TEMPORALLY SPECIFIC EXTINCTION OF PAVLOVIAN

CONDITIONED INHIBITION

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William Travis Suits

Certificate of Approval:

Jeffrey Katz
Associate Professor
Psychology

Martha Escobar, Chair
Associate Professor
Psychology

Christopher Correia
Associate Professor
Psychology

Ana Franco-Watkins
Assistant Professor
Psychology

George T. Flowers
Dean
Graduate School

TEMPORALLY SPECIFIC EXTINCTION OF PAVLOVIAN CONDITIONED INHIBITION

William Travis Suits

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CONDITIONED INHIBITION

William Travis Suits

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Signature of Author

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Date of Graduation

DISSERTATION ABSTRACT

TEMPORALLY SPECIFIC EXTINCTION OF PAVLOVIAN

CONDITIONED INHIBITION

W. Travis Suits

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(A.A., East Georgia College, 2001)

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In Pavlovian conditioned inhibition, conditioned stimulus A (CS A) is trained to predict the occurrence of an unconditioned stimulus (US), but when CS A is presented in compound with CS C, the US is omitted. Thus, CS C comes to predict the omission of the otherwise expected US; That is, CS C becomes a conditioned inhibitor. A Pavlovian conditioned inhibition procedure (i.e., A-US / AC-no US) was used in two experiments to test whether conditioned inhibition to CS C is dependent upon the excitatory value of its training excitor (CS A), and whether this inhibition is specific to the temporal location of the excitatory response potential of the training excitor. CS A was made excitatory in two separate temporal locations; its initial and final segments. In Experiment 1, the excitatory

potential of the initial segment of CS A was extinguished while the final segment remained unchanged. In Experiment 2, the excitatory potential of the final segment of CS A was extinguished while the initial segment remained unchanged. In both experiments, a retardation of acquisition training phase was used to determine whether manipulating the excitatory potential of training excitor, A, altered the inhibitory potential of conditioned inhibitor, C. CS C proved to be inhibitory in the segment paired with the segment of CS A that remained excitatory, but was abolished in the segment that was paired with the segment of CS A that received extinction. Thus the present results indicate that conditioned inhibition is dependent upon the excitatory potential of a training excitor and that this inhibition is temporally specific.

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INTRODUCTION

In Pavlovian conditioning (Pavlov, 1927), a stimulus that is originally neutral is paired with an unconditioned stimulus (US), which naturally elicits a response. As a consequence, the organism learns that there is a relationship between the two stimuli. When the relationship is one where the once neutral stimulus provides information about the occurrence or nonoccurrence of the US, the neutral stimulus becomes a conditioned stimulus (CS). When the CS predicts the occurrence of the US, it comes to elicit a conditioned response appropriate to the US and it is called an excitatory stimulus. In contrast, when the CS predicts the absence of an expected US, it comes to suppress a response and it is called an inhibitory stimulus.

Pavlov viewed inhibition as a process that actively suppressed a response (Pavlov, 1927). He amended this principle using a paradigm now known as the Pavlovian conditioned inhibition procedure. In this procedure, Pavlov trained a CS (A) to be excitatory by pairing it with a US (i.e., A-US). Training of the excitatory stimulus was interspersed with nonreinforced compound presentations of CS A and CS C (i.e., AC-no US), establishing CS C as a conditioned inhibitor. Responding to the AC compound was weaker than responding to CS A alone. Pavlov argued that a higher level of responding during the presentation of CS A alone indicated that the excitatory association had not been lost but merely suppressed by the presence of CS C.

Conditioned inhibition did not receive a great deal of attention in learning theory until the 1960's. Until that time, the main focus of associative learning was directed toward the procedures and parameters necessary to establish a conditioned stimulus as an excitor. With the proposal of his contingency theory, Rescorla (1966) renewed interest in inhibition phenomena. The basis of Rescorla's contingency theory is that the type of conditioning that occurs is determined by the contingency between the CS and US. That is, if the CS is trained to become a reliable predictor of occurrence of the US, there will be a positive contingency between the two stimuli and the CS will become excitatory. In contrast, if the CS is trained to predict the omission of the US or is an unreliable predictor, there will be a negative contingency between the two stimuli and the CS will become inhibitory.

It should be noted that Pavlov (1927) described excitation and inhibition as physiological processes that resulted in the production or suppression of an elicited response. However, Rescorla (1969) provided a new operational definition of excitation and inhibition based on behavioral observations and which has become commonplace in the literature today. According to Rescorla, conditioned excitation occurs when two requirements are satisfied. First, an operation that relates the CS and US is given in an arrangement in which a positive contingency exists. Second, behavior changes as a result of this operation. His definition of inhibition is the mirror image of his definition of excitation. In addition, the inhibitor should specifically affect the behavior controlled by the excitor (Rescorla, 1969). Rescorla's definition of inhibition suggests that excitation and inhibition are on opposite ends of a continuum of associative strength (Williams, Overmier, & LoLordo, 1992). That is, at any given time, a stimulus can only be an

excitor or an inhibitor. Building upon the ideas of Rescorla (1969), most classic associative learning theories adapted the idea of excitation and inhibition as opposite ends of a continuum.

Measurement of Conditioned Inhibition

It is difficult to assess conditioned inhibition because it is unclear whether conditioned inhibition is the product of a single active mechanism or a byproduct of other processes that result in a failure to respond or attend to the stimulus. Rescorla (1969) proposed a method to rule out alternative explanations based on attention deficits by introducing what has come to be known as the *two-test strategy* to determine whether a conditioned stimulus has inhibitory value. The two-test strategy implies that, to be considered inhibitory, a stimulus must 'pass' both a retardation test and a summation test. In a *retardation test*, a stimulus thought to be inhibitory is paired with the unconditioned stimulus. The number of trials that it takes for acquisition of excitatory responding to occur is then compared to the number of trials for acquisition of responding to a novel stimulus paired with the unconditioned stimulus. If more trials are necessary for acquisition of excitatory responding to the stimulus that had received inhibitory training, it is assumed to have inhibitory properties. Alternatively, a set number of trials are given and conditioned responding is assessed. If responding to the putative inhibitor is lower than responding to the neutral stimulus inhibition is assumed to have occurred. In a *summation test*, the presumed inhibitor is presented in compound with a trained excitor. The level of responding is compared to that of a compound presentation of the trained excitor and a novel stimulus. If the level of responding is lower in the inhibitor-excitor

compound than the responding in the novel stimulus-excitor compound, the presumed inhibitor is considered to be inhibitory.

According to Rescorla (1969), a stimulus should be assumed to be a conditioned inhibitor only if it passes both the summation and retardation tests. Both tests have been considered necessary because, in combination, their alternative explanations are mutually exclusive. An alternative explanation for conditioned inhibition in a summation test is that only a certain amount of attention can be given to the multiple stimuli presented in a compound. Therefore, the presentation of an inhibitor-excitor compound reduces the amount of attention that can be allotted to the excitatory stimulus, with the result being a decrease in responding. An alternative explanation for conditioned inhibition in a retardation test is that the intended inhibitory stimulus is redundant during training, thus it does not command attention. Therefore, the presentation of an inhibitor along with the US results in retarded acquisition because there is little attention given to the inhibitory stimulus. Based on these explanations, the summation test could potentially reflect that there is too much attention being given to the inhibitory stimulus and the retardation test could potentially reflect that there is not enough attention being devoted to the inhibitory stimulus. Passing a summation and retardation test allows for these alternative explanations to be rejected because it is impossible for any given training to simultaneously increase and decrease the amount of attention given to a stimulus (Rescorla, 1969; Cole, Barnet, & Miller, 1997).

Although the two-test strategy establishes a measurement standard for conditioned inhibition, Papini and Bitterman (1993) argued that the control treatments for many experiments have not been adequate because they fail to use the same controls for

summation and retardation tests. Thus, different training or testing procedures for summation and retardation may allow alternative explanations for what has been viewed as conditioned inhibition. Papini and Bitterman (1993) argued that, with the use of appropriate controls, a retardation test may be sufficient to determine that a stimulus is inhibitory. These controls would just need to include a treatment that results in similar potential attenuation of attention to the control stimulus. Note that Williams et al. (1992) suggested a different strategy, namely that summation tests be viewed as being more sensitive than retardation tests. This suggestion was based on the observation that inhibitors that have some degree of excitatory potential (see next section) can potentially fail a retardation test even if they pass a summation test.

Cole, Barnet, and Miller (1997) addressed the concerns of Papini and Bitterman (1993) regarding the use of inadequate controls in conditioned inhibition studies. In one experiment, they preexposed subjects to three different stimuli without reinforcement, CSs B, C, and D. After preexposure, they used a Pavlovian conditioned inhibition procedure in which CS A was reinforced when presented alone but not reinforced if presented in compound with CS C (i.e., A-US / AC-no US, where '/'denotes intermixed trials). Thus, CS C was trained to be a potential conditioned inhibitor and CS A was its training excitor. CS B, trained as a predictor of the US, served as a transfer excitor because it was paired with CS C during summation testing. After training, a summation test was given in which subjects were in one of three groups. Subjects were presented with either compound BC, for the inhibition group, BD, for the control group, or CS B, for the transfer excitor group. If inhibition occurred, responding should be $B \geq BD > BC$, meaning that the inhibitory CS C attenuated responding to transfer excitor B beyond any

possible attenuation produced by presenting B in compound with neutral CS D. A second experiment used an identical training procedure but employed a retardation test in which subjects were presented with either CS C in the inhibition group or CS D in the control group. If inhibition occurred, responding should be D > C, meaning that the inhibitory CS C became excitatory more slowly than the neutral CS D. The authors' findings were consistent with the previous literature on conditioned inhibition: a conditioned inhibitor passed both summation and retardation tests, but in a situation that controlled for the concerns brought forth by Papini and Bitterman (1993). Cole et al. (1997) concluded that the two-test strategy is still the best tool for assessing conditioned inhibition.

