

# Reward Magnitude, But Not Time of Day, Influences the Trial-Spacing Effect in Autoshaping With Rats

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THOMAS, B., D. HUNEYCUTT AND M. R. PAPINI. *Reward magnitude, but not time of day, influences the trial spacing effect in autoshaping with rats.* *PHYSIOL BEHAV* **65**(3) 423–427, 1998.—The arousal hypothesis of the trial-spacing effect suggests that spaced-trial training increases emotional arousal and thus invigorates Pavlovian behavior, relative to massed-trial conditions. Emotional arousal was manipulated by varying reinforcer magnitude during training (either one or five food pellets/trial, across groups). In addition, autoshaping training was administered either in the morning (0900 h) or in the evening (1700 h). Rats were housed in an enclosed colony room and exposed to a regular light:dark cycle (light from 0700 to 1900 h). Available evidence indicates that reinforcer magnitude and time of day are related to arousal levels. As expected, a larger reinforcer magnitude led to a highly significant trial spacing effect. Evening training led to a higher response rate than morning training, but the trial-spacing effect was equally strong whether training was administered in the morning or in the evening. These results provide partial support for the arousal hypothesis and are discussed in the context of research on schedule-induced behavior. © 1998 Elsevier Science Inc.

Reward magnitude    Trial-spacing effects    Autoshaping    Rats

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THE trial spacing effect (TSE) refers to faster Pavlovian conditioning with relatively long, rather than short, intertrial intervals (ITIs). The TSE challenges the sufficiency of temporal contiguity and, thus, all major conditioning theories offer an explanation of the TSE. These theories can be grouped according to whether they treat the TSE as a failure of acquisition (20,22), or as a failure of performance (10,13,16), but they share the view that the source of the TSE is the poorer acquisition or performance of the group trained under massed conditions. For example, according to some theories (10,20), contextual conditioning interferes with signal learning to a larger extent in the massed group than in the spaced group. The longer ITIs typical of spaced trials are thought to allow for substantial extinction of the context which, in turn, allows for better learning and performance to the signal.

An alternative possibility is that the TSE reflects, at least in part, enhanced performance in the spaced-trial condition. Such a possibility is suggested by experiments on schedule-induced behavior, which closely resemble trial-spacing manipulations except that the reinforcer is typically unsignaled. Schedule-induced behavior seems to reflect increased arousal produced

by the spaced presentation of the scheduled reinforcer, usually food [see review in (17)]. For example, rats exposed to relatively long interreinforcement intervals (IRIs) develop excessive drinking (8) or aggressive responses (4), provided water or a conspecific, respectively, are available. Moreover, the strength of schedule-induced behavior increases with increases in the IRI up to a point, and then decreases (21). Long IRIs that do not support substantial schedule-induced drinking can induce drinking with larger reward magnitudes (19). Physiological variables also suggest a link between schedule-induced behavior and arousal (23). For example, widely spaced food delivery induces increases in plasma levels of corticosterone relative to pre-session levels (5), and the magnitude of polydipsia is reduced by the administration of diazepam (14, 18), a benzodiazepine receptor agonist with anxiolytic effects.

On the basis of these results, it has been suggested that schedule-induced behaviors reflect the arousing effects of spaced reinforcement (5,17). According to this arousal hypothesis, the termination of a meal induces a state of primary frustration when it occurs in the context of a strong food expectancy. This idea is an extension of Amsel's (3) theory, ac-

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ording to which the surprising omission or reduction in reward magnitude causes a primary frustration response. It is assumed here that even a regular meal episode can induce primary frustration when it ends (17). Surprising food omissions can result in the invigoration of ongoing behaviors, including lever pressing in rats (6,7). It is plausible, therefore, that the TSE may reflect, at least in part, the invigorating effects on the conditioned response of a state of primary frustration induced by the termination of a reward in the context of the relatively long ITIs of the spaced-trial condition.

