

# In a daily time–place learning task, time is only used as a discriminative stimulus if each daily session is associated with a distinct spatial location

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**Abstract** It is difficult for rats to acquire daily time–place (TP) learning tasks. One theory suggests that rats do not use time of day as a stimulus signaling a specific response. In the present study, we tested rats' ability to use time of day as a discriminative stimulus. A fixed-interval procedure was used in which one lever provided reinforcement on a FI-5-s schedule in morning sessions, and the same lever provided reinforcement on a FI-30-s schedule in afternoon sessions. Because only one place was used in this paradigm, the rats could only use time of day to acquire the task. Mean responses during the first 5 s of the first trial in each session indicated that the rats did not discriminate between the two sessions. In Phase II, a different lever location was used for each of the two daily sessions, which meant that both spatial and temporal information could be used to acquire the task. The rats readily acquired the task in this phase, and probe trials indicated that the rats were using a combination of spatial and temporal information to discriminate between the two different trial types. When the spatial cue was removed in Phase III, rats no longer discriminated the two sessions, suggesting that time can only be used as a discriminative stimulus when each daily session is associated with a distinct spatial location.

**Keywords** Circadian · Time of day · Rats · Discriminative stimulus · Time–place learning · Time–place discrimination

The ability to learn the spatiotemporal variability of a resource is called time–place (TP) learning or time–place discrimination

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and has been of interest to comparative psychologists for decades. Laboratory investigations of daily TP learning generally involve training an animal that at one time of day food is available in one location and at another time of day food is available in a different location. For example, Saksida and Wilkie (1994) trained pigeons that the delivery of food rewards were contingent on responding to one key in the morning sessions and responding to another key in the afternoon sessions.

Although research has shown that birds readily learn daily TP learning tasks (e.g., Biebach, Gordijn, & Krebs, 1989; Saksida & Wilkie, 1994), research with rodents has been inconsistent (see Thorpe & Wilkie, 2006). Rodents can acquire daily TP learning tasks (Aragona, Curtis, Davidson, Wang, & Stephan, 2002; Carr & Wilkie, 1997; Deibel & Thorpe, 2013; Lukoyanov, Pereira, Mesquita, & Andrade, 2002; Mistlberger, de Groot, Bossert, & Marchant, 1996; Pizzo & Crystal, 2002, 2006; Van der Zee et al., 2008; Widman, Gordon, & Timberlake, 2000; Widman, Sermina, & Genismore, 2004), or similar episodic memory tasks that require the animal to learn at what time of day an event occurred (Zhou & Crystal, 2009, 2011). Conversely, in other situations rats have failed to acquire daily TP learning tasks (Aragona et al., 2002; Boulos & Logothetis, 1990; Lukoyanov et al., 2002; Thorpe, Bates, & Wilkie, 2003; Widman et al., 2000; Widman et al., 2004), or acquired them with great difficulty (Means, Ginn, Arolfo, & Pence, 2000). Rats readily learn daily go no-go tasks in which food is available at one time of day but not at another time of day (Means, Arolfo, Ginn, Pence, & Watson, 2000; Thorpe et al., 2003).

Two trends have emerged from this research. First, rats are more likely to learn a daily TP discrimination in free operant versions of the task such as lever pressing (Carr & Wilkie, 1997; Deibel & Thorpe, 2013; Mistlberger et al., 1996; Pizzo

& Crystal, 2006) and head entries (Pizzo & Crystal, 2002), than they are in discrete trials versions such as radial arm and T-maze paradigms, in which the animal is removed from the maze at the end of each trial (Means, Ginn, et al., 2000; Thorpe et al., 2003).

Second, for discrete trial versions, response cost – the effort associated with executing a response – is an important determinant in whether learning is observed (Widman et al., 2000). For example, in the water maze daily TP task, rats typically do not learn that the platform is hidden in one location in morning sessions and in another location in afternoon sessions because the response cost is low (Widman et al., 2004). However, if the response cost is increased, either by severely food restricting the rats (Lukoyanov et al., 2002) or by having the rats wear weighted vests (Widman et al., 2004), then evidence of learning is seen.

Similarly, Van der Zee and colleagues (2008) found that mice only acquired TP associations in a three-arm maze if the goal of the task was to avoid an arm that administered a shock. They hypothesized that the animals did not acquire the appetitive version of the task because mice like to explore food-providing environments (Van der Zee et al., 2008). The response cost hypothesis could also apply to these results, because the cost associated with receiving a shock was higher than the cost of simply having to choose another arm in the appetitive version of the task. Aragona et al. (2002) also suggested that their rats did not acquire a daily TP task that relied on food-anticipatory activity as the dependent measure until the experimenters made it more difficult for the rats to switch between locations (by placing the water source between the levers), and therefore increased the response cost.

An important question remains as to whether the failures to find evidence of TP learning are due to failures that can be attributed to insensitive dependent measures (i.e., performance) or learning. Several studies suggest that the issue may be a performance one. First, as was mentioned for the Van der Zee et al. (2008) study, the mice might have been aware of temporal information in the appetitive version of the task, but were not motivated to display TP associations unless shocks were administered. Second, Carr and Wilkie (1997, 1999) trained rats that one of four levers in an operant box provided reinforcement in morning sessions, and another lever provided reinforcement in afternoon sessions. They found that when rats are first placed in the box, they initially patrolled the box and pressed the levers. If these initial responses were included in the analysis, it was concluded that the rats had not learned the discrimination. If, however, a short nonreinforced period was included at the beginning of each session, in which rats' exploratory behaviors were not scored, then evidence of learning was found. Similarly, Deibel and Thorpe (2013) found that conclusions about learning varied on the basis of the dependent variables measured. Their rats were trained that one lever in a T-maze provided food in morning sessions and another lever provided food in afternoon

sessions. If one considered only first arm choices, no evidence of learning emerged; however, if one considered first lever presses, rats did show evidence of learning.

