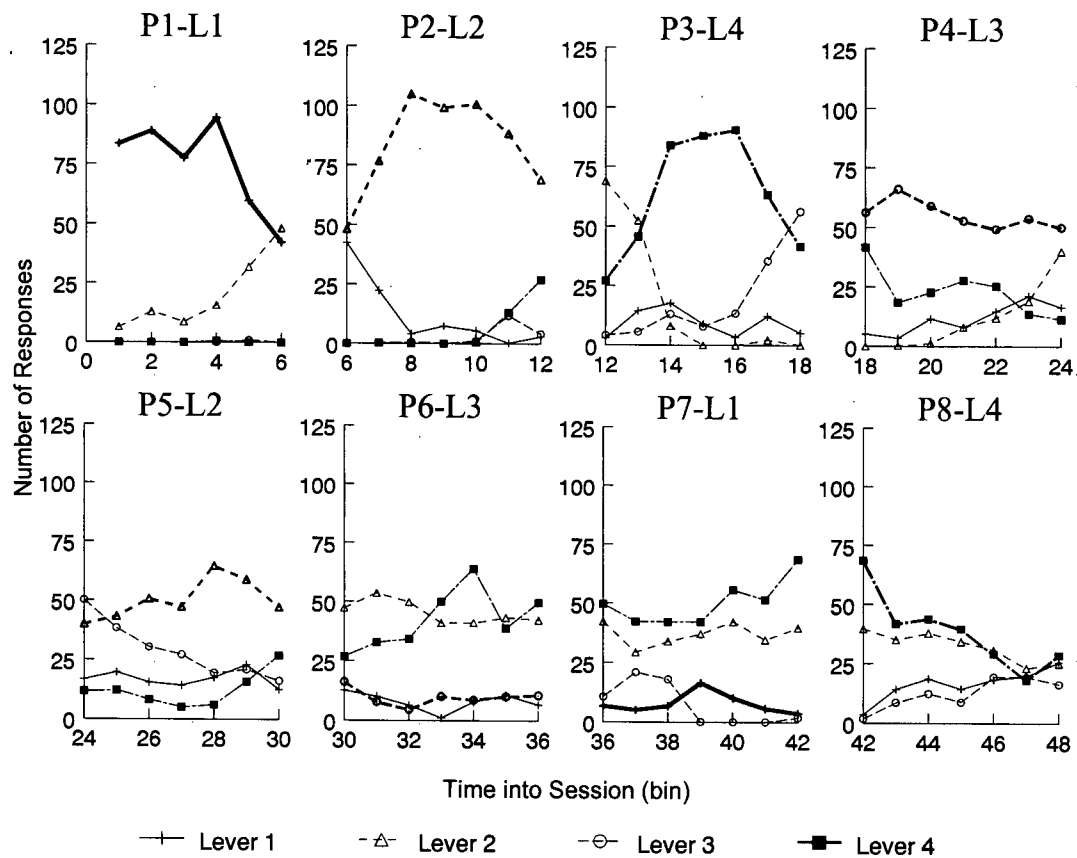
Figure 6.2. The average response rate distributions on Levers 1, 2, 3, and 4 during the 3 OHT sessions. Each period is represented in a separate graph. The labels along the top of the graph denote the period and lever that provided food (e.g., P3-L4 represents Lever 4 provided food during Period 3). The lever that provided food during each period in baseline sessions is bolded.

The average response distributions for the OHTs are shown in Figure 6.2. The rats did move from lever to lever at approximately the correct time even though there were no contingencies in place to necessitate their doing so. It is worth noting that by the fourth period there were more "errors" in where the rats responded.

To determine whether the rats were timing the entire session or the individual periods an analysis of the spread of the response distributions was conducted. The spread of the response rate distributions is taken as a measure of error. If the rats were using one timer that timed the entire session then the spread should increase across periods. If however the rats timed the individual periods (using either one clock that reset at the start of each new period or eight clocks that each timed one specific period), the spread should remain constant across periods. To calculate spread the period in which the lever provided food as well as the 6 bins prior to and after the period were analyzed. Gaussian curves were then fitted to these data as is commonly the case (for example, Roberts, 1981). This was done using the SlideWrite 5.0 graphing program (Advanced Graphics Software, Inc., Carlsbad CA). This program utilizes the Levenberg-Marquardt algorithm, which returns the coefficients which minimize the sum of the squared deviations. This fitting program also extrapolated the curves for Periods 1 and 8. Spread was defined as the width of the distribution at 50% of maximum responding. Figure 6.3 shows the spread for each period averaged across the 4 rats. The regression line ($y = 9.17 - 0.79x$) was found to have no significant slope ($F(1, 6) = .30, p = .602$) indicating that the error did not increase across periods.
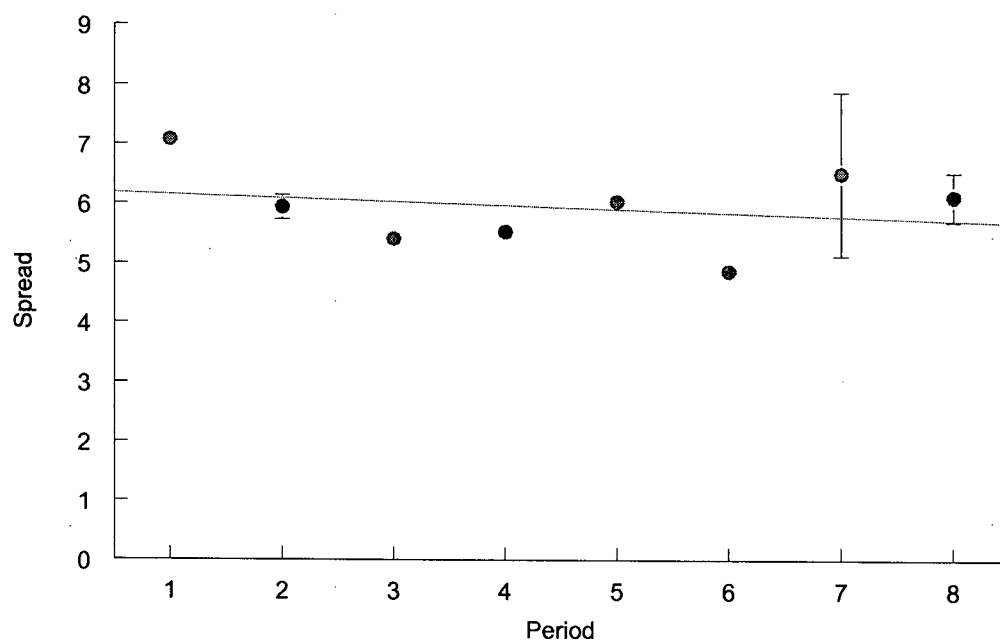
Figure 6.3. The average spread for each of the eight periods. The dashed line represents the regression line (y = 9.17 − 0.79x).

Discussion

To demonstrate knowledge of an interval TPL task, rats must learn when the levers provide food as well as the sequence in which they provide food. Rats were clearly able to learn an interval TPL task in which there were eight "places" as demonstrated by their tendency to anticipate the arrival and depletion of food on baseline sessions as well as their tendency to move from lever to lever during the OHTs. As noted in the Results section, on OHT sessions there were more "errors" in where the rats responded during Periods 6 through 8. Taken in isolation, this could suggest that the rats did not learn the spatiotemporal characteristics of these periods. However, if one also includes the data from the baseline sessions which show anticipation during these periods, it suggests that the rats did learn the spatiotemporal regularity of food presentation. It has been previously demonstrated that there is more variability in OHT sessions than in baseline sessions (Carr, Tan, Thorpe, & Wilkie, 2001). It may the case that there are more "errors" in the present experiment, particularly in the second half of the session, due to the increased complexity of the task.

It was previously shown on an interval TPL task with four locations that rats time each period rather than the entire session. If the rats were timing the entire session, the error should have increased linearly across the periods. If however, the rats were timing the individual periods, the error should have remained approximately constant across the periods. An analysis of the spreads in the current eight "place" task revealed that the error did not increase with increasing periods providing further evidence that the rats were timing the individual periods rather than the entire session. Unfortunately, we cannot determine whether the rats were using one timer for all periods that restarted at the

beginning of each period or alternatively if there were multiple timers that timed separate periods (Carr & Wilkie, 1998).

In addition to the timing aspect of the task, the rats also had to learn the sequence in which the levers provided food. The apparent increase in variability of responding during Period 6 might suggest that the rats did not know the sequence in which the levers provided food (at least in the second half of the session). While that would not be surprising given that one might expect some degree of interference or confusion due to the fact that each lever (except Lever 1) is succeeded by two different levers. However, if we look at the anticipatory responses we can see that the rats restrict their anticipatory responses to the lever that will next provide food – suggesting that they did know the order in which the levers provide food.

Pizzo and Crystal (2004) also found evidence recently suggesting that rats could learn an interval TPL task with eight places. An 8-arm radial maze was used, with each arm providing food in succession for 7-min. In this design, there were eight distinct locations, so there was no need for a revisiting strategy. They also found that the rats anticipated the onset and depletion of food. Rats are clearly capable of learning interval TPL tasks that contain up to eight locations, even when each location provides food on more than one occasion.