#### EXPERIMENT 1

If the TSE depends on arousal levels, then its size (i.e., the difference between performance of the spaced and massed groups) should be a direct function of arousal level. In the present experiment, arousal levels were manipulated by varying reinforcer magnitude. Reinforcer magnitude has been related to arousal levels in a variety of studies. For example, the finding that food delivery is followed by an increase in general activity directly related to the magnitude of the reinforcer has been interpreted as reflecting underlying changes in reward-induced arousal (12). Moreover, schedule-induced behavior develops more strongly with scheduled reinforcers of large size (9,19,21). Higher levels of polydipsia associated with large food reinforcers are consistent with the hypothesis that arousal levels are modulated by reinforcer magnitude.

In addition, training was administered either in the morning or in the evening, on the assumption that arousal levels are relatively low and high, respectively. Time of day is perhaps even more clearly related to arousal levels. The notion of arousal has historically been derived from the study of circadian rhythms of sleep and wakefulness (15). Rats are known to display circadian rhythms in feeding, drinking, and activity (2,11), although less is known about the relationship between these cycles and learned behavior. To our knowledge, neither reinforcer magnitude nor time of day have been previously studied as variables potentially affecting autoshaping in rats.

According to the arousal hypothesis, a greater TSE was predicted in groups trained with larger reinforcers and during the evening. The strategy was to select training parameters that are known on the basis of prior studies not to promote a significant TSE (16), and to determine if the TSE could be induced by increasing reinforcer magnitude and/or by training during the evening.

#### Method

**Subjects.** The subjects were 64 male 90-day-old Wistar rats, caged individually, with water freely available. The rats used in the present two experiments were derived from Wistar stocks but bred at the TCU vivarium facility. Rats were gradually deprived of food (Purina rat chow) until they reached 80% of their ad lib weight before the start of the experiment. Throughout training, supplemental food was provided 30–60 min after each session and in the cage to keep rats at a constant weight level. Rats were exposed to a 12:12 h light:dark cycle, with light beginning at 0700 h and ending at 1900 h, while enclosed in a room without access to natural light.

**Apparatus.** Four similar conditioning boxes, each enclosed in a sound-attenuating chamber, were used in this experiment. Internal dimensions were 20.1 cm wide, 28 cm long, and 20.5 cm high. Each box had a grid floor made with stainless steel bars of 0.4 cm in diameter and spaced 1.6 cm apart, from center to center. A speaker and a fan provided background noise (75 dB, SPL, scale B, measured in front of the feeder). Pellets

(45-mg Noyes rat formula) were automatically delivered into a feeder located in the center of one of the lateral walls, 2 cm above the floor. A retractable lever was installed 2 cm to the left of the feeder cup and 7 cm above the floor. The lever was made of aluminum and it was 4.8 cm wide and 1.9 cm deep when fully inserted into the box. Insertion (or retraction) of the lever took 0.2 s. The lever was adjusted so that minimal force was necessary to produce a detectable movement. A light bulb (GE 1820) was located 4.5 cm directly above the lever (11.5 cm from the floor), but it was not used in the present experiments. Diffuse illumination was constantly provided by a lamp (GE 1820) located on the ceiling of the sound-attenuating chamber and in the side opposite to that of the feeder.

**Procedure.** Rats were randomly assigned to eight groups of equal size ( $n = 8$ ), according to a factorial design involving ITI (15 vs. 90 s), reinforcer magnitude (one versus five pellets per trial), and time of day (0900 vs. 1700 h). For each time-of-day condition, there were four groups: M1, S1, M5, and S5 (M = massed, S = spaced). All subjects received two sessions of habituation to the conditioning box of equal duration to the training sessions of the following phase (5 min for massed groups, 18 min for spaced groups). No stimuli (lever or food) were presented during these sessions. Habituation was followed by a phase of acquisition training (delay conditioning procedure) that lasted for 20 daily sessions. In each session, rats received 10 lever-food pairings. The lever was presented for 10 s; lever retraction was followed by the response-independent delivery of food pellets (either one or five, depending on the group). When five pellets were administered, the computer was programmed to deliver them in rapid succession at intervals of 0.2 s. Any contact with the lever sufficient to close a circuit was recorded by a computer located in an adjacent room. The number of lever contacts was transformed to a responses per minute measure for the purpose of statistical analyses.

