

DO DYNAMIC STIMULI FACILITATE PERFORMANCE IN A PAVLOVIAN OCCASION  
SETTING PROCEDURE?

by

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A Thesis Proposed to the Graduate Faculty of the  
College of Science and Engineering  
Texas Christian University  
in partial fulfillment of requirements  
for the degree of

Master of Science

Fort Worth, TX  
August 2014



## ACKNOWLEDGEMENTS

To my advisor, Ken Leising, I am incredible grateful for the mentoring you have provided not only during my Thesis work but my entire graduate career. You are an excellent teacher and your patience and personality make you very approachable for any questions I may have, whether they be related to school or anything else. I would like to thank the TCU vivarium coordinator Lindy Bledsue for her excellent care of the animals used in my experiments. I would also like to thank Chad Ruprecht, my lab partner, as well as the undergraduates who helped me run the long days of experiments, especially Joe Leyva. To my parents and brother, without the example set by you I would never be the motivated and hardworking individual I am today. And most importantly to my wife, Gina, thank you for your support and putting up with my long nights at the lab and the weekends we have lost due to research. I love you very, very much!

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## **1. Introduction**

The animal kingdom is full of predators and prey that are locked in a continuous struggle for survival. For those animals with a long history of competition, genetic variation has led to the evolution of reflex mechanisms to respond stereotypically to consistent threats and learning mechanisms to respond more flexibly to new or changing threats. The influence of genetic variation and selective pressure is exemplified by the physical attributes and reflexes of a lioness and water buffalo. The lioness has evolved the biological equipment necessary for speed, strength, and group hunting techniques (e.g., coordinated stalking), whereas the water buffalo has the advantage of size, stamina, horns, and herd behavior (i.e., strength in numbers). In addition to the physical attributes of the prey, the success of the lioness depends on interspecies differences in the escape strategy of its prey (Van Orsdol, 2008). The ability of each animal to detect motion, and more importantly, act and react in response to the type and direction of motion detected in other animals underlies successful predation and evasion. Given that movement is ubiquitous in the animal kingdom, an object in motion should hold a privileged position in perception, as well as in learning and memory.

Support for the importance of object motion in animal behavior comes from both the observation of the natural behavior of animals and from experimental research on discrimination learning. The natural behavior of animals provides insight into the ecological niches in which motion detection mechanisms evolved, whereas, experimental research aims to isolate which properties of objects in motion are learned and how they come to influence behavior.

### **1.1 Motion in the natural behavior of animals**

All species within the animal kingdom are required to obtain nutrition from external sources. In most species, this has led to the evolution of motility and an arms race of sorts

between prey and predator. Though some animals move haphazardly, many animals detect and encode the positions of objects in space (including their own and that of other animals) to find food, evade predation, return home, or seek a mate. For example, lion prides hunting together will often herd their prey in the direction where more lions are waiting to ambush. If the prey is driven into the path of the waiting lions, it must react not only to the speed and direction of the stalking lion but also the speed and direction of the waiting lions. As the prey is evading the attacking lion, both predator and prey rely heavily on their ability to detect motion and respond in a way that best supports survival (Van Orsdol, 2008). The importance of motion detection in the animal kingdom can also be found in mating behavior.

Many of the mating displays and courtship rituals of animals rely on extravagant colors and displays of motion. Mate selection can be determined by how well an individual is able to react to and reciprocate complex behaviors of conspecifics (Gillard, 1952). For example, the visual displays of the gekkonid lizard, typically found in Guam and the Mariana islands, include color, posture, pattern, and movement and are used for predator threats as well as courtship displays and conspecific directed aggression (Marcellini, 1977; Sabath, 1981). Another example of the importance of motion detection is the courtship dance of the Superb Bird-of-paradise (*Lophorina superba*). After calling to nearby females, the male bird extends the feathers on his neck revealing a bright turquoise stripe display on a background of black feathers and performs an elaborate dance showcasing his genetic offering and mastery of complex movement patterns (Frith & Frith, 1987). Each of these motion effects requires the animal to possess a visual system to detect the movement of self and other animals.

The need to quickly respond to movement has led to development of reflexive object tracking, driven by the superior colliculus, which allows for some movement to be attended to



automatically in humans, non-human primates, and rodents (Anderson & Rees, 2011; Lau et al., 2011; Goodale & Milner, 1992). The visual pathways of most mammals have also branched off into two main pathways responsible for different visual tasks such as object identification (ventral visual pathway) and the perception of object spatial location and motion (dorsal visual pathway; Desimone, & Ungerleider, 1989). Motion activated processing in the dorsal pathway is critical for guiding spatial attention and aids in the process of differentiating objects in the visual field (Jackson, Shepherd, Mueller, Husain, & Jackson, 2006; Kellman, 1993). This two-pathway distinction is also present in the common laboratory rat (*Rattus norvegicus*).

Rats are vulnerable to predation from many species. As a result, rats and other prey species have developed laterally set eyes, which allow for the detection of visual information, including potentially threatening motion, from a wider visual field than forward-facing eyes. As nocturnal foragers, rats are active and vulnerable to large predators hunting in poorly-lit conditions. While rats have a large visual field, these conditions have also resulted in poor visual acuity. Rats have a visual acuity of about 1 - 1.5 cycles per degree of visual angle, compared to the 24 - 60 cycles per degree typical of humans (Prusky et al., 2002). Cycles per degree is a measurement of angular resolution, or how many different objects can be differentiated by an eye in terms of the visual angle at which they are being viewed. The poor visual acuity of rats has led some researchers to dismiss rats as acceptable subjects for studies involving visual components. However, despite the relatively poor visual acuity of the rat, visual information has been demonstrated to play a significant role in their escape (Morris, 1981; Morris, Hagan, & Rawlins, 1986) and foraging (Suzuki, Augerinos, & Black, 1980) behaviors. Furthermore, it stands to reason that motion may play a larger role in the visual system of the rat, given its poor capacity to determine the identity of the predator.

The ability to detect motion is widespread in the animal kingdom; however, its relevance to learning and memory across species is not well understood. While naturalistic examples provide evidence of the importance of motion and direction discrimination, research is needed to determine which aspects of motion are most important, as well as which procedures and paradigms will be enhanced by the inclusion of motion-based stimuli.

