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A visual safety signal improves learning of an auditory avoidance task

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A VISUAL SAFETY SIGNAL IMPROVES LEARNING OF AN AUDITORY AVOIDANCE TASK

by

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A thesis submitted in partial fulfillment
of the requirements for the Master of Arts
degree in Psychology
in the Graduate College of
The University of Iowa

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee for
the thesis requirement for the Master of Arts degree
in Psychology at the May 2016 graduation.

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ABSTRACT

Learning to escape aversive stimuli and effectively predicting the consequences of different cues provides animals with an increased chance of survival. Discriminative avoidance conditioning affords the opportunity to examine these specific behaviors. The present experiment investigated the influence of a visual safety signal on an auditory discriminative active avoidance conditioning task. Building on the work of Gabriel and colleagues (Freeman et al., 1997; Poremba and Gabriel, 1997, 1999), originally conducted in rabbits, an adaptation of the discriminative active avoidance paradigm was implemented using male rats. Animals were trained to avoid a signaled shock (US) by spinning a small wheel during an auditory cue, the positive conditioned stimulus (CS+). A second auditory cue signaled the absence of shock, the negative conditioned stimulus (CS-).

A visual safety signal was added following a correct response to the CS+ (successful avoidance of the shock). Three groups were formed based on experience with the visual safety signal: animals that never had training with the visual signal, animals that had the visual signal added during their training, and animals that began training with the visual signal. Animals trained with the visual safety signal showed a decrease in the number of days needed to reach criterion. When training included the addition of the visual signal, the percentage of animals that learned the task increased. These results suggest that a visual safety signal enhances learning during an auditory discriminative avoidance conditioning task. This task will be used to expand exploration of the active avoidance neural circuitry and investigate the circuitry underlying the visual safety signal.

PUBLIC ABSTRACT

Learning to escape aversive stimuli and effectively predicting the consequences of different cues provides animals with an increased chance of survival. Discriminative avoidance conditioning affords the opportunity to examine these specific behaviors. The present experiment investigated the influence of a visual signal on an auditory discriminative active avoidance conditioning task. Building on the work of Gabriel and colleagues (Freeman et al., 1997; Poremba and Gabriel, 1997, 1999), originally conducted in rabbits, an adaptation of the discriminative active avoidance paradigm was implemented using male rats. Animals were trained to avoid a signaled shock (US) by spinning a small wheel during an auditory cue, the positive conditioned stimulus (CS+). A second auditory cue signaled the absence of shock, the negative conditioned stimulus (CS-).

A visual safety signal was added following a correct response to the CS+ (successful avoidance of the shock). Three groups were formed based on experience with the visual safety signal: animals that never had training with the visual signal, animals that had the visual signal added during their training, and animals that began training with the visual signal. Animals trained with the visual safety signal showed a decrease in the number of days need to learn the task and the percentage of animals that learned the task increased. These results suggest that a visual safety signal enhances learning during an auditory discriminative avoidance conditioning task. This task will be used to expand exploration of the active avoidance neural circuitry and investigate the circuitry underlying the visual safety signal.

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INTRODUCTION

The ability to avoid aversive stimuli and experiences provides animals with an increased chance of survival. Successfully predicting the consequences of different cues in an animal's environment affords a level of advantage over conspecifics. An animal that recognizes the smell or sound of a predator and leaves the area avoids potentially becoming prey. Avoidance behavior is not only seen in the animal kingdom, but can also be observed in human behavior. Much of human behavior centers on avoiding aversive situations or consequences. Whether filling up on gas to avoid being stranded, completing an assignment on time to avoid a late penalty, or stopping at a red light to avoid a crash, we see that these types of avoidance behaviors occur frequently. Maladaptive avoidance responses can also be found as main symptoms in multiple mental illnesses (American Psychiatric Association, 2013). These responses can include inappropriate avoidance of stimuli, objects, or individuals. Given the prevalence of avoidance behaviors and maladaptive fear behaviors, it is crucial to determine the conditions and mechanisms underlying avoidance conditioning.

Avoidance behavior often follows the occurrence of some type of discriminative cue. The ability to distinguish between different cues in one's environment can promote survival by effectively utilizing resources. It would not be adaptive for an animal to avoid all environments, instead distinguishing between cues that signal safety and danger can allow them to avoid aversive stimuli and conserve energy. Discriminative avoidance conditioning provides a holistic investigation of animal responses to varying cues with distinct associations. Discriminative cues can cause different behavioral responses as well as produce differences in neural activity (Gabriel, 1993).

An extensive amount of work has been done by Michael Gabriel and colleagues (e.g., Freeman et al., 1997; Poremba and Gabriel, 1997, 1999) in examining the neural circuitry that underlies avoidance learning using a discriminative active avoidance conditioning task in rabbits. In their paradigm, rabbits learned to step in a running wheel in response to the presentation of a sound stimulus (CS+) that signaled an electric foot-shock, and to ignore the presentation of a different sound stimulus (CS-) that did not predict shock. In order to extend the findings of Gabriel and colleagues, as well as address several questions that remain unanswered from their studies such as where the long-term association memory is stored, we wanted to establish a similar paradigm in rodents.

Implementing a similar paradigm to Gabriel and colleagues would provide the best comparison between the two species in regards to conditioning and circuitry. To establish a discriminative avoidance paradigm in rodents, animals learned to respond to the presentation of a sound stimulus (CS+) that signaled an unconditioned stimulus, and to ignore the presentation of a second sound stimulus (CS-) that was not associated with the unconditioned stimulus. Due to differences in species behavior, the running wheel used for the rabbits was not as suitable for use in the rodent paradigm. Research has shown a tendency for rats to continuously run when provided a running wheel (Sherwin, 1998; Alfonso and Eikelboom, 2003), which could have inhibited learning. A small wheel that could be rotated with the front paws was implemented in place of a running wheel.

Discriminative avoidance conditioning utilizing an instrumental response that does not qualify as a species-specific defense behavior, such as wheel-turning or bar pressing, has been shown to be difficult for some animals to learn (Nakamura and Anderson, 1964; Brown et al., 1967; Bolles, 1970) and often leads to slower acquisition rates (Mogenson et al., 1965). During multiple pilot studies, it was found that the rodents had difficulty learning the paradigm or were slow to learn the discriminative behavior. Because of this, a visual signal was added following a correct response to the CS+, in the absence of shock. The addition of a light signal could be viewed as a safety signal or a secondary reinforcer.

