Rats Distinguish Between Absence of Events and Lack of Information in Sensory Preconditioning

Aaron P. Blaisdell, Kenneth J. Leising, and W. David Stahlman
University of California, Los Angeles, U.S.A.

Michael R. Waldmann
University of Göttingen, Germany

In two experiments, rats received sensory preconditioning treatment in which an auditory conditioned stimulus (CS) X was followed by visual CS A in Phase 1, and CS A was followed by an appetitive US (sucrose) in Phase 2. Rats also received presentations of auditory CS Y unpaired with other events. At test, rats looked for sucrose more following CS X than following CS Y on non-reinforced probe test trials only if the light bulb on which CS A had been presented during training was removed from the chamber at the time of testing. With the light bulb present (but unlit), rats showed no difference in amount of nose poking between CS X and CS Y. These results suggest that rats distinguish between the explicit absence of events and lack of information.

I recharge my cell phone battery each night on a recharging unit on the nightstand next to my bed. The indicator light that signals a charge in progress can be surprisingly bright in the middle of the night, and so I place a small book overtop so I can sleep better. Although I can no longer see the indicator light, I know that when I wake up the next morning, my phone battery will be completely recharged. Last summer, I installed a drip irrigation system to efficiently water my landscaping. Whenever I turned the system on, I saw tiny streams of water flow slowly from the emitters directly to the base of each plant. Recently I added a thick layer of mulch to the landscaping, thereby covering the emitters. Although I can no longer see the water issue forth from the emitters when I turn the system on, I know that the plants are still receiving their water. These two examples illustrate the effortless ability we have to represent events that exist in the absence of sensory feedback. The indicator light can operate and the emitters can function while being obscured from view. And I can safely (for the most part) assume their continued operation when the systems are engaged.

The attribution of the physical presence of events that are hidden from view or obscured from the other senses (hearing, smell, etc.) is called object permanence (Piaget, 1937/1954). Objects and event states should continue to exist even after they disappear from view, such as when I covered my cell phone with a book. Object permanence develops early in childhood, emerging by at least 3.5 months of age (Baillargeon & DeVos, 1991). Other species have also shown evidence of expecting an object to exist after it has become obscured, including primates (Call, 2007; Hauser, 2001; Santos & Hauser, 2002), cats and dogs (Dore, Fiset, Goulet, Dumas, & Gagnon, 1996), and even the domestic chick (Regolin, Vallortigara, & Zanforlin, 1995; Vallortigara, Regolin, Rigoni, & Zanforlin, 1998). Many of these species also show evidence for a related ability to represent the
visible (Collier-Baker, Davis, & Suddendorf, 2004; de Blois, Novak, & Bond, 1998; Dore et al., 1996) and invisible displacement of objects (Call, 2001; de Blois et al., 1998; Mendes & Huber, 2004; Pepperberg, Willner, & Gravitz, 1997; Schino, Spinozzi, & Berlinguer, 1990).

For the past few years, our lab has been investigating causal cognition in rats (Blaisdell, Sawa, Leising, & Waldmann, 2006; Leising, Wong, Waldmann, & Blaisdell, 2008). An early surprise that emerged during our investigation was the finding that rats seem to distinguish between observing an event and intervening on that same event. For example, rats received a sensory preconditioning treatment (Brogden, 1939; Rescorla, 1980) in which they first observed a visual conditioned stimulus (CS) A followed by an auditory CS X in Phase 1, and then observed CS A followed by an appetitive US (sucrose) in Phase 2 (Blaisdell et al., 2006 Experiment 2). After both phases of sensory preconditioning treatment, rats received non-reinforced probe trials of CS X alone. In this test condition rats showed an expectation of the sucrose US during CS X as evidenced by the large amount of feeder activity (nose poking into the feeder where sucrose had been delivered in Phase 2). This indicated that the rats were acting as if they reasoned backward from CS X to CS A, and then forward from CS A to sucrose. Another group of rats were given the opportunity to press a novel lever that had never been available to the rats prior to the test session. Presses on the novel lever resulted in the presentation of CS X. That is, the rats were provided with the opportunity to intervene on CS X. In contrast to the performance of rats in the observation test condition described above, the rats in this intervention test condition nose poked very little during CS X. These results were interpreted as supporting causal model theory, in which an intervention (e.g., a lever press) on an effect leads to discounting of prior causes of that effect (e.g., CS A) (see Waldmann & Hagmayer, 2005; Waldmann, Hagmayer, & Blaisdell, 2006; Waldmann, Cheng, Hagmayer, & Blaisdell, 2008; see Blaisdell, 2008 for a discussion of the role of cognitive processes of operant learning in causal inferences).

