PROPERTIES OF SIMULTANEOUS DISCRETE OCCASION SETTERS

A Thesis
by
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Abstract

PROPERTIES OF SIMULTANEOUS DISCRETE OCCASION SETTERS

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This series of experiments used 80 to120 day old water-deprived rats (*Rattus norvegicus*) to

investigate whether simultaneously presented discrete occasion setters shared two properties

of serially presented discrete occasion setters. First, serial occasion setters are able to

modulate behavior outside of their direct associations with the unconditioned stimulus.

Second, serial occasion setters are only able to transfer to other stimuli that have had a serial

occasion setter trained with them. The prevalence of these properties in simultaneous

occasion setters was tested in a series of four experiments. In the first experiment, the

strength of the reinstatement effect was assessed to aid in interpretation of the results and

design of Experiment 2. Results of Experiment 1 revealed that, regardless of training history,

all groups showed a strong reinstatement effect. In the second experiment target stimulus

was first trained as a predictor of shock and then a simultaneously presented occasion setter

to inhibit responding to the target stimulus. The occasion setter was then paired with a shock

and tested for whether it maintained its ability to inhibit responding to the original target

stimulus. Due to a limitation of the procedures, results from Experiment 2 were

inconclusive. The third experiment tested whether simultaneously presented occasion setters would only transfer to other targets that underwent occasion setting training. Two pairs of features and targets were trained and then tested for whether the feature not previously paired with a target would still be able to inhibit responding to that target. Results from Experiment 3 revealed strong contextual control over behavior that overshadowed the ability of the features to modulate responding to the targets. In a fourth experiment, the limitations of Experiment 3 that resulted in strong contextual control of behavior were addressed. Conditioning to one target occurred in context A while conditioning to the other target occurred in context B. During extinction training, the contexts were switched so that the target was paired with a feature in a different context than it was originally trained. Results from Experiment 4 revealed that simultaneous discrete occasion setters were unable to transfer to another target stimulus. Overall results from this experiment indicate that simultaneously presented cues do not act as occasion setters.

Keywords: animal learning, occasion setting, feature negative, simultaneous, phasic, transfer effects, context, renewal, reinstatement, sources of relapse

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Properties of Simultaneous Discrete Occasion Setters

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Abstract

This series of experiments used 80 to120 day old water-deprived rats (*Rattus norvegicus*) to investigate whether simultaneously presented discrete occasion setters shared two properties of serially presented discrete occasion setters. The prevalence of properties of serial occasion setters in simultaneous occasion setters was tested in a series of four experiments. In the first experiment, the strength of the reinstatement effect was assessed to aid in interpretation of the results and design of Experiment 2. Results of Experiment 1 revealed that all groups showed a strong reinstatement effect. In the second experiment target stimulus was first trained as a predictor of shock and then a simultaneously presented occasion setter to inhibit responding to the target stimulus. The occasion setter was then paired with a shock and tested for whether it maintained its ability to inhibit responding to the original target stimulus. Due to a limitation of the procedures, results from Experiment 2 were inconclusive. The third experiment tested whether simultaneously presented occasion setters would only transfer to other targets that underwent occasion setting training. Results from Experiment 3 revealed strong contextual control over behavior that overshadowed the ability of the features to modulate responding to the targets. In a fourth experiment, the limitations of Experiment 3 that resulted in strong contextual control of behavior were addressed. Conditioning to one target occurred in context A while conditioning to the other target occurred in context B. During extinction training, the contexts were switched so that the target was paired with a feature in a different context than it was originally trained. Results from Experiment 4 revealed that simultaneous discrete occasion setters were unable to transfer to another target stimulus. Overall results from this experiment indicate that simultaneously presented cues do not act as occasion setters.

Properties of Simultaneous Discrete Occasion Setters

In Pavlovian conditioning, the training histories of stimuli, and their arrangement with other stimuli, are what influence behavior. One of the most basic training procedures is excitatory conditioning. In excitatory conditioning a previously neutral stimulus (NS) is paired with an unconditioned stimulus (US) until the response elicited by the US, the unconditioned response (UR), is also elicited by the NS. The NS is now considered a conditioned stimulus (CS), and the response elicited by the CS is the conditioned response (CR; Pavlov, 1927/2009). The CS, when presented, will excite a memory of the US (CS$\rightarrow$US) and elicit the CR and is called an exciter. Once an exciter has been established, it is possible to train a separate NS as an inhibitor to the exciter through inhibitory conditioning. In inhibitory conditioning, the excitatory CS is paired with a separate NS without the US over numerous trials; eventually the NS will become a conditioned inhibitor (CI; Rescorla, 1969a; Rescorla, 1969b). When the CI is presented with the CS, the animal will not perform the CR. Both the exciter and the inhibitor have direct associations with the US (see Figure 1; Bouton, 2007). The exciter when presented will activate the memory of the US, and in contrast when the inhibitor is presented it will prevent activation of the US. Thus whenever the inhibitor is present, it should prevent the US memory from being activated by any other stimulus also directly associated with the US (Brooks & Bouton, 1994; Rescorla, 1991). Consequently, the CI should be able to transfer to other exciters connected with the same US.

In the 1980s research started to emerge suggesting that there was a separate classification of stimuli that differed from exciters and inhibitors; these stimuli were called occasion setters. Unlike exciters and inhibitors, occasion setters do not share a direct association with the US. Instead, the occasion setter provides information about whether

another CS, like an exciter, will be followed by the US (Bouton, 2007). Thus, the occasion

setter modulates the association between a CS and the US (see Figure 1) and produces a very

distinct set of properties that will be discussed later. Traditionally, a simultaneous

arrangement of the stimuli results in a conditioned inhibitor and a serial arrangement results

in an occasion setter. The current series of experiments further expands upon the conditions

under which occasion setting can occur and what properties those stimuli may have.

Sources of Relapse of the CR

A major area of interest in contemporary research of Pavlovian conditioning is

understanding extinction of conditioned behavior. Extinction is the process by which an

established CR is suppressed as a consequence of presentations of a CS without the

accompanying US. Studies of extinction have found that an extinguished CR is prone to

relapse and that return of responding comes from a variety of sources. Central to this idea is

the notion that extinction does not cause unlearning (Bouton, 2002). That is, that the

extinction treatment does not destroy the original association that a stimulus has, but instead

provides a second association for that stimulus. When confronted with an ambiguous

stimulus, an ambiguous stimulus being one that has more than one association, the animal

responds according to the association that best predicts what will happen next. It is thought

that the second learned information causes retroactive interference on the recall of the first

learned information, thus eliciting behavior consistent with the second learned association

(Bouton, 1993). There are, however, several circumstances under which relapse can occur.

Renewal. One source of relapse is the renewal effect. Renewal is the reemergence

of a CR due to a change in context (Bouton, 2002). First the animal learns an excitatory

association (CS→US) before it learns a second association (CS→No US) during extinction.

When the animal learns the second association (extinction), the information can become specific to the context in which the learning occurred and any departure from this context will result in a return of the original CR. There are several paradigms used to study renewal. "ABA renewal" is when conditioning occurs in context A, extinction in context B, and then the animal is returned to the conditioning context, A, for testing (Bouton & Bolles, 1979; Bouton & King, 1983; Harris, Jones, Bailey, & Westbrook, 2000). In "ABC renewal" conditioning and extinction occur in A and B, respectively. The animal is then tested in a novel context, context C, and the effect is smaller but still occurs in this paradigm. This paradigm allowed researchers to determine that it was the departure from the extinction context, and not the return to the conditioning context, that resulted in relapse (Harris et al., 2000).

Central to the current series of experiments is the finding that extinction reminder cues can help attenuate the renewal effect (Brooks & Bouton, 1994). In Brooks and Bouton's study, rats were first trained to press a lever for a food reinforcer. Interruption of this lever pressing served as the dependent measure. Rats then received an excitatory CS (CS1) immediately followed by shock in context A causing the animal to freeze and thus interrupt lever pressing. Because CS1 has been paired with shock, the rat will inevitably begin freezing when CS1 is present, a procedure known as conditioned suppression (of lever pressing). After conditioning, they received an extinction treatment in context B in which CS1 was no longer followed by the shock. A separate CS (CS2) preceded CS1 on 75% of these extinction trials. Following extinction of the conditioned freezing response, the rats were tested in either context A or B. Subjects tested with CS1 in context A showed a strong freezing response due to their return to the conditioning context; however, subjects tested

with the CS1 preceded by CS2 in context A demonstrated less freezing. These results

showed that during extinction CS1 acquires an inhibitory association with the US that

counters the excitatory association; however, since CS1 has been made ambiguous, the

inhibitory powers of CS1 are specific to the extinction context (context B). Any departure

from the extinction context results in relapse of the originally learned association. If a

second stimulus, CS2, is trained during extinction, then it is able to prevent expression of the

CR to CS1, even when tested outside of the extinction context. This is because CS2 has

never been made ambiguous and thus can generalize to other contexts.

      Reinstatement. Another potential source of relapse is the reinstatement effect.

Reinstatement is the reemergence of a CR due to exposure to the US following extinction. In

a reinstatement treatment, a CS is conditioned through pairings with a US. Responding to

the CS is then extinguished through multiple presentations of the CS alone. Following

extinction of the CS, the US is presented multiple times (typically 66% of the number of

times it took to originally condition the response), a procedure known as a reexposure

treatment, and when the subjects are tested with the CS, a return of the conditioned response

is observed (Bouton, 2002). Further research has determined that reinstatement effects are

largely dependent on context. For instance, if the animal is tested in a context outside of the

reexposure context, reinstatement does not occur (Bouton, 1984; Bouton & Bolles, 1979;

Bouton & King, 1983). In the work done by Bouton and Bolles (1979), rats were first

conditioned to have a freezing response to a CS (CS1) that was paired with a shock US. The

animals then underwent an extinction procedure in which responding to CS1 was reduced by

presenting CS1 without the shock US. Following extinction, another CS (CS2) was paired

with the shock US. During this stage, the conditions were differentiated by the context in

which the shocks were presented where some rats received shocks in their testing context, others in the conditioning context. At test, only the rats who received shock in the testing context showed a return of the conditioned freezing behavior to CS1.

Occasion Setters

In the studies previously mentioned, context is seen as modulating how the animal will respond to stimuli. Context is considered an occasion setter and "sets the occasion" for how an animal will respond; however, context as well as discrete stimuli can become occasion setters (Bouton & Swartzentruber, 1986; Miller & Oberling, 1998; Swartzentruber & Bouton, 1988). An occasion setting relationship is generally comprised of two parts: a feature stimulus, F, and a target stimulus, T. The feature acts as the occasion setter and traditionally precedes the target CS. Occasion setting can either involve a feature positive design, F→T+, T-, or a feature negative design, F→T−, T+, where "+" denotes reinforced trials in which the US is presented and "-" denotes nonreinforced trials in which the US is omitted. In a feature positive design the organism learns that on a trial where F is present, T will be reinforced and thus the strength of the CR will increase in the presence of F. In a feature negative design the organism learns that on a trial where F is present, T will not be reinforced and thus the strength of the CR will decrease in the presence of F (Bouton, 2007).

The current series of experiments investigated two unique properties of occasion setters. First, that occasion setters have distinctive summation properties that differ from traditional conditioned stimuli. Whereas an inhibitor transfers readily to any other exciter paired with the same US, an occasion setter is specific to the target with which it was trained. Holland and Lamarre (1985) conducted an experiment that investigated when transfer of inhibitory properties of a stimulus is observed using various configurations of cues. Rats

received separate presentations of two CSs (T1 and T2) which were each reinforced with a

shock US resulting in a freezing response. Over the next 48 trials, the rats received

alternating presentations of a reinforced T1 and either an unreinforced simultaneous CS

compound (F1T1) or an unreinforced serial CS compound (F1→T1). In the serial

presentations, rats first received the F1 stimulus followed by a 5-s gap; at the terminus of the

gap T1 was presented. All rats learned a differential response to the T1 based on whether F1

was present or not. At test the rats that received F1→T1 presentations were presented

F1→T2 and F1→T1. One group received simultaneous F1 and T1 compounds but the other

group experienced a serial procedure. The goal of this test was to see if the inhibitory powers

of the F1 stimulus would also modulate responding to T2 based on whether the animals had

received simultaneous or serial training. Holland and Lamarre (1985) found that when the

stimuli were trained serially, F1 was unable to prevent the freezing response to T2.

However, simultaneous training allowed the F1 to transfer well to T2 and inhibited the

freezing response to that stimulus. Holland and Lamarre concluded that the simultaneous

cues were acting as conditioned inhibitors, whereas the serially trained cues were acting as

occasion setters. The results of this study provided support for the hypothesis that occasion

setters must act on the association between a CS and the US and not the US itself and that

serial presentation of cues leads to occasion setting. Had the occasion setter been modulating

the US directly, then transfer to T2 would have been observed.

It is possible to observe transfer of the modulatory powers of an occasion setter on a

target stimulus to another stimulus paired with the same US. Lamarre and Holland (1987)

conducted an experiment that looked at the conditions under which the modulatory powers of

an occasion setter will transfer to other targets. In their experiment, rats were trained to press

a lever for food. They then received separate presentations of two CSs (T1 and T2) each

followed by a shock US to acquire a freezing response to each stimulus. The rats then had

the freezing response to both stimuli extinguished using an alternating procedure of one

reinforced trial and three nonreinforced trials in which T1 was preceded by a feature (F1) and

T2 was preceded by another feature (F2). Both compounds were presented serially. This

procedure is typical when not manipulating context for establishing discriminative inhibition.

At test the rats received F1 and F2 with their originally paired and transfer exciters (i.e.

F1$\rightarrow$T1 and F1$\rightarrow$T2). Unlike the results found in Holland and Lamarre (1985), this

experiment concluded that transfer of modulatory control to other exciters was possible

provided that the transfer exciter had undergone similar occasion setting training with a

different occasion setter.

Another property of occasion setters is that they modulate responding outside of their

direct associations with the US. A feature can have more than one associative path to the

US. It can signal that the US will occur, but also that another stimulus, the target, will not be

reinforced. For instance, if a feature negative occasion setter was established and then

separately reinforced (i.e., the feature is directly paired with the US), it would maintain its

ability to modulate responding to the original target. In an experiment by Holland (1984),

rats received pairings of a CS (T1) and a shock US so that T1 was able to elicit a freezing

response. Next, rats received alternating presentations of T1 followed by shock and a

feature/target compound that was either presented serially (F1$\rightarrow$T1) or simultaneously

(F1T1). Once the freezing response was extinguished, F1 was presented alone and

reinforced with a shock US. As a consequence of this training, the rats had an established

freezing response to both F1 and T1 when either was presented alone. At test, the rats were

presented with either T1 alone or an F1T1/F1→T1 compound. The compound was presented in the same manner as it was presented during the extinction phase (serially or simultaneously). Rats in the simultaneous condition expressed the conditioned freezing response to all stimuli, whereas rats in the serially trained condition did not freeze to F1→T1. Thus F1 had acquired two independent associative paths to the US. In one it directly predicted that the shock was imminent, and in the other it predicted that T1 would not be followed by shock. When the stimuli were presented serially, the CR to T1 was inhibited by F1 despite that F1 alone predicted the US; however, when the stimuli were presented simultaneously, no such effect was observed. The results of this experiment provide further evidence for occasion setters modulating the association between the CS and the US and not the US itself. If F1 in the serial condition had a direct association with the US, then pairing F1 with shock would have interfered with the original training.

