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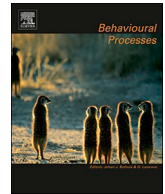
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Timing and the transition between modes in the defensive behavior system

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ABSTRACT

Antipredator defense is organized in a way that mirrors Timberlake's feeding behavior system because the goal of defense is to thwart predatory behavior. Each predatory mode has a corresponding antipredator mode. Like appetitive behavior systems, the defensive behavior system is organized around distinct modes along a spatio-temporal continuum we call the predatory imminence continuum. Behavior systems theory directs investigation toward the factors that lead to transitions between modes. In the feeding and sex systems the time between Conditional Stimulus (CS) and Unconditional Stimulus (US; e.g., CS-US interval or CS duration) is an important factor. Short CSs elicit conditional responses (CR) characteristic of more terminal modes and long CSs provoke CRs belonging to initial modes. Therefore, we asked if short CSs (10 s) would provoke CRs like the vigorous activity bursts and escape-like responses characteristic of the terminal mode of the predatory imminence continuum (Circa-Strike Behavior). Also, via analogy to appetitive systems, long CSs (3 min) were predicted to favor the intermediate mode, post-encounter behavior, which is characterized by freezing. Instead we found that both CSs produced freezing but not activity burst CRs and that freezing was actually greater with the short CS. We suggest that this difference between behavior systems flows from selection pressure that favors moving toward terminal modes in appetitive systems but away from terminal modes in the antipredator system. In addition, since appetitive reinforcers are more likely to be repeatedly experienced than predators, the learning of timing may be less relevant to defense. We also found that shock produced activity bursts and argue that when you are in the post-encounter mode (freezing) a sudden change in stimulation causes an immediate transition to circa-strike (terminal) behavior.

1. Introduction

Bill Timberlake saw behavioral systems as being organized around a sequence of modes consisting of functionally related behaviors. Each of these modes were anchored by their spatiotemporal distance from the commodity or functional goal that the system was exploiting (e.g., food in the predatory behavior system, (Timberlake, 1994). For example, in the case of predation the General Search Mode, the Focal Search Mode and the Handling/Consumption Mode are progressively closer to the food because you must search before you can procure food, and procure it before you consume. Each mode has a unique set of behaviors appropriate for its distance from the goal. The organization of predation has immediate implications for antipredator defensive behavior because the main objective of defense is to thwart predation. Each mode of predation should have a corresponding defensive mode because different behaviors would be needed to thwart search versus consumption.

The defensive behavior system is organized around what we call the predatory imminence continuum (PIC) anchored on one end by the

absence of threat, or as close to absence as the species under consideration enjoys (see Fig. 1, Fanselow and Lester, 1988). At this lowest level of predatory imminence, the animal engages in its preferred activity pattern optimally balancing the demands of energy needs, nest- and self-maintenance and reproductive activities. However, if some risk is associated with these activities, such as an increased possibility of encountering a predator while foraging for food, there will be modifications in these behaviors in a way that reduces the likelihood of such an encounter. When rats leave their nest for a potentially dangerous foraging excursion they do so with a stretched approach posture and adjust their meal size to more fully exploit a patch of food reducing the total time needed to forage (Fanselow et al., 1988). This phase of defense is called the Pre-Encounter Mode (Table 1).

Once an actual threat is detected, when things have moved from potential to present danger, behavior must change to thwart detection and attack. This is the Post-Encounter Mode and in Timberlake's terminology the dominant behavioral module in this mode is freezing. Freezing can be thought of as a behavioral module because it is not simply movement arrest (Fanselow et al., 1997; Sigmundi, 1997). When

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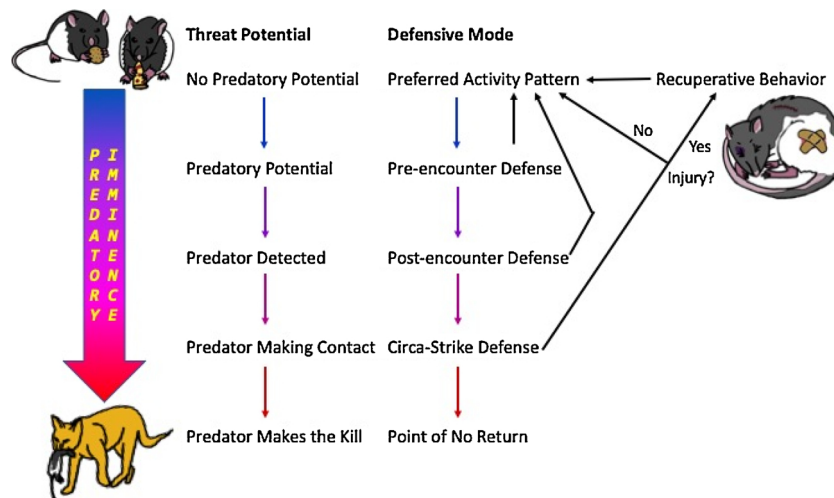


Fig. 1. The Predatory Imminence Continuum Model. Based on Fanselow and Lester, 1988.

Table 1

The organization of defense into modes.