## **1.2 Research on Motion Discrimination**

Early motion experiments with rats focused on determining whether rats were capable of learning discriminations between static and moving (i.e., dynamic) objects. In these experiments the dynamic and static objects were lamps used to signal which of two boxes contained a food reward. The dynamic lamp moved back and forth like a pendulum, whereas, the static lamp was motionless. In one of the earliest motion discrimination studies, Reeves (1917) conducted 700 experimental trials before all four rats had learned to discriminate between a dynamic and static lamp. Reeves concluded that the high number of trials needed to establish the discrimination indicated only a minor role for motion and visual stimuli in the daily functioning of a rat. This conclusion may have developed as a result of poor visual acuity of the albino strain used in the experiments. Visual acuity is poorer in non-pigmented rat strains than pigmented strains, which suggests visual stimuli may play a larger role in lives of pigmented strains (Prusky et al., 2002). Nonetheless, Reeves' (1917) was the first demonstration that rats could discriminate between motion (swinging lamp) and non-motion (static lamp).

More recently, Hupfeld and Hoffman (2006) evaluated the motion discrimination threshold of pigmented (Long-Evans) and albino (Wistar) rat strains. Rats were required to distinguish between dynamic noise and a coherent moving motion pattern in a moving random dot paradigm. Coherent movement was created by a proportion of dots vanishing and

reappearing in a new location and appearing to move to the right while the dynamic noise was created by an equal proportion of dots moving in random directions. The overall percentage of dots that appeared to be coherently moving among dynamic noise determined the percentage of coherence within a trial. The motion coherence threshold (MCT) was the percentage of coherent movement among random noise that rats were able to discriminate. Long-Evans (pigmented) rats had a MCT of 12% compared to the 30% in Wistars (non-pigmented). It is clear that the Long-Evans rats have a more highly developed motion perception system than the albino Wistar strain, as lower MCT imply better perception of coherent motion. This finding suggests that research on motion discrimination and the visual system of rats should be conducted with Long-Evans rats, or at least with a pigmented strain. These findings have helped to refute earlier research claiming that the visual system of the rat plays an unimportant role in behavior when in reality it looks as though visual acuity of a rat strain determines the degree to which visual stimuli will be effective (Reeves, 1917).

Additional research has demonstrated that rats not only have the ability to discriminate dynamic from static visual stimuli, but also discriminate the direction of the movement. In a place recognition task, Long-Evans rats were placed in a modified operant chamber inside the perimeter of a rotating elevated circular arena and lever presses were reinforced only while a 60° sector of the arena was in view (Klement & Bures, 1999). Rats quickly learned to respond only in the presence of the discriminative cue. Even more relevant to the directional motion detection literature, is the fact that the lever pressing rate started to increase before the rewarded zone and peaked either immediately before or after entry into the rewarded area. It was not simply the case that the viewing angle was driving the increase in responding. If the viewing angle alone was driving the increase in responding, there would be evidence of the increased rate of

responding regardless of the rotational direction (away from or toward the rewarded zone). This increase in responding was observed for clockwise and counter-clockwise directional movement for each rat. The transitions between clockwise and counter-clockwise directional rotation occurred at pseudorandom intervals making it impossible for the rat to rely solely on temporal cues

In a similar study conducted by Pastalkova, Kelemen, and Bures (2002) rats were placed in a rotating box around a stationary arena and rewarded for their first lever press when they entered the rewarded viewing area. Again a significant majority of lever presses occurred in the anticipatory quadrant before the rewarded 60° sector. Both of these experiments provide evidence for the ability of rats to utilize directional motion cues to make a response.

Advances in technology have allowed for motion discrimination tasks to be conducted within an operant box equipped with a computer monitor. Nekovarova and Klement (2005) conducted a study with a moving bar and stationary rectangle presented on a computer screen and the lever presses of rats were rewarded only if the moving bar contacted the stationary rectangle. Much like the rotational platform research, rats demonstrated anticipation and increasing rates of lever pressing as the moving bar approached the stationary rectangle. An unpredictable temporal relationship for the speed at which the bar approached the rectangle, suggests that timing and discriminations of rewarded versus non-rewarded periods were not sufficient for explaining the increase in lever press behavior. Similarly, Klement, Levčik, Duskova, and Nekovarova (2010) tested rats with a moving bar that continued across the rewarded zone to make contact with the edge of the monitor and return to the starting location to create a continuous movement paradigm. Lever pressing rates increased only as the bar was approaching the rewarded zone and not when the bar was in the same position on the screen but

moving away from the rewarded zone. Control conditions eliminated the explanation of behavior occurring solely due to timing or the discrimination of rewarded vs. non-rewarded positions of the bar on the screen. This study serves as the most compelling evidence thus far for directional movement of the bar as the essential cue supporting successful discrimination.

The most recent example of the rat's ability to detect motion comes from an experiment investigating whether rats perceive biological motion. MacKinnon, Troje, and Dringenberg (2010) designed a water-maze (modified Morris pool) visual discrimination task whereby a series of increasingly difficult visual discriminations signaled to the swimming rat the location of an escape platform. The final and most difficult discrimination was that of a human point-light display (HPLD). The configuration of coordinated movements of the pixels of a HPLD gave the appearance of either leftward or rightward movement, as if a human were walking on a treadmill viewed from the side. Based on the condition assigned to specific rats, a HPLD walking to the left indicated the platform location on the left of a divider, and vice versa. Rats, once well trained, chose the correct side of the divider based on the HPLD on 76% of trials, well above chance levels of responding. These results indicate that rats are able to detect and utilize information from directional motion.

### **1.3 Research on the Dynamic Superiority Effect**

There is some evidence suggesting that dynamic motion cues may also facilitate learning referred to as the dynamic superiority effect. In a study conducted by Dittrich and Lea (1993), pigeons were trained to discriminate between moving and static images of pigeons with half the pigeons receiving reinforcement for pecking during motion trials and half the receiving reinforcement for pecking during static trials. Pigeons allocated to the motion+/static- group quickly learned the discrimination while pigeons in the motion-/static+ group made no

improvements in the discrimination across trials, suggesting that motion may facilitate feature positive discrimination learning. In another study conducted by Cook and Katz (1999), pigeons displayed positive evidence of a facilitated discrimination where dynamic orthographic 3-D projections of a cube and pyramid were discriminated better than static ones. In a study investigating the effects of motion on learning with humans, Ostuka, Konishi, Kanazawa, Yamaguchi, Abdi, and O'Toole (2009) demonstrated that 3- to 4-month old infants were able to recognize faces that were previously unfamiliar with as little as 30 s of pre-test familiarization when the training face displayed was in motion during a video clip. However, it took infants nearly 90 s of pre-test familiarization to recognize a face during test when the training face displayed in the clip was static. This significant difference between the dynamic and static groups provides additional evidence of dynamic superiority.