Alternatively, some indirect evidence suggests that there is more to the failure to demonstrate learning than simply an insensitive dependent measure. Thorpe and Wilkie (2007) argued that, contrary to previous hypotheses that rats automatically encode time–place–event information into a single tripartite code (Gallistel, 1990; Wilkie, 1995), rats instead automatically encode this information into two separate bipartite codes (time–event and event–place). They further argued that only in certain situations—for example, high response cost—do rats form a single tripartite code. It is also possible that in these high-response-cost situations, rats form two bipartite codes, and that the high response cost allows these two codes to be bound together. In a traditional low-response-cost discrete-trials task, in which rats are trained that Place A contains food in morning sessions and Place B contains food in afternoon sessions, the bipartite codes created in morning sessions would be  $\text{Time}_1\text{--}E_{\text{food}}$  and  $E_{\text{food}}\text{--}Place_A$ ; in afternoon sessions, the bipartite codes created would be  $\text{Time}_2\text{--}E_{\text{food}}$  and  $E_{\text{food}}\text{--}Place_B$ . Because the events are the same, the rat is only able to learn a preference for the locations that provide food. That is, for example, in morning sessions the rat retrieves the code that signals that  $\text{Time}_1$  is associated with the event food. However, the food event has been equally associated with Places A and B, and as was demonstrated in our previous research (Thorpe et al., 2003), the rat chooses equally between Places A and B.

Thorpe and Wilkie (2007) showed that rats in a low-response-cost task, in which a large amount of food was given in Place A in morning sessions and a small amount of food was given in Place B in afternoon sessions (counterbalanced across rats), successfully learned the task. Thorpe and Wilkie argued that this was because the events were different. In morning sessions, rats encoded  $\text{Time}_1\text{--}E_{\text{lgfood}}$  and  $E_{\text{lgfood}}\text{--}Place_A$ . In afternoon sessions, they encoded  $\text{Time}_2\text{--}E_{\text{smfood}}$  and  $E_{\text{smfood}}\text{--}Place_B$ . Now, when the animal was placed in the maze in morning sessions, it retrieved from memory that  $\text{Time}_1$  was associated with large amounts of food and that large amounts of food were only associated with  $Place_A$ . Therefore, the rat successfully used time of day to predict the amount and location of food (Thorpe & Wilkie, 2007).

The results of go–no-go tasks (Means, Arolfo, et al., 2000; Thorpe et al., 2003) are also consistent with this bipartite hypothesis. In these tasks, rats are trained that food is available in both arms of a T-maze in one daily session, and no food is available in either arm in the other daily session. Rats quickly learn at what time of day food is available, as indicated by significantly shorter latencies on the food trials. Thorpe and Wilkie (2007) argued that rats solve this task because they encode that  $\text{Time}_1\text{--}E_{\text{food}}$  and  $\text{Time}_2\text{--}E_{\text{nofood}}$ . When placed in the maze at  $\text{Time}_1$  they quickly look for food because it has been associated with food, but do not go as quickly at  $\text{Time}_2$ .

Means and colleagues (Means, Arolfo, et al., 2000; Means, Ginn, et al., 2000) hypothesized, on the basis of these results, that rats use time of day as an occasion setter signaling that food is available, but not as a discriminative stimulus or “a signal for a specific response” (Means, Arolfo, et al., 2000, p. 13). However, if time of day can only be used to tell whether food is available, we would not have expected the rats in Thorpe and Wilkie’s (2007) study to learn the task given that food was available at both times, albeit in different amounts.

The purpose of the present study was twofold. First, we wanted to determine whether rats could use time of day as a discriminative stimulus when place was held constant. Second, we wanted to determine whether the event component in the time–event code could encode information in addition to the presence/absence and amount of food. To increase the likelihood of seeing evidence of learning, we used a free-operant task and included a nonreinforced period at the start of each session, as per Carr and Wilkie (1997, 1999). Because we used a free-operant procedure, it was not necessary to have a high response cost (Deibel & Thorpe, 2013), and therefore comparisons to discrete-trials tasks would be easier. Specifically, we modified the methodology used by Budzynski and Bingman (1999). In that study, pigeons were reinforced for keypecks according to an FI-5-s schedule in one daily session, and they were reinforced for pecks to the same key according to an FI-30-s schedule in the other daily session. The pigeons responded differently depending on the time of day. These results are in accordance with previous findings, which have demonstrated that pigeons easily learn daily TP tasks (Saksida & Wilkie, 1994).

Using a design similar to that of Budzynski and Bingman (1999), rats were trained on a task in which one lever provided food on an FI-5-s schedule in one daily session, and the same lever in the other daily session provided food on an FI-30-s schedule. That is, the spatial location was the same, but the contingency varied depending on the time of day. In this phase, only time of day could be used as a discriminative stimulus. If rats encoded the schedule of reinforcement (i.e., expected delay to initial access to food) in the event component of the time–event code, we would expect to see evidence of learning. In the second phase of the study, the same rats were trained with the same time-of-day contingency, but the spatial location differed as well. In this phase, rats could use either the spatial or the temporal information to solve the task. The third phase was identical to the first phase.

## Method

### Subjects and apparatus

Six male Long-Evans rats were obtained from Charles River (St. Constant, Quebec). The rats were experimentally naïve

and approximately 50 days old at the start of training. The rats were fed a standard rat diet (PMI Nutrition International, MO, USA) at approximately 5:00 p.m. each day, regardless of whether they were tested that day. Prior to starting the experiment, food intakes were restricted to maintain rats at 85 % of their free-feeding weight. They were then allowed to gain approximately 5 g per week to allow for continued growth. This level of deprivation was not considered extreme, since we have previously maintained rats in good health using this method. During training and experimental sessions, rats received 45-mg pellets (Bio Serv, Frenchtown, NJ) as reinforcers. Free access to water was available at all times, except during experimental sessions. Rats were maintained in strict accordance with Canadian Council on Animal Care (CCAC) guidelines.

Rats were housed individually in transparent plastic cages (45 × 25 × 21 cm) lined with aspen woodchip bedding (Necto Company, New York, NY). In their home cages, rats were given paper cups, wooden blocks, and bones (Nylabone, Neptune, NJ) for chewing and nest building. All rats were housed in a colony room maintained on a 12:12-h light:dark cycle, with light onset at 7:00 a.m. and offset at 7:00 p.m. In addition to the experimental sessions, rats received biweekly individual enrichment sessions of approximately 20-min duration. During enrichment sessions, rats were placed individually in a Plexiglas box (61 × 61 × 61 cm) lined with aspen woodchip bedding, which contained several plastic tubes, plastic containers, and a standard running wheel.