In avoidance conditioning, the absence of shock is a primary reinforcer and by pairing the light with the absence of shock, the light becomes a secondary reinforcer. Secondary reinforcement was originally described by Hull (1943) as a stimulus that acquires reinforcing properties when it is presented concurrently with a reinforcing state. Several theorists have added to the concept of secondary reinforcement, including Dinsmoor (1950) who stated that when a cue, such as a light stimulus, is produced by a subject's own behavior it should be referred to as a secondary reinforcer. Mowrer's (1956) revision of the two-factor theory also advanced the concept of secondary reinforcement to include stimuli in the absence of an aversive event. These stimuli would follow an avoidance response and acquire reinforcing properties through fear reduction.

While the visual signal could be labeled a secondary reinforcer, other terms may provide more specificity in regards to avoidance conditioning, such as a safety signal. Safety signals are generally discussed in reference to avoidance conditioning whereas the term secondary reinforcer could be used in multiple paradigms. Avoidance conditioning often pairs a stimulus with the presentation of shock, a warning signal. This implies that there could also be a stimulus paired with the absence of shock, a safety signal

(Dinsmoor, 1973). A safety signal consists of a discriminative stimulus or cue that occurs in the absence of a predicted aversive event. Seminal studies presented a signal during the absence of shock and found that signal could produce reinforcing effects (Rescorla, 1969; Weisman and Litner, 1969). These studies spurred further investigation of safety signals in avoidance conditioning, providing evidence that safety signals incur reinforcing properties (Berger and Brush, 1975; Morris, 1975; Dinsmoor, 2001). The light stimulus therefore could be termed a safety signal. Although this terminology has not been popular since the 1970s, there is a resurgence in work examining avoidance conditioning using safety signals (Fernando et al., 2014; Krypotos et al., 2015).

In pursuit of a consistent learning paradigm with replicable learning curves, we investigated the effect of the addition of a visual safety signal. This was conducted by examining animals that never had the visual signal during training, animals that had the visual signal added during their training, and animals that began their training with the visual signal. The paradigm will ultimately be transitioned to a trace avoidance conditioning task in order to develop a more complex task that comprises components of memory storage in establishing a behavior (Runyan et al., 2004). Establishing a robust paradigm would allow future studies to explore the neural circuitry underlying discriminative active avoidance conditioning in rodents utilizing lesion, inactivation, and recording studies. An efficient paradigm will also allow the investigation of the circuitry involving the effect of the visual signal.

METHODS

Subjects

Subjects were 39 male Long-Evans rats weighing 300-700grams. The rats were individually housed in Spence Laboratories of Psychology at the University of Iowa in the animal colony with *ad libitum* access to food and water, and maintained on a 12 h light/dark cycle. All procedures were in compliance with National Institutes of Health guidelines for care of laboratory animals and approved by the University of Iowa Institutional Animal Care and Use Committee.

Task Apparatus

Animals were trained in a Plexiglas conditioning box (27.9 cm x 14 cm x 7.4cm) which was housed in a darkened sound attenuation chamber (Lafayette Co., 77.5 cm x 50.5 cm x 49 cm). A small red light provided background light in the upper left-hand corner of the chamber and a fan generated white noise (52 dB) throughout training. A small running wheel 7.4 centimeters in diameter was situated in front of the animal (Figure 1). Access to half of the wheel was available for the animal to provide a behavioral response, moving the running wheel. The animals' tail rested along a 7.4 cm extension of Plexiglas at the back of the chamber. Tail movement was restricted using medical tape (0.6 cm-1.3 cm) strips at two sections about mid-point along the tail. Training was controlled using Experimentor Software (Experimentor, Canada).

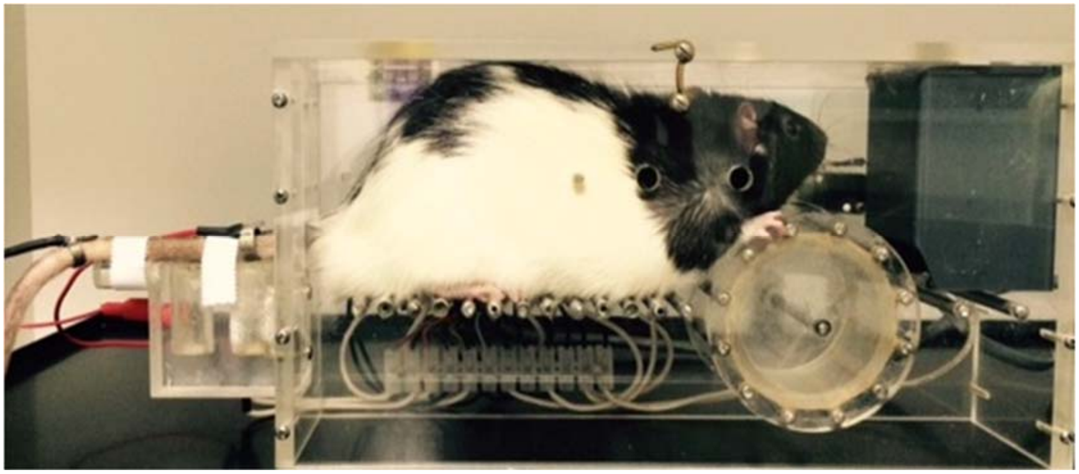


Figure 1. Photograph of a subject in the conditioning task apparatus. Animals were situated in a Plexiglas box and responses were made by turning a small running wheel 22.5°. Tail movement was restricted using medical tape strips. The unconditioned stimulus was delivered through metal floor rungs inside the box and metal rings placed on the tail.

Stimuli

Two conditional stimuli were used in this discriminative avoidance task. One tone, the positive conditional stimulus (CS+), signaled the occurrence of a mild foot and tail shock and the other tone, the negative conditional stimulus (CS-), signaled safety and the absence of shock. The CS+ consisted of two tones co-presented at frequencies: 3.52 kHz and 4.186 kHz. These roughly correspond to A7 and C8 notes, respectively, on a piano. The CS- also consisted of two tones co-presented at frequencies: 0.659 kHz and 0.698 kHz. These roughly correspond to E5 and F5 notes, respectively, on a piano. Tone pairs were implemented to increase salience by creating harmonic (CS+) and dissonant (CS-) pairs (Izumi, 2000). The CSs were 3 seconds in duration at 80±5 dB.

The unconditioned stimulus (US) consisted of a tail and foot shock at 0.4±0.2 mA. The foot shock was administered via stainless steel rungs inside the Plexiglas box. The tail shock was administered by two small stainless steel rings that were placed on the rat's tail. Electrode cream was applied directly to the tail to increase conduction. The maximum shock duration was 2 seconds. Shock was immediately terminated when a conditioned response was made. There was no minimum duration of the shock, it could be terminated immediately after onset. The conditioned response involved moving a small wheel with the front paws by 22.5 degrees of wheel rotation or more. Each time the wheel was rotated 22.5 degrees or more, a lever mechanism was initiated and the rotation was recorded as one conditioned response. Latency of response was measured as the duration between the onset of the CS and the first rotation of the wheel by 22.5 degrees or more.

