



## **The Reinforcement Magnitude of Flavored Stimulus Interferes with Omission Effects in Rats**

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Reinforcement omission effects (ROEs) have been interpreted as behavioral transient facilitation after nonreinforcement induced by primary frustration, and/or behavioral transient inhibition after reinforcement induced by demotivation or temporal control. According to frustration theory, the size of the ROEs should depend directly on the reinforcement magnitude: the behavioral facilitation after the reinforcement omission of larger magnitude should be greater than that observed after the reinforcement omission of smaller magnitude. However, studies involving operant paradigms have presented difficulty in demonstrating this relationship. Thus, the present study aimed to clarify the relationship between reinforcement magnitude and ROEs manipulating the magnitude linked to discriminative stimuli in a partial reinforcement fixed interval schedule. Rats were trained on a fixed-interval 12 s with limited hold 6 s signaled schedule in which correct responses were always followed by one of two reinforcement magnitudes (0.5 and 0.05 ml of a 0.15% saccharin solution). After acquisition of stable performance, the training was changed from 100% to 50% reinforcement schedules. The results showed that response rates were higher after omission than after reinforcement delivery. Besides, results showed that response rates were highest after the reinforcement omission of larger magnitude than of smaller magnitude. However, the findings did not support the hypothesis that the reinforcement omission of large magnitude induces greater behavioral facilitation than the reinforcement omission of smaller magnitude. The data were interpreted in terms of behavioral facilitation after nonreinforcement and behavioral transient inhibition after reinforcement.

The reinforcement omission effects (ROEs) indicated by greater response strength immediately after omission that after reinforcement delivery have been attributed to both motivational and attentional consequences of the surprising reinforcement omission. For instance, Amsel and Roussel (1952) reported that the introduction of partial reinforcement in the first goal of a double runway led to greater response in the second runway immediately after omission than after reinforcement delivery. This effect was explained as behavioral facilitation induced by primary frustration (Amsel, 1992; Amsel & Roussel, 1952; Papini, 2003; Papini & Dudley, 1997). However, ROEs can be also interpreted in terms of behavioral transient inhibition after reinforcement induced by demotivation or reset of internal clock (Seward, Pereboom, Butler, & Jones, 1957; Staddon, 1974).

According to frustration theory, proposed by Amsel and Roussel (1952), the behavioral facilitation after reinforcement omission of larger magnitude should be greater than that observed after reinforcement omission of smaller magnitude. Thus, the size of the ROEs should depend directly on the reinforcement magnitude. From this perspective, several studies investigated the effects of reinforcement magnitude on performance following reinforcement and its omission. Results obtained in both runway procedures (Bower, 1962; Daly, 1968; Hughes, Dunlap, & Dachowski, 1974; McHose & Ludvigson, 1965; Peckham & Amsel, 1967) and operant conditioning paradigms (Jensen & Fallon, 1973; Judice-Daher, Tavares, & Bueno, 2011, 2012) are divergent.

The results of the studies developed by Bower (1962), Hughes et al. (1974), and Peckham and Amsel (1967), for instance, corroborate that there is a relationship between behavioral facilitation followed by reinforcement omission and reinforcement magnitude. In the study of Peckham and Amsel (1967), rats learned in a double-runway apparatus to discriminate a large (8 pellets) or a small (2 pellets) magnitude of reinforcement in the first goal box; the reinforcement magnitude in the second goal box was always the same (2 pellet). In the test phase, the percentage of reinforcement in the first goal box was reduced from 100 to 50%. Results showed that larger frustration effects were found following non reinforcement in the alley associated with the larger reward. Similarly, the study of the Hughes et al. (1974) showed that the size of the frustration effect was directly related to reinforcement magnitude. In this experiment, the frustration effect (activity following non reinforcement compared with reinforcement) was measured in an alley whose goal-box floor was a stabilimeter.