An important note about the previous experiments described is that the ideal circumstance under which a cue acquires occasion setting properties is when the stimuli are presented serially (Bonardi, 2007; Bueno & Holland, 2008; Holland, 1984; Holland & Lamarre, 1985; Lamarre & Holland, 1987; Rescorla, 1987). When stimuli are presented in simultaneous compounds, the feature stimulus takes on properties of traditional associative stimuli (i.e., they become a conditioned inhibitor). Bouton (2007) offers a possible explanation for this difference. When the feature stimulus is presented simultaneously with an exciter, the feature has good contiguity with the anticipated US and could allow for the feature to develop a direct association with the US. In contrast, serially presented compounds traditionally involve a 5 to10-s gap between the feature and the exciter. Since the both simultaneous and serially presented cues are equated for contiguity, the reduction in

contiguity between the feature and the US must promote the feature acquiring information

about the anticipated relationship between the exciter and the US rather than a direct

association with the US.

The Current Study

The current series of experiments further examined the conditions under which

occasion setters form and the properties of those occasion setters. Data gathered by Jacobs,

Mason, and Denniston (2014) suggested that there may be additional procedures that form

occasion setters. Originally this series of experiments was intended investigate the ability of

extinction cues to attenuate the renewal effect, so the procedures used various contexts (A, B,

or C). In these experiments rats were trained to press a lever for a water reinforcer. With a

consistent high frequency behavior established, interruption of the lever pressing behavior by

the shock or any associated CSs was indicative of a freezing response. Experiment 1 was

conducted in three stages: conditioning, extinction, and test. In the conditioning phase,

subjects were presented with a pairing of a CS (T1) and a shock US in context A. Over the

course of six trials, all subjects reliably developed a freezing response to T1. In the next

phase, CS1 was paired with a simultaneous feature (F1) without a shock in context B, context

B was counterbalanced with context C, until the subjects no longer showed a freezing

response to the F1T1 compound. At test, subjects were either placed in the extinction

context or a novel context, B or C respectively, and either presented with the F1T1

compound or T1 alone. Regardless of place, if F1 was present, then subjects did not have a

return the conditioned freezing response. These results replicate the findings of Brooks and

Bouton (1994) which dealt with extinction reminder cues.

In Experiment 2 of Jacobs et al. (2014), researchers examined the ability of the extinction reminder cue to transfer inhibition to other exciters paired with the shock US. Like Experiment 1, there were three phases: conditioning, extinction, and test. Conditioning proceeded over eight trials in which there were four presentations of a CS (T1) and a separate CS (T2) followed by shock in context A. Subjects reliably acquired a freezing response to both of these stimuli. Extinction involved simultaneously pairing a feature (F1) and T1 without shock in context B. Extinction ended when all subjects no longer showed a freezing response to the F1T1 compound. At test subjects were either tested in the extinction context or a novel context, B or C, respectively. Additionally, subjects either received a F1T2 compound, or T2 alone. Regardless of testing context or testing stimulus used, all subjects showed a conditioned freezing response. These results are inconsistent with findings reported by Holland and Lamarre (1985) and Lamarre and Holland (1985). Those studies dealt with the transfer properties of serially and simultaneously presented stimuli. An important finding that was not replicated was that inhibitory stimuli (i.e., F1) will modulate responding to other exciters (i.e., T2) whereas serially presented inhibitors will not unless very specific criteria, like those described in Lamarre and Holland (1987) are met. Results from Lamarre and Holland (1987) suggests that, for serially presented stimuli, F1 should only have been able to modulate responding to T2 when it had been extinguished in the presence of another feature. For simultaneously presented stimuli, F1 should be able to inhibit responding to T2 provided that T2 has a direct association with the same US F1 is predicting will not occur. In Jacobs et al. (2014), stimuli were presented simultaneously and yet F1 failed to inhibit responding to T2. T2 was never extinguished in the presence of

another cue leading to the interpretation that the F1 stimulus must be acting as an occasion setter rather than a conditioned inhibitor.

Jacobs et al. (2014) had two key procedural deviations from Holland and Lamarre (1985), Lamarre and Holland (1985), and Lamarre and Holland (1987). First, Jacobs et al. provided training in multiple contexts. It is unclear at this time what role this may have played in the contrasting results. For this reason, the manipulation of context will be preserved in the proposed experiments to better replicate the conditions under which these findings originally occurred. Second, traditional occasion setting literature used alternating trials when presenting stimuli (e.g. Holland & Lamarre, 1985; Lamarre & Holland, 1985; 1987). Jacobs et al. (2014) used a phasic procedure in which the reinforced and nonreinforced trials were presented in sequential blocks instead of an interspersed pattern. Likewise, more investigation is needed to determine to what extent the magnitude of the contrasting results is accounted for by these differences. For the purposes of the proposed study, a phasic procedure was used so as to better replicate the conditions of Jacobs et al.

In the current series of experiments, a potential simultaneous occasion setter was tested for congruence with the unique properties of serial occasion setters. Namely, whether a simultaneous occasion setter was able to hold its inhibitory modulation of behavior after being reinforced with a shock, and whether the potential simultaneous occasion setter transferred to other stimuli that had also been trained with a separate simultaneous occasion setter.

General Method

Subjects

Subjects were water-deprived, experimentally naïve, 80-120 day-old, Long Evans rats (*Rattus norvegicus*) counterbalanced for sex within groups. Animals were bred and supplied by Rankin Science North Animal Care Facility and were kept on a 14 hr day and 10 hr night schedule. All animals were kept above 85% initial body weight and were monitored for changes in health. The protocol for use of animal subjects was approved by Appalachian State University's Institutional Animal Care and Use Committee on January 28, 2015.

Apparatus

Standard rodent experimental chambers (MED Associates) were housed in sound and light-attenuating enclosures. Each chamber could deliver the following stimuli: click, white noise, buzzer, tone, water via a dipper with a .04 cc cup, and a brief, mild foot shock (0.5 s, 0.4 mA). Auditory stimuli were presented approximately 8 dB (C-scale) above background noise levels. The frequency of the tone was 2200 Hz. All CSs were 60 s in duration. Physical contexts were manipulated in the following manner: Context 1 consisted of a standard operant chamber illuminated by a #1820 incandescent bulb and also contained an odor cue, one drop of methyl salicylate placed on a wooden block inside of the environmental enclosure, but outside of the experimental chamber. Context 2 had a striped pattern on the walls, and was illuminated by a #1820 incandescent light bulb. Context 3 had a different grid floor, level or staggered, than that used for context 2 and no illumination. Physical contexts 1, 2, and 3 will serve as contexts A, B, and C. Unless otherwise specified, physical context 1 always served as context A, and physical contexts 2 and 3 served as contexts B and C, counterbalanced by groups.

Procedure

All subjects were exposed to a progressive water deprivation schedule before the study began in which access to water was gradually reduced to 15 min per day. This level of deprivation was sufficient to provide adequate motivation to support conditioned lever pressing for water (the behavioral baseline) during each experimental session while at the same time allowing the animals to take in their normal daily ration of water (25 ml). Water was available for 15 min shortly following each daily session. Subjects were also able to earn water during each experimental session as reinforcement for the lever pressing behavior. Experimenters handled the animals daily as part of the experimental protocol and monitored animals for changes in health.

Prior to initiating the water deprivation schedule, all subjects were weighed in order to obtain pre-deprivation weights. Animal weights were checked on a weekly basis throughout the study and any animals falling below 85% of their pre-deprivation weight received additional water in order to ensure adequate hydration and nutrition.

In each experiment, all subjects received preliminary training in order to shape the lever press response. On Day 1 of pretraining, subjects obtained water reinforcers contingent upon each lever press response and also received non-contingent reinforcers on a variable-time (VT) 2 min schedule in order to condition an association between the sound made by the movement of the liquid dipper and the availability of water. Day 2 of pretraining involved training on a continuous reinforcement (CRF) schedule, in which all reinforcement was contingent upon lever pressing; every lever press earned reinforcement. On Days 3 and 4, rats maintained lever pressing on a progressive fixed ratio (FR) 2 schedule of reinforcement, in which subjects were required to make two responses in order to obtain reinforcement. If

the time between the last press and the preceding press was <1 s then the FR increased by

one with a maximum FR10 possible. On Days 5, 6, and 7 of pretraining subjects secured

reinforcement on a tandem variable interval (VI) 10 s /variable ratio (VR) 5 schedule, in

which subjects needed to make one response after a VI10 s (ranging from 1 s to 20 s) to

satisfy the variable interval schedule. The schedule then transitioned to a VR5 schedule

(ranging from 1 to 10 responses), which required an average of five responses before

securing reinforcement. This training developed a high, steady rate of lever pressing

behavior; disruption of which served as the dependent measure in these experiments. Each

pretraining session was 60 min in duration and alternated between contexts A and B.

Context C also received exposure in Experiment 3.

        The schedule described above was dependent on the behavior of the animals and

represents the ideal timing. Progression from the CRF schedule described on Day 2 required

that all rats performed at least 50 lever presses before proceeding to the progressive FR2

schedule. Advancement out of the progressive FR2 schedule was contingent on every rat

performing at least 200 responses per session. Progression out of pretraining required that all

rats performed at least 500 responses on the last day of the tandem VI10 s VR5 schedule.

Failure to meet any of these requirements resulted in all rats remaining on the same schedule

of reinforcement until the requirement for that stage was met.

In all experiments, subjects were randomly assigned into groups following acquisition of the

lever pressing behavior and counterbalanced by sex within groups. Interruption of lever

pressing served as the dependent variable. When the rat is exposed to a CS that elicits fear, it

will develop a freezing response that interrupts lever pressing. Thus, the rat's CR to each

stimulus can be quantified by calculating suppression ratios. The formula for calculating

these ratios is:

$$\frac{CS}{PreCS + CS}$$

Suppression ratios are calculated with the number of responses during the 1 minute

CS in the numerator and the sum of the number of responses one minute prior to the CS and

during the 1 minute CS. A value between .5 and 1 indicates increased responding elicited by

the CS whereas a value between 0 and .5 indicates suppressed responding elicited by the CS.

Values closer to 0 indicate a greater suppression of lever pressing. In the current series of

experiments, numbers closer to 0 indicate disruption of responding (greater fear) and

numbers closer to .5 indicate less disruption of responding (less fear). In each experiment,

when all subjects reached a threshold around .4 for two consecutive days during extinction,

then all subjects proceeded to test.

Experiment 1

The purpose of Experiment 1 was to assess the role that unsignaled and signaled

reinstatement would play in the Experiment 2. Signaled reinstatement occurs when there is a

previously unpaired stimulus present during the reexposure treatment. Following reexposure,

a recovery of the previously extinguished CR is observed. This effect was demonstrated in

the Bouton and Bolles (1979) study in which following extinction, subjects received

presentations of the US preceded by a clicking stimulus. Unsignaled reinstatement occurs

when the US is presented alone during reexposure. Much like signaled reinstatement, a

recovery of the previously extinguished CR is observed. Bouton and King (1983) tested this

mechanism by presenting shocks after an extinction treatment without a preceding stimulus

and found results similar to Bouton and Bolles (1979). In both experiments, reinstatement

only occurred when the reexposure and testing contexts were the same.

Experiment 1 investigated how the mechanisms of reinstatement would operate

within Experiment 2; an experiment which involved reexposing the animal to the US. This

experiment provided baseline measurements of levels of reinstatement expected with the

procedures used in Experiment 2. The procedures were a conceptual replication of those

found in Bouton and Bolles (1979) and Bouton and King (1983). This experiment used six

groups of subjects. The Unsignaled Reinstatement Same (URS) group provided an

experimental group that received an unsignaled exposure to the US after extinction; the

testing and reexposure contexts matched each other. This group was expected to demonstrate

a return of the CR. The Unsignaled Reinstatement Different (URD) group underwent the

same procedure as URS but was tested in a novel context. This group was expected to be

unlikely to show a return of the CR since its reexposure and testing context would not match.

The Signaled Reinstatement Same (SRS) and Signaled Reinstatement Different (SRD)

groups followed the same logic and procedures as the unsignaled reinstatement groups but a

60 s CS preceded the shock.

Two additional groups replicated the methods of Westbrook, Iordanova, McNally,

Richardson, & Harris (2002). Group Reinstatement in B Different (RB-D) received

reexposure to the US in context B, and returned to the conditioning and extinction context, A,

for test. Group Reinstatement in B Same (RB-S) received reexposure in context B and was

also tested in context B. The reasoning behind reexposing the animal in B is to test the

assertion that reinstatement can be attenuated if the animal is reexposed in a nonconditioning

context. Westbrook et al. found that rats that received conditioning and extinction in context

A, reexposure in B, but were then moved back to A for test spent very little time freezing. The design for the current experiment is summarized in Table 1.

This experiment tested two hypotheses. First, reinstatement only occurs when the testing and reexposure contexts match. Second, results of the signaled reinstatement conditions would not differ from the unsignaled reinstatement conditions.

Method

Subjects. This study utilized a sample of 48 experimentally naïve Long-Evans hooded rats as previously described ($n = 8$ subjects per group).

Apparatus. The same apparatus as previously described in the General Methods section was used.

Procedure. Prior to conditioning in Phase 1, the pretraining procedures previously described established lever pressing behavior for a water reinforcer. Subjects received one day of shaping in context A, one day of CRF training in context B, two days of progressive FR training in context B then context A, and three days of VIVR training in context B, then context A, then context B again. During each day of VIVR training, subjects received two presentations of each target stimulus alone, for a total of four total presentations each day, with the order randomized. Throughout the entirety of training and testing, subjects maintained lever pressing behavior on a tandem VI10 s VR5 schedule of reinforcement.

In Phase 1 all subjects received conditioning in which a target stimulus, T1, preceded a 0.5 s 0.4-mA foot shock US, indicated by a "+", in context A. T1 was a tone or white noise, counterbalanced. Subjects received three T1+ pairings during each of the two daily 60-min sessions, for a total of six conditioning trials.

In Phase 2 all subjects received an extinction treatment in which stimulus T1 presentations occurred in the absence of shock in context A. Extinction took place over seven daily 60-min sessions. Each session involved eight exposures to the T1 stimulus for a total of 56 exposures. Mean suppression ratios were calculated for the entire sample of subjects daily, and extinction ceased once the mean suppression ratio for the first presentation each day reached a value greater than or equal to .4 for two consecutive days.

In Phase 3 Groups URS and URD received four unpaired 0.5 s 0.4-mA foot shock US in context A during one 60-min session. Groups SRS and SRD received four pairings of T2, tone and white noise counterbalanced, and a 0.5 s 0.4-mA foot shock US in context A during one 60-min session. Groups RB-D and RB-S received four unpaired 0.5 s 0.4-mA foot shock US in context B during one 60-min session.