Modes of defense→	Pre-Encounter	Post-Encounter	Circa-Strike
Predatory Behavior Mode→	Foraging	Search & Procure	Handling & Consumption
Function of Defensive Mode→	Reduce the likelihood of encountering a predator	Decrease the likelihood of detection and attack	Survive direct contact with a predator
State	anxiety	fear	panic
Antecedent Stimuli→	Past experiences with predation or threats	Detection of a predator or signal for imminent threat	A striking predator is making or is about to make physical contact
Consequent Behaviors→	Stretched approach, alterations in meal patterns (less frequent larger meals), retreat to nest	Freezing and thigmotaxis	Audible vocalizations (scream), vigorous escape attempts. Protean movement, jumping, darting.

rats freeze they first invariably move to nearby objects and shelter and then freeze there; part of the freezing module is to move to a readily accessible optimal location for freezing (de Oca et al., 2007). In other words, freezing and a form of movement called thigmotaxis are part of the same response module (Grossen and Kelley, 1972). Indeed, rats freeze more if they have easy access to hiding places that provide optimal places to freeze (de Oca et al., 2007). This is not to say that thigmotaxis is always accompanied by freezing. Movement along walls is a different form of thigmotaxis that occurs during pre-encounter defense and is frequently seen in tests of anxiety such as the open field (Delprato et al., 2017). Rather, when freezing occurs it is always near a wall or object.

While most studied in rodents, freezing has been reported in species ranging at the least from humans (Roelofs et al., 2010) to frogs (Cooper et al., 2008). There are two reasons why freezing is so ubiquitous. First, most visual systems are optimized for the detection of motion. Second, for most predators the releasing stimulus for attack is movement and there is substantial evidence showing that motionless prey have a higher chance of survival than their moving counterparts (e.g., Herzog and Burghardt, 1974; Kaufman, 1974). If Post-Encounter defense fails the prey enters the final anti-predator mode, Circa-Strike Defense, which only occurs immediately before or during physical contact. Note that there has to be occasions when every defensive mode fails; otherwise the predator dependent on that prey would cease to exist. When circa-strike defense fails we reach the final anchor of the PIC and the predator consumes the prey.

A fundamental problem within a behavior systems approach is to define the conditions that trigger a shift between adjacent modes. For both sexual behavior and feeding the temporal interval between onset of a conditional stimulus (CS) and the presentation of the unconditional stimulus (US) appears to be an important factor (Akins et al., 1994; Silva et al., 1998). When the CS-US interval is short the CS produces behaviors characteristic of the more terminal modes (e.g.,

consumption) but when this interval is long the conditional response (CR) resembles the behaviors of more distal modes (general or focal search). For defensive behavioral topography the role of CS-US interval, or CS duration, has received less attention than in appetitive systems. If the rules from the appetitive behavior systems (feeding and sex) generalize to defensive behavior one might expect to see freezing with relatively long CSs and circa-strike behaviors with short CSs. While such a prediction was suggested by Bouton et al. (2001) it has not been empirically tested.

One thing that does cause a switch from freezing to the vigorous locomotion characteristic of circa-strike behavior is the footshock US used in fear conditioning (Fanselow, 1982). While CSs paired with footshock produce a freezing CR, the unconditional reaction (UR) to shock is vigorous activity. In one experiment, rats were placed in a conditioning chamber and after 3 min they received a series of footshocks. When the rats were placed into the same context the next day, they froze likely because of contextual fear conditioning. After 3 min a 0.75 s shock was introduced that immediately disrupted freezing. The activity burst behavior during that period was characterized by running (during the shock), locomotion, turning and rearing. Similar reactions were produced by a sudden presentation of a multimodal (light, sound & vibration) nonpainful stimulus (Fanselow, 1984). Such findings have led to the view that post-encounter defensive mode uses associative fear conditioning as its primary underlying mechanism (Fanselow, 2018). That is, post-encounter defense is mediated primarily by conditional responses such as freezing, while circa-strike defenses, such as activity bursts, are primarily unconditional responses to contact related stimuli (Fanselow, 1989). Such a view predicts that freezing should track associative strength. Since the acquisition of associative strength is, in part, inversely related to CS-US interval (Gibbon and Balsam, 1981; Rescorla and Wagner, 1972), this view of post-encounter defense suggests that freezing should actually be greater with short CS-US intervals.

