The current study served as an extension and replication of Balsam and colleagues (2006). They found that unsignalled unconditioned stimulus (US) presentations outside the conditioned stimulus (CS) did not retard acquisition of a Pavlovian response due to a breakdown of "associative strength", but because they altered the ratio of US rate during the CS and the US rate in the background (C/t ratio). If two experimental procedures arrange for the same ratio value, then acquisition should be the same even if unsignalled US presentations are given. This finding is contrary to traditional associative theories of Pavlovian conditioning and supports timing-based theories. The current experiment arranged three groups to replicate this effect and extend its finding to conditioned reinforcement in a new-response acquisition task. All groups in the present experiment underwent Pavlovian training in which head-entries into the food receptacle were measured. More entries during the CS versus the equivalent pre-CS period indicates acquisition of the Pavlovian response. After Pavlovian training, subjects underwent conditioned reinforcement testing, but that data is not analyzed here. Groups Extra and No Extra were arranged to have the same C/t ratio, but group Extra received unsignalled US presentations. Group Control received the same temporal arrangement of CS presentations as group No Extra, but received unsignalled US presentations. Group Control had a smaller C/t ratio than groups Extra and No Extra. To replicate Balsam and colleagues, group Control should show the worst Pavlovian performance, and groups Extra and No Extra should show similar performance.

Methods

Subjects & Procedure

Subjects were 29 Long-Evans rats assigned to three groups (Group Control, n = 9). Pavlovian training data was collected across 108 trials nested within 12 sessions, with nine trials per session. Additionally, each trial comprised of a CS and equivalent pre-CS period. For some

trials, the pre-CS period was not of the same duration as the CS, and therefore an offset was used to adjust for these discrepancies.

Measures & Variables

In the present experiment, the primary dependent variable of interest was response counts from head-entry into a food magazine. The data were nested within three levels; the rat was the third level, followed by session and trial as the second and first level, respectively. The level three independent variable was Group (i.e., Control, Extra, No Extra), and the level one independent variables were Period (i.e., pre-CS versus CS), and Time, with time referring to the 108 individual trials conducted across 12 evenly spaced sessions. There were no missing data points.

Analysis

All analysis was conducted with R version 4.2.1 (R Core Team, 2022) and generalized linear models were constructed using R package lme4 (Bates et al., 2015). Because the data was in the form of counts, generalized linear mixed modeling (GLMM) was used. Both a poisson and negative binomial model were constructed and then compared in order to determine the best distribution to model the data. Both models were constructed using a theory-driven approach. We hypothesized that group assignment should influence the acquisition of the pavlovian response, as measured by more responding during the CS than the pre-CS period. Therefore, both models were constructed to test a three-way interaction between Group, Time, and Period. Time was centered and divided by 10 in order to assist in model convergence. Because CS and pre-CS durations differed between groups, an offset of period duration in minutes was used in both models. Both full models were then compared using a likelihood ratio test. Fixed effect

significance was tested using Wald tests. Additionally, null models random intercept with a fixed effect of time were fit using both distributions in order to obtain the interclass correlations (ICC).

Results

Descriptive statistics for the first and last trial for each group are displayed in table 1. Figure 1 shows response rates (response count/period time) across pavlovian training. All groups of rats showed an increasing trend for CS responses. Group Control had an increasing trend across trials for the precue responses, while both other groups had decreasing trends.

Poisson GLMM

The random intercept null model indicated an ICC of $\rho = .097$ for session, indicating that 9.7% of the variance in response counts are from session-to-session variance. The ICC for individual rats was $\rho = .122$, indicating 12.2% of the variance in response counts was due to rat-to-rat variance. The full, theory driven model was compared to the null model using a LRT, which confirmed that the full model was superior, $\chi^2(10) = 2350.3$, $p < .001$. Model coefficients and Wald p-values are presented in Table 2. Figure 2 shows estimated marginal means for the model, indicating that all groups showed an increase in responding during the CS, and little to no increase in responding during the pre-CS period. Of primary interest were group differences in Pavlovian responding during the last trial of Pavlovian conditioning. Larger differences between CS and pre-CS responding indicate a greater degree of Pavlovian responding. In order to probe this relationship, interaction contrast statements were preformed to compare differences in responding during the CS versus the pre-CS period between groups. All group contrasts were significant ($p < .05$). The difference between CS and pre-CS responding was greatest for group No Extra, followed by group Control and group Extra.