We also conducted experiments in which we intended to establish a causal chain. Surprisingly, in our initial pilot studies we failed to find evidence that rats reasoned in a forward direction along a causal chain. During causal-chain training, a group of rats first observed CS X followed by CS A in Phase 1, and CS A followed by sucrose US in Phase 2. When the rats then observed CS X on subsequent non-reinforced probe test trials they did not show an expectation of sucrose (i.e., little nose poking). This was particularly surprising especially in light of the large body of literature showing second-order excitatory conditioning using similar procedures (e.g., Pavlov, 1927; Rescorla, 1980; Yin, Barnet, & Miller, 1994). Why did the rats in the causal-chain condition fail to anticipate sucrose in the presence of second-order CS X? Keith Holyoak (personal communication) suggested a possible explanation. If the rats had learned the X-A and A-sucrose associations, then when CS X (a tone) was presented at test they should have expected CS A to immediately follow. We did not present CS A (a light) during these test trials and so the rats may have attended to the explicit absence of the light. If the absence of the light violated their expectations, then they may have also abandoned their expectation of sucrose. It is interesting to note that rats that
had been trained on a common-cause model in which A preceded X and Food during training, thereby establishing A as a common cause of both events (X↔A→Food), showed strong excitatory responding to X at test despite the fact that CS A’s light bulb was present but not illuminated (Blaisdell et al., 2006). We speculated that there is a differential sensitivity to remembered versus perceived absence of CS A with the perceived absence being more salient.

To test this account of “breaking-the-causal-chain”, he proposed that we hide at test the light bulb from which CS A had been presented during training. With the light hidden, the rat should still expect CS A to follow CS X at test, but will not be able to see it because the source of CS A, the bulb, is hidden. If rats can reason that they expect to not see CS A because the light bulb is hidden, they may still believe that CS A has occurred following CS X and thus the causal chain would not be broken. With an intact causal chain of events (X→A→sucrose) rats should expect sucrose and thus we should observe high rates of nose poking. We are not aware that rats have been shown capable of this degree of cognitive sophistication, which motivated the following experiments.

**Experiment 1**

All subjects received sensory preconditioning treatment consisting of pairings of an auditory CS X with a visual CS A in Phase 1 followed by pairings of CS A with sucrose US in Phase 2 (see experimental design in Table 1). In addition to the Phase 1 X-A pairings, subjects also received unsignaled presentations of another auditory CS Y to serve as an unpaired CS against which to measure the excitatory nature of CS X. After Phase 2, subjects received non-reinforced test probes of CS X or CS Y. Orthogonal to this test treatment, for half of the subjects tested on each test cue the light bulb on which CS A had been presented during training was removed from the conditioning chamber and replaced with a blank aluminum plate. The light bulb was not removed for the remaining subjects in each test stimulus condition. Thus, subjects were tested either on CS X with the light bulb present (Group Paired-Present) or absent (Group Paired-Absent) or on CS Y with the light bulb present (Group Unpaired-Present) or absent (Group Unpaired-Absent). If the rats in our unpublished experiment described above did not nose poke during CS X at test because CS A was visibly absent, then removing the light bulb at test should increase uncertainty about the current state of this light, and hence result in an increase in nose pokes on test trials with CS X. Nose poking to CS X at test with the light bulb removed is expected to be higher than nose poking to CS X when the light bulb is present (and off) at test, as well as higher to nose poking to CS Y regardless of whether the light bulb is present or absent.
Table 1
Design of Experiments 1 & 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Test</th>
<th>Light at test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paired-Present</td>
<td>X→A / Y</td>
<td>A→ Sucrose</td>
<td>X</td>
<td>Present</td>
</tr>
<tr>
<td>Unpaired-Present</td>
<td>X→A / Y</td>
<td>A→ Sucrose</td>
<td>Y</td>
<td>Present</td>
</tr>
<tr>
<td>Paired-Absent</td>
<td>X→A / Y</td>
<td>A→ Sucrose</td>
<td>X</td>
<td>Absent</td>
</tr>
<tr>
<td>Unpaired-Absent</td>
<td>X→A / Y</td>
<td>A→ Sucrose</td>
<td>Y</td>
<td>Absent</td>
</tr>
</tbody>
</table>

A = Flashing light, X and Y = Tone and Noise, counterbalanced within group. ‘→’ indicates that the second event followed the first, trials on either side of the slash ‘/’ were distributed in an intermixed fashion within each session. Prior to testing in Groups Paired-Absent and Unpaired-Absent, the light bulb on which Stimulus A had been presented during Phases 1 and 2 of treatment was covered during test sessions. The bulbs remained present during testing for subjects in Groups Paired-Present and Paired-Absent.

Method

Subjects

Thirty-two experimentally-naïve female Long-Evans rats (Rattus norvegicus) obtained from Harlan Laboratories (Indianapolis, IN) served as subjects. Subjects were pair-housed in translucent plastic tubs with a wood shaving substrate in a vivarium maintained on a 12-hr dark/12-hr light cycle. All experimental manipulations were conducted during the dark portion of the cycle. A progressive food restriction schedule was imposed over the week prior to the beginning of the experiment, until each rat received 15 grams of food each day. All animals were handled daily for 30-s during the week prior to the initiation of the study. Subjects were randomly assigned to one of four groups (n = 8): Paired-Present, Unpaired-Present, Paired-Absent, Unpaired-Absent.

