Reducing shock imminence eliminates poor avoidance in rats

Lindsay C. Laughlin[2], Danielle M. Moloney[1,2], Shanna B. Samels[2], Robert M. Sears[1,2] and Christopher K. Cain[1,2]

[1]NYU School of Medicine, Department of Child & Adolescent Psychiatry, New York, NY 10016

[2]Nathan Kline Institute for Psychiatric Research, Emotional Brain Institute, Orangeburg, NY 10962

Corresponding Author:

Christopher Cain, PhD

Research Scientist

Emotional Brain Institute at NKI

Child & Adolescent Psychiatry at NYU Langone Health

140 Old Orangeburg Road

Orangeburg, NY 10962

Phone: (845) 398-6560

Email: Christopher.Cain@NKI.RFMH.org

Running Title: Reducing shock imminence improves avoidance

Abstract

In signaled active avoidance (SigAA), rats learn to suppress Pavlovian freezing and emit actions to escape threats and prevent footshocks. SigAA is critical for understanding aversively-motivated instrumental behavior and anxiety-related active coping. However, with standard protocols ~25% of rats exhibit high freezing and poor avoidance. This has dampened enthusiasm for the paradigm and stalled progress. We demonstrate that reducing shock imminence with long-duration warning signals leads to greater freezing suppression and perfect avoidance in all subjects. This suggests that instrumental SigAA mechanisms evolved to cope with distant harm and protocols that promote inflexible Pavlovian reactions are poorly-designed to study avoidance.

In the signaled active avoidance paradigm (SigAA), rats learn to suppress Pavlovian reactions (e.g. freezing) and emit instrumental actions (e.g. shuttling) to escape warning signals (WSs) and prevent painful unconditioned stimuli (USs, typically footshocks). Understanding the psychological and neural mechanisms of SigAA is critical for several reasons. It is the prototypical paradigm for studying aversively-motivated instrumental actions (Cain et al., 2013; Rescorla and Solomon, 1967). Maladaptive or excessive avoidance responses (ARs) contribute to every major anxiety disorder (APA, 2013). Lastly, adaptive ARs reduce emotional reactions and give subjects control over environmental threats (Boeke et al., 2017; Cain and LeDoux, 2007; Choi et al., 2010; Kamin et al., 1963), suggesting a potential role in proactive coping behaviors and resilience in humans (Collins et al., 2014; LeDoux and Gorman, 2001; Moscarello and Hartley, 2017; van der Kolk, 2006).

Despite its importance as a fundamental learning mechanism with clinical relevance, SigAA research has lagged behind research on Pavlovian threats and appetitive instrumental behavior (Cain, 2019; Krypotos et al., 2015; LeDoux et al., 2017). The phenomenon of “poor avoidance” has been one major obstacle to progress. Avoidance acquisition is typically slower than Pavlovian conditioning, but most animals learn to prevent >80% of scheduled shocks in two-way shuttlebox tasks. However, a significant subset of animals exhibit high freezing and rarely emit ARs (Choi et al., 2010; Galatzer-Levy et al., 2014; Keehn, 1967; Martinez et al., 2013). For some tasks, poor avoidance is the rule rather than the exception (Neffinger and Gibbon, 1975; Solomon and Brush, 1954). From a practical standpoint, avoidance studies are more costly and time-consuming because poor avoiders must be replaced. Pretraining loss-of-function

studies are also ill-advised with SigAA, since there is no reliable way to predict which animals will acquire ARs. Finally, the poor avoidance phenomenon raises questions about whether instrumental AR learning is a major component of defense worthy of study (Bolles, 1975; Fanselow, 1997; 2018). Animals evolved defensive mechanisms to cope with predators, not shocks, and it is difficult to see how a trial-and-error learning mechanism that often fails could have evolved under predatory pressure.

One simple explanation is that researchers have used suboptimal protocols for studying avoidance in the laboratory. SigAA is typically evaluated in small chambers with short-duration WSs and high-density shock protocols. These conditions are ideal for modelling a state of high predatory imminence that triggers hard-wired, stereotyped fear-like reactions (e.g. freezing) that are incompatible with ARs (Fanselow and Lester, 1988; Weiss et al., 1968). However, prey animals spend much more time in a state of low predatory imminence where encounters with predators are temporally distant or uncertain. Perhaps instrumental avoidance mechanisms evolved to deal with these anxiety-like states, where animals must balance conflicting needs (Cain, 2019; Diehl et al., 2019; Gray and McNaughton, 2000). Under these “pre-encounter” conditions, less rigid defensive behaviors may not interfere with AR learning.