In conclusion, the current view in the conditioned inhibition literature is that the two-test strategy is a sufficient means of assessing conditioned inhibition. However, if the proper controls have been used to rule out alternative explanations, passing a retardation test alone may sufficiently identify a stimulus as having inhibitory properties (but see Williams et al., 1992).

Excitation and Inhibition in a Conditioned Stimulus

On a theoretical level, conditioned inhibition has traditionally been considered to be at one end of an associative strength continuum, with conditioned excitation being the opposite end. For example, Rescorla and Wagner (1972) conceptualized inhibition as a negatively-valued association to the US. They proposed a model in which the associative strength (*V*) of all CSs present during a given trial changes as a consequence of pairings with the presence or absence of the US. The formula used to determine this change is

$$\Delta V_X = \alpha\beta(\lambda - \Sigma V)$$

where ΔV_X represents the change in associative strength for CS X, α is the salience of CS X, and β is the salience of the US. The parenthetical term (λ- ΣV) represents the total amount of associative strength that can be supported by the US, minus the total associative strength already accrued by all CSs present on that particular trial. Thus, changes in associative strength are inversely proportional to how much of the US is predicted by all CSs present on that particular trial. According to the Rescorla Wagner model, a stimulus gains excitatory strength (+ ΔV) if it is paired with the US and gains inhibitory strength (- ΔV) when the CS is present but an expected US is omitted. Therefore, a conditioned stimulus will either be excitatory (+V) or inhibitory (-V) but it can not be both excitatory and inhibitory at the same time. In addition, the term ΣV implies that, when multiple CSs are presented in compound, the available associative strength is shared among the multiple CSs. Because the Rescorla Wagner model assumes that stimuli are either excitatory or inhibitory in their entirety, the associative status of a given CS should not differ across CS duration. Nonetheless, there is mounting evidence that would challenge this view.

Under certain conditions, an individual CS can exhibit both excitatory and inhibitory properties (Droungas & LoLordo, 1994; Matzel, Gladstein, & Miller, 1988; Williams & Overmier, 1988). Williams and Overmier used a conditioned barpress suppression preparation experiment to investigate five inhibitory associative structures: differential, explicitly unpaired, standard, backward, or trace. In this procedure, subjects were initially trained with pairings of CS A and shock (i.e., A-US) in order to establish CS A as an excitor. In the next phase of training, CSs B and C were trained as inhibitors in one of the five procedures. That is, CS B was presented alone and intermixed with CS

A-US trials (i.e., A-US/B-no US; differential procedure), alone with unsignaled US presentations (i.e., US/B-no US; explicitly unpaired procedure), in compound with A (i.e., A-US/AB-no US; standard procedure), or in a backward or trace arrangement with the US (i.e., US → B or B → gap → US, respectively). An equivalent treatment was give to CS C. CS B was then extinguished (i.e., CS B-no US) while CS C remained unchanged. At test, suppression was measured with a summation test by comparing responding during the compound presentation of either AB or AC. Inhibition was evident with both the AB and AC compounds when B and C were trained with a differential or explicitly unpaired procedure. However, inhibition was not evident in the other three procedures unless extensive extinction was given (i.e., Inhibition was evident in the AB but not the AC test). Williams and Overmier concluded that treatments of standard, backward, or trace conditioning resulted in collateral excitation to the putative inhibitor and that inhibition was only evident if this excitation was extinguished. Presumably, collateral excitation can be enough to mask the inhibitory properties of a stimulus, causing it to fail a test for inhibition. Importantly, extinction treatments can modulate the excitatory structure of a stimulus but not the inhibitory structure of a stimulus (Lysle & Fowler, 1985; Williams & Overmier, 1988). Thus, extinction procedures can unmask inhibitory properties that had previously been unobservable because that stimulus had collateral excitation.

Research findings such as those presented here provide a sufficient argument that conditioned inhibition is not the opposite of conditioned excitation. Otherwise, a stimulus could not possibly simultaneously have both excitatory and inhibitory properties. However, the mechanisms determining how a behavioral response will be made in the

presence of a stimulus that possess both excitatory and inhibitory properties are still in question (Savastano et al., 1999).

Explanations for Conditioned Inhibition

There are varied theoretical explanations of conditioned inhibition. They can be grouped into theories that assume conditioned inhibition is the result of an acquisition deficit and those that assume it is a performance deficit.

In general, the assumptions that support conditioned inhibition as an acquisition deficit assume that inhibition and excitation are on opposite ends of an associative strength continuum as previously described. For example, the Rescorla Wagner model can explain a standard Pavlovian conditioned procedure (i.e., A-US/AC-no US) in the following terms. During A-US trials, CS A is assumed to acquire positive associative strength ($+\Delta V_A$). Once enough positive associative strength is acquired by CS A, it should begin to elicit a conditioned response appropriate to the US. During AC-no US trials, both CSs are expected to acquire negative associative strength ($-\Delta V_A$ and $-\Delta V_B$). CS A should continue to be excitatory because any inhibition acquired during the AC- trials should be countered by the excitation acquired during the A-US trials. However, CS C would only acquire negative associative strength and should remain inhibitory (Rescorla & Wagner, 1972). Due to their use of positive and negative values to represent excitation and inhibition, the Rescorla and Wagner model can easily accommodate summation and retardation tests. If the inhibitor has negative value in a summation test, then its presentation with an excitor should attenuate responding through a simple additive rule. In a retardation test, if the stimulus has negative associative value, then it must first gain enough positive associative strength to counter its negative associative

value and hence it should take longer for an inhibitor to become excitatory than it would a neutral stimulus (Rescorla & Wagner, 1972).

A different view of conditioned inhibition is proposed by performance-based accounts. For example, the comparator hypothesis (Miller & Matzel, 1988), views inhibition as a competition between excitatory associations at the moment of testing instead of assuming that stimuli acquire negative values (e.g., Urcelay & Miller, 2006). According to the comparator hypothesis, a response is made if the excitatory representation of the US directly activated by the target stimulus has more strength than the excitatory representation of the US indirectly activated by the target stimuli through its associates (the so-called comparator stimuli). In a Pavlovian conditioned inhibition procedure (e.g., A-US/AC-no US) CS A is trained as an excitor and CS C is trained as a potential inhibitor. In terms of the comparator hypothesis, CS C would be the target stimulus because the interest is in its response potential and CS A would be C's comparator stimulus because it was present during training of CS C. During training, CS C is never paired with the US resulting in an extremely weak connection to the representation of the US via the so-called Link 1 (see Figure 1). Presentation of CS C also activates a representation of its comparator stimulus, CS A, which in turn activates its own representation of the US via Links 2 and 3, respectively. During training, CS A develops a stronger association to the US than CS C because CS A and the US often occur together but CS C and the US do not. Therefore, Link 3 will be stronger than Link 1. CS C and CS A also form a strong association (Link 2) because the two are repeatedly presented together during training. At test, whether a behavioral response is observed is determined by a comparison of the strength of Link 1 and the product of the strength of

Links 2 and 3. Increases in the strength of Link 1 are positively related to conditioned responding, whereas increases in the strength of the product of Links 2 and 3 are inversely related to conditioned responding. Thus, conditioned inhibition will be behaviorally evident as the strength of the product of Links 2 and 3 grows in comparison to the strength of Link 1. In the Pavlovian conditioned inhibition procedure, presentation of CS C should result in attenuated conditioned responding.

Consistent with the comparator view, Lysle and Fowler (1985) suggested that conditioned inhibition is a slave process to excitation. That is, the inhibitory strength of a stimulus is dependent upon the excitatory strength of its training excitor. In terms of the Pavlovian conditioned inhibition procedure (e.g., A-US/AC-no US), CS C can only be inhibitory as long as CS A is excitatory. If the excitatory potential of CS A were extinguished, then this would also attenuate the inhibitory potential of CS C. However, if CS C was presented in extinction (CS C-no US) without manipulating the excitatory potential of CS A, there would not be a change in the inhibitory value of CS C. Indeed, Lysle and Fowler (1985) supported these assumptions by demonstrating that extinction of CS A attenuated inhibition of CS C and that the inhibitory potential of CS C did not extinguish if CS C was presented alone.

The comparator hypothesis predicts a loss of inhibitory control by CS C as a consequence of extinguishing its training excitor, CS A (Denniston, Blaisdell, & Miller, 2004). Recall that, following conditioned inhibition training, presentation of target stimulus C activates a representation of the US directly, as well as a representation of the US indirectly through CS A. Because the product of Links 2 and 3 is strong, excitatory responding is attenuated and CS C acts as a conditioned inhibitor (see Figure 1).

However, if CS A is extinguished, Link 3 loses effectiveness; consequently, the product of Links 2 and 3 will decrease thus increasing the potential for excitatory responding. Therefore, after extinguishing CS A, CS C will lose inhibition (i.e., behavior indicative of excitation will increase) without a direct manipulation of CS C.

In conclusion, there have been various attempts to describe the underlying structure of conditioned inhibition. Rescorla and Wagner (1972) proposed that excitation and inhibition were on opposite ends of a continuum and were mirror images of each other. Other research has demonstrated that excitation and inhibition do not have to be mutually exclusive, suggesting that a stimulus can simultaneously have excitatory and inhibitory response potentials (Droungas and LoLordo, 1994; Matzel, Gladstein, & Miller, 1988; Williams & Overmier, 1988).