Pre-training

After a 7 day period had elapsed for adaptation to living cages, animals were handled and acclimated to the conditioning apparatus for 2-3 weeks. Before discrimination training began, animals underwent one session of shock avoidance training. During this pre-training session animals received 34 trials of shock only, no tones were presented. The US was presented approximately every 50 seconds and had a maximum duration of 3 seconds. Shock was immediately terminated when an escape response was made. Pre-training sessions were used to establish a conditioned escape response in the animals and lasted 27±1 minutes.

Original Paradigm

In the original paradigm, CS presentations were not terminated when a response was made. During a CS+ trial there was a 2500 millisecond response window beginning when the CS+ was played. After the response window the shock (US) was presented and the CS+ continued to play for 500 ms to provide an overlap. The shock continued for 1500 ms after the tone ended. If the animals did not respond within the 2500 ms response window, the shock (US) was presented. When an animal responded within the 2500 ms response window, no shock was presented. Shock was immediately terminated when an escape response was made. There was no minimum duration of the shock, it could be terminated immediately after onset. Responses were made when the rat turned the wheel with their front paws by 22.5 degrees of wheel rotation or more. During a CS- trial the CS- was presented for 3 seconds and immediately followed by the intertrial interval. There were a total of 120 trials: 60 CS+ and 60 CS- on any given training day. Presentation of CS+ and CS- were pseudo-random with an intertrial interval of 20 seconds, no more than 10 consecutive trials of either stimulus type were presented. Total duration of each session was 46±2 minutes.

Addition of Visual Safety Signal

The final task consisted of identical parameters to those above with two major changes. 1) CS presentations were terminated when a response was made. At any point during a CS+ or CS- 3 second presentation a response could be made and the tone would be terminated. There was no minimal duration of the tone, it could be terminated immediately after onset. 2) Additionally when an animal responded within the 2500 ms response window, the shock would not turn on as in the original task and approximately 1 second after the initial response a 300 lumens clear white LED light (bare light bulb) on the right side of the apparatus turned on (Figure 2). Light duration was 1 second and fixed. The light was situated 6±1 inches from the animal's head.

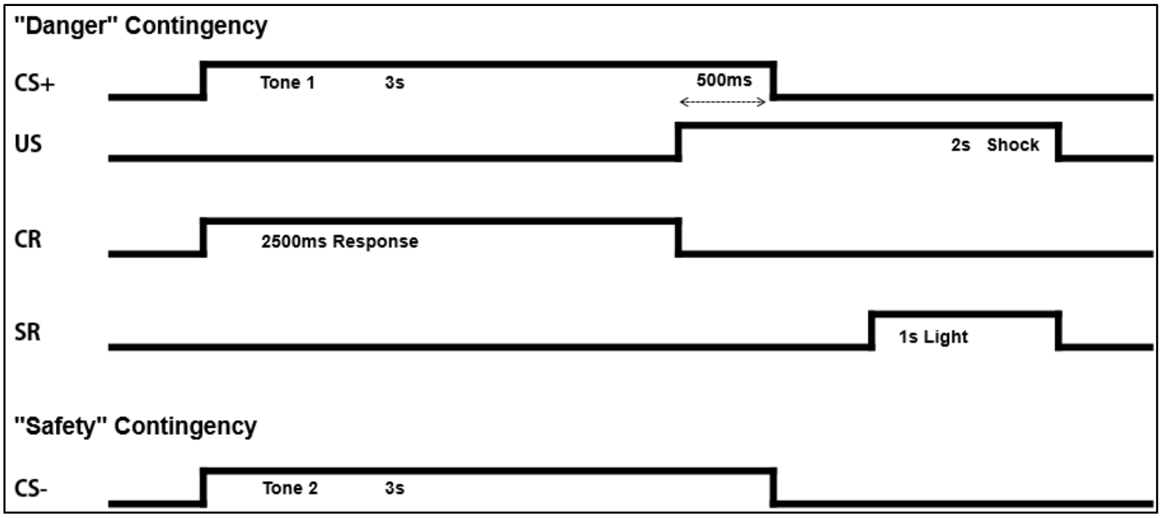


Figure 2. Line schematic of the conditioned parameters and visual safety signal (light) timing in the final training contingencies. The “Danger” contingency consisted of a presentation of the CS+ followed by the unconditioned stimulus, the shock. Animals could avoid the US with a conditioned response (CR) to the CS+. When the animal made a conditioned response to the CS+ a visual safety signal (SS) was presented: a 1 second light stimulus. In the “Safety” contingency a different sound stimulus was presented.

Training Groups

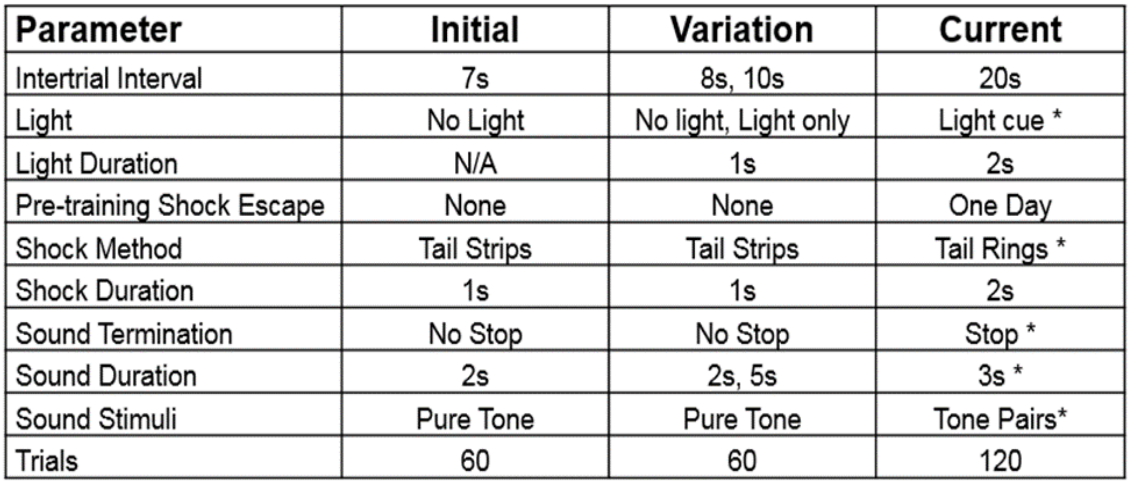
There were three training groups separated by their experience with the visual safety signal: animals that never had training with the visual safety signal were labeled as the without group (N=24), animals that had the visual safety signal added after multiple days of training (22-48 days) were classified as the switched group (N=7), and animals that began their training with the visual safety signal were labeled as the with group (N=4).