To solve the poor avoidance problem and optimize avoidance training, we designed two experiments to evaluate AR learning while systematically varying threat intensity. In the first, WS-US contingency was varied to test how US certainty affects AR learning. In the second, WS duration was varied to test how US imminence affects AR learning. In Pavlovian studies, reducing US certainty or imminence appears to promote anxiety over fear; freezing reactions are diminished and more flexible antipredator

strategies increase (Blanchard et al., 1989; Cain et al., 2005; Goode et al., 2019; Helmstetter and Fanselow, 1993; Kim and Jung, 2018; Mobbs et al., 2007; Rescorla, 1968; Waddell et al., 2006). Lesions that impair freezing rescue ARs in poor avoiders, suggesting that freezing reactions interfere with avoidance (Choi et al., 2010; Lazaro-Munoz et al., 2010; Moscarello and LeDoux, 2013). Pavlovian reactions also impair avoidance performance in humans (Rigoli et al., 2012). Thus, we predicted that both methods of reducing threat intensity would decrease Pavlovian freezing and improve AR learning.

Experiments were conducted on adult male and female Sprague-Dawley rats (Hilltop Lab Animals) weighing 300-350g on arrival. Rats were pair-housed by sex, had ad lib access to food and water, and were tested during the light phase of a 12:12-hour light:dark schedule. All procedures were approved by the NKI-IACUC.

All rats received 10 days of two-way SigAA training in standard shuttleboxes equipped with speakers, houselights, cameras, grid floors and infrared beams to detect shuttling (Coulbourn Instruments). Sessions included a 5-minute acclimation followed by 15 trials where warning stimuli (80dB white noise) preceded scrambled 0.5s footshocks (males: 1.0mA, females: 0.7mA). Session 1 always began with an inescapable Pavlovian trial, ensuring that all subsequent WS-shuttles occurred during threat of shock. For all subsequent trials, shuttling to the opposite chamber side terminated the WS, produced feedback (5s, 5kHz, 80dB tone), and cancelled the upcoming shock (if scheduled). Shuttling was automatically recorded by Graphic State software (Coulbourn Instruments) and freezing was recorded to video files for off-line analysis. Intertrial intervals (ITIs) averaged 2-minutes unless otherwise stated. Avoidance percentage was calculated for

individuals each session [(WS-shuttles/Trials)*100]. Avoidance latency reflects the time from WS onset to shuttle, with failures recorded as the full WS duration. Freezing was scored during the WS for select sessions by two experienced raters blind to group (inter-rater reliability correlation >0.9). To facilitate comparisons of freezing suppression between studies, Session 10 freezing was also analyzed as a percentage of Session 1 freezing (calculated for individuals and then averaged).

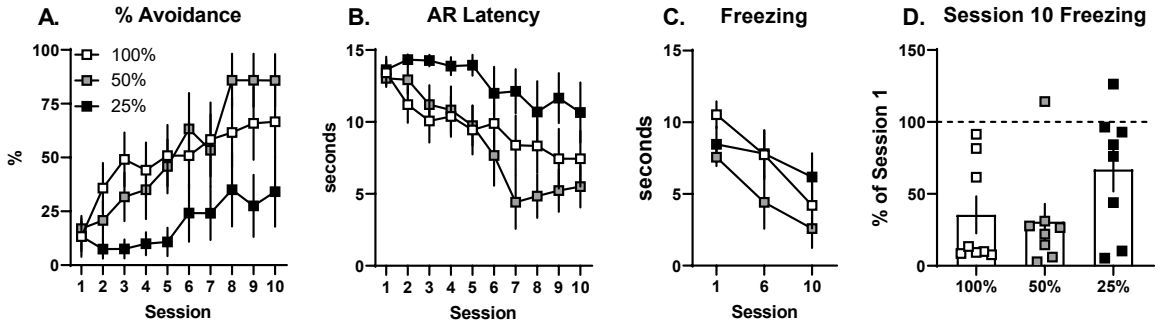

Figure 1. Reducing WS-US contingency does not improve avoidance. A) Mean percent avoidance by session. **B)** Mean avoidance response (AR) latency by session. **C)** Mean seconds freezing during warning signals for Sessions 1, 6 and 10. **D)** Mean Session 10 freezing expressed as a percentage of Session 1 freezing. Squares represent individuals. N=8/group (4 females, 4 males). Error bars = S.E.M. *p<0.05 vs. 100% WS-US contingency group

In Experiment 1, rats received SigAA training with a standard 15s WS except the likelihood of receiving a shock on failure trials was varied (100%, 50% or 25%; Figures 1A-B). Two-way ANOVAs (GraphPad Prism v8) indicate group differences in acquisition rate for both AR% and AR latency (Group x Session interactions: $F_{(18,189)}=1.8$, $p=0.02$; $F_{(18,189)}=2.0$, $p=0.01$), however, reducing WS-US contingency did not improve learning. These differences appear to be driven by a deficit in the 25% group, where rats shuttled

on average more slowly and less frequently. On average, freezing declined across avoidance training but there were no significant group differences. A two-way ANOVA revealed a significant effect for Session ($F_{(2,42)}$=21.6, p<0.01), but not for Group ($F_{(2,21)}$=0.26) or the Group x Session interaction ($F_{(4,42)}$=1.7; Figure 1C). Similarly, Session 10 freezing was lower on average than Session 1 but no group differences were observed (Figure 1D; One-way ANOVA: $F_{(2,21)}$=2.1).

