

1 Conditional Freezing, Flight and Darting?

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18

19 **Abstract**

20 Fear conditioning is one of the most frequently used laboratory procedures for modeling
21 learning and memory generally, and anxiety disorders in particular. The conditional response
22 (CR) used in the majority of fear conditioning studies in rodents is freezing. Recently, it has
23 been reported that under certain conditions, running, jumping or darting replaces freezing as the
24 dominant CR. These findings raise both a critical methodological problem and an important
25 theoretical issue. If only freezing is measured but rodents express their learning with a different
26 response, then significant instances of learning, memory, or fear may be missed. In terms of
27 theory, whatever conditions lead to these different behaviors may be a key to how animals
28 transition between different defensive responses and different emotional states. We replicated
29 these past results but along with several novel control conditions. Contrary to the prior
30 conclusions, running and darting were entirely a result of nonassociative processes and were
31 actually suppressed by associative learning. Darting and flight were taken to be analogous to
32 nonassociative startle or alpha responses that are potentiated by fear. On the other hand,
33 freezing was the purest reflection of associative learning. We also uncovered a rule that
34 describes when these movements replace freezing: When afraid, freeze until there is a sudden
35 novel change in stimulation, then burst into vigorous flight attempts. This rule may also govern
36 the change from fear to panic.

37

38 **Introduction**

39 Fear limits the behaviors available to an animal to its species-specific defense reactions
40 (SSDRs), thereby precluding more flexible voluntary behavior (Bolles, 1970). This characteristic
41 is one reason that conditions characterized by high fear levels such as anxiety disorders are so
42 maladaptive (Fanselow, 2018). It is also one reason that Pavlovian fear conditioning is so easy
43 to measure in the laboratory, one can simply measure innate defensive responses (i.e., SSDRs)
44 to diagnose fear and fear-related memory. This has made fear conditioning one of the major
45 rodent assays of learning, memory and anxiety disorders. Over the last four decades fear
46 conditioning studies have extensively used one of these defensive behaviors, freezing, more
47 than any other response (Anagnostaras et al., 2010; Bouton & Bolles, 1980; Do-Monte et al.,
48 2015; Fanselow & Bolles, 1979; Grewe et al., 2017; Kim & Fanselow, 1992; Kwon et al., 2015;
49 Nader et al., 2000; Roy et al., 2017). Freezing is a common and adaptive defensive behavior as
50 it reduces the likelihood of detection and attack by a predator (Fanselow & Lester, 1988).

51 However, if rodents have multiple defensive responses, an important theoretical question is
52 what are the conditions that select between different SSDRs (Fanselow, 1997). An influential
53 model of SSDR selection applied to both humans and rodents is Predatory (or Threat)
54 Imminence Continuum theory, which states that qualitatively distinct defensive behaviors are
55 matched to the psychological distance from physical contact with a life-threatening situation
56 (Bouton et al., 2001; Fanselow & Lester, 1988; Mobbs, 2018; Mobbs et al., 2007). Stimuli that
57 model particular points along this continuum elicit behaviors appropriate to that level of
58 predatory imminence. For example, rodents freeze when they detect a predator but show
59 vigorous bursts of activity to contact by the predator (Fanselow & Lester, 1988). The former,
60 labeled post-encounter defense, relates to fear-like states. The latter, referred to as circa-strike
61 defense, relates to panic-like states (Bouton et al., 2001; Perusini & Fanselow, 2015).

62 According to this account, in fear conditioning experiments the shock US models painful contact

63 with the predator and therefore invariably produces circa-strike activity bursts but not freezing
64 (Fanselow, 1982). On the other hand, stimuli associated with shock such as an auditory CS,
65 models detection of a predator and therefore invariably produces post-encounter freezing as a
66 CR but not activity bursts (Fanselow, 1989).

67 Recently, two reports challenge this view. Fadok et al. (2017) used a unique two component
68 serial CS consisting of a 10-sec tone followed immediately by a 10-sec white noise ending with
69 a 1 sec shock and found that the initial component (tone) produced freezing, while the second
70 component (noise) produced bursts of locomotion and jumping in mice. Gruene et al. (2015)
71 reported that in rats a tone CS resulted in a similar burst of locomotion, labelled darting. The
72 results were interpreted as a competition between “active” and “passive” defenses. These
73 findings not only challenge the above response selection rule but, also call for a “reinterpretation
74 of rodent fear conditioning studies” because if only one SDR is measured (e.g., freezing) but
75 the situation is characterized by a different SDR, fear and fear-related learning may be
76 misdiagnosed (Gruene et al., 2015). Also note that contrary to Predatory Imminence Theory,
77 Gruene et al. (2015) suggested that freezing and darting were competing CRs to the same level
78 of threat (Fanselow, 1989).

79 Both previous reports concluded that these activity bursts were CRs because they increased
80 over trials during acquisition when CS and US were paired and decreased during extinction
81 when the CS was presented alone (Fadok et al., 2017; Gruene et al., 2015). While these
82 behavioral patterns are certainly properties of a CR, they are not diagnostic of associative
83 learning as these changes could also result from nonassociative processes such as
84 sensitization and habituation (Rescorla, 1967). Additionally, no formal assessment was made of
85 what properties of the CS led to the alternate CRs (e.g., its serial nature, the ordering of the two
86 sounds, or stimulus modality). One subsequent study using this serial conditioning procedure in
87 mice has suggested that this white-noise elicited activity burst is mainly a result of the stimulus

88 salience or intensity of the white noise and does not depend on any particular temporal relation
89 to the US (Hersman et al., 2020). Another recent study using this procedure in rats has
90 suggested that this flight behavior only occurs in context in which fear has been established and
91 is a result of associative processes, but some of the metrics used to score this flight are
92 confounded with any potential freezing prior to the noise presentation (Totty et al., 2021).
93 Therefore, to better understand the associative nature of these flight responses, we embarked
94 on a series of experiments to test these theoretical views and assess the validity of these
95 concerns (Tables 1-4).

96 **Results**

97 **Experiment 1**

98 Experiment 1 was conducted as delineated in Table 1 (see Fig. 1 for a schematic representation
99 of the serial conditioned stimulus and the design for training and testing for Experiment 1). We
100 first conducted a nearly exact replication of the conditions used by Fadok et al. (2017), using
101 male and female mice (Replication Group). Briefly, animals received 10 pairings of footshock
102 and the two-component stimulus (10-sec tone followed by 10-sec white noise) over 2 days
103 before being tested on the third day with the two-component stimulus. We scored bursts of
104 locomotion and jumping with a Peak Activity Ratio (PAR; Fanselow et al., 2019) and the number
105 of darts (Gruene et al., 2015). PAR reflects the largest amplitude movement made during the
106 period of interest, while darts reflect the frequency of large movements during the same period
107 (see methods). We included two additional groups in this experiment to test the nature of any
108 observed behaviors. We asked whether any observed behavior occurred to the noise because
109 it was embedded in a serial compound or because of the brevity of the noise (10 sec). For one
110 group, we simply conditioned and extinguished a 10-sec white noise (CS Duration Group). A
111 third group of mice was trained with a 20-sec tone, but tested with the two-component serial
112 compound stimulus (Stimulus Change Group).

113 In a nearly exact replication of the conditions used by Fadok et al. (2017), using male and
114 female mice, we obtained nearly identical results with our Replication Group (Table 1, Fig. S2).
115 For this and all experiments described below, no effects of sex were observed in initial
116 comparisons/ANOVAs (see Discussion). Sex was thus removed as a factor in subsequent
117 statistical analyses. In the replication group, freezing to the initial tone progressively increased
118 over the course of conditioning. At the beginning of training, freezing increased to the white
119 noise but plateaued after a few trials. When freezing plateaued the noise elicited activity bursts,
120 and this pattern maintained throughout acquisition and extinction testing.

121 Then, we directly asked whether the plateau in freezing and increase in activity that occurred to
122 the noise was because of the brevity of the noise (10-sec) and its close temporal relation to the
123 US. We simply conditioned and extinguished a 10-sec white noise (CS Duration Group) and
124 found that freezing increased linearly during a 10 sec pre-noise period reflecting the acquisition
125 of contextual fear conditioning (Kim & Fanselow, 1992; Fig. S3). During testing the reaction to
126 onset of the white noise was almost a duplicate to what we saw when the noise was embedded
127 in the compound. In other words, activity bursts and darting in no way depended on the use of
128 a serial compound.

129 To probe the necessity of the compound during acquisition we trained a third group of mice with
130 a 20-sec tone instead of the compound but tested them with the serial compound stimulus
131 (Stimulus Change Group). During these shock-free tests the noise evoked a very similar PAR
132 and darting behavior to when training was with the compound (Fig. 2). What is striking about
133 this finding is that even though the noise was never paired with shock it still evoked an activity
134 burst. These findings strongly implicate nonassociative processes in the activity burst rather
135 than conditioning.

136 Overall in Experiment 1, we replicated findings that differential defensive behaviors develop to
137 separate components of a serial CS (Replication Group). This pattern of behavior holds true if

138 the noise is presented by itself during training (CS Duration group), and this pattern of behavior
139 at testing does not require the noise to be present during training (Stimulus Change Group).
140 Despite differences in behavioral procedures used across acquisition and extinction, we sought
141 to examine any differences in reactivity to the noise during extinction testing between these
142 three groups. We directly analyzed velocity data across the three groups (Fig. 3). We focused
143 on the first four trials of extinction testing as this is when the majority of the darting behavior
144 occurred, and we further narrowed our analyses to the 10s Noise period as all groups received
145 at least the 10s noise at test.

146 A mixed model ANOVA revealed a significant effect of Time, [$F(19,361)=8.203$, $p<.001$] as well
147 as a Group X Time interaction [$F(38,361)=1.497$, $p=.034$]. Generally, velocity peaked during the
148 first bins of the noise period and then quickly decreased to more stable levels. Post-hoc
149 analyses revealed that the Stimulus Change group trended to have the elevated velocity during
150 the first bin of the noise period with trends for higher velocity than the CS Duration group
151 ($p=.09$) and did have significantly higher velocity than the CS Duration group during the fifth bin
152 (~2.5 seconds into the noise; $p=.04$).

153 While the noise did not need to be within a serial compound stimulus or even need to be
154 presented during training in order to elicit flight, it is worth noting that the strongest noise-elicited
155 flight occurred for the group which received the serial compound stimulus at test and for which
156 the noise was novel at test.

157

158 **Experiment 2**

159 The mice that received the 20 sec tone during training but the compound during testing showed
160 darting to the noise embedded in the compound (Figures 2, 3, S3). Since the noise was not
161 paired with the shock, this suggests that the response to the noise was nonassociative.

162 However, it is possible that during the initial test trials the response to the noise occurred via
163 second-order conditioning as the noise was paired with the previously reinforced tone. This
164 seems unlikely because most darts were seen at the beginning of testing and decreased over
165 the session. A second-order conditioning interpretation suggests the opposite pattern.
166 Nonetheless, in a second experiment, we included classic controls to directly test for the
167 phenomenon of pseudo-conditioning (Table 2). Pseudo-conditioning is a form of sensitization
168 whereby mere exposure to the US changes behavior to the stimulus used as a CS (Underwood,
169 1966), and this appears to be what was observed in Experiment 1 (Stimulus Change Group;
170 Fig. 2). Two pseudoconditioned groups of mice simply received the same shock schedule used
171 in the prior study without any auditory stimuli (no CS). A third was merely exposed to the
172 chamber. The final group was a conditioning group that received noise-shock pairings. All
173 groups received tests with the 10 sec noise, except for one of the pseudoconditioning groups
174 that was tested with the tone.