Apparatus

Each of the eight experimental chambers, measuring 30 x 25 x 20 cm (l x w x h) was housed in separate sound- and light-attenuating environmental isolation chest (Med Associates, Georgia, VT). The side walls and ceiling of the chamber were constructed of clear Plexiglas. The front and rear walls were constructed of aluminum panels. The floor was constructed of stainless-steel rods measuring 0.5 cm in diameter, spaced 1.5 cm center-to-center. The enclosure was dimly illuminated by a 28-V, 100 mA shielded incandescent house light mounted on the top of the rear wall of the conditioning chamber, 2 cm below the ceiling. All experimental procedures were conducted with the house light on, except where otherwise noted. A diffuse light was located 13 cm above the floor, 1-cm below the house light. One stimulus consisted of the flashing (.25 s on / .25 s off) of the diffuse light. The house light was turned off during the duration of the flashing light presentation. Two speakers on the outside walls of the chamber could deliver a high-frequency tone (3000 Hz) 8 dB(A-Scale) above background and a white noise stimulus 8 dB above background. The tone and noise were counterbalanced within groups in their roles as CS X and CS Y. The flashing light served as CS A. Ventilation fans in each enclosure and a white noise generator on a shelf outside of the enclosures provided a constant 74-dB background noise. Each chamber was equipped with a dipper that could deliver sucrose solution (20%). When in the raised position, a small well (0.05 cc) at the end of the dipper arm protruded into the feeding niche. An infrared photo-detector was positioned across the entrance to the feeding niche. When a rat placed its nose into the feeding niche to lick the sucrose solution, the photo beam was disrupted. The computer measured the disruption of the photo beam which we refer to as a nose poke.
**Procedure**

*Magazine training.* On Day 1, sucrose was delivered for 10-s every 20 ± 15 s in a 60-min session to train rats to approach and eat the sucrose from the dipper. Actual intertrial intervals (ITI) were evenly drawn from the following distribution: 5, 10, 15, 20, 25, 30, and 35 seconds.

**Phase 1: Sensory Preconditioning.** On Days 2-5, all subjects received six daily X → A trials randomly interspersed among six daily Y- trials. On X → A trials, CS X was presented for 10-s followed by the presentation of CS A for 10-s, with the termination of X coincident with the onset of A. On Y- trials, CS Y was presented for 10-s. Trials were delivered with a discrete uniform distribution from 3-7 min in steps of 1 min during each 60-min session.

**Phase 2: Acquisition.** On Days 6 and 7, all subjects received 12 A → sucrose trials on which CS A was presented for 10-s followed by the presentation of sucrose for 10-s. Trials were delivered with a discrete uniform distribution from 2-6 min in steps of 1 min during each 65-min session. To measure acquisition of the A → sucrose association, we recorded the amount of time the rat spent inspecting the feeder (nose poking) during a 30-s Pre-CS period prior to the onset of CS A, and during the 10-s during which CS A was present. These measures allowed us to compute a discrimination ratio which was calculated as the number of nose pokes into the feeder during CS A divided by the sum of nose poking during CS A and during the Pre-CS period (divided by 3 to produce an equal interval of baseline responding) prior to the onset of CS A. A discrimination ratio above 0.5 indicates that the rats expected sucrose more during CS A than in the baseline interval prior to the onset of A.

**Test.** Subjects were tested on CS X (Groups Paired-Present and Paired-Absent) or CS Y (Groups Unpaired-Present and Unpaired-Absent) in the following manner. Prior to testing for subjects in Groups Paired-Absent and Unpaired-Absent, the light bulb on which CS A had been presented during Phases 1 and 2 of training was removed from the conditioning chamber and replaced with a blank aluminum plate. The light bulb was not removed for subjects in Groups Paired-Present and Unpaired-Present. Rather, the light bulb was present but off in the Present conditions. No sucrose was delivered during these sessions. On the first two test days (Days 8 & 9), we recorded all nose pokes during the 30-s Pre-CS period and during the 10-s presentation of the CS. After analyzing these data it became apparent that we needed to extend the data recording period on each trial for 20 s beyond the termination of the test CS. Thus, on Days 10-12, in addition to the above measures, we also recorded all nose pokes during a 10-s period immediately following the termination of the CS (designated as Post 1) and the 10-s period immediately following the end of the Post 1 interval (designated as Post 2). If rats in Groups Paired-Present and Paired-Absent had encoded the temporal interval between X and A in Phase 1 and between A and sucrose in Phase 2, then they might expect sucrose to occur during interval Post 2 (see Leising, Sawa, & Blaisdell, 2007). Following the procedure used by Leising et al. (2008), prior to graphing and data analysis, the Pre-CS score was divided by three to facilitate comparison of baseline rates of nose poking to the other intervals. This was done because the Pre-CS interval was 30-s in duration and the other three test intervals were only 10-s in duration.