Adding motion to static cues, as in the above experiments, may increase the salience of the stimulus entering into an association. According to the Rescorla-Wagner model (1972) the change in the associative strength of the CS during any given trial depends on the salience of the CS (*alpha*), the strength of the US (*beta*), and the difference between the maximum and current associative values of the CS. Adjustments to the salience of the CS in the model have the effect of increasing the rate of learning. Consequently, in discrimination and conditioning tasks, putting a stimulus in motion may make it more salient and thereby increase the rate of learning about a CS. In previous studies on the dynamic superiority effect, two explanations for the effect have been offered. Objects in motion reveal more features that can enhance the subsequent discrimination, or objects in motion increase attention to the objects and task.

Previous experiments have shown discrimination performance based on motion, and there is evidence suggesting dynamic stimuli facilitate performance on simple visual

discrimination tasks. However, to our knowledge there are no studies that evaluate whether dynamic stimuli facilitate performance on more complex, higher-order tasks, such as occasion setting. Occasion setting is a procedure in which one stimulus (feature) sets the occasion for a response (or another stimulus) to be followed by an outcome. This procedure attracted attention in the 1930s and again in the 1980s because this type of higher-order stimulus control was thought to be critical to understanding the organization of memory. The wealth of literature on occasion setting effects and on their critical role in memory suggests it may be a useful procedure to evaluate whether motion facilitates learning of higher-order stimulus control.

#### **1.4 Research on the Occasion Setting Phenomenon**

In Pavlovian conditioning paradigms, a conditioned stimulus (CS; e.g., a tone) may elicit a conditioned response if it signals the arrival of an unconditioned stimulus (US; e.g., food). In a different paradigm, a stimulus (feature, X) may come to control behavior because it signals that an otherwise ambiguous CS (target, A) will be followed by the delivery of the US. The stimulus in the first paradigm acts as a CS, whereas, the feature in the second paradigm is referred to as an “occasion setter” (Holland, 1983, 1992) or “facilitator” (Rescorla, 1985). In a feature-positive occasion setting procedure, the feature is the only stimulus that uniquely signals the upcoming outcome, and thus is referred to as a positive feature.

Although the mechanism by which a feature exerts control over responding is still a matter of debate (Baeyens, Vansteenwegen, Hermans, Vervliet, & Helen, 2001; Bonardi, & Jennings, 2009; Bonardi, Bartle, & Jennings, 2012), several procedures have been used to dissociate whether the feature is equivalent to a Pavlovian CS or exerts control over responding in some other manner (e.g., modulation). For example, a feature in a FP discrimination could come to control responding via a direct connection to the US (i.e., as a CS) or by entering into a

unique configuration with the target CS it was paired with. An occasion setter exhibits two properties that a standard CS does not show: (1) transfer of facilitation only to targets that have a history of training in a FP discrimination (XA+, A- to YB+/B-), and (2) extinction of the feature does not disrupt subsequent facilitation of responding to the previously trained target in the presence of the feature. While there is not yet a benchmark test or tests for evaluating the role of the feature, these tests are the most commonly used.

During feature-positive (FP) occasion setting training, trials in which the feature signals that the target will be followed by the delivery of the US are intermixed with trials in which the target is presented alone and no US is delivered. Because the target stimulus is only paired with the US when it is preceded by the feature, the target comes to elicit a stronger conditioned response on feature-target trials compared to target alone trials. Additional trials of another CS trained independently (the CS not trained in feature-target trial) followed by the US are typically intermixed with trials of feature-target and target alone during training. After training, responding to the additional CS can be extinguished and responding to the CS can be tested alone and in the presence of the feature. If the feature was eliciting a response as a result of a direct connection between the feature and the US, then the excitation elicited by the feature will summate with that of the extinguished CS to generate a partially recovered response. Previous research, however, has shown that a feature trained in a feature-target relationship does not elicit a response via a direct connection to the US, and consequently, results in very little summation when paired with the extinguished CS. The second test, following post-training extinction (repeated non-reinforced presentation) of the feature, also investigates the role of a direct feature-US connection. Post-training extinction trials of the feature should create new learning which inhibits any direct connection from the feature to the US from exerting behavioral control but



should not influence the feature's ability to modulate responding to the target on feature-target trials.

Holland (1995) utilized an occasion setting procedure to examine the transfer of occasion setting across stimuli and responses during operant FP discriminations that provide examples of both occasion setting test procedures used in the current research. In one experiment rats received FP discrimination training with two serial FP discriminations ( $X \rightarrow A+/A-/Y \rightarrow B+/B-$ ) as well as exposure to a third target (C) that was initially trained but then separately underwent extinction training. During initial training the FP trials as well as the C alone trials always provided an opportunity for reinforcement while the individual target trials never had an opportunity for reinforcement. Due to the procedure being operant in nature the delivery of reinforcement was contingent not only on the appropriate trial type but on a lever press response as well. Responding to C was extinguished and then the ability of features X and Y to control responding when paired with targets A, B, and C was examined during a transfer test. Brief retraining on the original discriminations followed the transfer test and then subsequent extinction of the features X and Y occurred before the post extinction test occurred. This final test examined post-feature extinction responding controlled by the features.

The results of the transfer test following the extinction training of C showed nearly perfect transfer of the feature's occasion setting properties to the targets that had also participated in previous FP discrimination while transfer to the separately trained and extinguished target was incomplete. Results from the post-feature extinction test showed that neither responding to the original FP compound nor the transfer to the other target trained in FP discrimination were affected by the extinction of the features. It is critical that the features continued to control responding during the subsequent feature target compounds despite extinction and that the

transfer to the separately trained and extinguished target (C+/ then C-) was minimal for features X and Y.