On the other hand, Daly (1968) and McHose and Ludvigson (1965) suggested that ROEs are unrelated with reinforcement magnitude. In the study by McHose and Ludvigson (1965), rats were trained in an L-shaped double-alley apparatus. In acquisition, one group of rats (Group 10) received 10 pellets in the first goal box on each trial, while other group (Group 2) received 2 pellets in first goal box. Both groups always received two pellets in the second goal box. In test (six post shift trials), some manipulations were made. The Group 2 received two pellets in first goal box on three trials, and no reinforcement on three trials; the Group 10 received 10 pellets on three trials, two pellets on one trial, and no reinforcement on two trials. The results showed that the running speed of the rats in the second alley had no relationship with the reinforcement magnitude.

Jensen and Fallon (1973) suggested that the relationship between behavioral facilitation followed by reinforcement omission and reinforcement magnitude is subtle and difficult to document. The authors submitted rats to a two-component multiple fixed-interval reinforcement schedule; a response at the end of the second component was always reinforced with an invariant reinforcement magnitude (0.75 s of licking time), while reinforcement magnitude and reinforcement omission were systematically varied in the first component (0.75, 1.50, and 3.00 s of licking time). Results showed that the response rate in the first component increased as magnitude of reinforcement in this component increased, but there was no change in response rate in the second component as magnitude of reinforcement in the first component increased. When reinforcement was omitted in the first component (50% reinforcement condition), there was an increase in response rate in the second component that was unrelated to reinforcement magnitude. According to the authors, the difficulty in producing an effect may be due to the reinforcement magnitude is not highly salient at the moment that reinforcement is omitted.

According to Judice-Daher et al. (2011), the study of Jensen and Fallon (1973) may have had some methodological difficulties. For instance, the performance followed by nonreinforcement was analyzed after a timeout period. Thus, the authors proposed an experimental design in which the ROEs could be examined immediately after nonreinforcement. Judice-Daher et al. (2011) submitted rats on a signaled fixed-interval 12 s with limited hold 6 s reinforcement schedules (FI 12 s LH 6 s) in which correct responses were always followed by one of two reinforcement magnitudes: 0.05 ml or 0.5 ml of water. After acquisition of stable performance, the training was changed from 100% to 50% reinforcement schedules. This procedure allowed that ROEs were examined in the same component in which the partial reinforcement was introduced (LH period). Also, the procedure employed allowed a reliable baseline since exteroceptive stimuli provided information about the reinforcement earned (Collwill & Rescorla, 1988). The different performance during the signaled FIs ensured the discrimination between the reinforcement magnitudes.

The results obtained by Judice-Daher et al. (2011), showed that the performance during the FI signaled by the stimulus associated with large magnitude of reinforcement was more effective than that observed during the FI signaled by the stimulus associated with small magnitude of reinforcement. When reinforcement omission was introduced, for both reinforcement magnitudes, the response rates were higher after omission

than after reinforcement delivery. Also, the response rates were highest after the reinforcement omission of larger magnitude than that of smaller magnitude. However, according to the authors, these findings did not support the hypothesis that the reinforcement omission of larger magnitude induces greater behavioral facilitation than the reinforcement omission of smaller magnitude. The difference in performance after the reinforcement omission of the larger and smaller magnitudes, in the LH period, reflected the one that already existed previously (at the end of the FI). Thus, the reinforcement omission did not eliminate the control of behavior by the signal associated with different reinforcement magnitudes. Therefore, the authors suggested that the reinforcement magnitude operate in omission, but not in behavioral facilitation.

Judice-Daher et al. (2012) employed the same procedure of that used by Judice-Daher et al. (2011) to evaluate whether the mechanisms underlying the ROEs depend on the different motivational properties attributed to the specific activation of the amygdala. According to the authors, the use of different magnitudes of reinforcement could contribute to a better understanding of the involvement of amygdala on ROEs modulation. Results obtained by Judice-Daher et al. (2012) were similar to those showed by Judice-Daher et al. (2011). There were response rates higher after the reinforcement omission of larger magnitude than of smaller magnitude, but this relationship was unrelated to behavioral facilitation.

Although Judice-Daher et al. (2011, 2012) used reinforcement magnitudes more discrepant than Jensen and Fallon (1973), this discrepancy may not have been high enough to produce different levels of behavioral facilitation after reinforcement omission of larger and smaller magnitudes. Thus, the results of the studies that employed operant conditioning do not support that the behavioral facilitation after reinforcement omission is related to reinforcement magnitude.

