TIME-OF-DAY ASSOCIATIVE LEARNING TO SPATIAL OR FEATURE INFORMATION IN HOMING PIGEONS (COLUMBA LIVIA)

Brittany A. Sizemore

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Committee:
Verner P. Bingman, Advisor
Michael E. Geusz
Richard B. Anderson
ABSTRACT

Verner P. Bingman, Advisor

The current study examined time-of-day associative learning to either spatial or feature information in homing pigeons in an open-field, laboratory setting. Homing pigeons are well known for their navigational abilities and typically have been shown to rely more heavily on spatial rather than non-spatial cues in identifying a goal. However, during goal localization homing pigeons have also been known to use non-spatial, feature information. Past work has demonstrated that homing pigeons are capable of using time-of-day information to guide their goal-seeking behavior in operant tasks and open-field environments. Additionally, avian time-of-day associative learning is thought to be influenced by two potential timing mechanisms, a circadian-based clock or an interval timer. While previous research has investigated avian time-of-day associative learning and cue utilization during goal localization, the current study aimed to blend these two research branches. Homing pigeons were divided into two groups, space or feature, and were trained to locate two time-of-day dependent food reward sites in open-field environments. We hypothesized that homing pigeons would develop a stronger or better time-of-day association with spatial information than feature information and that they would consult a circadian based clock rather than an interval timer during a temporal discrimination of reward availability. Homing pigeons that were able to use spatial information were more likely to find the correct goal location in comparison to homing pigeons that were only able to use feature or color information. Although the data are too few to draw any conclusion, the majority of homing pigeons in both groups seemed to use interval timers instead of circadian clocks during the task.
This thesis is lovingly dedicated to my sweet and playful feline friend, Mocha.
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INTRODUCTION

Time is an important cue that many animals, including birds, may rely on during activities such as resource acquisition, mate selection, home burrow localization, predator avoidance, migration, and offspring care (for reviews see Cassone & Westneat, 2012; Mulder, Gerkema, & Van der Zee, 2013). Animals may use time information to guide specific behaviors that occur annually, seasonally, or daily. Gallistel (1990) posited that animals are generally able to remember spatio-temporal properties of biologically significant events. Memories for these important events may be unique regarding their individual characteristics, their location in space and their temporal occurrence. Different species of animals have been demonstrated to use time-of-day information during resource acquisition and goal localization tasks in laboratory settings. This field of research, known as time-place learning (TPL), typically requires animals to remember the association between temporally sensitive parameters and specific spatial locations of some biologically significant event (Wilkie, 1995; Crystal, 2009; Mulder et al., 2013). In the present study, we wanted to further investigate TPL in homing pigeons and the potentially different impact of spatial, location-based or feature, color-based information.

In the early 1980’s, initial field observation work on kestrel, *Falco tinnunculus* (Rijnsdorp, Daan, & Dijkstra, 1981), foraging preceded development of time-place laboratory paradigms designed to examine temporal associations on a circadian (time-of-day) scale. Biebach, Gordijn, and Krebs (1989), in their seminal paper, were among the first researchers to provide evidence of time-of-day, time-place learning in a laboratory environment using garden warblers (*Sylvia borin*). Warblers were able to rely on time to find food rewards in different rooms of an experimental environment. Following this study, similar research has been conducted in other types of birds, including starlings (*Sturnus vulgaris*; Wenger, Biebach, &
Krebs, 1991) and homing pigeons (*Columba livia*; e.g. Wilkie, 1995) with consistent results. Homing pigeons are able to use time as a discriminative cue, either as short-intervals (i.e., seconds, minutes) or long-intervals (i.e., hours, days) in operant paradigms (e.g., Wilkie & Willson, 1992; Saksida & Wilkie, 1994; Wilkie, Saksida, Samson, & Lee, 1994; Wilkie, Carr, Galloway, Parker, & Yamamoto, 1997) or open-field environments (Petruso, Fuchs, & Bingman, 2007) to guide their key pecking or goal-seeking behavior. Recently, it has been shown that homing pigeons are able to associate time-of-day information with spatial or color information in operant tasks (Meyers-Manor, Overmier, Hatfield, & Croswell, 2013). However, it seems that not all animals are able to form time-place associations as easily as birds. Previous work demonstrates that while some rats are able to use time-of-day information (Thorpe, Bates, & Wilkie, 2003; Widman, Sermania, & Genismore, 2004; Thorpe & Wilkie, 2007), other studies provide contrasting evidence that many rats experience great difficulty in using time-of-day independently in time-place learning tasks (e.g., Means, Ginn, Arolfo, & Pence, 2000; Means, Arolfo, Ginn, Pence, & Watson, 2000; Thorpe et al., 2003).

In a time-place learning task, an animal is able to associate a goal location with a specific time-of-day, but how does one know what type of temporal information an animal may be relying upon? Phase-shifts (i.e., delays or advances) of the light-dark cycle have been used to distinguish between timing mechanisms birds may be relying upon during time-place learning tasks (e.g., Biebach, Falk, & Krebs, 1991). It has been suggested that animals may rely on different types of temporal mechanisms, such as circadian or interval timers, during daily time-place associations (Crystal, 2009; Cassone & Westneat, 2012; Mulder et al., 2013). In the context of time-of-day learning, a circadian timing mechanism is derived from endogenous oscillations that act as an internal clock, is based on an approximate 24 hour phase cycle, and is
self-sustaining. Circadian timers are entrained to exogenous cues, or Zeitgebers, the most important of which is the light-dark cycle. This entrainment is usually long lasting and remains stable for several days even when the light-dark cycle is manipulated (Aschoff, 1989). By contrast, interval timers act like an “hourglass” and require a start/finish activation to properly function. An interval timing mechanism may rely upon onset or offset of the light-dark cycle. During photoperiod manipulations, if an animal relied upon an interval timer, they would immediately shift their behavior with the newly established light-dark cycle.

As previously discussed, a small number of avian species are capable of using temporal information to find reward sites, however, research also suggests that some birds, such as songbirds, may rely on other types of environmental cues during goal-oriented tasks. In songbirds, some species may store or cache their food for later retrieval while others may be more opportunistic and find food when needed. This distinction between food-storing and non-food storing birds has led to research dedicated to better understand cue utilization, specifically the preferential use of spatial versus feature information, for learning to navigate to and/or recognize a goal location. Studies have demonstrated that some food-storing, avian species rely heavily on spatial cues during goal localization and recognition (e.g., Broadbeck, 1994; Clayton & Krebs, 1994; Broadbeck & Shettleworth, 1995). However, preference for feature or color based information has also been observed (LaDage, Roth, Fox, & Pravosudov, 2009).

Homing pigeons, while not food-storers, possess impressive spatial abilities that enable them to navigate across vast distances to return to their home loft (e.g., Wiltschko & Wiltschko, 2003; Wallraff, 2005). In both laboratory studies and field settings, the avian hippocampal formation (HF) has been found to be an important brain area during landmark-based navigation, homing, and spatial memory tasks (e.g., Bingman, 1992; Gagliardo, Ioalé, & Bingman, 1999;
Bingman & Able, 2002; Bingman, Hough, Kahn, & Siegel, 2003; Columbo & Broadbent, 2000). During such tasks, pigeons may create an allocentric “map-like” representation of their spatial environment, often referred to as a “cognitive map” (O’Keefe & Nadel, 1978). This map-like representation may be composed of multiple landmarks, objects, and their spatial arrangement to one another (O’Keefe & Nadel, 1978). In general, homing pigeons, like food-storing songbirds, have often been found to prefer spatial (Strasser & Bingman, 1996; 1999; Vargas, Petruso, & Bingman, 2004; Bingman, Erichsen, Anderson, Good, & Pearce, 2006; Nardi & Bingman, 2007; Kahn & Bingman, 2009) cues compared to feature cues for goal localization and recognition, although this is not always the case (Strasser & Bingman, 1996; 1997; Kelly, Spetch, & Heth, 1998).

Homing pigeons do not store food, but they generally rely more heavily on spatial information in recognizing a goal compared to feature information; a preference that may be related to their superior navigational abilities. However, it is uncertain if the observed “spatial advantage” would facilitate time-of-day associative learning to spatial cues compared to feature cues, as one might expect. Due to the importance of the avian hippocampus in cognition and its dedicated role in spatial memory, we hypothesized that time-of-day information would be more readily associated with spatial, location-based cues than with non-spatial, features-based color cues. In the current study we trained one group of pigeons to use time of day to discriminate among alternative spatial choices and another group to discriminate among alternative feature (color) choices. We predicted learning would proceed more rapidly or be in some way better when time-of-day was associated with spatial in comparison to feature information. While we were interested in how well pigeons would be able to associate spatial or feature cues with certain times of day, we also wanted to better understand what timing mechanism pigeons may
use in a temporal discrimination task. Given that several studies have demonstrated that circadian
timers are preferentially consulted upon in birds (Biebach et al., 1991; Wenger et al., 1991;
Saksida & Wilkie, 1994; Budzynski & Bingman, 1999; Petruso et al., 2007), we hypothesized
that homing pigeons in the current time-of-day associative learning task would also preferentially
consult an endogenous circadian timer rather than an interval timer.
METHODS

Subjects

Twelve unsexed adult homing pigeons (*Columba livia*) were obtained from local hobbyists or bred in the Bowling Green State Animal Facility’s on-site aviary. While some of the birds were previously involved in a perceptual probability task, none of the birds had prior training experience in a time-of-day memory task. Birds were individually housed in stainless steel cages (56 x 38 x 31 cm) in a temperature and humidity controlled colony housing room with a 14/10 light-dark cycle. Lights turned on at 8:00 local time and turned off at 22:00 local time. Birds were food-restricted to no less than 80% of their free-feeding weights and were supplementally fed daily at 21:00. Water was provided *ad libitum*. Pigeons were randomly assigned to one of two experimental conditions: spatial group (n=6) or feature group (n=6). All procedures were approved by the Bowling Green State University Institutional Animal Care and Use Committee.

Experimental Environment

The same experimental room was used during training and testing for both the spatial and feature groups, but the physical characteristics of the room differed for each group. The experimental room’s dimensions were approximately 3.6 x 2.4 m (Figure 1). There were eight florescent lights mounted on the ceiling, heating and cooling system vents were located along the East wall and a white sink was attached to the South wall. Additionally, there was a partition located at the room’s entrance (West wall) that served as the hidden observation area. The experimental room for the spatial group (Figure 2) was rich in 2-D visual cues and 3-D landmark objects; food bowls remained at fixed locations and were all blue. By contrast, the experimental room for the feature group (Figure 3) was surrounded with a gray curtain that made the sink,
heating/cooling vents and the partition not visible; the experimental room was also devoid of external visual cues. Food bowls, uniquely colored red, green or yellow, occupied three fixed locations, but the location of any given bowl changed across sessions.