Negative Binomial GLMM

The random intercept null model indicated an ICC of $\rho = .070$ for session, indicating that 7.0% of the variance in response counts are from session-to-session variance. The ICC for individual rats was $\rho = .104$, indicating 10.4% of the variance in response counts was due to rat-to-rat variance. The full, theory driven model was compared to the null model using a LRT, which confirmed that the full model was superior, $\chi^2(10) = 1831.4$, $p < .001$. Model coefficients and Wald p-values for the Negative Binomial model are also presented in Table 2. As can be seen in Table 2, model coefficients for the Poisson and Negative Binomial model were almost identical, so no further analysis was performed with the Negative Binomial model.

Discussion

These results are highly incongruent with theoretical predictions of both association and timing-based models of Pavlovian learning. By all accounts, the Control group should have performed worse than both the No Extra and the Extra groups. This incongruency with all available theories and with previous research suggests potential methodological or model issues. Residuals for the current model were checked, and no major assumptions were violated. There is reason to believe methodological concerns may have contributed to the results. First, rats in the current experiment were not experimentally naïve, and therefore may have been influenced by previous learning histories. Additionally, rats were not trained to drink from the liquid dipper used to deliver reinforcers. Because of this, groups Control and Extra had more opportunities to learn about reward delivery than group No Extra, which may have contributed to some of the observed pattern of results. Future work should utilize naïve rats and incorporate dipper training, so all rats have equal opportunity to learn about reinforcer delivery before Pavlovian training is commenced.

Table 1

Group Average Head-Entry Response Rates for the First and Last Trial of Pavlovian

Conditioning

|	Control-0	No Extra-0	Extra-0	Control-1	No Extra-1	Extra-1
	n = 18	n = 20	n = 20	n = 18	n = 20	n = 20
count/min						
	2.8 (5.5)	2.2 (5.7)	6.1 (13.4)	3.8 (11.7)	0.6 (2.0)	2.4 (6.3)

Note. Standard deviations are presented in parenthesis.

Table 2

Coefficients and 95% Confidence Intervals for the Poisson and Negative Binomial GLMM

	Poisson	Negative Binomial
(Intercept)	1.68 [1.47; 1.92] *	1.69 [1.48; 1.93] *
(time – 54)/10	1.03 [1.01; 1.05] *	1.03 [1.01; 1.05] *
periodDuring Cue	1.87 [1.78; 1.98] *	1.87 [1.77; 1.98] *
groupNo Extra	0.82 [0.68; 0.99] *	0.82 [0.68; 0.99] *
groupExtra	0.78 [0.65; 0.94] *	0.78 [0.65; 0.94] *
(time – 54)/10:periodDuring Cue	1.08 [1.06; 1.10] *	1.08 [1.06; 1.10] *
(time – 54)/10:groupNo Extra	0.96 [0.93; 0.99] *	0.96 [0.93; 0.99] *
(time – 54)/10:groupExtra	0.97 [0.94; 0.99] *	0.97 [0.94; 0.99] *
periodDuring Cue:groupNo Extra	1.20 [1.11; 1.29] *	1.19 [1.10; 1.29] *
periodDuring Cue:groupExtra	0.80 [0.74; 0.87] *	0.80 [0.74; 0.88] *
(time – 54)/10:periodDuring Cue:groupNo Extra	1.05 [1.03; 1.08] *	1.05 [1.02; 1.08] *
(time – 54)/10:periodDuring Cue:groupExtra	1.00 [0.98; 1.03]	1.00 [0.97; 1.03]

Note. Stars obtained from Wald t-tests.