### **1.5 Development and Design of iPad-Equipped Apparatus**

Recently, several alternatives to a traditional skinner box have been developed (Bussey, Padain, Skillings, Winters, Morton, & Saksida, 2008; Horner et al., 2013; Wolf, Urbano, Ruprecht, & Leising, 2014). Our lab has incorporated an iPad as the method of presenting visual stimuli. The use of an iPad-equipped apparatus has advantages over standard touchscreen operant chambers (TOCs) and has greater flexibility as to the type of stimuli it can present compared to the standard chamber lights which allows for the use of dynamic (left-to-right movement) visual stimuli. The iPad apparatus is also considerably more affordable than a prefabricated TOC system (approximately \$400 vs. \$8,000).

The development of the iPad-equipped apparatus consisted of 3 major phases: development of necessary mounting hardware, development of the software, the Touchscreen Behavioral Evaluation System (TBES) app, and conducting a battery of tests to determine if rats were capable of interacting with the iPad hardware and software and whether the iPad apparatus was a feasible and flexible alternative to the infrared touchscreen technology and traditional operant chambers.

The iPad-equipped apparatus uses a standard operant chamber retrofitted with an iPad replacing one wall of the chamber. A mount for the iPad was constructed from three pieces of black poster board held together with four sets of nuts and bolts. This mount allowed the iPad to replace the back wall of the chamber, opposite from the feeding niche. The mount provided the rats with an unimpeded view of the entire iPad screen.

To control response detection and stimulus presentation we developed software to be used with the iPad-equipped apparatus. The software includes two freely downloadable components: (1) the TBES App (Urbano, Westfall, & Leising, 2013), and (2) a server program written in a programming language capable of using TCP/IP sockets to communicate with the TBES App (Leising, 2013). For the server, we wrote, an application in Microsoft Visual Basic 6 (VB6; Redmond, WA). The TBES App used the TCP/IP sockets to exchange data with the PC running the Visual Basic program.

The feasibility of the iPad apparatus was evaluated with a battery of tests: response latency, iPad response sensitivity, whether a press (with paw) or poke with (nose) could meet the iPad's capacitive sensor criterion, and screen protection and durability. The battery of tests revealed that, the latency between signals was almost negligible ( $< 5$  ms), the iPad was highly sensitive to human responses (registering 100 of 100 presses), both presses and pokes were successful in meeting the capacitive criterion, and screen protectors were not only unnecessary, but actually encouraged scratching and gnawing at the screen. Rats were also successfully trained to interact with stimuli presented on the iPad screen. Lastly, we also trained rats to make a visual discrimination using clip art images displayed on the iPad. The results suggest the iPad-equipped apparatus can be used to investigate the role of motion in discrimination learning by presenting visual stimuli on the iPad.

## **1.6 Summary and Overall Hypotheses**

Recent occasion setting literature with rats has focused on many different aspects of the phenomenon, including transferability of stimuli used in occasion setting (Morell & Davidson, 2002), evidence for a hierarchical account of occasion setting (Bonardi & Jennings, 2009), the importance of US specificity in occasion setting (Morell & Davidson, 2002; Bonardi, Bartle &

Jenning, 2012), the use of the differential outcomes effect in occasion setting (Delamater, 2010), contextual control as an occasion setter, (Hall & Mondragon, 1998), and the role of the intertrial interval as a contextual stimulus in FP discriminations (Bouton & Hendrix, 2011). Despite the knowledge of the visual learning and memory capabilities of rats, there is no research exploring the use of dynamic (directional or simply non-static motion) vs. static stimuli in tasks more complex than simple discriminations, such as occasion setting. We utilized a modified version of Holland's (1995) FP discrimination training and testing procedure to test rats viewing stimuli presented on an iPad in a Pavlovian preparation. Holland's experimental parameters were easily adapted to our iPad apparatus while at the same time allowing us to substitute dynamic stimuli in place of traditional static visual stimuli such as panel lights in an attempt to demonstrate the effectiveness of motion as an occasion setter.

The current experiment utilized a conditioned magazine paradigm to examine the suitability of motion as a cue in higher-order learning. We expected that a dynamic visual stimulus would facilitate acquisition rate during FP discrimination training. Furthermore, we expected the dynamic feature to demonstrate properties of an occasion setter during transfer tests.

### **1.7 Feature Positive Discrimination Pilot Study**

While it is encouraging that earlier experiments utilizing the iPad-equipped apparatus have been successful, there is still much to learn about how best to use the iPad to investigate discrimination learning in rats (Wolf et al., 2014; Leising, Wolf, Ruprecht, 2013.) To better understand how a FP discrimination procedure would function in our iPad-equipped apparatus, we conducted an initial pilot study.

The FP discrimination pilot study ran for 36 sessions and followed a standard FP discrimination training procedure borrowed from Holland (1995), but altered slightly to accommodate our iPad-equipped apparatus. In the pilot study, half of the rats ( $n = 8$ ) received FP discrimination training with a static visual stimulus as the feature and an auditory cue as the target (Group Static,  $X \rightarrow A+/A-$ ; see Table 1). The other group of rats ( $n = 8$ ) received FP discrimination training with a dynamic visual stimulus as the feature and an auditory cue as the target (Group Dynamic,  $X \rightarrow A+/A-$ ). Trials of  $X \rightarrow A+$  consisted of a variable-time 15-s (range = 10-20 s) presentation of X and a 15-s presentation of A. The onset of A overlapped with the last 5 s of X and always terminated with 3-s access to the US. For the dynamic group, the feature always consisted of vertically-oriented black and white bars moving from left-to-right across the iPad screen at a constant rate of 10 pixels per second. For the static group, rats received the same black-and-white image as the feature but the image did not move. Trials of A alone consisted of a 15-s presentation of A without the US. Trials of C alone were conducted similarly, but always terminated with the delivery of the US. As in Holland's research, altering the proportions of each trial type after 15 sessions facilitated learning of the FP discrimination. Despite the facilitated performance following altered trial type proportions, learning progressed slower than expected (see Figure 1). We stopped the pilot study after 36 sessions because the rats had demonstrated steady but slow improvement. Possible reasons for the slow rate of learning include the duration of the visual feature prior to the onset of the target being too short, and minimal exposure to the feature (i.e. feature only on the top half of the iPad screen was not salient enough for the rats to learn the FP discrimination in the amount of time we expected). Additionally, we speculated that altering the proportion of trial types earlier in

training would lead to faster learning of the FP discrimination. All of these possible issues and modifications were taken into account in developing the procedure used in the experiment.