On the two days following Phase 3, all rats received a reshaping procedure in context A during which bar pressing was reinforced on the tandem VI10 sVR5 schedule of reinforcement to reestablish baseline responding. Reshaping took place during two 60-min sessions.

Testing varied by group and took place during one 60-min session with four exposures to the T1 stimulus. Subjects in Groups URS, RB-D, and SRS were tested in context A. Subjects in Groups URD, RB-S, and SRD were tested in context B.

Results and Discussion

The results of Experiment 1 are summarized in Figures 2-4. Figure 2 depicts that by the end of the six Phase 1 conditioning trials all subjects acquired a conditioned freezing response to the T1 stimulus because suppression ratios declined towards 0. Data is pooled across groups since all subjects received the same treatment in Phase 1. Figure 3 summarizes

the results of the extinction procedure provided during Phase 2. On the final two days of

extinction, the suppression ratios for all animals reached asymptote, and despite not meeting

the previously described threshold of .4, subjects progressed to testing in order to not over

train extinction. As with Figure 2, the data in Figure 3 is pooled across groups since all

animals received the same treatment in Phase 2.

Figure 4 depicts the testing in context A and B separated by group. A One-Way

ANOVA examined the effect of group on suppression ratios for the T1 stimulus. When

considered by group, there was not a significant effect of group for ratio 1 in context A, F (5,

41) = .08, p = .995, ω^2 <.001, or ratio 2 F (5, 33) = 1.15, p = .356, ω^2 = .02. When considered

by group, there was not a significant effect of group for ratio 1 in context B, F (5, 35) = 2.45,

p = .053, ω^2 = .02, or ratio 2, F (5, 30) = .51, p = .764, ω^2 <.001.

The results of Experiment 1 failed to replicate previous findings of Bouton and Bolles

(1979), Bouton and King (1983), and Westbrook et al. (2002) on reexposure and testing

context match and mismatch. When tested in a context that differed from the reexposure

context, subjects in the current experiment still showed a strong reinstatement effect.

Reinstatement is thought to occur due to the summation of a context → US association and

any remaining CS → US association (Bouton & King, 1983). During reexposure the animal

associates the US pairings with context and then when the animal is presented with the CS at

test, that CS activates the memory of the context and therefore also the US. So it follows that

testing in a context that is not the reexposure context, or reexposing the animal to the US in a

context that is not the one in which the animal has received the CS should attenuate the

reinstatement effect. In the current experiment, there was a procedural difference that may

have accounted for the results. Experiment 2 in Westbrook, Jones, Bailey, and Harris (2000)

found that testing in a context that had received preexposure may have activated a preexposure memory that could interfere with performance at test. In the current experiment, the subjects were preexposed to T1 and T2 in both of our experimental contexts and this may have created a CS → context association. When the animals were presented with the CS at test, there were CS → context associations already formed which may have facilitated retrieval of the US memory for all groups at test. Although previous research such as that by Bouton and Bolles or Bouton and King have used preexposure in their experiments, Westbrook et al. (2002) did not. Given that the current experiment used an extensive pretraining and preexposure procedure, this difference may be a source of this discrepant finding. Further research is needed to determine the role of preexposure to stimuli and context on reinstatement.

Despite the lack of significant differences, the current experiment provided some valuable information for the design of Experiment 2. First, data gathered from groups SRS-S and SRS-D allowed us to gauge the appropriate number of trials to use. Previous experiments (e.g., Bouton & Bolles, 1979; Bouton & King, 1983) used a ratio of two-thirds the amount of reexposure trials as were used in conditioning. For the current experiment, that same ratio was kept and used four reexposure trials based on our six conditioning trials; however, it seemed that on the third reexposure trial the subjects had reached a mean suppression ratio of .139 with only 68% of subjects responding. It could be that in the current experiment more reexposure training was provided than was appropriate for our procedures. The amount of training provided in this phase was adjusted in Experiment 2. Secondly, since preexposure to the targets may have influenced the results in the current

study, CS preexposure was eliminated from future studies. Upon reevaluation, CS

preexposure provided no foreseeable benefits to the future studies.

Experiment 2

Experiment 2 was designed to assess whether pairing a simultaneous occasion setter

with a shock US would disrupt the occasion setters ability to modulate responding to its

original target. One unique property established in the occasion setting literature is that a

single stimulus, a serially established occasion setter, is able to hold two separate associative

paths with the US; one that directly activates the US, and one that provides information about

whether the US will follow a target stimulus (Holland, 1984). Experiment 2 tested whether

this property applies to a potential simultaneous occasion setter, like the one seemingly

established in Jacobs et al. (2014).

The design of this experiment is outlined in Table 2. All groups received the same

acquisition and extinction treatment in Phases 1 and 2 respectively. In Phase 1, two targets

(T1 and T2) each preceded a shock in context A. In Phase 2, subjects received T1 in the

presence of a feature, F1, with the goal of extinguishing the CR to T1 in context B. In Phase

3 all groups received a second acquisition phase that differed by which stimulus, if any, is

reinforced before proceeding to test. In Group Feature 1 – Reinforced (F1-R), F1 preceded a

shock; subjects in this group were then tested with the F1T1 compound. If F1 was acting as

an occasion setter, then these subjects were expected show a weak CR at test, as F1 should

hold its ability to modulate responding to T1 despite a history of differential reinforcement.

Subjects in Group Feature 2 - Reinforced (F2-R) received a novel feature stimulus, F2, which

preceded a shock during Phase 3. Subjects in this group then received the F1T1 compound at

test and were expected to show a weak CR. Groups F1-R and F2-R had equated signaled

exposures to the shock and allowed for comparisons between these groups. Group F2-R was expected to show a weak CR, regardless of how the F1 stimulus operated, based on previous research done by Holland (1984), Rescorla (1969a), and Rescorla (1969b), whereas Group F1-R should show a weak CR only if F1 acted as an occasion setter. Group Summation (SUM) received a novel F2 stimulus preceding shock in Phase 3 but was tested with the F2T1 compound stimulus. This group was expected to show a strong CR due to the fact that it did not receive the negative occasion setter, F1, at test. Through summation effects of the F2 and T1 stimuli, this group was predicted to demonstrate well-established fear. The Unsignaled Reinstatement (USR) group received unsignaled presentations of the US during Phase 3 and subjects were tested with the F1T1 compound stimulus. If the F1 stimulus was able to overshadow the context for control of behavior, then Group USR was predicted to show a weak CR. Regardless, Group USR provided a valuable control group in determining the role of reinstatement in our results.

The training for Group F1-R was predicted to elicit the effect under investigation. If the F1 stimulus was acquiring the expected occasion setting properties, it should be able to reduce the strength of the CR despite the history of reinforcement of F1 in Phase 3. Testing was conducted in context C in order to minimize the influence of contextual fear on responding to the test stimuli. However, with this change in context ABC renewal effects could have potentially impacted our results.

Method

Subjects. This study utilized a sample of 32 experimentally naïve Long-Evans hooded rats as previously described (n = 8 subjects per group).

Apparatus. The apparatus was the same as that used in Experiment 1.

Procedure. Prior to conditioning in Phase 1, the pretraining procedures previously described established lever pressing behavior for a water reinforcer. Subjects received one day of shaping in context A, one day of CRF in context B, two days of progressive FR in context C then context A, and three days of tandem VIVR training in context C, then context A, and then context B. Throughout the entirety of training and testing, subjects maintained established lever pressing behavior on a tandem VI10 sVR5 schedule of reinforcement.

In Phase 1 all subjects received conditioning in which T1, a tone, preceded a 0.5 s 0.4-mA foot shock US in context A. Subjects received three T1-US pairings in each of two daily 60-min sessions for a total of six conditioning trials.

In Phase 2 all groups received an extinction treatment with simultaneous presentations of the F1 and T1 stimuli in context B in the absence of shock. F1 was a white noise or click, counterbalanced within groups. Extinction took place over four days during 60-min sessions; each subject received eight F1T1 or T1 pairings not followed by shock on each day for a total of 32 extinction trials. Departure from this phase was contingent on a consistent mean suppression ratio of .4 across two consecutive days.

In Phase 3, subjects in Group F1-R and F1-B received two F1-US pairings in context B. Groups F2-R and SUM received two F2-US pairings in context B. Group USR received two unsignaled presentations of the foot shock US in context B at the same intervals as the other groups. In total, each group received two US presentations during a one-day 60-min session.

Following the end of Phase 3 training, all subjects received two days of reshaping in context B before moving on to test. The 60-min reshaping procedure involved the subject pressing a lever on the tandem VI10 sVR5 schedule of reinforcement with no stimuli

presented throughout the session. The purpose of these sessions was to restore baseline

responding after Phase 3.

At test Groups F1-R, F2-R, and USR received six nonreinforced trials of the F1T1

compound in context B on the first day of testing and six nonreinforced trials in context C on

the second day. Group SUM received six, nonreinforced trials of the F2T1 compound in

context B on the first day of testing and six, nonreinforced trials of the F2T1 compound in

context C on the second day. Each test session lasted 60 min.

Results and Discussion

The results of Experiment 2 are summarized in Figures 5-7. Figure 5 summarizes

acquisition. By the last trial of Phase 1, all subjects had acquired a fear of T1 indicated by

responding declining towards a suppression ratio of 0. Figure 6 summarizes extinction in

Phase 2. After only four days, subjects consistently performed above the set .4 suppression

ratio threshold for two consecutive days before testing.

Originally it was planned to collect six ratios over the course of testing and examining

rates of extinction to the stimuli, but unfortunately this was untenable due to a large number

of subjects not responding after the first trial. Therefore, only the first ratio from each day is

reported. Figure 7 summarizes the results from testing on Day 1 in context B and Day 2

testing in context C. A One-Way ANOVA examined the effect of group on suppression ratio

for the F1T1 stimulus on each day. For the ANOVA for context B, the assumption of

homogeneity of variance was violated, *Levene F* $(3, 28) = 5.07$, $p = .006$, therefore Welch's

F is reported. There was a significant effect of group, $F(3, 11.67) = 6.35$, $p = .008$, $\omega^2 = .18$,

on ratio 1 of the test in B. Games-Howell post hoc tests were run to account for the violation

of the assumption of homogeneity of variance and indicated, $p = .044$, that subjects in group

F1-R (*M* = .002, *SD* = .004), 95% CI [-.002, .005] expressed more fear than subjects in group

USR (*M* = .25, *SD* = .21), 95% CI [.08, .26]. Subjects in groups F2-R (*M* = .31, *SD* = .31),

95% CI [.06, .58] and F2-S (*M* = .09, *SD* = .25), 95% CI [-.11, .30] did not differ

significantly from any other group. For the ANOVA run for testing in context C, the

assumption of homogeneity of variance was violated, *Levene F* (3, 26) = 4.43, *p* = .012,

therefore Welch's *F* is reported. There was a significant effect of group, *F* (3, 11.06) = 5.49,

p = .015, ω^2 = .14, on ratio 1. Games-Howell post hoc tests indicated, *p* = .044, that group

F1-R (*M* = .002, *SD* = .005), 95% CI [-.003, .006] expressed greater fear than group USR (*M*

= .25, *SD* = .21), 95% CI [.08, .26]. Groups F2-R (*M* = .30, *SD* = .33), 95% CI [-.005, .60]

and F2-S (*M* = .09, *SD* = .21), 95% CI [-.11, .30] did not differ significantly from any other

group.

The hypothesis that forming an association between the feature and the US would not

disrupt the modulatory powers of the feature on its target was not supported in the current

experiment. With serial occasion setters, the feature is able to provide information about

whether the target will be followed by the US. Since the feature is terminated prior to onset

of the target, the animal is able to express both fear to the feature but also no fear to the

target. Thus one very severe limitation of the preset procedure becomes apparent. In

previous studies that examined pairing serial occasion setters with the US (e.g. Holland,

1984), the suppression ratios were calculated from based upon the suppression of responding

to the target stimulus alone. Due to the serial presentation used by Holland, the CR to the

target was able to be isolated from the CR to the feature. In the current experiment both the

feature and the target were presented simultaneously, so the data gathered included

responding to the feature and the target combined, rather than isolated responding to the

target stimulus. So while group F1-R and F2-S did not significantly differ, it is unclear whether the expressed fear was the result of summation between F1 and T1 or by fear to the F1 stimulus alone. Future research into this property of simultaneous occasion setters would need to devise a procedure that could isolate what stimulus or compound of stimuli the animal was responding to. The current study's procedures fail to adequately assess the hypothesis.

Experiment 3

The purpose of Experiment 3 was to further elaborate on the properties of the F1 stimulus. This experiment tested the simultaneous occasion setter against the assumption that a feature should only transfer to targets that have undergone occasion setting training. Unlike Experiment 2, this experiment used an ABC renewal paradigm to reduce the influence of any associative value of context B. Since contexts can act as occasion setters, it was needed to depart from context B in order to eliminate the influence it holds as a contextual occasion setter (Bouton & Swartzentruber, 1986; Miller & Oberling, 1998; Swartzentruber & Bouton, 1988). The design for this experiment is outlined in Table 3. In Phase 1 all subjects received a pattern of interspersed T1 and T2 trials paired with a shock US. These stimuli served as the transfer exciters for each other's feature. During Phase 2 all groups received extinction in context B, but the groups differed by which stimuli underwent extinction.

Group Transfer (T) received both the F1T1 and F2T2 nonreinforced simultaneous compounds interspersed within the same extinction session. At test this group received F2T1 and F1T2 compounds, counterbalanced for test order effects. Should F1 have acted as an occasion setter then a weak CR in both presentations was expected. Should F1 have acted as a CI then a weak CR was also expected; another group, No Transfer (NT), provided

discrimination between these two possible mechanisms. NT received the F1T1

nonreinforced compound and the T2 nonreinforced stimulus during extinction. At test this

group received F2T1 and F1T2 compounds. If F1 acted as an occasion setter, then it was

predicted there would be no transfer to these two targets and the compounds would elicit a

strong CR; however, if F1 was acting as a CI, then the compounds would elicit a weak CR

comparable to that of all other groups. It was possible that a transfer between F1 stimulus and

context B could be observed, provided that transfer was possible between contextual and

discrete occasion setters when testing with the F1T2 compound. If this effect occurs, it will

warrant further investigation into the nature of such a mechanism. Group Occasion Setting

Control (OSC) received the same treatment as Group T during extinction, but at test this

group received the F1T1 and F2T2 compounds. Regardless of how F1 operated, this group

provided a control comparison to gauge proper occasion setting since each target was paired

with its original feature.   Finally, group Renewal Control (FC) indicated baseline CR due to

ABC renewal. Without any interactions of compounds at test, this group simply received

extinction to the two target stimuli in the presence of the features. Then subjects were tested

with the targets but without the features present. The results of this procedure should

produce a strong CR.

       In Experiment 3,the hypothesis that when a simultaneously presented feature negative

occasion setter is presented with a transfer target CS, the feature will only be able to

modulate responding to the transfer target if it has undergone a feature negative occasion

setting training history was tested.

Methods

Subjects. This study utilized a sample of 32 experimentally naïve Long-Evans hooded rats as previously described (*n* = 8 subjects per group).

Apparatus. The apparatus was the same as previously described in Experiment 1.