Both pretraining and discrimination sessions took place in a transparent Plexiglas operant box (48 × 48 × 37 cm) with four retractable levers (Model No. ENV-112CM, Med Associates Inc., St. Albans, VT), one centered on each of the four box walls. A pellet dispenser (Model ENV-203045, Med Associates, Inc., St. Alban, VT) was mounted to the top of each wall, to deliver pellets to food wells located 6 cm from the box floor and adjacent to each lever. Above each lever was a light (Dialco, 75 W 125 V). The operant box was lined with aspen woodchip bedding and was located on a tabletop in a small room (170 × 160 cm), which contained a cabinet, a radio, and a door.

### Procedure

*Pre-training* During a pretraining period, rats were shaped to leverpress. Rats were placed in pairs in the operant box for 20-min sessions to habituate to the boxes. To encourage rats to lever press, levers were coated in a food mash. Once rats were consistently pressing the levers they began individual training, which consisted of gradually shifting rats from a continuous reinforcement schedule to a variable ratio (VR) schedule. Rats began Phase I once they were consistently pressing on a VR-30-s schedule.

**Discrimination training** Discrimination training was composed of running rats in three experimental manipulations; Phases I, II, and III. Rats were run in Phase I for 42 days, Phase II for 35 days, and Phase III for 35 days. Rats were tested 5 days a week and received two sessions per day: a morning session (starting at 8:30 a.m.) and an afternoon session (starting at 2:30 p.m.). During each session rats were tested individually, and the order always remained the same. All rats remained in their home cages on a cart outside of the experimental room while waiting to be tested in the experiment. To begin each session, the rat was placed in the operant box, and a corresponding computer program for the session was started immediately. A laptop computer outside the experimental room recorded the reward delivery and timing of each lever press to 0.2-s accuracy. A data file was composed in which the lever press, time of press, and number of rewards were recorded. In the experimental room, the overhead light was off and a radio was playing during all trials.

For the first 2 min of all sessions, rats were given a nonreinforced period in which the lights above the levers remained off; however, they had access to some of the levers. The purpose of this nonreinforced period was to provide an opportunity for the rats to exhibit species-typical behavior by exploring other lever choices. Carr and Wilkie (1997, 1999) only found evidence of TP learning if each session started with a brief nonreinforced period in which exploratory species-typical behaviors could be executed without penalty. To increase the likelihood of seeing evidence of learning, this initial nonreinforced period was also included in the present study. In Phases I and III, rats had access to three levers that would not be active during the session. For example, if Lever 1 was to be the lever providing reinforcement, then during the nonreinforced period, Levers 2, 3, and 4 were extended. In Phase II, rats only had access to one lever during the nonreinforced period. This lever was the one that never provided reinforcement during any of the phases. We did not want to provide a cue in Phase II as to which lever would be available before the session started, because of the importance of spatial location in this phase.

The lights above the reinforced lever came on after the 2-min exploration period to indicate the start of a trial. Each session consisted of 12 trials, 80 % of which were normal trials, while 20 % were peak trials. In peak trials, a light was illuminated above the correct lever for 90 s; however, no reward was available. At the end of each trial, a variable intertrial interval between 10 and 60 s was given. For all phases, rats were trained that in one of two daily sessions lever presses would be reinforced on a 5-s fixed-interval (FI) schedule, and in the other session, a FI-30-s schedule. The lever location and FI schedule were counterbalanced across rats, and the reinforcement schedule did not change for rats when phases were changed.

During Phase I, once the 2-min nonreinforcement period ended, only one lever was extended, and this lever provided

reinforcement in both morning and afternoon sessions. A light illuminated the correct lever and was turned off during the intertrial interval. In this phase, time of day acted as the sole discriminative stimulus for the reinforcement schedule. If rats could discriminate between sessions, we would expect to see an increased rate of responding during the first 5 s of a FI-5-s session, in comparison to a FI-30-s session.

Once rats had been run in Phase I for 42 days, they were switched to Phase II. In Phase II, the spatial location of the correct lever alternated in morning and afternoon sessions. The lever locations in Phase II were different from those in Phase I (e.g., if, in Phase I, Lever 2 provided reinforcement in morning and afternoon sessions, in Phase II, Lever 1 provided food in morning sessions, and Lever 3 in afternoon sessions). As in Phase I, only one lever was extended during a session, and the light above this lever was turned on. This provided rats with spatial location as a second discriminative stimulus. The purpose of this phase was to determine whether rats would use spatial and/or temporal information to guide their lever choices. At the end of Phase II, rats received four days of conflict probes over two weeks. For the first 2 min of each conflict probe, the levers were extended as normal (i.e., the nonreinforced period was included as in nonprobe sessions); however, at 2 min the lever that was reinforced during the opposite time of day was extended. During conflict probes, rats received only a single peak trial (i.e., lights remained on for 90 s, and no reinforcement was provided). For example, if during Phase II a rat received an FI-5-s schedule on Lever 2 in morning sessions and an FI-30-s schedule on Lever 4 in afternoon sessions, then on conflict days, in the morning Lever 4 would be extended, and in the afternoon Lever 2 would be extended. If rats were using temporal information as the discriminative stimulus, then they should respond as they normally would at that time of day (i.e., in our example, in morning conflict sessions the rat would respond as if the schedule in effect was an FI 5 s, and in afternoon conflict sessions the rat would respond as if an FI-30-s schedule was in effect). If they were using spatial information, however, they should respond as if it were the other session (i.e., in our example, in morning conflict sessions the rat would respond as if the schedule in effect was an FI 5, and in afternoon conflict sessions it would respond as if an FI-5-s schedule was in effect). After Phase II, rats were run in Phase III. Phase III was identical to Phase I (i.e., the same lever location was used for both times of day).