**Results and Discussion**

**Phase 2: Acquisition.** Prior to the factorial analysis, an outlier analysis was conducted to remove from the experiment subjects with a discrimination ratio two standard deviations from their respective group means pooled across the last six trials on the final day of acquisition. Data from two subjects (one from Group Present-Paired and one from Group Absent-Paired) below their group means were removed [because low discrimination scores (undefined and 0.22, respectively) indicate failure to learn the A → sucrose relationship]. All groups acquired the discrimination with the following mean discrimination ratios (± SEM): Group Paired-Present (Mean = 0.69 ± 0.02), Group Unpaired-Present (Mean = 0.78 ± 0.04), Group Paired-Absent (Mean = 0.77 ± 0.01), Group Unpaired-Absent (Mean
= 0.78 ± 0.03). A two-way analysis of variance (ANOVA) conducted on discrimination ratios from the last six trials of the second day of acquisition with Light (Present or Absent) and Cue (Paired or Unpaired) as factors revealed no main effects nor interaction, $F_s(1, 26) < 1$.

**Testing.** No group differences were found for discrimination ratios pooled across the first two days of testing (Days 8 & 9): Group Paired-Present (Mean = 0.82 ± 0.05), Group Unpaired-Present (Mean = 0.71 ± 0.10), Group Paired-Absent (Mean = 0.77 ± 0.02), Group Unpaired-Absent (Mean = 0.82 ± 0.02). This observation was confirmed by a two-way ANOVA conducted on discrimination ratios from test trials pooled across the first two days of testing with Light (Present or Absent) and Cue (Paired or Unpaired) as factors revealed no main effects nor interaction, all $F_s < 1.48$, $p_s > 0.23$.

We next analyzed data from Days 10-12 (the last three days of testing) for which we recorded an expanded range of intervals during each test trial. Figure 1 shows the data pooled across Days 10-12 separately for the Light Present conditions (top panel) and Light Absent conditions (bottom panel). No clear differences were observed between tests on the CS X (Paired) or CS Y (Unpaired) with the light bulb present during testing, while a difference started to emerge between X and Y during the last interval (Post 2) with the light bulb absent during testing. A two-way ANOVA conducted on mean discrimination ratios with CS (X or Y) and Light (Present or Absent) as factors revealed only a main effect of interval, $F(3, 78) = 33.41, p < 0.001$. Although no differences were found across conditions, inspection of the right panel of Figure 1 reveals a hint of a difference between X and Y when the light bulb was absent from testing. Appreciable extinction may have accrued during the first two days of testing. It is possible a significant difference may have been observed if we had recorded nose pokes prior to the third test session.

**Figure 1.** Experiment 1: Mean nose pokes during test trials for each of four intervals: Pre-CS = a 30-s period prior to the onset of the CS (score divided by 3), CS = the 10-s period during which the CS was presented, Post 1 = the 10-s interval following the termination of the CS, and Post 2 = the 10-s interval following the termination of interval Post 1. Nose poke responses at each interval shown separately for second-order CS X and unpaired CS Y for with the light bulb present (left panel) and absent (right panel). Error bars represent standard errors of the mean.
Experiment 2

Experiment 2 served as an exact replication of the design and procedure of Experiment 1 except that we recorded nose poke responses during the Pre-CS period, CS, Post 1 interval, and Post 2 interval beginning on the first test day. Because rats are expected to acquire both X-A and A-sucrose intervals and integrate these intervals to form an \( \text{X} \rightarrow \text{A} \rightarrow \text{sucrose} \) temporal map (Leising et al., 2007), differences in mean nose pokes between CS X and CS Y should be maximal during the time when the rats expected food in the presence of X (but not Y). Thus, we focused our analysis on the Post 2 interval which is when the rats tested on X should expect food. To compare across groups, we calculated a discrimination ratio for each subject based on Pre-CS responses compared to responses during Post 2 interval.

Method

Subjects and Apparatus

Thirty-two rats of the same type and maintained as in Experiment 1 were randomly assigned to one of four groups, Paired-Present, Unpaired-Present, Paired-Absent, and Unpaired-Absent, \( n = 8 \) per group. The apparatus was the same as that used in Experiment 1.

Procedure

Magazine training, Phase 1, and Phase 2. On Days 1-8, the procedures used were identical to those described for Experiment 1 except that one additional Phase 2 session was conducted because discrimination scores were relatively low at the end of the second Phase 2 acquisition session. Thus all subjects received three Phase 2 acquisition sessions (Days 6-8) with 12 A\( \rightarrow \text{sucrose} \) pairings presented in each daily session in the same manner as in Experiment 1.

Test. On Days 9 and 10, rats received test treatment as in Days 10-12 of Experiment 1. On both test days, we recorded all nose pokes during the 30-s Pre-CS period, during the 10-s CS, during the 10-s period immediately following the termination of the CS (called Post 1), and the 10-s period immediately following the end of interval Post 1 (called Post 2). To assess the excitatory nature of the response during Post 2, we calculated a discrimination ratio by dividing the number of responses during Post 2 by the sum of responses during Post 2 and during the Pre-CS interval. Prior to calculating the discrimination ratio, the Pre-CS score was divided by three to facilitate the comparisons among equal intervals because the Pre-CS interval was 30-s in duration and the Post 2 interval was only 10-s in duration.