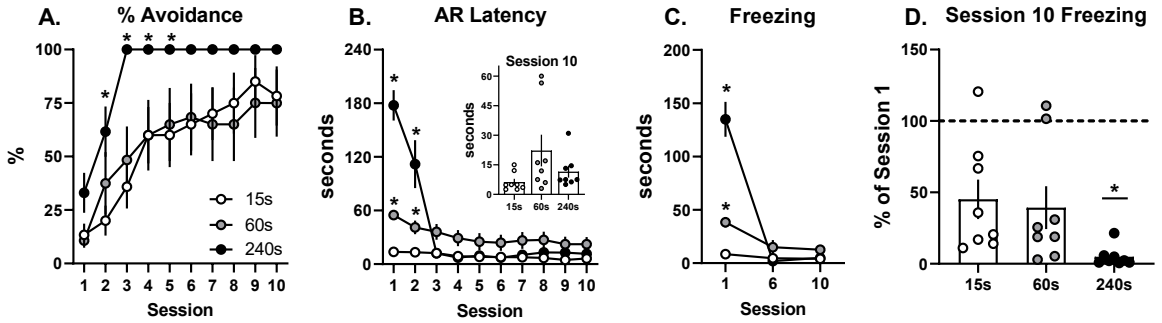

Figure 2. Reducing US imminence leads to perfect avoidance. A) Mean percent avoidance by session. **B)** Mean avoidance response (AR) latency by session. ***inset*:** mean AR latency for individuals during Session 10. **C)** Mean seconds freezing during warning signals for sessions 1, 6 and 10. **D)** Mean Session 10 freezing expressed as a percentage of Session 1 freezing. Dots represent individuals. N=8/group (4 females, 4 males). Error bars = S.E.M. *p<0.05 vs. 15s-WS group

In Experiment 2, rats received avoidance training with 100% WS-US contingency (shock delivered on every failure trial), but the WS duration was varied (15s, 60s or 240s; Figures 2A-B). AR% increased across training (Session: $F_{(9,189)}$=21.1, p<0.01) and there was a significant effect of WS duration (Group: $F_{(2,21)}$=4.7, p=0.02), however the pattern of change over time did not differ between groups (Group x Session: $F_{(18,189)}$=0.9). The effect of WS duration was driven mainly by the 240s group, where AR% was higher than

the 15s group for Sessions 2-5 (Dunnett's tests). Remarkably, every rat in the 240s group showed perfect avoidance from Session 3 until the end of training (no failures). As expected for different WS durations, large differences in AR latency were observed across training (Group x Session: $F_{(18,189)}=21.9$, $p<0.01$). These differences are not very informative early in training when failures were common and WS-duration determined AR latency. Interestingly, AR latencies were very similar by the end of training, even though rats in the 60s and 240s groups had much more time to emit ARs (Figure 2B, inset). Large freezing differences were also apparent across training (Figure 3C; Groups x Session: $F_{(4,42)}=37.7$, $p<0.01$). This may be partly explained by the different WS durations. Rats in all groups froze for most of the WS early in training and freezing declined to similarly low levels as ARs were acquired. Dunnett's post tests revealed that rats in the 240s and 60s groups froze more than rats in the 15s group during Session 1 only (p values <0.01). Rats in the 240s group showed the strongest suppression of freezing by Session 10 (Figure 2D; one-way ANOVA: $F_{(2,21)}=3.4$, $p=0.05$; Dunnett's test vs. 15s group: $p<0.05$). This appears to reflect more than the programmed differences in WS duration; unlike the other groups, no rats in the 240s group maintained or increased freezing across training (as occurs in poor avoiders; Lazaro-Munoz et al., 2010).

One potential criticism of the long WS is that apparent ARs reflect locomotor activity not instrumental shuttling. To address this, we replicated AR training with the 240s WS (n=8; 5 females, 3 males) and included Yoked controls (n=8; 4 females, 4 males). All rats in the 240s-WS group again attained perfect avoidance (not shown). Yoked controls shuttled far less frequently during the WSs than Master rats (<0.2 shuttles/trial on average

by training end; Group x Session interaction ($F_{(9,126)} = 8.3$, $p<0.01$). This supports the notion that WS shuttles represent instrumental ARs even with long-duration WSs.