Procedure. Prior to conditioning in Phase 1, the pretraining procedures previously described established lever pressing behavior for a water reinforcer. Subjects received one day of shaping in context A, two days of CRF training in context B, then context C, two days of progressive FR training in context A then context C, and three days of VIVR training in context A, then context B, and then context C. Throughout the entirety of training and testing, subjects maintained established lever pressing behavior on a tandem VI10 sVR5 schedule of reinforcement.

Rats were randomly assigned to one of four conditions counterbalanced for sex within groups. The four conditions are Transfer (T), Occasion Setting Control (OSC), No Transfer (NT), and Renewal Control (FC).

Counterbalancing was be the same as in Experiment 2 with the addition that T1 and T2 were a tone and white noise stimulus. F1 and F2, were a clicking stimulus and a flashing light. Prior to conditioning in Phase 1, the previously described pretraining procedures were used.

In Phase 1 all subjects received conditioning in which T1 and T2 were each separately paired with the foot shock US in context A. All subjects received two T1+ and two T2+ pairings over three days of conditioning, for a total of six reinforced trials for each stimulus. Each session consisted of both T1+ and T2+ presentations. The order was varied

daily to control for primacy and recency effects. The orders were: T1, T2, T1, T2; T1, T2,

T2, T1; and T2, T1, T1, T2. Sessions were 60 min in duration.

In Phase 2, Groups T, OCS, and FC received four nonreinforced presentations of the

F1T1 compound and four nonreinforced presentations of the F2T2 compound over the course

of six days for a total of 24 trials per stimulus in context B. Group NT received four

nonreinforced presentations of the F1T1 compound and four nonreinforced presentations of

the T2 stimulus over six days for a total of 20 trials per stimulus in context B. The extinction

procedure was concluded after the mean suppression ratio on the first extinction trial reached

a mean of .4 on two consecutive days. All sessions were 60-min in duration.

After Phase 2, testing occurred in separate sessions across two days. All subjects

received four test presentations within a single session. Groups T and NT received two

presentations of the F2T1 and F1T2 compounds for a total of four presentations in context C,

counterbalanced for test order across sessions. Group OCS received two presentations of the

F1T1 and F2T2 compounds in context C, counterbalanced for test order. Group FC received

two presentations of stimuli T1 and T2 in context C, counterbalanced for test order.

Results and Discussion

The results of Experiment 3 are summarized in Figures 8-12 Figure 8 depicts the

results of acquisition in phase 1. Mean suppression ratios for T1 and T2 are combined across

groups. By the end of phase 1 all rats had acquired a fear to both the T1 and T2 stimulus.

Figure 9 depicts the results from the extinction treatment in phase 2. After six days, mean

suppression ratios for all subjects stabilized around .4 and subjects proceeded to test.

As was done in Experiment 2, additional ratios were collected in the hope that

differences in rates of extinction could be observed, but this analysis was made untenable due

to a large number of subjects missing data for the later ratios (i.e., subjects stopped

responding). For analysis, the ratios were organized according to what target is present

during that ratio. For instance group T received the F1T2 and F2T1 stimuli so stimulus

compound F2T1 is included in the T1 ratio and F1T2 is included in the T2 ratio. Figure 10

depicts the testing results from the first ratio of each stimulus on Day 1 and Figure 12 depicts

the testing results from the first ratio of each stimulus on Day 2. A One-Way ANOVA was

conducted with group as the single factor on the suppression ratios obtained during testing.

There was a significant effect of group, F (3, 25) = 6.02, p = .003, ω^2 = .34, on the

presentations of the T1 stimulus on Day 1. Tukey's HSD post hoc tests revealed, $p < .05$,

groups T (M = .12, SD = .12), 95% CI [.02, .22] and NT (M = .04, SD = .07), 95% CI [-.04,

.12], showed significantly more fear than group FC (M = .33, SD = .20), 95% CI [.13, .26].

Group EC (M = .25, SD = .13), 95% CI [.13, .37] did not differ significantly from any other

group. There was no significant effect of group on presentations of the T2 stimulus on Day 1,

F (3, 26) = 2.14, p = .119, ω^2 = .10, T1 stimulus on Day 2, F (3, 24) = 1.50, p = .240, ω^2 =

.05, or on T2 on Day 2, F (3, 22) = .934, p = .441, ω^2 = .0.

The hypothesis that features will only transfer to stimuli that have also undergone

occasion setting training was not supported in the current experiment. The suppression ratio

for our critical experimental group, T, was not significantly different from either the EC or

NT groups. It was expected that T should not be different from EC, indicating successful

transfer, and would be different from group NT, which would have defined the conditions

under which transfer occurs.

The control group FC had significantly less fear than both group T and NT. Group

FC was intended to demonstrate fear. At test, group FC received the T1 and T2 stimuli,

absent of any discrete or contextual occasion setters. Without the features present during extinction, and since the test was in a novel context, it is unclear as to why this group did not express fear. Conceptually, this control group is similar to the groups that received only their conditioning stimulus at test from Jacobs et al. 2014. In that study, when the conditioning stimulus was presented alone, the animals expressed fear regardless of place (including in the extinction context). In order to gather more data on why group FC did not respond as expected, an additional day of testing in context A was ran. During this test session, groups T, NT, and FC received four presentations of the T1 and T2 stimuli and group EC received four presentations of the F1T1 and F2T2 stimuli. The results of this test are summarized in Figure 12. Regardless of stimulus used, all groups showed a strong fear response. Clearly there was strong contextual control of responding in the current experiment.

It is possible that procedural differences may account for why responding was more context dependent in the present study than in previous research. Previous research done on occasion setting only provided training in one context (Holland & Lamarre, 1985; Lamarre & Holland, 1987). In these experiments, the features were the only valid stimuli to differentiate the ambiguity associated with the targets. In other words, it wasn't possible for the context to overshadow the features for control of behavior. Additionally, Jacobs et al. (2014) provided training to two CSs in context A, similar to that provided in the current experiment; however, in Jacobs et al., only one of the stimuli received extinction training whereas both stimuli received extinction treatments in the current experiment. Perhaps since all stimuli were conditioned in context A and responding to all stimuli was extinguished in context B, context became a better, and easier, predictor to disambiguate the outcome of the various stimulus presentations. In other words, the presence of the features simply provided redundant

information that was already handled by the context therefore reducing attention to the features. Still, even with this possible explanation, more research is needed to determine why ABC renewal was attenuated in this circumstance.

Experiment 4

The purpose of Experiment 4 was to address the limitations of Experiment 3 and test the same hypothesis. Since results from Experiment 3 revealed strong contextual control over behavior, Experiment 4 was designed to reduce the predictive value of the context. In this experiment, T1 and T2 were trained in either context A or context B; for instance, T1 was trained in context A and T2 was trained in context B. During extinction, the animal received an extinction treatment to a stimulus in the opposite context in which it was originally trained. In the present example, T1 was presented with F1 in context B and T2 was presented with F2 in context A. Previous research has shown that rats are capable of such a reversal (Harris et al., 2000). By making both training contexts predictive of the US, the animal should instead rely on F1 and F2 to disambiguate the target stimuli. An additional change from Experiment 3 was that training for each stimulus or stimulus set occurred in blocks within a single context. For instance, instead of interspersing T1 and T2 or F1T1 and F2T2 trials within a single context, rats received a block of T1 or F2T2 trials in A and a block of T2 or F1T1 trials in B.

The test phases, groups, and purpose for each group remained the same as in Experiment 3. Group T was intended to show transfer of simultaneous occasion setters, Group NT was intended to demonstrate a lack of transfer without an appropriate training history, Group OSC provided a control group to show adequate extinction and modulatory control of the occasion setters, and Group FC provided a control group to demonstrate the

CR to T1 and T2 when no occasion setter was present at test. The hypotheses from

Experiment 3 remained unchanged; when a simultaneously presented feature negative

occasion setter is presented with a transfer target CS, the feature would only be able to

modulate responding to the transfer target if it has undergone a feature negative occasion

setting training history.

Subjects. This study utilized a sample of 32 experimentally naïve Long-Evans

hooded rats as previously described ($n = 8$ subjects per group).

Apparatus. The apparatus was the same as in Experiment 1, with one exception.

Since rats received two experimental sessions per day in different contexts, using physical

context 1 as context A was unfeasible due to lingering odors caused by the odor cue. For this

reason, physical contexts 2 and 3 served as context A and B, counterbalanced, and physical

context 1 served as context C. Additionally, contexts A and B needed to be counterbalanced

to ensure that one context didn't affect responding more than the other since acquisition and

extinction occurred in both of these contexts.

Procedure. Prior to conditioning in Phase 1, the pretraining procedures previously

described established lever pressing behavior for a water reinforcer. Subjects received one

day of shaping in context A, two days of CRF training in context B then context C, two days

of progressive FR training in context A then context C, and four days of VIVR training in

context A, then context B, then context C, and then context B. Throughout the entirety of

training and testing, subjects maintained established lever pressing behavior on a tandem

VI10 sVR5 schedule of reinforcement.

Subjects were randomly assigned rats to one of four conditions counterbalanced for

sex within groups. The four conditions were Transfer (T), Occasion Setting Control (OSC),

No Transfer (NT), and Renewal Control (FC). Counterbalancing was the same as in

Experiment 3.

   In Phase 1 all subjects received pairings of T1 or T2 and the US in context A and the

other target (T1 or T2) was paired with the US in context B, counterbalanced within group.

Subjects received conditioning in both contexts within a single day, over two days, for a total

of four total conditioning sessions, counterbalanced for order within group. Daily sessions

were separated by an hour break during which the subjects were placed back into their home

cages. During each session, subjects received three pairings of either the T1 or T2 stimulus

and the US for a total of six trials for each stimulus and 12 trials overall.

   In Phase 2, Groups T, OCS, and FC received eight presentations of F1T1 or F2T2 in

one of the training contexts (A or B) that was not the context in which the target was

originally trained and the received eight presentations of the other stimulus compound (F1T1

or F2T2) in the other training context. For instance, if a subject received conditioning to T1

in A and T2 in B, that rat would receive eight presentations of F1T1 in B and eight

presentations of F2T2 in A. Group NT followed the same procedure as the other three

groups but were presented the F1T1 compound and the T2 alone stimuli. Subjects received

two extinction training sessions per day separated by a one hour break during which they

were returned to their home cages over six days. In total each stimulus arrangement was

presented 48 times and each subject received a total of 96 extinction trials. The extinction

procedure was concluded after the mean suppression ratio on the first extinction trial reached

a mean of .4 on two consecutive days.

   After Phase 2, testing occurred in separate sessions across two days; all testing

occurred in context C. All subjects received two test presentations of a single stimulus

arrangement within a single session. Groups T and NT received two presentations of the

F2T1 or F1T2 compounds within a single session, counterbalanced for test order. Group

OSC received two presentations of the F1T1 or F2T2 compounds in a single session,

counterbalanced for test order. Group FC received two presentations of stimuli T1 or T2 in a

single session, counterbalanced for test order.

Results and Discussion

Results of this experiment are summarized in Figures 13-17. Figure 13 depicts

acquisition of the T1 stimulus and Figure 14 depicts acquisition of the T2 stimulus.   By the

end of Phase 1, all subjects had acquired a conditioned fear response to both stimuli. Figures

15 and 16 depict the extinction treatments from Phase 2 for F1T1 and F2T2/T2 respectively.

Over the course of six days, all rats reached asymptotic responding just under the established

.4 threshold and proceeded to test

The mean suppression ratios from the two days of testing are combined into Figure

17. Figure 17 represents the first presentation of each stimulus separated by group. Just as in

Experiment 3, the data for each group was organized and analyzed according to the target

stimulus they received. A One-Way ANOVA was conducted with group as the single factor

on the suppression ratios obtained during testing. There was a significant effect of group, F

$(3, 28) = 5.19$, $p = .006$, $\omega^2 = .261$, on the T2 suppression ratios. Tukey's HSD post hoc tests

revealed, $p < .05$, group EC ($M = .21$, $SD = .20$), 95% CI [.05, .38], expressed significantly

less fear than groups NT ($M = .05$, $SD = .08$), 95% CI [-.02, .11], and FC ($M = .003$, $SD =$

.008), 95% CI [-.004, .01]. Group T ($M = .06$, $SD = .07$), 95% CI [.002, .12], did not differ

significantly from any other groups. There was not a significant effect of group, $F (3, 28) =$

.554, $p = .650$, $\omega^2 = 0$, on the T1 stimulus presentation.

The hypothesis that features will only transfer to targets that have undergone occasion setting training remains unsupported in Experiment 4. Group T was not different from any other group resulting in a failure to reject the null hypothesis. As with Jacobs et al. (2014), the feature was unable to transfer to another CS regardless of whether the other CS had undergone a similar procedure. The results of both studies could be explained through generalization decrement. Generalization decrement of the CR occurs when a stimulus or stimulus arrangement is sufficiently different compared to when it was trained. Generalization decrement is greater when the stimulus or stimulus arrangement is more different than the original training. In the case of the fear control group (or element groups that received the target alone at test in Jacobs et al.) the testing stimulus is more different from the extinction arrangement than it is from conditioning arrangement. In other words T1 alone at test is more similar to the T1 alone conditioning than it is to the F1T1 training received during extinction. As a result, the animal acts on the memory from conditioning rather than the memory from extinction. The same sort of pattern can be applied to the transfer groups in both Experiment 2 of Jacobs et al. and the current experiment. When a feature was presented with a target with which it has not been trained, perhaps the arrangement was more similar to the original conditioning memory than it was to the extinction memory. The subject is not in an extinction context, and the pairing is novel. This explanation gains strength when the modality of the stimuli is considered. In the current experiment, the features were a click and flashing light stimulus. Supposing F1 was a light and F2 was a clicking sound, when F2 is presented with T1, not only is the animal receiving a different stimulus arrangement, but that stimulus arrangement is activating different sensory systems. Perhaps this mismatch in the modality resulted in greater generalization

decrement than if the modalities had matched. Further research is needed to investigate this explanation.

General Discussion

The current series of experiments tested the assumption that occasion setters only form when stimuli are presented serially. Previous research done by Jacobs et al. (2014) provided some preliminary support for the idea that simultaneously presented cues may also form occasion setters. The procedures of the current series of experiments were intended to demonstrate two unique properties of occasion setters, they can hold associations outside of a direct association with the US, and have unique summation properties. Experiment 2 tested the former while Experiments 3 and 4 tested the latter. Experiment 1 served as a pilot study to hone the procedures used in Experiment 2.

Experiment 1 tested the hypothesis that the reinstatement effect could be mitigated by testing in a context other than the reexposure context. Results from this experiment did not provide support for the hypothesis. Regardless of testing context, all groups showed fear to the originally trained CS. Despite the lack of support for the hypothesis, Experiment 1 did provide some valuable information which improved the procedures used in Experiment 2. Future research will be aimed at assessing why this experiment failed to replicate findings of previous research (e.g. Bouton & Bolles, 1979; Bouton & King, 1983; Westbrook et al., 2002). It is possible that preexposure to the stimuli and contexts used in the current experiment may have been responsible for the discrepant findings.