175 Figures 4 and 5 summarize the test results from Experiment 2 (see Fig. S4 for trial-by-trial data).
176 As would be expected for a CR, freezing to the noise was greatest in the mice that received
177 noise-shock pairings [$F(3,28) = 11.76, p < .001$]. Significant associative learning was indicated
178 by more noise-elicited freezing in the paired group than the shock-only trained group tested with
179 the noise. Interestingly, the No Shock group that was tested with the noise gradually increased
180 freezing over the course of noise testing (Fig. S4) suggesting that the 75dB noise itself was
181 aversive to the mice and could support some conditioning of freezing (i.e., it was a weak US).

182 The test session data were very different for activity bursts (Figs. 4 and 5). The greatest PAR
183 occurred in the pseudoconditioned control (shock only during training) that was tested with the
184 novel noise [$F(3,28) = 20.085, p < .001$]. The pseudoconditioned control tested with the novel
185 noise showed the most darting behavior. Furthermore, these results are supported by a direct
186 analysis of velocity data during the 10s CS period at test (Fig. 5).

187 A mixed model ANOVA on the averaged velocity measures during the CS period for the first 4
188 trials of the test session revealed significant effects of Group [$F(3,28)=5.796$, $p=.003$] and Time,
189 [$F(4.06,113.69)=6.038$, $p<.001$] as well as a Group X Time interaction [$F(12.18,113.69)=2.695$,
190 $p=.003$]. Generally, velocity again peaked during the first bins of the noise period and then
191 quickly decreased to more stable levels. Post-hoc analyses revealed that the Shock Only-Noise
192 Test group had the highest velocity during the second bin of the noise period (the first second of
193 the CS) with significantly higher velocity than the No Shock-Noise Test ($p=.03$), Shock Only-
194 Tone Test ($p=.004$) and, importantly, the Noise Shock-Noise Test groups ($p=.007$).

195 Pseudoconditioning is indicated by more activity during the noise test in the previously shocked
196 mice than the no-shock controls tested with the same noise. Note that for both of these groups
197 the noise was novel during testing so it had no association with shock. Another striking finding
198 is that while the group that received noise-shock training showed an elevated PAR, the level
199 was significantly less than the pseudoconditioning control ($p<.001$). Not only are activity bursts
200 not conditioned, they are actually suppressed by conditioning! In other words, flight and darting
201 are a result of nonassociative processes and are not CRs.

202

203 **Experiment 3**

204 In a third experiment, we included a control group in which the shock and noise were explicitly
205 unpaired to again test for the phenomenon of pseudo-conditioning but in a situation where
206 exposure to the CS is equated during training (Table 3). One group was again a conditioning
207 group that received noise-shock pairings, and one group was again a pseudoconditioned group
208 that only received shocks without any CS. One group received equal numbers of noise and
209 shock presentations but in an explicitly unpaired manner. An additional control group received

210 presentations of only the white noise CS to examine whether or not the CS alone was able to
211 support conditioning and/or activity bursts.

212 Acquisition and test results are summarized in Figures 6 and 7. As seen in the prior
213 experiments, across training freezing to the white noise rises, and then plateaus in the Paired
214 and Unpaired groups, at which point the noise begins to elicit activity bursts. In the CS only
215 group white noise alone supported low, but consistent levels of freezing but in the shocked
216 groups the noise disrupted freezing to the context. During training, the Paired and Unpaired
217 groups showed elevated PAR to the noise [$F(3,28)=29.94$, $p<.001$ for Day 1; $F(3,28)=75.18$,
218 $p<.001$ for Day 2], and increased darting to the noise [$F(3,28)=8.187$, $p<.001$ for Day 1;
219 $F(3,28)=22.538$, $p<.001$ for Day 2]. Interestingly, for darting, the Paired group showed elevated
220 responding on Day 2 compared to the Unpaired group ($p=.026$). During testing, activity bursts
221 (measured as both PAR and darting) to the noise were elevated in all groups which received
222 shock [$F(3,28) = 13.35$, $p<.001$ for PAR; $F(3,28) = 8.302$, $p<.001$ for darting]. Again, similar to
223 during training, darting was the most elevated in the Paired group on Trial 1 of testing ($p=.001$).

224 While overall darting was elevated in the Paired group [during acquisition and on the first trial of
225 testing], the velocity traces during testing (Fig. 7) reveal that the magnitude/frequency of the
226 initial activity burst to the noise appears to be reduced in the Paired group, and that increased
227 levels of activity bursts during the latter portion of the CS account for any differences in overall
228 numbers of darts. Indeed, a direct analysis of the velocity data during the 10s Noise CS period
229 at test revealed significant effects of Group [$F(3,28)=9.733$, $p<.001$], Time,
230 [$F(5.15,144.22)=9.614$, $p<.001$] as well as a Group X Time interaction [$F(15.45,144.22)=2.045$,
231 $p=.02$]. Generally, as seen in prior experiments, velocity again peaked during the first bins of
232 the noise period and then quickly decreased to more stable levels. In the Paired group
233 specifically, there is an additional peak of activity in the latter half of the stimulus period. Post-
234 hoc analyses revealed that the Unpaired group had the highest velocity during the first bin of the

235 noise period (the first second) with significantly higher velocity than the CS Only Group
236 ($p=.007$). Additionally, in the 16th and 17th bins towards the end of the CS period, the Paired
237 Group showed the most activity with significantly higher velocity than the CS Only Group
238 ($p=.002$ & $p=.001$), the Shock Only Group ($p=.001$, $p=.02$), and the Unpaired Group ($p<.001$,
239 $p=.003$)

240 That pairing noise and shock altered the timing of the activity bursts is an interesting fact worth
241 considering and suggests that pairing noise and shock may have primarily resulted in a
242 conditioned freezing response which in fact competes with/reduces any initial non-associative
243 activity/bursting to the white noise. Taken together, this and the prior experiment using control
244 groups to assess pseudoconditioning reveal that a large portion, if not all, of the noise-elicited
245 activity bursts observed are due to non-associative processes which result in an increase in
246 darting behavior to the noise following shock exposure, regardless of any direct training history
247 of the noise with shock. There does appear to be evidence that pairing noise with shock may
248 further increase or alter the timing of this behavior, but by no means is pairing noise with shock
249 necessary to produce these activity bursts.

250

251 **Experiment 4**

252 The experiments thus far have suggested that much of the white-noise-elicited activity bursting
253 is a non-associative process. We have also shown that novelty of the CS at test may increase
254 this noise-elicited activity (Figs. 3 & 4). In a final, fourth experiment, we explicitly tested whether
255 habituation to the white noise stimulus prior to noise-shock training would be able to reduce
256 noise-elicited activity bursts. If increased levels of novelty of the CS are driving noise-elicited
257 activity bursts, then prior habituation should reduce the levels of darting to the noise CS. In this
258 experiment, we had four groups which differed in whether they received an additional two days

259 of habituation to the white noise stimulus (5 noise presentations each day) and whether they
260 received noise-shock pairings during training or just shock only (Table 4). One comparison of
261 particular interest was between the habituated or non-habituated Shock Only groups as these
262 groups would directly compare whether prior experience with the CS would decrease darting at
263 test compared to a group for which the CS was completely novel.

264 Figure 8 shows the results of Experiment 4 during testing (see Figure S5 for trial-by-trial results
265 for freezing, PAR, and darting across habituation, training, and testing). During the two days of
266 habituation, interestingly, we found that within groups which received habituation, a low level of
267 darting to the white noise alone without any shock decreased across day one [$F(4,48) = 2.887$,
268 $p=.026$] and increased by the end of the second day of habituation [$F(4,48) = 2.793$, $p = 0.36$]
269 (Fig. S5). Concurrently, freezing to the white noise increased over habituation trials, again
270 showing that this white noise stimulus alone can act as a US. It is interesting that darting
271 occurred to the white noise at the start of habituation when the CS was very novel, and at the
272 end of habituation once the white noise alone was able to support some level of fear.

273 Comparing the two Shock Only groups during test, the noise disrupted freezing more than tone.
274 In this regard noise seems to act like a weak shock US (Fanselow, 1982). Like shock it disrupts
275 freezing (Fig S5) and like shock it supports conditioning of freezing (Fig 6).

276 Within Paired groups (H-Paired and C-Paired), we found that throughout acquisition and
277 particularly on the second day of training (Fig. S5), prior habituation to the white noise increased
278 freezing [$F(1,24)=5.701$, $p=.025$] and decreased noise-elicited darting [$F(1,24) = 5.130$, $p=.033$],
279 as predicted if prior exposure to the CS functions to reduce any partially novelty-driven darting.
280 We again saw that freezing to the white noise initially increased during acquisition, but as the
281 darting response begins to become more apparent, freezing decreases to medium levels. At
282 test (Figs. 8 & S5), for freezing, we found a main effect of pairing [$F[1,24] = 11.306$, $p=.003$],
283 such that animals who received white noise paired with shock froze more than animals who only

284 received shock during acquisition, again indicative that noise-elicited freezing is a conditional
285 behavior that results from associative learning. For darting behavior, we found a Habituation X
286 Pairing interaction [$F(1,28)=4.939$, $p=.035$] such that pairing white noise with shock increased
287 darting within habituated animals ($p=.033$), and that habituation reduced darting within animals
288 who only received shock during training ($p=.045$). These results reveal multiple points of
289 interest. First, and as shown in prior experiments, the white noise acts as a US on its own and
290 need not be paired with shock to produce darting at test. Merely experiencing the shock is
291 enough to produce darting to the white noise at test (pseudoconditioning due to sensitization).
292 Furthermore, prior experience with the white noise, through habituation, actually reduced this
293 darting at test. Additionally, in this experiment, we do again show evidence that pairing white
294 noise with shock can further increase darting behavior at test, at least within animals who have
295 already experienced the noise during habituation. Again, as with Experiment 3 (Fig. 7) the
296 timing of the darting response in Paired groups is fundamentally altered compared to Shock
297 Only groups (Fig. 8). The magnitude/frequency of the initial activity burst to the noise appears
298 to be reduced in the Paired groups, and increased levels of activity bursts during the latter
299 portion of the CS account for any differences/increases in overall numbers of darts.

300 Indeed, a mixed model ANOVA with Pairing, Habituation, and Time as factors on the averaged
301 velocity traces for each trial revealed significant effects of Time [$F(56,1568)=17.420$, $p<.001$], a
302 Habituation X Pairing interaction [$F(1,28)=4.696$, $p=.04$], and a Pairing X Time interaction
303 [$F(56,1568)=3.036$, $p=.01$]. Generally, once again, velocity peaked during the first bins of the
304 noise period and then quickly decreased to more stable levels. As seen in the experiments
305 above, again, this initial peak in velocity was most apparent in the Shock Only groups, with the
306 Paired groups showing an initially smaller peak in velocity. Post-hoc analyses revealed that the
307 Shock Only groups had significantly higher velocity during the first three bins of the noise than
308 the Paired groups (p 's=.02, .03, .005 respectively). Post-hoc analysis on the Pairing X

309 Habituation interaction reveal that within the non-habituated groups, pairing noise and shock
310 significantly reduced the velocity throughout test trials ($p < .001$). Additionally, within Shock Only
311 groups, habituation reduced the velocity throughout test trials ($p < .001$). These results are
312 exactly what would be predicted if exposure to the noise CS (through pre-exposure and/or
313 through pairing CS and US) in fact reduces noise-elicited activity bursts and flight/darting
314 behavior, that is, darting is enhanced by novelty.

