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The temporal pattern of responding in conditioned bar-press suppression: The role of the context switch and training mode

Jeremie Jozefowicz,

U.F.R. de Psychologie, Université Charles de Gaulle-Lille3 & Escola de Psicologia, Universidade do Minho, Braga, Portugal

James E. Witnauer, and

Department of Psychology, State University of New York, SUNY-Brockport, Brockport, New York

Ralph R. Miller

Department of Psychology, State University of New York, SUNY-Binghamton, Binghamton, New York

Abstract

The present study examined the temporal pattern of responding in a conditioned bar-press suppression task in rats. Rats were exposed to either a 30-s or a 120-s conditioned stimulus (CS) followed by a footshock. Training took place either while the rats were lever-pressing for water (online), or with the lever removed from the box (offline). They were then exposed to the CS while they were lever-pressing for water, either in the training context or in a different context. Bar-press suppression during the CS was constant across the duration of the CS during training, but was restricted to the initial portion of the CS at the time of testing, especially when subjects were tested in a different context. Those results replicate the reactive (as opposed to anticipatory) pattern observed in a lick suppression procedure by Jozefowicz, Witnauer, & Miller (2011) and indicate that a change in context at the time of testing might be critical for its expression.

Keywords

Fear conditioning; conditioned suppression; conditioned responding; unconditioned responding; pseudoconditioning

In conditioned bar-press suppression (Estes & Skinner, 1941), fear triggered by a conditioned stimulus (CS) that was previously paired with an aversive unconditioned stimulus (US), such as a footshock, is assessed by measuring the degree to which the CS disrupts baseline bar-pressing for food or water delivered on a variable-interval (VI) schedule of reinforcement. While many experiments report a constant rate of suppression during the CS, some studies have observed an inhibition-of-delay pattern, in which suppression is more pronounced at the end of the CS, when the US is more imminent, than at the beginning (see Hammond & Maser, 1970; Hendry & Van-Toller, 1965; Holmes, Jackson, & Byrum, 1971; Labarbera & Church, 1974; Libby & Church, 1975; Rosas &

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Correspond with: Dr. Jeremie Jozefowicz, U.F.R. de Psychologie, Université Charles de Gaulle-Lille3, Domaine Universitaire de Pont de Bois, B.P. 60149, 59653 Villeneuve d'Ascq Cedex, France, jeremie.jozefowicz@univ-lille3.fr.

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Alonso, 1996, 1997a, 1997b; Schachtman, Channell, & Hall, 1987; Zielinski, 1966). Moreover, at least two studies (Rescorla, 1968; Bouton, M. E., Frohardt, R. J., Sunsay, C., Waddell, J., & Morris, R. W., 2008) have reported a paradoxical temporal pattern of suppression in bar-press suppression, in which suppression was more pronounced at the beginning of the CS than at the end of it.

Recently, Jozefowicz, Witnauer, and Miller (2011) have reported a similar reactive pattern in a lick-suppression procedure. Suppression to a tone CS was stronger at the beginning than at the end of the tone. This pattern was observed only in rats exposed to a shock US, but did not depend on the contingency of the tone with the shock. Jozefowicz et al. concluded that the behavior of the rats in lick-suppression was the sum of two independent components: A conditioned, time-independent (except in cases in which sufficient training with a sufficiently long CS occurs to produce inhibition of delay) component caused by the pairing of the CS and the US and an unconditioned reactive component, caused by mere exposure to the US. The latter component does not seem to be limited to lick suppression per se, because Rescorla (1968) and Bouton et al. (2008) observed it in a bar-press suppression task. In contrast, many other studies of the temporal pattern of suppression have reported either an inhibition-of-delay pattern or a constant amount of suppression during the CS, as opposed to a reactive pattern. Rosas and Alonso (1996), for instance, observed that constant suppression preceded the development of inhibition of delay in their study. They did not report a reactive pattern.