Experiment 2 was intended to test the hypothesis that the simultaneous occasion setter should be able to modulate responding outside of its direct association with the US. Unfortunately, the procedures of Experiment 2 did not address this hypothesis. Previous

research was able to isolate the responding to the target stimulus in a serial procedure and the

data gathered only incorporated responses to the target stimulus and not the feature (Holland,

1984). It is very possible, and likely, that the subjects in that study would express fear to the

feature and not to the target. Likewise it is possible in the current experiment that the

subjects were expressing fear to the feature and no fear to the target, but since the two stimuli

were presented simultaneously it was impossible to isolate responding to the target.

Consequently, the results of Experiment 2 did not provide support for the hypothesis;

however, it may still be possible that simultaneous occasion setters possess multiple

associations in relation to the US. Future research will need to devise a procedure that still

presents the stimuli simultaneously but is able to isolate which stimulus the subject is

expressing fear towards.

Experiment 3 tested the hypothesis that simultaneous occasion setters will only

transfer to other targets that have undergone a similar occasion setting training history. The

data from this experiment indicated that the context was modulating behavior and not the

features. In previous literature investigating the transfer of occasion setters, experiments

were only performed in one context, so there was no possibility of context overshadowing

the discrete cues for control of behavior (Holland & Lamarre, 1985; Lamarre & Holland,

1987). This result is contradictory to a similar experiment run by Jacobs et al. (2014) in

which the modulatory power of the context was overshadowed by an extinction cue. A key

difference from that experiment was that all targets in the current experiment underwent

extinction. It seems that by extinguishing all the targets in a single context, the more

parsimonious cue to disambiguate the meaning of a target was the context and thus the

transfer properties of the features were not apparent. Experiment 4 was devised to correct for this finding.

Experiment 4 tested the same hypothesis as Experiment 3; however, changes to the procedure involved using both contexts for acquisition and extinction. Results from this experiment indicated that the change in procedure was effective at reducing the ability of context to overshadow the features for control of behavior. Results from this experiment indicated that the features were still not able to transfer to other stimuli with a similar training history as in Holland and Lamarre (1985) and Lamarre and Holland (1987). The hypothesis remained unsupported in Experiment 4. Future research should devise procedures which limit the amount of generalization decrement that is possible by presenting all stimuli as the same sensory modality.

In conclusion, the idea that simultaneous occasion setters are possible did not receive any support from the current series of experiments. Another possibility for the identity of this extinction cue investigated in the current series of experiments and in Jacobs et al. (2014) is that the extinction cue may have only been acting as a weak or partial inhibitor. In the Rescorla-Wagner model, cues are thought to have a value that is bound between -1 and 1 (Rescorla & Wagner, 1972). Positive values are acquired when the animal is not yet fully expecting the US (indicated by a value of 1) upon being presented with a CS. The CS is then followed by the US until the animal fully expects the US to follow the CS. Negative values are acquired when the animal is expecting the US to follow a CS, but another stimulus is also present and the animal does not receive the US. This other stimulus gains negative, or inhibitory, value and the CS should concurrently lose some of its excitatory value. When the

sum of the two stimuli is 0, then no more learning occurs and the values of the stimuli have reached asymptote.

In theory, using an interspersed procedure, rather than a phasic procedure, would produce very different inhibitory values for an extinction cue (Rescorla & Wagner, 1972). During an interspersed procedure, like those used in Holland and Lamarre (1985), and Lamarre and Holland (1987), a simultaneous extinction cue would reach a value of -1 (inhibitory) since both the CS and the extinction cue start out at a value of 0 (neutral). For every nonreinforced trial which brings the value of the CS closer to 0, a reinforced trial regains the lost value and adds some. Meanwhile the extinction cue is only present on nonreinforced trials and so it only loses value until it reaches -1. The value of -1 from the extinction cue counteracts the value of 1 from the CS to inhibit the CR. Thus when that extinction cue is paired with any other cue, a transfer CS, it should counteract the excitatory value of that transfer CS to also sum to 0. In contrast, during a phasic procedure, first the CS reaches a value of 1 (excitatory) before being paired with an extinction cue. When paired with an extinction cue, which starts off neutral at 0, during extinction, the value of the CS should fall to .5 due to the nonreinforced presentations and no reinforced presentations to recover the lost value, and the extinction cue would likewise fall to a value of -.5. Once the extinction cue has a value of -.5 and the CS a value of .5, the sum of the two will equal 0 and no more learning will occur for the extinction cue. Thus, when paired with a full strength exciter, such as in the current series of experiments or in Jacobs et al. (2014), the extinction cue does not have sufficient inhibitory power to counteract the strength a fully conditioned transfer CS. Future research involving pairings with partially reinforced or relatively weak targets may provide useful data exploring this possibility. Further research is needed to

better define the mechanism and properties of the cue, but for now continued research into its

occasion setting properties lacks merit.

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Table 1

Experiment 1 Design Table

Group	Phase 1	Phase 2	Phase 3	Reshaping	Test
URS	T1+ in A	T1- in A	+ in A	BP in A	T1 in A
URD	T1+ in A	T1- in A	+ in A	BP in A	T1 in B
RB-D	T1+ in A	T1- in A	+ in B	BP in A	T1 in A
RB-S	T1+ in A	T1- in A	+ in B	BP in A	T1 in B
SRS	T1+ in A	T1- in A	T2+ in A	BP in A	T1 in A
SRD	T1+ in A	T1- in A	T2+ in A	BP in A	T1 in B

Note. The groups are, in order, Unsignaled Reinstatement Same, Unsignaled Reinstatement Different, Extinction Control, AAB Renewal, Signaled Reinstatement Same, and Signaled Reinstatement Different. Target 1 (T1) is a tone and Target 2 (T2) is a white noise. BP is representative of bar pressing procedure, absent of any stimuli. A "+" indicates a reinforced trial and a "-" indicates a nonreinforced trial. A and B represent the context.

Table 2

Experiment 2 Design Table

Group	Phase 1	Phase 2	Phase 3	Reshaping	Test 1	Test 2
F1-R	T1+ in A	F1T1- in B	F1+ in B	BP in B	F1T1 in B	F1T1 in C
F2-R	T1+ in A	F1T1- in B	F2+ in B	BP in B	F1T1 in B	F1T1 in C
SUM	T1+ in A	F1T1- in B	F2+ in B	BP in B	F2T1 in B	F2T1 in C
USR	T1+ in A	F1T1- in B	+ in B	BP in B	F1T1 in B	F1T1 in C

Note. The groups are, in order, Feature 1 - Reinforced, Feature 2 - Reinforced, Summation, and Unsignaled Reinstatement. A or B indicates what context will be used. Feature 1 (F1) and Feature 2 (F2) are a click and white noise, counterbalanced. Target 1 (T1) is a tone. BP is representative of bar pressing procedure, absent of any stimuli. A "+" indicates a reinforced trial and a "-" indicates a nonreinforced trial.

Table 3

Experiment 3 Design Table

Group	Phase 1	Phase 2	Test 1	Test 2
T	T1+/T2+ in A	F1T1-/F2T2- in B	F2T1/F1T2 in C	F1T2/F2T1 in C
OSC	T1+/T2+ in A	F1T1-/F2T2- in B	F1T1/F2T2 in C	F2T2/F1T1 in C
NT	T1+/T2+ in A	F1T1-/T2- in B	F2T1/F1T2 in C	F1T2/F2T1 in C
FC	T1+/T2+ in A	F1T1-/F2T2- in B	T1/T2 in C	T2/T1 in C

Note. The groups are, in order, Transfer, Occasion Setting Control, No Transfer, and Renewal Control. A or B indicates what context will be used. Feature 1 and Feature 2 are a click and white noise, counterbalanced. Target 1 and Target 2 are a tone and a pulsing tone, counterbalanced. A "+" indicates a reinforced trial and a "–" indicates a nonreinforced trial. A "/" indicates the trials are interspersed within a single session.

Table 4

Experiment 4 Design Table

Group	Phase 1	Phase 2	Test 1	Test 2
T	T1+ in A/	F1T1- in B/	F2T1/F1T2 in C	F1T2/F2T1 in C
	T2+ in B	F2T2- in A		
OSC	T1+ in A/	F1T1- in B/	F1T1/F2T2 in C	F2T2/F1T1 in C
	T2+ in B	F2T2- in A		
NT	T1+ in A/	F1T1- in B/	F2T1/F1T2 in C	F1T2/F2T1 in C
	T2+ in B	T2- in A		
FC	T1+ in A/	F1T1- in B/	T1/T2 in C	T2/T1 in C
	T2+ in B	F2T2- in A		

Note. The groups are, in order, Transfer, Occasion Setting Control, No Transfer, and Renewal Control. A or B indicates what context will be used. Feature 1 and Feature 2 are a click and white noise, counterbalanced. Target 1 and Target 2 are a tone and a pulsing tone, counterbalanced. A "+" indicates a reinforced trial and a "–" indicates a nonreinforced trial. A "/" indicates the trials are interspersed within a single session.
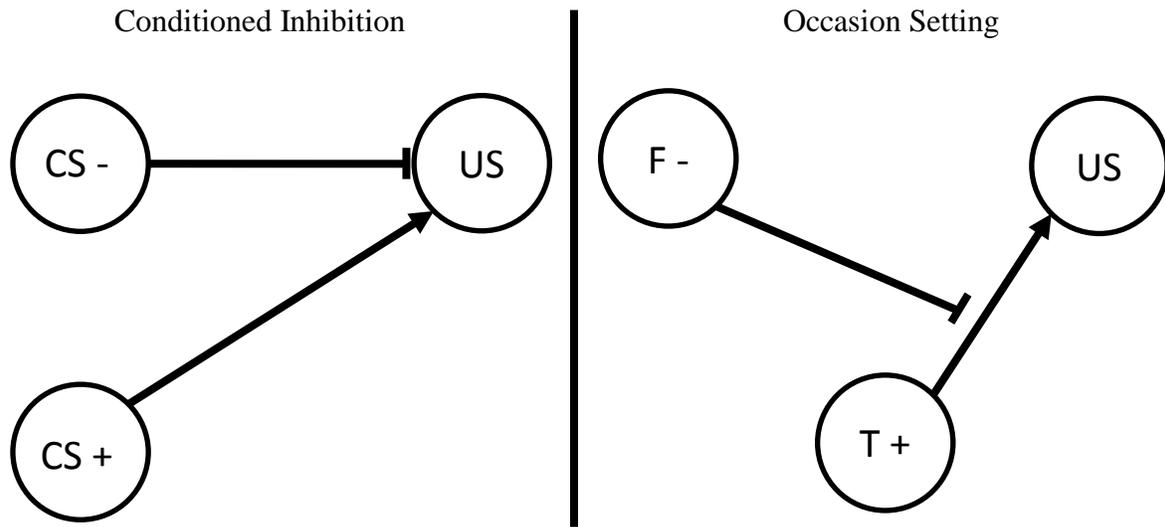
Figure 1. Diagram of the memory nodes active during a conditioned inhibition trial and an occasion setting trial. "CS+" denotes an excitatory conditioned stimulus, "CS-" denotes an inhibitory conditioned stimulus, "F-" denotes a negative feature, "T+" denotes an excitatory target, and "US" denotes the unconditioned stimulus. An arrow indicates an excitatory association and a capped line indicates an inhibitory association. On the left side of the figure, CS+ has an excitatory association with the US and CS- has an inhibitory association with the US. On the right side of the figure, T+ has an excitatory association with the US and F- has an inhibitory association with the excitatory association between T+ and the US. Traditionally, a simultaneous arrangement results in a conditioned inhibitor and a serial arrangement results in an occasion setter.
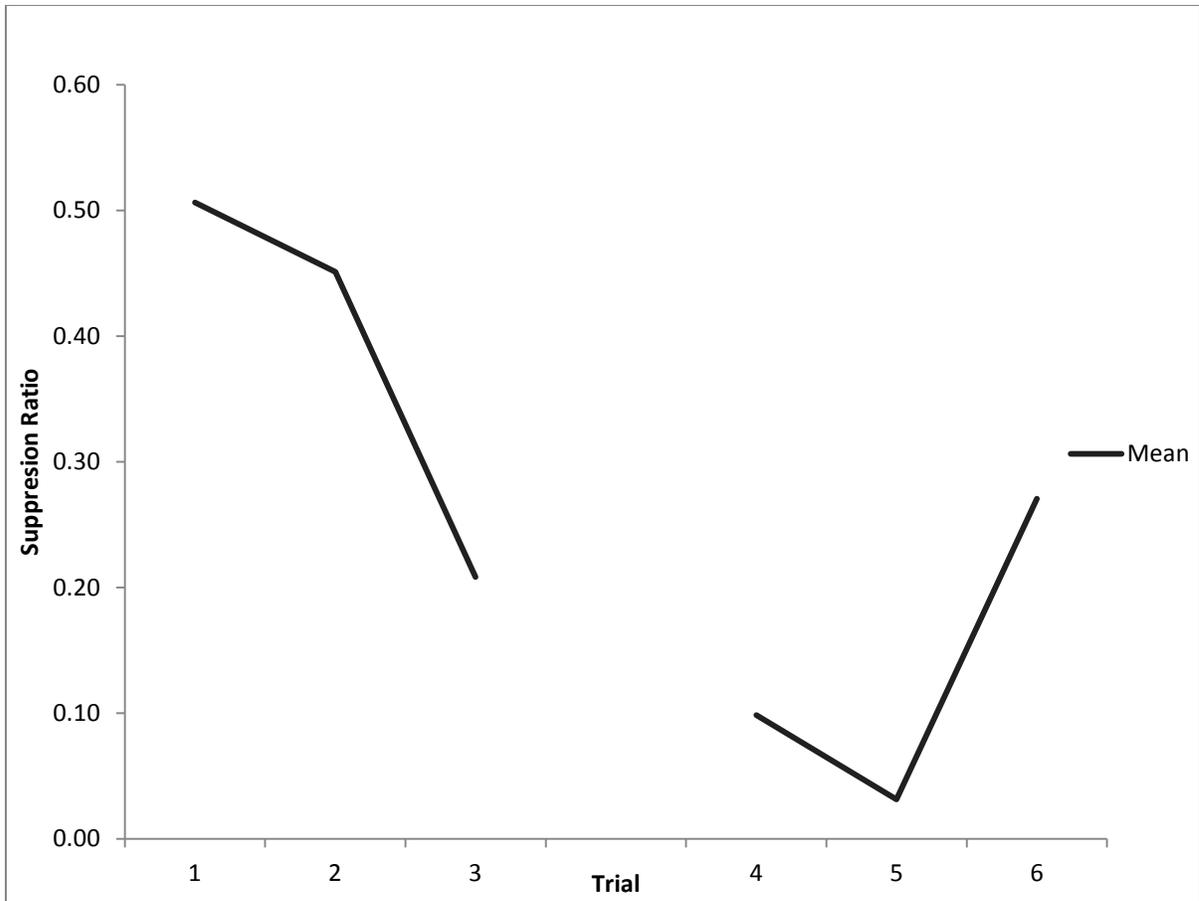
Figure 2. Acquisition graph for Experiment 1. Subjects received three presentations to T1 over the course of two days.
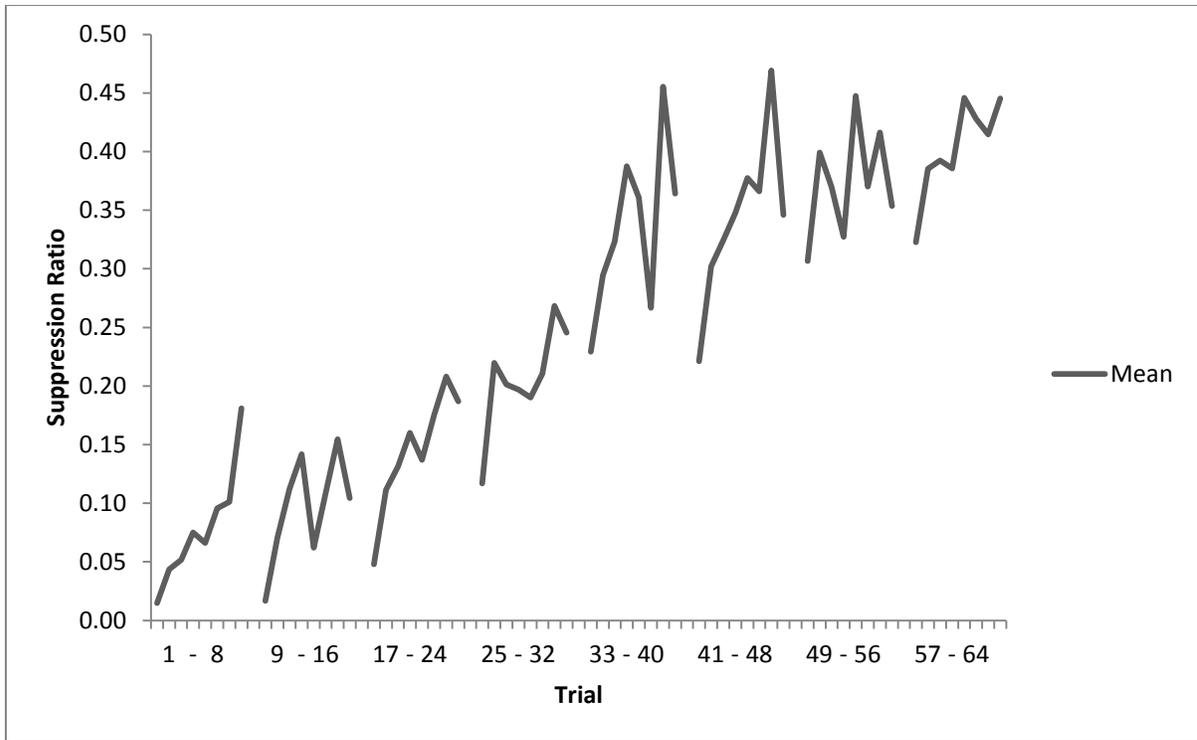
Figure 3. Extinction graph for Experiment 1. Subjects received eight presentations of T1