315

316 **Discussion**

317 Prior work reported that contact/pain-related stimuli (e.g., shock) disrupt freezing and provoke
318 panic-like circa-strike defensive behaviors (Fanselow, 1982). The current results suggest a
319 modification of the rules governing a transition between these behavioral states. The rule is that
320 when you are in the post-encounter mode (fear) a sudden change in stimulation, particularly the
321 onset of an intense novel stimulus, can cause an immediate transition to the circa-strike mode
322 (panic). Indeed, the vast majority of the activity bursts/darting behavior occurred at the onset of
323 the stimulus (Figs. 3, 5, 7, 8). The effectiveness of this transition depends on the qualities of the
324 stimulus. Stronger shocks cause a greater disruption of freezing and a longer activity burst, yet
325 the same stronger shocks simultaneously condition more freezing to the prevailing cues
326 (Fanselow, 1982). The current data call for an expansion of this rule to nonnociceptive stimuli.
327 While both tone and noise disrupted ongoing freezing, the noise did so for longer than the tone
328 (Fig. S6) and noise on its own was able to support a minimal level of fear conditioning (Figs. 6,
329 S4, S5). The rule is: when in a state of fear (Post-encounter defense) sudden stimulus change
330 provokes panic-like circa-strike defenses proportional to stimulus intensity and novelty.

331 As the majority of the experiments presented here and in most prior studies conduct both
332 training and testing in the same context (Fadok et al., 2017; Gruene et al., 2015, Hersman et al.,

333 2020), these animals would already be in a high state of fear or post-encounter defense (from
334 any learned contextual fear during training), thus endowing the presentation of the white noise
335 to be a particularly startling stimulus change which can provoke these panic-like flight
336 responses. Novelty of the stimuli is an important factor and familiarity with the CS during
337 conditioning and/or habituation reduced CS novelty for the test. In the experiments presented
338 here, the mice that received noise-shock pairings and were tested with noise showed lower
339 flight to the noise than mice trained only with shock and then received noise for the first time.
340 Additionally, prior habituation to the noise or experience with the noise during training further
341 reduced noise-elicited flight at test.

342 Another important factor to consider is the timing of the activity burst with respect to CS and US
343 onset. With poorly timed and sustained conditional responses such as freezing the CR tends to
344 fill the entire CS-US interval and spill over beyond the time of expected US delivery (e.g., Ayres
345 & Vigorito, 1984; Gale et al., 2004). However, shorter duration ballistic responses such as the
346 darting response allow a clearer assessment of when the CR occurs with respect to CS and US
347 delivery and such CRs are expected to anticipate US delivery. Hull (1934) cautioned
348 conditioning researchers that it is important to distinguish true conditional responses from
349 unconditional responses to the CS, which he named alpha responses. These alpha responses
350 occur at the onset of the CS, rather than the time of the expected US. Alpha responses have
351 been most studied with the Pavlovian conditional eyeblink response, where the true CR is well-
352 timed to US delivery (McCormick & Thompson, 1984, Perrett et al,1993). Blinks that occur to
353 CS onset are classified as alpha responses, which are considered to be nonassociative startle
354 responses to the CS and not CRs (e.g., Gerwig et al., 2005; Nation et al., 2017; Schreurs and
355 Alkon, 1990, Woodruff-Pak et al., 1996). Typically, in eyeblink studies alpha responses are
356 excluded from analysis by omitting any responses that occur at the beginning of the CS. Our
357 darting responses almost exclusively occurred at CS onset and there were never any US

358 anticipatory-like responses. Thus, traditional Pavlovian analyses for ballistic CRs would have
359 categorized darting as an unconditional alpha response and not a bona fide CR. Consistent
360 with this analysis is that darting occurred to the noise during the first few trials of the habituation
361 session in Experiment 4 (Fig S5)

362 Our interpretation that noise unconditionally elicits a ballistic activity burst bears some
363 relationship to the unconditional acoustic startle response. Loud noises will elicit an
364 unconditional startle response that wanes with repeated presentations of that noise (i.e.,
365 habituation; e.g., Davis, 1980; Hoffman & Fleshler, 1963; Leaton, 1976). While our 75 dB noise
366 stimulus is less intense than the 98-120 dB noise used in typical acoustic startle studies, we are
367 observing an unconditional noise elicited response that also decreases with habituation
368 (Experiment 4). Furthermore, our data and those of Totty et al. (2021) indicate that these
369 responses require a fearful context in order to occur. Fear is well known to potentiate startle
370 responses (Brown, et al., 1951; Davis, 1989). Perhaps the low intensity noise is below
371 threshold to elicit a startle response on its own, but a fearful context potentiates this response
372 and brings it above threshold. Additionally, there appears to be considerable overlap in the
373 neuroanatomy that supports this circa-strike behavior and fear potentiated startle. Totty et al.
374 (2021) found that inactivation of the Central Nucleus or the Bed Nuclei of the Stria Terminalis
375 disrupts the flight response. These two regions have been shown to be important mediators of
376 fear's ability to potentiate startle (e.g., Campeau & Davis, 1995; Davis & Walker, 2014).
377 Furthermore, Fadok et al. (2017) reported that it is corticotropin releasing hormone (CRH)
378 expressing cells, but not somatostatin expressing cells, within the Central Nucleus that support
379 flight behavior. Again, there is extensive data implicating CRH and fear potentiated startle (Lee
380 & Davis, 1997).

381 It is of note that the relationship between startle (circa-strike defense) and freezing (post-
382 encounter defense) was described by Fanselow & Lester (1988) when accounting for how rats

383 rapidly transitioned between these behaviors when a detected predator launches into attack. “It
384 is as if the freezing animal is tensed up and ready to explode into action if the freezing response
385 fails it. This explosive response probably has been studied in the laboratory for over 30 years
386 under the rubric of potentiated startle...It seems that the releasing stimulus for this explosive
387 motor burst is a sudden change in the stimulus context of an already freezing rat (Fanselow &
388 Lester, 1988, p 202).”

389 Neither Fadok et al. (2017) nor Gruene et al. (2015) included any controls for nonassociative
390 behavior, which is something required in order to conclude that a response is conditional
391 (Rescorla, 1967). Both of these research groups concluded from their single group experiments
392 that flight/darting was a CR because the behavior increased with successive shocks during the
393 shock phase and decreased with shock omission during the test phase, likening these
394 behavioral changes to acquisition and extinction. While acquisition and extinction are
395 characteristics of a CR, learning theorists have never taken these as diagnostic of a CR. For
396 example, increases in responding with successive shocks could arise via sensitization and
397 decreases in responding when shocks are omitted could arise from habituation. Indeed, that is
398 exactly what we believe caused these behavioral changes that we also observed in our study.
399 Shocks, by conditioning fear to the context, sensitize or potentiate the darting response and
400 repeated presentations of the noise alone cause the response to habituate. The behavior of our
401 pseudoconditioning control provides clear evidence of this. Just giving shocks conditioned fear
402 to the context such that when the noise was presented for the first time during test it caused a
403 strong activity burst. The behavior gradually decreased during testing because repeated
404 presentations of the noise led to habituation of this unconditional response.

405 Given our argument that the flight/darting behavior is nonassociative, Totty et al.'s finding that
406 noise-shock paired rats showed more noise elicited activity burst behavior than rats that had
407 unpaired noise and shock requires additional comment. Since both unpaired and paired rats

408 were exposed to noise during acquisition those exposures could lead to habituation of the
409 unconditional response to the noise. However, it would be expected that habituation would be
410 greater in the unpaired group because pairing a stimulus (noise in this case) with another
411 stimulus (shock in this case) is known to reduce the magnitude of habituation (Pfautz et al.,
412 1978). This reduction in habituation is observed even if the second stimulus is not an
413 unconditional stimulus (Pfautz et al., 1978). Additionally, pairing a habituated stimulus with a US
414 can also cause a return of the habituated alpha response and this loss of habituation is not
415 observed when the two stimuli are not paired (Holland, 1977). Thus, the difference between the
416 paired and unpaired groups reported by Totty et al. (2021) are likely due to differential
417 habituation of the noise during training. This effect of habituation was probably enhanced by
418 Totty et al. including a noise habituation phase prior to training.

419 Initial reports suggest a sex difference in this noise-elicited flight behavior such that female rats
420 show more of this behavior than males (Gruene et al., 2015). Within each experiment, we found
421 no such sex differences between male and female mice for the PAR and darting measures of
422 flight behavior, and Totty et al. (2021) similarly found no sex differences in such behavior in
423 male and female rats. To further increase the power of such an analysis for sex differences, we
424 pooled all of the groups across the four experiments which received noise-shock pairings. In this
425 analysis, again, we saw no sex differences in flight to the white noise across the two days of
426 acquisition for both PAR [Day1: $F(1,29)=.323$, $p=.58$; Day 2: $F(1,29)=.507$, $p=.48$], and darting
427 [Day1: $F(1,29)=.009$, $p=.92$; Day 2: $F(1,29)=3.752$, $p=.06$], and we observed no sex differences
428 across testing to the white noise in extinction for both PAR [$F(1,20)=.099$, $p=.76$] and darting
429 [$F(1,13)=1.397$, $p=.258$]. Perhaps initial reports of sex differences could be explained by
430 differences in handling and stress provided to females as a result of monitoring estrous phase, a
431 potentially stressful procedure for the animals for which there is not an ideal control in males.

432 Some have characterized freezing as a passive response that occurs because no other
433 response is available (Blanchard & Blanchard, 1969; Fadok et al., 2017; Gruene et al., 2015; Yu
434 et al., 2016). However, because motion is often the releasing stimulus for predatory attacks it is
435 the best thing for a small mammal like a rat or a mouse to do when a predator is detected and
436 will only be replaced if there is a change consistent with contact (Fanselow & Lester, 1988).
437 Rodents choose locations in which to freeze such as corners or objects (thigmotaxis) (Grossen
438 & Kelley, 1972). The current data show that the freezing rodent also prepares to react to
439 sudden stimulus change. There is nothing passive about it.

440 **Methods and Materials**

441 **Subjects**

442 Subjects for all experiments included 120 C57BL/6NHsd mice (Experiment 1, n=24; Experiment
443 2, n=32; Experiment 3, n=32; Experiment 4, n=32), aged 9-11 weeks of age and purchased
444 from Envigo. This C57BL/6NHsd strain was chosen to match that of Fadok et al. (2017). Each
445 group consisted of 4 male and 4 female mice. A necessary/powerful group sample size of 8 was
446 calculated based both on years of data in our lab which suggests n=8 is sufficient to detect such
447 behavioral differences in fear conditioning studies and on the recent articles in the literature
448 which are using this procedure. Mice were group-housed four per cage on a 12-hr light/dark
449 cycle with ad libitum access to food and water. Across each experiment, mice in each cage
450 were randomly assigned to one of the groups, ensuring that every group had a representative
451 from each cage to avoid any cage effects. All experiments were conducted during the lights-on
452 phase of the cycle. Animals were handled for 5 days prior to the start of experiments. Subjects
453 were all treated in accordance with an approved protocol from the Institutional Animal Care and
454 Use Committee at the University of California-Los Angeles following guidelines established by
455 the National Institute of Health.