Additional Parameters

Due to the difficult nature of this paradigm, pilot studies were used to optimize task contingencies and parameters for learning in rats before establishing the initial task. Multiple variations were tried with limited success. Initial parameters, variations, and final parameters are outlined in Table 1. One manipulation included changes in the conditioned stimuli. Initially pure tones at 1 and 8 kHz were used but animals were unable to reach learning criterion. The length of the tone was also varied, previous parameters included tone lengths of 2, 2.5, and 5 seconds.

Delivery of the unconditioned stimulus was altered to improve unconditioned responses. Pilot studies using two metal prongs to deliver the tail shock did not provide a consistent unconditioned response. Metal rings placed on the animals' tail were added to increase the unconditioned response and consistency of the unconditioned stimulus delivery. In addition to shock method, duration of the shock was changed and increased from one second to two seconds.

Variations of the light stimulus were implemented with limited success. Training sessions with light as the only conditioned stimulus did not result in improved performance. Other parameters implemented were intertrial intervals of 8 and 10 seconds. Both were an insufficient duration between trials to reduce arousal preceding the onset of the next trial. Intertrial intervals were increased to 20 seconds to decrease the arousal created from the unconditioned stimulus prior to the onset of the subsequent trial. Trial number was also increased from 60 trials to 120 trials to increase contingency presentations of the CS+ and CS- within sessions and to mirror parameters used in Poremba and Gabriel, 1997.



Parameter	Initial	Variation	Current
Intertrial Interval	7s	8s, 10s	20s
Light	No Light	No light, Light only	Light cue *
Light Duration	N/A	1s	2s
Pre-training Shock Escape	None	None	One Day
Shock Method	Tail Strips	Tail Strips	Tail Rings *
Shock Duration	1s	1s	2s
Sound Termination	No Stop	No Stop	Stop *
Sound Duration	2s	2s, 5s	3s *
Sound Stimuli	Pure Tone	Pure Tone	Tone Pairs*
Trials	60	60	120

Table 1. Parameter changes made during multiple pilot studies. * Indicates variations that improved learning and performance. Initial and current parameters are included.

An additional group of animals (N=4) was trained on a similar paradigm utilizing trace conditioning. Identical stimuli for the CS+ and CS- were used. During a CS+ trial, the CS+ was presented for 3 seconds, after which there was a 500 ms delay before the unconditioned stimulus (shock) was presented. When an animal responded within the 3500 ms response window, the shock would not turn on and approximately 1 second after the initial response a LED light on the right side of the apparatus turned on. If the animals did not respond during the CS+ presentation or the 500 ms delay period, the shock (US) was presented. When an animal responded during the 3 second presentation of the CS+ or during the 500 ms delay period, no shock was presented. The duration of shock was 2 seconds and was immediately terminated when an escape response was made. There was no minimum duration of the shock, it could be terminated immediately after onset. During a CS- trial the CS- was presented for 3 seconds and immediately followed by the intertrial interval. CS presentations were also terminated when a response was made.

Responses were made when the rat turned the wheel with their front paws by 22.5 degrees of wheel rotation or more.

RESULTS

Analysis began by examining the behavioral responses to the CS+, which predicted the onset of the unconditioned stimulus (shock), and the CS-, which predicted safety. Conditioned responses to the CS+ and CS- were calculated by dividing the responses to the sound stimulus by the total number of presentations of the CS+ or CS- within a given session. Difference scores were computed by subtracting the CS- percent response from the CS+ percent response. Analyses for the CS+ and CS- conditioned responses were conducted and separate analyses were conducted for the difference scores. Response latency was also analyzed by subtracting the time stamp of the first rotation of the wheel by 22.5 degrees from time stamp of the onset of the CS.

Learning Curves

Learning curves were analyzed for three groups of trained animals. The first group consisted of animals without any experience with the visual safety signal (Without). The second group consisted of animals who had the visual safety signal added during their training (Switched). The final group consisted of animals who had the visual safety signal throughout their entire training (With) (Figure 3).

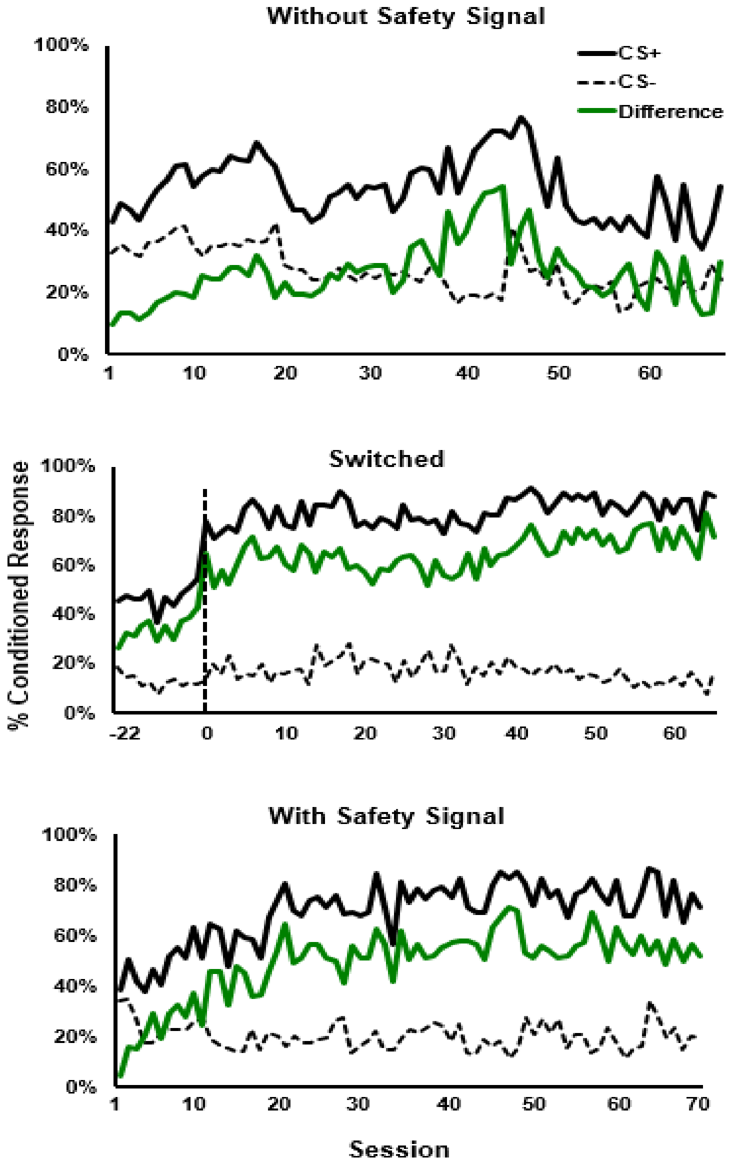


Figure 3. Learning curves for each group. Curves were created by averaging data across 2 day intervals. Three groups were examine: animals that never received the visual safety signal (Without), animals that switched to the visual safety signal during training (Switched), and animals that began training with the visual safety signal (With). For the Switched animals 0 separates the training difference and indicates the switch over to using the safety signal.