The present experiment aimed to clarify whether or not the relationship between reinforcement magnitude and the omission effects involves different levels of behavioral facilitation after omission of different magnitudes of reinforcement. Compared to the study of Judice-Daher et al. (2011), the same behavioral procedure was employed, but we introduced another motivational variable, saccharin solution, as the reinforcer. It is possible that manipulating the reinforcement quality using the flavor of saccharin would produce an incentive value which could be sufficient to show the behavior facilitation in the omission reinforcement condition. This hypothesis is supported by previous studies showing that animals prefer the sweetened flavors than conventional reinforcers (for example, Boyer, Russin, & Cross, 1971; Papini, Ludvigson, Huneycutt, & Boughner, 2001).

## **Method**

The study was approved by the Ethics Committee on Animal Use of the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo at Ribeirão Preto, Brazil (protocol no. 11.1.1390.53.0).

### **Subjects**

Fifteen experimentally naive male Wistar rats, 90 days old at the beginning of the experiment and weighing 416-433 g, were used in the study. Throughout the experiments, the animals were singly housed in steel cages in the laboratory colony room on a 12 h/12 h light/ dark schedule (lights on 8:00 AM to 8:00 PM). The rats were maintained on a food deprivation schedule at 85% of their ad libitum body weight by limiting access to food. Water was freely available in their cages.

### **Apparatus**

The experiment was conducted in operant chambers (Lafayette model 80201) equipped with a speaker, which delivered a 1000 Hz, 30 dB tone; a 5 W house-light lamp; a 5 W lamp which delivered white-light; a retractable 5 cm lever. Each chamber was in a soundproof wooden box provided with a transparent acrylic window which was hold in soundproof experimental rooms. An

electrical interface (MRA-Electronic Equipment, Ribeirao Preto, Brazil) connected the experimental chambers to a PC. This system used a program prepared with Microsoft QuickBasic 4.0 designed for this experiment, which controlled the reinforcement mechanisms registered and recorded lever presses.

### **Behavioral Training Procedures**

**Lever press training.** Lever press training was carried out over two sessions. In the first session, each rat was placed into the operant chamber and trained to lever press for one 0.05 ml drop of a 0.15% saccharin solution (1000 ml of water/ 1.5 mg of saccharin). Each rat performed a total of 50 lever presses in this first session. The following session consisted of continuous reinforcement (CRF training, with a single drop of a 0.15% saccharin solution delivered with each lever press), for a total of 100 lever presses. Each session lasted a maximum of 1 hour.

**Acquisition training.** The rats went through a total of 30 sessions of acquisition training lasting approximately 50 minutes each. In the first phase (10 sessions) of acquisition training, rats were trained in two fixed-interval 12 s with limited hold 6 s signaled schedules (FI 12 s LH 6 s), one in each session on alternate days. One schedule was presented simultaneously with a light stimulus of 18 s (L18 s) and the other, with an auditory stimulus of 18 s (T18 s). For half the rats, the FI 12 s LH 6 s signaled by L18 s indicated that the correct response – the first lever press occurred between 12 s and 18 s – resulted in delivery of the 0.5 ml of a 0.15% saccharin solution; the FI 12 s LH 6 s signaled by T18 s indicated that the correct response resulted in delivery of the 0.05 ml of a 0.15% saccharin solution; this relation was counterbalanced for the other rats. All rats received 28 training trials per session; each trial was interpolated with variable timeout periods (TO; mean: 75 s).

In the second phase of acquisition training (20 sessions), two FI 12 s LH 6 s schedules were randomly presented in the same session. All rats received 28 training trials per session, half of them consisted of the presentation of the FI 12 s LH 6 s schedule signaled by L18 s, and other half consisted of the presentation of the FI 12 s LH 6 s schedule signaled by T18 s. Each trial was interpolated with variable timeout periods (TO; mean: 75 s).