These differences in the temporal pattern of suppression probably result from procedural differences. The lick suppression procedure used by Jozefowicz et al. (2011) differs considerably from most bar-press suppression procedures, such as the one used by Rosas and Alonso (1996), in at least three major ways: a) The baseline operant response is typically reinforced on a VI schedule in bar-press suppression, whereas a continuous reinforcement schedule prevails in lick suppression. b) Pavlovian training is usually done online in the bar-press suppression studies that demonstrate anticipatory temporal regulation (i.e. an inhibition-of-delay pattern), with the baseline response available while the CS and US are being paired. In lick suppression, training is usually done offline with the baseline response re-introduced at the time of testing. c) Training and testing are usually done in the same context in bar-press suppression, whereas testing often involves a change of context in lick suppression. The last two features might be critical. For instance, Rescorla (1968) also trained his rats offline, exposing them to the Pavlovian contingency between the CS and the shock in the absence of the lever, while the lever, and hence access to food, was reintroduced at the time of testing. Note that, when the lever is present, its mere presence but also the presence of food or water and the responding by the rat de facto creates a testing context radically different from the training one. Hence, training offline and testing online might be equivalent to a context switch, explaining why both Rescorla and we observed a reactive pattern of responding at testing.

In the present experiment, we assessed the impact of the online/offline nature of training and the change of context at the time of testing, to determine if they could explain differences in the temporal distribution of responding between bar-press and lick suppression. We did this by testing whether offline training and a change in context between training and testing would induce a reactive pattern of temporal regulation in a bar-press suppression procedure as opposed to the more commonly observed anticipatory pattern.

Method

Subjects

The subjects were 24 female and 24 male Sprague-Dawley, experimentally naive, young adult rats, bred in our colony. Body weight was between 177 and 260 g for the females and 261 and 356 g for the males. Subjects were individually housed and maintained on a 16-hr light / 8-hr dark cycle with experimental sessions occurring roughly midway through the light portion. All subjects were handled for 30 s three times per week from weaning until the initiation of the study. Subjects had free access to food in their home cages. One week prior to initiation of the experiment, water availability was progressively reduced to 20-min per day, given within two hours following the end of any daily treatment.

Apparatus

The bar-press apparatus consisted of twelve chambers, each measuring $30 \times 30 \times 27$ cm (length $\times$ width $\times$ height). The side walls of each chamber were made of stainless steel sheet metal, and the front and back wall as well as the ceiling of each chamber were made of clear Plexiglas. The floor was constructed of 0.3-cm diameter rods, spaced 1.3 cm center-to-center, and connected by NE-2 neon bulbs that allowed a constant-current footshock to be delivered by means of a high voltage AC circuit in series with a 1.0-M Ω resistor. Each chamber was housed in an environmental isolation chest, which was dimly illuminated by a houselight (1.12-W, #120 incandescent bulb) mounted on the left wall of the experimental chamber.

Within these chambers, two contexts could be created. Context 1 (training context) was distinct from Context 2 (nontraining context). In Context 1, the houselight was on and there was a grid floor. By contrast, in Context 2, the houselight was off and the floor was covered with a sheet of Plexiglas. Moreover, for each rat, a different instance of the chambers was used for Context 2 and Context 1.

The CS was a complex tone composed of two frequencies, 3000 and 3200 Hz, presented simultaneously. It was delivered 6 dB (C scale) above background by a speaker on the inside wall of the chamber. Ventilation fans in each chest provided a constant background noise of 74 dB (C scale). The US consisted of a 0.3-s footshock, which co-terminated with the CS (see below for the magnitude of the shock).

Procedure

Table 1 summarizes the experimental design. The four groups in this study constituted a 2 (CS duration [30 s vs. 120 s]) $\times$ 2 (Training [Online vs. Offline]) design. All subjects were tested in both the training context and a distinctively different one. The subjects were randomly assigned to one of four experimental groups ($n = 12$), counterbalanced within groups for sex: 30 s-online, 30 s-offline, 120 s-online, and 120 s-offline. The four groups were trained using a bar-press procedure. Prior to conditioning, an 8-day acclimation and shaping regimen established stable rates of bar-pressing for a 0.04-ml water reward. Each of these acclimation days involved at least one 60-min session. To facilitate magazine training, the onset of the water delivery was accompanied by the onset of a 0.5-s buzzing sound. On Days 1 and 2, a fixed-time 2-min schedule of noncontingent reward was concurrent with a continuous reinforcement schedule. Rats that made fewer than 10 bar-presses were given an extra 30-min session later that day. On Day 1, bar-press training took place in the training context; on Day 2, it took place in the nontraining context. On Days 3 and 4, the noncontingent water delivery was discontinued, and subjects were trained on the continuous reinforcement schedule alone. All subjects that emitted fewer than 50 responses on any of these days were later placed back in the chambers and hand-shaped through successive

approximation for 30 min. Acclimation and shaping took place in the training context on Day 3 and in the nontraining context on Day 4. The sessions on Days 5 to 8 provided water on a VI 20-s schedule, which prevailed throughout the remainder of the experiment, whenever bar-pressing was permitted, including during testing. On Days 5 and 7, the rats were exposed to the training context; on Days 6 and 8, the rats were given equivalent exposure to the nontraining context.