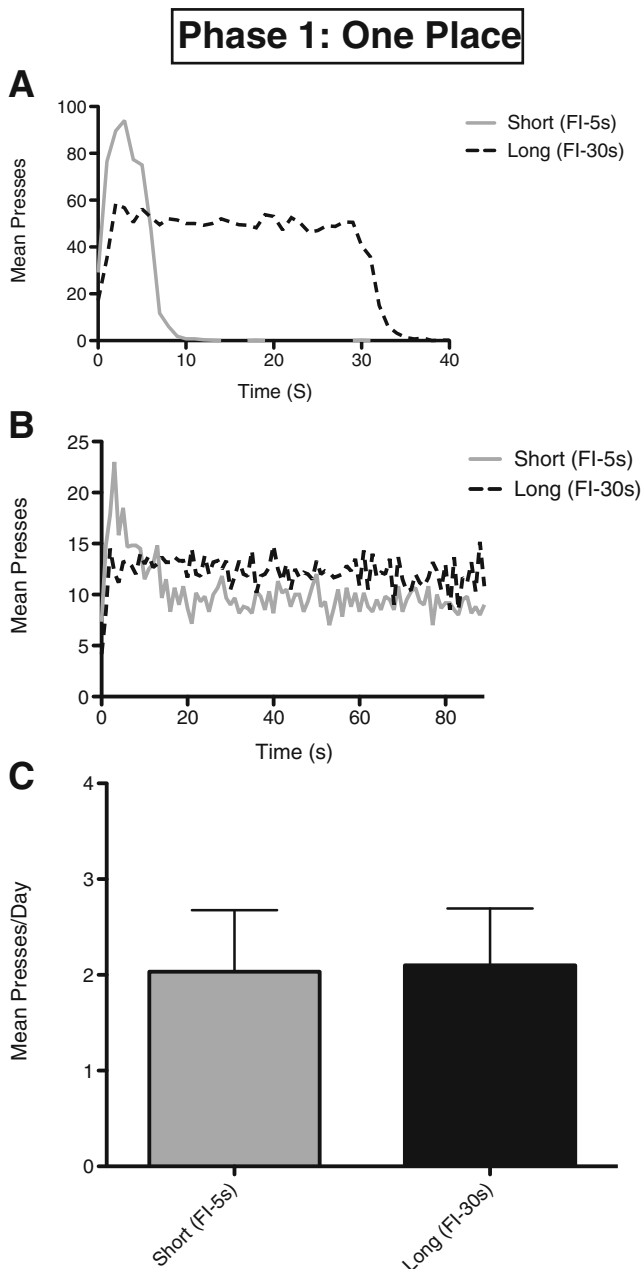
## Results and discussion

On any given day, data were excluded from the analyses for the entire day if data could not be obtained for any one session (AM or PM) on that day. Such instances were rare, occurring an average of 2.8 sessions per rat over the entire experiment, due to experimenter or mechanical error. Furthermore, only

one rat missed a session during the last ten days of any of the phases (Phase II).

### Phase I

For the last 10 days of Phase I, the mean number of presses for each second of baseline trials was calculated across rats separately for the FI-5-s and FI-30-s sessions. As is depicted in



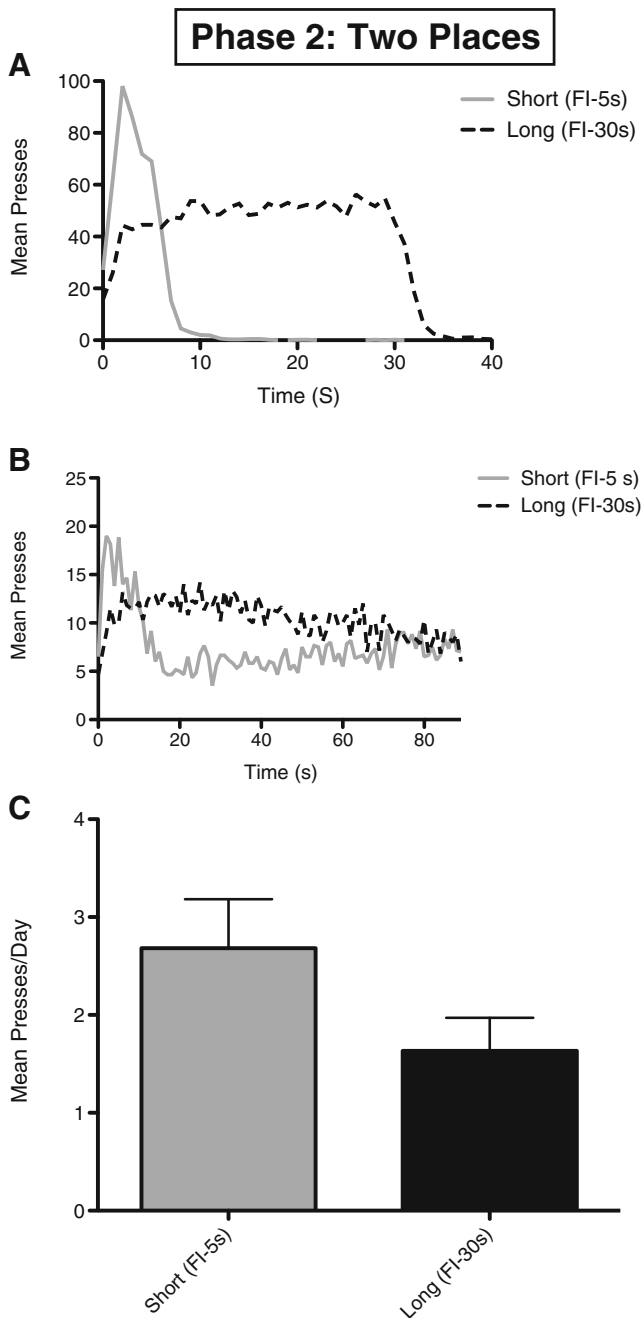
**Fig. 1** (A) Mean numbers of presses per rat during the last 10 days of Phase I for normal trials for both short (FI-5-s) and long (FI-30-s) sessions. (B) Mean numbers of presses per rat during the last 10 days of Phase I for peak trials for both short (FI-5-s) and long (FI-30-s) sessions. (C) The mean presses per day for the first 5 s of the first trial each session for the last 10 days. Error bars represent the standard errors of the means

Fig. 1A, the distributions of responding are clearly different in FI-5-s and FI-30-s sessions. Rats' response rates were initially much higher in FI-5-s than in FI-30-s sessions, but FI-5-s responding then decreased more rapidly. A similar pattern of responding can also be seen on peak trials (Fig. 1B), where the initial rate of responding is higher for the FI-5-s than for the FI-30-s sessions. In addition, responding decreases earlier in FI-5-s than in FI-30-s sessions. Together, these data suggest that rats are able to discriminate which sessions are reinforced on an FI-5-s versus an FI-30-s schedule. However, these data are confounded, because in all trials except the first trial of a session, the rats may use the timing of the first reward (i.e., 5 vs. 30 s) as a cue for when the subsequent rewards in that session will occur. Therefore, to determine whether rats were using time of day as the discriminative stimulus, only data from the first trial of each session should be analyzed. Figure 1C shows the mean numbers of presses per day for the first 5 s of the first trial of each session for the last 10 days. Only the first 5 s were analyzed because the rate of responding would decrease once the reinforcement was given, and therefore it would appear that the rate of responding was lower in the FI-5-s than in the FI-30-s sessions if the data from the entire trial were included. Paired-sample *t* tests confirmed a lack of difference in the numbers of responses during the first 5 s between the two sessions,  $t(5) = 0.241$ ,  $p = .819$ . If rats used time of day as a discriminative stimulus, more presses should have occurred on the FI-5-s trials than on the FI-30-s trials. These data suggest that rats did not use time of day as a discriminative stimulus.