ITIs were on average either 15 s (range: 10–20 s) or 90 s (range: 60–120 s). These ITI values were chosen on the basis of prior research that indicated that the TSE fails to occur when a single pellet is administered as the reinforcer (16). A value of 90 s was also found to lead to both autoshaping and polydipsia in rats with access to both a retractable lever and water (1). The groups were trained over a 2-h period, either from 0900 to 1100 h, or from 1700 to 1900 h. The order in which the squads of four rats were run was randomized across days. Any particular rat was assigned the same Skinner box throughout training.

#### Results

The main results of this experiment are presented in Fig. 1, for each group across the 20 sessions of acquisition training. Three animals became ill during the experiment and the data they generated were not included in the analyses. Groups M5 in the morning, and M5 and S1 in the evening were left each with  $n = 7$ ; all the other groups remained with  $n = 8$ . The results were analyzed with an ITI  $\times$  magnitude  $\times$  time  $\times$  session analysis of variance, with session as a repeated-measure factor. The software package used to analyze the results (AB-Stat, from AndersonBell Corp.) uses a method of harmonic cell means to handle unequal sample size. As expected on the basis of prior results (16), the groups trained with a single pellet (S1 and M1, at 0900 or 1700 h) exhibited no evidence of the TSE. However, the groups trained with five pellets (S5 and M5, at 0900 or 1700 h) exhibited a strong TSE in terms of higher response rates in the spaced than in the massed condition. This was supported by a significant ITI  $\times$  magnitude  $\times$

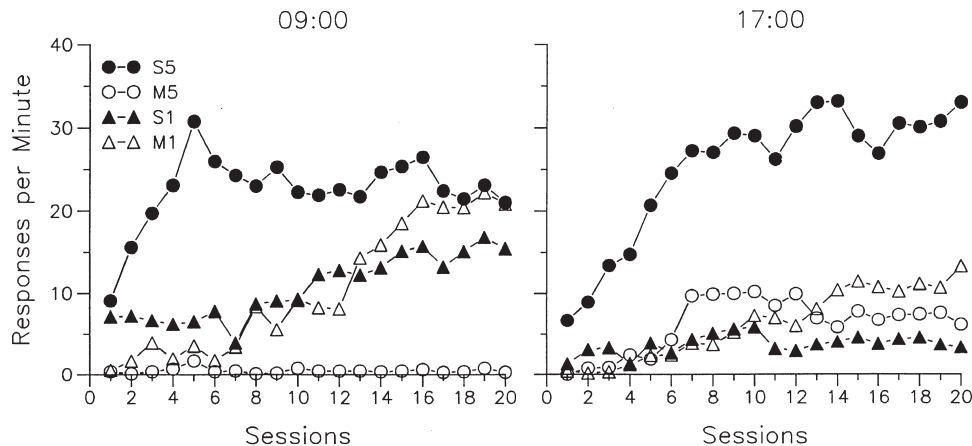


FIG. 1. Response rate as a function of 10-trial sessions in eight groups of rats trained either in the morning (0900 h) or in the evening (1700 h). "S" refers to spaced training and "M" to massed training; "1" and "5" refer to the number of pellets delivered in each autoshaping trial.

session triple interaction,  $F(19, 1007) = 2.77, p < 0.0001$ , and a significant ITI  $\times$  magnitude double interaction,  $F(1, 53) = 8.83, p < 0.01$ . There was also an overall TSE reflected in a significant simple effect for ITI,  $F(1, 53) = 7.00, p < 0.05$ .

Contrary to what was expected, time of training did not influence the size of the TSE. No interactions involving ITI and Time achieved a significant level. If anything, the morning groups trained with one pellet (S1 and M1) actually exhibited higher response rates than their evening counterparts. The only significant interaction involving the circadian factor was the magnitude  $\times$  time  $\times$  session triple interaction,  $F(19, 1007) = 2.53, p < 0.0001$ , reflecting the increasing rates observed in the morning one-pellet groups across sessions. There was also a significant main effect of session,  $F(19, 1007) = 8.88, p < 0.0001$ . All other effects were nonsignificant.