Behavioral Training

Shaping. During shaping, birds were gradually introduced to the default experimental room (see Figure 1) over the course of several days. The room lacked extraneous visual cues and landmark objects except for the ceiling lights, heating and cooling system vents, white sink, and partition. At this stage, birds were haphazardly placed at different locations in the room with three white bowls filled with grit. Initially, the room’s lights were turned off, but once a bird was in the room and the researcher was behind the partition, the lights were turned on for the entire duration of a shaping trial and were turned off again at the end of a trial. In the beginning, food was scattered across the floor and was placed on top of each bowl. Once a bird became more familiar with the room and ate all the food on the floor, the availability of food was gradually reduced until all food was buried under grit in each bowl. Shaping trials continued until a bird could find buried food in all three bowls within five minutes.

Training. The experimental room was now transformed into either the spatial (see Figure 2) or the feature (see Figure 3) condition. In the spatial condition, three blue bowls were placed in three spatially distinct locations, which did not change across sessions. As noted above, there were numerous two-dimensional (posters) and three-dimensional (a metal seat, a black napkin carrying case, and Styrofoam pillars) objects dispersed throughout the room with the intent of creating a spatially rich environment that could be used to represent in memory the locations of the three food bowls. In the feature condition, three colored bowls (red, green, and yellow) were placed as a linear array in the middle of the room. The location of the array did not change across
sessions although the location of any given bowl changed within the array (see Figure 3). As noted above, a gray curtain was suspended from the room’s ceiling masking the two-dimensional objects along the wall described earlier. The three-dimensional objects were also removed. All birds were trained at two different times of day: in the morning at 11:00 local time and in the evening at 17:00 local time. For both the morning and evening sessions, only one bowl per time-of-day was baited with a food reward. For example, a pigeon in the spatial group only received food at Location 1 during morning sessions and only received food at Location 2 during evening sessions. Similarly, a pigeon in the feature group only received food from the green bowl during morning sessions, whereas in the evenings, food was only available in the red bowl. These temporal food associations were counterbalanced across all pigeons.

Pigeons completed six trials during each experimental session, which were run six days per week. There were a total of thirty sessions for each time-of-day, and any given day could have consisted of either morning only, evening only, or combined morning and evening sessions. Sessions were pseudo-randomly determined with the constraint that pigeons could not experience the same time-of-day session three sessions in a row. The session-type order was designed to exclude the possibility that the pigeons could have used something other than time of day (e.g., an alternation strategy) to determine which of the two potentially baited food bowls was correct. At the beginning of each trial, a systematic dice roll determined where a pigeon was placed on the floor at the middle of one of the walls (1, North; 2, South; 3, East; 4 West); the same start location could not be experienced consecutively and each direction had to be used at least once per session. Once a pigeon was on the floor, the researcher closed the experimental room door, turned on the lights and recorded all bowl choices and their latencies with a stopwatch. Only one bowl out of the three contained a food reward. A choice was defined as a
peck into a bowl and only the first choice was scored as correct or not. Pigeons were allowed to continue to search until they found the correct bowl baited with a food reward for each trial. If a pigeon did not make a choice after five minutes, the trial was terminated. Once a pigeon had made a correct choice or did not choose at all after five minutes, the lights were turned off and the pigeon was removed from the room. Occasionally, if a pigeon was making only incorrect choices within the five minutes, the trial was extended up to ten minutes and was then terminated. After five/ten minutes, if a pigeon still did not make a correct choice, the trial was restarted. During the two minute inter-trial interval, a pigeon was placed back in its housing cage and the researcher cleaned the floor of debris and re-baited the correct bowl. After thirty sessions at each time-of-day, a phase-shift manipulation was implemented to investigate the timing mechanism a pigeon may have used to make the time-of-day discrimination.

*Phase-shift Probe Trials.* After completing the 60 total training sessions, a pigeon was immediately subjected to a 6-hour, phase delay of the light-dark such that lights on occurred at 14:00 local time instead of 8:00 local time. Pigeons were now supplementally fed at 3:00 local time, one hour before lights were turned off. The day immediately following the phase-shift, pigeons were trained at 17:00 local time. Probe trials were unreinforced (bowls did not contain food rewards) and each pigeon experienced one probe trial. A systematic dice roll (see above) determined the directional location where a pigeon was placed on the floor during probe trials. Once settled on the floor, the researcher turned on the lights and recorded a pigeon’s first trial choice. Immediately after the first choice, the lights were turned off and the pigeon was removed from the room and placed back in its housing cage. The researcher then cleaned debris from the floor and prepared for the next bird.
For the session immediately following the delayed phase-shift (which resulted in a long night), we hypothesized that if a pigeon was relying on a circadian timing mechanism to determine which bowl contained a food reward, the previously experienced evening bowl should be chosen because the existing circadian rhythm would still be regulated by the previous light-dark cycle, and not yet re-entrained to the newly manipulated photoperiod. By contrast, if the pigeons were relying on an interval timer calibrated to “lights on”, they would be expected to choose the previously rewarded morning bowl during the first probe trial.

Statistical Analyses

In order to assess evidence of time-of-day associative learning, the total number of correct choices across the training sessions were compared using a 2 (time-of-day: morning, evening) x 2 (group: spatial, feature) x 3 (blocks: first 10, second 10, and the last 10 sessions) mixed model ANOVA. When necessary, post-hoc analyses were conducted using t-tests. Soon after training began, pigeons routinely chose from the two bowls that potentially contained food, essentially ignoring the bowl that never contained food. As such, the expected chance probability was set at 50% for first trial correct choices for each session. In order to determine if first trial choices, which were not influenced by any within session learning, were different from the expected chance of 50%, one-sample t-tests were performed independently for each group. A chi-square test was used to assess if there were between-group differences regarding probe trial (morning or evening bowl) choices. Binomial tests were conducted to determine if pigeons’ choices differed from the expected chance of 50% during probe trials. The majority of analyses were performed using SPSS Statistics, Version 20.0 (Armonk, NY, USA: IBM Corp.), while the binomial tests were performed using an online website (http://vassarstats.net/binomialX.html). Criterion for significance was $p < .05$. 
RESULTS

Learning Performance

Overall, and as expected, the pigeons in both groups improved their performance across training and there was no difference in either groups’ performance with respect to morning compared to evening training. There was no main effect of time-of-day on correct choice performance \((F(1, 10) = .001, p > .05)\) nor was there a significant interaction between time-of-day and group \((F(1, 10) = .003, p > .05)\). Additionally, there was not a significant two-way interaction between time-of-day and blocked sessions \((F(1.25, 20) = 1.231, p > .05, \text{Greenhouse-Geisser estimate})\) and the three-way interaction between time-of-day, blocked sessions, and group was not significant as well \((F(1.25, 20) = 1.163, p > .05, \text{Greenhouse-Geisser estimate})\). There was, however, a main effect of blocked sessions \((F(2, 20) = 42.992, p < .001)\). Post-hoc analyses revealed that percent correct first choices during BLOCK 1 \((M=56.24, SE=1.91)\) was significantly less than during BLOCK 2 \((M=70.89, SE=2.76, t(11) = -4.675, p < .001)\) and BLOCK 3 \((M=78.88, SE=2.88, t(11) = -6.907, p < .001)\). Correct choices during BLOCK 2 \((M=70.89, SE=2.76)\) were also significantly less than during BLOCK 3 \((M=78.88, SE=2.88, t(11) = -3.384, p = .003)\).

As presented in the Introduction, we went into this study with the hypothesis that time-of-day associative learning would in some way be superior or easier when coupled with spatial differences in candidate goal locations compared to feature differences. Of particular interest, therefore, was the observation of a significant interaction between blocked sessions and group \((F(2, 20) = 4.891, p = .019; \text{Figure 4})\). Post-hoc analyses indicated that during BLOCK 1, the spatial \((M=55.41, SE=3.01)\) and feature \((M=57.08, SE=2.59)\) groups were not significantly different with respect to correct first choices \((t(10) = .418, p > .05)\). During BLOCK 2, it was becoming
apparent that the time-of-day/space learning was proceeding more rapidly than the time-of-
day/feature learning. During Block 2, the spatial group’s mean correct first choices ($M=75.83, SE=3.10$) was close to significantly different ($t(10)=-2.02, p<.10$) compared to the feature
group ($M=65.96, SE=3.76$). During BLOCK 3, the spatial group’s performance ($M=85.74, SE=3.68$) was now significantly better than the feature group ($M=72.36, SE=2.41; t(10)=-2.96, p=.014$).

First Trial Choices

Because we were interested in the enduring memory of the time-of-day associations, the
first trial of each session was of particular importance. Only on the first trial could we separate
what the animals were remembering across sessions in contrast to what they may transiently
learn within a session. Therefore, to assess how well the animals were remembering the learned
associations across sessions, we examined the percent correct choices of the pigeons on the first
trial of each session (Figure 5). As mentioned earlier in the Statistical Analyses section, soon
after training began the birds were essentially choosing from two bowls (i.e., morning or
evening) and were not interested in the remaining third bowl. Therefore, we conservatively set
chance at 50% correct because the birds were essentially choosing between two bowls. During
the last fifteen individual morning sessions, first trial correct performance for the spatial birds
was above but not significantly greater than expected chance ($M= 65.55, SE=10.52), t (5) =1.47,
p = .200), whereas the feature birds’ first trial performance was significantly above chance
($M=70.00, SE=6.83; t (5) = 2.928, p < .03$). During the last fifteen individual evening sessions,
the spatial birds’ first trial correct performance was significantly greater than expected by chance
($M=70.00, SE=7.04; t (5) = 2.83, p < .04$) as was the feature birds’ ($M=61.11, SE=4.00); t (5) =
2.77, p < .04 (Figure 5)). Overall, during the last 15 training sessions the pigeons from both
groups were preferentially choosing the correct food bowl on the first trial of a session, reflecting that the time-of-day associative representations were encoded in a long-term memory applicable across sessions.