Therefore, in the present experiments we compared a long and short CS to test between these hypotheses. The first being that, by analogy with the feeding and reproductive systems, long CSs would produce relatively more freezing and shorter CSs would produce relatively more activity burst-like behavior. The second being, that if post-encounter defense reflects conditional responses to contact-relevant stimuli and circa-strike defense reflects unconditional responses to contact-relevant stimuli, then freezing should be greater with a short CS but neither CS should produce circa-strike behaviors (Fanselow, 1989). For the long CS we chose 3 min with an 18 min intertrial interval (ITI) because Fanselow and Lester (1988) using those intervals found strong evidence for freezing as a CR despite several opportunities for other behaviors such as escape. Additionally, in the activity burst experiments described above (Fanselow, 1982, 1984) shock was administered 3 min after placement in the conditioning context corresponding to a 3 min contextual CS duration. For the shorter CS we chose 10 s because the multimodal stimulus used previously to generate an activity burst caused a 12 s disruption of freezing (Fanselow, 1984). Shock-elicited activity bursts last between 7 and 45 s depending on shock intensity (Fanselow, 1982). Therefore, the short CS–US interval was well within the window for circa-strike behaviors. For the short CS condition the ITI was reduced to 60 s to keep the CS-US interval to ITI ratio similar to the long CS parameters because the ratio of CS duration to intertrial interval is an important determinant of conditioning (Gallistel, 1990; Gibbon and Balsam, 1981) and can also influence the topography of behavior (Burns and Domjan, 2001). A group that received only the shock during training but was tested with the CS was included as an associative control so that potential nonassociative factors contributing to CS-induced responding could be assessed. We chose this control over an unpaired control because unpaired controls may underestimate the presence of such nonassociative factors because of habituation or the development of conditioned inhibition.

2. Material and methods

2.1. Subjects

Adult female and male Long Evans rats (90–120 days old at start of experiment) from our in-house breeding colony were used in the current experiment. Rats were pair housed and maintained on a 12-h light/dark cycle with ad libitum food and water. All rats were handled for 5 days (approx. 1 min/day) prior to behavioral testing. All procedures were conducted with approval from the University of California Los Angeles Institutional Care and Use Committee and were in compliance with the National Institutes of Health Guide for the Care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

2.2. Apparatus

All behavioral testing was conducted in two rooms with sets of four identical fear conditioning chambers (30 × 25 × 25 cm; Med Associates) equipped with an automated VideoFreeze behavior scoring system (Med Associates). Percent time freezing to auditory stimuli and context were recorded and measured by this system, which was calibrated to a trained observer (VideoFreeze; Med Associates). A motion index was also collected by the software for an analysis of motion characteristic of an activity burst (elaborated below).

2.3. Procedures

Rats were pseudo-randomly assigned to one of three conditioning groups that varied in the CS duration during conditioning (Long CS, Short CS, and Shock Only Control) such that the total n for each group consisted of 4 male and 4 female rats. Following a 180 s baseline period, all groups received 3 conditioning trials with 2 s/0.75 mA footshock. Auditory CSs were 2900 Hz/75 dB tones. The Long CS group's CS

duration was 180-sec with an 18-min ITI (shock onset to shock onset). The Short CS group's CS duration was 10-sec with a 60-sec ITI. Thus, the ratio of CS–US interval to ITI was 0.167 for both conditioning groups. For both CS groups shock onset was simultaneous with CS termination. The Shock Only group received the same shock presentations as the Short group, but without auditory CSs.

2.3.1. Fear conditioning and testing

One day prior to fear conditioning, rats were habituated to transport where they were moved from the vivarium to the testing room in the same manner as they were transported during the experiment proper. On day 1 (training), rats were transported to the chamber and underwent fear conditioning based on group assignment (Long CS, Short CS, or Shock Only). Thirty seconds following the last shock, all rats were removed from the chamber and returned to the vivarium.

The following day, all groups were tested for tone CS fear in a novel context. The novel context differed by transport method, physical location, chamber shape, lighting, odor, and flooring. All groups were tested the same with 180-sec baseline period followed by a single 180-sec tone and were removed from the chamber 30-sec following the end of the tone.

Four hours later, rats were transported to the same context where they received conditioning and tested for context fear and tone fear memory under the same parameters as the first tone test (180-sec CS duration).

2.4. Measures and analyses

2.4.1. Freezing

Freezing was scored automatically by Med Associates VideoFreeze Software. The system compares successive videoframes at 30 frames a second and sums the total grayscale change in each pixel between frames. If that value does not exceed a threshold value for one second the rat is scored as freezing for that time period. The threshold was determined so that the automated system's output closely approximated that of a highly trained observer. The data are plotted as the percentage of time scored as freezing during a period of interest.

2.4.2. Activity Burst

In terms of behavior in the face of an actual predator, the rodent circa-strike activity burst would be characterized by sudden and rapid flight and/or jumps in an attempt to escape the clutches of the predator. To capture this type of activity we took the peak activity score during a period of interest (e.g., the first 10 s of CS presentation = During) and calculated a ratio of that level of activity to a similar score derived from a preceding control period of equal duration (e.g., 10 s before presentation of the tone = PreStim) of the form During/(During + PreStim). We call this the Peak Activity Ratio (PAR) where a 0.5 indicates that during the time of interest there was no instance of activity greater than that observed during the control period (PreStim). PARs approaching 1.0 indicate an instance of behavior that far exceeded baseline responding. The video analysis system compares adjacent video frames, which are collected at 30 frames a sec. The peak score is taken from the pair of video frames that has the greatest degree of pixel change during the interrogated interval. This method is excellent at capturing a single instance of ballistic movement such as a jump or dart that others have suggested might occur during intense fear (Fadok et al., 2017; Gruene et al., 2015). The measure is insensitive to activity that occurs between frames other than those used to calculate the peak score (e.g., only the biggest jump or dart matters). We also used a similar ratio calculated on the average pixel change score during the entire interval of interest. That measure produced very similar results and no change in conclusions. However, the average measure was strongly influenced by any freezing that occurred during the interval (the system defines freezing as pixel change below a very low threshold that filters out video noise) and therefore the average score was less of a

pure activity burst measure.