#### EXPERIMENT 2

Experiment 1 failed to provide evidence for a relationship between circadian factors and the size of the TSE, but such failure could be attributed to the effects of extensive training at a particular time of day on arousal levels. Research on the conditioning of circadian rhythms indicates that signals preceding the onset of ambient light can elicit changes in cellular (activation of suprachiasmatic nucleus neurons responsible for the circadian pacemaker), physiological (changes in body temperature), and behavioral variables (shifts in activity) (2). It is possible that the feeding schedule and training procedure used in this experiment led to a readjustment that prevented normal circadian arousal rhythms to influence the TSE. For example, the rats assigned to training at 0900 h received feeding (both in the conditioning chamber and in their cages after the daily session) during the morning, and thus, their regular circadian cycles of arousal might have been shifted accordingly. One way to avoid such a problem would be to train rats in the evening and test them in the morning. Such a shift in time of day would occur presumably fast enough to prevent the influence of conditioning effects on the circadian rhythms. This was attempted in Experiment 2.

#### Method

**Subjects and apparatus.** Sixteen male Wistar rats served as subjects. Their general characteristics and maintenance condi-

tions were the same as in Experiment 1. The same conditioning boxes described previously were used in the present experiment.

**Procedure.** Rats were randomly assigned to groups M5 and S5 ( $n = 8$ ). In Days 1–2, rats were habituated to the conditioning boxes. No stimuli were presented during these sessions. Animals in group M5 and S5 received habituation sessions lasting 5 min and 18 min, respectively, a duration that was approximately that of the training sessions scheduled for the following phase.

Training started on Day 3 and lasted until Day 12, for a total of 10 acquisition sessions. These acquisition sessions were exactly as those described in Experiment 1 for Groups S5 and M5; a strong TSE was expected by the end of this phase. Daily training sessions in the habituation and acquisition phases were run between 1700 and 1900 h.

The critical test sessions were scheduled for Days 14 and 16, according to the following design. A rest day was interpolated before each of these two training sessions (Days 13 and 15) for the purpose of equating the time between the last meal and the training session across groups. On Day 14, half of the rats in each group received training at 1700–1900 h, as in acquisition, whereas the rest of the rats in each group received training at 0900–1000 h. Subgroups were determined by matching subjects in terms of response rate during Day 12, the last acquisition session, and then randomly assigning pair members to one of two possible training sequence: 0900–1700 or 1700–0900. Each animal received the last meal 23 h before the session. Animals trained at 0900 h on Day 14 were fed at 1000 h on Day 13 (a rest day), whereas animals trained at 1700 hr on Day 14 were fed at 1800 hr on Day 13. Day 15 was also a rest day, during which the feeding schedule was reversed for each subgroup of four animals. On Day 16, the assignment to training at 0900 h or at 1700 h was the opposite to that of Day 14 in each of the two subgroups. Rats previously trained at 0900 h were now trained at 1700 h, and vice versa. For the purpose of statistical analyses, each rat contributed a response rate score for sessions ran at 0900 h and at 1700 h. Other aspects of this design were the same as in Experiment 1.

#### Results

The results are presented in Fig. 2, again in terms of responses/min and separately for the initial 10 sessions of acquisition (left panel) and for the testing at 0900 or 1700 h (right

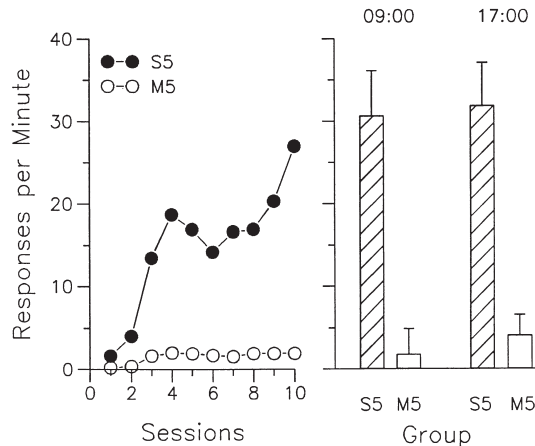


FIG. 2. Response rate during the initial 10 10-trial sessions of acquisition (left panel) for groups trained under massed, "M," or spaced, "S," training, with five pellets as the US per trial. Acquisition training was administered at 1700 h. The right panel shows the results of the test sessions at either the same (1700) time of day, or during the morning (0900). Each group (S5: crosshatched bars; M5: open bars) was trained at both times in a counterbalanced fashion. Brackets represent the standard error of the mean.

panel). As expected, a strong TSE emerged during the acquisition sessions that replicates the results of the prior experiment. A group  $\times$  session analysis of variance indicates that group S5 performed significantly above group M5,  $F(1, 14) = 9.25$ ,  $p < 0.01$ , that the acquisition effect was highly significant,  $F(9, 126) = 4.85$ ,  $p < 0.001$ , and that the groups significantly diverged from each other across the 10 sessions of acquisition,  $F(9, 126) = 3.53$ ,  $p < 0.001$ .