Temporal Factors in Conditioned Inhibition

One factor that has a critical role in determining whether a stimulus will become inhibitory is the temporal relationship between the would-be inhibitor and its training and transfer excitors. If an organism acquires not only the predictive information between two stimuli but also the temporal information, then the organism learns not only that a US will occur in the presence of a CS but also when it will occur (Savastano & Miller, 1998).

Barnet and Miller (1996) reported a study which supported the view that temporal information plays a vital role in the development of inhibitory conditioning. Their subjects received Pavlovian conditioned inhibition training (i.e., A-US/AC-no US) but the temporal information was different across groups. Excitatory training involved the presentation of CS A, which was either simultaneously (i.e., A|US) or forward-paired (i.e., A → US) with the unconditioned stimulus. After excitatory training had occurred,

CS C was paired but not reinforced with CS A in either a simultaneous (i.e., C|A) or forward-paired (i.e., C → A) presentation. Barnet and Miller observed that, if CS C was presented in the same temporal arrangement as CS A during excitatory training, then inhibition occurred. However, if CS C did not predict the absence of the US at the same temporal location in which CS A predicted its occurrence, inhibition did not occur. This study provides evidence that temporal information plays a vital role in associative learning; otherwise, inhibition should have occurred even in situations in which there were unequal times for expectation of US presentation and omission.

Denniston, Cole, and Miller (1998) extended the findings of Barnet and Miller (1996) regarding the relevance of temporal relationships in conditioned inhibition. Denniston et al. manipulated the relationship between the excitor (CS A) and the US instead of the relationship between the inhibitor (CS C) and the US. They found that inhibition occurred when CS C predicted the absence of the US at the same time that CS A predicted the occurrence of the US. This study supports that the consistency between the predicted expectation and omission of a US is important for conditioned inhibition to occur. That is, the temporal information about expectation and omission of the US has to match up for inhibition to develop. This provides support for the view that temporal relationships play a vital role in the development of conditioned inhibition.

The two previously described studies utilized summation tests for conditioned inhibition in order to investigate the importance of the temporal relationship between the training and transfer excitors, inhibitor, and US. Burger, Denniston, & Miller (2001) provided additional support that the temporal arrangement of stimuli is important by using retardation tests for conditioned inhibition. They trained CS C as a Pavlovian

conditioned inhibitor (A-US/AC-no US). The training excitor, CS A, was trained to predict the occurrence of the US at stimulus offset. During the retardation test, CS C was presented and followed by the US, either immediately after CS C offset or five seconds after CS C offset. Burger et al. (2001) found that if the US occurred in the same temporal location as its omission was predicted, inhibition was maximized. However, if the US occurred at a time different than its omission was predicted, inhibition did not occur. This study provided further support to the view that temporal information about time of occurrence of the CS and US is encoded as part of the association.

Williams, Johns, and Brindas (2008) recently provided additional support that the temporal relationship plays a critical role in the development of conditioned inhibition. They trained three different excitors (CSs A , D, and E) that lasted for 30 s. CS A was reinforced 10 s after stimulus onset, CS D was reinforced 30 s after stimulus onset, and CS E was reinforced at both 10 s and 30 s after stimulus onset. Training also consisted of the presentation of two different potential inhibitors (CSs B and C) in compound with the excitors. During training, compound presentations of AB resulted in the omission of the expected US at 10 s, whereas compound presentations of DC resulted in the omission of the expected US at 30 s. Thus, CS B was trained to predict omission of the US at 10 s and CS C was trained to predict omission of the US at 30 s. After training, a summation test consisting of presentations of E, EB, and EC was given. The authors found that inhibition was specific to the temporal location in which the US was omitted. For example, presentation of EB resulted in inhibited responding during the initial segment of the stimulus but excitatory responding during the final segment of the stimulus. A subsequent retardation test was given in order to support the findings of temporally specific

conditioned inhibition. Training was consistent with that described for the summation test. At test, inhibitors (CSs B and C) were reinforced at the time in which they predicted the omission of the US. Acquisition of excitatory conditioning was compared to when CSs B and C were reinforced in a temporal location that did not match the predicted omission of the US. This training resulted in the groups that received reinforcement in a temporal location that matched the omission of the US to have inhibited acquisition on excitatory conditioning.

Several studies have demonstrated the importance of temporal factors in the establishment of conditioned inhibition. In all of these studies, inhibition appears to only occur when the inhibitor predicts the omission of the US at a time in which the excitor predicts that occurrence of the US (Burger et al. 2001; Denninston, Blaisdell, & Miller, 1998; Denniston, Blaisdell, & Miller, 2004; Williams et al. 2008).

The Purpose of the Present Studies

This project combined the idea that inhibition is a process dependent upon the excitatory value of the training excitor and the expectation that this slave process would be temporally specific. This was achieved by investigating whether the inhibitory value of a stimulus could be manipulated by altering the excitatory value of its training excitor at different temporal locations.

Escobar, Suits, and Rahn (2009) established a procedure used to test for conditioned inhibition at different temporal locations across the duration of a long-delay conditioned stimulus. They presented CS A for 60 s and presented the US 55 s after stimulus onset. They assessed whether the initial segment of CS A had acquired inhibitory value by using a retardation test in which CS A was presented and US delivery

occurred at 10 s after stimulus onset. When acquisition of responding to CS A was compared to acquisition of responding by a novel CS, Escobar et al. (2009) found that the initial segment of CS A passed a retardation test. This study also provides evidence that different segments of a long stimulus can have different associative values.

The proposed experiments used a procedure similar to that of Escobar et al. (2009), but the stimulus was trained to be excitatory in two different temporal locations. That is, CS A was presented for 60 s and the US was delivered both at 10 seconds and 55 seconds after CS A onset. This should have established the initial and final segments of this stimulus as being excitatory. CS C was presented together with CS A, and the compound did not signal US delivery. Thus, CS C should have been established as a conditioned inhibitor for the initial and final segments of CS A.

After completion of this training, the excitatory value of CS A was manipulated at one of the two temporal locations in which it predicted US delivery. Specifically, CS A was presented and predicted US delivery at only one of the previously reinforced locations, thereby extinguishing the excitatory value of the other previously reinforced location. CS C was then presented and paired with the US in one of the two locations in which it should have been inhibitory. A retardation test was used to determine whether CS C was inhibitory across its entire duration. If inhibition is a slave process and the temporal information of events is encoded as part of the association as has been suggested, extinction of excitation in one segment should have attenuated the inhibitory potential of CS C during that same segment but not during the alternative segment. That is, if the initial segment of the CS A was extinguished, then the initial segment of CS C should not be inhibitory. However, the final segment of CS C should be inhibitory

because CS A continued to be excitatory in its final segment. If temporal information was not encoded as part of the association, there should be a direct association between the excitatory potential of CS A and the inhibitory potential of CS C. Consequently, any extinction treatment of CS A should have nondifferentially disrupted inhibition in CS C.

EXPERIMENT 1 INTRODUCTION

Experiment 1 used a retardation of acquisition test to determine whether the inhibitory potential of a stimulus was dependent upon the temporally-specific excitatory potential of another stimulus. Inhibition was assessed with a retardation test: If acquisition of excitation by the inhibitor was retarded in the non-extinguished segment but not retarded in the extinguished segment, we could conclude that the inhibitory potential of the stimulus was attenuated when the excitatory potential of its training excitor was extinguished.

In this experiment, CS A was a 60-s CS trained as a predictor of US (sucrose pellets) occurrence at 10 s and 55 s after CS onset. The times of US delivery were selected to ensure that subjects used interval timing to determine the time of delivery of the US, as opposed to using CS onset or termination as cues. A second CS, C, was trained as a predictor of US nonoccurrence. Once excitation was established in the initial (10 s) and final (55 s) segments of CS A, subjects began a new phase of training.

In the second phase of training, only CS A was presented. However, presentation of CS A only resulted in US delivery during the final segment of the stimulus presentation. This was done in order to extinguish excitation during the initial segment of CS A. This phase was followed by assessment of the inhibitory potential of CS C.

During the retardation test, subjects were separated into four groups. Two groups were presented with CS C predicting US delivery either during its initial segment (Group

Inhibition-Extinction) or during its final segment (Group Inhibition-NoExtinction). The other two groups were the control groups and were presented with a novel CS, B, predicting US delivery during either its initial segment (Group Control-Extinction) or during its final segment (Group Control-NoExtinction). The groups were named in reference to whether reinforcement occured during test in the same temporal location as the excitatory segment extinguished during the second phase of training (Condition Extinction) or during the alternative segment (Condition NoExtinction).

If acquisition of excitation during the initial segment of CS presentation was not different for the Inhibition-Extinction and Control-Extinction groups, we could conclude that the inhibitory potential of a prospective conditioned inhibitor was attenuated when the excitatory potential of its trained excitor was extinguished. If acquisition of excitation during the final segment of CS presentation was different for the Inhibition-Extinction and Control-Extinction groups, we could conclude that CS C was effectively trained as a conditioned inhibitor during the first phase of training. In addition, if retardation of acquisition was evident in the final segment of CS C in the Inhibition-NoExtinction group, we could conclude that inhibition is temporally specific and that for a stimulus to be inhibitory, a trained excitor must have excitatory potential at the same temporal location as that in which inhibition is assessed. In addition, this difference should indicate that extinction of excitation did not attenuate the overall inhibitory potential of a stimulus, but instead attenuated inhibition only at that specific temporal location.