## **2. Occasion Setting in a Pavlovian Preparation**

Holland (1995) found that a feature previously trained in a FP occasion setting relationship (XA+, A-) was only able to moderately elevate responding when paired with a different target cue (C+) not previously trained in a FP occasion setting relationship. Furthermore, the feature (X) maintained control over responding during the target it was paired with during training (XA) even following training designed to extinguish responding to the feature alone (X-). However, following extinction of X transfer to C (not trained in FP) was reduced. As previously discussed, X in this task would qualify as an occasion setter because 1) facilitation from X transferred to B, which had a history of training in a FP discrimination (YB+, B-) but not to C, which had no history of FP training (C+), and (2) extinction of X did not disrupt subsequent facilitation of responding to A. It is interesting that transfer to C was reduced following extinction of X in Holland's study. This suggests that X acquired some associative strength during feature-positive training (XA+, A-), but extinction would have reduced its associative strength. Following the reduction, X was still able to facilitate responding to A, which indicates that simple summation of the associative strength of X and A was not driving the increased responding. We investigated whether rats trained on a FP discrimination with a feature that was dynamic would exhibit more rapid acquisition than a group with a static feature, and conducted a transfer test and extinction of the feature to determine whether rats solved the task using occasion setting and whether motion would facilitate transfer to a CS not trained in a FP discrimination.

One group of rats was trained on a FP discrimination training with a static visual stimulus as the feature and an auditory cue as the target (Group Static,  $X \rightarrow A+/A-$ ; see Table 1). Another group of rats received the same training but with a dynamic visual stimulus as the feature and an auditory cue as the target (Group Dynamic,  $X \rightarrow A+/A-$ ). Visual stimuli were vertically-oriented black and white bars that either moved left-to-right across the iPad screen for rats in Group Dynamic or persisted as a static image for rats in Group Static. All rats also received trials where a second auditory target, C, was presented alone and always ended with the delivery of sucrose (C+).

In Experiment 1, we expected faster acquisition of the discrimination by Group Dynamic than Static. During transfer testing, we predicted that feature X would result in minimal summation when paired with target C, which lacked prior FP training. Following feature extinction training, we expected feature X to continue to facilitate control of responding to A but further decrease summation when paired with target C.

## **2.1 Method**

### **2.1.1 Subjects**

The subjects were 8 male and 8 female Long-Evans rats bred in the TCU vivarium from parents obtained from Harlan Laboratories (Indianapolis, IN). Subjects were pair-housed in translucent plastic tubs with a substrate of wood shavings in a vivarium maintained on a 12 hr dark/12 hr light cycle. All experimental manipulations were conducted during the light portion of the cycle. A progressive food restriction schedule was imposed over the week prior to the beginning of the experiment, until each subject reached 80-85% of its free feeding weight. All animals were handled daily for 30 s for a week prior to the initiation of the study.

### **2.1.2 Apparatus**

All tests occurred within a standard operant chamber measuring 30 x 25 x 20 cm (l x w x h) housed within a sound- and light-attenuating environmental isolation chest (Med Associates). The walls and ceiling of the chamber were composed of clear Plexiglas and the floor was constructed of stainless-steel rods measuring 0.5 cm in diameter, spaced 1.5 cm center-to-center. The chamber was equipped with a dipper capable of delivering sucrose solution (18% w/v). When in the raised position, a small well (0.05 cc) at the end of the dipper arm protruded up into the feeding niche. Breaks to a single infrared beam positioned over the dipper measured the duration of time spent in the feeding niche. The back wall of the operant chamber was replaced by an iPad touchscreen. The iPad was capable of presenting visual stimuli during the procedure. Ventilation fans in each enclosure and a white-noise generator on a shelf outside of the enclosure provided a constant 74-dB (A) background noise. The auditory stimuli were an 80 dB (A-scale) 80 hz tone and an 80 dB white noise.

### **2.1.3 Procedure**

During magazine training, rats learned to drink sucrose in an iPad-equipped operant chamber. No stimuli were present on the iPad, but rats received 30 unsignaled presentations of sucrose on a fixed-interval schedule of 60 s. When sucrose was delivered, the dipper arm elevated and remained elevated until the subject interrupted the infrared beam located over the feeding niche. Once interrupted, the arm lowered 3 s later. The houselight remained off during the trial and while the dipper was elevated, but turned on when the dipper lowered and remained on for the duration of the variable ITI.

Feature-positive discrimination training followed magazine training and continued until all rats achieved a .7 mean elevation ratio (ER = responding during A on XA+ trials/ responding



during A on XA+ trials plus responding during A on A alone trials) during the target A on X→A+ trials across 8 sessions. All rats received three different trial types randomly presented during each 55-min session. Table 1 displays the different trial types during each phase of training and testing. During the first 5 sessions of FP training, all rats received 8 X→A+ trials and 8 each of A- and C+. For sessions 6-44, rats received 6 each of X→A+ and C+ trials, as well as 12 A- trials. We modified the proportions of each trial type earlier for the experiment based on the results of the pilot. More trials of the non-reinforced A- should lead the rats to more quickly learn that A alone was an unreliable predictor of reward. Feature X was always the visual stimulus presented on the iPad and trained in a feature-target relationship with Target A. Targets A and C were always auditory stimuli and the assignment of each stimulus (noise or tone) to the Targets was counterbalanced. Trials of X→A+ consisted of a variable-time 15-s (range = 10 to 20 s) presentation of X and a 15-s presentation of A. The onset of A overlapped with the last 5 s of X and always terminated with 3-s access to the US. For half the rats ( $n = 8$ ), the feature always consisted of vertically-oriented black and white bars moving from left-to-right across the iPad screen at a constant rate of 10 pixels per second (Group Dynamic). The remaining rats ( $n = 8$ ) were in Group Static, which received the same black-and-white image as the feature but the image did not move. Trials of A alone consisted of a 15-s presentation of A without the US. Trials of C alone were conducted similarly, but always terminated with the delivery of the US.

**Table 1** Design of Experiment

Group	FP Disc (44)	Ext of C (10)		Test 1 (1)	Retraining (8)	Ext of X (4)	Test 2 (1)
		Early	Late				
Dynamic	X→A+/A-/C+	C-	X→A+/A-/C-	X→A- X→C-/A-/C-	Same as Late Ext of C	X-	X→A- X→C-/A-/C-/X-
Static	X→A+/A-/C+	C-	X→A+/A-/C-	X→A- X→C-/A-/C-	Same as Late Ext of C	X-	X→A- X→C-/A-/C-/X-

**Table 1** displays the trial types during each of the training and testing phases. FP trials are indicated by X→A. Delivery of sucrose is indicated by (+) while no sucrose delivery is indicated by (-). Target C extinction alternated between early and late extinction sessions. The numbers of sessions are indicated in parentheses.