The right panel shows a small difference in response rate, which was higher at 1700 h than at 0900 h, although the size of the TSE was very similar at both times. A group  $\times$  time-of-day analysis, with repeated measures for the latter factor, indicated again a highly significant TSE,  $F(1, 14) = 12.07$ ,  $p < 0.01$ . Although numerically small, the higher response rate in the evening sessions was consistent enough across animals to reach a significant level,  $F(1, 14) = 4.87$ ,  $p < 0.05$ . Notice that this is a within-group comparison, and thus the error bars only show variation among conditions, not necessarily overlap of scores across subjects. The interaction, however, was not significant ( $F < 1$ ), suggesting that the time of testing did not affect the size of the TSE.

#### GENERAL DISCUSSION

These results are partially consistent with the arousal hypothesis, that is, the view that the TSE originates, at least in part, as a result of higher arousal levels in the spaced-trial condition than in the massed-trial condition. Meal termination is assumed to induce a state of primary frustration. Increases in arousal levels in the spaced training condition were

hypothesized to result from a combination of frustration and relatively long periods of nonreinforcement. Arousal level is one important determinant of the strength with which dominant behaviors express themselves in a particular situation. The availability of water may result in polydipsia, the presence of a conspecific may induce aggressive behavior, and the presence of a lever may elicit invigorated approach and contact responses (17).

The TSE failed to occur when a single pellet was used as the reinforcer (16). However, a reinforcer magnitude of five pellets led to a strong TSE. Such large-reinforcer TSE was due to both a higher performance level of the spaced group, and a lower performance level of the massed group. It had been suggested, on the basis of research on schedule-induced polydipsia (17), that an increase in reinforcer magnitude should lead to particularly high arousal levels in the spaced-trial condition, thus causing relatively high response rates. By contrast, the density of food delivery in the massed condition would keep arousal levels to a minimum, thus concomitantly reducing response levels.

Other theories of the TSE do not predict a direct relationship between reinforcer magnitude and the size of the TSE. The model proposed by Rescorla and Wagner (20) predicts that an increase in reinforcer magnitude leads to greater strength for both signal and contextual cues. Depending on the parameters, large reinforcers may actually reduce the TSE, compared to small reinforcers, because extinction of the context during the ITI can be compensated by a higher increase in contextual strength after reinforcement. Performance models (13) suggest that the increase in strength of the signal in the large reinforcer condition would be compensated by a proportional increase in the strength of the context, thus leading to a similarly strong TSE under both magnitude conditions.

It was also predicted that arousal levels would vary as a function of time of day. Based on independent evidence on circadian rhythms in rats, it was anticipated that evening training should be associated with a relatively stronger TSE than morning training. The results were uniformly negative, even when an attempt was made to control for the potentially shift in arousal levels as a result of extensive training and post-session feeding during the morning hours (see Fig. 2).

Time of day did influence lever contact performance, although the results were equivocal. In Experiment 1, the groups trained with five pellets (Groups S5 and M5) performed significantly higher in the evening sessions than in the morning session, a result consistent with the expectation of higher arousal levels at 1700 h than at 0900 h. Experiment 2 replicated the enhancing effects on lever-contact behavior of evening training with groups S5 and M5, both receiving five pellets per trial. Unlike in Experiment 1, however, this case involved a within-group comparison. However, the opposite result was obtained in Experiment 1 in the groups trained with one pellet (groups S1 and M1), that is, lever-contact responses were more frequent in the morning sessions than in the evening sessions. The source of this complex interaction of reinforcer magnitude and circadian variations in lever-contact responses is not clear at this time.

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