EXPERIMENT 1 METHODS

Subjects

The subjects were 32 male (429-573 g) albino rats (Holzman stock, Harlam Labs) that had previously participated in a conditioned suppression preparation using stimuli different from those used in this study. The 32 subjects were randomly assigned to one of four groups, Inhibition-Extinction, Inhibition-NoExtinction, Control-Extinction, or Control-NoExtinction (*n*s = 8). Subjects were housed in pairs in standard plastic cages with wire lids in a vivarium maintained on a 12-hr light/12-hr dark cycle. All experimental manipulations occurred during the light portion of the cycle. Cagemates were assigned to different groups. Water was available *ad lib* to all subjects. A food deprivation schedule was imposed during the week preceding the initiation of the experiment so that animals were gradually brought down and maintained at 12.5 grams of regular rat chow per day. Food was provided approximately one hour after completion of the daily experimental sessions. For the time between completion of their previous study and initiation of this study, animals were handled for 30 s every other day.

Apparatus

The apparatus consisted of eight Med Associates standard rat chambers (30.5 cm long x 24.1 cm wide x 21.0 cm high). The sidewalls of each chamber were made of aluminum sheet metal, and the front and back walls as well as the ceiling of the chamber were made of transparent polycarbonate. The floor was constructed of 4.8-mm stainless

steel rods, spaced 1.6 cm center-to-center. Each chamber was housed in a melamine sound attenuation cubicle equipped with an exhaust fan that provided a constant 70 dB background noise. All sound pressure level measurements were made in the (A) scale.

All chambers were equipped with a pellet dispenser located on the right side wall. This dispenser delivered 45-mg sucrose pellets in a cup located inside a niche (5.1 cm long x 5.1 cm wide x 5.1 cm high). The niche was placed 1.5 cm above the grid floor and equipped with infrared photo beams, which were used to detect the number of head entries into the niche when the beams were disrupted. Head entries were used as the dependent variable. All chambers were also equipped with a speaker mounted above the pellet dispenser and a speaker mounted on the opposite wall. These speakers could produce an 80 dB, 2,900-Hz tone and an 84 dB, 800-Hz tone, respectively. A 1.12-Watt (#1820) flashing houselight (0.20 s on/0.20 s off) was used as a visual stimulus.

Procedure

Figure 2 presents the critical aspects of Experiment 1. CSs A and C were the 2,900-Hz tone and flashing houselight, counterbalanced within groups. CS B was the 800-Hz tone. When presented, CSs A, B, and C were 60 s in duration. The US consisted of the delivery of two 45-mg sucrose pellets delivered at 10 s and 55 s after CS A onset. All session durations were 120 minutes. Also, the chamber was dark (i.e., the houselight will be off) during the sessions with the exception of when the flashing houselight stimulus was presented.

Acclimation. On Day 1, all subjects were acclimated to the experimental context and retrieving pellets from the niche. Sucrose pellets were delivered on a fixed-time (FT) 5 min schedule. During this session, subjects were exposed to two presentations of all

stimuli used in the experiment to enhance their discriminability. The order of presentations was: houselight, 800-Hz tone, 2,900-Hz tone, 800-Hz tone, houselight, and 2,900-Hz tone, with a mean intertrial interval of 19 minutes.

Pavlovian Conditioned Inhibition Training. On Days 2-21, all subjects received 16 daily compound presentation of CSs A and C (320 total). They also received 4 daily CS A-US pairings, with US delivery occurring at 10 s and 55 s after CS A onset (80 total). Thus, CS A was trained as a conditioned excitor and CS C was trained as a conditioned inhibitor. Two schedules of training were used on alternate days. In Schedule 1, the 3rd, 6th, 12th, and 17th trials were designated as A-US trials. In Schedule 2, the 2nd, 9th, 16th, and 20th trials were designated as A-US trials. In both schedules, the mean intertrial interval was 6 (± 3) min. Probe trials (presentation of the CS without US delivery) were included to test for acquisition of the response without contamination from US presentation. The 16th, 32nd, 48th, 64th, and 80th presentations of CS A were designated as probe trials.

Extinction of Excitatory Conditioning Training. On Days 22-29, all subjects received 20 daily presentations of CS A (160 total). In each of these presentations, the US was delivered at 55 s after stimulus onset (i.e., the US presented at 10 s after CS onset during Pavlovian conditioned inhibition training was omitted). Two schedules of training were used on alternate days. Both schedules of training resembled the schedules used during the excitatory and inhibitory training phase with the following exceptions: (1) CS C was never presented during this phase of training and (2) the probe trials were the 20th trial of the stated days for both schedules. Probe trials were given on days 23, 25, 27, and 29.

Retardation Training. On Days 30-33, all subjects received 20 daily pairings of either CS C-US (Inhibition Groups) or novel CS B-US (Control Groups). Presentation of the US occurred at either 10 s (Extinction groups) or 55 s (NoExtinction groups) after CS onset. This ensured that the Inhibition or Control groups received US presentation during the segment that remained excitatory in CS A (final segment; condition NoExtinction) or during the segment that was extinguished during the extinction of excitation phase of training (initial segment; condition Extinction). Two schedules of training were used on alternate days. Both schedules of training resembled the schedules used during the excitatory and inhibitory training phase with the following exceptions: (1) CS A was never presented during this phase of training and (2) the probe trials were the 20^{th} trial of each session for both schedules.

Data Analyses. Number of head entries during all training and test sessions were recorded in 5-s bins. For purpose of analysis, the first three bins and last three bins (i.e., the first and last 15 s) of the 60-s CS were used as a measure of responding during the initial and final segments of the CS. In Phase 1, the last presentation of compound CSs AC was compared to the last probe trials (i.e., CS A) in order to assess whether conditioned inhibition had been established. In Phase 2, the initial and final segment of the last presentation of CS A was assessed to determine whether excitation had been extinguished in the initial segment. Retardation was assessed by analyzing the initial and final segment of the second retardation probe trial.

EXPERIMENT 1 RESULTS

Upon completion of the Phase 1 conditioned inhibition training, subjects responded as expected: subjects in all groups exhibited more responding during the presentation of CS A than during presentation of the AC compound (see Figure 4). Extinction of excitation occurred by the end of training in the second phase as expected, with subjects in all groups exhibiting lower responding during the initial segment than the final segment of CS A (see Figure 5). The data of greatest interest were obtained during the retardation training phase. Conditioned responding to the initial segment of novel CS B did not differ from conditioned responding to the initial segment of inhibitory CS C in either the Extinction or NoExtinction conditions. Additionally, conditioned responding during the final segment of novel CS B was higher than conditioned responding to the final segment of inhibitory CS C in the NoExtinction condition (see Figure 6). In contrast, conditioned responding to novel CS B did not differ from conditioned responding to the final segment of inhibitory CS C in the Extinction condition. That is, CS C appeared to be inhibitory where conditioned inhibition was assessed in the segment that was manipulated , the segment of CS A that continued to be reinforced during Phase 2 (final segment), but not where it was assessed in the segment of CS A that had undergone extinction during Phase 2 (initial segment). The following analyses support these conclusions.

The effectiveness of Phase 1 treatment was assessed with a 4 (group, between groups factor) X 2 (stimulus: A vs. AC, within-subjects factor) X 2 (segment: initial vs. final, within-subjects factor) X 5 (probe trial, within-subjects factor) analysis of variance (ANOVA). This analysis revealed main effects of stimulus and segment, *Fs*(1, 28) = 46.18 and 33.91, *MSEs* = 17.93 and 6.65, respectively, both *ps* < .01. There was also a Stimulus X Segment interaction $F(1, 28) = 52.06$, $MSE = 7.63$, $p < .01$. This analysis also revealed no main effect of trial, $p > .11$. However, trial interacted with stimulus and segment, *Fs*(4, 112) = 7.81 and 2.79, *MSEs* = 4.26 and 3.58, *ps* < .01 and .05, respectively. In addition, there was a three-way Stimulus X Trial X Segment interaction $F(4, 112) = 2.95$, $MSE = 3.27$, $p < .05$. Thus, responding tended to be higher in the initial than final segment of CS A (Stimulus X Segment interaction). However, the final segment of CS A became excitatory as trials progressed but responding did not increase in the presence of AC across any of the trials (Trial X Segment and Stimulus X Trial X Segment interactions). Importantly, conditioned inhibition progressed equivalently across groups, as suggested by the lack of a main effect of group and the lack of an interaction between groups and any of the other factors.

Asymptotic responding at completion of Phase 1 training was assessed with a 4 (group) X 2 (stimulus) X 2 (segment) ANOVA conducted on responding during the last probe presentation of CS A and compound AC. This analysis revealed main effects of stimulus and segment, *Fs*(1, 28) = 37.24 and 29.23, *MSEs* = 9.16 and 4.31 respectively, both *ps* < .01. The analysis also revealed an interaction of Stimulus X Segment, $F(1, 28) = 37.56$, $MSE = 4.49$, $p < .01$. No other main effects or interactions were significant, all *ps* > .08. The lack of a main effect of group as well as the lack of significant interactions

between group and any of the other factors led us to conduct planned comparisons collapsing across the group factor. Planned comparisons revealed that responding was higher during the initial and final segments of CS A than the equivalent segments of compound AC, *Fs*(1, 28) = 47.19 and 4.74, *MSEs* = 10.49 and 3.17, *ps* < .01 and .05, respectively. Thus, at the end of the first phase of training, CS A was excitatory at both the initial and final segments and CS C effectively attenuated responding to CS A during both segments.