In both acquisition training phases the sessions began and ended with TOs in which no response was reinforced and only ambient lighting was maintained. At the end of each session, the rats were returned to their cages and given water sufficient to maintain their planned body weight schedule. The rats were food-deprived for approximately 23 hours before the beginning of each session.

At the end of the acquisition training, rats attained a stable baseline performance, indicated by the following criteria: (1) average response higher during the signaled schedule than during inter-trial intervals; (2) average response higher at the end of the FI 12 s LH 6 s schedule signaled by the stimulus associated with reinforcement of larger magnitude than at the end of the FI 12 s LH 6 s schedule signaled by the stimulus associated with reinforcement of smaller magnitude; (3) 80% accuracy for session, three consecutive days.

**Testing: Partial reinforcement.** The test phase (4 sessions) began with the introduction of a 50% reinforcement schedule. The rats were submitted to the same conditions of acquisition training (first phase). All rats received 28 training trials per session, but the reinforcement was not delivered after the correct response in half of the trials. For half the rats, even-numbered sessions involved the reinforcement omission of a larger magnitude (0.5 ml of a 0.15% saccharin solution) and odd-numbered sessions involved the reinforcement omission of a smaller magnitude (0.05 ml of a 0.15% saccharin solution); this was counterbalanced for the other rats. Reinforced and nonreinforced events were randomly distributed during the session using the criteria of up to three subsequent intervals with the same schedule.

### **Statistical Analysis**

For statistical analysis, the average response during the FI 12 s LH 6 s was grouped into 3 s periods (3 s FI, 6 s FI, 9 s, FI 12 s, 3 s LH, 6 s LH). These data were analyzed using two-way analysis of variance (ANOVA), with reinforcement magnitude (larger and smaller conditions) and period (3 s FI; 6 s FI; 9 s FI; 12 s FI; 3 s LH; 6 s LH) as intra-group factors. Significant effects in the ANOVA were followed by the Newman-Keuls post hoc test. Values of  $p \leq .05$  were considered statistically significant.

## **Results**

The data of the last sessions of the second phase of the acquisition training indicate that there was a gradual increase in responding across both signaled schedules, indicating that performance was under the

temporal control function. Also, the responding was higher at the end of the FI Lm schedule, indicating that there was discrimination between the two reinforcement magnitudes (Figure 1). ANOVA analyzed the average response during the presentation of FI 12 s LH 6 s schedules signaled by the stimuli linked with larger (Lm) and smaller (Sm) reinforcement magnitudes in the last three sessions of the acquisition training, revealing significant effects of reinforcement magnitude,  $F(1, 100) = 44.475, p < 0.001$ , period,  $F(5, 100) = 69.205, p < 0.001$ , and reinforcement magnitude x period,  $F(5, 100) = 30.562, p < 0.001$ . Newman-Keuls post-hoc tests showed that: (1) For Lm condition, all periods were significantly different from each other (except 3 s FI and 6 s LH, and 6 s FI and 3s LH); (2) For Sm condition, 3 s FI and 9 s FI periods, 3 s FI and 12 s FI periods, 9 s FI and 6 s LH periods, and 12 s FI and 6s LH periods were significantly different from each other. Newman-Keuls post-hoc tests also showed significant differences between the two 6 s FI periods, the two 9 s FI periods, the two 12 s FI and the two 3 s LH periods from presentation of FI 12 s LH 6 s Lm schedule to the presentation of FI 12 s LH 6 s Sm schedule.