Once rats ( $n = 11$ ) reached the elevation ratio criterion (.70 during A on X→A+ trials across 8 sessions), they received 10-min sessions of extinction training designed to extinguish responding to the auditory target C, which had previously been followed by the delivery of the US. Target C extinction training consisted of alternating sessions of 12 C- trials where C was presented for 15 s without the delivery of the US (Early Extinction C) and sessions where C- trials replaced C+ trials in the original FP discrimination training. Rats received a total of 5 Early Extinction C sessions and 5 Late Extinction C sessions.

Following Target C Extinction training, rats received 1 34-min session of transfer testing consisting of four presentations each of X→A-, X→C-, A- and C- trials.

Following the first test session all rats were retrained on the original FP discrimination for 8 sessions with the modification of C- replacing C+ trials. After the retraining all rats received 4 sessions of X extinction training where X was presented on the iPad screen for 15 seconds with no US.

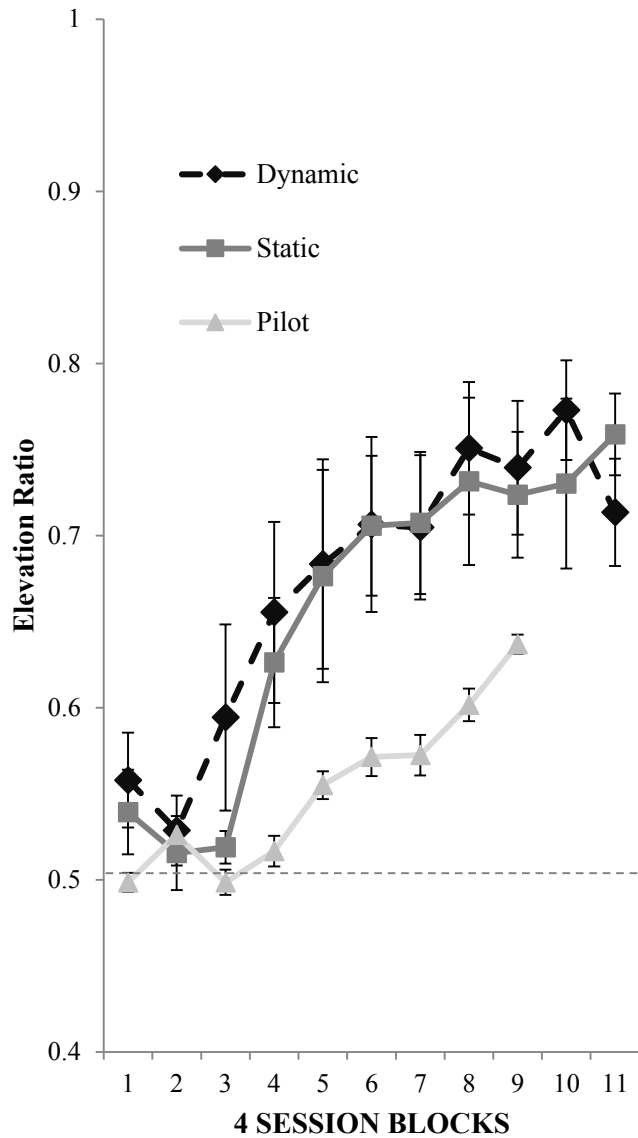
Following X extinction training, all rats received an additional test session consisting of four presentations each of X→A-, X→C-, A-, C-, and X trials.

#### 2.1.4. Data analysis

We recorded the duration of time (100 ms resolution) spent in the feeding niche as an index of food seeking behavior for the 15 s preceding each trial type (pre-trial period), as well as during each stimulus. To evaluate performance we calculated an ER for each session of acquisition by dividing the time spent in the magazine during Target A on  $X \rightarrow A+$  trials by the time spent in the magazine during Target A- alone trials plus Target A during  $X \rightarrow A+$  trials. Elevation ratios across acquisition were broken into 4-session blocks and analyzed using a repeated measures ANOVA with group (e.g., Dynamic vs. Static) as the between-subject factor and block as the within-subjects factor. Repeated measure ANOVAs and independent  $t$ -tests were used where appropriate to evaluate time spent in the magazine during Tests 1 and 2.

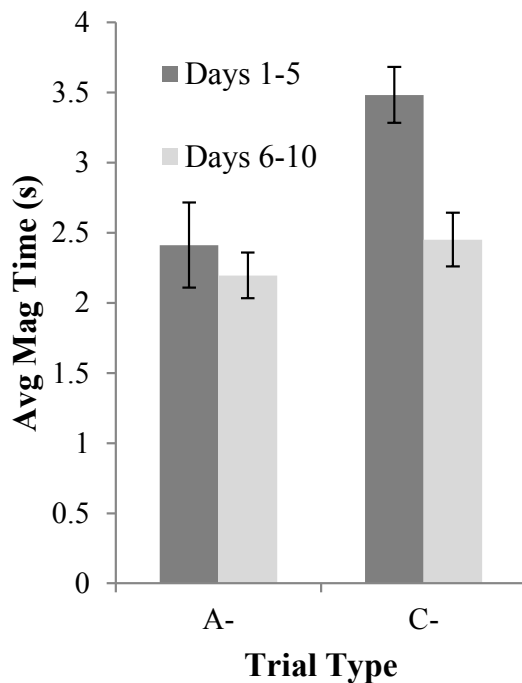
#### 2.1.5. Results

**Acquisition:** Figure 1 displays FP discrimination training acquisition data as an ER broken into 4-session blocks. Rats in both Group Dynamic and Group Static learned the FP discrimination. However, 5 of the 16 rats failed to reach the elevation ratio acquisition criterion. As a result, those rats that showed poor evidence of learning the FP discrimination were excluded from analysis ( $n = 5$ ) and the following analyses were conducted on the rats that met the elevation ratio criterion ( $n = 11$ ). A 2 (Group: Dynamic, Static) by 11(Block) mixed-design ANOVA, with Block as the repeated measure, revealed a main effect of Block,  $F(10, 90) = 23.9$ ,  $p < .001$ ,  $\eta^2 = .73$ , but no interaction between Group and Block,  $F(10, 90) = .83$ ,  $p > .1$ ,  $\eta^2 < .1$  and no main effect of Group,  $F(1, 9) = .002$ ,  $p > .1$ ,  $\eta^2 < .01$



**Figure 1.** Displays elevation ratios across 4-session blocks during FP Discrimination training during the experiment (Dynamic and Static lines) and the pilot study (Pilot line). Chance responding level is indicated by the dashed gray line. During the pilot study, the trial type proportions were altered after day 15 (middle of block 4) and altered after day 5 during the experiment (beginning of block 2).