The effectiveness of the second phase of training was assessed with a 4 (group) X 2 (segment) X 4 (probe trial) ANOVA. This analysis revealed a main effect of segment, $F(1, 28) = 19.31$, $MSE = 11.27$, $p < .01$. No other main effects or interactions were significant, all *ps* > .08. Additionally, responding at the completion of Phase 2 training was assessed with a 4 (group) X 2 (segment) ANOVA on the number of head entries recorded during the last probe trial of CS A. This analysis revealed a main effect of segment, $F(1, 28) = 10.61$, $MSE = 12.46$, $p < .01$, indicating that responding was higher in the final segment than the initial segment of CS A in all groups (see Figure 5). Neither the main effect of group nor the interaction were significant, *ps* > .83, which suggests equivalent acquisition across groups.

Retardation was assessed with a 2 (condition: inhibition vs. control) X 2 (extinction: extinction vs. noextinction) X 2 (segment: initial vs. final) ANOVA performed on the number of head entries recorded during the second test stimulus probe trial. The analysis revealed a main effect of segment, $F(1, 28) = 5.38$, $MSE = 11.89$, $p < .05$, and a Segment X Condition interaction, $F(1, 28) = 8.83$, $MSE = 11.89$, $p < .01$. No other main effects of interactions were significant, all *ps* > .14. Planned comparisons

revealed no differences in conditioned responding between groups Inhibition-Extinction and Control-Extinction in neither the initial segment nor the final segment of the test stimulus, *Fs*(1, 28) < 1. Groups Inhibition-NoExtinction and Control-NoExtinction did not differ in responding during the initial segment of the test stimulus, $F(1, 28) < 1$. However, conditioned responding during the final segment of the test stimulus differed between these two groups, $F(1, 28) = 4.38$, $MSE = 14.63$, $p < .05$. That is, retardation was evident in the final segment of CS C only in the condition in which the equivalent segment of training excitor A remained excitatory.

In conclusion, subjects responded as expected in all phases of training. In Phase 1, subjects exhibited more responding during the presentation of CS A than during presentation of the AC compound. During Phase 2, extinction of excitation to the initial segment occurred as expected, with subjects in all groups exhibiting lower responding during the initial than the final segment of CS A. In the retardation training phase, conditioned responding during the initial segment did not differ between novel CS, B, and the inhibitory CS, C. However, conditioned responding during the final segment was higher in novel CS B than the inhibitory CS C. This signifies that extinction of an excitatory temporal segment in training excitor, A, attenuated behavior indicative of conditioned inhibition to its conditioned inhibitor, C, but conditioned inhibition continued to be evident to the segment of inhibitor C that matched the still excitatory segment of the training excitor.

EXPERIMENT 2 INTRODUCTION

Experiment 2 sought to confirm and extend the findings from Experiment 1, which indicated that conditioned inhibition is temporally specific and relies upon the excitatory potential of the training excitor. The same design was used as in Experiment 1, with the exception that the final segment, as opposed to the initial segment, of the excitatory stimulus was extinguished in Phase 2.

EXPERIMENT 2 METHODS

Subjects and Apparatus

The subjects were 32 male (324 – 397 g) albino rats (Holzman stock, Harlam Labs) that had previously participated in a conditioned suppression preparation using different stimuli from those used in this study. The 32 subjects were housed and treated in the same manner as they were during Experiment 1. The apparatus was the same as in Experiment 1.

Procedure

Figure 3 presents the critical aspects of Experiment 2. The design and procedure were the same as in Experiment 1, except that the extinction of excitation phase of training was changed so that the excitatory potential during the final segment of the CS was extinguished as opposed to the initial segment that was extinguished during Experiment 1. Group names were kept in a manner to correspond to the excitatory segment of CS A that received extinction training. Therefore, Inhibition-NoExtinction and Control-NoExtinction refer to the groups in which retardation trials involved presentations of the US during the initial segment of CSs B and C. Inhibition-Extinction and Control-Extinction refer to the groups in which retardation trials involved presentations of the US during the final segment of CSs B and C.

Data Analyses. Number of head entries during all training and test sessions were recorded in 5-s bins. For purpose of analysis, the first three bins and last three bins (i.e.,

the first and last 15 s) of the 60-s CS were used as a measure of responding during the initial and final segments of the CS. In Phase 1, the last presentation of compound CSs AC was compared to the last probe trials (i.e., CS A) in order to assess whether conditioned inhibition had been established. In Phase 2, the initial and final segment of the last presentation of CS A was assessed to determine whether excitation had been extinguished in the initial segment. Retardation was assessed by analyzing the initial and final segment of the first probe trial. The first probe trial was analyzed instead of the second probe trial (as analyzed in Experiment 1) due to the (later) observed faster acquisition of excitatory responding during the initial than final segment of CS A. The potential reasons for this faster acquisition will be analyzed in the Results section.

EXPERIMENT 2 RESULTS

Upon completion of the Phase 1 conditioned inhibition training, subjects responded as expected: subjects in all groups exhibited more responding during the presentation of CS A than during presentation of the AC compound (see Figure 7). Extinction of excitation occurred by the end of training in the second phase as expected, with subjects in all groups exhibiting lower responding during the final segment than the initial segment of CS A (see Figure 8). The data of greatest interest were obtained during the retardation training phase. Conditioned responding to the final segment of novel CS B did not differ from conditioned responding to the final segment of inhibitory CS C in either the Extinction or NoExtinction conditions. Additionally, conditioned responding to the initial segment of novel CS B was higher than conditioned responding to the initial segment of inhibitory CS C in the in the NoExtinction condition (see Figure 9). In contrast, conditioned responding to novel CS B did not differ from conditioned responding to the initial final segment of inhibitory CS C in the Extinction condition. That is, CS C appeared to be inhibitory where conditioned inhibition was assessed in the segment of CS A that continued to be reinforced during Phase 2 (initial segment), but not when it was assessed in the segment that of CS A that had undergone extinction during Phase 2 (final segment). The following analyses support these conclusions.

The effectiveness of Phase 1 treatment was assessed with a 4 (group, between groups factor) X 2 (stimulus: A vs. AC, within-subjects factor) X 2 (segment: initial vs.

final, within-subjects factor) X 5 (probe trial, within-subjects factor) analysis of variance (ANOVA). This analysis revealed main effects of stimulus and segment, *Fs*(1, 28) = 81.52 and 34.09, *MSEs* = 17.89 and 10.65, respectively, both *p*s < .01. There was also a Stimulus X Segment interaction *F*(1, 28) = 36.79, *MSE* = 7.07, *p* < .01. This analysis revealed no main effect of trial, *p* > .43. However, trial interacted with stimulus, *F*(4, 112) = 6.29, *MSE* = 5.67, *p* < .01. The three-way Stimulus X Trial X Segment interaction was also significant, *F*(4, 112) = 8.67, *MSE* = 3.90, *p* < .01. Thus, responding tended to be higher in the initial than final segment of CS A (Stimulus X Segment interaction). However, the final segment of CS A became excitatory as trials progressed (Stimulus X Trial X Segment interaction) but responding did not increase in the presence of AC across any of the trials. Importantly, conditioned inhibition progressed equivalently across groups, as suggested by the lack of a main effect of group and the lack of interaction between group and any of the other factors.

Asymptotic responding at completion of Phase 1 treatment was assessed with a 4 (group) X 2 (stimulus) X 2 (segment) ANOVA conducted on responding during the probe presentation of CS A and compound AC. This analysis revealed main effects of stimulus and segment, *Fs*(1, 28) = 39.67 and 20.67, *MSEs* = 12.01 and 4.83, respectively both *ps* < .01. The analysis also revealed an interaction of Stimulus X Segment, *F*(1, 28) = 28.17, *MSE* = 6.66, *p* < .01. No other main effects or interactions were significant, all *ps* > .41. The lack of a main effect of group as well as the lack of significant interactions between group and any of the other factors led us to conduct planned comparisons collapsing across the group factor. Planned comparisons revealed that responding was higher during the initial and final segments of CS A than the equivalent segments of

compound AC, *Fs*(1, 28) = 54.08 and 4.72, *MSEs* = 11.67 and 7.00, *ps* < .01 and .05, respectively. Thus, at the end of the first phase of training, CS A was excitatory at both the initial and final segments and CS C effectively attenuated responding to CS A during both segments.

The effectiveness of the second phase of training was assessed with a 4 (group) X 2 (segment) X 4 (probe trial) ANOVA. This analysis revealed main effects of trial and segment, *Fs*(1, 28) = 2.85 and 108.62, *MSEs* = 6.30 and 33.77, *ps* < .05 and .01, respectively. Additionally, responding at the completion of Phase 2 training was assessed by using a 4 (group) X 2 (segment) ANOVA on the number of head entries recorded during the last probe trial of CS A. The analysis revealed a main effect of segment, *F*(1, 28) = 47.79, *MSE* = 15.40, *p* < .01, indicating that responding was higher in the final segment than the initial segment of CS A in all groups (see Figure 8).