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CHAPTER SEVEN: INTERVAL TIME-PLACE LEARNING BY RATS: VARYING REINFORCEMENT CONTINGENCIES[6]

Many biologically significant events such as food availability vary spatiotemporally in a predictable manner. The ability to learn this variability, known as time-place learning, confers an advantage to an animal. There are numerous examples of animals in the field and the laboratory demonstrating knowledge of circadian variability in resources (e.g., Biebach et al., 1989, garden warblers; Daan & Koene, 1981, oystercatchers; Reebs, 1996, golden shiners; Saksida & Wilkie, 1994, pigeons; Widman et al, 2000, rats). Work in our laboratory has recently focused on another type of time-place learning, called interval time-place learning. Here the time frame of interest is much shorter – in the range of minutes. In the typical experiment, the animal (rat or pigeon) is placed in a clear, square, transparent operant box, which rests on a tabletop in a well-lit room. On each of the four walls is a light, a food hopper, and an operandum (key or lever). All four lights are illuminated at the start of the session and remain lit for the duration of the session. Each operandum provides reinforcement on an intermittent schedule, e.g., variable ratio (VR) 15 schedule, in succession, for a constant interval of time. For example, Lever 1 provides food for the first 5-min period, Lever 2 for the second 5-min period, and so on. Only one lever provides reinforcement at any given time and the levers provide food in the same order each day.

We are confident that the rats are timing the duration of food availability (rather than simply discriminating when a lever has stopped providing reinforcement) for two reasons. First, rats begin pressing on a lever just before it starts providing reinforcement.

This is known as anticipation (Carr & Wilkie, 1997). Second, rats continue to move to the correct lever at approximately the correct time on probe tests in which there are no contingencies in effect to necessitate their doing so. So called "Open Hopper Tests" (OHT, in which all levers provide reinforcement on a VR15 for the entire session) are given once rats have mastered the time-place task. If the rat was relying solely on a win-stay/lose-shift strategy to solve the time-place contingency, it would be expected to press the first lever for the entire OHT session because it would continue to receive reinforcement for pressing that lever. While the rat continues to move from lever to lever at approximately the correct time, there is often an increase in the variability. This increase in variability suggests that the rats are relying on a strategy, in addition to timing, to keep error (non-rewarded responding) to a minimum. Perhaps the most likely error-management strategy is to monitor the availability of food (Thorpe & Wilkie, 2001). For example, if the rat has not received reinforcement for a period of X seconds (or X responses), then it should respond on the next correct lever. In addition to keeping error levels down, the presence of the "error checker" also allows the animal flexibility to deal with environmental changes. The availability of biologically significant events such as food often varies predictably. However, this does not mean that there is a perfect correlation in the spatiotemporal availability of food. Occasionally, food does not occur when expected, and it is advantageous for the animal to know when to "give up" and try a new patch. An error checker allows an animal to know when expected food is not occurring.

While many of the characteristics of interval time-place learning have been uncovered in the laboratory, these discoveries have been in experiments in which only

one lever provides reinforcement at any given time (with the exception of the OHT probe sessions) and in which all levers provide reinforcement at the same rate. One can imagine that a more ecologically valid design would employ the use of various reinforcement contingencies. In nature, it is probably unlikely that patches provide food in an all-or-none manner. And it is unlikely that all patches provide the same rate of food encounters. Accordingly, in the present experiments, two changes were made to increase the validity of the time-place experiment designs. This is important given that, to the best of our knowledge, no field studies have looked at interval time-place learning. In Experiment 1 all levers provided reinforcement at all times, but on different schedules. For the first 5-min period, Lever 1 provided food on a VR8 while the other three levers provided food on a VR35. During the second 5-min period Lever 2 provided food on a VR8 while the other three levers provided food on a VR35. Lever 3 and Lever 4 provided food on a VR8 for the third and fourth periods respectively.

It was hypothesized that rats will be able to learn this task. Rats are able to discriminate between VR8 and VR35 schedules. Furthermore, optimal foraging theory suggests that they will maximize their efficiency and minimize their energy costs by responding on the levers that provide food on a VR8 rather than on those that provide food on a VR35. It is also hypothesized that rats' timing will be demonstrated through their performance on OHT and through anticipation on baseline sessions. Furthermore, if as suggested above, rats use the absence of food to decrease error in the task, then there should be more error in the present design in which all levers provide reinforcement at all times than on the typical design in which only one lever provides reinforcement at any given time. To measure error, a Gaussian curve was fitted to the normalized response rate

distribution. The width (i.e., spread) of the distribution when the response rate was at 50% of maximum was taken as a measure of error.

In Experiment 2, rats were initially trained on a typical interval time-place task in which only one lever provides reinforcement at any given time according to a VR15 schedule. Once this was mastered, probes were conducted in which Lever 2 provided reinforcement on a VR5 rather than a VR15 schedule. According to one model of timing literature, it would be expected that this increase in rate of reinforcement will lead to an increase in clock speed used in timing, thereby causing the rat to move to the next lever prematurely (Killeen & Fetterman, 1988). However, from an "ecological" point of view it might be expected that rats would stay on a lever that provided food at a higher than expected rate of reinforcement rather than move to a lever that probably provides food at a lower rate. If this is the case, we would expect to see rats perseverate on Lever 2.

Following these probes, rats were trained for 32 consecutive days with Levers 1 to 4 providing reinforcement on a VR15, VR 8, VR 15, and VR 30, respectively. They then received a further 32 days of training with Levers 1 to 4 providing reinforcement on a VR 15, VR 30, VR 15, and VR 8, respectively. According to the timing literature, there is no reason to expect any differences in behaviour on each of the levers. However, according to an "ecological" perspective one might expect to see greater anticipation on the levers that provide higher rates of reinforcement, irrespective of their position in the sequence. We also sought to see if the rats would perseverate on the richer levers.

Experiment 1

Experiment 1 attempts to determine if rats are capable of learning an interval time-place task in which all levers provide reinforcement but according to different schedules.

Method

Subjects and Apparatus. The subjects were five experimentally naive, male Long Evans hooded rats obtained from Charles River (St. Constant, Quebec). At the start of training the rats were approximately 70 days old. The rats were weighed once per week and were allowed to gain 5-10 g per week until they reached a maximum weight of approximately 450 g. The rats received 45 mg Noyes A/I pellets (P. J. Noyes Company, Inc., Lancaster, NH) during test sessions. At the end of test days, and on non-test days, they received standard rat diet (PMI Feeds, Inc., St. Louis, MO). Rats had free access to water except during test sessions.

Rats were housed individually in opaque, plastic cages (46 x 26 x 20 cm) lined with Bed o' Cobs (Andersons, Maumee, OH) bedding. Each week the rats were given paper products with which to build nests. Four to five times weekly, the rats received 20-min sessions of behavioural enrichment which consisted of placing the rats, in groups of two or three, in a large clear Plexiglas chamber containing various tubes, ladders, and toys. Colony lights were turned on at 0730 and off at 1930. Throughout the duration of the experiment, the rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

During experimental sessions the rats were tested in a transparent Plexiglas chamber (31 x 46 x 46 cm) located on a bench (90 cm height) in a small, well lit room

(159 x 210 x 275 cm). Several distal room cues were visible from the chamber (e.g., posters, door, etc.). The floor of the chamber was covered with 2 cm of Bed o' Cobs bedding. Centred on each of the four walls was a food cup, small cue light (24 V DC), and a lever (Scientific Prototype Mfg. Corp., New York, NY, Model No. E2103). Lever presses were recorded by the closure of a micro-switch mounted on each lever. Four pellet hoppers (Scientific Prototype Mfg. Corp., New York, NY, Model No. D700) were mounted on top of the chamber. When operated, the hoppers dispensed one 45 mg Noyes A/I reward pellets into the food cups next to the levers. A C++ program running on a networked computer carried out data collection and equipment control.

Procedure. Initially all five rats were placed into the testing chamber as a group. During this phase all levers provided food reinforcement on a continuous reinforcement (CRF) schedule. The experimenter did not shape lever pressing, rather one rat would accidentally press a lever and obtain reinforcement and through observation all of the rats eventually began pressing. This training usually required 4 to 5 days. Once all rats were consistently lever pressing individual training began. During individual training rats were gradually shifted from a CRF to a variable ratio (VR) 15 schedule (i.e., on average every 15th response on a lever was reinforced).

When the rats were consistently pressing on a VR15, they began training on the interval time-place task. Rats were placed into the testing chamber one at a time and a 2-min non-reinforced period began. Responses had no consequence during this time. The purpose of this non-reinforced period was to allow the rats sufficient time to patrol and inspect the chamber before testing began. Previous research has shown that initial responses may be due to causes other than forgetting or non-learning (for example, the

rats may simply be patrolling or investigating the area). To reduce the likelihood of intrusion of these errors Wilkie, Willson and Carr (1999) introduced a short non-reinforced period prior to the start of the experiment. After the non-reinforced period the cue lights came on and the session proper began.

During all sessions a data file was generated in which the time each response occurred was recorded, in addition to which lever was pressed, and whether the response resulted in the delivery of a food pellet.

At the end of the session, rats were transported back to their home cages. At approximately 1600 hr the rats were fed the remaining amount of their daily food ration. Rats received a single daily session 5 – 7 days per week. The order in which the rats were tested varied randomly across days.

There were three different types of sessions:

(1) VR8VR35 Sessions: Sessions were 20 min long and were divided into four equal periods. During each period only one lever provided food according to a VR8, while the other three levers provided food according to a VR35. For the first period, the lever on the East wall provided reinforcement on a VR8 while the other levers provided reinforcement on a VR35. Next, the lever on the North wall provided reinforcement on a VR8 while the other levers provided reinforcement on a VR35. The lever on the West wall then provided reinforcement on a VR8 while the other levers provided reinforcement on a VR35. Finally, the lever on the South wall provided reinforcement on a VR8 while the other levers provided reinforcement on a VR35.