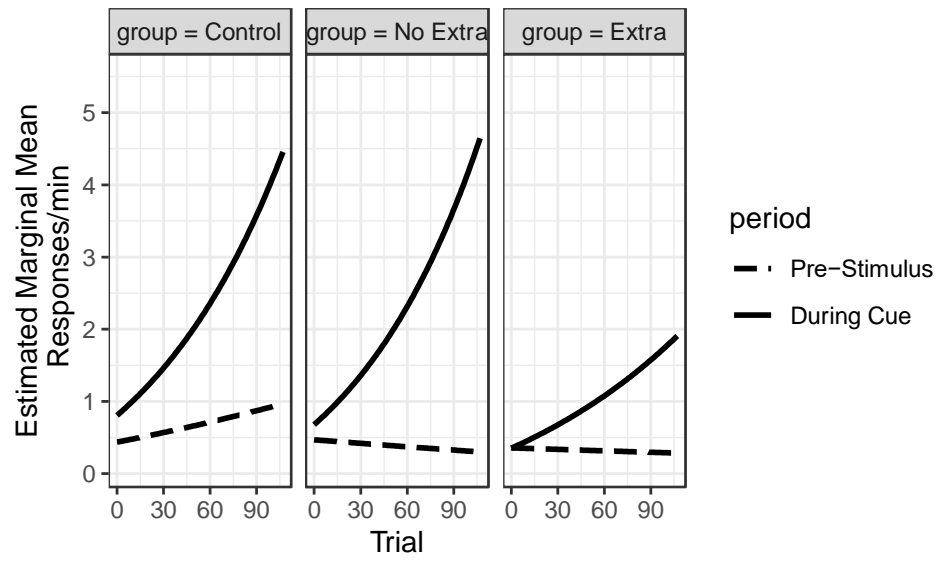* p < .05.

Figure 1

Group Average Head-Entry Response Rates Across Trials
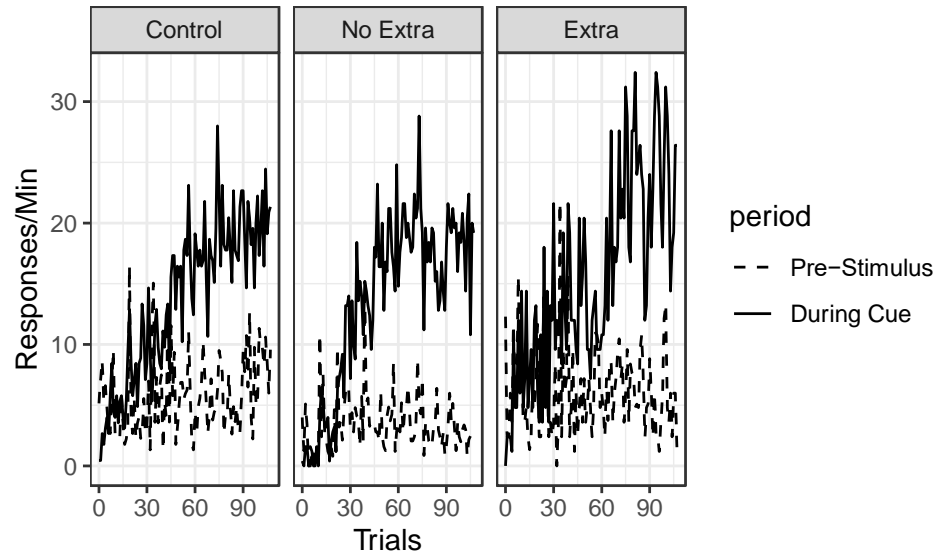
Note. Data paths represent group averages for each trial.

Figure 2

Grouped Estimated Marginal Means for Poisson GLMM

Note. Shaded gray region represents a 95% confidence interval.

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