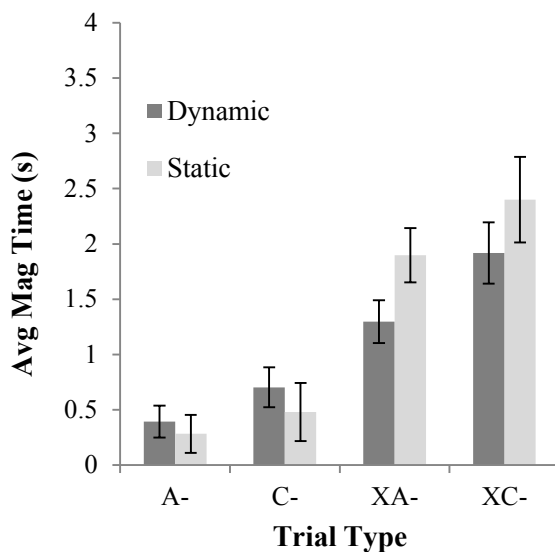
**Target C Extinction Training:** Figure 2 displays the average magazine duration for the auditory targets A and C during extinction training. Mean magazine time during C was lower across the second 5 sessions ( $M = 2.41, SD = .8$ ) than first 5 sessions ( $M = 3.48, SD = 1.2$ ) ( $t(15) = 4.5, p < .001$ ). These results indicate the responding to C was successfully extinguished by the end of extinction training.



**Figure 2** displays average magazine duration for the trial types during Target C Extinction Training.

**Test 1:** Figure 3 displays the mean magazine duration for the targets during the 4 testing trial types. A 2 (Group: Dynamic, Static) by 4(Trial Type: A-, C-, XA-, and XC-) mixed design ANOVA with Trial Type as the repeated measure revealed a main effect of Trial Type,  $F(3, 27) = 28.7, p < .001, \eta^2 > .76$ , but the main effect of Group and the interaction were non-significant, with the greatest non-significant effect occurring at the interaction of Group X Trial Type, all  $F_s < 1.7$ , all  $p_s > .19$ , all  $\eta^2 < .16$ . Planned comparisons, with a correction for multiple comparisons,

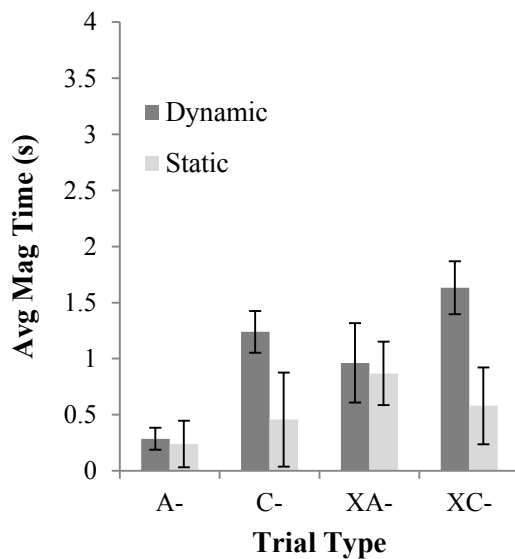
were used to compare trial types directly to one another. These comparisons revealed that rats spent more time in the magazine during trials of XA than during trials of A,  $F(1, 9) = 27.8, p < .001, \eta^2 = .76$ , spent more time in the magazine during trials of XC than trials of C,  $F(1, 9) = 60.2, p < .001, \eta^2 = .87$  but did not differ in amount of time spent in the magazine during trials of XC and XA,  $F(1, 9) = 4.6, p > .05, \eta^2 = .34$ , or during trials of A and C,  $F(1, 9) = 3.1, p > .05, \eta^2 = .27$ .



**Figure 3** displays average magazine duration for the different trial types during Post Target C Extinction test.

**Feature X Extinction Training:** Feature X extinction training reduced the amount about time spent in the magazine during trials of X-. A paired-samples t-test revealed that mean time spent in the magazine during X for session 4 ( $M = .45, SD = .2$ ) of X extinction was significantly lower than the mean time spent in the magazine during X for session 1 ( $M = .79, SD = .37$ )  $t(15) = 2.87, p < .05$ . While the average time spent in the magazine decreased across trials, it is worth noting that average magazine time was comparatively low during the first session of X extinction training relative to C during extinction of C.

**Test 2:** Figure 4 displays the average magazine duration for the targets during the 4 testing trial types. A 2 (Group: Dynamic, Static) X 4 ( Trial Type: A-, C-, XA-, and XC-) mixed design ANOVA, with Trial Type as the repeated measure, revealed a main effect of Trial Type,  $F(3, 27) = 8.7, p < .001, \eta^2 > .5$ , but the main effect of Group and the interaction were non-significant, all  $F_s < .25$ , all  $p_s > .62$ , all  $\eta^2_s < .01$ . Planned comparisons revealed that rats spent more time in the magazine during trials of XC than during trials of XA,  $F(1, 9) = 5.7, p < .05, \eta^2 = .39$ , and spent more time in the magazine during trials of C than trials of A,  $F(1, 9) = 12.8, p < .05, \eta^2 = .59$ , but did not differ in magazine time on trials of XA and A,  $F(1, 9) = 3.8, p > .05, \eta^2 = .3$  or trials of XC and C,  $F(1,9) = 4.9, p > .05, \eta^2 = .35$ .