(2) *Open Hopper Test (OHT) Sessions:* OHT were performed to confirm that rats were timing the period of food availability. The OHT were only begun once the rats were

performing consistently, that is, once the performance appeared stable upon visual inspection. Rats received two OHT sessions which were interspersed among baseline sessions in a quasi-random fashion. During OHT, all the levers provided food for the entire 20-min session on a VR15. The rats could therefore press any lever throughout the session and continue to receive food. A VR15 schedule was chosen because it was approximately midway between the VR8 and VR35 schedules used in training.

(3) *VR15EXT Sessions:* On VR15EXT sessions only one lever provided food on a VR15 schedule per period while the other levers did not provide food. The levers provided food on a VR15 schedule during the same period they provided food on a VR8 in the VR8VR35 condition. This was done to determine if there were any differences between performance on the VR8VR35 and the regular interval time-place sessions typically run.

Order of Sessions: Rats received 130 sessions of the VR8VR35 program. Interspersed within these sessions they received two OHT sessions. Next rats received 75 sessions of the VR15EXT program. Again interspersed within these sessions were two OHT sessions. To rule out possible order effects, rats were again given 60 sessions of the VR8VR35 program (i.e., A-B-A design).

Results and Discussion

Results are reported for the last 15 sessions of the VR8VR35 and VR15EXT conditions. Each rat's response rate on each of the four levers during each of the 40 30-s recording bins that comprised the 20-min sessions was computed. Any differences in overall response rates across the levers were removed by normalizing each rat's response

rate distribution for each lever. This was accomplished by expressing each rat's mean response rate on a given lever during each bin as a percentage of the rat's maximum response rate per bin on that lever. This yielded normalized mean response rates that ranged from 0 to 100.
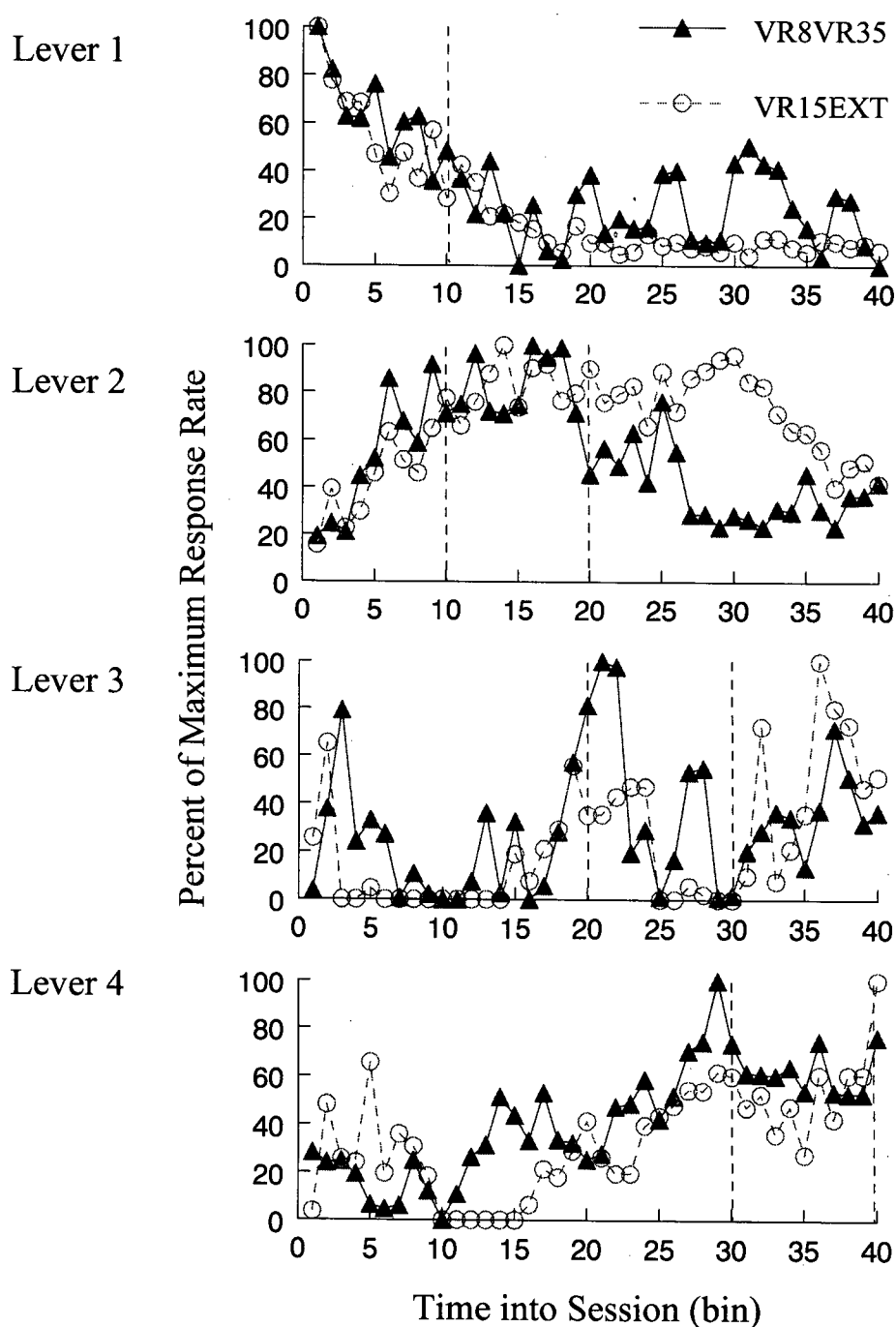
An overall response rate distribution was then computed for each lever. This was accomplished by finding the average for each bin across animals. This was again normalized, so that a maximum of 100 existed for each lever. The normalized response rate distributions for each of the 40 30-s bins are presented in Figure 7.1 for the last 15 sessions of both rounds of VR8VR35. The rats restricted the majority of their responding on all four levers to the period in which the levers provided food on the VR8. Rats received food on both VR8 ($M = 1544.4$ pellets, $SD = 226.3$) and VR35 ($M = 250.0$ pellets, $SD = 78.41$) schedules. There do not appear to be any major differences between the first and second round of VR8VR35.

To verify that rats were timing, the overall normalized response rate distribution for the two OHT given during the first round on VR8VR35 was compiled (Figure 7.2; also shown are the data for the two OHT given during VR15EXT). While the data are obviously much noisier than during baseline sessions, the rats do move from lever to lever in the correct order rather than staying at one lever for the entire session, indicating that they are in fact timing.

Figure 7.3 shows the normalized response rate distributions for both the first round of VR8VR35 and VR15EXT phases of the experiment. Overall performance was similar in the two conditions – rats restricted the majority of their responding to the lever
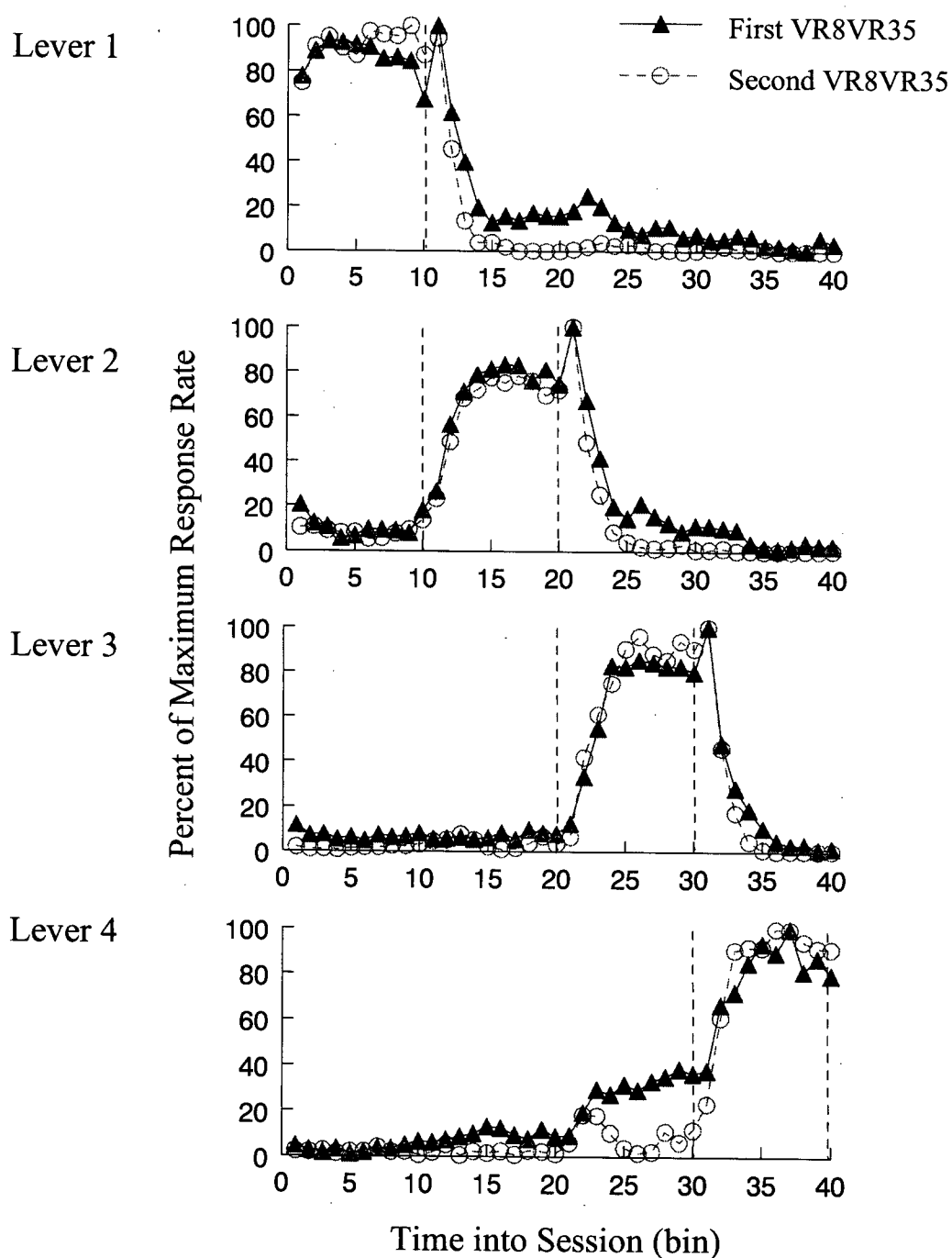
Figure 7.1. The normalized response rate distributions for the last 15 sessions of both rounds of VR8VR35 program on Levers 1, 2, 3 and 4. Responses on each lever were reinforced on a VR8 during the period bounded by the vertical dashed lines and on a VR35 at the other times.