2.4.3. Analyses

When group variances were homogenous the data were analyzed by ANOVA. In the one instance where there was significant heterogeneity of variance we used nonparametric analyses. The acquisition session was analyzed by Group X Trials factorial ANOVA. Subsequent pairwise contrasts were considered significant when they exceeded $p < .050$ using a Bonferroni correction to hold alpha constant. Test session data were analyzed by one-way ANOVA with subsequent pairwise contrasts using Tukey's Test for multiple comparisons. Since the data were in terms of percentages (freezing) or ratios (Activity Bursts) all data for the parametric tests were subjected to an Arc Sine transformation $\{2(\arcsine(\sqrt{x}))\}$. However, for the sake of comparison to other published data all the figures present mean and SEM of the original percent or ratio measures. Our initial analyses included sex as a factor. There were no sex differences or interactions, so we reanalyzed all data collapsed over this factor.

3. Results

3.1. Freezing

3.1.1. Acquisition Session

Data for acquisition are presented in Fig. 2. Rats did not freeze upon placement into the chamber nor during the first CS presentation. Freezing increased in all groups after the first shock and throughout acquisition resulting in a significant main effect of trials, $F(2,42) = 120.40$, $p < .001$. There was also a significant main effect for groups ($F(2,21) = 6.24$, $p = .007$). Over successive CS presentations (or an equivalent preshock period in the Shock Only group) freezing increased most rapidly in the Short CS group resulting in a significant trials x group interaction ($F(4,42) = 11.54$, $p < .001$). The Short CS group froze significantly more than either the Long CS or the Shock Only group on trial 2 (contrasts made with Bonferroni correction, significance at $p < 0.001$). The Long CS group froze less than the other two groups on trial 3 ($p < 0.050$).

3.1.2. Novel Context Test Session

3.1.2.1. Baseline. Prior to CS presentation in the test environment, baseline freezing was different between groups, $F(2, 21) = 7.96$, $p = .003$ (see Fig. 3, first panel). Contrasts between groups were made with Tukey's test for multiple contrasts to hold $p < .050$ as the

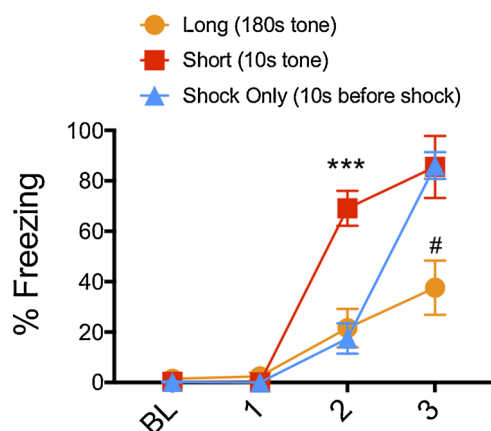


Fig. 2. Acquisition: The mean (\pm S.E.M) percent time spent freezing during the training session. Baseline (BL) is the 3 min prior to shock. Data show freezing during the 3 tones presented during training. The shock only group shows 10 s that corresponded to the time when the short CS group received the tone. *** $p < 0.001$ Short vs. Long and Shock Only; # $p < 0.050$ Long vs. Short and Shock Only.

threshold for significance for all between group ANOVA's. The Long CS group had less baseline freezing than either the short ($p = .016$), or Shock Only ($p = .003$) groups. Baseline freezing in this novel environment reflects generalized contextual fear (Jacobs et al., 2010) and was likely lower in the Long CS group because the shocks were distributed over a longer acquisition session allowing for more extinction of contextual fear during the ITI.

3.1.2.2. First 10 s of tone test. When averaged over the first 10 s of the test tone, or an equivalent period in the Shock Only group, there was a significant main effect for group, $F(2,21) = 4.54$, $p = .023$. The Short CS group froze more than the Long CS ($p = .046$), or the Shock Only group ($p = .038$; see Fig. 3, middle panel).

3.1.2.3. Three min tone test. When freezing was analyzed for the entire 180-sec tone presentation the difference fell just short of significance, $F(2,21) = 3.43$, $p = .051$ (see Fig. 3, 3rd panel). The pattern was similar to that obtained during the first 10 s with greater freezing in the Short CS group.

3.1.3. Training Context Tone Test

3.1.3.1. Baseline. Albeit greater, the pattern of freezing during the baseline period in the original training context was similar to that in the novel context with a significant between groups difference, $F(2,21) = 10.55$, $p = .001$. Again, the Long CS group froze less than the Short CS ($p = .009$), or Shock Only ($p = .001$) groups (see Fig. 4, first panel).