Preliminary analyses of the retardation data revealed that acquisition progressed extremely fast when reinforcement was provided during the initial segment of the test stimuli. This observation is consistent with the observation of higher levels of responding to the initial than final segment of training excitor A during Phase 1 training (see Figures 4 and 7), as well as the higher levels of responding to A when reinforced in its initial segment (Figure 8) than its final segment (Figure 5). In consequence, the first (rather than the second) retardation probe trial was used for all analyses. Retardation was assessed with a 2 (condition: inhibition vs. control) X 2 (extinction: extinction vs. noextinction) X 2 (segment: initial vs. final) ANOVA performed on the number of head entries recorded during the first test stimulus probe trial. The analysis revealed main effects of condition and segment, *Fs*(1, 28) = 6.39 and 6.64, *MSEs* = 13.02 and 13.96, *p* < .05 and *p* < .01,

respectively. There was also a Segment X Condition interaction, $F(1, 28) = 5.02$, $MSE = 13.96$, $p < .05$. No other main effects of interactions were significant, $ps > .08$. Planned comparisons revealed no differences in conditioned responding between groups Inhibition-Extinction and Control-Extinction in either the initial segment or the final segment of the test stimulus, $Fs(1, 28) = 1.72$ and 3.01, $MSEs = 20.98$ and 6.00, $ps > .20$ and .09, respectively. Groups Inhibition-NoExtinction and Control-NoExtinction did not differ in responding during the final segment of the test stimulus, $F(1, 28) = 2.04$, $MSE = 6.00$, $p > .16$. However, conditioned responding during the initial segment of the test stimulus differed between these two groups, $F(1, 28) = 6.30$, $MSE = 20.98$, $p < .05$. Thus, retardation was evident in the initial segment of CS C only in the condition in which the equivalent segment of CS A remained excitatory. Note that conditioned responding during the final segment of inhibitory CS C in the Inhibition-Extinction group was lower than predicted. This observation is consistent with the slower acquisition of conditioned responding to the final segment of the CS observed in this preparation (see above). Responding during this segment did increase as retardation training progressed, from a mean of 0.25(± 0.71) in Probe trial 1 to a mean of 2.63 (±2.56) in Probe trial 2.

In conclusion, subjects responded as expected in all phases of training. In Phase 1, subjects exhibited more responding during the presentation of CS A than during presentation of the AC compound. During Phase 2, extinction of excitation to the final segment occurred as expected, with subjects in all groups exhibiting lower responding during the final than the initial segment of CS A. In the retardation training phase, conditioned responding during the final segment did not differ between the novel CS B and the inhibitory CS C. However, conditioned responding during the initial segment was

higher in novel CS B than in inhibitor CS C. This signifies that extinction of an excitatory temporal segment in training excitor, A, attenuated behavior indicative of conditioned inhibition but conditioned inhibition continued to be evident to the segment of inhibitor C that matched the still excitatory segment of its training excitor.

DISCUSSION

Two experiments were performed to test whether conditioned inhibition is dependent upon the excitatory value of the inhibitor's training excitor, and whether this inhibition is specific to the temporal location of the excitatory response potential of the training excitor. I hypothesized that if the excitatory value of the training excitor was extinguished at a certain temporal location, inhibition would also be lost at the same location. Conversely, inhibition should be observed in the same temporal location as excitation was observed to the training excitor.

The data indicate that our training was successful in establishing CS A as a conditioned excitor and endowing CS C with response attenuating properties in both experiments. This is evident in the comparison of responding during the last presentation of CS A and the last presentation of compound AC. Our training was also successful in extinguishing the excitatory response potential to the initial segment of training excitor A in Experiment 1 and the final segment of training excitor A in Experiment 2. That is, on the last presentation of CS A, there was little responding during the initial segment (Experiment 1) or the final segment (Experiment 2) of CS A in a manner consistent with the second phase of training. Taken together, the data indicate that training was successful and the results of the retardation training support the hypothesis that conditioned inhibition was attenuated in the temporal location that matched the excitatory

segment of CS A that was extinguished in Phase 2, but remained inhibitory in the temporal location that matched the temporal location of the excitatory segment of CS A.

The findings of these experiments indicate that conditioned inhibition is a slave process dependent upon the excitatory value of the inhibitor's training excitor (Lysle & Fowler, 1985). In Experiment 1, the initial and final segments of 60-s CS A were trained to be excitatory. The excitatory response potential of the initial segment of CS A was then extinguished, and C was trained as an excitor in either its initial or final segment. Experiment 1 indicated that, when the initial segment of a training excitor CS A was extinguished, behavior indicative of conditioned inhibition during the initial segment of CS C was attenuated (i.e., no retardation was observed). Importantly, the final segment of training excitor CS A remained excitatory, and the retardation test revealed behavior indicative of conditioned inhibition during the final segment of CS C. In Experiment 2, animals received analogous training with the exception that excitation was extinguished in the final, rather than the initial, segment of the CS A. Consistent with the results of Experiment 1, Experiment 2 indicated that, when the final segment of a training excitor CS A was extinguished, behavior indicative of conditioned inhibition during the final segment of CS C was attenuated (i.e., no retardation was observed). Importantly, the initial segment of training excitor CS A remained excitatory, and the retardation test revealed behavior indicative of conditioned inhibition during during the initial segment of CS C. Taken together, these two experiments support the hypothesis that conditioned inhibition is a process that is dependent upon conditioned excitation. In addition, these experiments support the hypothesis that conditioned inhibition (i.e., knowledge about US

omission) is temporally specific to the time of reinforcement of the training excitor (i.e., knowledge of US delivery).

This series of studies contradicts the predictions made by associative learning models that view excitation and inhibition as a property that is acquired to a stimulus as a unitary whole. For example, the Rescorla-Wagner model (1972) predicts that a stimulus is either excitatory or inhibitory in its entirety, and that the associative status of a stimulus should not differ across CS duration. However, the present studies indicate with a retardation test that inhibition can accrue to one segment of a CS but not the other. These findings support the ever-growing reports in the conditioned inhibition literature that conditioned inhibition and conditioned excitation are not mutually exclusive, but both can co-exist across the duration of a CS (Droungas & Lolordo, 1994; Escobar et al., 2009; Matzel et al., 1988; Williams et al., 2008; Williams & Overmier, 1988).

Time as a Relevant Variable in Associative Learning

Timing as part of an association has been widely studied in the associative learning literature. Indeed, there have been multiple theories that have been developed that focus on how temporal information is used in stimulus representation (Gallistel & Gibbon, 2000; Gibbon & Balsam, 1981; Roberts & Church, 1978). However, only one timing theory has been applied to conditioned inhibition, the Temporal Coding Hypothesis (TCH; Matzel, Held, & Miller, 1988). The main assumptions of the temporal coding hypothesis can be summarized in four principles. First, contiguity is necessary and sufficient for the formation of associations. Second, the temporal relationship between paired events is automatically encoded as part of the association. Third, temporal information plays an important role in the nature, magnitude, and timing of a conditioned

response. Fourth, organisms can integrate temporal information from training if there are common elements in the temporal map (Barnet & Miller, 1996; Burger, Denniston, & Miller, 2001; Savastano & Miller, 1998).

The assumptions of the TCH have been demonstrated in several studies investigating conditioned inhibition. For example, Barnet and Miller (1996) trained subjects in a Pavlovian conditioned inhibition procedure with excitatory training occurring either simultaneously (i.e. A|US) or forward-paired (i.e., A → US). After excitatory conditioning had occurred, CS C was paired with CS A in either a simultaneous (C|A) or forward-paired (C →A) manner. According to the TCH, if temporal map integration occurred and the maps predicted US occurrence and omission in the same temporal location, conditioned inhibition would be observed. That is, when the training excitor was simultaneously paired with the US and the inhibitor was simultaneously paired with the training excitor, US occurrence and omission would be predicted in the same temporal location. However, if the training excitor was simultaneously paired with the US, but forward paired with the inhibitor, the temporal location for US occurrence and omission would not overlap. Therefore, conditioned inhibition would not be observed. Indeed, they found that if A-US and A-C associations were trained with the same temporal arrangement (i.e., both CSs paired simultaneously or both CSs forward-paired), conditioned inhibition was observed. However, if the A-US and A-C associations were trained with different temporal arrangements (i.e., one CS paired simultaneously and the other forward-paired), conditioned inhibition was not observed. This suggests that the temporal information was used in order to determine whether responding would occur, as predicted by the TCH.

In a related study, Denniston et al. (1998) manipulated the relationship between the excitor and the US (instead of between the inhibitor and the US [Barnet & Miller, 1993]). They found that if CSs A and C were paired in such a way that CS C predicted US absence in the same location as CS A predicted its occurrence, conditioned inhibition was observed but not when CS C predicted US absence in a different temporal location than CS A predicted its occurrence. This series of studies further suggests that when the temporal maps are integrated, expectation and omission of the US have to overlap in order for conditioned inhibition to be evident.

In conclusion, there is sufficient support that temporal information plays a vital role in associative learning. Although only one timing theory, the TCH, has been applied to conditioned inhibition, it seems that there is adequate research that demonstrates that inhibitory behavior is at least partially determined by the temporal expectancies of US occurrence developed during training (see Denniston & Miller, 2007, for a review). However, the TCH has limited explanatory power for the present experiments because it assumes that all stimuli are encoded as unitary events. In contrast with this assumption, it seems that different segments of a stimulus can have different response potentials. The studies of Williams et al. (2008) and Escobar et al. (2009) discussed in the Introduction also demonstrate that time to US omission is a relevant factor that is included as part of inhibitory associations. Williams et al. found that conditioned inhibition is specific to the temporal location in which an expected US is omitted. Likewise, Escobar et al. found that a stimulus can have different associative values across its duration and that there is temporal specificity regarding when excitation and inhibition occur. These observations are most consistent with componential theories of learning.