Figure 7.2. The normalized response rate distributions for the two OHT given during the first round of VR8VR35 (triangles) and the two OHT given during VR15EXT (circles). The vertical dashed lines represent where the rats received reinforcement on a VR8 (VR8VR35) or VR15 (VR15EXT) on baseline sessions.
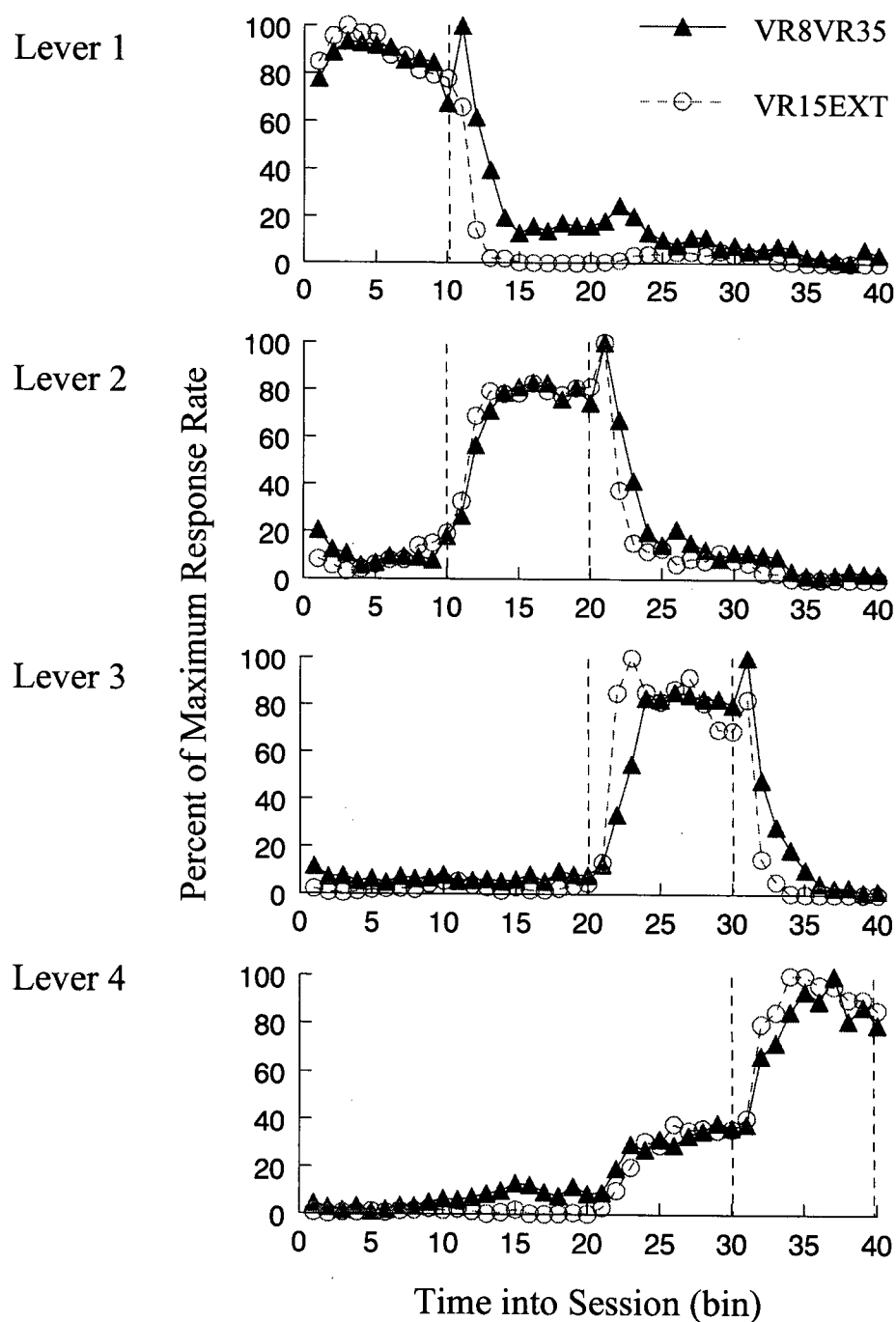
Figure 7.3. The normalized response rate distributions for the last 15 sessions of the first round of the VR8VR35 (triangles) and VR15EXT (circles). The vertical dashed lines represent where the rats received reinforcement on a VR8 (VR8VR35) or VR15 (VR15EXT).

that provided food on the highest density. Rats were timing as demonstrated by their performance on OHT (Figure 7.2) and by their tendency to anticipate when a lever would provide food. The anticipation effect can be seen in Figure 7.3 but is more clearly visible in Figure 7.4A which shows rats' responses on a lever for the 10 bins immediately prior to the lever providing food on both VR8VR35 and VR15EXT sessions. Responses are averaged across Levers, 2, 3, and 4. These lines have a significant positive slope (VR8VR35: $F(1,8) = 53.94, p < .001, R^2 = .871$; VR15EXT: $F(1,8) = 216.14, p < .001, R^2 = .964$), demonstrating an increased rate of responding as time of food availability on the next lever approaches.

While previous studies on interval time-place learning have shown the anticipation effect this experiment is the first to show a spike in responding after the lever stopped providing food. While the spike in response rate at the start of a lower rate of reinforcement period can be seen in Figure 7.3 it is shown more clearly in Figure 7.4B. Figure 7.4B shows the rate of responding on the last rewarded bin and on the first non-rewarded bin (VR15EXT) or less rewarded bin (VR8VR35) averaged across Levers 1 to 3. There is a significant increase in responding on the non-rewarded bin for the VR8VR35 ($t(4) = 3.39, p = .028$), but not for the VR15EXT ($t(4) = 1.818, p = .143$). In the VR8VR35 condition, this high rate of responding on a lever continued for another couple of bins after it had stopped providing food. This tendency to stay on a lever after it has stopped providing reinforcement is known as perseveration. To measure perseveration, the area under the response rate distribution for the 3 bins immediately following when a lever stopped providing food was calculated. Figure 7.5 shows the average perseveration for the VR8VR35 and VR15EXT groups. As can be seen, there is a

A
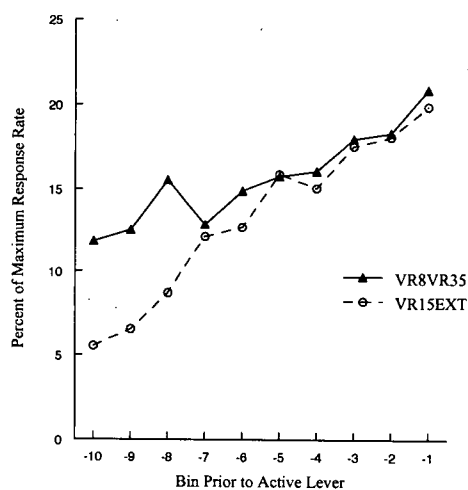
B
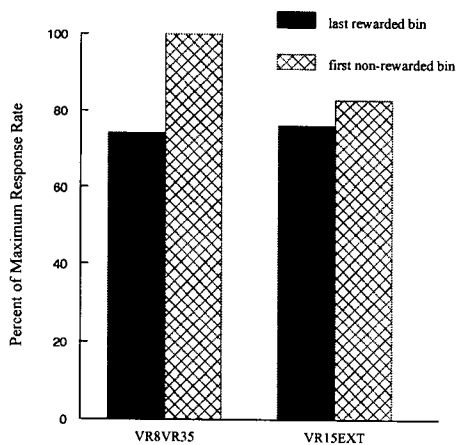
Figure 7.4. Characteristics of the last 15 sessions of the VR8VR35 and VR8EXT trials. Panel A shows the percent of maximum response rate for the 10 bins prior to a lever providing reinforcement for correct responding. Triangles represent VR8VR35; circles represent VR15EXT. Panel B shows the percent of maximum response rate for the last rewarded bin and the first non-rewarded bin averaged across levers.
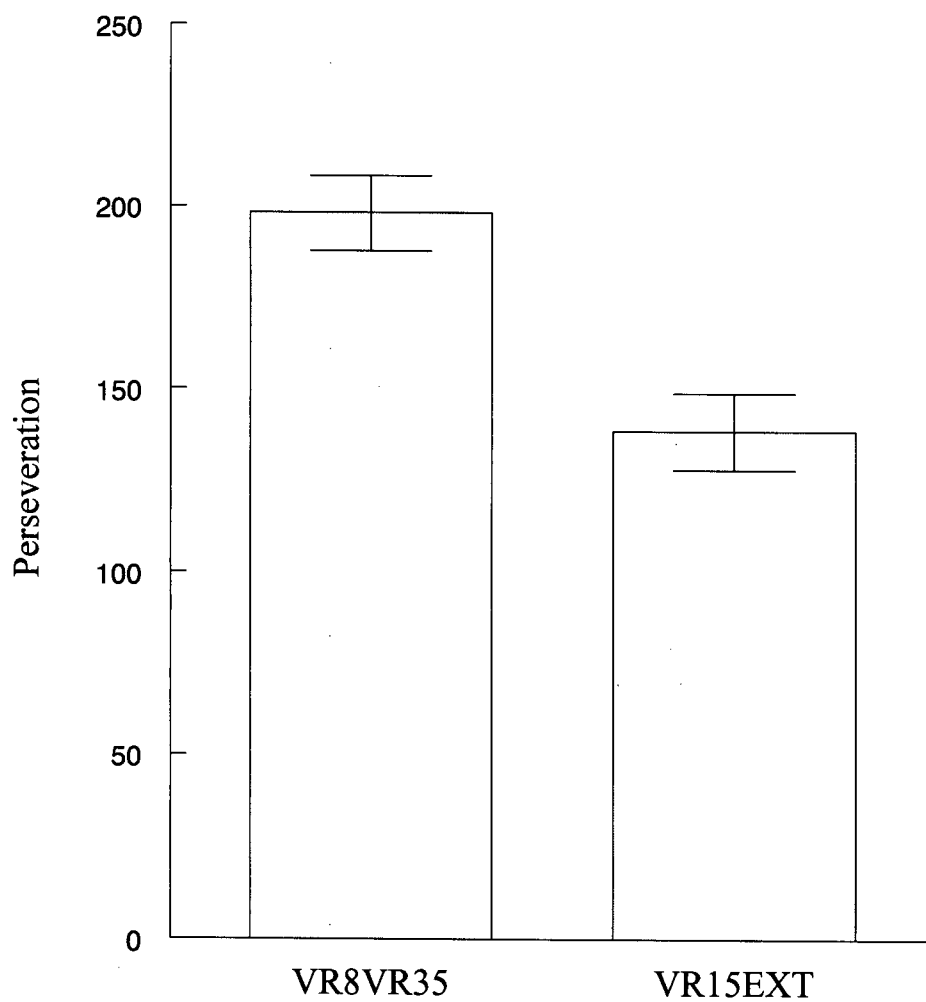
Figure 7.5. Average perseveration for the last 15 VR8VR35 and VR15EXT sessions. Perseveration was measured as the area under the response rate distribution for the 3 bins immediately following when a lever stopped providing food.