3.1.3.2. First 10 s of tone test. The freezing data during the first 10 s of the tone CS in the training context paralleled that for the novel context test but the overall group difference was not significant via ANOVA, $F(2,21) = 3.23$, $p = .060$. This was probably related to marked heterogeneity of variance where all but one of the rats in the Short CS group froze 100% of the time and the other two groups were quite variable (see Figure, 2nd panel). Indeed, the Brown-Forsythe test for heterogeneity of variance indicated significant heterogeneity of variance, $F(2,21) = 6.04$, $p = .009$, for only this particular data set. Therefore, we applied a nonparametric analysis to these data. An overall Kruskal-Wallis analysis indicated a significant difference between groups, $H(2,24) = 7.07$, $p = .029$. Follow-up contrasts using Mann-Whitney's pairwise comparisons indicated that the Short CS group (median = 97.81) froze significantly more, $U(8,8) = 7$, $p = .010$, than the Long CS group (Median = 47.56). The Shock Only group showed an intermediate level of freezing (median = 65.14) that was not significantly different from either of the tone trained conditions.

3.1.3.3. Three min tone test. When freezing was analyzed for the entire 180 s tone presentation the main effect for groups was significant, $F(2,21) = 4.95$, $p = .017$. Again, the Short CS group froze more than the Long CS group ($p = 0.014$, see Fig. 4, last panel). No other group differences were significant.

3.2. Activity Bursts

3.2.1. Activity burst during the novel context test session

3.2.1.1. Initial 10 s of CS presentation. Fig. 5 (first panel) shows the Peak Activity Ratio (PAR) at the initial onset of the tone CS (first 10 s) with respect to 10 s immediately prior to the tone. The two conditioned groups had ratios slightly below 0.5 indicating that the tone if anything decreased activity. The Shock Only group, for which the tone was novel, had a ratio slightly over 0.5. The differences between groups fell short of statistical significance, $F(2,21) = 1.60$, $p = 0.226$. Thus, there is no indication that either conditioning procedure resulted in a conditioned circa-strike-like activity burst at CS onset.

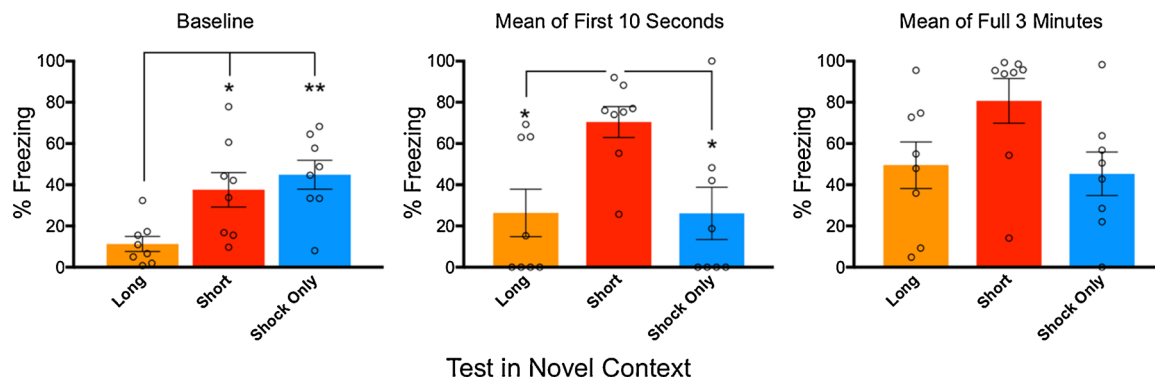


Fig. 3. The mean (\pm S.E.M) freezing during the test session in the novel context. Panel 1: Freezing (percent time) during the initial 3 min baseline period. Panel 2: Freezing during the first 10 s of the tone. All groups received a 3 min tone regardless of training condition. Panel 3: The percent time spent freezing averaged over the full 3 min of the CS test. * $p < 0.050$; ** $p < 0.010$.

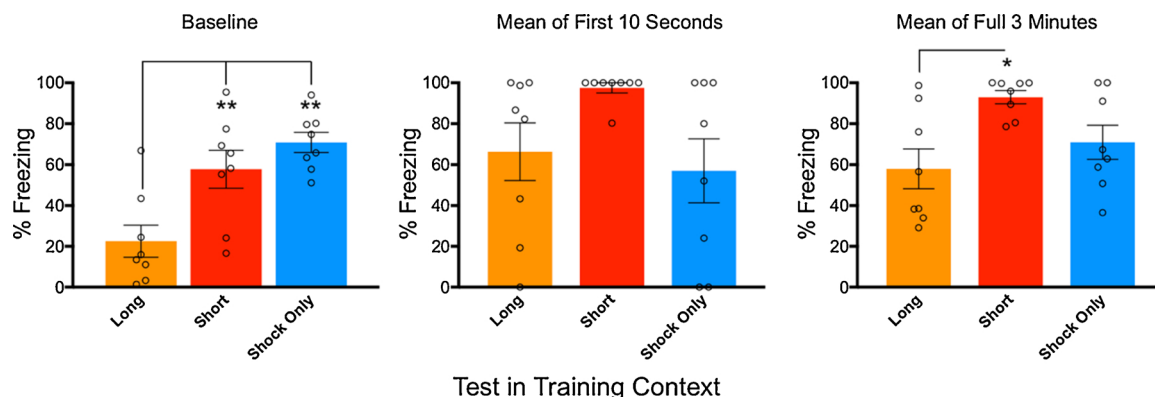


Fig. 4. The mean (\pm S.E.M) freezing during the test session in the training context. Panel 1: Freezing (percent time) during the initial 3 min baseline period. Panel 2: Freezing during the first 10 s of the tone. All groups received a 3 min tone regardless of training condition. Panel 3: The percent time spent freezing averaged over the full 3 min of the CS test. * $p < 0.050$; ** $p < 0.010$.