Data were analyzed with a mixed design analysis of variance with one between-subject variable (Group: Without, Switched, With) and two within-subject variables (Session and CS: CS+ and CS-) with sixty-five sessions analyzed. Significant main effects were found for CS ($F(1,9) = 257.76$, $p < 0.001$) and Session ($F(64,576) = 2.69$, $p < 0.001$). Interaction effects were also found for Session by Group ($F(128,576) = 2.25$, $p < 0.001$), CS by Group ($F(2,9) = 13.85$, $p = 0.002$), and Session by CS ($F(64,128) = 5.27$, $p < 0.001$).

Pairwise comparisons using the Fisher LSD test were conducted to examine the effect the CS+ and CS- response across groups. For the CS+ there were no significant differences between the groups, but for the CS- the Without group was significantly higher than the Switched group ($p = 0.014$) and neared significance in comparison to the With group ($p = 0.053$). The Switched and With groups were not significantly different from one another ($p = 0.429$). This indicates a higher response to the CS- for the Without group than animals in the Switched or With group.

Number of Days to Criterion

The number of training days necessary for the animals in each group to reach criterion was analyzed with criterion defined as two consecutive days of 50% or greater difference between the CS+ and CS- percent conditioned response. Animals that never reached criterion were assigned the maximum number of days that it took an animal in the Without condition to reach criterion (i.e., 83 days). A one-way ANOVA was conducted and indicated a significant main effect of group ($F(2,15) = 9.43$, $p = 0.002$). Post hoc comparisons using the Fisher LSD test revealed that the mean score for the Without group (M = 77.14, SD = 12.68) was significantly higher than the Switched group

(M = 47.71, SD = 33.38) and also was significantly higher than the With group (M = 16, SD = 5.66). The Switched group was also significantly higher than the With group (Figure 4). These results suggest that the addition of the visual safety signal decreased the number of days needed for an animal to reach criterion in this auditory discriminative avoidance task.

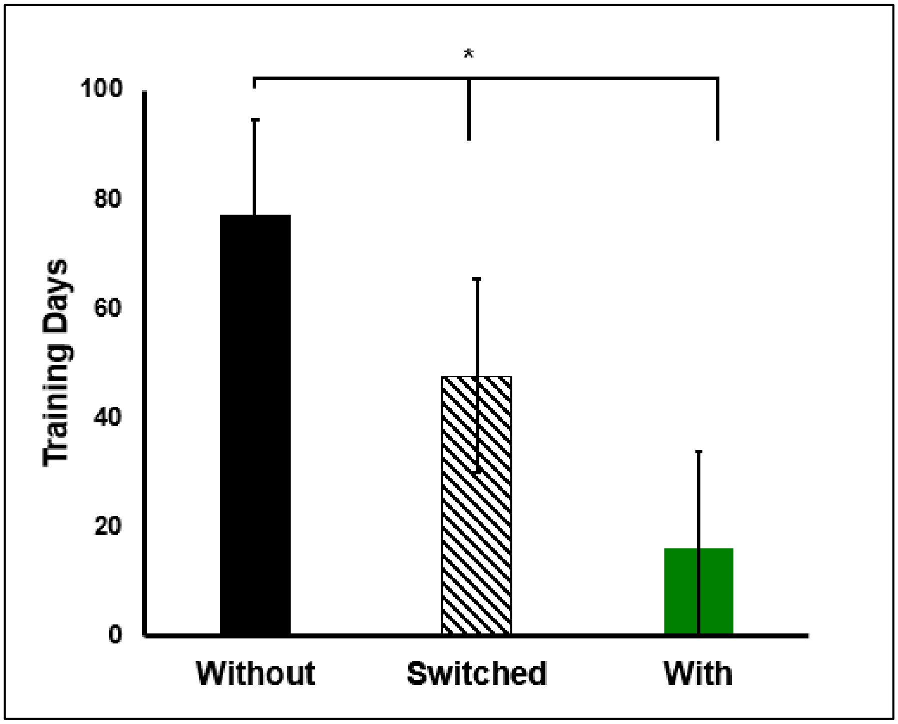


Figure 4. Average number of days to criterion across groups: animals without the visual safety signal, those that switched to the visual safety signal, and those that began training with the visual safety signal. Animals that did not reach criterion were assigned the maximum number of days that an animal took to learn in the Without group. Each group was significantly different from one another. *p <.05.

Percentage of Animals Who Learned the Task

To examine the consistency of the paradigm for future use with imaging and lesions or inactivation studies, we examined how many animals in each group attained criterion and were classified as having learned the task (Figure 5). For the Without group, twenty-four animals were trained with three of them reaching criterion for a 12.5% success rate. In the Switched group, seven animals were eventually trained with the visual safety signal added during their training and four animals attained criterion for a 57% success rate. For the With group, four animals have been trained at this point and all have reached criterion. An additional group of four animals was trained on a trace paradigm with a visual safety signal and all have reached criterion for a 100% success rate when using a visual safety signal from the onset of training.

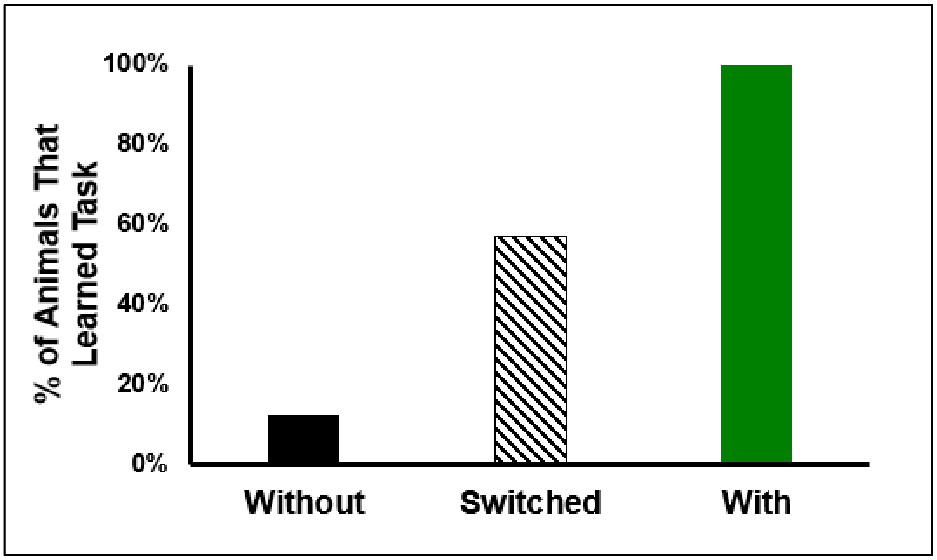


Figure 5. Percentage of animals trained that reached criterion and successfully learned the discriminative active avoidance task with and without the visual safety signal. An increase in percentage of animals who learned with the visual safety signal is shown.