456 **Apparatus and Stimuli**

457 All experiments were conducted in standard MedAssociates fear conditioning chambers (VFC-
458 008; 30.5 x 24.2 x 21 cm), controlled by Med Associates VideoFreeze software (Med
459 Associates, St. Albans, VT). For each experiment, the same context was used for training and
460 testing (see Discussion). The context was wiped down between each mouse with 70%
461 isopropanol and 3 sprays of 50% Windex were added to the pans below the shock grid floors to
462 provide an olfactory cue/context. The US consisted of a 1 second 0.9 mA scrambled shock
463 delivered through a MedAssociates shock scrambler (ENV-414S). Each of the CSs were

464 delivered using a MedAssociates speaker (ENV-224AM-2). The tone was 7.5kHz. Both the
465 tone and the white noise were 75dB inside the chamber. The inter-trial interval varied between
466 150 seconds and 210 seconds with an average length of 180 seconds.

467 **Design and Procedure**

468 Mice were handled for 5 days for approximately 1 minute per day prior to beginning the
469 experiment. At the beginning of each day of the experiment, mice were transported in their
470 home cages on a cart to a room adjacent to the testing room and allowed to acclimate for at
471 least 30 minutes. Mice were individually placed in clean empty cages on a utility cart for
472 transport from this room to the testing room and promptly returned to their home cages after the
473 session was over. These transport cages were wiped down with StrikeBac in between
474 trials/sessions.

475 Experiment 1 was conducted as delineated in Table 1 (see Fig. 1 for a schematic representation
476 of the serial conditioned stimulus and the design for training and testing for Experiment 1). The
477 Replication group was trained on each of the two days with 5 presentations of a 10 second tone
478 immediately followed by a 10 second noise, which was immediately followed by a 1 second
479 shock. On Day 3 it was then tested with 16 presentations of a 10 second tone immediately
480 followed by a 10 second noise. These parameters were chosen to match those of Fadok et al
481 (2017) except that we did not include a session of unreinforced CS preexposure prior to
482 conditioning as such treatment is known to reduce conditioned behavior (Lubow & Moore, 1959;
483 we did add such a treatment to Experiment 4 as an experimental factor). The CS Duration group
484 was trained on each of the two days with 5 presentations of a 10 second noise, which was
485 immediately followed by a 1 second shock. It was tested with 16 presentations of the 10 second
486 noise. The Stimulus Change group was trained on each of the two days with 5 presentations of
487 a 20 second tone immediately followed by a 1 second shock. It was tested with 16
488 presentations of a 10 second tone immediately followed by a 10 second noise (i.e., the

489 compound used in the replication group). Two mice were excluded from this study due to
490 experimenter error, one female in the Replication group and one female in the Stimulus Change
491 group.

492 Experiment 2 was conducted as delineated in Table 2. The Pseudoconditioned Noise and
493 Pseudoconditioned Tone groups were trained on each of the two days with 5 presentations of a
494 1-sec shock without any sound using the same schedule for shocks as Experiment 1. The No
495 Shock Control was merely allowed to explore the context for the same length of time as the
496 other groups without receiving any shock or auditory stimuli throughout the two days of
497 acquisition. The final Noise-Shock Conditioning group was trained on each of the two days with
498 5 presentations of a 10-sec noise, which was immediately followed by a 1-sec shock. As
499 Experiment 1 revealed that similar behavior was observed in groups which received compound
500 stimulus-shock pairings or just noise-shock pairings, we utilized simple noise-shock pairings in
501 this and some of the following experiments to more specifically assess the associative nature of
502 any white noise-driven behavior. All groups received tests with 16 presentations of the 10-sec
503 noise in extinction, except for one of the pseudoconditioning groups that was tested with the 10-
504 sec tone.

505 Experiment 3 was conducted as delineated in Table 3. The Paired Noise-Shock (Conditioning)
506 group was trained on each of the two days with 5 presentations of a 10 second noise, which
507 was immediately followed by a 1 second shock. The Unpaired Noise/Shock group was
508 presented with the same number and length of noise and shocks, but they were explicitly
509 unpaired in time. The Noise-CS Only group received 5 presentations of a 10 second noise
510 without receiving any shocks on each of the two days. The Shock Only (Pseudoconditioning)
511 group received 5 presentations of a 1 second shock on each of the two days. As the main
512 behavioral responses and differences between groups occurred primarily in the first few trials of
513 the previous experiments, and in order to more readily complete all of the testing within one

514 day's light cycle, for this and the following experiments we reduced the number of test trials
515 presented to the animals. Thus, at test for this experiment, all groups received two
516 presentations of a 10 second noise.

517 Experiment 4 was conducted as delineated in Table 4. Prior to training with shock, all groups
518 underwent 2 days of additional training with either habituation to the white noise or merely
519 exposure to the context. The habituated groups, Habituation/Shock Only (H-Shock) and
520 Habituation/Noise-Shock Pairing (H-Paired), were trained on each of the two days with 5
521 presentations of a 10-second noise, while the two non-habituated groups, Context
522 Exposure/Shock Only (C-Shock) and Context Exposure/Noise-Shock Pairing (C-Paired)
523 received only equivalent exposure to the context. The following two days, and as in the
524 Experiments above, all groups received 10 footshocks. The Paired groups (H-Paired and C-
525 Paired) were trained on each of the two days with 5 presentations of a 10-second noise,
526 followed immediately by a 1-second footshock. The Shock Only groups (H-Shock and C-Shock)
527 were trained on each of the two days with only 5 presentations of a 1-second footshock. At test,
528 all groups received 3 presentations of the 10-second noise.

529 **Data and Statistics and Analysis**

530 Freezing behavior for Experiments 1-3 was scored using the near-infrared VideoFreeze scoring
531 system. Freezing is a complete lack of movement, except for respiration (Fanselow, 1980).
532 VideoFreeze allows for the recording of real-time video at 30 frames per second. With this
533 program, adjacent frames are compared to provide the grayscale change for each pixel, and the
534 amount of pixel change across each frame is measured to produce an activity score. We have
535 set a threshold level of activity for freezing based on careful matching to hand-scoring from
536 trained observers (Anagnostaras et al., 2010). The animal is scored as freezing if they fall
537 below this threshold for at least a 1-sec bout of freezing.

538 For Experiment 4, due to a technical error, videos for the first 4 days of the experiment could not
539 be accurately assessed for freezing behavior using VideoFreeze. Therefore, we alternatively
540 measured and scored freezing behavior using EthoVision. Briefly, videos were converted to
541 MPEG, as described above, and analyzed using the Activity Analysis feature of Ethovision.
542 Thresholds for freezing were again determined to match hand-scoring from trained observers.
543 Two different measures of flight were used. We scored bursts of locomotion and jumping with a
544 Peak Activity Ratio (PAR); Fanselow et al., 2019) and the number of darts (Gruene et al., 2015).
545 To determine PAR, we took the greatest between frame activity score during a period of interest
546 (e.g., the first 10 s of CS presentation = During) and calculated a ratio of that level of activity to a
547 similar score derived from a preceding control period of equal duration (e.g., 10 s before
548 presentation of the tone = PreStim) of the form $\text{During}/(\text{During} + \text{PreStim})$. With this measure, a
549 0.5 indicates that during the time of interest there was no instance of activity greater than that
550 observed during the control period (PreStim). PARs approaching 1.0 indicate an instance of
551 behavior that far exceeded baseline responding. This measure reflects the maximum movement
552 the animal made during the period of interest.

553 Darting was assessed as in Gruene et al. (2015). Video files from VideoFreeze were extracted
554 in Windows Media Video format (.wmv) and then converted to MPEG-2 files using Any Video
555 Converter (AnvSoft, 2018). These converted files were then analyzed to determine animal
556 velocity across the session using EthoVision software (Noldus), using a center-point tracking
557 with a velocity sampling rate of 3.75 Hz. This velocity data was exported, organized, and
558 imported to R (R Core Team, 2018). Using a custom R code (available as source code 1), darts
559 were detected in the trace with a minimum velocity of 22.9 cm/s and a minimum interpeak
560 interval of 0.8 s. The 22.9 cm/s threshold was determined by finding the 99.5th percentile of all
561 baseline velocity data analyzed, prior to any stimuli or shock, and this threshold was validated to
562 match with manual scoring of darts, such that all movements at that rate or higher were

563 consistently scored as darts. See Figure S1 for representative traces of velocity across Day 1
564 of acquisition for a mouse in the Replication group of Experiment 1. The PAR measure reflects
565 the maximum amplitude of movement, while the dart measure reflects the frequency of
566 individual rapid movements.

567 Trial-by-trial Measures of freezing and flight were analyzed with a repeated measure
568 multifactorial analysis of variance (ANOVA) and post hoc Tukey tests. Baseline freezing, and
569 overall responding which were collapsed across session when appropriate, were analyzed with
570 a univariate ANOVA test. To directly compare each groups' activity and the magnitude of any
571 flight behaviors during extinction testing, velocity data was binned into .533s bins and
572 subsequently analyzed using repeated measures ANOVA in R. Whenever violations of
573 sphericity were found, the Greenhouse-Geisser correction was used to produce corrected
574 degrees of freedom and p-values. Significant effects and interactions were followed up with
575 simple main effects and Bonferroni-corrected pairwise t-tests. A value of $p < .05$ was the
576 threshold used to determine statistical reliability. For all experiments described above, no
577 effects of sex were observed in initial comparisons/ANOVAs. Sex was thus removed as a factor
578 in subsequent statistical analyses.

579

580

581 **Tables**

582 Table 1: Design of Experiment 1

Group	Training Treatment: 10 CS-US Pairings (5 per day)	Testing Treatment (5 on one day)
1) Replication	10-sec Tone→10-sec Noise→1-sec Shock	10-sec Tone→10-sec Noise
2) CS Duration	10-sec Noise→shock	10-sec Noise
3) Stimulus Change	20-sec Tone→shock	10-sec Tone→10-sec Noise

583

584 Table 2: Design of Experiment 2

Group	2 Day Training Treatment:	Testing Treatment
1) Pseudoconditioned Noise	10 Shocks (1-mA, 1-sec, 150-210s intertrial interval)	5 Noise Presentations (10-sec)
2) Pseudoconditioned Tone	10 Shocks (1-mA, 1-sec, 150-210s intertrial interval)	5 Tone Presentations (10-sec)
3) No Shock Control	Context exposure Only (17-min & 15-sec per day)	5 Noise Presentations (10-sec)
4) Noise-Shock Conditioning	10 Noise (10-sec)→shock pairings	5 Noise Presentations (10-sec)