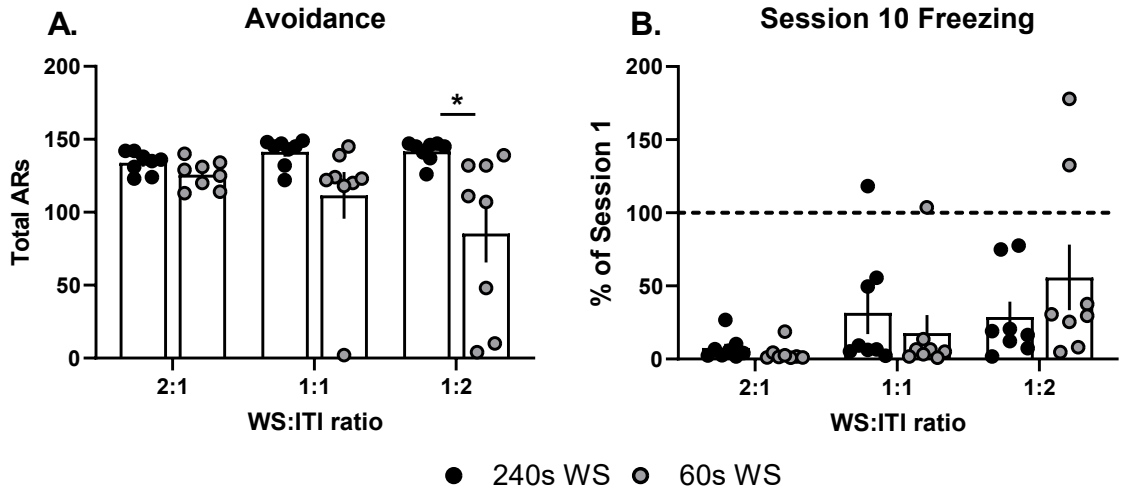

Figure 3. Reducing the WS:ITI ratio fails to impair avoidance with a 240s warning signal. A) Total avoidance responses emitted across 10 sessions of training. **B)** Mean Session 10 freezing expressed as a percentage of Session 1 freezing. N=8/group (4 females, 4 males). Bars represent separate groups. Bar height indicates group mean. Dots represent individuals. Error bars = S.E.M. *p<0.05 for 240s vs. 60s WS groups

Experiment 2 was designed to test the effect of US imminence on avoidance learning. However, because WS duration was varied while the ITI was held constant, another explanation is possible. In Pavlovian studies, the conditioned stimulus (CS) to ITI ratio has a strong impact on performance of Pavlovian reactions (reviewed in Balsam et al., 2010). Specifically, higher CS:ITI ratios weaken responding, perhaps because the signal loses informational value relative to the background context (Gibbon and Balsam, 1989). Thus, it is possible that our long WS enhanced avoidance because it increased the WS:ITI ratio and weakened competing freezing reactions. To address this, we

evaluated avoidance and freezing with WS:ITI ratios of 2:1, 1:1 and 1:2 using two different WS durations (60s and 240s). We trained four new groups of rats: 60s-WS:30s-ITI (2:1), 60s-WS:60s-ITI (1:1), 240s-WS:480s-ITI (1:2), and 240s-WS:240s-ITI (1:1). The remaining groups for the analysis came from Experiment 2: 60s-WS:120s-ITI (1:2) and 240s-WS:120s-ITI (2:1). Figure 3A depicts total (cumulative) ARs across training. A two-way ANOVA revealed a significant effect of WS-duration ($F_{(1,42)}$=12.9, $p<0.01$) and a non-significant trend towards a WS-duration x Ratio interaction ($F_{(2,42)}$=2.6, $p=0.09$). The main effect for Ratio was not significant ($F_{(2,42)}$=1.3). Thus, reducing the WS:ITI ratio failed to impair ARs and perfect avoidance was achieved for every rat trained with the 240s-WS. The same manipulation may reduce avoidance with a shorter WS; total ARs declined as the WS:ITI ratio dropped for the 60s WS. Further, in the 2:1 condition, rats in the 60s group avoided less than rats in the 240s group (planned Sidak's comparison). Suppression of freezing was more sensitive to the WS:ITI ratio (Figure 3B). Session 10 freezing increased as the WS:ITI ratio dropped (Ratio: $F_{(2,42)}$=0.03, $p=0.03$), but this effect was not modulated by WS-duration (WS-duration: $F_{(1,42)}$=0.09; WS-duration x Ratio: $F_{(2,42)}$=1.4). This suggests that the WS:ITI ratio is not a major determinant of AR acquisition with very long WSs. However, there are indications that reducing this ratio promotes freezing and impairs AR learning with shorter WSs. The reverse also appears true; using a 2:1 ratio led to very low Session 10 freezing and perfect avoidance for 7 of 8 rats trained with the 60s-WS. Exploring a wider range of WS:ITI ratios may help clarify these findings.