### Phase II

As in Phase I, the mean numbers of presses for FI-5-s and FI-30-s sessions were calculated for both normal trials (Fig. 2A) and peak probe trials (Fig. 2B) for the last 10 days. As in Phase I, it appears that in FI-5-s sessions, rats tended to have a higher initial rate of responding and to slow their responding earlier than in FI-30-s sessions. Again, these data were confounded, because the rats could use working memory of the first trial to complete the subsequent trials. Therefore, we analyzed performance on only the first trial of each session for the last 10 days of Phase II (Fig. 2C). A paired-sample *t* test comparing the mean numbers of presses showed that rats responded more during the first 5 s in FI-5-s sessions than in FI-30-s sessions,  $t(5) = -3.732$ ,  $p = .014$ . This suggests that rats were able to discriminate the FI-5-s and FI-30-s sessions by using either a combination of spatial and temporal information or just spatial information.

To determine whether rats were using spatial location or time of day as the discriminative stimulus, conflict probes were given in which the afternoon lever was presented in morning conflict sessions, and the morning lever was presented during afternoon conflict sessions. If rats were using spatial location as the main discriminative stimulus, then we should



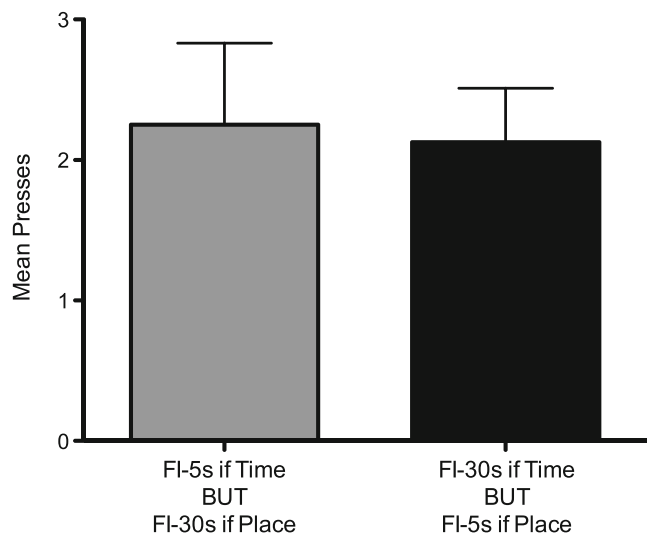
**Fig. 2** (A) Mean numbers of presses per rat during the last 10 days of Phase II for normal trials for both short (FI-5-s) and long (FI-30-s) sessions. (B) Mean numbers of presses per rat during the last 10 days of Phase I for peak trials for both short (FI-5-s) and long (FI-30-s) sessions. (C) The mean presses per day for the first 5 s of the first trial each session for the last 10 days. Error bars represent the standard errors of the means

see more responding on the lever location that was associated with the FI-5-s schedule. Likewise, if rats were using time of day as the main discriminative stimulus, then we should see more responding at the time of day associated with the FI-5-s schedule. The mean number of presses for the first 5 s was calculated for each lever per day (Fig. 3). A paired-sample  $t$  test confirmed no significant difference between the numbers

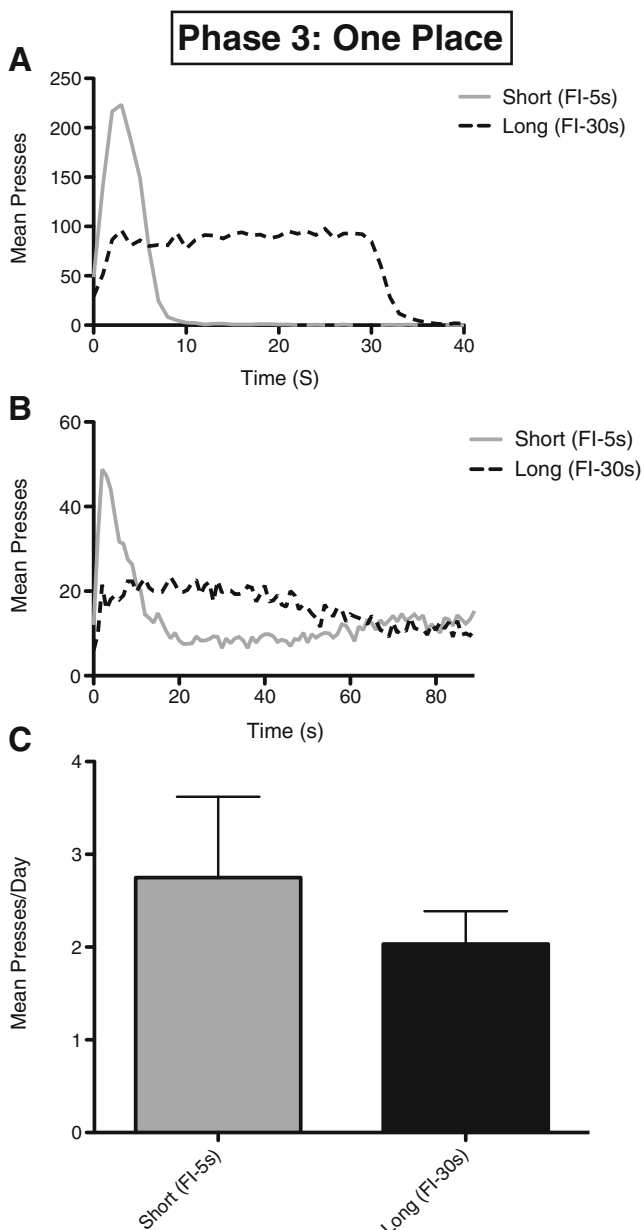
of presses in different sessions,  $t(5) = 0.149$ ,  $p = .887$ , suggesting that neither the spatial location nor the time of day was a more salient discriminative stimulus. Paired-sample  $t$  tests were used to compare the morning and afternoon conflict probe sessions with the average numbers of presses on the FI-5-s and FI-30-s sessions during the last 10 days of Phase II (i.e., cf. Figs. 2C and 3). We found no significant differences between the baseline FI-5-s sessions (i.e., gray bar Fig. 2C) and either of the conflict probes used to determine whether time was serving as a discriminative stimulus [i.e., FI-5-s schedule (gray bar in Fig. 3):  $t(5) = 0.842$ ,  $p = .438$ ; FI-30-s schedule (black bar in Fig. 3):  $t(5) = 0.688$ ,  $p = .522$ ]. Likewise, there were no significant differences between the baseline FI-30-s sessions (i.e., black bar in Fig. 2C) and either of the conflict probes used to determine whether time of day was serving as the discriminative stimulus [FI-5-s schedule (gray bar in Fig. 3):  $t(5) = -2.382$ ,  $p = .063$ ; FI-30-s schedule (black bar in Fig. 3):  $t(5) = -1.480$ ,  $p = .199$ ]. These analyses further confirmed that neither time of day nor spatial location was a more salient discriminative stimulus.