over the course of eight days.
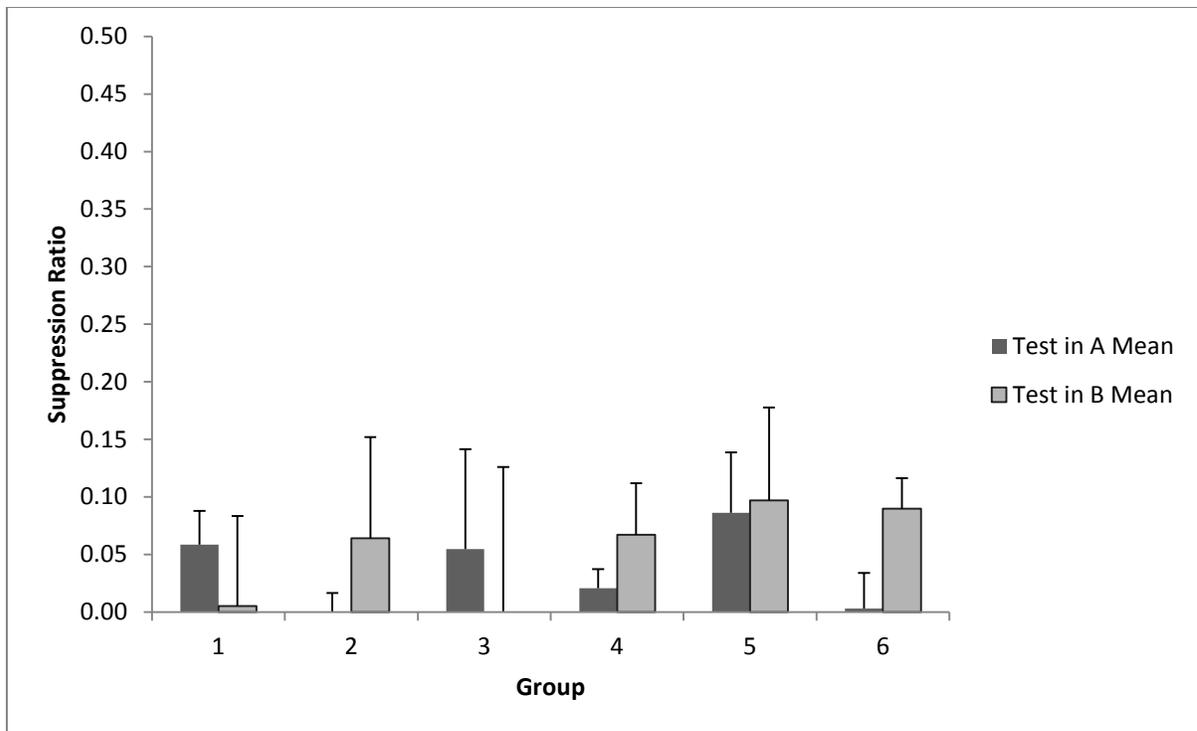
Figure 4. Suppression ratios for the first presentation of T1 in contexts A and B for each

group. Subjects in the "same" conditions received their first exposure to T1 in the

reexposure contexts. Subjects in the "different" conditions received their first exposure to T1

in a different context than where reexposure occurred. All rats eventually received exposure

to T1 in both contexts.
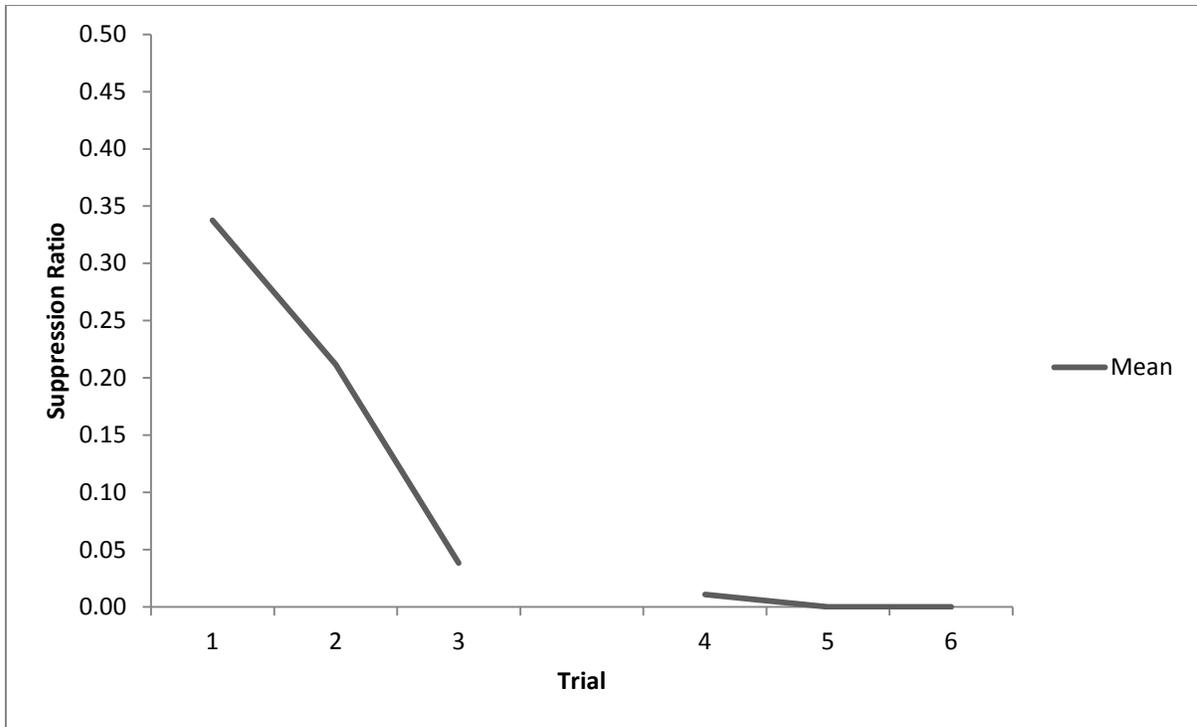
Figure 5. Acquisition in Experiment 2. Subjects received three exposures to T1 in each of

two daily sessions.
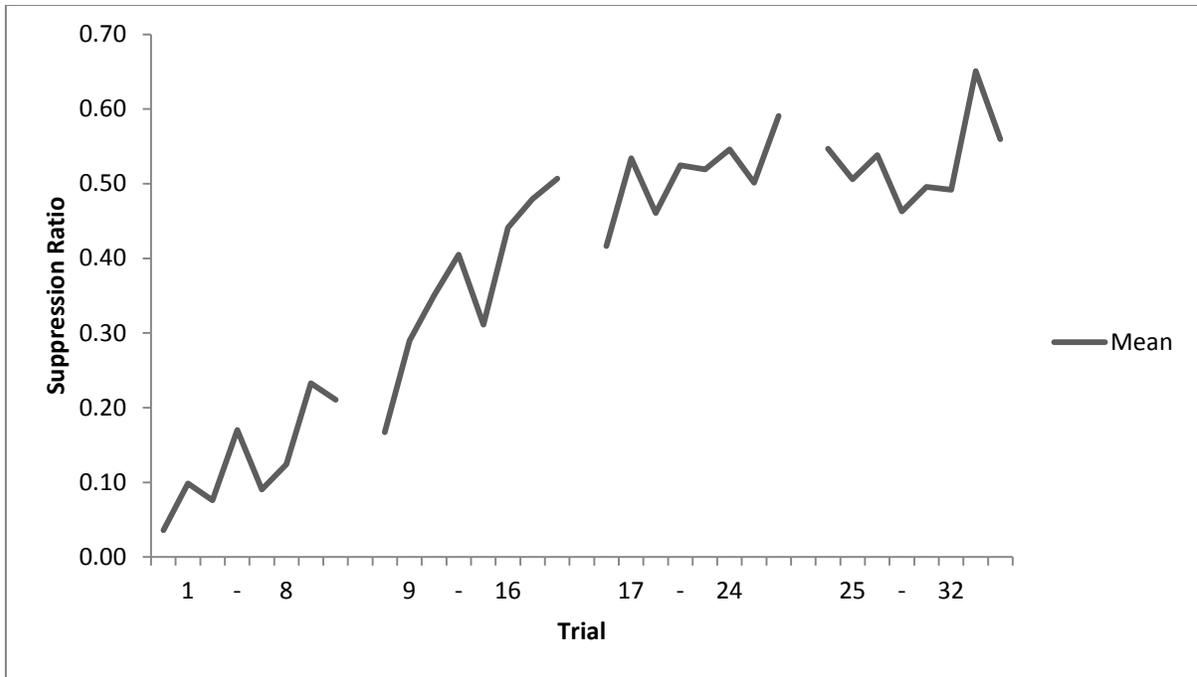
Figure 6. Extinction in Experiment 2. Rats received eight presentations of F1T1 in each of

four daily sessions.
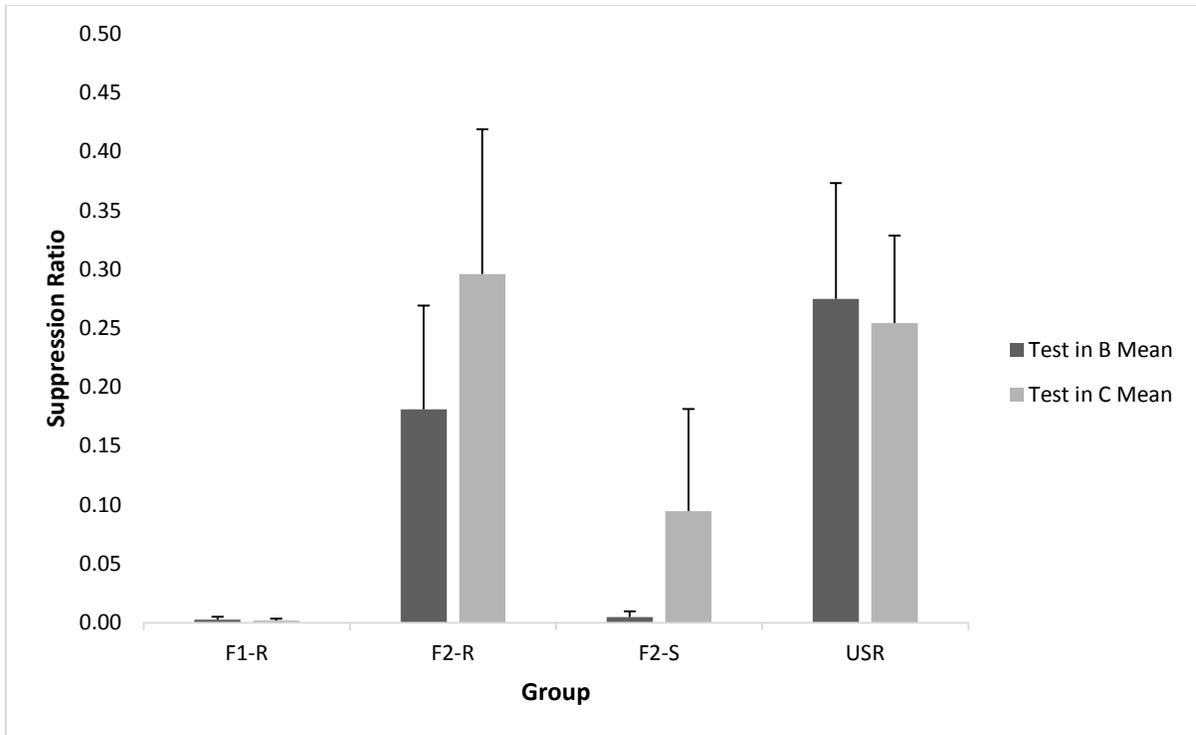
Figure 7. Days 1 and 2 of testing in Experiment 2. All rats first received testing in Context B

and then Context C. This graph depicts the first presentation of F1T1 (or F2T1 in group F2-