On Day 9, conditioning was initiated in the training context. All subjects were exposed to three CS-US pairings per session: The mean intertrial interval (ITI) was 10 min (range: 8–12 min). Mean session duration was 30 min. Subjects in the two short CS groups were exposed to a 30-s CS while subjects in the two long CS groups were exposed to a 120-s CS. The 0.3-s footshock US, initially 0.50-mA, was delivered during the last 0.3 s of the CS. For the two offline groups, the manipulandum was retracted during training, whereas it remained present for the subjects in the two on-line groups.

Each day, the rate of bar pressing by the two online groups was recorded for the 3 min preceding each of the three CS presentations. If, at the end of a session, any of these three rates fell below 50% of baseline (i.e., the last day of bar-press shaping in the training context), the CS-US pairing phase was suspended and all four groups of rats received at least two more days of reshaping (one 60-min session per day, in the training context). The CS-US pairings did not resume until responding was at least equal to what it was on Day 6 of shaping. Responding fell below 50% of baseline only once during the study. On the second day of training, the rats in all groups greatly reduced responding during the remainder of the session once the first US had been presented. It took three days of reshaping to restore them to baseline. When training resumed, the shock intensity was lowered from 0.50 mA to 0.35 mA to reduce disruption of baseline responding. The rats subsequently stayed on baseline for the remainder of the experiment. They were still perceiving the shock as indicated by the fact that lever pressing continued to be suppressed during the CS in the online groups (see below). Overall, the rats were trained for 29 days: The 2 first days with a 0.50-mA footshock, the remaining 27 days with a 0.35-mA footshock. The online groups received water (but no buzzer with the water) on the VI-20-s schedule.

Once training was completed, the rats entered a reshaping stage, which consisted of a 1-h session where lever-pressing was reinforced with water according to a VI 20-s schedule. For half of the rats (counterbalanced across sex within groups), this was done in the training context, and, for the other half of the rats, this was done in the nontraining context. For the first test, the rats were placed for 10 min in the test context in which they had been reshaped. Bar-pressing was still reinforced with water according to a VI 20-s schedule. Five minutes into the session, the CS was presented for 30 s to rats in the 30-s groups, and for 120 s to rats in the 120-s groups. To assess baseline responding, the rate of bar-pressing was recorded in the minute immediately preceding the CS presentation. Responding during the CS was recorded during twelve 2.5-s bins for a 30-s CS and twelve 10-s bins for a 120-s CS, so that the temporal pattern of responding during the CS could be plotted in relative time. This permitted us to test for any timescale invariant property of responding (Gibbon & Balsam, 1981), if any inhibition of delay occurred. Subjects were then given two more days of reshaping. This stage was identical to the previous reshaping stage except that the rats that were reshaped previously in the training context were now reshaped in the nontraining context and vice versa. Finally, the rats were tested a second time. This test stage was identical to the previous test stage except for the change in context.

Results

For each day of training, we looked at how the suppression ratio evolved during the CS. CS_{ij} is the rate of responding for a rat for temporal bin number j during the i th presentation of the CS ($i = [1, 2, 3]$). $preCS_i$ is the rate of lever-pressing in the minute preceding the presentation of CS_i (we did not adjust the duration of the baseline to the duration of the CS, as it is used to assess the suppression ratio over 2.5-s or 10-s bins, not over the whole CS duration). The suppression ratio for that rat for bin number j is

$$sr(j) = \frac{\sum_{i=1}^3 CS_{ij}}{\sum_{i=1}^3 CS_{ij} + \sum_{i=1}^3 preCS_i} \quad (1)$$

Figure 1 shows the mean suppression ratio for the online condition as a function of relative time in the CS and total CS duration for the last six days of conditioning. As can be seen, suppression remained fairly constant during the whole CS for both CS durations. A mixed design ANOVA using relative time in the CS and CS duration as factors found only a main effect of CS duration, $F(1, 22) = 31.26$, $p < 0.01$. Neither an effect of relative time nor an interaction was detected.