### Phase III

Again, the mean numbers of presses for FI-5-s and FI-30-s sessions were calculated for both normal trials (Fig. 4A) and peak probe trials (Fig. 4B) for the last 10 days. A similar



**Fig. 3** Mean numbers of presses per day in the first 5 s on each lever during the four days of conflict probes. The gray bar represents the lever associated with an FI-5-s schedule if time of day was the discriminative stimulus and the lever associated with an FI-30-s schedule if place was the discriminative stimulus. The black bar represents the lever associated with the FI-30-s schedule if time of day was the discriminative stimulus, and the lever associated with an FI-5-s schedule if place was the discriminative stimulus. If time of day was acting as the discriminative stimulus, more responses would appear on the gray bar than on the black bar. If place was acting as the discriminative stimulus, more responses would appear on the black bar than on the gray bar. Error bars represent the standard errors of the means



**Fig. 4** (A) Mean numbers of presses per rat during the last 10 days of Phase III for normal trials for both short (FI-5-s) and long (FI-30-s) sessions. (B) Mean numbers of presses per rat during the last 10 days of Phase III for peak trials for both short (FI-5-s) and long (FI-30-s) sessions. (C) Mean presses per day for the first 5 s of the first trial in each session for the last 10 days. Error bars represent the standard errors of the means

pattern of responding was found to that in Phases I and II. Namely, rats' response rates were initially much higher in FI-5-s than in FI-30-s sessions. When examining only the first 5 s of the first trial of each session (Fig. 4C), as in Phase I no differences appeared between the FI-5-s and FI-30-s sessions. Paired-sample *t* tests revealed no significant difference between the numbers of presses during the first 5 s in FI-5-s and FI-30-s trials,  $t(5) = -1.262$ ,  $p = .262$ . Furthermore, as is depicted in Fig. 5, when Phase III was divided into five blocks of seven days, a 5 (Block)  $\times$  2 (Session) repeated measures

analysis of variance revealed no main effects of block [ $F(4, 20) = 0.615$ ,  $p = .65$ ] or session [ $F(1, 5) = 1.82$ ,  $p = .235$ ]. We also found no Block  $\times$  Session interaction [ $F(4, 20) = 1.522$ ,  $p = .234$ ], and planned comparisons indicated that for any of the five blocks, the number of presses was not significantly greater in the FI-5-s trials, as compared to the FI-30-s trials.

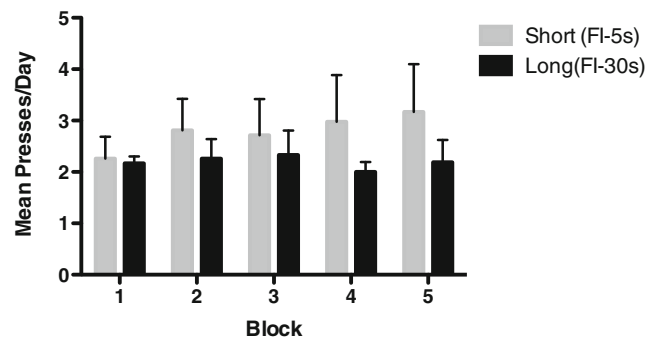
It should be noted that despite the lack of evidence for time being used as a discriminative stimulus in Phase III, as is demonstrated in Fig. 5, there is a trend, which suggests that the animals were responding more in the FI-5-s sessions during the later blocks of Phase III. Furthermore, qualitatively, Fig. 4C is more akin to Fig. 2C (evidence of learning) than to Fig. 1C (no evidence of learning). Although, in Phase III, the mean number of presses was higher for FI-5-s trials than for FI-30-s trials (Fig. 4C), the variance for short trials was much higher during Phase III ( $SD = 21.333$ ) than during the previous two phases (Phase I  $SD = 15.744$ , Phase II  $SD = 12.254$ ). The increased short-trial variance during Phase III appears to be due to two rats that were pressing almost twice as much during the FI-5-s trials than during the FI-30-s trials. Although statistics could not be conducted to evaluate the performance of individual rats, two of the rats possibly acquired discriminations in Phase III.

## General discussion

In Phases I and III, in which only time of day could be used as a discriminative stimulus, rats did not discriminate the two sessions. Although the patterns of responding on the two FI schedules were different if all of the trials were included, this likely reflected the ability of rats to use working memory of the first trial of each session to determine which schedule of reinforcement was in effect on subsequent trials. Thus, the most important dependent variable was the number of presses during the first 5 s of the first trial of each session; and using this measure, no significant difference emerged between the two sessions. However, with the addition of spatial location as a discriminative stimulus in Phase II, the rats readily solved the task, as demonstrated by more frequent pressing during the first 5 s of FI-5-s sessions, relative to the first 5 s of FI-30-s sessions.

We found hints that some rats might be able to use time of day in this task. For example, in Phase III the patterns of lever pressing of two rats suggested that they had learned the discrimination, even though the only available discriminative stimulus was time of day. However, in the absence of spatial cues this discrimination is not robust.