Results and Discussion

Phase 2: Acquisition. Prior to the factorial analysis, an outlier analysis was conducted to remove from the experiment subjects with a discrimination ratio two standard deviations from their respective group means on the final day of acquisition. No subjects were removed. All groups acquired the discrimination with the following mean discrimination ratios (± SEM): Group Paired-Present (Mean = 0.65 ± 0.05), Group Unpaired-Present (Mean = 0.66 ± 0.04), Group Paired-Absent (Mean = 0.57 ± 0.04), Group Unpaired-Absent (Mean = 0.63 ± 0.04). A two-way analysis of variance (ANOVA) conducted on discrimination ratios from the last six trials of the third day of acquisition with Light (Present or
Absent) and CS (X or Y) as factors revealed no main effects nor interaction, $F_{s(1, 28)} < 1.70, p > 0.20$.

**Testing.** Figure 2 shows the data pooled across Days 9-10. In the Light Absent condition (right panel) responding to X was higher during the CS and Post CS intervals than during the Pre-CS interval indicating an expectation of food elicited by the CS. Response was also higher during and following the onset of CS X than the onset of CS Y, establishing the necessity of the X-sucrose pairings for the higher rate of responding to X. These differences were not observed in the light present conditions (left panel). Although compelling, these observations were not confirmed by the three-way ANOVA which only found a main effect of interval, $F(3, 84) = 6.32, p < 0.001$. Neither other main effects nor interactions achieved significance. Inspection of Figure 2 indicates that the lack of interactions among factors was likely due to both the large standard errors and the shifting levels of performance within subject (especially the fluctuating levels of performance among the X and Y tests for the Light Present condition). The large standard errors are particularly detrimental with the low power resulting from having only eight subjects per condition. Nevertheless, the results of Experiment 1 suggested that the presence or absence of the light at test affected performance most during interval Post 2, which is when the food should be expected to occur following the presentation of the tone at test. Thus, we conducted a separate analysis of the data from Post 2 in comparison to baseline responding prior to the onset of the test trial. We calculated a discrimination ratio for each subject by dividing the number of nose pokes during Post 2 by the sum of all nose pokes from Post 2 and the Pre-CS period. Prior to this calculation, Pre-CS scores were divided by 3 to equate the recording intervals. For example, if a subject made a mean of 12 nose pokes during the 30-s Pre-CS interval across trials and a mean of 28 nose pokes during the 10-s Post 2 interval across trials, then the discrimination ratio for this subject would be $= 28/(12 + 28) = 0.70$.

![Figure 2](image-url)

**Figure 2.** Experiment 2: Mean nose pokes during test trials for each of four intervals: Pre-CS = a 30-s period prior to the onset of the CS (score divided by 3), CS = the 10-s period during which the CS was presented, Post 1 = the 10-s interval following the termination of the CS, and Post 2 = the 10-s interval following the termination of interval Post 1. Nose poke responses at each interval shown separately for second-order CS X and unpaired CS Y for with the light bulb present (left panel) and absent (right panel). Error bars represent standard errors of the mean.
Figure 3 shows the discrimination ratios for interval Post 2 (relative to the Pre-CS baseline) pooled across Days 9 and 10. With the light bulb present at test, discrimination ratios in Groups Paired-Present (X) and Unpaired-Present (Y) did not differ from each other and were both around a value of 0.5 which indicates no change from baseline response levels. When the light bulb was removed at test, however, a large difference emerged between responding to X (Group Paired-Absent) and to Y (Group Unpaired-Absent). In Group Paired-Absent (X) discrimination ratios were well above baseline, but were relatively below baseline in Group Unpaired-Absent (Y). These qualitative conclusions were supported by statistical analyses. A two-way ANOVA conducted on mean discrimination ratios with CS (X or Y) and Light (Present or Absent) as factors revealed only a CS X Light interaction, \( F(1, 28) = 4.92, p < 0.05 \). Planned comparisons using the error term from the two-way ANOVA were conducted to isolate the source of the interaction. Discrimination ratios for Group Paired-Present did not differ from those of Group Unpaired-Present, \( F(1, 28) < 1 \). Discrimination ratios for Group Paired-Absent, however, were much higher than for Group Unpaired-Absent, \( F(1, 28) = 6.81, p < .02 \). Paired-Absent showed marginally higher discrimination ratios than Group Paired-Present, \( F(1, 28) = 4.02, p = 0.055 \). Unpaired-Absent and Unpaired-Present did not differ, \( F(1, 28) = 1.28, p > 0.25 \).

![Figure 3. Experiment 2: Mean discrimination ratios for nose poke responses during test trials with second-order CS X and unpaired CS Y. Error bars represent standard errors of the mean.](image-url)
Single-sample t-tests were conducted to compare each group to 0.5, which would indicate no difference in responding during Post interval 2 relative to the Pre-CS baseline. Only Group Paired-Absent was reliably above 0.5, \( t(7) = 4.33, p < 0.005 \). The other three groups did not differ significantly from 0.5, \( ts(7) < 1.1, ps > 0.30 \). Thus, rats showed evidence of expecting sucrose following the presentation of CS X only when the light bulb was absent during testing.