S) on each day. Testing sessions consisted of six presentations during each daily session.
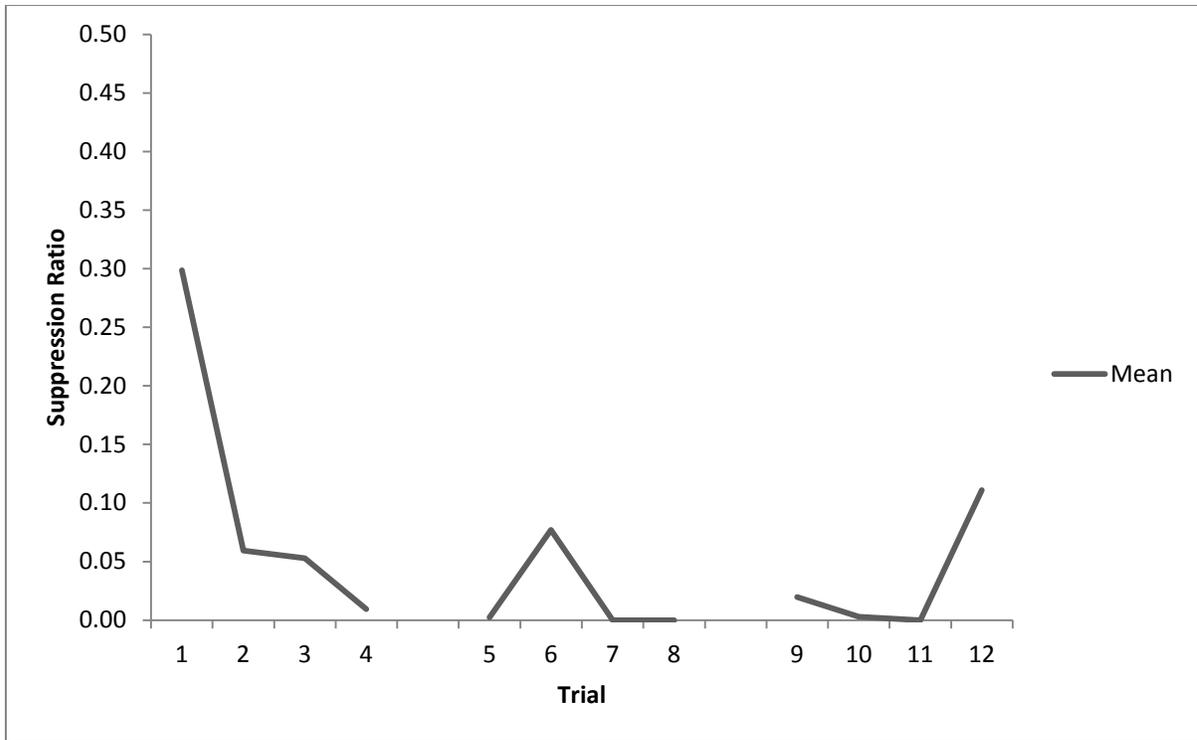
Figure 8. Acquisition in Experiment 3. Stimuli T1 and T2 are combined into the same graph.

Subjects received six total presentations to T1 and six total presentations to T2 across three

daily sessions. The order of the presentations was randomized each day.
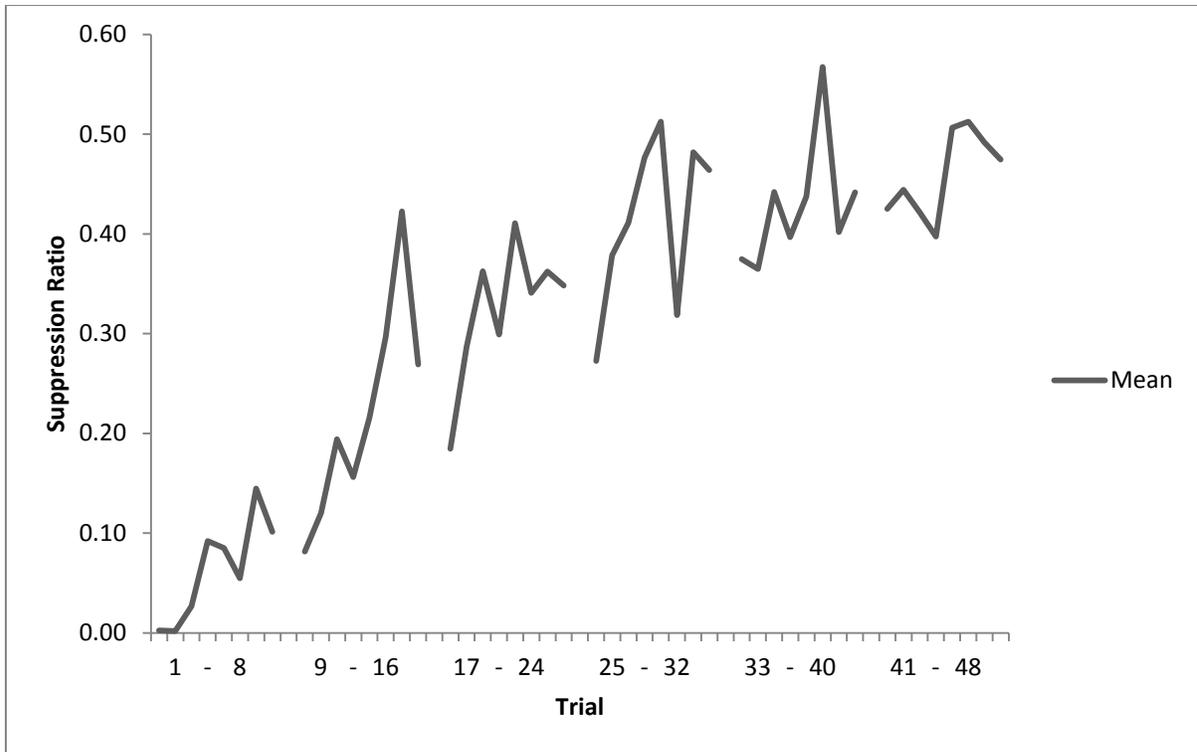
Figure 9. Extinction in Experiment 3. Stimuli F1T1 and F2T2/T2 are combined into the

same graph. Subjects received eight daily sessions in which each stimulus arrangement was

presented four times. There were two possible orders for stimuli which were

counterbalanced across days.
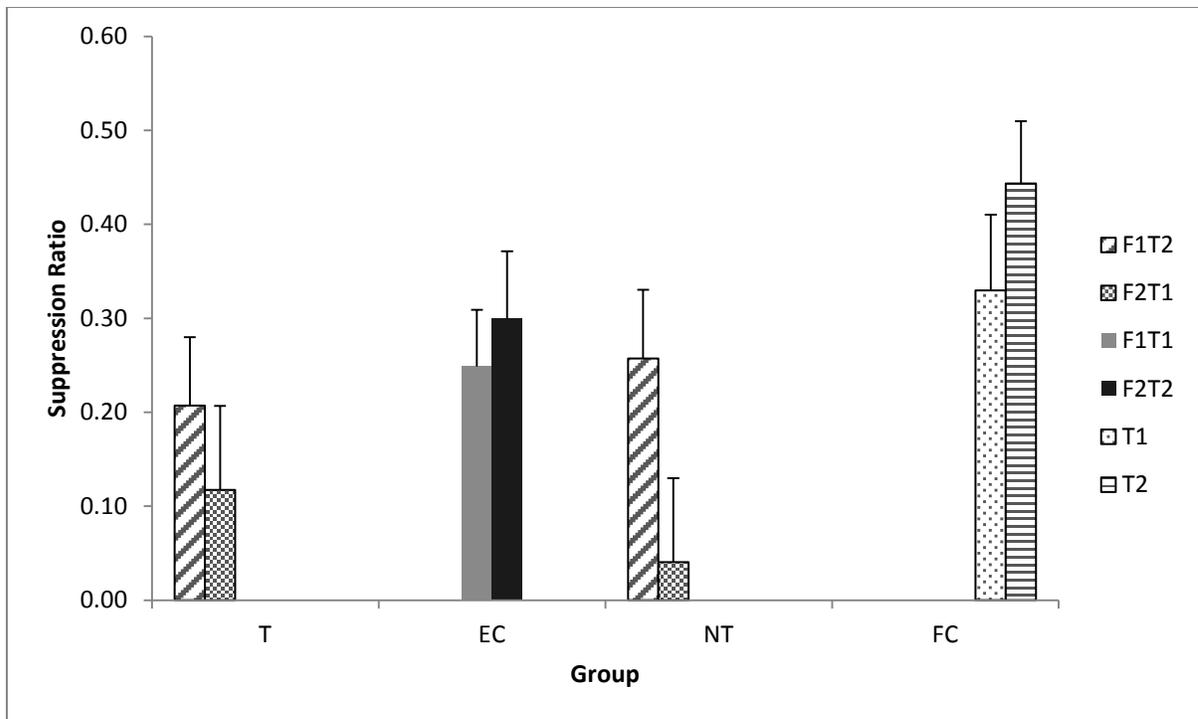
Figure 10. This graph depicts the first test trial of each stimulus on Day 1 for each group in context C for Experiment 3. The order of stimuli was counterbalanced within groups and there was no interaction of test order. Each subject received two presentations of their assigned stimuli per day across two days.
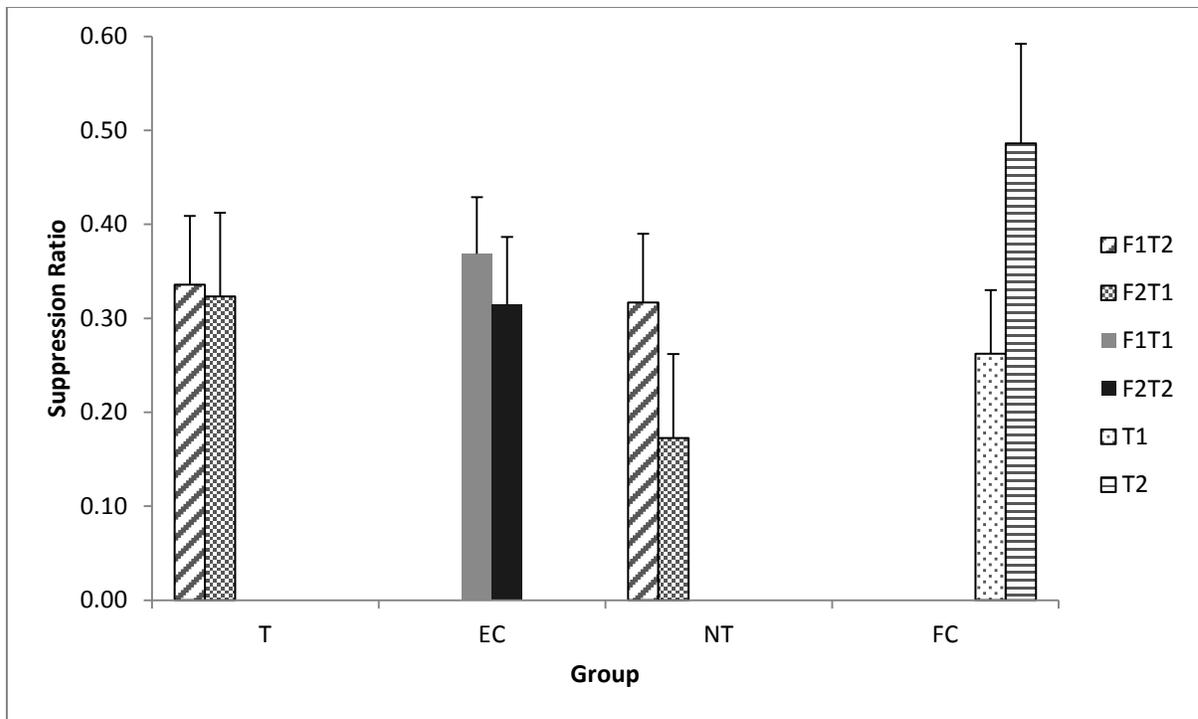
Figure 11. This graph depicts the first test trial of each stimulus on Day 2 for each group in context C for Experiment 3. The order of stimuli was counterbalanced within groups and there was no interaction of test order. Each subject received two presentations of their assigned stimuli per day across two days.
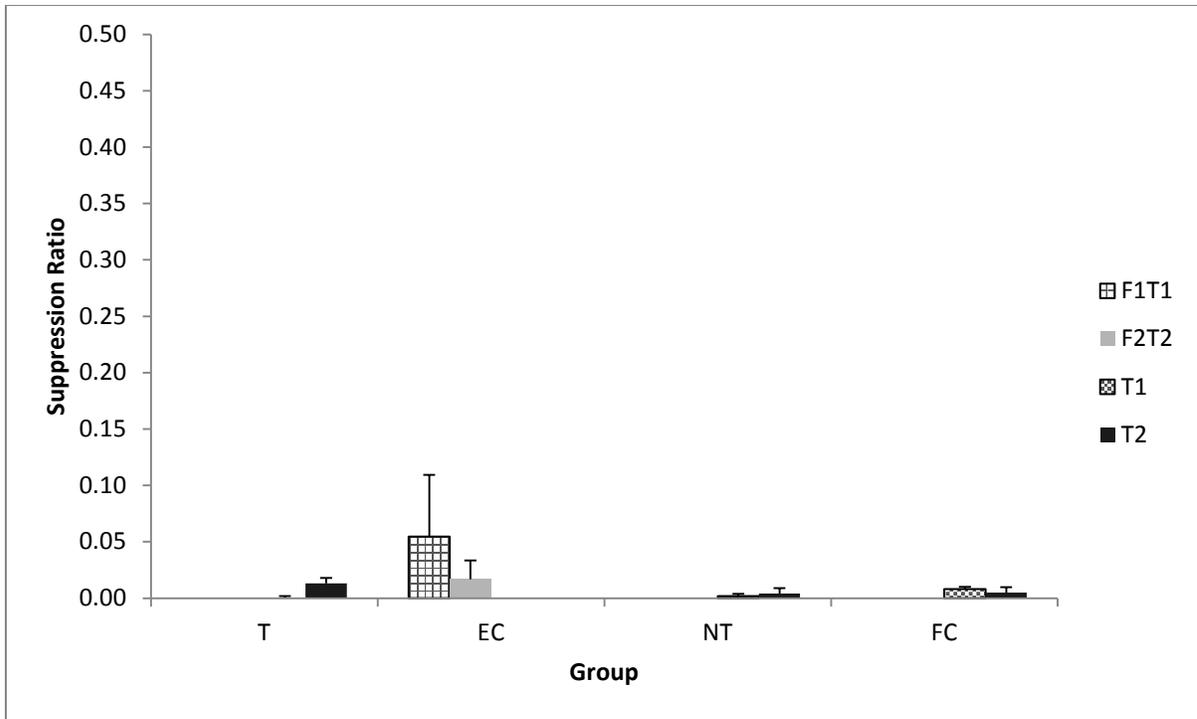
Figure 12. This graph details the exploratory test performed after the study proper had concluded in Experiment 3. Testing occurred in context A. This graph depicts the first trial of each stimulus. Groups T, NT, and FC received T1 and T2 while EC received F1T1 and F2T2.
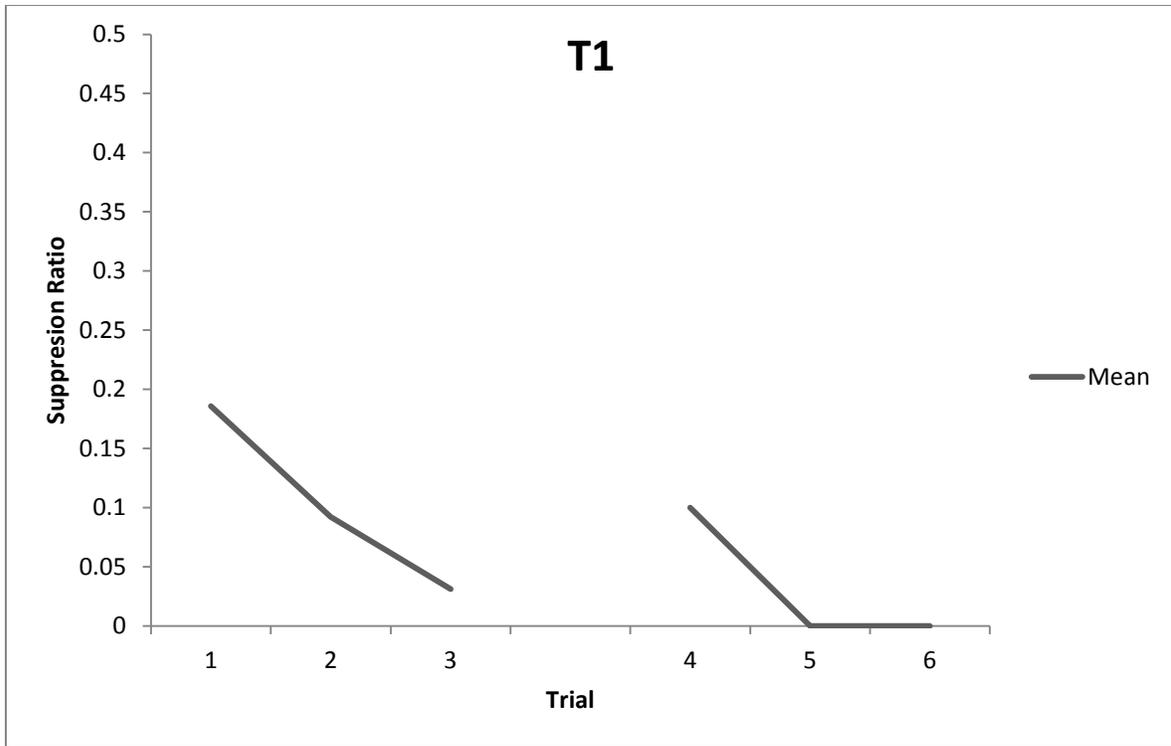
Figure 13. Acquisition to the T1 stimulus in Experiment 4. Each block of three trials contained the same stimulus before shifting contexts to the other context (A or B) where T2 was presented.
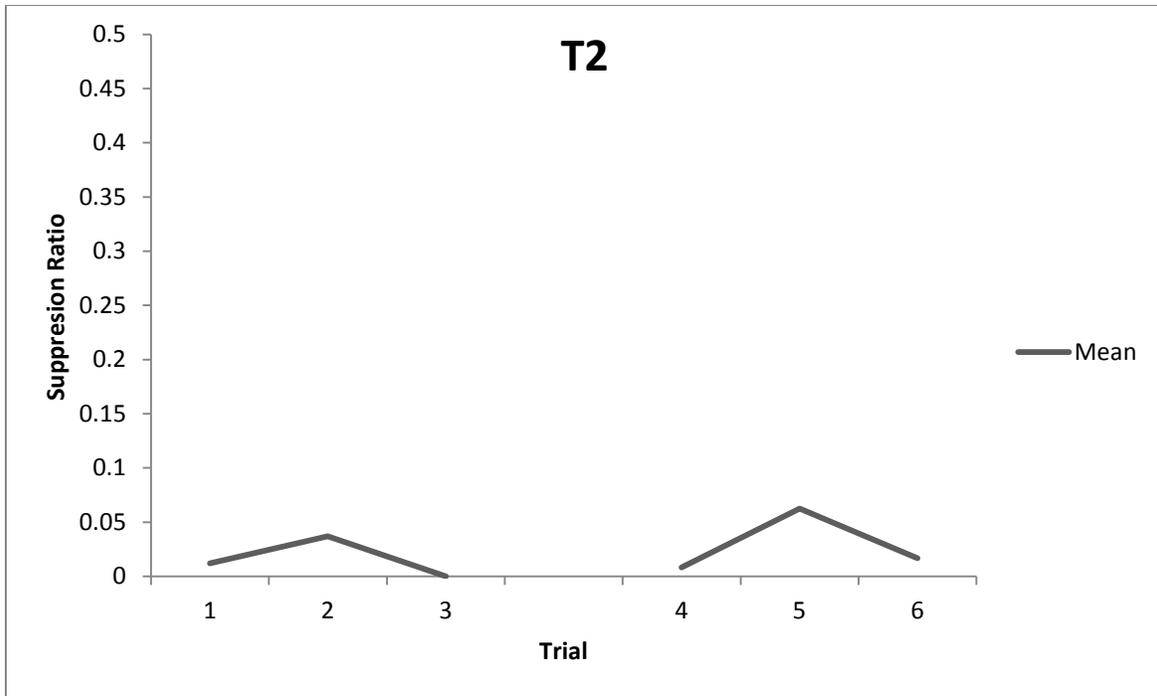
Figure 14. Acquisition for stimulus T2 in Experiment 4. Each block of three trials contained