On each day of testing, we recorded the rate of bar-pressing in the minute preceding CS onset and conducted a mixed design ANOVA on the baseline rate of responding using CS duration (30 s vs. 120 s), training mode (on-line vs. off-line), context (training context vs. retraining context), and order of testing (first tested in the training context vs. first tested in the retraining context) as factors to check baseline differences between the groups prior to the CS presentation. This analysis found a significant interaction between context and CS duration, $F(1, 40) = 6.61$, $p < 0.05$, and among context, CS duration, and training mode, $F(1, 40) = 7.01$, $p < 0.01$. This last interaction revealed a rather complex pattern in the data, which was not reflected in any way in the analysis of suppression ratios. Hence, it is unlikely that baseline differences influenced our critical results.

Figure 2 depicts mean suppression ratios during testing as a function of time in the CS, groups, and context of testing. The suppression ratio in bin j for each rat was computed with CS_j being the rate of responding for a rat for relative time bin number j during the CS, and $preCS$ being the response rate during the last minute before the CS presentation.

$$sr(j) = \frac{CS_j}{CS_j + preCS} \quad (2)$$

Comparing Figure 1 to Figure 2, it seems that the overall level of suppression for the two tests was similar to the pattern during training although somewhat lower for the 30-s groups in Figure 2. The mean suppression ratio was approximately 0.1 (vs. approximately 0.2 in Figure 1) for the groups exposed to a 30-s CS, independent of whether training occurred online or offline, and around 0.4 for the groups exposed to a 120-s CS, independent of whether training occurred online and offline. The change of context did not seem to have any effect on the two 30-s groups. Suppression remained constant during the entire CS, independent of the context of testing. In contrast, for the two 120-s groups, although suppression seemed roughly constant during the CS when the test took place in the training context (even though suppression appears a little bit higher during the first 10 seconds of responding), the reactive pattern clearly appeared when the rats were tested in the nontraining context, that is, suppression decreased as a function of time in the CS. This effect was more obvious for the group trained offline than for the group trained online. An

ANOVA limited to the 120-s groups using relative time in the CS, context, training mode, and order of testing as factors detected a main effect of relative time into the CS, $F(11, 220) = 2.42$, $p < 0.01$, and an interaction between time into the CS and context of test, $F(11, 220) = 2.01$, $p < 0.05$, with the interaction making the main effect of relative time into the CS inconsequential. Further analysis found an effect of relative time into the CS for the 120-s groups tested in the training context, $F(11, 220) = 2.08$, $p < 0.05$, but this effect was entirely due to the slightly lower suppression ratio for the first bin of responding (see Figure 2). If this data point is removed, the effect of relative time disappears, $F(10, 220) = 1.02$, $p = 0.43$). In contrast, the effect of relative time into the CS found for the 120-s groups tested in the nontraining context, $F(11, 220) = 2.24$, $p < 0.05$, did not disappear when the first data point was excluded from the analysis, $F(10, 220) = 2.38$, $p < 0.05$. The order of testing was never part of any main effect or interaction. Hence, overall, this analysis confirms what is apparent in Figure 2. Relative time into the CS had little effect on the pattern of suppression during the CS in the long CS condition when the rats were tested in the training context, at least past the first ten seconds of responding, whereas a reactive pattern appeared when the rats were tested in the nontraining context.

Discussion

Two common procedural features of lick suppression procedures not found in most bar-press suppression studies are offline training and a training-test context shift. The present experiment was designed to determine whether these differences could be responsible for the emergence of the reactive pattern of responding observed in lick suppression by Jozefowicz et al. (2011) by assessing their effect on the temporal distribution of responding in a bar-press suppression procedure.

As Figure 1 shows, the reactive pattern was not observed during training in the conditioned bar-press suppression procedure. Although we failed to obtain inhibition of delay, the observed constant level of suppression seen across the CS is the pattern commonly reported before anticipatory temporal regulation develops. The actual number of CS-US pairings necessary for inhibition of delay to appear in bar-press suppression is not clear and seems to vary greatly across studies. Overall, our rats received 87 CS-US pairings. Although in some studies (i.e., Rosas & Alonso, 1996), this number has proven sufficient to obtain anticipatory timed behavior, it was not with our procedure and parameters nor was it in some other studies (e.g., Hendry & Van-Toller, 1965; Libby & Church, 1975, in which more than 300 pairings were necessary to obtain inhibition of delay).