It is also possible that the failure to find evidence of discrimination in Phases I and III was due to several limitations. First, since only a small portion of the data were analyzed, more subtle effects might have been found if longer FI schedules had been used, so that more data would be available to compare. Similarly, only data from the first trial of each



**Fig. 5** Mean numbers of presses per day in the first 5 s of each block for FI-30-s and FI-5-s trials in Phase III. Performance did not improve across the five blocks, nor were there significantly more presses in the FI-5-s trials in any of the five blocks

session were used for analyses, and it is possible that there could have been a first-trial performance decrement. Nonetheless, in a similar operant conditioning daily TP learning task, we observed learning in measures that gauged performance before the first reinforcer was received in each session (first leverpress and prereinforcement presses; Deibel & Thorpe, 2013). Regardless, despite using the present restricted intervals and focusing the analysis on data collected from the first trial, we were able to find differences in responding on FI-5-s and FI-30-s sessions in Phase II, suggesting that this measure is sensitive enough when a more salient discriminative stimulus is used.

The results of the present study can shed some light on the nature of the hypothesized bipartite/tripartite codes. In Phases I and III, the rats did not discriminate which schedule was in effect when the only available discriminative stimulus was time of day. Given that previous work has shown that rats can successfully perform a daily go–no-go task (e.g., Means, Arolfo, et al., 2000; Thorpe et al., 2003), it would appear that the time–event codes contain information about the presence or absence of food, but not more specific information such as the schedule under which the reinforcement is given. If rats were encoding the information as tripartite codes (e.g.,  $T_1-P_3-E_{\text{food}}$  and  $T_2-P_3-E_{\text{food}}$ ), then these tripartite codes also did not contain schedule information.

Interestingly, when spatial location was included as a discriminative stimulus in Phase II, the rats readily learned the discrimination. At first glance, it would appear that this is evidence for the use of bipartite place–event codes that contained schedule information. It is also possible that the rats were using tripartite codes in Phase II (e.g.,  $T_1-P_1-E_{\text{food+FI5s}}$  and  $T_2-P_2-E_{\text{food+FI30s}}$ ), since the results of the conflict probes suggest that rats were aware of the spatial and temporal contingencies in effect. When the spatial and temporal cues were dissociated in the conflict probes, the rats did not appear to favor the use of one cue over the other. If rats were using only the spatial cues as the discriminative stimulus, we would have expected them to respond more quickly on the lever associated with the FI-5-s schedule, regardless of the time of

day. Conversely, if the rats were using temporal cues, they should have increased their responding at the time of day associated with the FI-5-s schedule, regardless of the spatial location of the lever. Instead, responding during the conflict probes did not offer support for either strategy, suggesting that both spatial and temporal information are important in this task. Therefore, on the basis of the conflict probes, it is likely that rats were using tripartite codes by the end of Phase II. It may have been that different spatial locations were needed to help bridge or bind the two bipartite codes. It is also possible that the results may simply have been due to the fact that three cues were better than two.

In Phase III, when the spatial discriminative stimulus was removed and the only remaining discriminative stimulus was time of day, the rats no longer discriminated the two sessions. If the rats were using tripartite codes in Phase II, then the new tripartite codes in Phase III would have been different from those in Phase II and would have required new learning, therefore explaining why discrimination was no longer seen. However, it is somewhat surprising that we found no statistically significant improvement in performance during Phase III. A very clear finding is that the time–event bipartite codes did not allow the rats to discriminate the schedules as quickly as the place–event codes. Throughout the entire study (112 days), the time–event codes were constant, and even at the end of training, rats did not exhibit significantly differential responding on FI-5-s versus FI-30-s sessions. In contrast, within 35 days of spatial location acting as the discriminative stimulus, rats were showing differential responding.

The notion that place–event codes may contain more information (such as the schedule of reinforcement) than the time–event code (which may be limited to information about the presence or absence of food) is consistent with the hypothesis of Means and colleagues (Means, Arolfo, et al., 2000; Means, Ginn, et al., 2000) that time of day can be used as an occasion setter, but not as a discriminative stimulus. That is, time of day is used to tell an animal that a particular event is likely to occur, but not what the animal must do to obtain the food. Instead, a combination



of temporal and local cue information for the specific locations modulates responding. Therefore, in the present study, time of day indicated that food would be present, but not the particular response that was required (i.e., which FI schedule was in effect). Because the local cues in Phases I and III were identical (due to both sessions occurring at the same place), the rats did not respond differentially on the basis of the two schedules. In Phase II, however, the local cues at the two spatially distinct locations allowed the rats to differentiate the response rate that was most efficient for each schedule.

If additional information, such as the schedules of reinforcement, is encoded with the place code, then it may be that all of the information encoded with time–place–event codes cannot be accessed unless the rat is in the correct place at the correct time of day. This might allow us to better explain the conflicting results of free-operant and discrete-trials tasks. When the rat is at the start arm of either free-operant or discrete-trials tasks, it only has access to the bipartite codes of time–event and event–place. Therefore, the rat would be unable to solve the task unless a distinguishing feature separated the event codes (e.g., different amounts of food in morning and afternoon sessions). However, once the rat is in the reinforced area, it can access the time–place–event code and respond appropriately. If the behavior was scored as incorrect prior to entry into that location (as in discrete-trials tasks), the rat would appear to have not learned the task. However, if the behavior was scored after entry into the location (as in lever pressing in free-operant tasks), the rat would be assumed to have learned the task.

An unexpected finding in the present study was that, although there was a difference in responding between the FI-5-s and FI-30-s schedules when all of the trials in a session were included, the distributions of mean responding did not have peaks at the appropriate times. This was particularly true for FI-30-s sessions, in which the rate of responding plateaued for the majority of the 30-s interval. This was unexpected, given that the rats were able to use working memory to dictate performance after the first trial in each session. Rats can change their responding patterns, depending on which of two different stimuli is presented in a peak-time procedure. For example, S. Roberts (1981) observed average peaks in responding at the appropriate times when FI-20-s and FI-40-s schedules were signaled by either a tone or a light, respectively.