Lastly, all experiments included both female and male subjects. Avoidance learning with the 240s WS was nearly identical between the sexes as measured by AR%

(Session: $F_{(9,126)}$=41.0, p<0.01, Sex: $F_{(1,14)}$=2.3, Session x Sex: $F_{(9,126)}$=1.0) and AR latency (Session: $F_{(9,126)}$=39.3, p<0.01, Sex: $F_{(1,14)}$=3.6, Session x Sex: $F_{(9,126)}$=0.6). Freezing across training was also very similar (Session: $F_{(2,28)}$=81.5, p<0.01, Sex: $F_{(1,14)}$=0.29, Session x Sex: $F_{(2,28)}$=0.08). Sex differences were difficult to evaluate in the other conditions due to poor avoiders, which appear to occur randomly (equally likely in both sexes).

Our major finding is that reducing US imminence by extending WS duration greatly facilitates SigAA learning. In four separate groups trained with the long-duration WS, every rat learned and performed the task perfectly (no subsequent failures), sometimes in fewer than 30 trials. The benefits of the long-duration WS also resisted manipulations of the WS:ITI ratio that promote competing freezing reactions. Several observations also argue against the concern that shuttling during long-duration WSs reflects exploration rather than instrumental ARs. First, exploration was severely depressed early in training where rats froze for more than 60% of the WS. Second, once the response was acquired, ARs were emitted with short latencies (usually <15s). Third, yoked controls shuttled during the WS at a far lower rate than master rats.

What might explain the enhanced efficiency of SigAA with long-duration WSs? Though there are some reports of improved SigAA learning with slightly longer or more complex WSs (Archer et al., 1984; Coll-Andreu et al., 1993; Levis and Stampfl, 1972; Satorra-Marin et al., 2001; Solomon and Brush, 1954; Terburg et al., 2018), this has not been systematically studied. There are far more studies of US imminence using Pavlovian paradigms. These suggest that threats activate different components of the survival circuit depending on proximity to harm (modeled by CS-US delay; Davis, 1998; Goode et al.,

2020; Mobbs et al., 2009; Mobbs et al., 2007; Sullivan et al., 2004; Waddell et al., 2006; Walker and Davis, 1997). Short-duration CSs recruit amygdala and periaqueductal gray to emit short-latency, inflexible, hard-wired responses that function to prevent threat escalation (e.g. freezing, a post-encounter response) or escape harm (e.g. flight, a circa-strike response). Long-duration CSs recruit bed nucleus of the stria terminalis and prefrontal cortex to flexibly reorganize behavior (e.g. thigmotaxis, altered meal-patterns), presumably to prevent threat escalation and prepare the organism to defend against distant or uncertain harm. Importantly, high US-imminence restricts behavior to species-specific defense reactions whereas low US-imminence balances defense with other behaviors like exploration and reward procurement (Blanchard and Blanchard, 1989; Bolles, 1970; Fanselow, 2018; Gray and McNaughton, 2000; Mobbs et al., 2015; Mobbs and Kim, 2015). Thus, long-duration WSs likely trigger less intense defensive strategies and allow for active responses like shuttling. This is consistent with observed patterns of freezing; though 240s-WS rats froze significantly early in training (~62%), they had more time to emit the AR and experience the instrumental contingency than rats in the other groups. Freezing appeared to be more easily suppressed in this condition too. Thus, optimal trial-and-error SigAA mechanisms may have evolved under low threat conditions, where errors (failures to emit the AR) lead to more intense threats and not necessarily harm.

Though our hypothesis about lowering threat intensity to improve avoidance was supported by the US-imminence experiment, it was not supported by reducing WS-US contingency. Rats receiving shocks on only 25% of failure trials did not perform better than rats in the 100% contingency condition. We see two possible explanations for this.

First, SigAA learning depends, at least in part, on omission of expected US presentations (Bolles et al., 1966; Cain, 2019; Hunter, 1935; Kamin, 1956). So even if 25% WS-US contingency reduces certainty and competing freezing reactions, this may have been offset by degradation of an important reinforcement signal. Second, 10 sessions of SigAA training may have been too few to observe the benefit of reduced WS-US contingency. Additional work is needed to clarify these points. We found only two other studies that varied shock delivery on failure trials (Boren and Sidman, 1957; Neffinger and Gibbon, 1975). Though these were conducted quite differently (contingency manipulated after standard training, poor avoiders eliminated, no-ITI protocols, bar-press avoidance etc.), both confirm that reducing the likelihood of shock on failure trials leads to an avoidance decrement.