the same stimulus before shifting contexts to the other context (A or B) where T1 was
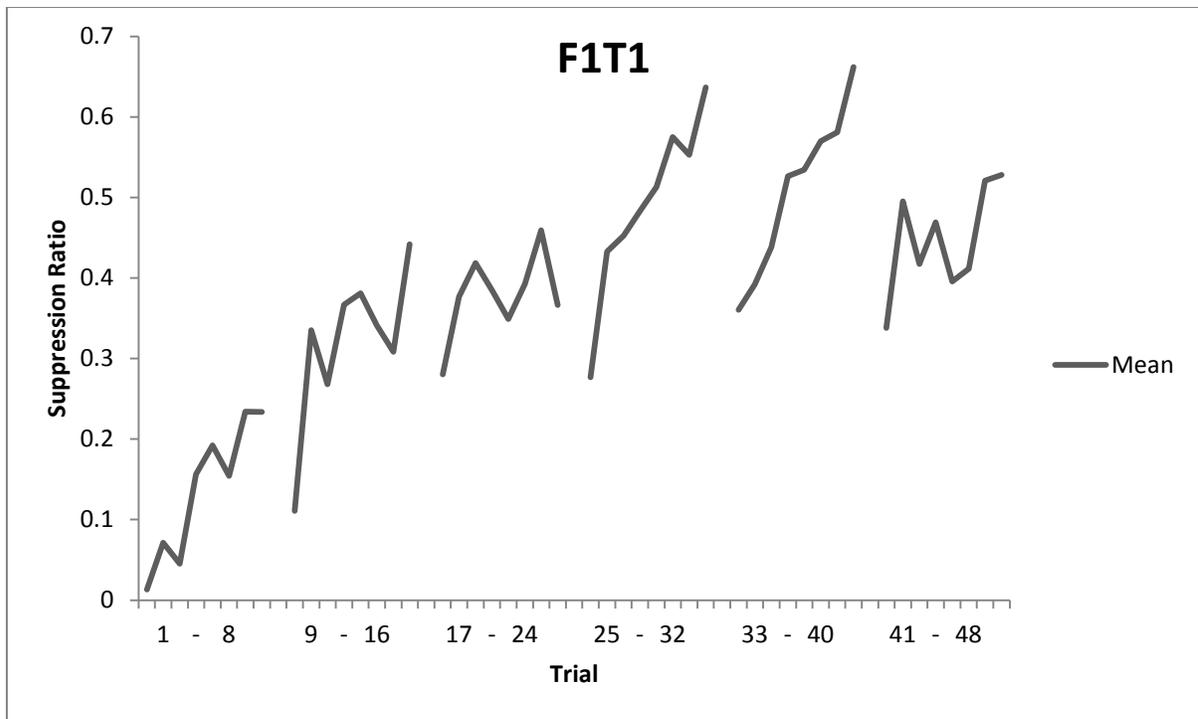
presented.

Figure 15. Extinction to the F1T1 compound in Experiment 4. Extinction to F1T1 occurred

in the context that was not the conditioning context for T1. Data from both contexts are

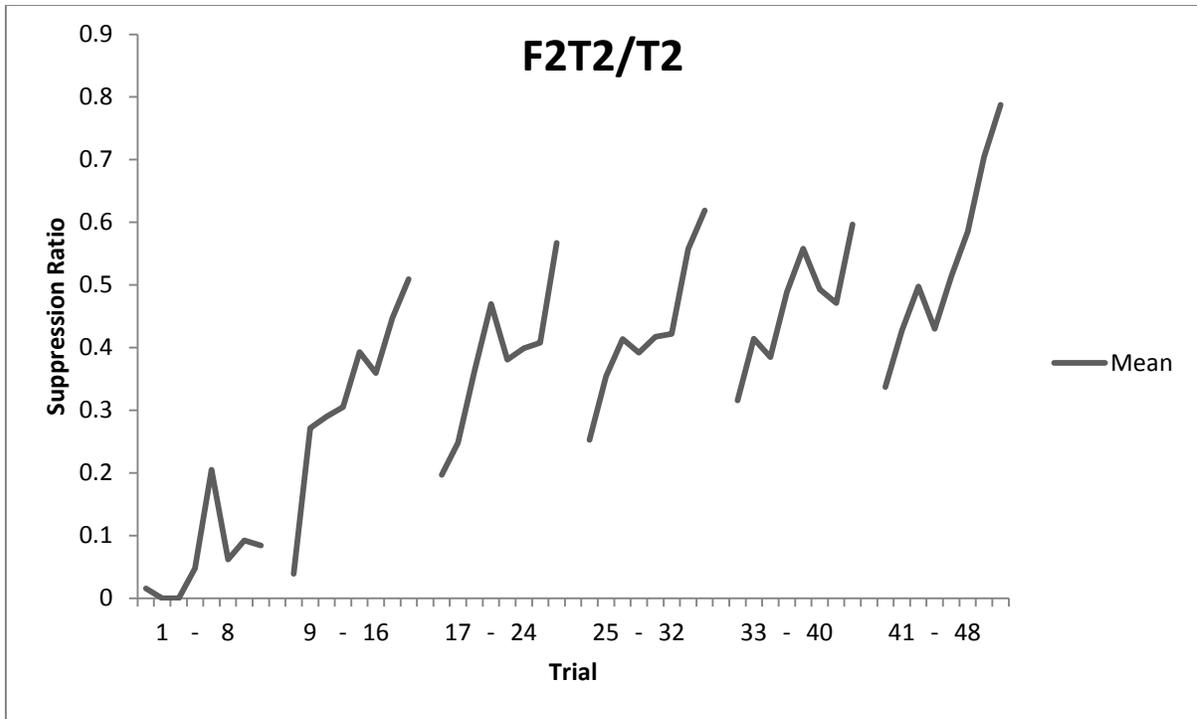combined in this graph. Each subject received 48 total trials of the F1T1 stimulus.
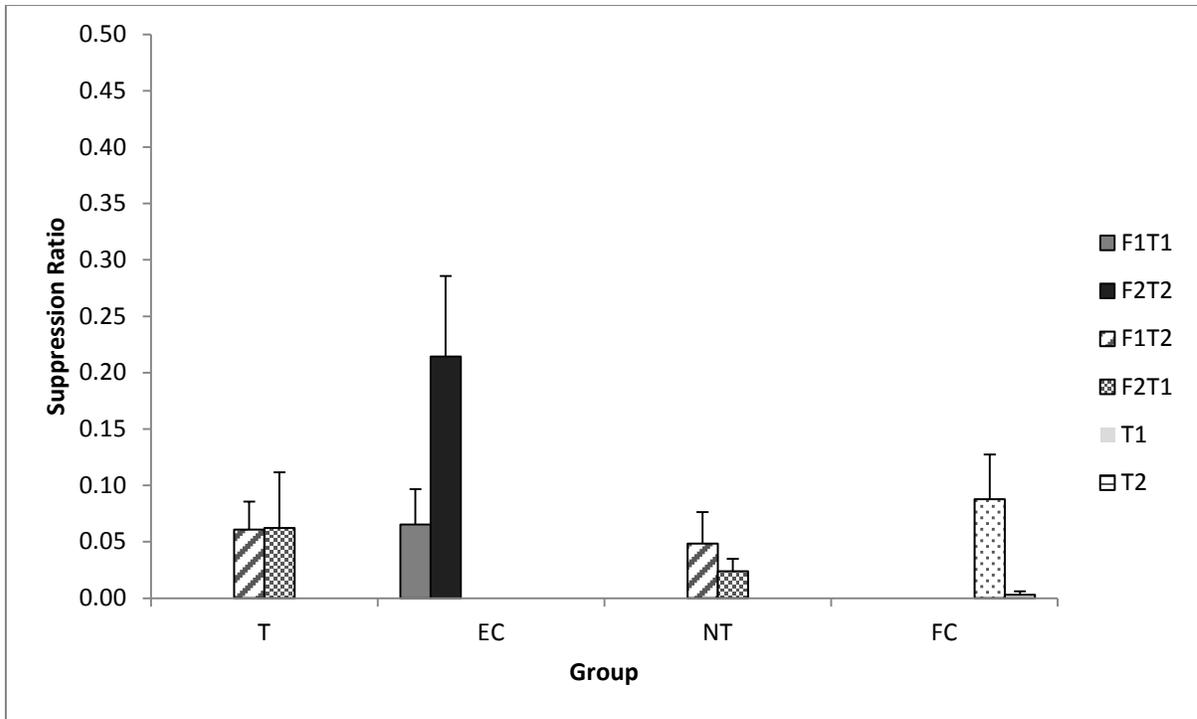
Figure 16. Extinction to the F2T2 stimulus compound in Experiment 4. Extinction to F2T2 occurred in the context that was not the conditioning context for T2. Data from both contexts are combined in this graph. Each subject received 48 total trials of the F2T2 stimulus.

Figure 17. This graph combines ratio 1 for each test stimulus for each group across the two days of testing in Context C for Experiment 4. There was no interaction of test order on the result, so the data was combined.

TO: Dr. Jim Denniston
Professor and Chair of Psychology

FROM: Dr. Ted Zerucha, Chair

Institutional Animal Care and Use Committee

DATE: January 28, 2015

SUBJECT: Institutional Animal Care and Use Committee

Request for Animal Subjects Research

REFERENCE: Transfer Properties of Discrete and Contextual Setters

IACUC Reference #15-07

Initial Approval Date – January 28, 2015

End of Approval Period – January 27, 2017

The above referenced protocol has been approved by the IACUC for a period of two years.

Best wishes with your research.

TZ/rst

Vita

       Ian Randolph Jacobs was born in Norfolk, V.A. to Mary and Fred Jacobs. He graduated from Appalachian State University with a B.S. in Psychology in 2013. The following fall he was accepted at Appalachian State University to obtain a M.A. in Experimental Psychology. Once completed he plans on pursuing a Ph.D. in Behavioral Pharmacology.