significant difference between the two groups in perseveration ($t(4) = 4.554, p = .010$). This increase in perseveration may reflect the fact that it is more difficult to distinguish between VR8 and VR35 than between VR15 and extinction. The ability to detect this difference may underlie the built-in error checker.

To determine if there were any differences in the error, Gaussian curves were fitted to these data as is commonly the case (e.g., Roberts, 1981) using the SlideWrite 5.0 graphing program (Advanced Graphics Software, Inc., Carlsbad, CA). This program utilizes the Levenberg-Marquardt algorithm to minimize the sum of the squared deviations. This fitting program also extrapolated the curves for Lever 1 and Lever 4. The spread of each curve was then calculated. Spread is defined as the width of the distribution at 50% of maximum response rate and is taken as a measure of error. As expected the spread was greater for the VR8VR35 ($M = 12.56, SD = 1.86$) than the VR15EXT ($M = 10.69, SD = .97$) condition. However, a paired sample t-test showed no significant difference between spread in the VR8VR35 and VR15EXT conditions ($t(4) = 1.996, p = .117$). Because of low power we cannot conclude that there is no difference in the spread of the VR8VR35 and VR15EXT conditions.

Experiment 2

Experiment 2 sought to determine how rats respond to an interval time-place task in which the levers provide reinforcement at different densities. The main measures of interest were anticipation and perseveration.

Methods

Subjects and Apparatus. The subjects were four experimentally naive, male Long Evans hooded rats obtained from Charles River (St. Constant, Quebec) aged approximately 70 days old at the start of training. Rats were housed and maintained in a similar fashion as in Experiment 1.

The experimental chamber was slightly smaller (40 x 40 x 40 cm) than that used in Experiment 1 but was otherwise identical. Again several distal room cues were visible from the chamber.

Procedure. The rats used in this experiment were trained to lever press in a similar manner as in Experiment 1. Rats received two daily experimental sessions 5 – 7 days per week. Again each session began with a 2-min non-reinforced period in which responses had no consequence. Rats were trained for 234 sessions in which Lever 1 provided food on a VR15 for the first 3-min period, followed by Lever 2 for the second 3-min period, Lever 3 for the third 3-min period, and finally Lever 4 for the fourth 3-min period. Only one lever provided food at any given time and the order in which the levers provided food was constant across sessions. Interspersed between these baseline sessions were OHTs similar to those described in Experiment 1.

Following completion of the 4 OHT, six VR5 probes were introduced. These probes were separated by at least 7 days. They were identical to baseline sessions except that Lever 2 provided food according to a VR5 (rather than a VR15).

Next, rats were trained for 64 sessions, so that Lever 1 provided reinforcement on a VR15 for the first 3-min period, Lever 2 on a VR8 for the second 3-min period, Lever 3 on a VR15 for the third 3-min period, and Lever 4 on a VR30 for the fourth 3-min period.

Following this, rats were trained for a further 64 sessions in which Lever 1 and 3 continued to provide food on a VR15, but Lever 2 switched to a VR30 schedule and Lever 4 to a VR8 schedule.

Results and Discussion

Normalized response rate curves were found for the baseline and OHT sessions in the same manner as described in Experiment 1. These results are shown in Figure 7.6. As can be seen from these data, the rats have learned the interval time-place task and are timing the 3-min periods. There is considerably less noise in the OHT in Experiment 2 than in Experiment 1. This is probably due to the simpler contingencies in effect in Experiment 2.

Figure 7.7 shows the normalized response rate distributions for both the baseline data and the six probe sessions in which the second lever provided reinforcement on a VR5 schedule. The rats did not leave Lever 2 to move to Lever 3 earlier on the probe sessions as would be expected from one account of how animals time (Killeen & Fetterman, 1988). According to this model, the increase in rate of reinforcement would have lead to an increase in clock speed which would then result in the rat leaving the lever early. This is not what happened. In fact, the maximum response rate for Lever 2 for the bin immediately following reinforcement was significantly higher on probe tests than on baseline tests ($t(3) = 5.711, p = .011$). From an ecological perspective it is not surprising that the rat perseverated on a lever that provided a higher than normal density of food.
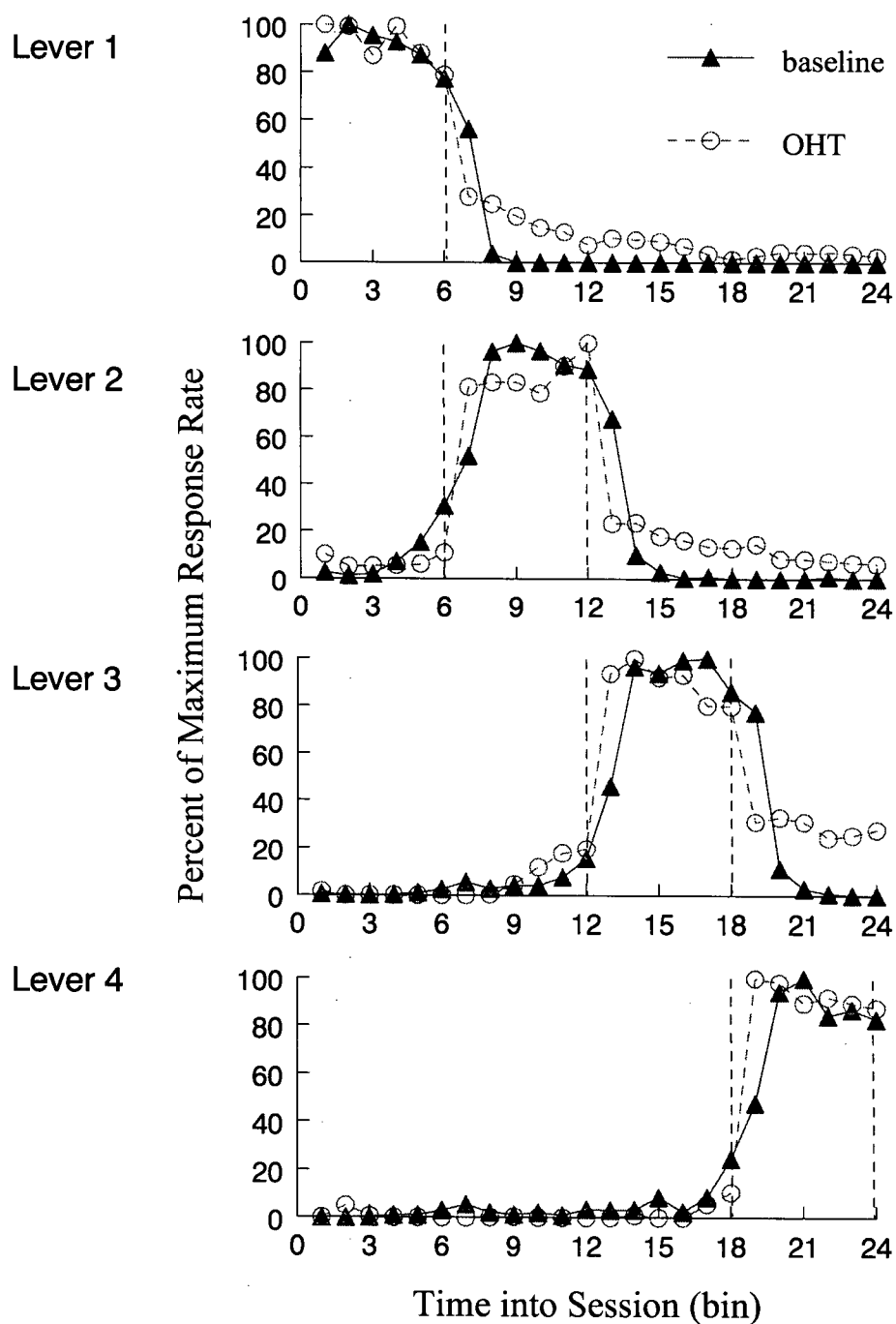
Figure 7.6. Normalized response rate distribution for baseline (triangles) and OHT (circles) sessions. The vertical dashed lines bound the period in which reinforcement was available during baseline sessions.