Assessment of the Switched Group Before and After Visual Signal Addition

In order to examine the effect of the visual safety signal on behavioral conditioned responses for CS+ and CS-, subsequent comparisons of the Switched group performance were carried out using a mixed design analysis of variance. The twenty sessions prior to the addition of the visual signal and the sequential twenty sessions after the visual signal was introduced were analyzed (Figure 6). This created a two block (with and without visual safety signal) by twenty sessions by two CS-mixed design assessing the effect of the addition of the visual safety signal within the Switched group. A main effect of the addition of the light, i.e., block (*F*(1,3) = 19.99, *p* = 0.021) and a main effect of CS (*F*(1,3) = 123.21, *p* = 0.002) was found. An interaction effect of block, i.e., the addition of the visual signal, by session by CS (*F*(19,57) = 1.78, *p* = 0.049) was also found. For evaluating whether a change in response to the CS+ and CS- occurred in the absence or addition of the visual signal, pairwise comparisons were conducted and revealed a larger difference in response to the CS+ versus CS- with the addition of the visual signal (*p* = 0.001) than without the visual signal (*p* = 0.040).

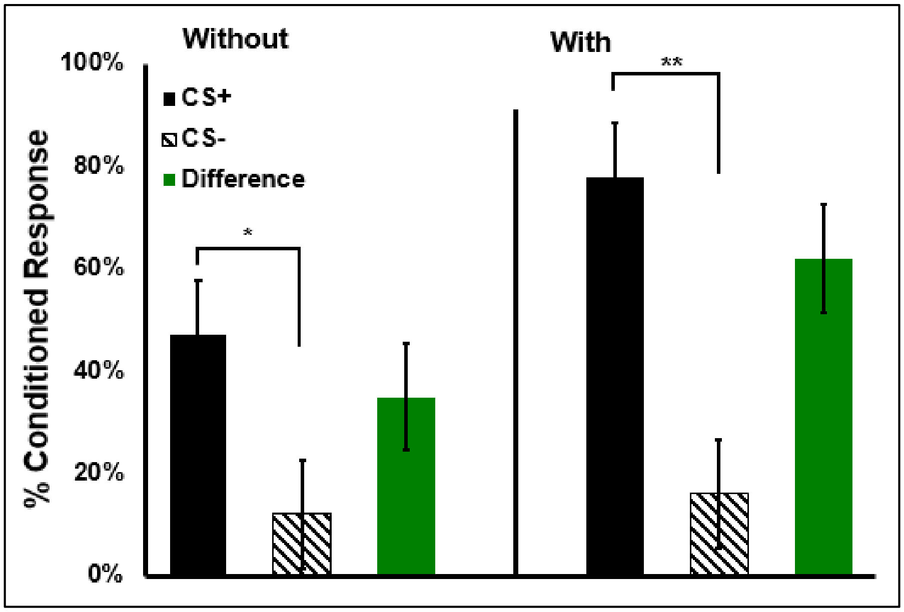


Figure 6. Average percent conditioned response for the CS+ and CS- without the safety signal (no light) and with the safety signal (light) in the switched group. Difference scores were calculated by subtracting the percent response to the CS- from the percent response to the CS+. The difference score represents the ability of the animal to discriminate between the CS+ and CS-. *p <.05, **p <.01.

We also examined whether this change in discrimination with the addition of the visual safety signal was due to a change in response to the CS+, CS-, or a combination of both. Pairwise comparisons were conducted looking at the effect of the visual signal addition on the response to the CS+ and the CS- separately. The CS+ neared significance at $p = 0.059$ and the CS- was not significant at $p = 0.210$. Thus the increase in discrimination seems more likely to be the result of an increase in response to the CS+ after the addition of the visual signal and not a change in the response to the CS-.

Additional analyses were conducted examining the difference scores for animals in the switched group before and after the addition of the visual signal. The twenty sessions prior to the addition of the visual and the sequential twenty sessions after the visual signal was introduced were analyzed. This created a two by forty mixed designed examining the effect of the addition of the visual signal by session. Although there appeared to be an increase in the difference score after the addition of the visual safety signal, no main effect of visual safety signal addition was found for the difference scores ($F(1,3) = 4.25$, $p = 0.131$).

Response During Initial Training Sessions Using the Visual Safety Signal

In a follow-up analysis, we examined whether there was a difference in conditioned responses to the CS+ and CS- in the initial sessions of training for the Switched and With group. The first 22 sessions of training for the Switched and With group were examined, with the aim of including all subjects in the analysis. During the first 22 sessions, the Switched group lacked the visual safety signal and the With group included the visual safety signal throughout training (Figure 7). The analyses indicated a significant main effect of CS (*F*(1,6) = 52.18, $p < 0.001$) but no interaction effects were observed. In order to determine at what point in the training the CS+ response significantly differed from the CS- response within each group, we examined each group by session for the With and the Switched group. We found that the first significant difference between responses to the CS+ and CS- occurred in session 14 for the Switched group, before the addition of the visual safety signal. For the With group, the first significant difference between the CS+ and CS- occurred in session 4. While there was no significant interaction in the group comparison, a significant difference between responses to the CS+ and CS- occurs earlier for the With group than for the Switched group.