**General Discussion**

Rats received CS X→CS A pairings and presentations of CS Y alone in Phase 1 followed by CS A→sucrose pairings in Phase 2. Rats were subsequently tested on the second-order CS X or the unpaired CS Y during non-reinforced probe tests. For half of the subjects tested on each CS, the light bulb that had been present during training was removed from the conditioning chamber prior to test, while the light bulb was present for the remaining subjects. In Experiment 1, we found a hint towards greater responding to CS X than to CS Y only when the light bulb was absent at test, although group differences made these patterns inconclusive. One possible reason for the lack of a significant difference may have been that we failed to record behavior until the third day of extinction testing from the intervals after CS X had ended—which correspond to the time when sucrose should have been most expected. Thus, in Experiment 2 we recorded the intervals following the test CSs on the first two days of testing, and found a reliable difference in performance. Second-order conditioning could only be detected in the test phase when the light in the middle of the chain was removed at test as compared to being present but off.

These results raise a number of suggestions. First, they replicate other findings that rats can acquire temporal intervals between paired events and integrate those events based on an associate common to both associations (Leising et al., 2007; see review by Savastano & Miller, 1998). For example, Leising et al. (2007) used an appetitive sensory preconditioning procedure in which rats received pairings of a 60-s tone CS with a 10-s light CS in Phase 1 with the light occurring either early or late during the tone. All rats then received simultaneous presentations of the 10-s light CS and 10-s sucrose US in Phase 2. When subsequently presented with the tone CS on non-reinforced test trials, rats nose poked most at the time at which they would have expected sucrose given the tone-light and light-sucrose intervals experienced during training. Our current results provide evidence consistent with associative temporal integration.

Moreover, we found that the increase in nose poking during CS X relative to a Pre-CS baseline was only apparent when the light bulb had been removed prior to test. Rats tested on CS X with the light bulb present (but not turned on) did not show excitatory nose poking (compared either to the Pre-CS baseline or to responding to unpaired CS Y). The implication is that rats had encoded X-A and A-sucrose associations, and that the presentation of CS X at test elicited the expectation that CS A would follow. If the rats then looked at the light bulb on which CS A had been presented during training, they would have observed the explicit absence of CS A. This violation of their expectation that CS A should have
occurred may thus have decreased their expectation of sucrose as well. If the bulb had been removed prior to test, however, then although the rats would have expected the bulb to turn on, there was no bulb present and so their expectations may not have been violated thus preserving the expectation of sucrose. [Note, however, that this line of reasoning assumes that the rats did notice that the light bulb was absent during testing. The difference in responding to CS X depending on the presence or absence of the light bulb supports this assumption.]

It is possible that removal of the light bulb at test created a different context from the training context. To the degree that both excitatory and inhibitory properties are acquired during a sensory-preconditioning procedure (McLaren & Mackintosh, 2000; Stout, Escobar, & Miller, 2004; Wagner, 1981), a context switch at test may allow the excitatory properties to generalize more effectively than the inhibitory properties. Although this analysis seems logical on the surface, a number of reasons can be marshaled against it. First, we used training parameters optimized to produce excitatory rather than inhibitory second-order conditioning (Yin et al., 1994). Thus, the inhibitory properties accrued to CS X should be minimal. Second, removal of a single element such as the light is insufficient support for contextual discrimination by rats (Bucci, Saddoris, & Burwell, 2002; Michael Fanselow, personal communication). Furthermore, both instrumental discriminations (e.g., Hearst & Wolff, 1989) and relational concept learning (e.g., Blaisdell & Cook, 2005b) are difficult when the absence or removal of a feature defines the target response. Still, these arguments don’t entirely rule out the potential role of context shifts and therefore we are directing future experiments to address this issue.

Although our explanation of these results may seem quite sophisticated for a rat (and many other vertebrates), upon closer consideration, our analysis may not be as large a leap in the rat’s cognitive capacities as one may think. Let us systematically deconstruct the behavior to its necessary component processes. These components include the following abilities: associative integration (e.g., sensory preconditioning and second-order conditioning), encoding and integration of inter-event intervals (i.e., temporal maps), conditioning of orienting responses to visual stimuli, surprise at the omission of anticipated events, and finally the lack of surprise at the omission of events when those events are occluded from view (i.e., hidden). There is evidence in the literature on rat behavior for all but the last component.