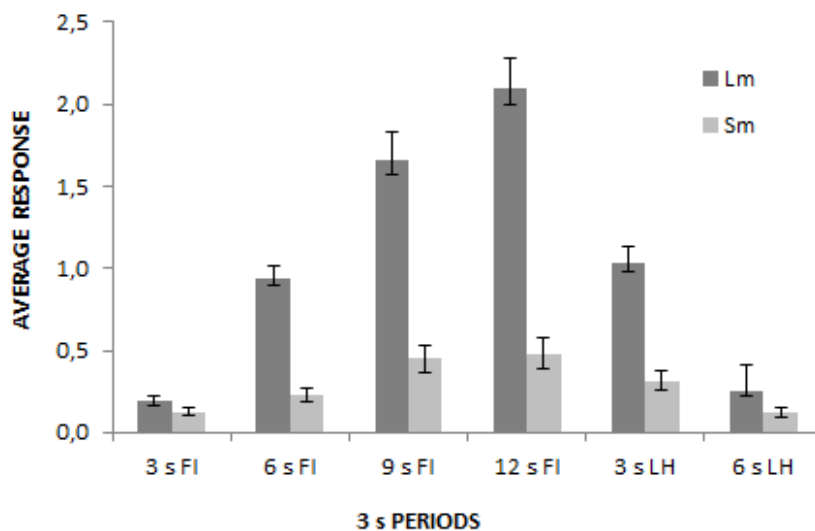


Figure 1. Acquisition training. Average response in the last three sessions of the acquisition training grouped in 3 seconds periods during the periods preceding the delivery of larger (Lm) and smaller (Sm) reinforcement magnitudes (3 s FI, 6 s FI, 9 s FI, and 12 s FI), and during the periods after the delivery of Lm and Sm (3 s LH and 6 s LH). FI: fixed-interval; LH, limited hold. Error bars indicate  $\pm$  SEM.

The data of the test phase confirm that there were ROEs for both Lm and Sm conditions. Moreover, the difference in responding during Lm and Sm conditions after nonreinforcement (3 s LH N and 6 s LH N) indicated that the magnitude of reinforcement interfered with ROEs. Figure 2 shows the average response, in the four sessions of the test phase, during the periods preceding and following the omission or delivery of larger and smaller reinforcement magnitudes. ANOVA revealed significant effects of reinforcement magnitude,  $F(1, 140) = 86.844, p < 0.001$ , period,  $F(7, 140) = 43.939, p < 0.001$ , and reinforcement magnitude x period,  $F(7, 140) = 75.074, p < 0.001$ . Newman-Keuls post-hoc tests showed that: (1) For Lm condition, 3 s LH N and 3 s LH R periods, and 6 s LH N and 6 s LH R periods were significantly different from each other; (2) For Sm condition, 6 s LH N and 6 s LH R periods were significantly different from each other. As for the differences in performance during presentation of the signaled schedules by stimuli linked to the larger and smaller reinforcement magnitude (FI 12 s LH 6 s Lm and Sm schedules), Newman-Keuls post-hoc tests indicated significant differences between the 6 s FI of both schedules, as well as for the 9 s FI, 12 s FI, 3 s LH N, 6 s LH N, and 3 s LH R periods.

Results also showed that there was no increase in responding after nonreinforcement, but there was a

decrease in it after reinforcement. Newman-Keuls post-hoc tests showed that the performance during the periods preceding the omission or delivery of Lm reinforcement (9 s FI and 12 s FI) was not different from that following the omission (3 s LH N and 6 s LH N), but was different from that following the delivery of reinforcement (3 s LH R and 6 s LH R). The same results were obtained for the Sm reinforcement condition.