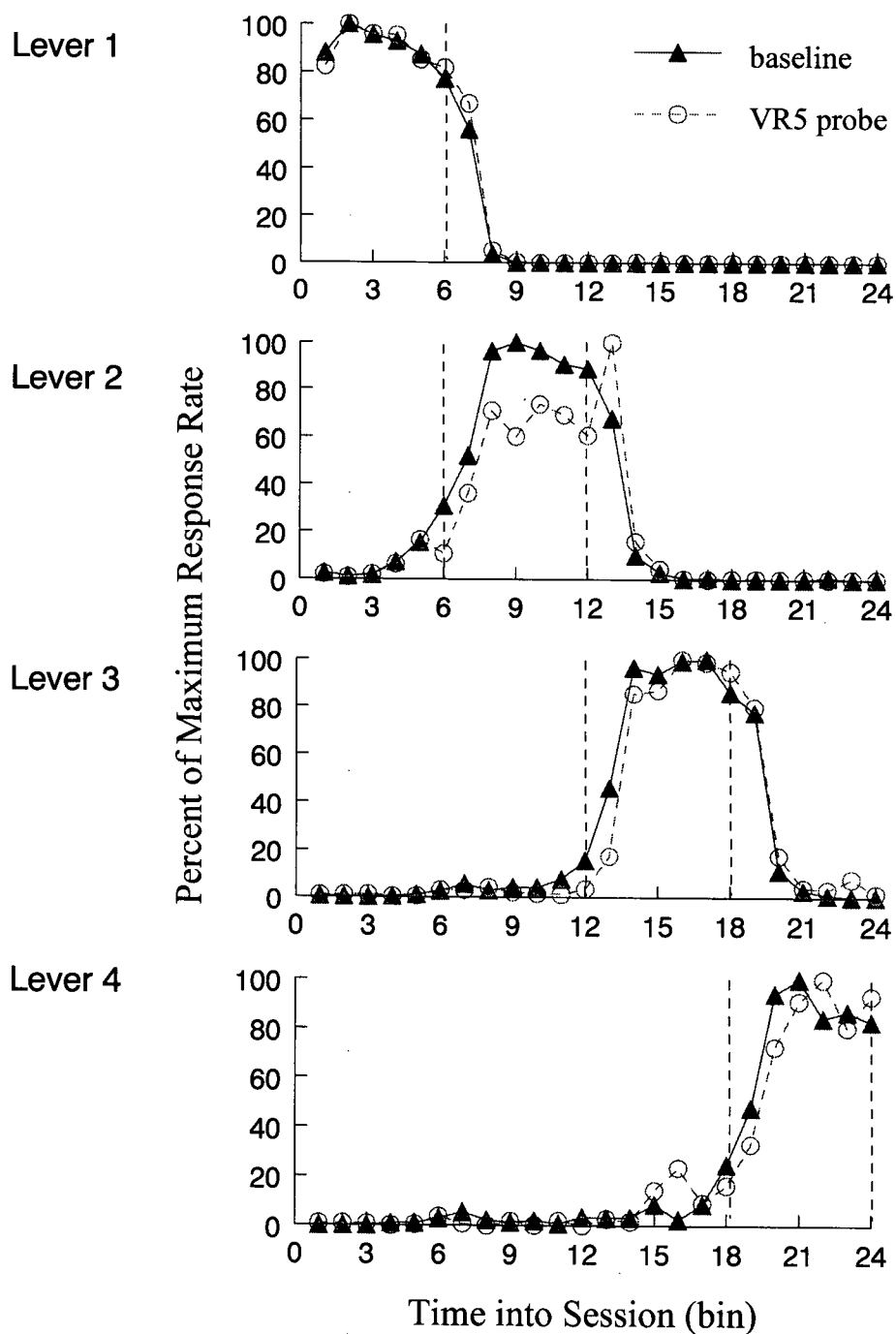*Figure 7.7.* Normalized response rate distributions for the baseline (triangles) and VR5 probe (circles) sessions. Area bounded by vertical dashed lines represents period in which levers provided reinforcement.
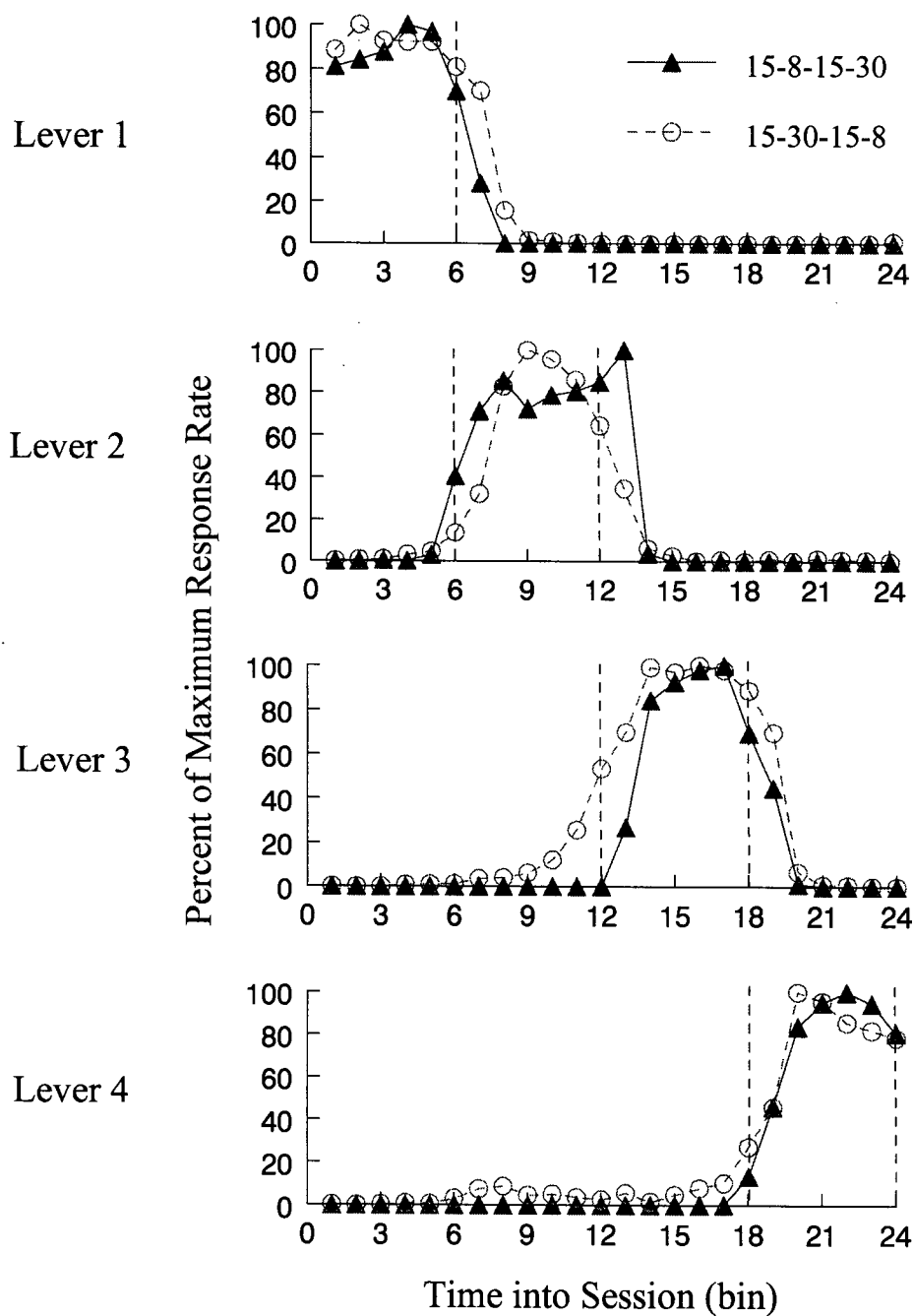
Figure 7.8. Normalized response rate distributions for the 15-8-15-30 (triangles) and 15-30-15-8 (circles) sessions. Area bounded by vertical dashed lines represents period in which levers provided reinforcement.

The normalized response rate distributions for the last 30 sessions of the 15-8-15-30 and 15-30-15-8 phase of the experiment is shown in Figure 7.8. There does not appear to be any differences in amount of anticipation on levers that provided higher densities of food reinforcement. There is a trend to perseverate longer on Lever 2 when a VR8 is in effect than when a VR30 is in effect ($t(3) = 2.697, p = .074$). This tendency to perseverate on levers providing higher densities of food is similar to that found above.

General Discussion

Until now, interval time-place learning has been studied under a restricted set of parameters. In the typical interval time-place study only one lever at a time provides reinforcement, most often on a VR15 schedule. Other levers do not provide food (extinction, EXT). The purpose of the present experiments was to study time-place learning under a different set of parameters, which are more like the conditions encountered in a natural environment. Because patches in natural environments probably do not often provide food in an all-or-none manner, but rather have food densities that vary in a graded manner, Experiment 1 was an important generalization of time-place behavior. In that experiment rats learned to track the time-place variation in high vs. low density, rather than all or nothing, food availability.