585

586

587 Table 3: Design of Experiment 3—Paired vs Unpaired Noise-Shock

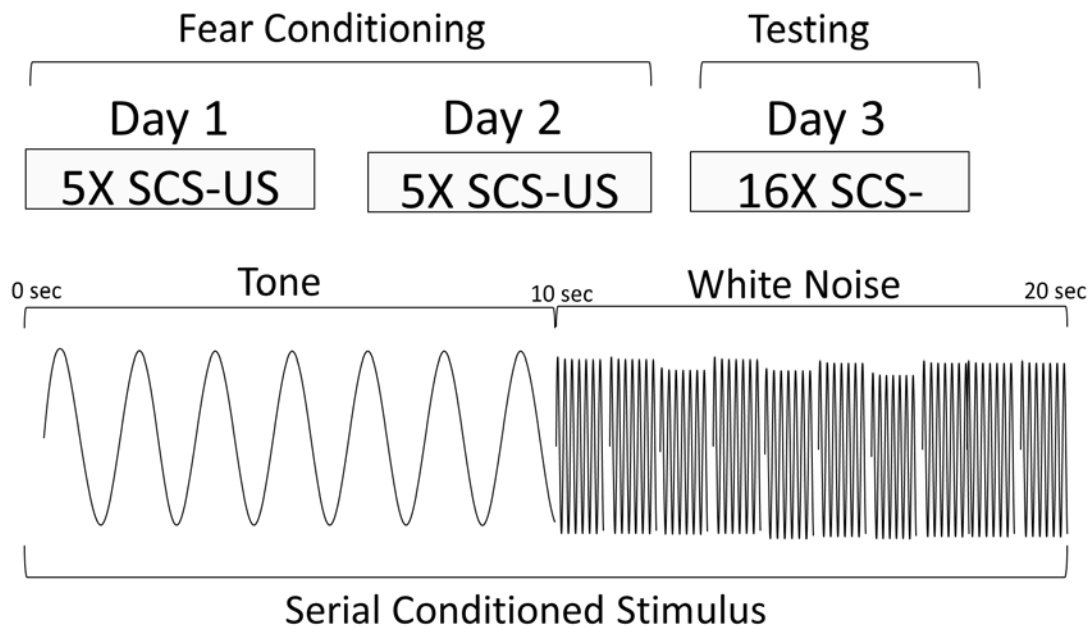
Group	Training Treatment: 10 CS-US Pairings (5 per day)	Testing Treatment (2 on one day)
1) Paired Noise-Shock (Conditioning)	10-sec Noise→1-sec Shock	10-sec Noise
2) Unpaired Noise/Shock	10-sec Noise & 1-sec Shock – Unpaired	10-sec Noise
3) Noise - CS Only	10-sec Noise	10-sec Noise
4) Shock Only (Pseudoconditioning)	1-sec Shock	10-sec Noise

588

589 Table 4: Design of Experiment 4—Tested the Effect of Habituation to the White Noise

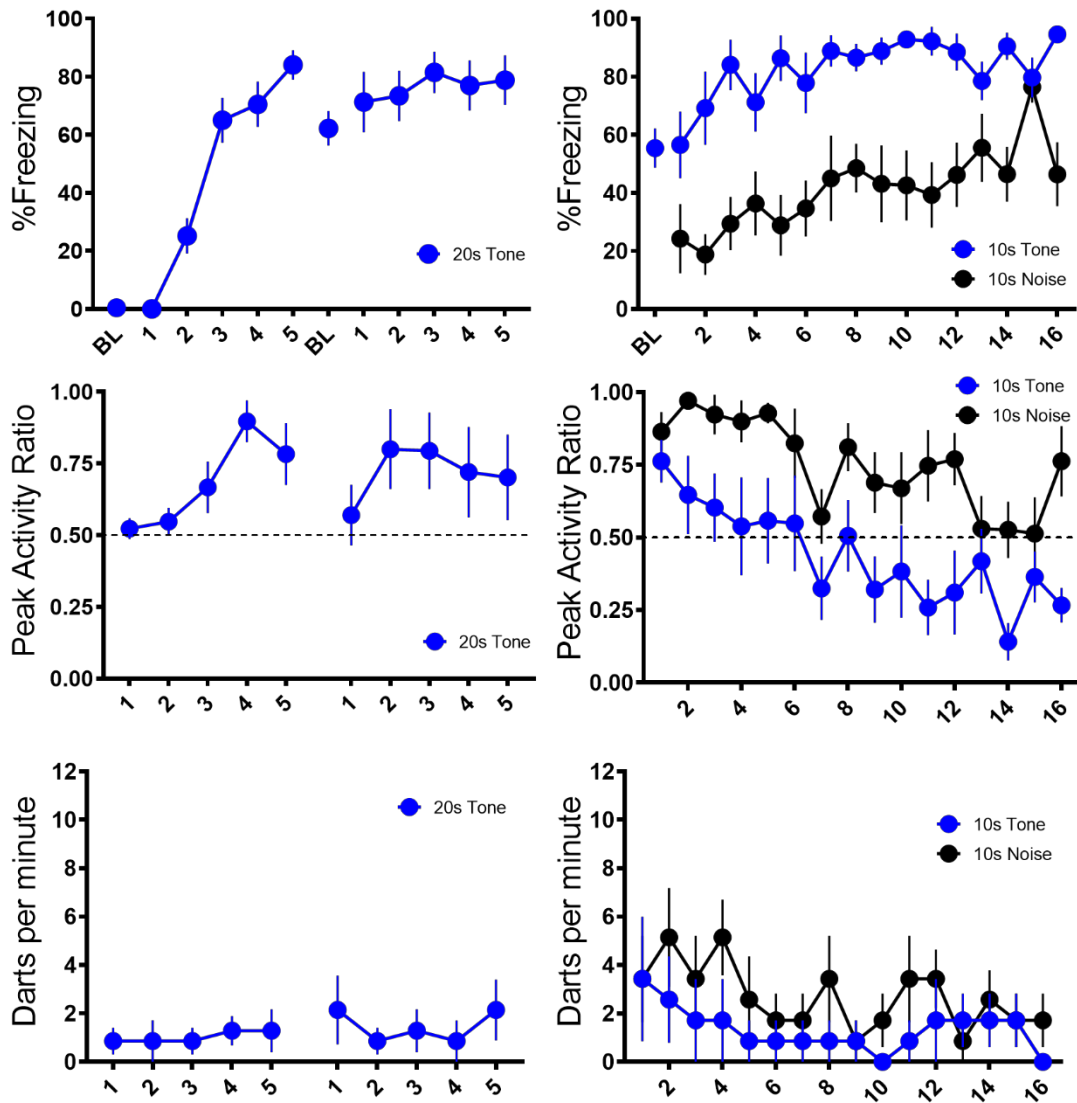
Group	Habituation Treatment: 10 CS Exposures (5 per day)	Training Treatment: 10 CS-US Pairings (5 per day)	Testing Treatment (3 on one day)
1) Habituation/Shock Only (H-Shock)	10-sec Noise	1-sec Shock	10-sec Noise
2) Habituation/Paired Noise-Shock (H-Paired)	10-sec Noise	10-sec Noise→1-sec Shock	10-sec Noise
3) Context Exposure/Shock Only (C-Shock)	Context Exposure	1-sec Shock	10-sec Noise
4) Context Exposure/Paired Noise-Shock (C-Paired)	Context Exposure	10-sec Noise→1-sec Shock	10-sec Noise

590 **Figures**



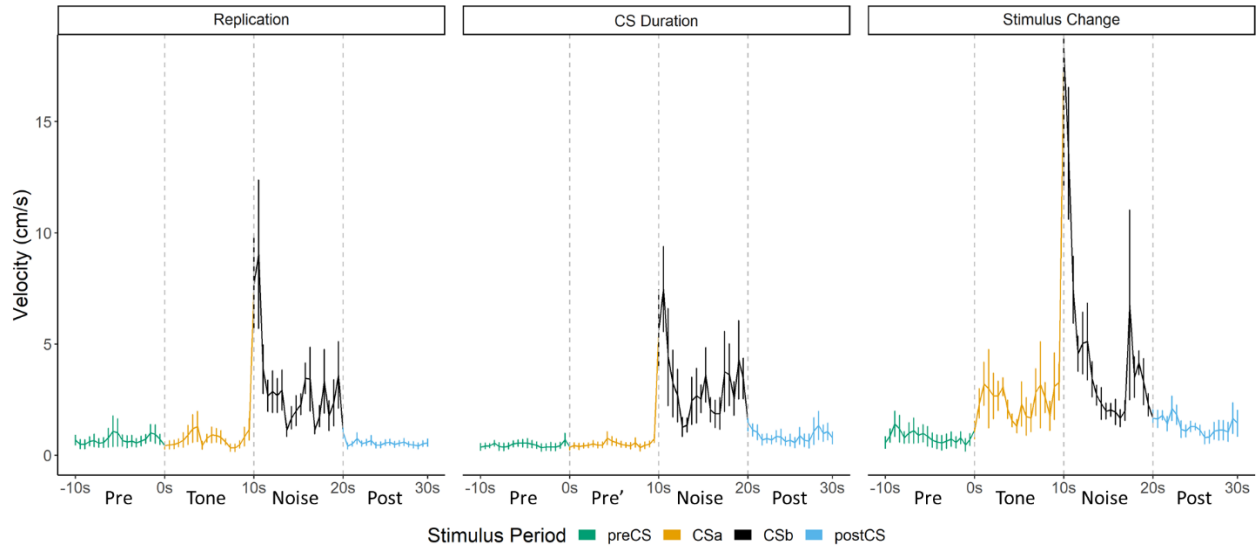
591

592 Figure 1 Behavioral design and schematic representation of the serial compound conditioned
593 stimulus (SCS) used for the Replication Group in Experiment 1. During training, animals were
594 given two days each of 5 SCS-US pairings. The SCS consisted of a 10s pure tone (7.5 kHz)
595 followed by a 10s white noise (75 dB). Immediately upon termination of the white noise/SCS, a
596 mild footshock US (1s, 0.9 mA) was delivered. On Day 3, the animals were tested with 16
597 presentations of the SCS without delivering any shocks.



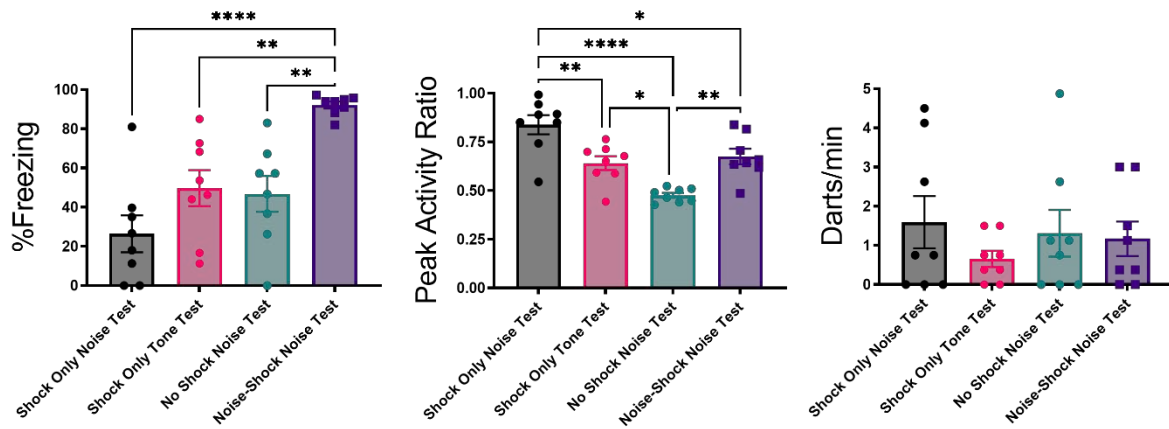
598

599 Figure 2 Trial-by-Trial Mean (\pm SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darts
600 per minute throughout all stimulus presentations during training (left panels) and testing (right
601 panels) for the Stimulus Change Group in Experiment 1.