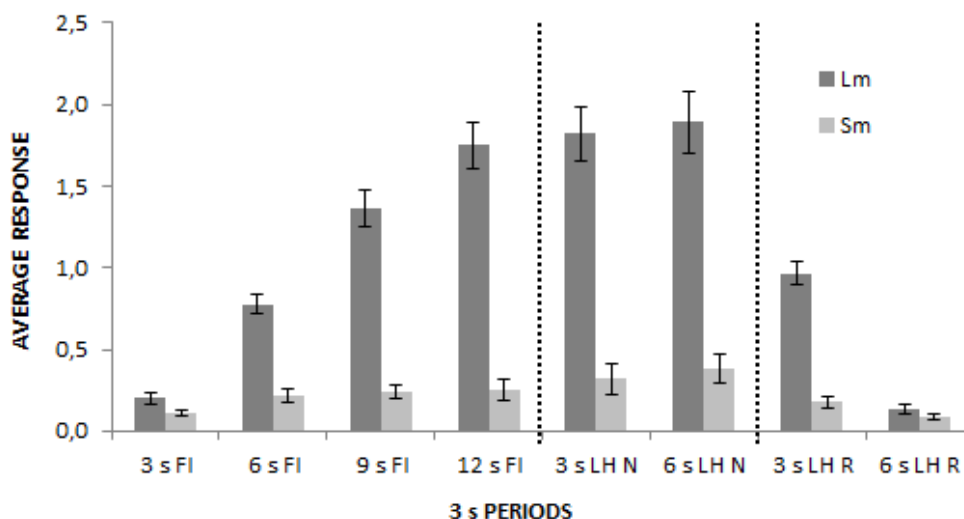


Figure 2. Testing: partial reinforcement. Average response in the four sessions of the test phase grouped in 3 seconds periods during the periods preceding the omission or delivery of larger (Lm) and smaller (Sm) reinforcement magnitudes (3 s FI, 6 s FI, 9 s FI, and 12 s FI), during the periods after the delivery (3 s LH R and 6 s LH R), and after the omission (3 s LH N and 6 s LH N) of Lm and Sm. FI: fixed-interval; LH, limited hold; R, reinforcement; N, nonreinforcement. Error bars indicate  $\pm$  SEM.

## Discussion

The data of the acquisition training were similar to those obtained by Judice-Daher et al. (2011, 2012). There was a discriminative control during the signal, producing different response distributions depending on the reinforcement magnitude anticipated. The performance during the FI signaled by the Lm stimulus was more effective than that observed during the FI signaled by Sm stimulus. The response during FI Lm schedule was higher than during FI Sm schedule when recorded in the last seconds of FI, but smaller in the first seconds. These data indicated the effect of the magnitude and the enhancement of timing during larger signaled magnitude. According to Crespi (1944), after some experience with different amount of incentive, the animals begin to exhibit varying levels of anticipatory excitement at the perspective of its acquisition. This different anticipatory excitement for different amounts of incentive generates differences in the performance curves. The greater the amount of incentive, the greater the anticipatory excitement and motivation to respond.

Although the behavior pattern of the rats during the signaled schedules was similar to that obtained by Judice-Daher et al. (2011, 2012), in the present study the difference between the response rates during FI signaled by the Lm stimulus and during the FI signaled by Sm stimulus seems to be more salient. Compared to Judice-Daher et al. (2011, 2012), the same ratio between the reinforcing stimuli was employed (1:10), but another motivational variable (saccharin solution) was introduced.

The relationship between the reinforcement magnitude and animal performance confirms data from the literature (Bower, 1962; Jensen & Fallon, 1973; McHose & Ludvigson, 1965; Peckham & Amsel, 1967; Pellegrini, Seal, & Papini, 2008) and can be explained by evoked expectation of reinforcement (Colwill &

Rescorla, 1988; Schultz, 1998). Studies involving both runway procedures and operant conditioning paradigms showed that the performance of animals is positively related to reinforcement magnitude. For instance, Pekcham and Amsel (1967) showed that the running speed of the rats in the first alley of a double-runway apparatus was higher for the larger magnitude of reinforcement (8 pellets) than for the smaller one (2 pellets). Also, Jensen and Fallon (1973) showed that the response rate in the first FI component of a multiple schedule (FI FI) increased as magnitude of reinforcement in this component increased (0.75, 1.50, and 3.00 s of licking time).

Larger magnitudes of reinforcement also seem to favor the learning compared with smaller magnitudes. O'Connell and Rashotte (1982) used a second-order conditioning procedure to investigate the effects of reinforcement magnitude on conditioned key pecking in pigeons. Results showed that the conditioned stimulus previously paired with an unconditioned stimulus of larger magnitude was more effective in conditioning second-order key-peck behavior to a second-order conditioned stimulus than the conditioned stimulus paired with an unconditioned stimulus of smaller magnitude.

The term ROEs can be considered empirically and from a theoretical point of view (Judice-Daher et al., 2011; Judice-Daher & Bueno, 2013a). Assuming that ROEs are empirically defined by the difference in responding after omission relative to that following reinforcement, then the results of the present study showed an omission effect in Lm condition for 3 s LH and 6 s LH periods. Although the overall response rates to Sm were lower than Lm condition, there was an omission effect for Sm condition in 6 s LH period.