Experiment 2 was also an attempt to arrange time-place contingencies more similar to those encountered in natural environments. In that experiment, rats learned a time-place task in which the different patches (levers) provided food at different rates.

In the standard VR15EXT task, one typically sees anticipation of food availability. Rats respond in a graded manner on a lever that is about to provide food: the closer the time of food becoming available, the more vigorous is the responding. In the standard task, one often also sees anticipation of depletion (Carr & Wilkie, 1997). Responding on a lever that is providing food wanes as the period of food availability expires. One can see hints of this effect in the VR15EXT condition in Experiment 1, especially on Levers 1, 3, and 4. Interestingly, this anticipation of depletion was not seen in the VR8VR35 condition. Indeed, rats tended to actually perseverate on the high density lever after it had changed to the low density condition. Thus, anticipation of depletion does not seem to occur when the boundaries of food density changes are blurred. This result suggests that foragers may over-stay their time in a patch when patch densities are not all or nothing. Rats also tended to over stay their time on a lever that provided a higher rate of reinforcement (Experiment 2). These effects may stem from animals' attempt to maximize reinforcement rate.

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CHAPTER EIGHT: DISCUSSION

The findings of this dissertation can be broken down into two portions. Chapters Two and Three show that while rats are capable of learning daily TPL tasks under some conditions, there are clearly instances in which they appear to not learn spatiotemporal contingencies. Chapter Three discusses how this finding violates Gallistel's (1990) theory that time-place-event information is automatically encoded into a tripartite code, and offers an alternative bipartite theory. This is discussed below in the Daily Time-Place Learning section.

The second portion examines the properties of interval TPL using more ecologically relevant tasks and procedures. Each of Chapters Four through Seven introduced modifications of the typical interval TPL paradigm to determine the properties of interval TPL. The major findings are discussed below in the Interval Time-Place section.

The major findings of each Chapter are summarized in Table 8.1.

Daily Time-Place Learning

Despite the appealing power and simplicity of Gallistel's (1990) view of memory, recent experiments have shown that this theory has constraints. In particular, there is empirical evidence questioning the automatic nature of time-place-event memory formation. Demonstrating robust time-place behaviour in rats and fish has been somewhat difficult.

The research conducted as part of my dissertation has added to the growing list of research demonstrating that rats do not readily demonstrate evidence of daily TPL. Rats

Table 8.1

Summary of Major Findings of Chapters Two through Seven

Chapter	Timer	Major Findings
Two	Daily	Rats failed to demonstrate knowledge of a TPL contingency using a semi-aversive water maze and food rewarded place preference task, radial arm maze task and T-maze task.
Three	Daily	Rats learned a TPL contingency when differing amounts of food were given during each session. Alternative bipartite theory proposed.
Four	Interval	Rats kept track of spatial information following distraction with cheese, but their clock appeared to stop. Further evidence for bipartite codes, rather than tripartite code.
Five	Interval	Rats learned TPL task in which each lever provided food for differing amounts of time. Evidence suggests rats were timing.
Six	Interval	Rats learned TPL task in which there were 8 "places" and a revisiting strategy was required. Evidence suggests rats were timing.
Seven	Interval	Rats learned to restrict the majority of responding to levers providing food at highest density when multiple levers provided food simultaneously. Evidence suggests rats were timing.

failed to demonstrate knowledge of a daily TPL contingency using a semi- aversive water maze and food rewarded place preference task, radial arm maze task and T-maze task (Chapter Two). These findings are obviously not meant in any way to suggest that rats are strictly incapable of learning daily TPL tasks. Other researchers, including myself, have shown that rats do have this ability. However, while rats may be able to learn daily TPL tasks under certain situations they seem *unprepared* to do so. Seligman (1970) proposed that animals are prepared to learn some associations (e.g., taste-illness), but not others (e.g., tone-illness). The fact that rats are unprepared to learn the association of Time-Place-Event is further evidence against Gallistel's theory.

In the past few years, there have been three theories formulated to explain the inconsistencies in the daily TPL literature. The first was by a group who suggested that the strain of rat used might be a critical determinant in daily TPL. Cain, Ko, Chalmers, and Ralph (2004) trained two strains of rats (Long Evans and Wistar) on a conditioned place preference task. During training they were always run at the same time of day. On test days they were placed in the apparatus at either the same or different time of day. Testing the rats at a different time of day resulted in Wistar rats having a decreased preference for the food rewarded place, whereas the Long Evan rats were unaffected by the change in time of testing. This finding suggests that the Wistar rats encoded circadian information, while the Long Evans rats did not. While this theory seems intriguing, it is inadequate to explain most discrepancies in daily TPL. There have been instances in which Long Evans rats have displayed daily TPL (e.g., Chapter Three of current dissertation; Carr & Wilkie, 1997, 1999; Pizzo & Crystal, 2002). Furthermore, Widman, Gordon, and Timberlake (2000), using Sprague-Dawleys, have sometimes found

evidence for daily TPL and sometimes have not. Response cost was a determining factor in success (see below), not strain.

Lukoyanov, Pereira, Mesquita, and Andrade (2002) noted that to date the only demonstrations of daily TPL had been in food rewarded tasks. This, combined with the fact that Mistlberger, de Groot, Bossert, and Marchant (1996) found that ablations of the suprachiasmatic nucleus (SCN; the brain area involved in light-entrained circadian timing) did not result in the inability of rats to learn a daily TPL task, suggested to Lukoyanov et al. that the involvement of a food-entrained oscillator may be a requirement for daily TPL. They trained two groups of rats on a version of the Morris water maze in which the location of the platform depended on the time of day. The food restricted rats received only 60% of the food that the ad libitum rats consumed. The food restricted rats learned the task while the ad libitum rats did not. In addition, corticosterone data suggested that the ad libitum rats' oscillator was light-entrained (serum levels of corticosterone were elevated only in the afternoon), while the food restricted rats' oscillator was food-entrained (serum levels of corticosterone were elevated in the morning and afternoon). The researchers theorized that in order for daily TPL to occur there must be activation of the food-entrained oscillator. Unfortunately, this theory has been ruled out by Widman et al. (2000).

Widman et al. (2000) have shown that by increasing the response cost, the success rate of daily TPL is also increased. Rats were trained to go to one of two locations for food reward depending on the time of day. The food reward was located at the top of a vertical maze. By increasing the height the rats had to climb (i.e., increased effort), they increased the success rate. This theory is also able to explain the results of the Lukoyanov

study described above. Widman, Sermania, and Genismore (2004) hypothesized that by restricting the food intake of the rats, the researchers also increased the response cost due to the increased metabolic needs of the rats. To test this hypothesis they put weights on half of the rats in a Morris water maze. (None of the rats were food restricted.) The rats that were weighted (i.e., increased response cost) were more successful at learning the spatiotemporal contingencies than were the non-weighted rats.

While this theory is extremely appealing and at first glance seems able to explain the discrepancies in the literature, it is lacking the ability to a priori determine if a group of rats will learn the task. Within a particular task, it can predict which rats are more likely to learn the TPL contingencies. For example, rats that have to climb higher towers for food are more likely to learn the task. But it does not specify at what height the towers must be for learning to occur. More importantly however, is its inability to quantify response cost between tasks. For example, how does one compare the relative response cost of the Morris water maze and lever pressing? A final concern with the response cost theory is that without a proper operational definition of response cost, it is inseparable from success and a circular definition results. If rats learn a daily TPL task it is because the response cost was sufficiently high; if they fail to learn it was because it was too low. Is requiring a rat to lever press according to a variable ratio 15 (VR15) considered low or high response cost? The answer must be high because Carr and Wilkie's (1997) rats learned when placed on this contingency. Is requiring a rat, deprived to 90% of its free-feeding weight, to swim in a Morris water maze for a maximum of 60 s considered low or high response cost? The answer must be low because Thorpe, Bates, and Wilkie (2003) did not find evidence of daily TPL. Unless an operational definition of low and high

response cost can be proposed, this theory will have no predictive ability and is therefore inadequate.

Gallistel proposed that whenever a biologically significant event occurred a tripartite memory code is automatically formed that includes the nature of the event and the time and place in which it occurred. This is clearly not the case. Under some situations (perhaps those with "high" response cost), it does appear that the animal has access to all three parts of the code. However, if the rat automatically stored the three components it would not make sense for it to ignore that information under some conditions. I hypothesize that, rather than automatically encoding a tripartite (Time-Place-Event) code, the rat encodes two bipartite codes (Time-Event and Event-Place; See Figure 8.1). These bipartite codes allow rats to learn a place preference for those locations that have provided food (Event-Place) in daily TPL tasks. It also explains how rats readily learn go no-go discrimination tasks (Time-Event).

In low response cost daily TPL situations in which the event (i.e., food) is the same, there is confusion as to which place the animal should go at a certain time of day. For example, if food (Event) has been found at both $Place_1$ and $Place_2$, then when it wants food it will equally sample $Place_1$ and $Place_2$ (because it does not encode the time and place in the same code). This is in fact what is found (Thorpe et al., 2003, Means, Ginn, Arolfo, & Pence, 2000). If however, the events are dissimilar at different times and places, the rat is capable of learning a daily TPL task. In Chapter Three, rats were trained in a T-maze that contained a large amount of food in one spatial location at one time of day, and a small amount of food in another spatial location at another time of day. Rats

HIGH REPONSE COST SITUATION

(as proposed by Gallistel (1990))

(T-P-E tripartite code)

Memory   *Decision*

T_1-E-P_1 T_1-E-P_1

T_2-E-P_2

LOW RESPONSE COST SITUATION

(T-E and E-P bipartite codes)

Normal Daily TPL

Memory   *Decision*

T_1-E   E-P_1 T_1-E but E-P_1 ⎫
                                                                   ⎬ Randomly
T_2-E   E-P_2 E-P_2 ⎭ choose P_1,P_2

Differential Food Daily TPL

Memory   *Decision*   ⎡ Only P_1
                                                                   | associated with
T_1-E_1 E_1-P_1 T_1-E_1 E_1-P_1 ⎬ E_1, choose P_1

T_2-E_2 E_2-P_2

Figure 8.1. Theorized memory codes and decision processes involved in high and low response cost daily TPL situations. The low response cost situations are further divided into those with the same event (i.e., equal amounts of food) and those with different events (i.e., different amounts of food). T=Time, P=Place, E=Event. The hypothesized memory codes are shown on the left side. The decision process that would occur at T_1 are shown on the right side.

were able to learn this task. Here, $Time_1$ was associated with $Event_1$, and $Event_1$ was only ever associated with $Place_1$. Therefore, there was no confusion as to the place to search. (Some additional evidence for the existence of bipartite memory codes will also be discussed in the interval TPL section).