602

603 Figure 3 Averaged traces of velocity (cm/s) across the first 4 trials of extinction during testing
604 for Experiment 1. Data is averaged across all animals per group and binned into ~.5s bins
605 (0.533s) and presented as means plus/minus standard error (Mean \pm SE). These within-subject
606 error bars are corrected for between-subject variability using methods as described in Rouder
607 and Morey (2005). During this test, the Replication Group and the Stimulus Change Group
608 received the serial conditioned stimulus (SCS) in which a 10s tone was followed by a 10s noise.
609 The CS Duration group was only tested with a 10s noise.

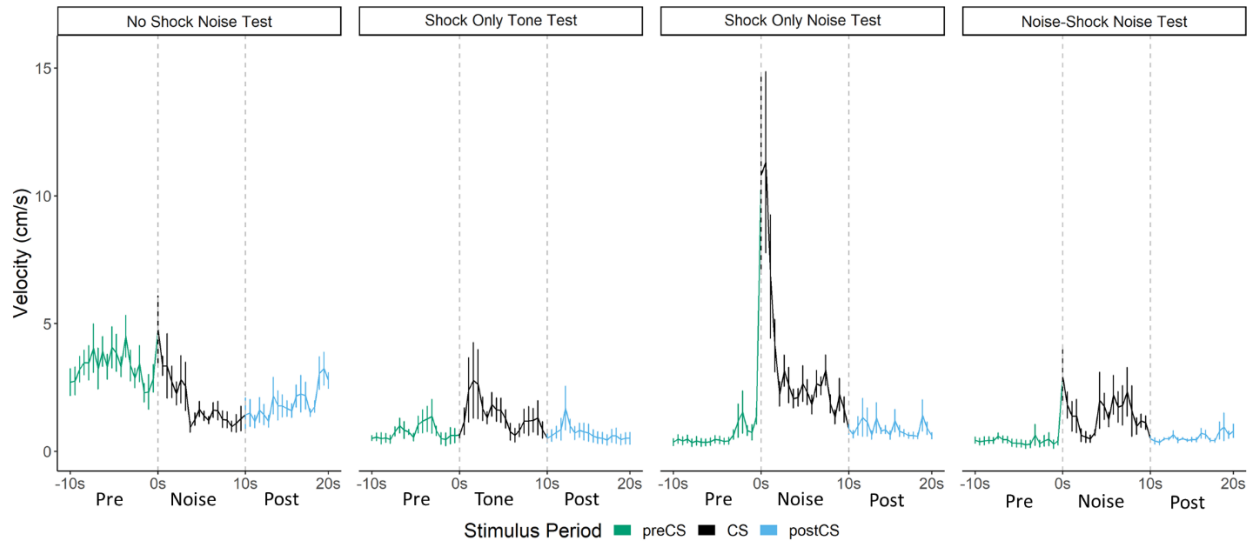


610

611 Figure 4 Mean (\pm SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darting for the test

612 session for Experiment 2. Values are averaged across the 16 trials of extinction during test.

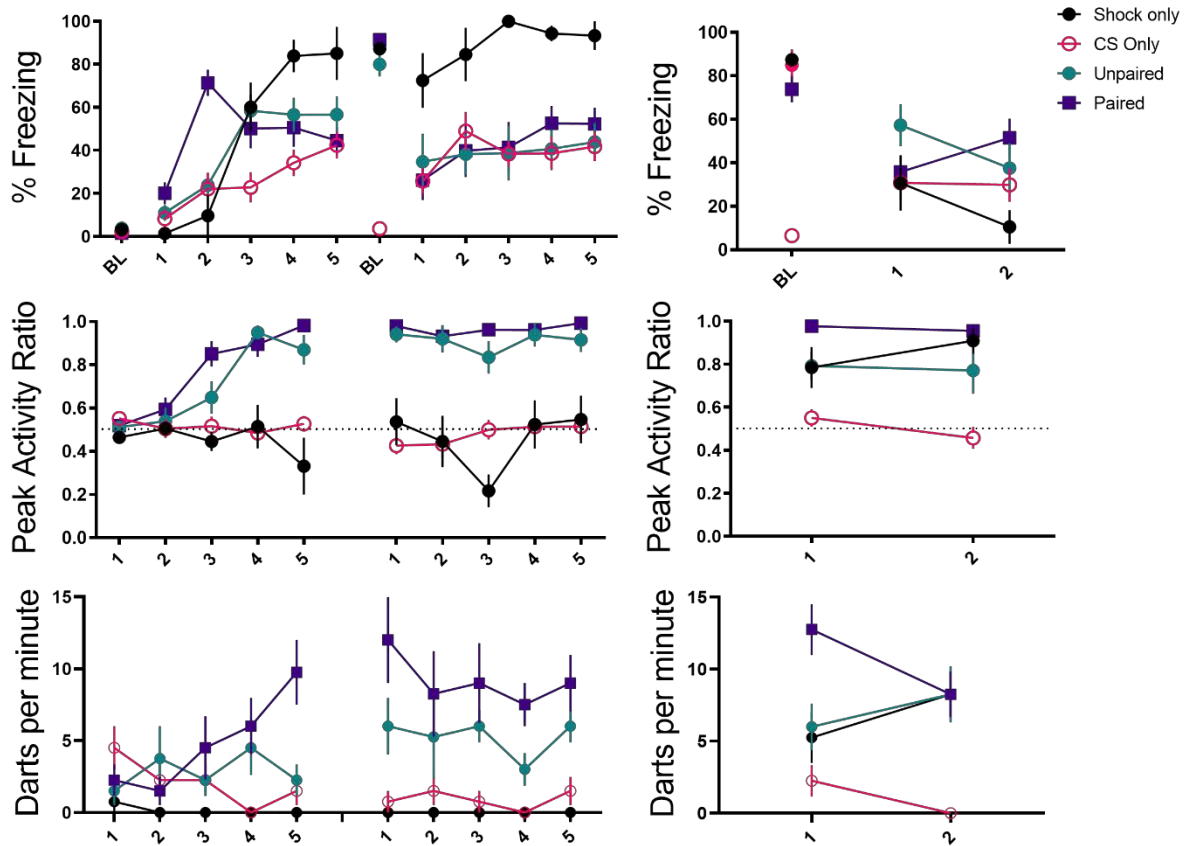
613 *p < .05, **p < .01, ****p < .0001



614

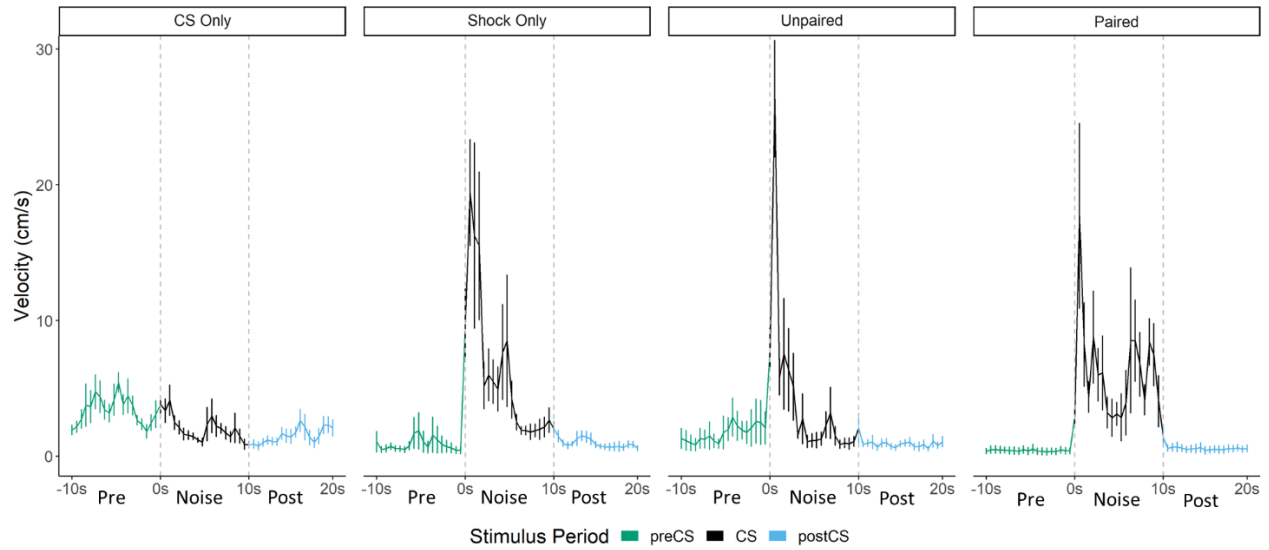
615

616 Figure 5 Averaged traces of velocity (cm/s) across the first 4 trials of extinction during testing
617 for Experiment 2. Data is averaged across all animals per group and binned into ~.5s bins
618 (0.533s) and presented as means plus/minus standard error (Mean \pm SE). These within-subject
619 error bars are corrected for between-subject variability using methods as described in Rouder
620 and Morey (2005). During this test, the No Shock-Noise Test, Shock Only-Noise Test, and
621 Noise-Shock Noise Test groups were tested with a 10s noise. The Shock Only-Tone Test group
622 was tested with a 10s tone.

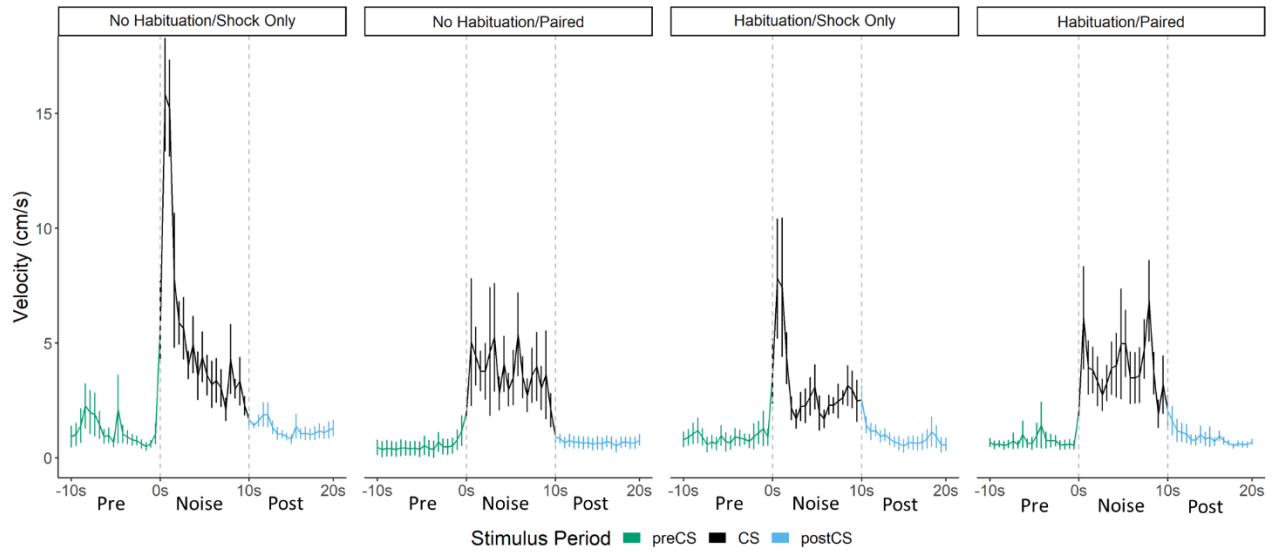


623

624 Figure 6 Trial-by-trial Mean (±SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darting
625 per minute throughout all stimulus presentations during training (left panels) and testing (right
626 panels) for Experiment 3.