Besides, data in the trials after non reinforcement showed that the responding was higher after the larger reinforcement omission than after the smaller one, pointing to the effect of the magnitude of reinforcement on ROEs. These results manipulating the reinforcement quality with the use of flavor of saccharin are similar to results obtained by Judice-Daher et al. (2011, 2012) with water reinforcement. These results also support previous data obtained in studies involving runway procedures (Bower, 1962; Hughes et al., 1974; Peckham & Amsel, 1967) showing the relationship between reinforcement magnitude and omission effects.

From a theoretical point of view, it is necessary to consider that response rate differences between after-nonreinforcement and after-reinforcement conditions can be driven by multiple processes (Judice-Daher et al., 2011; Judice-Daher & Bueno, 2013a). One interpretation is in terms of frustration, evidenced by an increase in responding after nonreinforcement (Amsel, 1992). Another interpretation is in terms of behavioral transient inhibition after reinforcement induced by demotivation or reset of internal clock (Seward et al., 1957; Staddon, 1974). According to Staddon (1974), in intermittent schedules of reinforcement, each reinforcement delivery acts as a time marker that resets the animal's internal clock, inhibiting the response rate. After nonreinforced trials, the absence of the resetting would not inhibit responses rates that remain higher after reinforced trials compared to nonreinforced ones. These effects can explain the differences between post-nonreinforcement and post-reinforcement trials.

Consistent with the behavioral transition inhibition hypothesis, the data obtained in the present study showed that the response rates in the period following reinforcement omission (Lm or Sm conditions, 3 s LH N and 6 s LH N) were not different from those presented in the period preceding it (Lm or Sm conditions, 12 s FI). However, the response rates in the period following reinforcement delivery (Lm or Sm conditions, 3 s LH R and 6 s LH R) were lower from those presented in the period preceding it (Lm or Sm conditions). Thus, in this study the ROEs could be explained by the inhibition after reinforcement hypothesis (Bueno, Judice-Daher & Tavares, 2013; Judice-Daher & Bueno, 2013a, 2013b; Judice-Daher et al., 2011, 2012).

The behavioral procedure introduced by Judice-Daher et al. (2011), and employed in this study, allows for the analysis of the relationship between ROEs and the level of anticipating reinforcement magnitude. The animal responding more vigorously during Lm than Sm anticipating conditions showed higher rate of response during Lm than Sm after nonreinforcement. The reinforcement magnitude linked to the schedule remains operating in the omission, and the ROEs can be attributed to tracking of discriminative control, or in another terms, the greater the reinforcement anticipated, the greater the omission effect. This possibility is not necessarily contradictory to the after non reinforcement facilitation hypothesis. It should be noticed that the temporal control and frustration accounts are not mutually exclusive, because temporal control may lead to frustration if the reinforcement fails to occur at the expected time (see Papini & Hollingsworth, 1998, for peak shift FI cycled schedules).

The results obtained with the manipulation of reinforcement quality using the flavor of saccharin corroborate the hypothesis that the reinforcement magnitude can operate in omission effect, since the reinforcement omission did not eliminate the control of behavior by the signal associated with different reinforcement magnitudes. However, an increase in the response rates during trials after non reinforcement compared with those immediately preceding ones was not obtained. These results did not support the behavioral facilitation approach of ROEs. Also, the animals showed higher rate of responses in some FI periods of the acquisition phase than that recorded during the test phase (Lm or Sm conditions). These data indicate the impossibility that a ceiling effect could prevent the recording of behavior facilitation effect in the periods after omission (Lm or Sm conditions, 3 s LH N and 6 s LH N) compared to the responses during FI periods. These data are similar to that obtained in other studies involving omission effect in operant behavior (Judice-Daher et al., 2011, 2012; Tavares, Judice-Daher, & Bueno, 2014).

Other studies manipulating the different parameters involved in ROEs (as suggested by Stout, Boughner, & Papini, 2003; Stout, Muzio, Boughner, & Papini, 2002) can clarify more precisely the multiple process hypotheses.

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