Interestingly, our follow-up experiment suggests another possible way to improve SigAA efficiency: increase the WS:ITI ratio. Pavlovian studies show that increasing the CS:ITI ratio impairs Pavlovian reactions (Balsam et al., 2010; Delamater and Holland, 2008; Stein et al., 1958). This is likely a result of the CS losing informational value relative to the background context (Gibbon and Balsam, 1989), making the CS a weaker threat. We see a similar pattern in freezing suppression during SigAA training; increasing the WS:ITI ratio produced weaker Session 10 freezing and near-perfect avoidance with the shorter 60s-WS. If replicated, this protocol could ensure good avoidance in all subjects with significantly shorter session durations.

In summary, we describe two simple procedural methods to improve SigAA learning and eliminate poor avoidance: increase the WS duration and/or the WS:ITI ratio. This removes a major obstacle to SigAA research that has dampened enthusiasm for the

paradigm over decades. Experiments requiring pre-training manipulations can be used with confidence if controls reliably learn and perform ARs. The explanation for enhanced avoidance with low-intensity threats also aligns with functional behavior systems theories of defensive behavior and Pavlovian studies of US-imminence (Fanselow and Lester, 1988; Mobbs and Kim, 2015; Waddell et al., 2006), but is inconsistent with two-factor "fear" theories of avoidance that assume avoidance positively correlates with threat intensity (Levis, 1989; Miller, 1948; Mowrer and Lamoreaux, 1946). This work may also help explain how strong avoidance responses may be acquired in human anxiety even when harm is not imminent.

Figure Captions

Figure 1. Reducing WS-US contingency does not improve avoidance. A) Mean percent avoidance by session. **B)** Mean avoidance response (AR) latency by session. **C)** Mean seconds freezing during warning signals for Sessions 1, 6 and 10. **D)** Mean Session 10 freezing expressed as a percentage of Session 1 freezing. Squares represent individuals. N=8/group (4 females, 4 males). Error bars = S.E.M. *p<0.05 vs. 100% WS-US contingency group

Figure 2. Reducing US imminence leads to perfect avoidance. A) Mean percent avoidance by session. **B)** Mean avoidance response (AR) latency by session. ***inset*:** mean AR latency for individuals during Session 10. **C)** Mean seconds freezing during warning signals for sessions 1, 6 and 10. **D)** Mean Session 10 freezing expressed as a percentage of Session 1 freezing. Dots represent individuals. N=8/group (4 females, 4 males). Error bars = S.E.M. *p<0.05 vs. 15s-WS group

Figure 3. Reducing the WS:ITI ratio fails to impair avoidance with a 240s warning signal. A) Total avoidance responses emitted across 10 sessions of training. B) Mean Session 10 freezing expressed as a percentage of Session 1 freezing. N=8/group (4 females, 4 males). Bars represent separate groups. Bar height indicates group mean. Dots represent individuals. Error bars = S.E.M. *p<0.05 for 240s vs. 60s WS groups

Acknowledgements

This work was supported by the National Institute of Mental Health of the National Institutes of Health under award numbers [R01MH114931] to C. Cain and [R21MH116242] to R. Sears. Additional funding for this project was provided by The William S. McIntyre Foundation to R. Sears. The authors thank Peter Balsam and Michael Fanselow for helpful discussions about the data.

References

APA (2013) *Diagnostic and statistical manual of mental disorders*, American Psychiatric Publishing, Arlingont, VA.

Archer T, Ogren S and Johansson G (1984) Stimulus conditions affecting the rate of acquisition in a computer-operated version of the two-way active avoidance procedure. *Scandinavian journal of psychology* **25**:89-95.

Balsam PD, Drew MR and Gallistel CR (2010) Time and Associative Learning. *Comp Cogn Behav Rev* **5**:1-22.

Blanchard RJ and Blanchard DC (1989) Antipredator defensive behaviors in a visible burrow system. *J Comp Psychol* **103**:70-82.

Blanchard RJ, Blanchard DC and Hori K (1989) Ethoexperimental approaches to the study of defensive behavior., in *Ethoexperimental Approaches to the Study of Behavior,* (Blanchard RJ, Brain PF, Blanchard DC and Parmigiani S eds) pp 114-136, Kluwer Academic Publishers, Dordrecht.

Boeke EA, Moscarello JM, LeDoux JE, Phelps EA and Hartley CA (2017) Active Avoidance: Neural Mechanisms and Attenuation of Pavlovian Conditioned Responding. *J Neurosci* **37**:4808-4818.

Bolles RC (1970) Species-specific defense reactions and avoidance learning. *Psychol Rev* **77**:32-48.

Bolles RC (1975) *Theory of Motivation*, Harper & Row, New York.

Bolles RC, Stokes LW and Younger MS (1966) Does CS termination reinforce avoidance behavior? *Journal of Comparative and Physiological Psychology* **62**:201-207.

Boren JJ and Sidman M (1957) Maintenance of avoidance behaviour with intermittent shocks. *Can J Psychol* **11**:185-192.

Cain CK (2019) Avoidance Problems Reconsidered. *Curr Opin Behav Sci* **26**:9-17.