While the default is to encode bipartite time-event and place-event information, under some conditions rats do encode time-place-event information into one code. More research needs to be conducted to determine exactly under which conditions a tripartite code is used and how the two bipartite codes are "bound" together into a tripartite code. This "binding problem" has received the most attention in studies of perception. For example, a visual feature of an object such as shape must be correctly associated with other features such as colour or location for a unified representation of that object to exist. Similarly, in TPL tasks, the spatial and temporal information must be associated with the biologically significant event for a complete understanding of the spatiotemporal characteristics of these events.

A second debate surrounding daily TPL concerns the timing mechanisms used in this task. It is extremely important to rule out the use of a non-timing strategy such as alternation when conducting daily TPL tasks. To determine the type of timing mechanism, skip sessions are used. If the animal is relying on an alternation strategy then following skip morning and afternoon sessions, the animal will respond incorrectly. Pizzo and Crystal (2004) recently found evidence showing that their rats that were trained on an eight arm radial maze TPL task relied on an alternation strategy to solve the task. Unfortunately, many researchers still do not conduct these skip session probes. Those few studies that have conducted the probes have reported conflicting results. Carr and Wilkie

(1997) found evidence suggesting that an ordinal timer was used by their rats. Ordinal timers allow an animal to predict the order in which events occur within a particular time period. The ordinal timer is reset by some, as yet unknown, event. These researchers also found that rats were reluctant to use a circadian timer even when a circadian timer better predicted the location of food (Carr & Wilkie, 1999).

Other researchers (e.g., Pizzo & Crystal, 2002) have found evidence showing that circadian timers are used in daily TPL. When circadian timers are used, evidence suggests that the timer is food-entrained, rather than light-entrained. This evidence comes from the Mistlberger et al. (1996) and Lukoyanov et al. (2002) studies discussed previously.

It would thus appear that rats are capable of using either a food-entrained circadian timer or an ordinal timer in daily TPL tasks. In Chapter Three, within one experiment, it was found that some rats used a circadian timer while others used an ordinal timer.

Interval Time-Place Learning

Prior to the start of my PhD research, investigations of interval TPL were conducted using a very restricted range of parameters. All levers provided reinforcement for the same duration and at the same rate. There was also no variation in the number of places involved, and the same place never provided food for more than one period within a session. This thesis has stretched the limits of interval TPL and thus sought to make the task more ecologically valid. In the process, some interesting characteristics of interval TPL have been uncovered.

First and foremost, the ability of rats to learn interval TPL is very robust to manipulations of the task. Rats easily learned the spatiotemporal contingencies to maximize their reinforcements. They learned to restrict the majority of their responding to the reinforced lever even when the levers provided food for differing amounts of time (Chapter Five). When multiple levers provided food simultaneously, rats learned the spatiotemporal variability of the levers providing reinforcement at the highest density (Chapter Seven). Finally, when levers provided food on more than one occasion, rats were able to keep track of the sequence in which they were to visit the arms and were able to keep the error from accumulating (Chapter Six).

Secondly, evidence from Chapter Four suggests that as in daily TPL, spatiotemporal information is unlikely to be represented in a tripartite memory code. When rats were presented with a highly preferred food during the TPL task, they were able to keep an accurate record of the spatial location of the correct lever (i.e., in most instances, they returned to the lever that had been providing food prior to the presentation of the distraction) suggesting that their spatial knowledge was intact. However, this retention was not the case for temporal knowledge. The rats' internal clock appeared to stop during the food presentation. This provides further support for the idea that temporal and spatial information are represented by two separate entities; namely the bipartite Time-Event and Place-Event codes.

This thesis also adds to the growing list of research showing that there are violations of scalar timing (Weber's Law). Scalar Timing Theory states that the longer the duration being timed, the greater the error in that timing (Gibbon, 1991). In Chapter Five the levers provided reinforcement for 6, 4, 2, and 8 minutes respectively. It was

found that the relative response rate curves for the different durations did not superimpose, and the coefficient of variation (standard deviation/mean) was not constant for the different durations. Crystal and Miller (2002) suggest that simultaneously processing temporal and spatial information results in violations of Weber's Law. Other researchers contend that Weber's Law does not hold in situations where there is a sequence of intervals to be timed or in situations where there is extensive training (Grondin, 2001). Both of these hypotheses are consistent with the present data.

A final point worthy of comment is that rats are exceedingly good at maximizing their reinforcement intake. They are able to detect differences in reinforcement rate and track the time-place variation in high versus low reinforcement density places. Rats also tend to overstay their time on levers that provide a higher rate of reinforcement, which probably stems from their attempt to maximize reinforcement rate. In addition to capitalizing on reinforcement, rats are also quite adept at keeping error to a minimum. During baseline sessions it is hypothesized that the rats discriminate when a lever stops providing reinforcement. If there is a conflict between the timing mechanism and the expected rate of reinforcement, the rat will eventually switch to a new lever. This can help explain why there is more variability on probe tests in which all levers provide food at all times (open hopper tests). Evidence also suggests that rats time individual periods rather than the entire session. This helps to minimize errors, especially in cases such as an eight location, TPL task (Chapter Six).

Concluding Remarks

The current research program has important implications for theories of learning and memory. As previously discussed, the difficulty in obtaining daily TPL in rats under some conditions suggests that Gallistel's (1990) theory of memory needs revision. I have suggested that rather than automatically forming a tripartite Time-Place-Event memory code, rats form two bipartite memory codes (Time-Event and Event-Place). This is the default memory mechanism – only under certain conditions do rats form a tripartite code. Perhaps the best theory explaining when rats form tripartite codes is Widman et al.'s (2000) response-cost theory. However, as discussed above this theory has limitations. Unfortunately, there is no clear alternative that better predicts when daily TPL will occur. The next logical step in this line of research is to continue investigating the properties of daily TPL so that an alternative hypothesis – one that more completely accounts for and predicts learning – may present itself.

One caveat that must be acknowledged in the proposed theory of TPL (and all laboratory studies) is that our results can only be generalized to rats raised in typical laboratory conditions. It is possible that if rats were raised in a more naturalistic environment in which they had early exposure to spatiotemporal regularities they might more readily learn TPL tasks. There are at least two possible methods for examining this. First, a field experiment could be conducted in which wild rats or rats raised in a more naturalistic setting are given the opportunity to learn a TPL contingency. Second, rats could be trained on spatiotemporal contingencies from the time of weaning. If there were a critical period for the ability to learn TPL tasks, then we might see evidence of TPL in rats trained from an earlier age.

Given that spatiotemporal characteristics of stimuli are liable to be encoded separately, it would be interesting to determine how animals learn spatiotemporal contingencies. Two possible studies come to mind. First, rats could be trained on an interval TPL task and analysis of the learning curves for both the location (percent correct lever choices) and timing (spread/error) could be conducted. If these two curves reached asymptote at different times it would provide further evidence that time and place are represented separately in memory. Secondly, an experiment could be conducted to tease apart the roles of temporal and spatial information. One group of rats could be trained with the duration that the levers provide food remaining constant across sessions while the sequence in which the levers provide food changes across sessions. Hence the rats have the opportunity to learn the Time-Event code. Another group of rats could be trained with the sequence in which the levers provide food remaining constant across sessions, while the duration that the levers provide food changes across sessions. Hence these rats would have access to the Place-Event codes. When both groups of rats have learned the tasks they will be trained on a typical TPL task in which the temporal and spatial information remains constant across sessions. Analysis of the learning curves could provide further insight into how the rats learn interval TPL tasks.

As a final point I would like to discuss the characteristics of TPL in relation to human memory, in particular the potential of TPL to contribute to the study of episodic-like memory in animals. Human episodic memory contains Time-Place-Event information. For example, last week (Time) I had a snowball fight (Event) in front of the Kenny building (Place). Are animals capable of the same type of memory? This question

has recently generated much interest (see Clayton & Dickinson, 1998, Roberts, W. A., 2002, Tulving, 1972, 1984).

Throughout this thesis many examples of animals' Time-Place-Event memory abilities have been given. Are the rats' memories of receiving food in the left arm of the T-maze yesterday morning the same as my memory of the snowball fight? I would argue not. A snowball fight in Vancouver is an extremely distinctive event. It is a *unique* Time-Place-Event combination. The rats in the TPL tasks experience the same Time-Place-Event combinations every day. If I had a snowball fight in front of the Kenny building every day it would surely lose its place as an episodic memory. I would remember it no better than I remember what I had for breakfast the morning of the snowball fight. Instead, the ability of animals to learn the spatiotemporal regularities of biologically important events may be considered equivalent to human semantic memory. So while it might be tempting to use TPL as a paradigm for the study of episodic memory, we must curb our enthusiasm.

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