**Associative Integration**

Evidence emerged over a century ago for what may be termed higher-order associative conditioning. Pavlov’s (1927) seminal writings contain the first reference to second-order conditioning. Dogs first acquired a conditioned response to a CS (e.g., a light) by pairing it with a food US. After dogs had developed the conditioned response (CR) to the light, he then paired another CS (e.g., a sound) with the light CS. The sound which was called a second-order CS by virtue of the fact that it bore a second-order relationship to the food US elicited the same CR (in this case, salivation) as did the first-order light CS. Brogden (1939) reported a
similar phenomenon he called sensory pre-conditioning in which the order of the two conditioning treatments was reversed. In a sensory preconditioning procedure, subjects first receive pairings of two neutral CSs (e.g., sound-light pairings) and subsequently receive conditioning to the second of the two CSs (e.g., light-food pairings). After both treatments, presentations of the sound (a second-order CS) will elicit a similar CR as did the first-order CS (though often of lesser magnitude). These phenomena have been shown in a wide range of species, including invertebrates (Hussaini, Komischke, Menzel, & Lachnit, 2007), and using a variety of conditioning procedures. Moreover, second-order associations (acquired through either second-order conditioning or sensory preconditioning procedures) have been suggested to play an important role in many phenomena, such as acquired equivalence (Hall, 1996; Ward-Robinson & Hall, 1999), mediated taste aversions (Holland, 1981; 1990), conditioned reinforcement (Williams, 1994), and the integration of spatial (Blaisdell & Cook, 2005a; Sawa, Leising, & Blaisdell, 2005) and temporal (Leising et al., 2007; Savastano & Miller, 1998) maps. Furthermore, higher-order associations also play a central role in some theories of learning and performance such as the extended comparator hypothesis (Blaisdell, Bristol, Gunther, & Miller, 1996; De Houwer & Beckers, 2002; Denniston, Savastano, & Miller, 2001; Stout & Miller, 2007), as well as associative models of retrospective-revaluation (Dickinson & Burke, 1996; Dwyer, Mackintosh, & Boakes, 1998).

The necessary role of higher-order conditioning in our effect can be most easily seen in the work by Holland on mediated conditioning (reviewed in Holland, 1990). Holland has shown, using the conditioned taste aversion procedure, that an association established between two events can allow each event to retrieve a representation of its associate. In a conditioned taste-aversion procedure, consumption of a flavor followed by the onset of gastric malaise can often establish an aversion to the flavor. This type of learning has been demonstrated experimentally in rats by pairing a novel flavor with injection of lithium chloride into the stomach, which results in gastric distress. On a subsequent presentation of the flavor, the rat rejects it—demonstrating the acquired aversion. Holland’s unique contribution was to show that prior pairings of a sound with the flavor allowed the sound itself to positively mediate a conditioned taste aversion to the flavor. In one study (Holland, 1981), rats were allowed to drink flavored water from a lick tube in the presence of a tone. On a subsequent presentation of only the tone the rats were then injected with LiCl. On a final test in which rats had access to the lick tube, rats consumed the same amount of water in the presence as in the absence of the tone. This is entirely consistent with a body of literature showing that audiovisual stimuli do not condition well when gastric distress is used as the aversive US (e.g., Garcia & Koelling, 1966). When Holland presented the rats with lick tubes containing water with the flavor that had been paired with the tone, however, rats showed a conditioned aversion to that flavor. Thus, the rats must have formed flavor-tone and tone-gastric illness associations which together allowed the tone to positively mediate (i.e., transfer) a conditioned taste aversion to the flavor. This demonstration, while highly interesting in its own right, also shows that stimuli that are absent are conditionable, that is, that they can enter into new
associations, so long as a representation of the physically absent event is present during the conditioning episode (see also Dickinson & Burke, 1996; Van Hamme & Wasserman, 1994 for similar accounts of negative mediation).

**Encoding and Integration of Temporal Maps**

There is a significant literature showing that animals from a variety of species can encode regular intervals between events (Shettleworth, 1998). While most of this work has been conducted in operant conditioning procedures, some evidence also comes from Pavlovian conditioning procedures. Honig (1981) was perhaps the first to extend the concept of a spatial map to the temporal domain to account for timing behavior in Pavlovian situations and in working memory. Not only can subjects form temporal maps between paired events, two maps can be integrated into a higher-order map if they both contain a common, linking element. For example, if the subject learns temporal maps \( A \rightarrow B \) and \( B \rightarrow C \), it can join the two maps together into an \( A \rightarrow B \rightarrow C \) map which would allow it to compute the expected time of occurrence of event C given that event A has occurred. We have already described above one such study from our own lab (Leising et al., 2007; see also Savastano & Miller, 1998) and thus will not dwell further on this issue.

**Conditioned Orienting Responses**

Many stimuli used in conventional conditioning procedures initially elicit unconditioned orienting responses. For example, presentation of an auditory stimulus will often cause a rat to jerk its head back and forth, presumably in an attempt to better localize the sound (Holland, 1977). Likewise, Holland observed that presentation of a visual stimulus, such as a light, would often cause the rat to rear on its hind legs and orient towards the light. Moreover, these behaviors which typically habituate with repeated presentations were maintained if these stimuli became established as CSs that reliably signaled the delivery of food. In our own lab we have even observed rats to approach and make physical contact with a light bulb that had been established as a signal for food (sign tracking). If the presentation of a visual CS can elicit an orienting response, then perhaps it is possible for the expectation of the delivery of a visual CS to elicit an anticipatory orienting response to the location from where the CS is expected to occur. Setlow, Gallagher, and Holland (2002) reported that pairings of an auditory CS with a visual CS that previously was paired with a food US not only established goal-tracking (food-cup behavior) but also a rearing orienting response to the second-order auditory CS. We will turn to this issue below.