**Phase-shift Probe Trials**

As previously discussed in the Introduction, time-of-day associative learning is thought to be influenced by the ability of an animal to use a timing mechanism to guide its goal localization behavior. On the first day after the phase-shift manipulation, we expected that if a pigeon was relying on a circadian timing mechanism the previously experienced “evening” bowl should be chosen. By contrast, if a pigeon was relying on an interval timer, the “morning” bowl should be chosen instead. A chi-square test determined that there was not a significant association between group and probe trial bowl choices ($\chi^2 = .444, p = .505$); the spatial group’s choices were not statistically different from those of the feature group’s. As such, data from both groups were combined and nine out of twelve birds chose the “morning” bowl on the probe trial compared to only three for the “evening” bowl, which suggests that the majority of birds were relying on an interval timer (binomial tests with the expected chance of .50, 9/12, $Z = 1.44$, $p = 0.053$). In sum, after a phase-delay, the majority of birds from both groups seemed to rely upon an interval timing mechanism during the probe trial.
DISCUSSION

Previous research has demonstrated that some birds are capable of forming time-place associations to acquire food resources in operant chambers and open-field environments (e.g., Biebach et al., 1989; Wilkie, 1995; Petruso et al., 2007). Additionally, some avian work has investigated the relationship between time, space and color cues (e.g., Meyers-Manor et al., 2013), but to the best of our knowledge, untested was the relative facility with which birds could use spatial compared to feature (color) cues to form associations with temporal cues in open-field environments. The intent of the current study was to test the hypothesis that time-of-day associative learning with respect to spatial cues is in some way superior to time-of-day associative learning with respect to feature cues (see Introduction). Although initially there were no group differences regarding overall correct choice performance, as training progressed the spatial group became increasingly more accurate than the feature group in their goal-directed behavior. At the end of training, while both groups were performing significantly greater than expected by chance, the spatial group clearly outperformed the feature group (see Figure 4). The observation that at the end of training the pigeons were performing better than chance on the first trial of each session (see Figure 5) further suggests that the discrimination displayed by both groups was based, at least in part, on enduring (long-term) representations of the time-of-day associations.

As revealed in Figure 4, the spatial group was better able to form associations with time-of-day information in comparison to the feature group. This finding is consistent with previous bird studies in which space enabled better correct choice performance than non-spatial cues in memory tasks when both cues were simultaneously available. In food-storing birds, results have revealed preferences for spatial rather than non-spatial cues in food-searching memory tasks
(Broadbeck, 1994; Clayton & Krebs, 1994; Broadbeck & Shettleworth, 1995). Additionally, homing pigeons often rely on spatial cues during goal localization or recognition (Strasser & Bingman, 1996; 1999; Vargas et al., 2004; Bingman et al., 2006; Kahn & Bingman, 2009). As such, we were also interested in how time-of-day information could be used independently with spatial or feature cues to facilitate first-trial correct performance. Examination of Figure 5 demonstrates that all pigeons were routinely making correct first trial choices during the last fifteen morning and evening sessions. Although the spatial group’s first-trial correct performance during morning sessions was not greatly above expected chance, results suggest that overall pigeons were still able to use time-of-day information during first trials of morning sessions and demonstrated significant preferences that exceeded expected chance during evening sessions. The feature group’s first trial correct performance greatly exceeded expected chance for both morning and evening sessions. This finding compares well with previous work that suggests that food-storing birds (LaDage et al., 2009), homing pigeons (Kelly et al., 1998), and hippocampal-lesioned pigeons (Strasser & Bingman, 1997; 1999; Vargas et al., 2004; Bingman et al., 2006; Nardi & Bingman, 2007; Kahn & Bingman, 2009) may also use feature information to find food reward sites.

The successful time-place learning of the current study naturally opens the question of the timing mechanism employed. Because of experimental constraints, addressing the question of a circadian or interval timing mechanism came down to one trial for each pigeon (the one trial session immediately following clock-shift), and therefore, only 12 data entries. Nonetheless, the preliminary data of the current study suggest that the majority of pigeons were relying on an interval timer and not on any endogenous circadian rhythm. However, three birds did display preferences for evening bowls, perhaps demonstrating an endogenous circadian clock bias.
Whereas previous avian work has demonstrated overt preferences for circadian clocks (Biebach et al., 1991; Wenger et al., 1991; Saksida & Wilkie, 1994; Budzynski & Bingman, 1999; Petruso et al., 2007), our results are not consistent with this trend, and to the best of our knowledge, suggests for the first time that homing pigeons are able to rely on interval timers during temporal discrimination tasks.

Although our results suggest that homing pigeons may employ interval timing mechanisms, there are other considerations regarding time-of-day information that need to be addressed. In the current study, we phase-delayed the light-dark cycle by six hours and then we conducted probe trials on the immediate day following the light-dark cycle manipulation. Based on previous research, it has been suggested that alterations to the light-dark cycle need to be carried out for several days in order to lessen the effects of transients (Pittendrigh, 1960; Pittendrigh & Bruce, 1959; Pittendrigh, Bruce, & Kaus, 1958) and masking (e.g., Mrosovsky, 1999). Additionally, it is necessary to generate free-running periods (FRPs) and phase-response curves (PRCs) for animals used in experimental studies that examines chronobiology-based aspects of time-of-day information, circadian rhythms, and locomotor activity (as reviewed by Johnson, Elliott, & Foster, 2003).

While some FRPs and PRCs have been conducted in house sparrows, *Passer domesticus* (Menaker, 1968; Binkley & Mosher, 1987; Chabot & Menaker, 1992) and homing pigeons, *Columba livia* (Abe & Sugimoto, 1987; Rashotte & Stephan, 1996; Chabot & Menaker, 1992; Rashotte, Basco, & Henderson, 1995), they have only been measured under 12:12 light-dark cycles or other variations, but not 14:10 light-dark cycles as was used in the current study. Future research should address this need by determining FRPs and PRCs for homing pigeons in 14:10 light-dark cycles. Moreover, while the majority of PRCs have been formulated for day-active
animals during daytime (light-pulse) hours (e.g., Beersma, Daan, & Hut, 1999; Comas, Beersma, Hut, & Daan, 2008), dark-pulse PRCs for diurnal animals is lacking and has only been conducted in bats, *Taphozous melanopogon* (Subbaraj & Chandrashekaran, 1978) and hamsters, *Mesocricetus auratus* (Boulos & Rusak, 1982). Due to experimental constraints, we were only able to conduct one probe trial per bird. Even though the evidence suggests that overall homing pigeons were relying on interval information to guide their choices, it would advantageous for future research to distinguish between circadian and interval timers based on overt behavior that can be measured easily, internal oscillations such as FRPs, and limits of entrainment to light-pulse and dark-pulse PRCs (Dunlap, Loros, & DeCoursey, 2004).

In summary, the current study provides evidence that spatial-goal information is more readily associated with time of day information compared to feature-goal information. Although both types of information allowed homing pigeons to discriminate the location of food at different times of day, learning the discrimination proceeded more rapidly when relying on space. Perhaps surprisingly, the majority of homing pigeons demonstrated preferences for an interval timer for the temporal discrimination.
REFERENCES


discrimination. *Behavioural Processes, 52*(1), 11-20. doi: 10.1016/S0376-6357(00)00109-1


APPENDIX A. TIME-PLACE LEARNING IN ANIMALS

*Birds*

Field Studies

In an observational field study, Rijnsdorp, Daan, and Dijkstra (1981) observed flying patterns and hunting strategies of kestrels (*Falco tinnunculus*) over the course of two years. The researchers found kestrels to be active hunters that cache their prey (usually voles) in the morning and then retrieve these food-caches later in the evening. They were also found to hunt more often in previously rewarded locations in comparison to locations where voles were not plentiful. Across multiple observation days, kestrels usually followed a time-of-day pattern (hunt and food-cache in the morning, retrieve in the evening) at specific locations, even when weather conditions were not favorable or constant. The researchers interpreted this finding to suggest that kestrels maintain “time-memory” of hunting areas in relation to hours of the day and rely upon circadian information to help maximize their food intake by only seeking out locations that had been previously rewarded (Rijnsdorp et al., 1981).

In another field study, Wilkie, Carr, Siegenthaler, Lenger, Liu, and Kwok (1996) examined time-place behavior in scavenging birds. Based on previous knowledge of time-place learning demonstrated by Biebach, Gordijn, and Krebs (1989, see below) and the observational insights from kestrels (Rijnsdorp et al., 1981), the authors were interested in how scavenging birds (e.g., pigeons, gulls, starling, sparrows, and crows) use time-of-day in order to acquire food resources. To study this, they counted the number of birds, people, and feedings at three different locations (i.e., marketplace, student union, control site) and times of day (i.e., morning, midday, afternoon) in 20 minute intervals over the course of several months. At the control site, where few people congregated and ate outside, birds were most often observed in the morning, but not during midday or the afternoon. For the other two sites, which tended to have more people, the
number of birds gradually increased during the morning, peaked by midday, and significantly decreased by the afternoon. The researchers concluded that scavenging birds of different species were apparently able to use time-of-day information to acquire food resources in this observational field study (Wilkie et al., 1996).

Laboratory Studies

In their seminal paper, Biebach, Gordijn, and Krebs (1989) investigated time-place learning in a laboratory setting using garden warblers (Sylvia borin). Throughout the entire experiment, the birds resided in a central box area with four connecting arms to other rooms. Before testing began, the birds explored the experimental area, received training on how to acquire food rewards, and also learned how to enter and exit of all of the connecting rooms. Following this acclimation period, training and testing procedures began. At the beginning of each session, birds were placed in the central box area for an interval of 280 seconds. After this interval, all of the room doors were opened and the birds were able to move freely within the experimental chamber (Biebach, et al., 1989).

Each room at the end of the connecting arms had one feeder in which food was available only during a specific time-of-day (i.e., Room 1: 0600-0900; Room 2:0900-1200; Room 3: 1200-1500; Room 4: 1500-1800). If a bird opened the correct feeder for a specific time-of-day, it was given access to the food reward for twenty seconds and the other three feeders would not be opened. If a bird opened an incorrect feeder, it was not given a reward and all feeders remained closed. The termination of a trial required a bird to return to the central box area before the start of a new trial could commence and each session consisted of four separate trials. Birds quickly learned the task and routinely found food rewards in the correct room based on the appropriate time-of-day and learned to avoid incorrect rooms. After reaching a predetermined learning
criteria, the birds began test sessions during which all rooms were open from 0600-1800 and the birds had free access to explore the experimental space. Room preference was found to be significantly influenced by time-of-day. Specifically, the birds independently chose rooms based on the familiar time-place association they had previously experienced. This suggests that the garden warblers were able to use time-of-day information in reference with specific spatial locations to find food rewards, thus demonstrating the ability to form time-place associations (Biebach et al., 1989).

Wilkie and Willson (1992) sought to extend the findings of Biebach and colleagues (1989) by investigating whether time-place associations could also be formed based on short time intervals (e.g., minutes, seconds). Using pigeons in an operant chamber task, Wilkie and Willson (1992) trained the birds to peck at specific keys within a box. In the first experiment, food rewards were available for thirty seconds in one of three different spatial locations (pecking keys). Trials lasted for ninety seconds and each key was optimized to offer a food reward for two periods of 15 seconds each. The first key provided food rewards during periods 1 and 2, the second key provided rewards during periods 3 and 4, and the final key three provided food during the last two periods, 5 and 6. Results indicated that pigeons’ pecking increased at specific keys based on the time period. Pigeons also learned to avoid ineffective keys that did not provide food rewards based on the specific time period (Wilkie & Willson, 1992).