628 Figure 7 Averaged traces of velocity (cm/s) across 2 trials of extinction during testing for
629 Experiment 3. Data is averaged across all animals per group and binned into ~.5s bins (0.533s)
630 and presented as means plus/minus standard error (Mean \pm SE). These within-subject error bars
631 are corrected for between-subject variability using methods as described in Rouder and Morey
632 (2005). During this test all groups were tested with a 10s Noise CS.



633

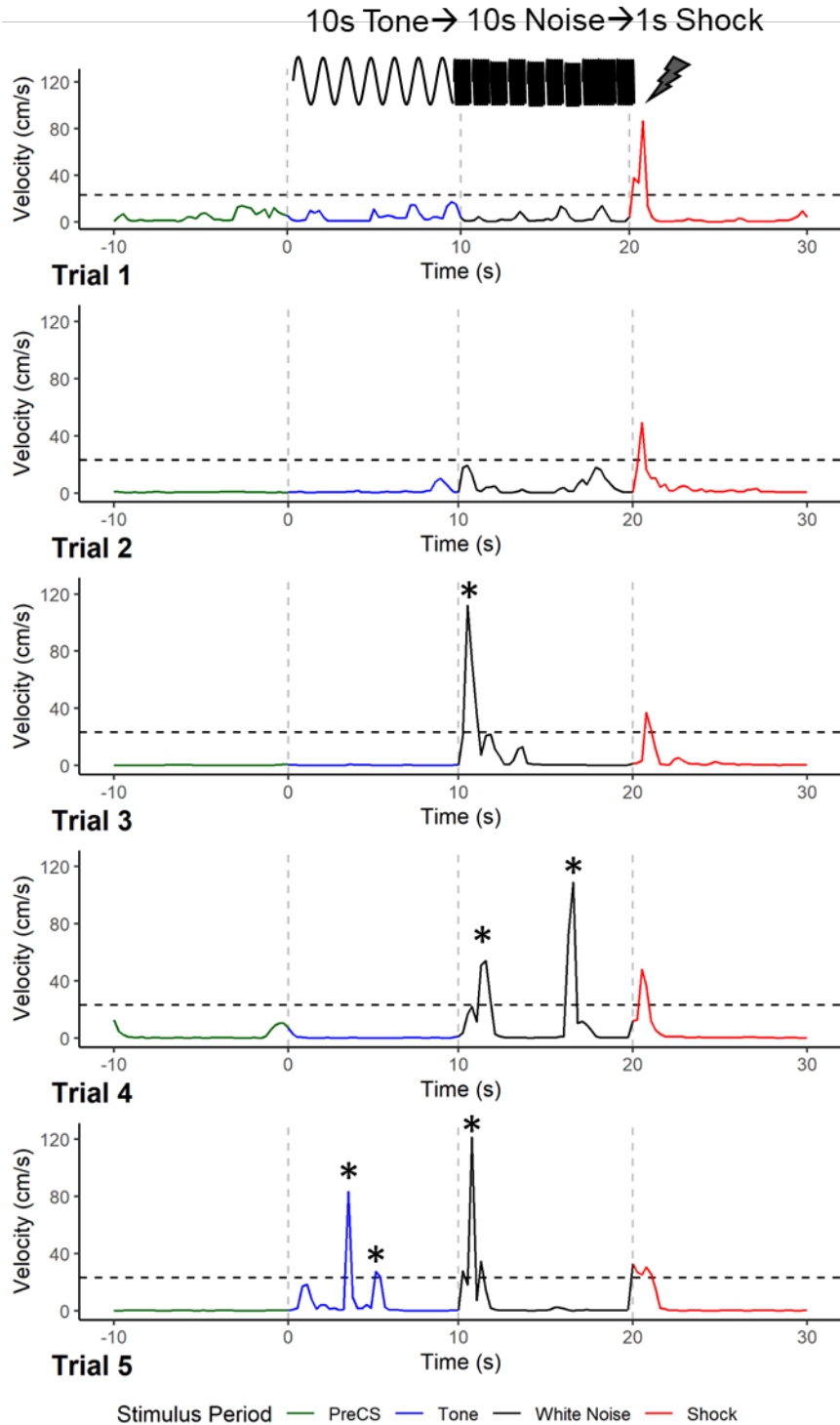
634 Figure 8 Averaged traces of velocity (cm/s) across 3 trials of extinction during testing for

635 Experiment 4. Data is averaged across all animals per group and binned into ~.5s bins (0.533s)

636 and presented as means plus/minus standard error (Mean \pm SE). These within-subject error bars

637 are corrected for between-subject variability using methods as described in Rouder and Morey

638 (2005). During this test all groups were tested with a 10s Noise CS.



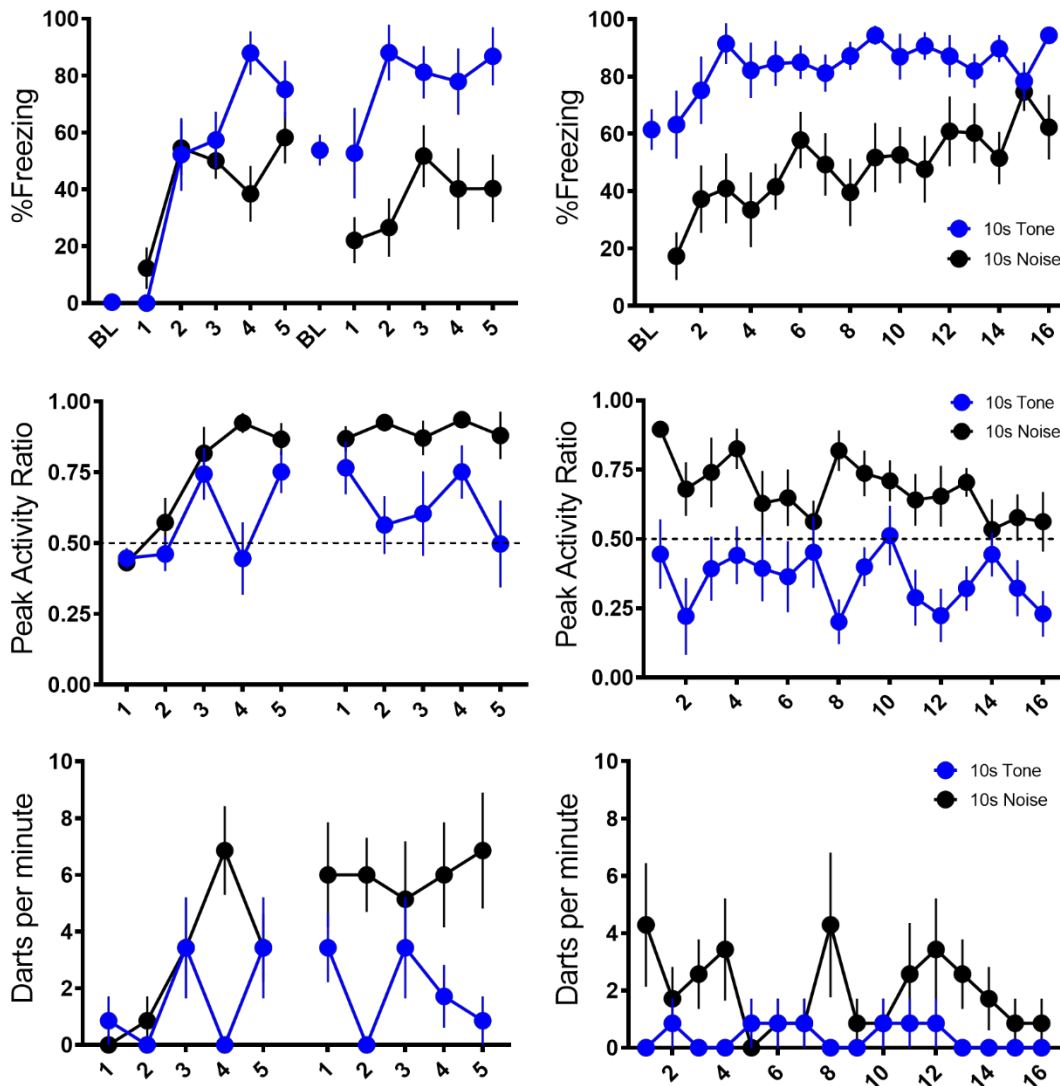
639

640 Figure S1 Example traces of velocity (cm/s) measurements obtained via EthoVision across five

641 trials on the first day of training for a mouse in the Replication Group of Experiment 1. Vertical

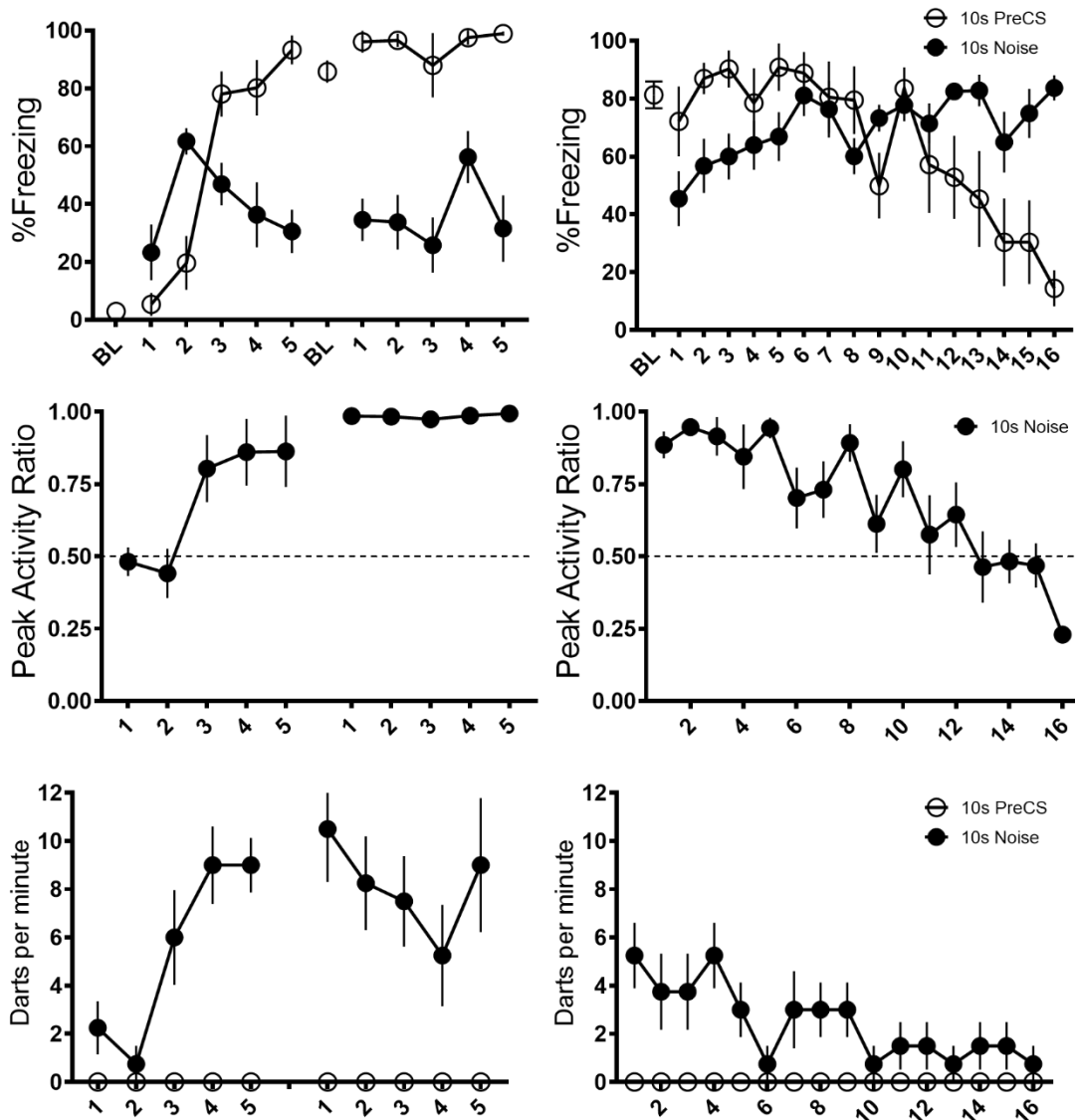
642 dotted lines denote stimulus onset times and the horizontal dotted line is the threshold for
643 scoring behavior as a dart (22.9 cm/s). Darting episodes are marked with an *.