3.2.1.2. *Final 10 s of CS presentation.* This period corresponds to when the Long CS group might have expected shock. Again, both trained groups had PARs below 0.5 suggesting a complete absence of conditioned activity bursts (Fig. 5, 2nd panel). The Shock Only group had a ratio slightly above 0.5. There was a significant difference between groups, $F(2,21) = 7.62$, $p = .003$ and Tukey’s test indicated that this was because the Short CS group had a lower PAR than the Shock Only controls ($p = 0.002$). This is likely because of freezing in the Short CS group, which is interesting because this freezing was occurring despite the fact that the period interrogated was long after an expected shock. In any case, the data provide no support for a CS-elicited circa-strike response. Indeed, the observation with the short CS

was in the opposite direction from that expected if there was an activity burst CR.

3.2.2. *Activity burst during the training context tone test*

3.2.2.1. *Initial 10 s of CS presentation.* The two tone trained groups showed PARs substantially below 0.5, while the Shock Only controls were closer to 0.5 (see Fig. 6, first panel). The group differences fell short of statistical significance, $F(2,21) = 3.19$, $p = 0.062$. What is again clear is that we failed to detect any vigorous activity during tone onset. Indeed, rats trained with the Short CS showed a marked suppression of the peak activity, which probably reflects their very high level of freezing.

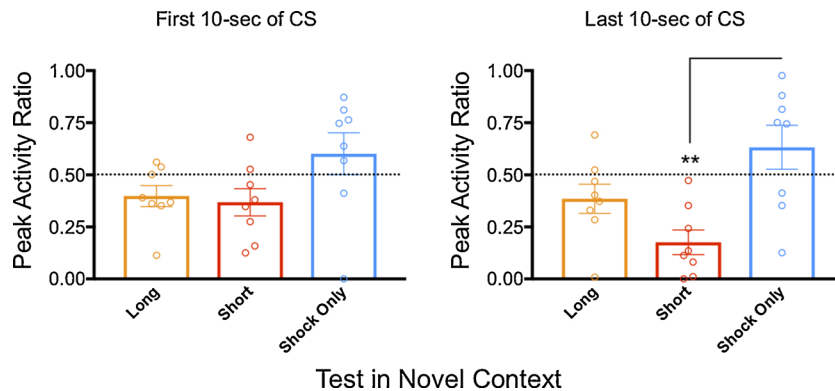


Fig. 5. The activity burst, expressed as the mean (\pm S.E.M) Peak Activity Ratio (PAR), during the tone test in the novel context. Panel 1: PAR for the first 10 s of the 3 min tone test. Panel 2: PAR for the last 10 s (170–180 sec) of the 3 min tone test. Ratios are with respect to the 10 s period prior to tone onset. *** $p < 0.010$.

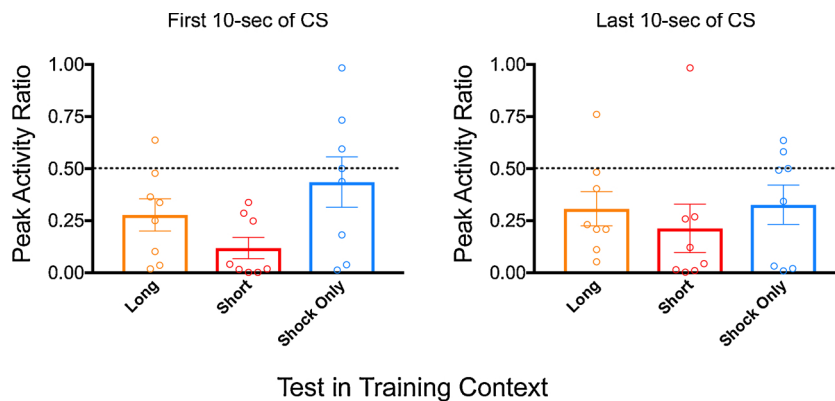


Fig. 6. The activity burst, expressed as the mean (\pm S.E.M) Peak Activity Ratio (PAR), during the tone test in the training context. Panel 1: PAR for the first 10 s of the 3 min tone test. Panel 2: PAR for the last 10 s (170–180 sec) of the 3 min tone test. Ratios are with respect to the 10 s period prior to tone onset.