The second experiment used a longer time interval (15 minutes) and spatially unique and distant pecking keys. Similar to the first experiment, pigeons were required to peck at four different keys based on the 15 minute time period. Pigeons were found to generally peck at key 1 during period 1, key 2 during period 2, key 3 during period 3, and key 4 during period 4. Overall, pigeons used temporal information, either 15 seconds or 15 minutes, to guide their pecking
behavior towards the spatially correct pecking key (Wilkie & Willson, 1992). This study stands as the first to demonstrate time-place learning in pigeons and contributes the important finding that short time-interval associations (i.e., seconds, minutes) can be learned just as readily as longer time-interval associations (e.g., Biebach, Gordijn, & Krebs, 1989).

Building on these findings, Saksida and Wilkie (1994) were interested if pigeons could also use time-of-day information or long intervals of time (e.g., 24 hour cycles) in a time-place task. In order to study this, they created an operant box similar to that used by Wilkie & Willson (1992). Pigeons received training sessions during the mornings and the afternoons, with one pecking key providing a food reward in the morning and another providing a food reward in the afternoon. All pigeons quickly learned to associate specific keys with food rewards in the morning or in the afternoon sessions. Therefore, this study was the first to demonstrate that pigeons are capable of using time-of-day information in a time-place associative task (Saksida & Wilkie, 1994).

Following the work conducted by Wilkie and Willson (1992), Wilkie, Saksida, Samson, and Lee (1994) wanted to further examine time-place learning in pigeons using short-time intervals (e.g., seconds, minutes) where goal locations were not adjacent to each other. In the task developed by Wilkie and Willson (1992), the pecking keys were positioned side by side. While the results of the study suggest that pigeons can form time-place associations, arguably the pigeons may have remembered to peck at individual keys by maintaining a counter-clockwise pattern of responding. Wilkie et al. (1994) addressed this limitation by adding additional pecking keys to the operant box for a total of nine. Pigeons were trained to peck at four spatially distinct key locations over the course of 60 minutes and each key provided a food reward for a 15 minute period. The researchers found that pigeons were able to use spatial and temporal cues to guide
their pecking key behavior, even when the keys were not adjacent to each other (Wilkie et al., 1994).

Wilkie, Carr, Galloway, Parker, and Yamamoto (1997), used pigeons in a conditional time-place learning study. Based on the experimental design of Wilkie and Willson (1992), pigeons were again tested on short intervals of time (i.e., minutes) in comparison to longer, time-of-day intervals (e.g., Biebach, Gordijn, Krebs, 1989). In contrast to previous experiments, this study was designed to determine if pigeons could use conditional stimuli in association with temporal information. Pigeons were trained and tested in an operant test box that had four walls, each with its own pecking key and food hopper. Outside of the chamber box, lamps affixed to the north and south walls served as the conditional discriminative stimuli (Wilkie et al., 1997).

During some sessions, the north lamp was illuminated which required pigeons to peck a specific key in order to receive food for 10 out of 30 minutes. Other sessions followed the same procedure with the south lamp and a different pecking key. Results indicate that pigeons’ pecking rates increased to specific keys during appropriate time periods based on which lamp was turned on. In the second experiment, the lamps were replaced with colored disks, thus enabling all keys to be either green or red. During sessions, rewarded key patterns were determined by color in that green and red had different patterns. Results suggest that pigeons were able to use short-intervals of time (i.e., 10 minutes) to form associations with specific colored keys (green and red) to gain access to food rewards in a 30 minute session. Taken together, pigeons were successfully able to form conditional time-place associations in an operant task in that they found rewarded pecking keys during the appropriate time period based on the presence of illuminated lamps or colored disks (Wilkie et al., 1997).
Petruso, Fuchs, and Bingman (2007) investigated time-space learning in homing pigeons. While previous work (see above) successfully demonstrated that pigeons can form time-place associations, the researchers were interested in learning if pigeons could also use another form of space, orientation angle to an artificial light source, in relation to temporal information during goal localization. In the study, pigeons were trained in an octagonal arena in which two food cups (each at a distinct angular/directional relationship to the light source) were available with food at different times of day (morning and evening). There were three different training phases in which pigeons learned to find food cups based on the time-of-day in relation to the position of the artificial light source. Once the training phases had been successfully completed, three different probe sessions were implemented. Results of the first probe session suggested that pigeons did not interpret the artificial light source as a surrogate sun, but instead as a fixed landmark. The results of the other two probe sessions are discussed later (see Temporal Mechanisms). Birds were able to associate different orientation angles based on two different times of day (Petruso et al., 2007).

Recently, time-place learning has been examined in the context of episodic-like memory in homing pigeons by Meyers-Manor, Overmier, Hatfield, and Croswell (2013). During five different experiments, pigeons were trained to peck at colored keys in spatially distinct locations in order to obtain a food reward. In the first experiment, key pecks would elicit qualitatively different (i.e., flavors) food rewards. Pigeons were trained to peck at specific keys during morning and afternoon sessions. It was found that pigeons altered their pecking behavior to individual colored keys at different times of day, and that they also changed where they pecked based on the experienced time-of-day. During skipped sessions, pigeons did not simply use an alteration or ordinal strategy, but instead relied upon time-of-day information to determine which
key to peck at based on either location or color cues. In the remaining four experiments, results
demonstrated that pigeons’ key pecking behavior was influenced by time-of-day information.
This study provided further evidence that pigeons are capable of relying on time-of-day
information in association with either spatial or features cues within an operant task (Meyers-
Manor et al., 2013).

Summary

Based on the evidence presented, it is clear that many birds have the capacity to form
time-place associations. Field studies demonstrate the influence of time-of-day during food
acquisition and caching and laboratory operant chamber paradigms illustrate that birds associate
temporal information (short or long intervals) with spatial locations or environmental features
during food-searching tasks. Many birds were able to use time as a reference cue to help guide
their goal-seeking behavior. However, no existing avian work has examined to what extent time-
of-day could be independently associated with either spatial or feature cues in an open-field
environment.

Rats

Boulos and Logothetis (1990) were among the first researchers to investigate time-place
learning in Long-Evans rats, specifically by exploring the role played by the of the
suprachiasmatic nucli (SCN). Previous research on lesioned SCN rats demonstrated the
importance of this brain area for circadian rhythms and light-entrainment, but not for food-
anticipation (Boulos & Terman, 1980). The researchers used two groups of rats: intact SCN rats
and lesioned SCN rats. During the study, intact rats were subjected to both constant dim light
conditions (LL) and a 12/12 light-dark cycle, whereas lesioned rats were only tested under LL
conditions. Experimental sessions were conducted in clear acrylic cages affixed with two
different food-pressing levers. The study involved two 20-day conditions: one in which two levers each provided food rewards at different times of day, and a second in which the same lever provided food twice per day. Both groups of rats were food-deprived for 3-5 days following each condition. Additionally, three intact rats were also food-deprived for 3 days after their restricted feeding sessions during the light-dark cycle (Boulos & Logothetis, 1990).

Results suggest that during the food-deprived condition, some of the intact rats in LD demonstrated food-anticipatory responses to individual levers at their previously experienced times of day. The dark phase was associated with better overall lever pressing performance for all rats in comparison to the light phase in which only 2/4 rats demonstrated food-anticipatory lever presses. By contrast, in the LL condition only 2/5 rats made more correct than incorrect food-anticipatory responses, with only one of those rats correctly anticipating both daily feeding times. The remaining 3 rats of the LL condition correctly anticipated one daily feeding time, but not both. For the SCN lesioned rats, the majority of rats seemed to anticipate food-responses and pressed specific lever bars during the previously experienced times of day. However, only some rats were able to correctly press both bars at the appropriate different times of day when they experienced food-deprivation for 5 days. Additionally, 1/2 rats that had to press the same bar at two different times of the day was only able to do so for a period of 5 days and afterwards only demonstrated a preference for one of the two different feeding times. Taken together, these findings suggest that while rats do seem to experience difficulty in forming time-place associations, they are perhaps capable of doing so by using a food-entrainable pacemaker, even during light-dark cycle manipulations and following SCN lesions (Boulos & Logothetis, 1990).

Carr & Wilkie (1997) used rats in a lever pressing study over the course of six different experiments. Throughout different experimental procedures, rats were enclosed in a clear acrylic
chamber with a lever bar and food hopper on each of its four walls. Rats were trained to press specific levers at different hours of the day. Results indicated that rats were able to discriminate between two different locations (one associated with the morning and the other with the afternoon) that offered food. Skipped and probe sessions supported the hypothesis that rats were using an ordinal strategy to decide which lever bar to press. The researchers interpreted these findings as support for the idea that rats were accurately and efficiently able to find rewards based on an ordinal strategy instead of circadian information or an interval timer. In other words, rats would always go to location A first, regardless of experimental manipulation, and then go to location B (Carr & Wilkie, 1997). Two other similar studies by the same research group further investigated time-place learning strategies rats. In both studies, rats were trained during three different types of sessions: mornings only, afternoons only, and a combination of mornings and afternoons. Results demonstrated that rats were again using an ordinal mechanism instead of circadian information or an interval timer to guide their behavior towards specific locations and food rewards (Carr & Wilkie, 1999; Carr, Tan, & Wilkie, 1999).

Means, Ginn, Arolfo, and Pence (2000) examined the ability of Sprague-Dawley rats to discriminate between two different times-of-day. The study involved four experiments, each using an elevated T-maze surrounded by landmark and color cues. In the first experiment, the authors were interested if rats could use time-of-day as a discriminative stimulus in comparison to other cues, including visual-tactile, orientation-position and turn responses. Rats were trained in the morning and in the late afternoon. Results indicate that rats that could only use time-of-day took the longest time to reach criterion levels, whereas rats that could use other cues were much faster in learning the task. It is noteworthy, however, that some rats using only time-of-day were still able to find goal locations in either arm of the T-maze. In the second experiment, some
rats were subjected to a normal 12/12 light-dark cycle while other rats experienced constant light. The majority of rats in both groups were able to discriminate between morning and afternoon sessions and reached criterion levels (Means et al., 2000).

The third experiment used skipped sessions to determine if rats were relying on an alteration strategy during time-of-day discrimination. Rats were trained until they attained 70% correct choice performance throughout 24 trials and twice daily testing followed a slightly altered schedule. Skipped sessions occurred once every seven days for 21 days. Results suggest that this task was more difficult for the rats: less than half of the rats were able to attain 90% correct choice performance within 96 trials and only slightly more were able to achieve this within 192 trials. During the first trials following skipped sessions rats attained 70% correct choice performance based on time-of-day information. This result supports the conclusion that the rats were not simply relying upon an alternation strategy, but instead were capable of incorporating time-of-day into their choices (Means et al., 2000).