Cain CK, Godsil BP, Jami S and Barad M (2005) The L-type calcium channel blocker nifedipine impairs extinction, but not reduced contingency effects, in mice. *Learn Mem* **12**:277-284.

Cain CK and LeDoux JE (2007) Escape from fear: a detailed behavioral analysis of two atypical responses reinforced by CS termination. *J Exp Psychol Anim Behav Process* **33**:451-463.

Cain CK, Sullivan GM and LeDoux JE (2013) The Neurobiology of Fear and Anxiety: Contributions of Animal Models to Current Understanding, in *Neurobiology of Mental Illness* (Charney DS, Buxbaum JD, Sklar P and Nestler EJ eds) pp 549-566, Oxford University Press, New York.

Choi JS, Cain CK and LeDoux JE (2010) The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learn Mem* **17**:139-147.

Coll-Andreu M, Marti-Nicolovius M, Portell-Cortes I and Morgado-Bernal I (1993) Facilitation of shuttle-box avoidance by the platform method: effects of conditioned stimulus duration. *Physiol Behav* **53**:349-352.

Collins KA, Mendelsohn A, Cain CK and Schiller D (2014) Taking action in the face of threat: neural synchronization predicts adaptive coping. *J Neurosci* **34**:14733-14738.

Davis M (1998) Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry* **44**:1239-1247.

Delamater AR and Holland PC (2008) The influence of CS-US interval on several different indices of learning in appetitive conditioning. *J Exp Psychol Anim Behav Process* **34**:202-222.

Diehl MM, Bravo-Rivera C and Quirk GJ (2019) The study of active avoidance: A platform for discussion. *Neurosci Biobehav Rev* **107**:229-237.

Fanselow MS (1997) Species-Specific Defense Reactions: Retrospect and Prospect, in *Learning, Motivation, and Cognition: The Functional Behaviorism of Robert C Bolles* (Bouton ME and Fanselow MS eds) pp 321-341, American Psychological Association, Washington, D.C.

Fanselow MS (2018) The Role of Learning in Threat Imminence and Defensive Behaviors. *Curr Opin Behav Sci* **24**:44-49.

Fanselow MS and Lester LS (1988) A functional behavioristic approach to aversively motivated behavior: predatory imminence as a determinant of the topography of defensive behavior., in *Evolution and Learning* (Bolles RC and Beecher MD eds) pp 185-211, Erlbaum, Hillsdale, N.J.

Galatzer-Levy IR, Moscarello J, Blessing EM, Klein J, Cain CK and LeDoux JE (2014) Heterogeneity in signaled active avoidance learning: substantive and methodological relevance of diversity in instrumental defensive responses to threat cues. *Frontiers in systems neuroscience* **8**:179.

Gibbon J and Balsam P (1989) Spreading associations in time, in *Autoshaping and conditioning theory* (Locurto CM, Terrace HS and Gibbon J eds) pp 219-253, Academic, New York.

Goode TD, Acca GM and Maren S (2020) Threat imminence dictates the role of the bed nucleus of the stria terminalis in contextual fear. *Neurobiol Learn Mem* **167**:107116.

Goode TD, Ressler RL, Acca GM, Miles OW and Maren S (2019) Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. *Elife* **8**.

Gray JA and McNaughton N (2000) *The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System*, Oxford University Press, New York.

Helmstetter FJ and Fanselow MS (1993) Aversively motivated changes in meal patterns of rats in a closed economy: The effects of shock density. *Animal Learning and Behavior* **21**:168-175.

Hunter WS (1935) Conditioning and extinction in the rat. *British Journal of Psychology* **26**:135-148.

Kamin CJ, Brimer CJ and Black AH (1963) Conditioned suppression as a monitor of fear of the CS in the course of avoidance training. *J Comp Physiol Psychol* **56**:497-501.

Kamin LJ (1956) The effects of termination of the CS and avoidance of the US on avoidance learning. *J Comp Physiol Psychol* **49**:420-424.

Keehn JD (1967) Running and bar pressing as avoidance responses. *Psychological reports* **20**:591-602.

Kim JJ and Jung MW (2018) Fear paradigms: The times they are a-changin'. *Curr Opin Behav Sci* **24**:38-43.

Krypotos AM, Effting M, Kindt M and Beckers T (2015) Avoidance learning: a review of theoretical models and recent developments. *Front Behav Neurosci* **9**:189.

Lazaro-Munoz G, LeDoux JE and Cain CK (2010) Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated Pavlovian processes. *Biol Psychiatry* **67**:1120-1127.

LeDoux JE and Gorman JM (2001) A call to action: overcoming anxiety through active coping. *Am J Psychiatry* **158**:1953-1955.