3.2.2.2. *Final 10 s of CS presentation.* This period corresponds to the time when the Long CS group might be expected to anticipate the shock. There was no significant between groups difference in the PAR, $F(2,21) = 0.38$, $p = 0.690$. Critically, all ratios were below 0.5 indicating that the rats were engaged in post-encounter freezing rather than circa-strike activity bursts (Fig. 6, 2nd panel).

3.2.3. *Shock-elicited activity bursts*

We found no evidence of tone elicited activity bursts to the auditory stimuli. By using the peak activity measure we should have been able to capture any sudden increase in activity even if it was very short. To ascertain if our measure was sensitive to such activity we determined if shock itself was capable of producing an activity burst that was reflected in the PAR. We did this in two ways. One was a within-subject test on the Shock Only group where we calculated PAR prior to the first shock to that including and following the first shock. The other was to compare PAR to the first tone onset in the tone trained animals to the first shock in the Shock Only rats. Both measures showed clear evidence for shock-elicited activity bursts.

In Shock Only rats we calculated a PAR for the 10 s immediately prior to shock and for 10 s that included the 2 s of shock and the following 8 s (see Fig. 7, first panel). All rats had a greater PAR during the shock period than the preceding 10 s, $t(7) = 5.86$, $p < .001$. Notice also that all rats had a PAR in response to shock that exceeded 0.5, indicating an increase in peak activity during the shock period.

Fig. 7 (2nd panel) shows the same shock period data for the Shock Only group but now contrasts it to the PAR in the combined tone groups where the ratio was calculated between the first 10 s of tone and the 10 s preceding the tone. Since the short and long groups were not differentially treated at this point we combined their data. For the tone PAR hovered around 0.5, while the mean PAR for the Shock Only group was 0.72. This difference was significant, $t(22) = 4.96$, $p < .001$. These two measures show that when an activity burst occurs it is reliably detected with the PAR.

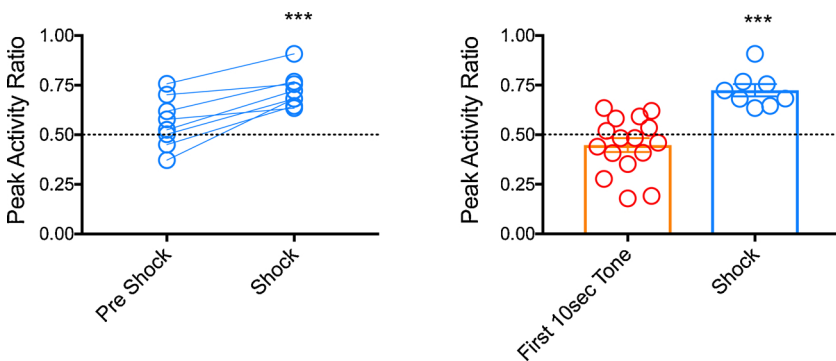


Fig. 7. Shock-elicited activity bursts expressed as the mean (\pm S.E.M) Peak Activity Ratio. Panel 1: PAR comparing the 10 s prior to shock to the subsequent 10 s that began with a 2 s shock. Panel 2: A comparison of the PAR to the first tone onset (both short and long CS groups are combined) to the PAR from the first shock presentation (Shock Only group). $***p < 0.001$.

4. Discussion

Freezing was consistently greater for the short CS than the long CS. This is perhaps most striking when the data were averaged over the full 3 min of the tone test presentation (Fig. 4, panel 3). Generalization decrement would have favored greater freezing in the long group as they were tested with the same CS used during training. For the Short CS group, the much longer stimulus used in testing compared to training should have resulted in considerable generalization decrement after the initial 10 s. Also, for this group there was a greater opportunity for within-session extinction during the test with the equivalent of 18 unreinforced CSs (Drew et al., 2017). Despite these factors freezing was greater in the Short CS group.

Based on applying principles of appetitive behavior systems to these procedures we predicted less freezing in the Short CS group, a pattern that was not only strongly disconfirmed by the data but went in the opposite direction. As stated previously, the Short CS was within the duration of the activity bursts generated by other stimuli such as shock. The long CS was chosen because in previous studies it produced freezing even when there were flight opportunities present (Fanselow and Lester, 1988). Still the findings were clearly in the opposite direction of that predicted from appetitive systems.

One could always argue that perhaps an even shorter CS to US interval than used here would fail to support freezing. The shortest CS onset to US onset interval occurs with simultaneous conditioning where both stimuli begin at the same time. The few data sets available with simultaneous conditioning are contradictory. Albert and Ayres (1997) observed significant freezing with one-trial simultaneous conditioning but they did not measure activity bursts. On the other hand, Esmoris-Arranz et al. (2003) reported activity bursts and not freezing with simultaneous conditioning. Several unique features of the Esmoris-Arranz et al study may have led to the outcomes they obtained. The CS in that study was a loud complex tone compounded with a light. We have previously shown that such stimuli elicit activity bursts that

unconditionally disrupt freezing in much the same way shock does (Fanselow, 1984). Additionally, Esmoris-Arranz et al used 30 pairings of this CS with a 15 s shock. Above, we argued that conditioning preparations using few trials are better models for antipredator behavior, so the Albert and Ayres (1997) study seems more applicable to both the present study and to our analogy to antipredator behavior. Furthermore, the comparisons in Esmoris-Arranz et al. (2003) were to an explicitly unpaired control. Thirty unpaired CS presentations afford the opportunity for significant habituation to the unconditional properties of the CS and therefore may have reduced any unconditional activity bursts to the control stimulus. Also, 30 explicitly unpaired presentations of the CS, which each predicted a minimum of 105 s of safety, might be sufficient for the development of conditioned inhibition. If inhibition developed to the CS the comparison to the simultaneous condition could be misleading (Rescorla, 1967). Certainly, how simultaneous conditioning affects behavioral topography is a topic in need of further study.