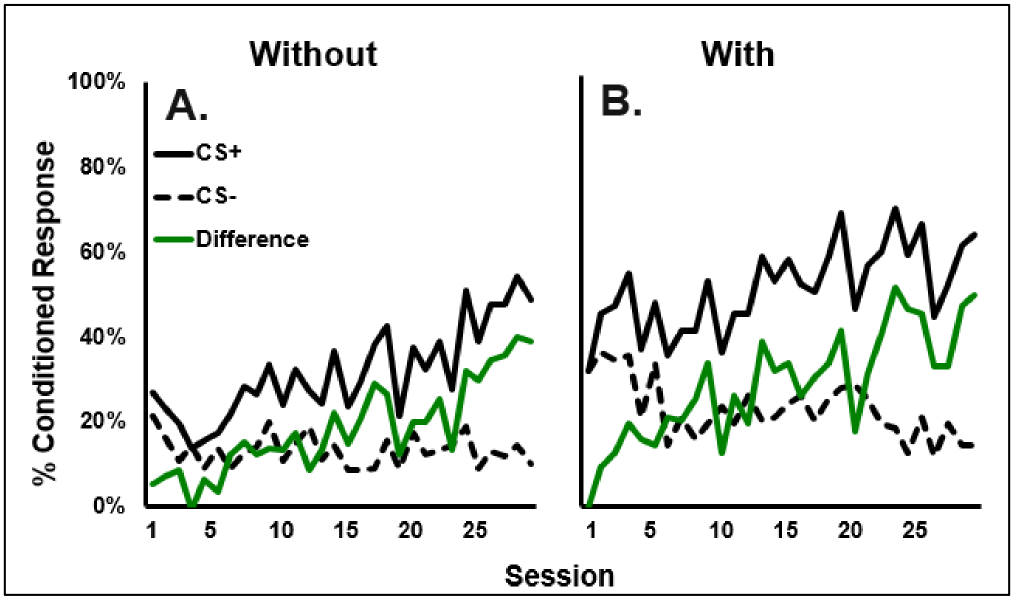


Figure 7. A. Average conditioned response to the CS+ and CS- in the first 30 sessions of training for animals in the Switched group without the safety signal (light). B. Average response to the CS+ and CS- in the first 30 sessions of training for animals with the safety signal (light). A significant difference between responses to the CS+ and CS- occurs earlier for the With group than for the Without group.

Speed of Discrimination Change During First Day Using Safety Signal

Once it was established that the addition of the visual safety signal improved performance we were interested in determining when this change in behavior occurred for the animals in the Switched condition. We analyzed the first 20 trials of the first session in which the animals in the Switched group were exposed to the visual safety signal. The first 20 trials were broken down into four groups of five trials each. We conducted a four by two repeated measures ANOVA examining block by CS. There was a significant main effect of CS ($F(1,9) = 27.50$, $p = 0.013$) but no main effect of block ($F(3,9) = 1.13$, $p = 0.389$) or interaction ($F(3,9) = 0.79$, $p = 0.530$). Although no significant interaction was obtained we planned to examine in which block the first significant difference between the CS+ and the CS- occurred. This would indicate when the animals' behavior shifted with to a larger response to the CS+ over the CS-. Pairwise planned comparisons using Fisher's LSD of block by CS revealed that the first block with a significant difference between the CS+ and CS- was block 4 ($p = 0.003$), which corresponded to trials 16-20 of the first session with the visual safety signal (Figure 8).

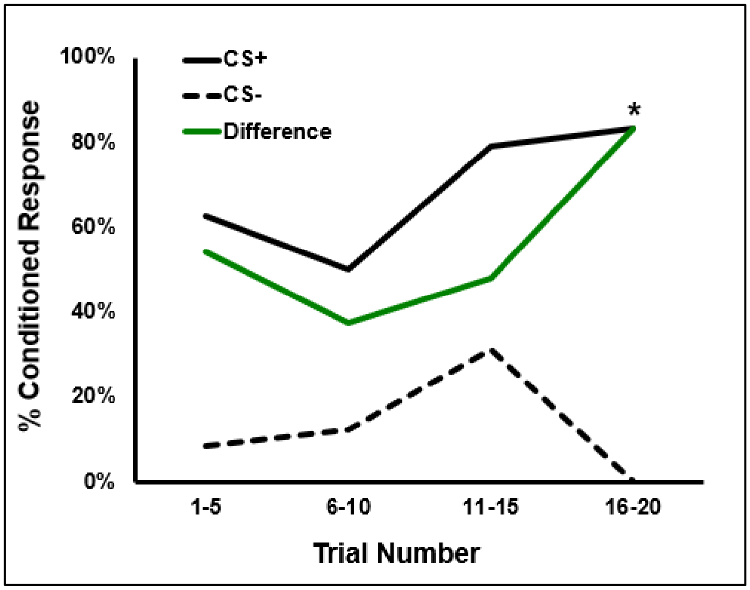


Figure 8. Average behavioral performance for the first 20 trials on Day 1 of visual safety signal training for animals in the switched group. Changes in percent response to the CS+ can be seen in the last five trials. *Indicates a significant difference between the CS+ and the CS-. $p < .05$.

Latency

The final analysis looked at the difference in response latency to the CS+ and the CS- in animals who had reached criterion. A paired samples t-test was conducted to compare the response latency for conditioned responses to the CS+ and CS-. There was a significant difference for CS+ (M=1.56, SD=0.13) and CS- (M=1.29, SD=0.21) responses; t(4)=4.77, $p = 0.009$. These results indicate that the average response time to the CS+ was longer than the average response time to the CS-.

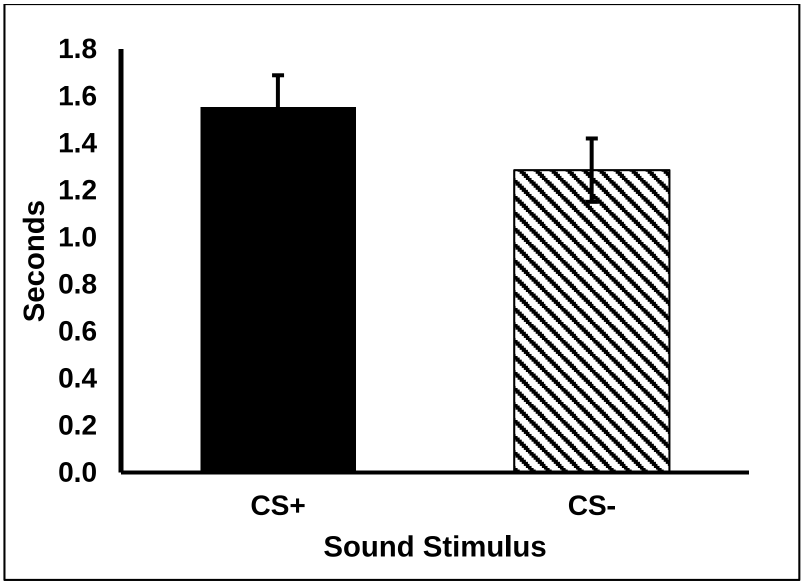


Figure 9. Latency to respond for each sound stimulus. The CS+ predicted the onset of the unconditioned stimulus and the CS- predicted safety. A significant difference was found between response latency for the CS+ and response latency for the CS-. $p < .05$.