In the final experiment, the researchers were interested in knowing if different experimenters could act as a potential cue for rats to use during time-of-day discrimination. While one experimenter handled and trained the same group of rats during morning and evening sessions, another group of rats was handled and trained by two different experimenters based on the time-of-day (mornings: Experimenter 2; evenings: Experimenter 3). Results indicate that the majority of rats from both groups were able to reach criteria within 96 trials and their performance did not differ greatly from one another, thus demonstrating that rats were not relying on the experimenters as cues during time-of-day discrimination. While rats were able to form time-of-day discriminations, they did so with great difficulty and routinely struggled to use time as a discriminative stimulus in comparison with other cues. The authors interpreted these
findings to suggest that rats may use time-of-day as an “occasion setter or contextual stimulus” instead of a discriminative stimulus. In other words, time-of-day may influence rats’ anticipatory behavior and allow them to be in the general correct location or explore relevant levers at the appropriate time, but not to use time independently to guide their goal-seeking behavior (Means et al., 2000).

Means, Arolfo, Ginn, Pence, and Watson (2000) conducted a study to determine if rats could use time-of-day as an occasion-setting stimulus (a cue that would enable food-seeking responses at the appropriate time-of-day in the correct general location) rather than as a signal for a specific response (with time-of-day as the sole cue) in a go/ no-go discrimination. Using an elevated T-maze across two experiments, the first involved one control group and five different experimental groups (distinct choice arm, two-trial, dark/light, perseveration extinction, and natural light) that were used to assess if rats could form daily, time-place associations. One maze arm provided food during morning sessions while the other provided food during the afternoons. Overall, only 39 out of the 49 total rats used in all groups were found to attain criteria within 120 trials. These results lend support to the idea that rats experience difficulty consistently obtaining time-place food rewards. (Means et al., 2000).

In the first part of the second experiment Means and colleagues (2000) subjected one group of rats to a go/ no-go discrimination task in which both maze arms provided food rewards during one daily session and neither provided food during the other daily session. Rats seemed to display longer latencies during no-go sessions than during go sessions, thus demonstrating they knew food was not readily available. Rats also acquired the task more easily and reached criterion in significantly fewer sessions than the majority of groups used in the first experiment. In the second part of the second experiment, rats were again tested on a go/ no-go task but with
irregular feeding times and constant white noise and light, followed by a return to normal training conditions. Rats were still able to acquire the go/no-go discrimination even when feedings were random. In constant noise and light conditions, rats did not perform better than chance during the first trial block, but did so by the second and third blocks. Even when returned to normal lighting and feeding conditions, rats were still performing above chance on the go/no-go discrimination task. Altogether, the data supports the authors’ original hypothesis that rats make better use of time-of-day as an occasion or situational setter that provides general information about where and when food may be available based on the go/no-go task, rather than using time-of-day used as a discriminative stimulus or as the sole signal for a specific choice response (Means et al., 2000).

Thorpe, Bates, and Wilkie (2003) attempted to demonstrate time-place learning in rats using novel experimental procedures. The researchers questioned the validity of Gallistel’s (1990) original theory about time and space being automatically encoded together with the characteristics of some biologically significant events, particularly among rats. Thorpe et al. (2003) alternatively proposed that rats may not be able to use the so-called tripartite memory code (event-place-time), but may instead rely more heavily upon two sources of information either event-place or time-place. In the first experiment, rats experienced a place preference task. One side of the acrylic box contained food rewards during morning sessions while the side provided food during afternoon sessions. After training rats were tested during morning and afternoon sessions. During these tests the rats had free access to the entire box but food was not available. Rats were found to have no preference based on time-of-day (Thorpe et al., 2003).

In the second experiment, rats were subjected to an aversive time-place Morris water task in which they were trained to associate one platform with the morning and another with the
afternoon. Rats were not able to use time-of-day to discriminate between each platform, but they were found to spend more time on the morning and afternoon platforms rather than other locations in the maze. The authors interpreted this finding to suggest that rats learned a place-event association, but not a time-event or time-place-event association. The third experiment placed rats in a radial arm maze where one arm was rewarded during morning sessions and a different arm was rewarded during afternoon sessions. Rats were not able to use time-of-day to find food rewards in either arm, but they were more likely to choose arms that had been previously rewarded rather than those that had not (Thorpe et al., 2003).

In the fourth experiment used different lighting conditions (bright light, standard fluorescent overhead lights, dark light, and a lamp). For three rats, morning sessions occurred in the bright light condition and afternoon sessions occurred during the dark light condition; for the remaining two rats, these associations were switched. Once rats were consistently performing well above 50%, the light conditions were altered and rats were either tested in all light or all dark. Subsequently, the rats’ overall performance dropped significantly below 50% during morning and afternoon sessions. The fifth experiment used a go/no-go discrimination task. For some rats, both arms of a T-maze were baited with food during morning sessions but not afternoon sessions, and other rats experienced the opposite. Rats were able to acquire the go/no-go task based on time-of-day information. During morning and afternoon skipped sessions, one rat demonstrated reliance upon an alternation strategy, two rats used an ordinal strategy, and the remaining four rats used a circadian timer. These findings add further support to the hypothesis that rats experience difficulty in forming daily time-place associations, but are capable of doing so in a go/no-go task (Thorpe et al., 2003).
Widman, Sermania, and Genismore (2004) investigated daily, time-place learning in rats without using food reinforcement. In this study, two experiments were conducted using a Morris water maze in a room rich in spatial and visual cues. Rats were given free access to food in their home cages. The initial training phase of the first experiment included morning and afternoon sessions during which one platform corresponded to either time-of-day. In the testing phase, the platforms were removed. Results suggest that rats were not able to correctly discriminate between morning and afternoon locations and did not spend significantly more time in areas where platforms had been present in comparison to areas free of platforms. Rats, while granted free access to food, did not form daily, time-place associations (Widman et al., 2004).

The second experiment followed the same procedure but the rats were harnessed with weighted Velcro belts. Results revealed that the rats were capable of discriminating between morning and afternoon platform locations. Rats tended to spend significantly more time in correct locations during morning and afternoon sessions in comparison to incorrect locations, thus suggesting that discrimination errors were significantly lower for morning and afternoon sessions in comparison to the first experiment. This study presents important findings that suggest that rats can form time-place associations when faced with a higher metabolic (i.e., energy, nutrients) or risk-injury (i.e., fatigue, drowning) related response cost (i.e., effort from the additional weight of the Velcro belt in the water maze). Furthermore, the results indicate that time-place associations can be formed even when food does not act as a source of entrainable information (Widman et al., 2004).

Although Widman et al. (2004) previously demonstrated that rats can form time-place associations when costs are high (i.e., metabolic, risk-injury, effort), Thorpe and Wilkie (2007) sought to understand if this trend persists during low costs contexts (not abnormally stressful to
the animal, weighted Velcro vests were not used, and a water maze was not employed) with differential amounts of food. Similar to Thorpe et al. (2003), Thorpe and Wilkie (2007) also argued that Gallistel’s tripartite memory code theory (1990) is not compatible with rats’ normal behavior and that some animals are only capable of forming bipartite memory codes of time-event or event-place information. Rats were trained in an elevated T-maze to associate different amounts of food (i.e., large, small) in different maze arms based on the time-of-day. Skipped sessions occurred once rats consistently performed above 75% correct choice performance for both times of day and latencies for larger amounts of food were 2 seconds shorter than that of smaller amounts of food. Skipped sessions allowed the researchers to determine what potential strategies (i.e., circadian, alternation, ordinal) rats may have used to discriminate between morning and afternoon sessions. Results indicate that rats’ latencies were significantly faster on trials that had larger amounts of food than on trials with smaller amounts. All rats performed significantly above chance for morning and afternoon sessions. Although rats seemed to use a circadian timing mechanism based on latency results, data of correct choice percentages remained inconclusive. This study demonstrates that rats can form daily, time-place associations based on differential amounts of food when so-called “costs” are minimal (Thorpe & Wilkie, 2007).

**Summary**

Taken together, these reviewed studies offer contradictory evidence regarding the ability of rats to form time-place associations and/or discriminate between different times of day. While the majority of this literature suggests that rats find it difficult to use time-of-day information independently to acquire food resources, some research has successfully demonstrated intact
time-place learning in rats. Rats may rely on ordinal or alternation learning strategies to find food resources and may use time as circadian information or as an occasion-based setter.
APPENDIX B. TEMPORAL MECHANISMS

As discussed by Biebach, Gordijn and Krebs (1989) and demonstrated by Biebach, Falk and Krebs (1991, see below) with birds, daily time-place associations can be based on at least two different types of timing mechanisms: a circadian clock/timer or an hourglass/interval timer. Based on an almost 24 hour phase cycle, circadian clocks are regulated by endogenous oscillations that act as internal clocks and are self-sustaining in that they do not rely on external cues for cyclical activity. By contrast, hourglass timers do not rely on internal self-sustaining oscillations but instead require external cues, called “Zeitgebers,” to function which serve to entrain an internal oscillator to ambient cycles (Biebach et al., 1989). For example, if a bird is given food between 0600-0900 in Room 1, 0900-1200 in Room 2, 1200-1500 in Room 3, and 1500-1800 in Room 4 over the course of training, it may remember this time-place association via circadian time-of-day information. However, it may also remember the same time-place association based on the onset/offset of the light-dark cycle and may use the number of hours since the lights have turned on to determine which room would have food available.

In order to determine which timing mechanism a bird may be using, an experimental manipulation of the light-dark cycle is usually required. The light-dark cycle may be phase-shifted as an advance (i.e., lights are turned on earlier than normal) or as a delay (i.e., lights are turned on later than normal) or maintained at constant dim conditions (Biebach et al., 1989; Biebach et al., 1991; Wenger, Biebach, & Krebs, 1991). Phase-delays are less likely to cause transients (similar to the experience of jet-lag) compared to phase-advances (Pittendrigh, 1981; Johnson, 1992). After a phase-shift, experimental birds usually experience test sessions to determine which temporal mechanism they may have used during their previously learned time-place associations. Crucially, a circadian timer would not immediately shift or re-entrain to a
new light-dark cycle manipulation. Rather, it is a gradual process that typically takes about one
day for the previously established cycle to re-entrain to a new light-dark cycle per one phase-
shifted hour (e.g., Budzynski & Bingman, 1999; Petruso, Fuchs, & Bingman, 2007; Schmidt-
Koenig, 1960). In contrast, an hourglass or interval timer would immediately and permanently
shift behavior in accordance to the new light-dark cycle (Biebach et al., 1991; Daan, 1987).