**Object Permanence**

Object permanence is the ability to represent the continued existence of an object that is temporarily hidden from the senses, typically vision (Piaget, 1937/1954). Although humans and perhaps all primates seem to show the most sophisticated object permanence abilities (Gomez, 2005), most active vertebrates,
such as birds and mammals, at least have the capability of representing the location of an object after it disappears from view—a skill necessary for the tracking of moving prey behind visual occlusion and the retrieving of previously cached food items. Consistent with this, rats accurately return to locations where they had buried food (Bird, Roberts, Abroms, Kit, & Crupi, 2003), and like some birds (Clayton & Dickinson, 1998, 1999) even remember what types of foods were hidden in different places (see also Babb & Crystal, 2005).

**Surprise at Omission of Anticipated Events**

In Tolman’s lab, Tinklepaugh (1928) did an experiment where he placed a piece of banana under one of three boxes while a macaque monkey watched. He then placed an opaque screen in front of the boxes and after a short delay removed the screen to let the monkey look for the piece of banana. On some trials, however, the monkey found a piece of lettuce—a less preferred food item—instead. In these cases the monkeys showed frustration behavior, such as rejecting the piece of lettuce which it would otherwise have accepted (e.g., if the monkey had observed the experimenter hiding lettuce). The monkey’s frustration provided evidence that the monkey had held an expectation of banana after it had been hidden, and compared this expectation to what it actually received after making its choice. It turns out that many mammalian species—including rats—show surprise or frustration at a downgrade in the value of an expected outcome, termed negative anticipatory contrast (or incentive downshift), whereas non-mammalian vertebrates do not (see review by Papini, 2006). Incentive downshift or the altogether omission of an expected outcome can have multiple behavioral effects, such as invigoration of ongoing behavior (Dudley & Papini, 1995; Papini & Dudley, 1997), as well as supporting new learning, such as resistance to extinction of a partially reinforced CS (the partial reinforcement extinction effect or PREE; e.g., Chen & Amsel, 1980). The effects of violations of expectation clearly demonstrate that rats hold expectations of events (e.g., a US or outcome), that these expectations can be elicited by the presentation of associated events (e.g., a CS), and that a violation of the expected event can elicit emotional reactions by the rat.

**Insensitivity to the Omission of Anticipated Events that are Hidden**

We have reviewed above evidence that rats are capable of associative integration, encoding and integration of temporal maps, acquiring conditioned orienting responses, show at least early levels of object permanence, and are surprised by reward omission. It is these abilities that allow the rats in our experiments that had received X-A pairings to expect CS A (a light) to occur following the presentation of CS X (a sound) at test. In Group Paired-Present which is tested in the presence of the light bulb on which CS A had been presented during training, the failure of the light to turn on after CS X had been presented violates the rat’s expectation. Because CS A had been established as a signal for sucrose (in Phase 2 A-sucrose pairings), the omission of the anticipated CS A may have been treated as an omission of sucrose itself. Sucrose omission can cause
frustration effects in rats, and as a consequence should disrupt goal tracking (i.e., nose poking in the feeder for sucrose). To our knowledge, however, our study constitutes the first demonstration that rats are less sensitive to the ambiguous absence of an anticipated event produced by the removal of the physical source of that event than to the explicit absence of the event when the physical source of the event has not been removed or hidden. That is, in Group Paired-Absent for which the light bulb was removed prior to testing, presentation of CS X should have elicited an expectation for CS A to occur on the light bulb. Without the light bulb present, however, the rats continued to goal track (nose poke for sucrose) despite the absence of CS A. The most logical conclusion from this is that although the rats expected CS A to follow CS X, the presence of CS A was made ambiguous by hiding its source (the light bulb). One may even conjecture that by hiding CS A’s light, the rats expected to not be able to observe CS A. The expectation that CS A should not be observable matched the actual outcome of not observing CS A, and thus there should be no violation of expectations. In the absence of a violated expectation, the rats should have continued to hold the expectation that sucrose would be delivered—which is consistent with the excitatory level of nose poking observed at the time following CS X at which sucrose should have occurred. It is this final inference that offers a glimpse at the sophisticated degree of cognitive processing in the rat. This analysis begs a host of questions that we are currently addressing in our laboratory related to the representational capacities of rats for hidden anticipated events and the theoretical mechanisms that account for inferences about hidden events. These studies may shed light on the kind of representation-level mechanisms that best account for the manner in which a given species approximates normative models of causal reasoning (Fodor, 2003; Mitchell, De Houwer, & Lovibond, in press; Shanks, 2007).

For example, one obvious restriction of our studies is that we manipulated access to information about the middle event of the chain (light) at test but not during learning. A well known puzzle in second-order conditioning is that, at least with few trials, rats seem to integrate the separately learned associations between A and X, and X and the US, into a chain although A and the US never co-occur (Yin et al., 1994). Similar to our reasoning for the test phase we could suspect that second-order conditioning of chains should be easier to get when the event that is currently not presented (e.g., sucrose in the A→X trial) is inaccessible rather than explicitly absent. Explicit absence is inconsistent with a chain whereas inaccessibility may be consistent if during learning the presence of the inaccessible event is inferred by the rats. It would be interesting whether removing informational access to events improves learning, and how manipulations of access in learning and testing interact.

References