DISCUSSION

In the current study we designed a discriminative active avoidance task in rodents, expanding upon the work conducted in rabbits by Gabriel and colleagues (e.g., Freeman et al., 1996; Poremba and Gabriel, 1997, 1999). In order to develop a replicable paradigm, a visual safety signal was added at different times during training and its addition improved learning for animals whether added during training and or present from the beginning of training. The number of days to reach criterion significantly decreased for the group in which the visual signal was added during training (Switched) and the group that began training with the visual signal (With). This demonstrates that the visual signal impacted the acquisition of this paradigm, decreasing the sessions necessary to reach criterion. Animals that had the visual safety signal added during their training (Switched) showed a rapid change in their behavioral responses when the visual safety signal was added: their response to the CS+ became significantly higher than their response to the CS-. This change emerged within the first twenty trials of the first session in which the visual safety signal was added, suggesting that the influence of the visual safety signal occurs relatively quickly. The percentage of animals that learned the task also significantly increased suggesting that the visual safety signal improved overall performance and lead to a higher success rate for the paradigm. By introducing the visual safety signal, we have been able to establish a robust discriminative active avoidance paradigm in rodents.

When examining the animals that had the visual safety signal added during their training (Switched), a significant increase in response to the CS+ was found, while only small changes in response to the CS- occurred with the addition of the light stimulus. This suggests that the light stimulus affected the response to the CS+. It was also found, however, that in comparing across all groups the CS- response was higher for those without the visual safety signal (Without) than for animals trained with the visual safety signal (Switched and With). The higher CS- response for those without training with the visual safety signal could have been the result of increased variability within the group. It is possible that the light stimulus may only affect the CS- response, but we found that the effect of the light stimulus was more general and impacted the CS+ response as well as the CS- response. The change in response to the CS+ was also greater than the change in response to the CS- when the light stimulus was added. The increased response to the CS+ could be the result of the visual safety signal acquiring reinforcing properties.

Previous research has suggested that light itself contains reinforcing properties (Kish, 1955; Forgays and Levin, 1958). The onset of a light stimulus will reinforce any response it follows and will increase the probability of that response. In this sense, light appears to be inherently reinforcing and would not fall into the category of a neutral stimulus (Murch, 1967). The reinforcing properties of light have been hypothesized to be due to positive feedback facilitation, in which the probability of repeating the previous behavior is increased (Berlyne et al., 1964).

In addition to the reinforcing properties of light, by signaling the absence of the aversive stimulus, safety signals are thought to reduce fear and reinforce behavior (Krypotos et al., 2015). Early studies provided evidence that the introduction of a safety signal could be reinforcing (Rescorla, 1969; Weisman and Litner, 1969; Berger and Brush, 1975). They failed to address, however, whether there could be any distinction between the termination of the warning signal, the CS+, and the onset of the safety signal. The presence of the CS+ would always be associated with the presence of the safety signal (Dinsmoor, 2001). This problem was addressed in Dinsmoor and Sears, 1973 and revealed that the safety signal does produce a positive reinforcing effect that is separate from the reinforcing effect of the termination of the CS+. It has also been demonstrated that duration of the safety signal does not impact its reinforcing properties (Brennan, 2003). This suggests that the primary function of the safety signal does not consist of fear reduction but instead relies on its informational role. A large body of research has provided further evidence that a discriminative stimulus that is paired with the absence of an expected unconditioned stimulus (safety signal), produces a conditioned positive reinforcing effect and improves learning (Bolles and Grossen, 1970; Morris, 1975; Dinsmoor, 2001; Fernando et al., 2014). These reinforcing properties of the visual safety signal may stem from changes in the level of dopamine release. Several studies have shown that dopamine modulates the mechanisms involved in the reinforcing properties of conditioned reinforcers (Taylor and Robbins, 1984; Cador et al., 1990; Kelley and Delfs, 1991) and safety signals (Fernando et al., 2014).

Aside from the reinforcing properties of the visual safety signal, the light stimulus may also increase the salience of the CS+. If modality is disregarded, the CS+, which signals danger, becomes a rare occurrence in comparison to the combination of the CS- and the light stimulus which both signal safety. This could have improved learning through the increased salience of the CS+ given its rare occurrence in the training sessions. Targeted rare events have been shown to produce signal increases in various regions of the brain (Clark et al., 2000). As the salience of the CS+ increases, this could lead to increased arousal and an enhanced release of acetylcholine. Salient and behaviorally relevant stimuli have been shown to increase acetylcholine release and arousal (Inglis and Fibiger, 1995; Acquas et al., 1996; Parikh et al., 2007; Klinkenberg et al., 2011). An increase in acetylcholine release and arousal may have enhanced attentional processing resulting in the improved performance within this task.

Previous studies have shown that an animal's ability to terminate the CS+ influences learning in avoidance conditioning (Bolles, 1966). This creates a confound within the present study given that the addition of tone termination after the avoidance response occurred simultaneously with the addition of the visual safety signal. However, in our multiple pilot studies tone termination was utilized but little evidence of learning was found. While it is possible that the termination of the tone, and not the addition of the visual safety signal, influenced the change in behavior, it is unlikely given the low success rate of previous pilot studies with tone termination alone.

We have shown that the response latency to the CS+ was longer than the response latency to the CS-. This is in contrast to several similar paradigm studies that have shown the response latency to the CS+ is faster than to the CS- (Gabriel, 1990; Poremba & Gabriel, 1999). Other studies have found that with extensive training the response latency to the danger signal (CS+) may lengthen (Zieliński et al., 1993; Zieliński et al., 1995). In avoidance conditioning after multiple pairings of the CS+ and US, the animal may acquire a fear response to the CS+. This fear response and subsequent increase in response latency could develop from Pavlovian inhibition of delay (Pavlov, 1927; Rescorla, 1967; Lynch, 1973). Given our extensive training and numerous CS-US pairings it's plausible that inhibition of delay resulted in the increased response latency to the CS+. Responses to the CS- could also be considered as trial errors and human studies have found that error responses are often faster than correct responses (Ratcliff, 1998; Pailing et al., 2002).

Collectively our data have shown that the addition of a visual safety signal improves learning and performance in a discriminative active avoidance paradigm. Time to criterion significantly decreased across animals that utilized the visual safety signal and the percentage of animals who successfully learned the task increased when the visual safety signal was added. This indicates the visual safety signal influences the acquisition and maintenance of responses in this task. In addition, we found that this paradigm can be used not only in delay conditioning but also in trace conditioning. The transition to trace conditioning will allow for further investigation of the effect of memory storage within this paradigm.

Future work will utilize a low frequency positive conditioned stimulus (CS+) and a high frequency negative conditioned stimulus (CS-) in order to counterbalance the CS presentations. Currently only male rodents have been used, but future studies will examine the differences, if any, between females and males within this paradigm. Other studies will investigate whether the visual safety signal is still necessary after animals have reached a high level of performance, with robust discrimination between the CS+ and CS-. It is plausible that the visual safety signal would no longer be necessary later in training as fear would be reduced due to the low occurrence of shock (Mowrer, 1947). Additionally, we will establish a reversal paradigm using a visual active avoidance task with an auditory safety signal. Future studies will also look at both the neural circuitry underlying this discriminative active avoidance paradigm as well as the circuitry underlying the effect of the visual safety signal.

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