LeDoux JE, Moscarello J, Sears R and Campese V (2017) The birth, death and resurrection of avoidance: a reconceptualization of a troubled paradigm. *Molecular psychiatry* **22**:24-36.

Levis DJ (1989) The case for a return to a two-factor theory of avoidance: The failure of non-fear interpretations, in *Contemporary Learning Theories: Pavlovian*

Conditioning and the Status of Traditional Learning Theory (Klein SB and Mowrer RR eds) pp 227-277, Lawrence Erlbaum Ass., Hillsdale.

Levis DJ and Stampfl TG (1972) Effects of serial CS presentation on shuttlebox avoidance responding. *Learning and Motivation* **3**:73-90.

Martinez RC, Gupta N, Lazaro-Munoz G, Sears RM, Kim S, Moscarello JM, LeDoux JE and Cain CK (2013) Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. *Learn Mem* **20**:446-452.

Miller NE (1948) Studies of fear as an acquirable drive: I. Fear as motivation and fear reduction as reinforcement in the learning of new responses. *Journal of Experimental Psychology* **38**:89-101.

Mobbs D, Hagan CC, Dalgleish T, Silston B and Prevost C (2015) The ecology of human fear: survival optimization and the nervous system. *Front Neurosci* **9**:55.

Mobbs D and Kim JJ (2015) Neuroethological studies of fear, anxiety, and risky decision-making in rodents and humans. *Curr Opin Behav Sci* **5**:8-15.

Mobbs D, Marchant JL, Hassabis D, Seymour B, Tan G, Gray M, Petrovic P, Dolan RJ and Frith CD (2009) From threat to fear: the neural organization of defensive fear systems in humans. *J Neurosci* **29**:12236-12243.

Mobbs D, Petrovic P, Marchant JL, Hassabis D, Weiskopf N, Seymour B, Dolan RJ and Frith CD (2007) When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* **317**:1079-1083.

Moscarello JM and Hartley CA (2017) Agency and the Calibration of Motivated Behavior. *Trends Cogn Sci* **21**:725-735.

Moscarello JM and LeDoux JE (2013) Active avoidance learning requires prefrontal suppression of amygdala-mediated defensive reactions. *J Neurosci* **33**:3815-3823.

Mowrer OH and Lamoreaux RR (1946) Fear as an intervening variable in avoidance conditioning. *J Comp Psychol* **39**:29-50.

Neffinger GG and Gibbon J (1975) Partial avoidance contingencies. *J Exp Anal Behav* **23**:437-450.

Rescorla RA (1968) Probability of shock in the presence and absence of CS in fear conditioning. *J Comp Physiol Psychol* **66**:1-5.

Rescorla RA and Solomon RL (1967) Two process learning theory: Relationships between Pavlovian conditioning and instrumental learning. *Psychological Review* **74**:151-182.

Rigoli F, Pavone EF and Pezzulo G (2012) Aversive pavlovian responses affect human instrumental motor performance. *Front Neurosci* **6**:134.

Satorra-Marin N, Coll-Andreu M, Portell-Cortes I, Aldavert-Vera L and Morgado-Bernal I (2001) Impairment of two-way active avoidance after pedunculopontine tegmental nucleus lesions: effects of conditioned stimulus duration. *Behav Brain Res* **118**:1-9.

Solomon RL and Brush ES (1954) Experimentally derived conceptions of anxiety and aversion., in *Nebraska Symposium on Motivation* (Jones MR ed) pp 201-305.

Stein L, Sidman M and Brady JV (1958) Some effects of Two Temporal Variables on Conditioned Suppression. *J Exp Anal Behav* **1**:153-162.

Sullivan GM, Apergis J, Bush DEA, Johnson LR, Hou M and LeDoux JE (2004) Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not a specific cue-conditioned fear stimulus. *Neuroscience* **128**:7-14.

Terburg D, Scheggia D, Triana Del Rio R, Klumpers F, Ciobanu AC, Morgan B, Montoya ER, Bos PA, Giobellina G, van den Burg EH, de Gelder B, Stein DJ, Stoop R and van Honk J (2018) The Basolateral Amygdala Is Essential for Rapid Escape: A Human and Rodent Study. *Cell* **175**:723-735 e716.

van der Kolk BA (2006) Clinical implications of neuroscience research in PTSD. *Ann N Y Acad Sci* **1071**:277-293.

Waddell J, Morris RW and Bouton ME (2006) Effects of bed nucleus of the stria terminalis lesions on conditioned anxiety: aversive conditioning with long-duration conditional stimuli and reinstatement of extinguished fear. *Behav Neurosci* **120**:324-336.

Walker DL and Davis M (1997) Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J Neurosci* **17**:9375-9383.

Weiss JM, Krieckhaus EE and Conte R (1968) Effects of fear conditioning on subsequent avoidance behavior and movement. *J Comp Physiol Psychol* **65**:413-421.