Componential theories assume that associations are formed between a CS and US through some sort of stimulus sampling process (cf. Estes & Burke, 1953). For example, Wagner's (1981) SOP model suggests that the components of a stimulus sampled in any given trial can be activated in one of two different states: A1 and A2. A1 is an active state and there is an excitatory association formed when components of the CS and US are simultaneously represented in this level. A2 is a state of decayed activity and stimulus components are represented here when they are associatively activated but not physically present. If components of the CS and US are in different states (i.e., CS components in A1 and US components in A2) an inhibitory association is formed between them. According to SOP, the components that enter the A1 or A2 state are randomly sampled. However, a recent extension of this model (C-SOP; e.g., Wagner & Brandon, 2001; Vogel, Brandon, & Wagner, 2003) assumes that the sampling of CS components is not random, but determined by a temporal process. That is, components of the CS that are closer to the US in terms of temporal location will become more strongly associated with the US than components that are further away. Thus, a CS could potentially have excitatory and inhibitory components at different temporal locations. However, C-SOP does not assume that integration of information is a fundamental determinant of responding. A possibility would be to integrate the assumptions of TCH and C-SOP assumptions. An integration of these assumptions would allow the TCH to be applied to the present series of studies in the following way. If temporal information is encoded as part of the association, then the initial and final segments of CS A should be encoded as excitatory. In addition, presentation of compound AC should result in segments of CS C being encoded as inhibitory because of the omission of the US expected at specific

temporal locations during CS A presentation (CS elements would be in state A1 and US elements would be in state A2). Subjects could potentially integrate the temporal maps created for CS A and the AC compound in Phase 1 and for A in Phase 2, using CS A as a common element allowing for that integration. According to the third tenet of the TCH, this temporal information would serve to determine whether or not a behavioral response is to be made.

Inhibition as a Slave Process to Excitation

As stated in the Introduction, conditioned inhibition has been hypothesized to be a slave process to conditioned excitation (cf. Lysle & Fowler, 1985). That is, the inhibitory strength of a stimulus is dependent upon the excitatory strength of its training excitor. In terms of the Pavlovian conditioned inhibition procedure (e.g., A-US/AC-no US), CS C can only be inhibitory as long as CS A is excitatory. If the excitatory potential of CS A were extinguished, then the inhibitory potential of CS C should be attenuated.

The present studies can be used to support the predictions of the comparator hypothesis (Miller & Matzel, 1988), which views inhibition as a competition between excitatory associations at the moment of a test instead of assuming that stimuli acquire negative values. The comparator hypothesis predicts a loss of inhibitory control by CS C as a consequence of extinguishing its training excitor. This was supported in the present studies and the work of Lysle and Fowler (1985). The present series of studies further extend this prediction in that there is a loss of inhibition in CS C to a specific temporal location as a consequence of extinguishing a similar temporal location in the training excitor (CS A).

Limitations of the Present Research and Future Directions

Although the present data allow us to conclude that conditioned inhibition is a slave process to the excitatory properties of a trained excitor, further research in the development of conditioned excitation and inhibition across the duration of a CS is necessary. In the excitation and inhibition phase of both experiments, responding during the initial segment of the training excitor probe trial was higher than responding during the final segment. This high level of responding during the initial segment could indicate that subjects are using the first presentation of the US as an indicator that a second US will occur at a later time. It is possible that the overall low levels of responding observed during the final segment of the test CS in Experiment 2 (in which the final segment of CS A was nonreinforced during the second phase of training) were the result of this expectation. For example, it is possible that when the first expected US delivery was omitted responding consistently decreased across the entire CS duration because the nonreinforcement became a signal that the US delivery in the final segment of the stimulus would be omitted. This problem could be addressed in a study in which training is similar to that of the present experiments, but in which the training excitor is reinforced only during either its initial or final segments. This would prevent subjects using the first US delivery as a signal for the second US delivery. If all other aspects of training and testing were to remain the same, similar findings to those presented here would provide additional support that conditioned inhibition is a slave process to conditioned inhibition and that information about specific temporal locations is used across the duration of the CS.

A second limitation of the present studies is that they only used a retardation test to assess whether conditioned inhibition occurred. Although it is generally accepted that both tests of the two-test strategy are necessary to determine whether conditioned inhibition is present, some authors have argued that passing a retardation test is sufficient evidence for conditioned inhibition (Papini & Bitterman, 1993; Williams et al., 1992). However, it is suggested that further research be conducted and the present training procedures be used and tested in a summation test.

A third limitation is that the studies did not use a full counterbalancing of the physical stimuli used as CSs A and C. The stimuli in the present studies were selected because they have proven to result in similar acquisition of excitatory response potential in previous studies. However, the possibility exists that they resulted in unequal rates of acquisition and, thus, retardation may reflect a stimulus rather than a treatment effect. The lack of differences in responding between B and C in the extinction condition would argue against this, but a future study needs to demonstrate that these 2 stimuli are indeed equivalent.

In conclusion, the present data suggest that specific temporal information is encoded as part of an association and that conditioned inhibition is a slave process to the excitatory potential of a training excitor in a Pavlovian conditioned inhibition procedure. Although there is sufficient evidence that conditioned inhibition was attenuated as a result of a manipulation of excitation in a training excitor, further research is needed in order to support and extend these findings.

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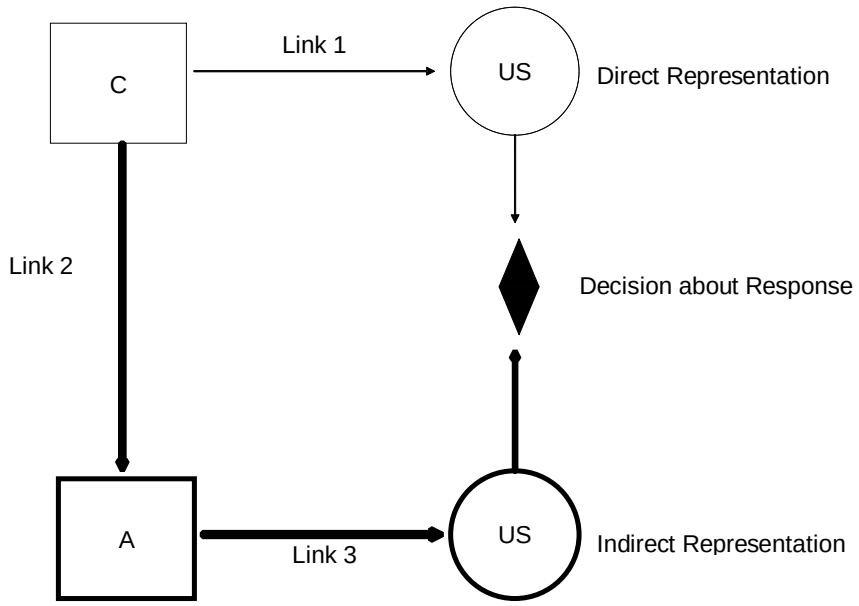


Figure 1. The Comparator Hypothesis as applied to conditioned inhibition

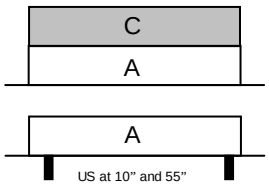
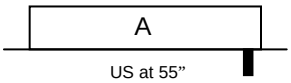
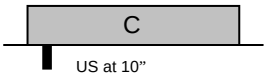
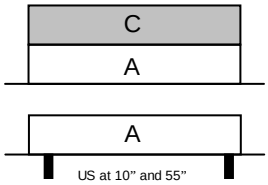
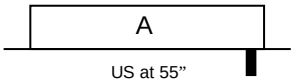
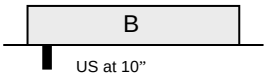
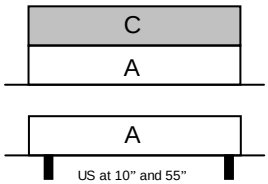
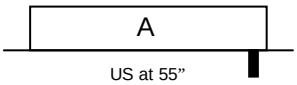
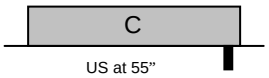
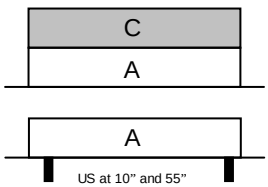
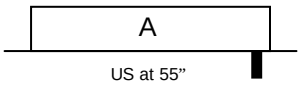
Group	Phase 1	Phase 2	Test
Inhibition-Extinction			
Control-Extinction			
Inhibition-No Extinction			
Control-No Extinction			

Figure 2. Design of Experiment 1.

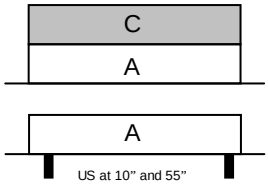
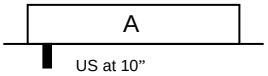
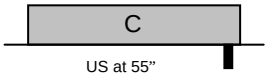
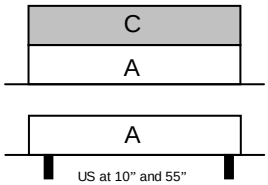
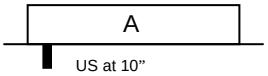
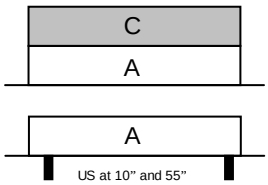
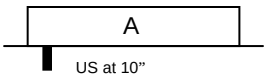
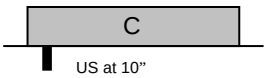
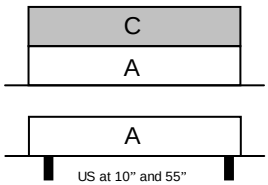
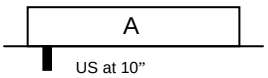
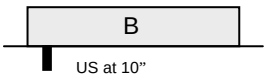
Group	Phase 1	Phase 2	Test
Inhibition-Extinction			
Control-Extinction			
Inhibition-No Extinction			
Control-No Extinction			

Figure 3. Design of Experiment 2.