644



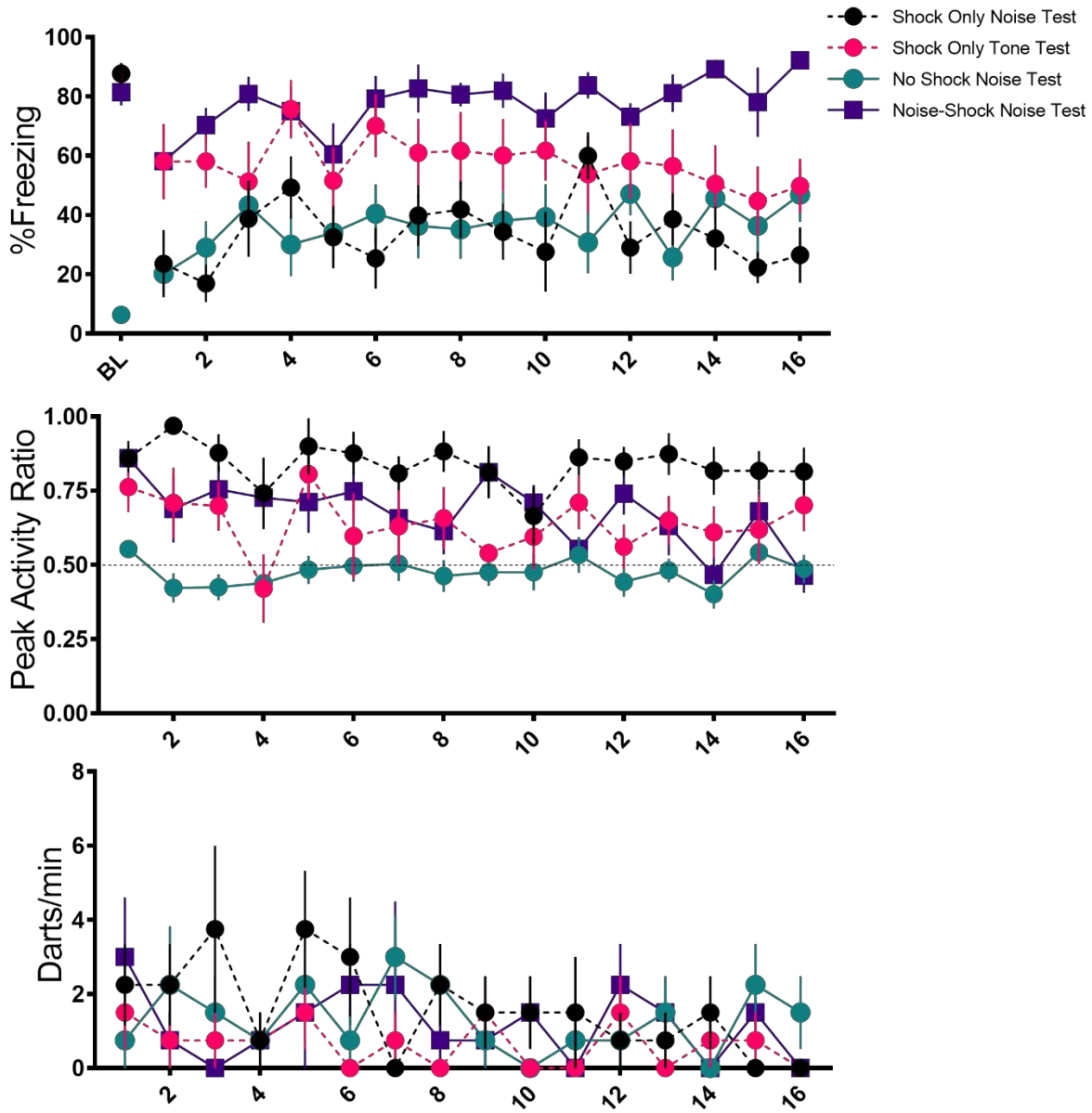
645

646 Figure S2 Mean (\pm SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darts per minute
647 throughout training (left panels) and testing (right panels) for the Replication Group of
648 Experiment 1. Responding during the tone is represented with filled in grey circles, responding
649 during the noise is represented with filled in black circles.



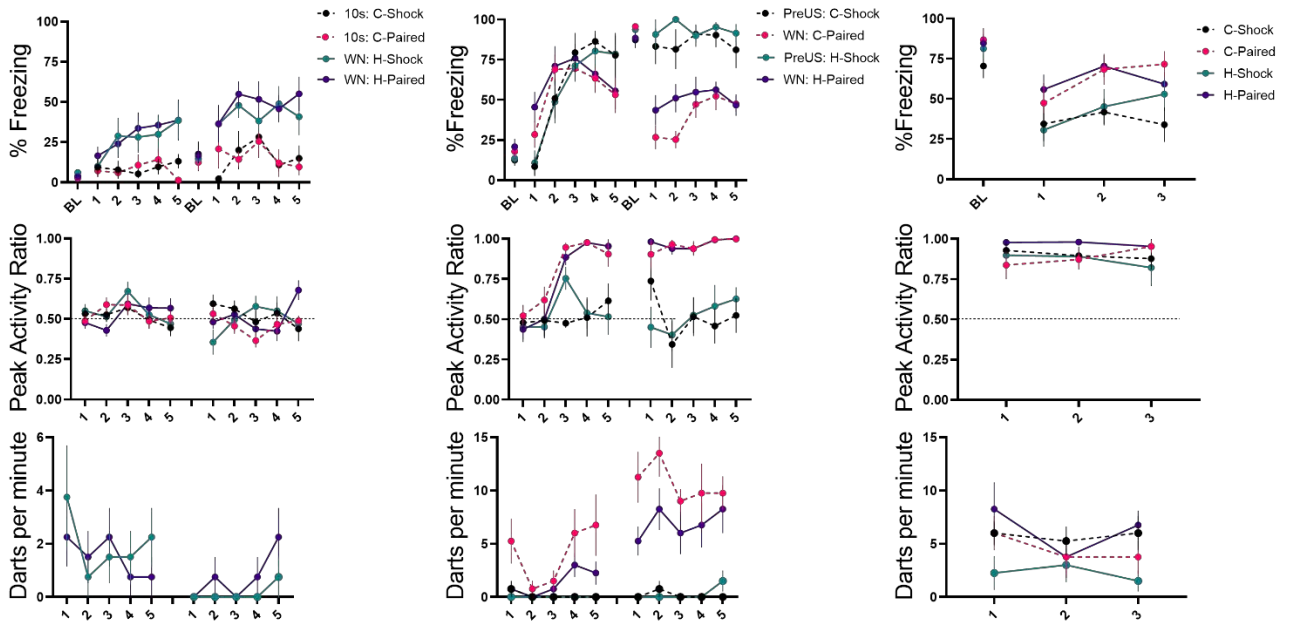
650

651 Figure S3 Mean (\pm SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darts per minute
652 throughout training (left panels) and testing (right panels) for the CS Duration Group of
653 Experiment 1. Responding during the 10s preCS period is represented with open circles,
654 responding during the noise is represented with filled in black circles.



655

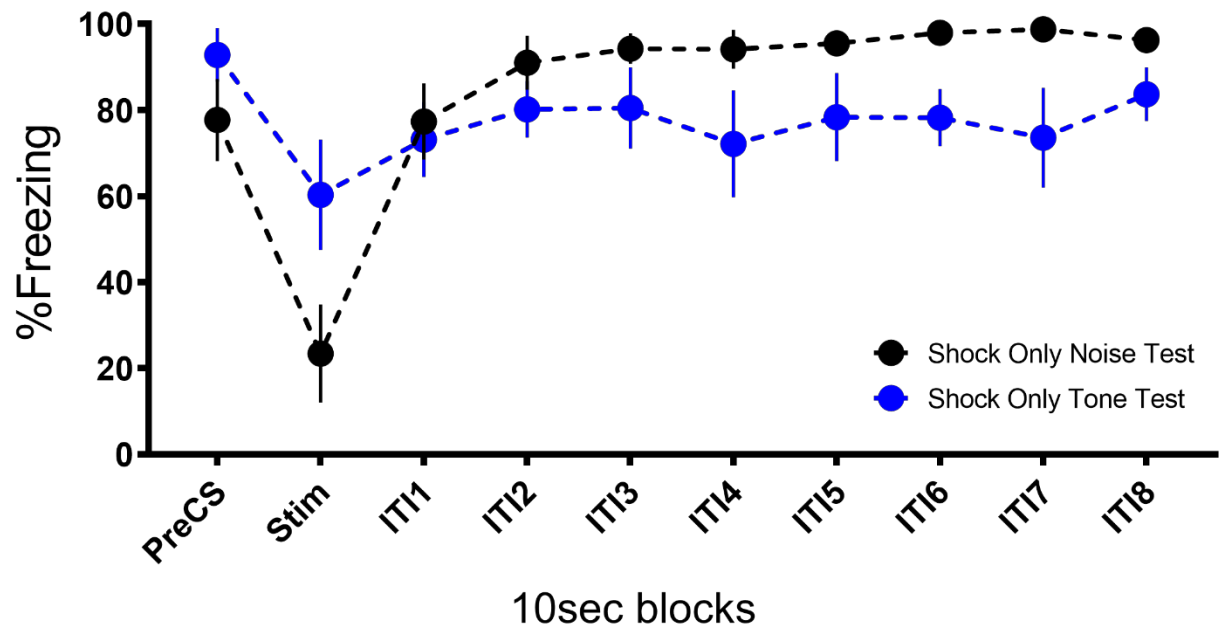
656 Figure S4 Trial-by-Trial Mean (\pm SEM) Percent Freezing, Peak Activity Ratio (PAR), and Darts
657 per minute throughout 16 trials of testing for Experiment 2.



658

659 Figure S5 Trial-by-trial Mean (\pm SEM) Percent Freezing, Peak Ratio (PAR), and Darting per
660 minute throughout all stimulus presentations during habituation (left panels), training (middle
661 panels), and testing (right panels) for Experiment 4.

662



663

664 Figure S6 Mean (\pm SEM) Percent Freezing during extinction/testing for Experiment 2 showing
665 that the occurrence of the stimuli at test disrupt freezing to the context and that the noise
666 disrupts freezing to a greater extent than the tone.

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