Besides freezing, we also obtained measures (peak activity ratio) that should detect conditional activity burst responses. The measure clearly detected activity bursts elicited by shock (Fig. 7). However, no such activity bursts were detected as a CR to either the Long or the Short CS, in that the PARs during the CS test were similar to those that occurred in the untrained control (Shock Only group). Indeed, if anything the Shock Only control showed more flight-like activity in response to the tone than the two conditioned groups (PARs greater than 0.5). Instead, the only CR we found evidence for was freezing. Obviously other CRs accompany freezing such as autonomic arousal but in terms of overt defensive activity that might impact the success of a predator the only CR detected here was freezing. This should not be taken to mean that we think the only defensive behavior of the rat is freezing. We have argued that nonfreezing defensive behaviors occur in situations that model the pre-encounter and circa-strike phases of defense. It is the post-encounter mode that favors freezing. Fear conditioning procedures seem to effectively model post-encounter defense. One reason for this may be that the post-encounter mode operates through fear conditioning even in natural environments. Elsewhere, we have argued that fear conditioning has several unique features that make it an effective way to recognize threats (Fanselow, 2018). Even a single trial supports strong conditioning and conditional responding is observed immediately after the US because of strong contextual fear conditioning (Fanselow, 1980, 1982; 1986).

Why is a basic rule found in other behavior systems not applicable for defensive behavior? There are several features of defense that make it somewhat different than appetitively driven behavior systems such as predation and reproduction that may lead to these differences in the operating characteristics between behavior systems. Moving between modes is very different for appetitive behavior systems (feeding and sex) than antipredator behavior. In appetitive systems movement toward the terminal modes (e.g., consumption) is the ultimate goal. Success at focal search is movement to the handling mode. For defense it is the exact opposite, any movement toward the terminal modes is inherently more dangerous and imparts a greater cost. Success in the post-encounter mode is NOT moving to the circa-strike mode. Even if circa-strike behavior is successful any injury incurred would require a period of recuperation that could compromise appetitive activities (see Fig. 1). The nature of transitions between modes may be quite different for the defense and appetitive systems; in appetitive systems there is a pull toward the terminal phases but in defense there is a push away from the terminal phases. While a specific process in the operation of behavior systems is different, that overall organization still reflects the nature of the specific problems needed for survival and reproductive fitness.

A second reason for the difference in behavior systems may be how multiple experiences impact behavior. Food is something that must be experienced repeatedly and such repeated experiences, especially if a similar patch is re-investigated, may provide the necessary experience

to learn timing relationships. However, threats need to be avoided and animals that repeatedly expose themselves to the same danger are unlikely to survive. So, repetition of specific aversive events should be very infrequent and therefore learning about specific timing relationships is not particularly useful. To be consistent with this ecological consideration we used just 3 training trials.

So what controls the transition from post-encounter to circa-strike behavior? Previously, we have shown that when a freezing rat, which is presumably in the post-encounter phase, receives a sudden intense or novel stimulus, freezing is immediately disrupted and replaced by vigorous circa-strike-like activity. Shocks produce activity bursts whose duration is proportional to the intensity of shock (Fanselow, 1982). Nonpainful, but sudden intense stimuli, such as a novel tone, flashing light and vibration compound also produces activity bursts in the freezing rat (Fanselow, 1984). We found a similar result here in that shock produced an activity burst as measured by our Peak Activity Ratio. The rule may be that once a threat is encountered, rats freeze and hold that freeze until there is a sudden dramatic change in stimulation. Given that freezing removes the releasing stimulus for attack, freezing until attack is acutely imminent would be effective. Sudden stimulus change may provide a reliable indicator that attack has been released and a different strategy is urgently needed.

In the traditional learning theory analysis of behavior, the interest and focus has been almost exclusively on associative processes and considerable effort is put into including controls that rule out any contributions of nonassociative processes. At the same time, the studies rarely include controls to see if these nonassociative processes exist and if they contribute to behavior. Timberlake's behavior systems approach has a very different focus; it has us ask how do animals solve biologically important problems? This broadening of the investigator's perspective opens the window of investigation so that we search for any process that is an important determinant of behavior. In the study described here we find that an important behavioral transition was not controlled by associative learning. Rather, a nonassociative process triggered by sudden stimulus change controls the switch between two very different behavioral modes. Future investigation is needed to understand the nature of how stimulus change drives behavior in fear related situations and to what extent similar processes participate in other behavior systems.

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