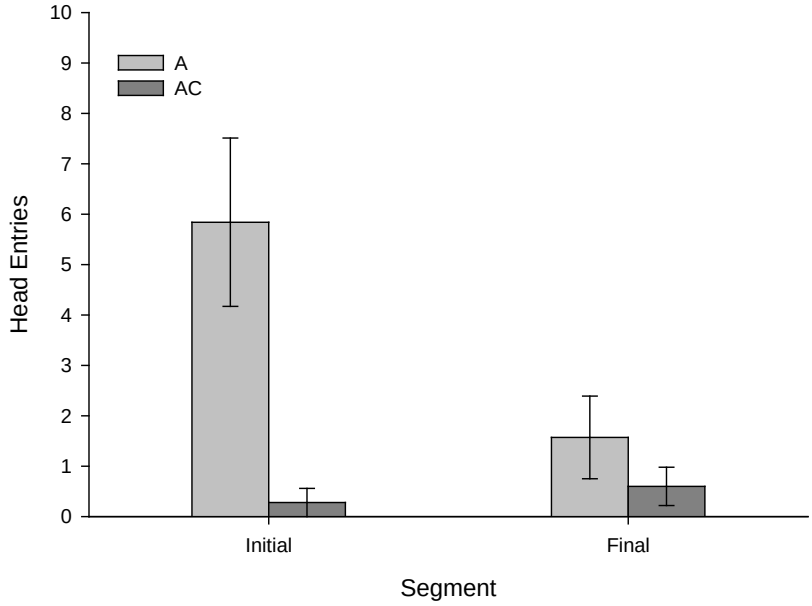


Figure 4. Experiment 1 Phase 1.

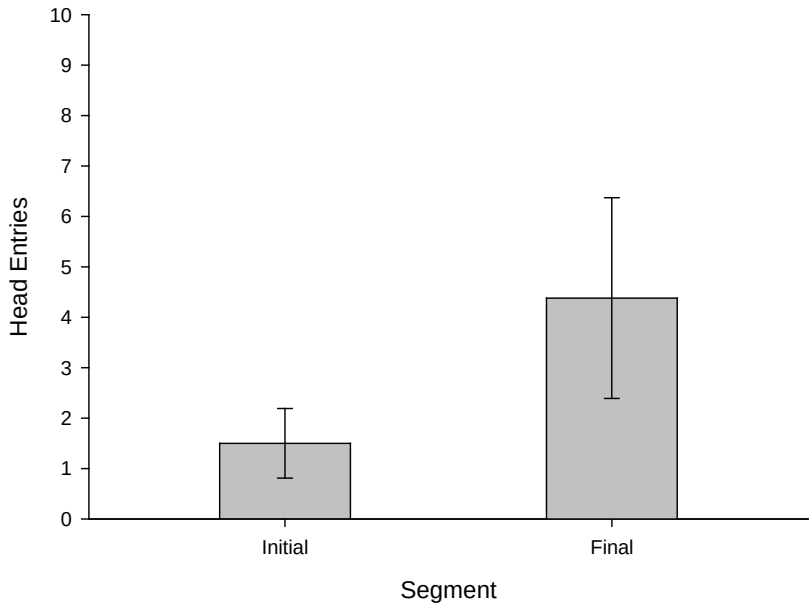


Figure 5. Experiment 1 Phase 2.

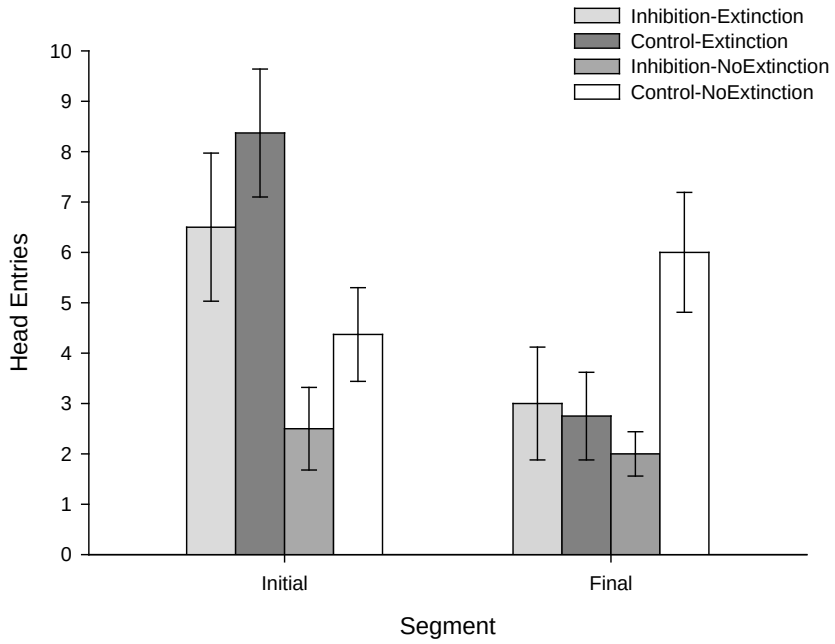


Figure 6. Experiment 1 Retardation Training.

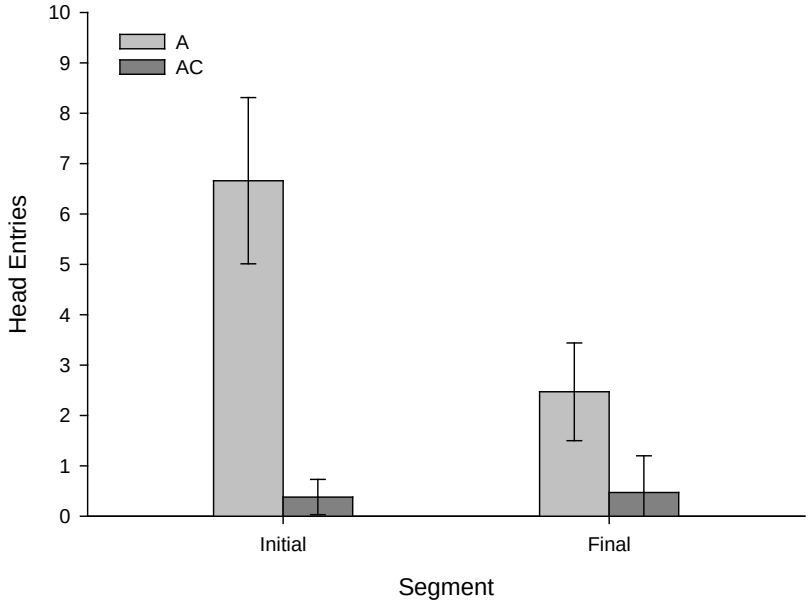


Figure 7. Experiment 2 Phase 1.

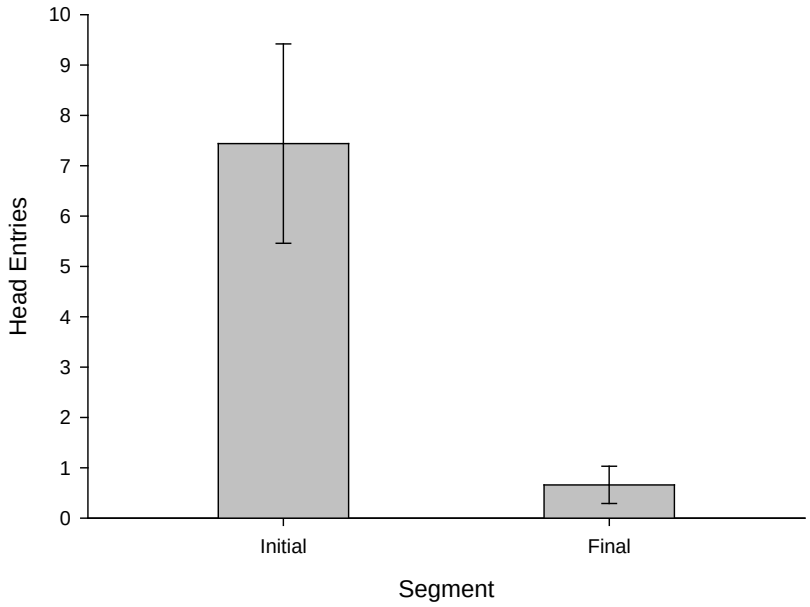


Figure 8. Experiment 2 Phase 2.

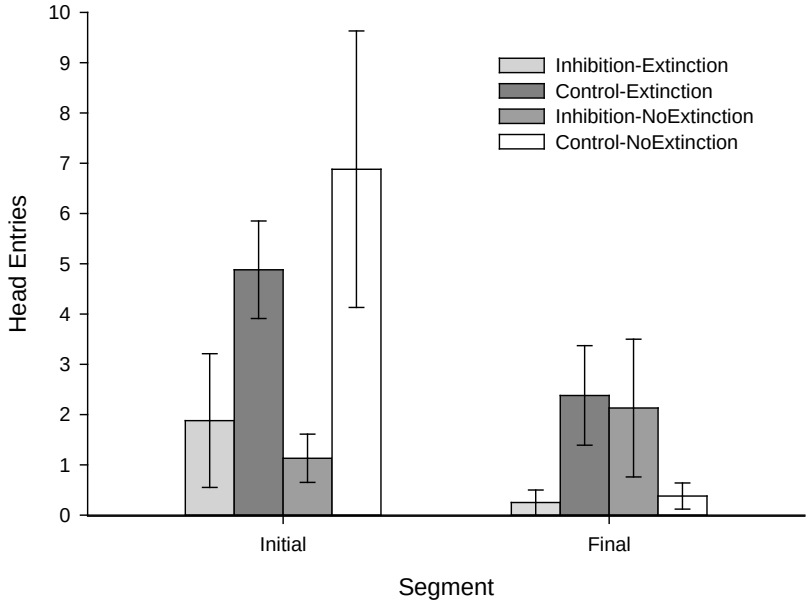


Figure 9. Experiment 2 Retardation Training