Biebach, Falk, and Krebs (1991) conducted a study to determine what temporal
mechanisms garden warblers (Sylvia borin) may have used in a time-place learning task similar
to Biebach, Gordijn, and Krebs (1989). Warblers were placed in a central box location with four
connecting rooms, each with its own food dispenser and door. At the beginning of each test day,
warblers had to wait 280 seconds in the central room before they were free to explore all four
rooms. Each room had food available during a different time block (i.e., Room 1, 0600-0900;
Room 2, 0900-1200; Room 3, 1200-1500; Room 4, 1500-1800). If a warbler correctly chose a
feeder at the appropriate time they were given access to food for 20 seconds. After each choice, a
warbler was required to return to the central box location and wait for the next trial to begin.
Warblers quickly learned to associate specific room locations with food at different time blocks
and usually avoided rooms when food was not available in them (Biebach et al., 1991).

Biebach and colleagues (1991) originally hypothesized that the behavior of warblers
relying on interval timers would shift by at least six hours immediately following a six hour
phase-shift of the light-dark cycle. However, if relying on a circadian clock, warblers’ behaviors
would shift by less than six hours. Following the previously described time-place learning task,
the warblers were subjected to three additional light-dark cycle manipulations. One group of
warblers continued their normal time-place associative training in constant dim light conditions.
The second group experienced a phase-advance of the light-dark cycle and the remaining group
experienced a phase-delay. The phase-advance was achieved by reducing the dark period from twelve to six hours and the phase-delay extended the light cycle from twelve to eighteen hours, followed by six hours of darkness. The researchers were interested in the warblers’ subsequent first choices during 0600-0900 (Biebach et al., 1991).

Results were consistent with the proposed circadian clock hypothesis rather than an hourglass interval timing mechanism. Within the phase-shift conditions, the warblers’ behavior was shifted by 2.6 hours earlier than normal during the phase-advance condition and was negatively delayed by almost half an hour in the phase-delay condition. Predictions consistent with the hourglass timer hypothesis were not supported due to the fact that the warblers shifted their behavior by more than zero but less than six hours. Based on these results, it follows that warblers consulted their internal circadian clock to guide their goal orientated behavior during phase-shift conditions (Biebach et al., 1991).

Wenger, Biebach and Krebs (1991) investigated potential temporal mechanisms used by starlings (*Sturnus vulgaris*) in a time-place learning study. While previous research reveals circadian clock usage during light-dark cycle manipulations among garden warblers (Biebach et al., 1991), Wenger and colleagues (1991) were interested in seeing if other birds, such as starlings, demonstrate a similar behavior. Using the same experimental setup as Biebach et al. (1989), starlings were housed in an experimental chamber with one central room and four connecting arms, each with their own food hopper and door. Food was available in each hopper for a specific duration of time throughout a day (i.e., Room 1: 0600-0900; Room 2: 0900-1200; Room 3:1200-1500; Room 4:1500-1800). At the beginning of each trial, starlings were granted access to all four rooms, but they received food (for 20 seconds) only if they chose the correct hopper. After each choice, starlings returned to the central room and waited five minutes before
the next trial began. Once all starlings reached at least 75% correct choice performance, three different tests were administered to determine to what extent circadian information could be used and remembered under different light-dark cycle manipulations (Wenger et al., 1991).

Food was available in all four hoppers during twelve hour periods, but the accompanying light-dark cycles were different for each test. The first test used 300 lux lights during lights-on and 0 lux lights for lights-off for the 12/12 light-dark cycle. This was used to compare previous training performance when food was only available during specific times of day to when food was freely available. In the second test, the 12/12 light-dark cycle used 7 lux lights during both the light and dark cycle in order to lessen the potential masking effects of a circadian clock due to input from bright lights. The final test used 7 lux lights during lights-on and 0 lux lights during lights-off for 12/12 light-dark cycle to see how long starlings retained the previously learned time-place association (Wenger et al., 1991).

In the first test starlings were found to visit the correct room based on the previously learned time-place association for 2-4 days. In the second test starling maintained their previously learned time-place association for 2-6 days. In the third test, the starlings maintained their time-place associations up to three days, but it should be noted that only two birds were involved in this test. However, their internal feeding rhythms remained intact for five days and started to free-run afterwards. The researchers interpreted these findings to suggest that starlings, like garden warblers, are capable of forming circadian-based time-place associations (Wenger et al., 1991).

As previously described in Appendix 1, Saksida and Wilkie (1994) used pigeons in a time-of-day discrimination task in which specific pecking keys were associated with different times of day. Across three experiments pigeons quickly learned to only peck the keys that
provided food in the morning and in the evening, thus demonstrating intact time-place learning.

Additional experiments were conducted to determine which temporal mechanisms (e.g.,
circadian or interval timer) pigeons may have used during time-of-day discriminative tasks
(Saksida & Wilkie, 1994).

In the fourth experiment, pigeons were subjected to a six-hour phase-advance of the light-
dark cycle. Pigeons were tested during both morning and evening sessions on the day
immediately following the phase shift and again, six days later. On the first test day, pigeons’
key pecking behavior was not influenced by the phase-shift and they responded normally based
on their previously established time-of-day learned association. Rather than depend on the
altered light-dark cycle to help them to decide which keys to peck during the morning or evening
session, pigeons appeared to rely on their circadian internal representation of time. Six days after
the phase-advance, only two birds (out of four) were able to correctly peck at appropriate keys
based on the newly established light-dark cycle. In the fifth and final experiment, pigeons were
subjected to constant dim light conditions. Following this, all pigeons were again tested in the
morning and in the evening. Results indicate that three of the four pigeons pecked the correct
keys well above chance levels and their performance did not differ from that of baseline sessions.
This suggests that the majority of birds relied upon circadian information to determine which
keys to peck during morning and evening sessions. Altogether, results from experiments four and
five suggest that pigeons were using circadian information during time-of-day discriminative
learning (Saksida & Wilkie, 1994).

As described previously in Appendix 1, time-space learning was investigated in homing
pigeons by Petruso, Fuchs, and Bingman (2007). Briefly, pigeons were trained during morning
and evening sessions in an octagonal arena in which two food cups (each at a distinct
angular/directional relationship to the artificial light source) were baited with food at different times of day. Pigeons successfully learned to find food-cups based on the time-of-day in relation to the position of the artificial light source. In order to determine what timing mechanism (i.e., circadian, interval) pigeons may have consulted during temporal discriminations, the researchers manipulated the light-dark cycle with a ten-hour phase-advance. Late afternoon probe sessions were conducted on the day immediately following the phase-advance and once more eight or nine days later. For the first probe session, if pigeons were relying on an endogenous circadian timer they should have oriented themselves to the previously trained late afternoon angle. The circadian timer would still be intact and would not have time to re-entrain to the altered photoperiod. On the other hand, if pigeons were relying on an hourglass or interval timer, they should have oriented themselves to the previously trained morning angle because an hourglass or interval timer would immediately update itself to the altered photoperiod. Day one probe sessions results were consistent with a circadian timing system in that birds used time-of-day information based on endogenous circadian rhythms during a temporal discrimination task (Petruso et al., 2007).
Appendix C. Avian Cue Use During Goal Localization

Songbirds

A large number of studies have been conducted on the differences between food-storing and non-food storing species of songbirds. Generally, food-storing species live in environments where they must forage and cache their acquired food items for later retrieval. Food retrieval can occur several days, weeks, or months after initial caching episodes. Based on this knowledge, researchers have proposed that food-storing birds may rely more heavily on spatial information rather than feature or object-centered information for memory encoding. Relatedly, food-storing birds tend to have a larger hippocampus than related non-storing birds (e.g., Krebs, Sherry, Healy, Perry, & Vaccarino, 1989; Sherry, Vaccarino, Buckenham, & Herz, 1989). In non-food storing species, foraging behavior tends to be more opportunistic and based on daily environmental demands. Rather than cache and retrieve food items, these birds take food whenever available. Additionally, these birds may be more likely to rely on feature or object-centered cues during food memory tasks. Comparative studies that have used food-finding experiments to observe differences between food-storers and non-food-storers are detailed below.

Previous research has demonstrated that food-storing black-capped chickadees tend to rely substantially on spatial cues (e.g., Sherry, 1992) rather than object-centered or feature cues. While black-capped chickadees (Parus atricapillus) and dark-eyed juncos (Junco hyemalis) are not closely related, they share similar living habitats yet use different feeding strategies to survive the winter season. Broadbeck (1994) compared cue utilization differences between food-storing black capped chickadees and non-storing dark-eyed juncos in five different experiments.
In all experiments, four uniquely colored feeders were placed in spatially distinct locations, with one feeder baited with a food reward (Broadbeck, 1994).

The first experiment used a food-storing task (caching and retrieval) in order to determine what cues black-capped chickadees used to remember where they stored their food. In this experiment, one feeder was available as a food storage site instead of being actually baited with a food reward. During unrewarded test trials, two feeders were switched so that the candidate color and spatial goal positions were dissociated from each other. Black-capped chickadees were significantly more likely to select the spatially correct feeder as their first choice and the correctly colored feeder as their second choice during unrewarded test trials. In sum, space was found to be more salient than color for black-capped chickadees during a food-storing task (Broadbeck, 1994).

The second experiment was a food-finding task that used a similar experimental apparatus and procedure. Results again demonstrated a superior reliance on spatial cues with object-centered cues referenced secondary. In the third experiment all feeders were shifted to demonstrate the important of absolute spatial position in comparison to color/pattern cues and array position cues. During probe trials, the originally baited feeder was emptied and switched places with another randomly chosen feeder. As such, the second feeder would now be correct with respect to global spatial information, but incorrect with respect to feeder array position. The feeder nearest to the absolute spatial location was most commonly selected as the first choice, followed by choices to the correct feeder with respect to array position and object-centered cues (Broadbeck, 1994).

In the fourth experiment, all three types of cues (absolute spatial, array position, and object-centered colors/patterns) were dissociated from one another. During food-retrieval,
chickadees could go to the correct feeder based on absolute spatial position, feeder-array position, or color/pattern information. Chickadees’ first choices were most often reserved for absolute spatial position, followed by feeders with the correct array position, and finally to feeders with correct colors/patterns. Broadbeck (1994) followed the same procedures in their fifth and final experiment, but this time with dark-eyed juncos. These birds were found not to favor one type of cue (absolute spatial position, array position, or colors/patterns) during dissociation tests. Juncos seemed to rely equally on spatial and feature cues during first, second, and third choices. Based on these findings, it would seem that food-storing chickadees tend to prefer global space in comparison to features and non-storing juncos do not prefer one cue over another, instead relying on both types of information equally (Broadbeck, 1994).

Following Broadbeck (1994), Clayton and Krebs (1994) wanted to further compare cue utilization between food-storing and non-storing birds in a one-trial associative memory task. In this study, comparisons between birds within the same families (parids: storing marsh tit [Parus palustris] and non-storing blue tit [Parus caeruleus]; corvids: storing jay [Garrulus glandarius] and non-storing jackdaw [Corvus monedula]) were used to provide additional insight regarding preferences for spatial and non-spatial cues. During phase one, birds could eat from one of four baited feeders (each colored differently and positioned in a unique spatial location). In phase two, after eating a peanut, birds were given a five minute retention interval and were then allowed to find the hidden food in the previously rewarded baited feeder (Clayton & Krebs, 1994).