No effect of the mode of training nor of the testing context was observed in the 30-s groups, probably because of a floor effect. Otherwise, a reactive pattern replaced the constant rate of suppression observed during training for the rats tested in the nontraining context. This effect was somewhat more pronounced for the rats trained offline rather than online, although inferential statistics did not fully support this conclusion. A suggestion of a reactive pattern was also observed in the long CS duration groups tested in the training context, as the suppression was slightly (although not statistically) higher during the first 10 seconds of the CS.

This suggests that a change in the context, either physical or temporal, is critical for the expression of the reactive pattern. Given that at test, the rats were exposed to the CS for the first time after a retention period of three days, there is a potential change in the temporal context, which might explain the weak reactive pattern observed in the rats tested in the training context, even though there was no trace of it during training (see Bouton, 1993). The reactive pattern was stronger for the rats exposed to both a physical and a temporal context change, that is to say the rats tested in the nontraining context. The (non-significant)

effect of the mode of training observed in those rats could also be explained by an effect of the context change. For rats trained online, the new test context shares with the training context the lever, responses on the lever, and water delivery with the training context. Consequently, it is less novel than for the rats trained offline for which the lever was never present in the training context. This fits with the results of Rescorla (1968), which yielded a reactive pattern after training rats offline. However, Bouton et al. (2008) observed a reactive pattern even though their animals were trained online and tested in the training context. Hence, it seems that there are still unidentified variables that might be relevant to the expression of the reactive pattern.

Overall, those data replicated in a bar-press suppression procedure the reactive pattern observed by Jozefowicz et al. (2011) in a lick suppression procedure. Jozefowicz et al. argued that the reactive component of responding was an unconditioned component, caused by the mere exposure to the US, driven by variations in nonassociative learning (habituation and sensitization). As changes in the perceptual properties of a habituated stimulus are known to result in elevated unconditioned responding to the stimulus relative to baseline (i.e. sensitization can be observed to a previously habituated stimulus after a change in the perceptual properties of the stimulus), this might explain the effect of the context switch observed in the present experiment.

Further research is necessary to better understand the nature of this unconditioned component as well as the processes underlying it and the variables, besides context change, which affect it. In the meantime, the present research is a cautionary tale for researchers who might be using procedures involving context switch and whose dependent variables might be affected by the reactive pattern. For instance, Rosas and Alonso (1996, 1997) reported that, in rats that displayed inhibition of delay in a bar-press suppression paradigm, testing after a retention interval and/or in a new context flattened the temporal pattern of suppression during the CS, mainly because the rats suppressed more at the beginning of the CS. They concluded that the memory for the CS-US interval was weaker and more prone to interference than the memory for the CS-US association. An alternative explanation, suggested by the present data, is that, by switching the physical and/or temporal context, Rosas and Alonso induced an unconditioned reactive pattern in their animals. This might explain the higher suppression at the beginning of their CS and, hence, the apparent flattening of the response curve, which could have been mistaken for a loss of temporal control.

Highlights

- Rats were exposed to a conditioned bar-press suppression.
- Three variables were manipulated: (1) The CS duration (30 vs 120 s), (b) the mode of training (online vs. offline), (c) the testing context (training vs. nontraining context).
- Suppression in the 120-s group was a decreasing function of time in the CS when the animals were tested in the nontraining context.
- This clarifies some of the conditions necessary to obtain a reactive pattern of suppression in bar-press suppression previously reported by Jozefowicz, Witnauer, & Miller (2011).

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Figure 1.

Bar-press suppression ratio as a function of relative time in the CS, and CS duration for the two online groups during the last 6 sessions of training. Bars represents standard errors.

Figure 2. Bar-press suppression ratio as a function of relative time at test in the training and nontraining context. Bars represents standard errors.

Table 1

Experimental design

Group	Training	Training mode
30 s-online	30-s X+	Online
30 s-offline	30-s X+	Offline
120 s-online	120-s X+	Online
120 s-offline	120-s X+	Offline

Note: X was a complex tone. + indicates footshock at the end of the CS.