One possible explanation for the failure to see peaks at the expected times is that the current task was more difficult, because each session only contained one trial type and these sessions were separated by 6 h. This may have made it more difficult for the rats to discriminate the two different trial types. In the S. Roberts (1981) study, the trials were interleaved, so the rats received equal numbers of trials with each stimulus per session. Therefore, it was easier to compare the two durations, because only a very small amount of time

elapsed between the presentations of these schedules. Because the stimulus was time of day in the present study, only one FI schedule was used for the entire session, and the rats were not exposed to the second FI schedule for another 6 h. Additionally, different times are likely much harder cues for a rat to differentiate than discrete cues, such as a tone and a light. However, pigeons, in addition to acquiring peak procedures with interleaved trials (e.g., Cheng & Roberts, 1991; W. A. Roberts, Cheng, & Cohen, 1989), were also able to acquire a time-of-day version of the task in which only one schedule was presented per session, depending on the time of day (Budzynski & Bingman, 1999).

This experiment has demonstrated yet another difference between pigeons and rats with respect to timing. Although Budzynski and Bingman (1999) showed that pigeons learned the time-of-day discrimination when the spatial location was held constant, the present experiment demonstrated that rats did not learn a similar discrimination. Although when comparing results across species, variables such as the number of trials, motivation, and the details of the apparatuses need to be considered, it is possible that the impairment in the present task was a result of rats' unreadiness to use time of day as a discriminative stimulus. These findings parallel other research showing that, whereas pigeons readily acquire daily TP learning tasks, rats sometimes do not (Thorpe & Wilkie, 2006).

Importantly, the results of this study suggest that rats' difficulty using time of day as a discriminative stimulus is due to learning, rather than performance, issues. With the exception of an additional place in Phase II, Phases I and III were identical to Phase II. Therefore, it seems unlikely that some procedural detail would affect performance only when time of day was used as the discriminative stimulus.

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## References

- Aragona, B. J., Curtis, J. T., Davidson, A. J., Wang, Z., & Stephan, F. K. (2002). Behavioral and neurochemical investigation of circadian time–place learning in the rat. *Journal of Biological Rhythms*, *17*, 330–344.
- Biebach, H., Gordijn, M., & Krebs, J. (1989). Time–place learning by garden warblers, *Sylvia borin*. *Animal Behaviour*, *37*, 353–360.
- Boulos, Z., & Logothetis, D. E. (1990). Rats anticipate and discriminate between two daily feeding times. *Physiological Behavior*, *48*, 523–529.
- Budzynski, C. A., & Bingman, V. P. (1999). Time-of-day discriminative learning in homing pigeons, *Columbia livia*. *Animal Learning & Behavior*, *27*, 295–302.

- Carr, J. A. R., & Wilkie, D. M. (1997). Rats use an ordinal timer in a daily time–place learning task. *Journal of Experimental Psychology: Animal Behavior Processes*, *23*, 232–247. doi:10.1037/0097-7403.23.2.232
- Carr, J. A. R., & Wilkie, D. M. (1999). Rats are reluctant to use circadian timing in a daily time–place task. *Behavioural Processes*, *44*, 287–299.
- Cheng, K., & Roberts, W. A. (1991). Three psychophysical principles of timing in pigeons. *Learning & Motivation*, *22*, 112–128.
- Deibel, S. H., & Thorpe, C. M. (2013). The effects of response cost and species-typical behaviors on a daily time–place learning task. *Learning & Behavior*, *41*, 42–53. doi:10.3758/s13420-012-0076-4
- Gallistel, C. R. (1990). *The organization of learning*. Cambridge, MA: MIT Press.
- Lukoyanov, N. V., Pereira, P. A., Mesquita, R. M., & Andrade, J. P. (2002). Restricted feeding facilitates time–place learning in adult rats. *Behavioural Brain Research*, *134*, 283–290.
- Means, L. W., Arolfo, M. P., Ginn, S. R., Pence, J. D., & Watson, N. P. (2000a). Rats more readily acquire a time-of-day go no-go discrimination than a time of day discrimination. *Behavioural Processes*, *52*, 11–20.
- Means, L. W., Ginn, S. R., Arolfo, M. P., & Pence, J. D. (2000b). Breakfast in the nook and dinner in the dining room: Time of day discrimination in rats. *Behavioural Processes*, *49*, 21–33.
- Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., & Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research*, *739*, 12–18.
- Pizzo, M. J., & Crystal, J. D. (2002). Representation of time in time–place learning. *Animal Learning and Behavior*, *30*, 387–393.
- Pizzo, M. J., & Crystal, J. D. (2006). The influence of temporal spacing on time–place discrimination. *Learning & Behavior*, *34*, 131–143.
- Roberts, S. (1981). Isolation of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes*, *7*, 242–268.
- Roberts, W. A., Cheng, K., & Cohen, J. S. (1989). Timing light and tone signals in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, *15*, 23–35.
- Saksida, L. M., & Wilkie, D. M. (1994). Time-of-day discrimination by pigeons, *Columba livia*. *Animal Learning & Behavior*, *22*, 143–154.
- Thorpe, C. M., Bates, M. E., & Wilkie, D. M. (2003). Rats have trouble associating all three parts of the time–place–event memory code. *Behavioural Processes*, *63*, 95–110.
- Thorpe, C. M., & Wilkie, D. M. (2006). Properties of time–place learning. In T. R. Zentall & E. A. Wasserman (Eds.), *Comparative cognition: Experimental explorations of animal intelligence* (pp. 229–245). Oxford, UK: Oxford University Press.
- Thorpe, C. M., & Wilkie, D. M. (2007). Rats acquire a low response-cost daily time place task with differential amounts of food. *Learning & Behavior*, *35*, 71–78.
- Van der Zee, E. A., Havekes, R., Barf, R. P., Hut, R. A., Nijholt, I. M., Jacobs, E. H., & Gerkema, M. P. (2008). Circadian time–place learning in mice depends on *Cry* genes. *Current Biology*, *18*, 844–848.
- Widman, D. R., Gordon, D., & Timberlake, W. (2000). Response cost and time–place discrimination by rats in maze tasks. *Animal Learning & Behavior*, *28*, 298–309.
- Widman, D. R., Sermina, C. M., & Genismore, K. E. (2004). Evidence for time–place learning in the Morris water maze without food restriction but with increased response cost. *Behavioural Processes*, *67*, 183–193.
- Wilkie, D. M. (1995). Time-place learning. *Current Directions in Psychological Science*, *4*, 85–89.
- Zhou, W., & Crystal, J. D. (2009). Evidence for remembering when events occurred in a rodent model of episodic memory. *Proceedings of the National Academy of Sciences*, *106*, 9525–9529.
- Zhou, W., & Crystal, J. D. (2011). Validation of a rodent model of episodic memory. *Animal Cognition*, *14*, 325–340.