During phase two dissociation tests none of the feeders provided food rewards. Two of these feeders were rearranged to a new spatial location while their object-centered cues remained unchanged. Birds were required to choose between spatial or non-spatial/object-centered cues
during dissociation tests. All birds, regardless of family, differed significantly from random during first choices in that they went to the correct spatial position or the correct colored feeder before going to those that never provided a food reward. Food-storing marsh tits and jays tended to search for food first at the feeder in the correct spatial position and then relied on object-centered cues second. Non-storing blue tits and jackdaws demonstrated no preferences during first and second choices for either cue and relied on both spatial and non-spatial cues equally before choosing at random. This influential study demonstrated that even within the same family, food-storing birds rely more heavily on spatial cues in comparison to non-storing birds that use spatial and non-spatial cues equally (Clayton & Krebs, 1994).

Broadbeck and Shettleworth (1995) compared food-storing (black-capped chickadees) and non-storing (dark-eyed juncos) birds in operant delayed-matching-to-sample (DMTS) tasks. In Experiment 1A, chickadees and dark-eyed juncos experienced an adaptation of Broadbeck’s (1994) aviary task in an operant chamber. Birds could peck at three different colored patches on a touchscreen. During the study phase of a trial, birds were reinforced with a food reward for pecking at a black dot in one of the three colored patches. After a short retention interval, birds again received food if they pecked at the correct sampled patch where the black dot was previously located. Test trials included one of three arrangements: correct spatial location as the sample but a different color, the same color as the sample but a different spatial position, or no relation to the sample. Chickadees’ first choices most often tended to be the patch in the location of the sample whereas dark-eyed juncos chose spatial and feature cues more or less equally (Broadbeck & Shettleworth, 1995).

Experiment 1B used the same DMTS task, but with only a stimulus during phase one and across more trials per session. Similar to Experiment 1A, chickadees chose space first while
dark-eyed juncos equally chose space and color first during dissociation trials. Experiment 2 again followed the same DMTS task, but used only spatial or color information during test trials. Chickadees performed better on test trials that only required spatial information in comparison to color cues while dark-eyed juncos performed equally well on both types of trials. As with previous studies (e.g., Broadbeck, 1994; Clayton & Krebs, 1994), this operant DMTS task demonstrated that food-storing birds (black-capped chickadees) rely more on spatial cues than non-spatial cues and that non-storing birds (dark-eyed juncos) rely on spatial and non-spatial cues equally (Broadbeck & Shettleworth, 1995).

LaDage, Roth II, Fox and Pravosudov (2009) conducted a study to determine if food-storing mountain chickadees (Poecile gambeli) prefer spatial or non-spatial cues in an associative learning task. The experimental room held two artificial trees constructed of wooden perches and blocks. Inside each block was a hole hidden by a string with a knot tied at the end of it. During training trials, one block was taped with a specific color (blue, red, yellow, green, orange, pink, or purple) while the other blocks were covered with brown tape. In the first training phase, one block was baited with a food reward while the remaining blocks were empty. At the beginning of a trial, a bird had five minutes to locate a pine nut inside a colored block. Following this, they could peck at the pine nut for three seconds before the lights were extinguished. After a retention interval of five minutes, the second training phase began. A bird was then allowed to reenter the testing room and given five minutes to locate the previous food reward. This time however, all blocks were covered string knots to ensure that the bird was relying on its memory of the food reward rather than the mere sight of it (LaDage et al., 2009).

Unrewarded dissociation tests, which separated spatial and feature cues, were administered after the second training phase. In this session the previously rewarded block was
brown and that adjacent to it was distinctly colored. Birds could then go to the correct spatial position of the previously rewarded block or to the currently correct colored block. Mountain chickadees did not demonstrate a preference for spatial cues over color cues. Surprisingly, the majority of mountain chickadees first chose the adjacent colored block more often than the previously experienced spatial location block. Additionally, they chose the correct cue (either space or color) before choosing at random. This study suggests that not all food-storing birds rely as heavily on space over color as previously thought. Mountain chickadees were able to use both spatial and color cues in the food finding task, and, perhaps surprisingly, they seemed to rely more on color than space (LaDage et al., 2009).

Homing Pigeons

Strasser and Bingman (1996) investigated homing pigeons’ ability to use spatial and non-spatial cues in both a field and laboratory study. In the first experiment, pigeons were trained in a home loft recognition task. The study used two lofts, a green one that faced east and a red one that faced west. During training, pigeons were taken to locations ranging from 0.8 to 4.8 km away from their home lofts and then released. Experimental test sessions began once birds correctly returned home. During test sessions, the colored boards were switched between the lofts so that the previously experienced color cues were now in different spatial locations. Birds were then released in novel directions (northeast or northwest) from their home lofts. The majority of homing pigeons returned to the loft that was correct with respect to location instead of color; color cues were seemingly ignored. This finding suggests that during conflict test trials, free-flying pigeons rely on spatial information in contrast to color (Strasser & Bingman, 1996).

In the second experiment involved a food location task in an open-field laboratory setting. The room contained four different colored bowls arranged in spatially distinct locations
with a landmark beacon always placed next to the baited bowl. Pigeons could rely on the color of a bowl (feature cue), the position of a bowl with respect to the landmark beacon (proximal spatial cue) or the position of a bowl with respect to its location in the room (distal spatial cue) during goal localization. Through training, pigeons learned to associate the colored bowl next to the landmark beacon with a food reward. Following this, three different test trials (color bowl shift; landmark beacon shift; color bowl and landmark beacon shift) were administered to determine cue preference during goal localization. Results suggest that during test trials pigeons did not uniformly rely on any one type of cue. Pigeons did not demonstrate an overwhelming preference for color or spatial cues, but did choose the correct colored bowl that was next to the landmark beacon if they were moved together. Based on these findings, the authors argued that homing pigeon cue utilization may be similar to other non-storing birds as no overt preferences for distal or proximal spatial cues were found in this study (Strasser & Bingman, 1996).

Kelly, Spetch, and Heth (1998) investigated how homing pigeons use featural and geometric cues differently within a rectangular enclosed environment. Pigeons were divided into two groups, feature or geometry, before training occurred. In the feature group, pigeons were trained in an environmental space that had either landmark objects or colored panels in each of the four corners, one of which provided a food reward. In the geometric group, pigeons were initially trained with no featural components, but two geometrically equivalent corners of the rectangular enclosure provided food reinforcement. After a series of tests were administered, the geometric group was re-trained with featural information and only one corner provided a food reward. Results suggest that both groups were able to use featural information to find the correct rewarded corner, but initial training experience did influence performance across both groups. The feature-only group seemed to rely more heavily on featural information to find the correct
corner and did not use geometric information, whereas birds in the geometric group were equally likely to use geometry and feature-based cues in choosing a correct corner. Based on previous training experience, some pigeons preferred to use only one cue to locate reward sites while others were able to synthesize two cues together. Taken together, this study demonstrated that homing pigeons are capable of using feature and geometry-based cues within a spatial environment (Kelly et al., 1998).

Summary

Differences between storing and non-storing avian species regarding cue use during goal localization are well documented in the previously discussed studies. The majority of food-storing birds, except mountain chickadees, apparently rely on spatial information more heavily than non-spatial cues. By contrast, most non-storing birds seem to acknowledge features or object-centered cues and space equally, although there are some contradictory research findings from homing pigeons.
Homing Pigeons

Homing pigeons, while not food-storing birds, possess remarkable spatial skills (e.g., Wiltschko & Wiltschko, 2003; Wallraff, 2005). These skills are considered to be a form of spatial memory that enables pigeons to navigate across familiar and unfamiliar terrain, find food resources, and ultimately survive and reproduce. In both laboratory studies and field settings, the avian hippocampal formation (HF) has been found to be an important brain area during landmark-based navigation, homing, and spatial memory tasks (e.g., Bingman, 1992; Gagliardo, Ioalé, & Bingman, 1999; Bingman & Able, 2002; Bingman, Hough, Kahn, & Siegel, 2003; Columbo & Broadbent, 2000). During such tasks, pigeons may create an allocentric “map-like” representation of their spatial environment, often referred to as a “cognitive map” (O’Keefe & Nadel, 1978). This map-like representation may be composed of multiple landmarks, objects and their spatial arrangement to one another (O’Keefe & Nadel, 1978). Space can be defined by a goal location’s spatial representation within an area or enclosure by global or distal environmental cues, local or positioned-based information, and by the relative geometry of the shape of an environment (Columbo & Broadbent, 2000; Bingman & Able, 2002; Cheng, Spetch, Kelly, & Bingman, 2006). Non-spatial features, or object-centered cues, such as colors or patterns can also serve as a reference for a goal’s location within an enclosure or environment (Columbo & Broadbent, 2000; Bingman & Able, 2002; Cheng et al., 2006). Environments (either inside a laboratory or outside in a natural field setting) can be rich in both spatial and non-spatial cues and it has been suggested that animals, including homing pigeons, are able to use both types of information to find goal locations. In the following reviewed studies, additional
information regarding the role of the avian hippocampus during spatial memory tasks will be discussed.

While Strasser and Bingman (1996) demonstrated that pigeons were capable of using different types of environmental cues to identify goal locations, Strasser and Bingman (1997) investigated the role of the avian hippocampus in a similar food finding task. Again, pigeons could rely on three different types of information (bowl color, position with respect to a landmark beacon, and position with respect to the room) to guide their goal seeking behavior towards a rewarded bowl. Some birds received hippocampal lesions while other were used as controls. During training, pigeons gradually learned to associate specific colored bowls adjacent to the landmark beacon with food rewards. Afterwards, unrewarded test trials (color vs. location information; landmark beacon vs. location information; color vs. landmark beacon; conflict) occurred (Strasser & Bingman, 1997).

Results of the test trials suggest that control pigeons chose equally among all the candidate bowls available during the color vs. location and landmark beacon vs. location trials. By contrast, hippocampal-lesioned pigeons overwhelmingly chose the correct colored candidate bowls during the color vs. location trials. They showed no significant preference for candidate bowls near the landmark beacon or in the correct spatial location during landmark beacon vs. location trials, but chose those bowls more often the previously non-rewarded bowls. However, during color vs. landmark beacon trials, control and hippocampal-lesioned pigeons chose the correct colored bowl in comparison to other bowls. During conflict trials, controls and hippocampal-lesioned pigeons demonstrated individual preferences: some chose the correct colored bowl, the bowl associated with the beacon, or the bowl associated with the room information. These results provide additional support for the findings of Strasser and Bingman.
(1996) in that control pigeons with intact hippocampi did not prefer one single cue over another, while hippocampal-lesioned pigeons consistently relied on feature information more often than spatial cues. Pigeons were capable of using both spatial and non-spatial cues to recognize previously experienced food sites, thus demonstrating the importance of the avian hippocampus in spatial memory (Strasser & Bingman, 1997).

Although previous comparative research in food-storing and non-storing birds has investigated cue preference in one-trial associative learning tasks, Strasser and Bingman (1999) also conducted a one-trial food association task in homing pigeons. This task used three groups of pigeons: controls, lesion controls, and hippocampal-lesions. Pigeons were trained to find hidden food rewards in specific bowls in unique spatial locations after varying retention intervals (10 mins, 1 hr, 7 hr, and 24 hr). This study differed from Strasser and Bingman (1996; 1997) in that rather than receiving extensive training across repeated trials, pigeons had only one initial acquisition exposure in which they could locate food. Then, they had to wait for a predetermined amount of time before they could search for the now hidden food. During test trials, the previously rewarded bowl was moved to a new spatial location and replaced with a different bowl. Pigeons could have relied on non-spatial, feature cues (bowl color) or spatial cues (location with respect to the experimental room) to guide their goal-seeking behavior towards a food site (Strasser & Bingman, 1999).

Results suggest that all pigeons generally chose one of the two previously rewarded bowls as their first choice more often than would be expected by chance. Control pigeons chose the bowl in the correct spatial location after the 10 minute interval, whereas the hippocampal-lesioned pigeons chose the correct bowl with respect to color. Across all retention intervals, control pigeons’ first choices were generally to the correct bowl with respect to spatial location,
whereas hippocampal-lesioned pigeons’ first choices tended to be towards the correct bowl with respect to color. This trend was also apparent for total choices by both all groups. In a one-trial associative learning task, control pigeons were more likely to choose spatial information over features, whereas hippocampal-lesioned pigeons chose differently and used featural information more often than space (Strasser & Bingman, 1999).

Vargas, Petruso, and Bingman (2004) examined the role of the avian hippocampus during feature-based and geometric-based goal seeking tasks. This experiment used hippocampal-lesioned and surgical control homing pigeons in a food finding task within a rectangular arena. In the rectangular arena in which a red poster board served as the feature cue while the relative geometric shape of the arena was considered the geometric or spatial cue. Pigeons were trained to find one baited food bowl that was always in the same location with respect to the colored board and geometry of the rectangular arena. All pigeons quickly learned the task. Subsequently, unrewarded probe trials were administered to determine cue preference and to observe if learning strategies differed between control and lesioned pigeons (Vargas et al., 2004).

For geometry-only probes, the red poster board was removed from the arena. For the feature-only probes, all the walls were made to be the same size, a square. Finally, during dissociation probes, the red poster board was affixed to a long wall instead of a short wall, putting both cues in conflict with each other. Results suggest that during geometry only trials, all control pigeons were able to use the geometry-based information to find the correct food bowl. Lesioned pigeons did not demonstrate a preference for geometrically correct food bowls. During feature-only trials, over half of the control pigeons were able to use only the red poster board to find a correct bowl, while the majority of lesioned pigeons were able to rely exclusively on the red feature cue to find a correct bowl. However, during dissociation trials, the majority of
control pigeons chose the geometrically correct bowl instead of the correct bowl with respect to feature cues. By contrast, lesioned pigeons demonstrated a robust preference for the featurally correct bowl in comparison to the correct bowl with respect to geometry cues. Together, these findings demonstrate that control pigeons were able to use spatial and non-spatial cues while lesioned pigeons exhibited an overreliance on non-spatial, featural cues during goal localization. There was a distinct control pigeon preference for spatial, geometry-based information in comparison to lesioned pigeons’ preference for features (Vargas et al., 2004). Interestingly, Nardi and Bingman (2007) also found similar results with their control and hippocampal-lesioned pigeons in a rectangular arena. Control and right hippocampal-lesioned pigeons seemed to favor spatial, geometry-based cues rather than the non-spatial color cues that left hippocampal-lesioned pigeons seemed to prefer. However, all three groups could use either cue independently to find a correct food bowl (Nardi & Bingman, 2007).

Bingman, Erichsen, Anderson, Good, and Pearce (2006) investigated spatial (global environmental shape, geometry) and non-spatial (colored cards) cue reliance in homing pigeons with a food finding task. Pigeons were divided into two groups: hippocampal-lesions and controls. One corner of a rectangular arena provided food reinforcement and was made to look distinct from the other three corners. Each corner contained a pair of green and pink cards in a specific spatial arrangement (green, right side; pink, left side), with this arrangement reversed in the corner that provided food. Pigeons were trained to associate the distinct card assortment with geometric information about the arena to identify a correct food site. After training, two different types of test trials (conflict and geometry-only) commenced (Bingman et al., 2006).
In conflict tests, the distinct card assortment was placed in a corner that had not previously been rewarded, whereas in geometry-only tests, the distinct card assortment was removed and the normal card (green on right, pink on left) layout was used in all four corners. Results indicate that the majority of control pigeons’ first choices during conflict trials were towards the corner that would have been geometrically correct with respect to their training experience, whereas hippocampal-lesioned pigeons’ first choices were mainly towards the correct corner defined by its featural properties. Almost all of the control and hippocampal-lesioned pigeons also chose the correct corner when only geometric information was available. These findings demonstrate the relative importance of the avian hippocampus during goal recognition via geometric cues or feature-based cues. Intact control pigeons exhibited a robust preference towards geometrically correct food bowls, whereas hippocampal-lesioned pigeons did not demonstrate the same preference and instead seemed to rely on featural information. Hippocampal-lesioned pigeons could use geometric cues independently when needed, thus demonstrating that although hippocampal-lesions may deter pigeons from developing robust preferences for geometric information, this damage does not make it impossible (Bingman et al., 2006).

Recently, Kahn and Bingman (2009) provided additional information about homing pigeons’ cue preference in an open-field laboratory study. Before the start of behavioral training and testing, pigeons were divided into two groups: controls or hippocampal-lesions. Goal locations (bowls) differed with respect to food quality (preferred vs. non-preferred), color, and spatial location. Throughout training, pigeons learned to associate specific bowls with either their preferred or less preferred food. After reaching a learning criteria, unreinforced probe trials occurred. During space-only trials, all bowls were identical with respect to color and in color-
only trials, different colored bowls were positioned in a row in the middle of the experimental room. Finally, during conflict trials, previously rewarded color bowls changed locations with non-rewarded colored bowls. All of these probes were carried out to elucidate to what extent pigeons in both groups were using spatial and non-spatial cues to find food rewards (Kahn & Bingman, 2009).

Results from the spatial-only probes indicate that control pigeons relied on spatial information to guide their behavior, whereas hippocampal-lesioned birds did not demonstrate this same robust ability. During color-only trials, control pigeons appear to show no preference for bowls based on previous food associations. By contrast, hippocampal-lesioned birds were able to use color cues to find previously rewarded bowls. In conflict trials, control pigeons relied on spatial cues while hippocampal-lesioned pigeons relied more on color cues to identify previously rewarded bowls. This study highlights the importance and potential preferences and perhaps preference for spatial cues in comparison to non-spatial cues among homing pigeons with intact hippocampi. It also illustrates that homing pigeons with lesioned hippocampi seem to demonstrate a strong preference for non-spatial, feature-based color cues. By demonstrating the tendency of pigeons to rely on non-spatial information in the face of hippocampal damage, this study further cements the relationship between the avian hippocampus and spatial memory (Kahn & Bingman, 2009).

Summary

The avian hippocampus is an important brain area involved in spatial memory. The majority of control pigeons demonstrated a robust preference for spatial or geometric-based information rather than features. While some hippocampal-lesioned pigeons also seem capable of
relying on these different types of information, many seem to prefer non-spatial, feature-based color cues.
Figure 1. Shaping Environment. The experimental room’s dimensions were 3.6 x 2.4 m. There was one door entrance to the room from the West (W) wall and a heating/cooling vent was positioned above the floor alongside the East (E) wall. On the upper right portion of the South (S) wall, there was a white sink fixture, and along the North (N) wall, a partition served as the hidden observation area. Three white bowls (W) that changed position across sessions were used to initially shape the birds.
Figure 2. Spatial Training Environment. Three blue (B) bowls were consistently positioned at three stable, spatial locations. Two-dimensional wall posters (X), various three-dimensional objects (MS, a metal seat; BL, a black napkin carrying case; SL, a silver metal cylinder; BR, brown cardboard box; R, a red broom), three Styrofoam pillars (PS, purple and pink stripes; Y, yellow; GR, green), and a partition covered with a blue-red (BR) sheet provided a rich landmark composition, which could be used to represent a goal location.
Figure 3. Feature Training Environment. Three bowls, R (red), Y (yellow), and G (green), were centrally arranged in the middle of the room, but the position of any given bowl changed across sessions. A gray rectangular curtain suspended from the top of the ceiling eliminated visual access to the sink, heating/cooling vent, and the sink. The dimensions of the environmental space within the enclosed curtain were 2.3 x 2.4 m. The landmark cues used in the spatial condition were absent.
Figure 4. Mean (±SEM) percent of correct choices for each group (morning and evening sessions combined). There was a significant interaction between blocked sessions and group. Post-hoc analyses indicated that during BLOCK 1, there was not a significant group difference with respect to correct first choices, but by BLOCK 2, the spatial group’s mean correct first choices was close to becoming significantly different compared to the feature group. During BLOCK 3, the spatial group’s performance was now significantly better than feature group. + p < .10; ** p < .01.
Figure 5. Mean (±SEM) percent correct first choices during the last fifteen sessions for morning and evening sessions separated by group. During the morning sessions, the spatial group’s first trial correct performance, although well above 50%, failed to reach statistical significance, whereas the feature group’s performance was significantly greater than expected chance; during the evening sessions, both the spatial and feature group’s first trial correct performance was significantly greater than expected chance. * p < .05.
APPENDIX F. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

LETTER

Office of Research Compliance
309A University Hall
Bowling Green, OH 43403-0183
Phone: (419) 372-7710
Fax: (419) 372-6018
E-mail: hsrb@bgnet.bgsu.edu

February 7, 2014

Dr. Verner Bingman
Bowling Green State University

Re: IACUC Protocol 14-002

Title:
Time Place Learning and Spatial Memory

Dear Dr. Bingman:

On February 6, 2014 the above referenced protocol received final approval after review of the requested modifications by Designated Member Review. The modifications have been incorporated into the official copy of your protocol (see modifications below).

This approval expires on February 5, 2015, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol. Please also remember to keep a copy of the approved protocol in the animal facility room(s) in which your animals are housed and in any associated procedure rooms (contact the UAF staff for assistance in this regard).

Please consult with the staff of the Animal Facility about your requirements to get started on this project. Good luck with your project.

Sincerely,

Hillary Snyder, Ph.D.
IACUC Administrator