**Figure 4** displays average magazine duration for the different trial types during Post Feature Extinction test.

### 2.1.6. Discussion

The goal of the present experiment was to determine whether a dynamic visual feature would facilitate FP discrimination learning better than a static feature stimulus and to determine if the visual feature displayed the properties of an occasion setter. Using what we learned from the FP Pilot study, we were able to design a successful FP discrimination procedure in the

current experiment evidenced by the amount of learning across the subsequent sessions of acquisition training. While there was evidence of learning in both Group Dynamic and Group Static, there were 5 rats that failed to reach the acquisition criterion. This left us with only 11 of the original 16 rats to include in the analyses. Of these rats, Group Dynamic ( $n = 6$ ) and Group Static ( $n = 5$ ) demonstrated similar acquisition patterns in the task. Following acquisition, extinction of C was successful in reducing the amount of magazine time during the auditory target C. Despite low magazine time during C on Target C alone test trials during Test 1 (Post-C Extinction Test), rats increased the amount of time in the magazine during C on novel trials of XC to the same degree as during A on trials of XA. Furthermore, following extinction of X, rats failed to demonstrate significantly more magazine responding during A on XA trials than on trials of A alone. Rats did spend significantly more time in the magazine during trials of XC and C compared to trials of XA and A, respectively. However, the amount of time spent in the magazine did not differ between trials of XC and C. We also expected the terminal levels of FP discrimination to be the same for rats in Group Dynamic and Group Static, we also expected to see facilitation of acquisition in Group Dynamic. Terminal levels of FP discrimination were the same for both groups, however, no significant facilitation of the discrimination was found for Group Dynamic compared to Group Static.

As previously mentioned, an alternative to FP discrimination is that feature X could be used a predictive cue (i.e., a CS) to control magazine responding directly, rather than by acting as an occasion setter to facilitate responding to A. As previously discussed, an occasion setter, but not a CS, exhibits two properties that a standard CS does not: (1) transfer of facilitation only to targets that have a history of training in a FP discrimination (XA+, A-), and (2) extinction of the feature does not disrupt subsequent facilitation of responding to the previously trained target in



the presence of the feature. We conducted both of these tests, and thus evaluation of those data should provide evidence for one or the other interpretation of the observed dynamic superiority effect.

During Test 1, the average amount of time spent in the magazine during the different test trials was compared to evaluate the extent to which feature X influenced magazine time during the previously trained XA relationship and the novel XC relationship after extinction of C. We expected that magazine time during A on XA trials would still be high, because neither the X→A relationship, nor X by itself, had receive additional training that may alter the properties of X. At test, average magazine time during A on XA- trials was significantly higher than magazine time during A alone trials. No significant difference was found between magazine time during XC and XA trials. According to the occasion setting literature, an occasion setter should only transfer its facilitation to a target that was trained in a similar relationship, namely the FP trial type. Following FP training, Holland (1995) did find elevation of responding during C on XC trials, but it was lower than that observed on XA trials. Our results indicate no difference between responding during C and A on XC and XA trial types, respectively. These results indicate that X is influencing C in a way not expected of an occasion setter. If X has established a direct connection to the US, like a standard CS (generalized excitor), then the excitation elicited by X would sum with that produced by C, which is consistent with the result we observed on XC trials. If X was functioning as a CS, then extinction of X should have a marked effect on facilitation of responding to A on XA trials and transfer to C on XC trials.

Test 2 examined the extent to which feature X extinction training affected magazine time during the different test trial types. According to the literature on occasion setting, a feature should still drive responding to A on XA trials following feature extinction training because the

feature is thought to facilitate the relationship between the target and the US, which is independent of any direct connection it may have with the US. Extinction of X should reduce any direct connection to the US, but leave intact the modulatory connection to A and the US. The results of Test 2 added further support to the idea that X is not acting as an occasion setter. We observed no difference in responding during XA and A trials following extinction of X. If X was an occasion setter, the difference between XA and A should have persisted. If X was acting as a CS to elicit responding directly, then extinction of X should also have reduced responding on XC trials during Test 2. Consistent with this prediction, responding during C on XC trials and C alone trials no longer differed.

Interestingly, Test 2 also revealed high levels of responding during both the XC- and C-trial types. Magazine time was also higher for trials of XC than XA and higher for trial of C than A. There is clearly evidence for spontaneous recovery of responding to target C. Responding during trials of C during Test 1 revealed successful extinction of C, and additional extinction trials of C were given following Test 1. However, there were 4 sessions of feature X extinction training between C extinction and Test 2. Responding to an extinguished CS is frequently observed to recover simply with the passage of time (spontaneous recovery; Pavlov, 1927). In our task, four sessions was long enough to elicit spontaneous recovery of responding to C during testing. With the increase in responding to C, it is likely that the difference in responding to C on XC and A on XA trials is driven by the difference already present between A and C, at the level of the individual targets. The high levels of responding during trials of C presented both alone and following feature X highlight the importance of intermixing sessions of extinction of C with sessions of extinction of X to avoid spontaneous recovery during testing.

Responding at Test 1 and Test 2 revealed that rats solved the feature-positive discrimination largely by learning the predictive value of X. During training, X was the cue that most reliably predicted the delivery of the US, and as a result, increased responding during any cue (A or C) that followed X during testing. Consequently, the results of FP discrimination training and subsequent test sessions revealed that a dynamic visual feature did not facilitate FP discrimination learning during any portion of acquisition training. Had any facilitation been observed, it is likely that it would not have been as a result of enhanced occasion setting. Instead, the directional motion of the cue would likely have elicited more attention than the same cue presented statically. This type of result would be consistent with that predicted by the Rescorla-Wagner model (Rescorla & Wagner, 1972) based on the relationship between learning rate and CS salience. The model predicts that the salience of a stimulus acts on the rate of learning, rather than the asymptote. In the current experiment, rats in Group Dynamic progressed at the same rate and to the same level of terminal performance as the static group. In previous studies on the dynamic superiority effect, the two potential causes of the effect were confounded. Objects in motion reveal more features of the objects that can be used to enhance subsequent discrimination, but they also increase attention to the objects and task. In this experiment, the dynamic motion of the black and white grating visual stimulus did not reveal more visual information that could be used to solve the discrimination. Consequently, any differences between the groups, would have been driven by increases in attention alone. Consequently, this experiment may serve as weak support for the enhanced discrimination, rather than enhanced attention, explanation of the dynamic superiority effect.

A few other parameters have been shown to discourage the formation of a direct connection between the feature and US. One such parameter is temporal priority (Holland,

1986). The feature must precede the target. However, we suggest that additionally, the feature must be sufficiently removed from the US as to render the target a significantly more proximal predictor of the US. In our task, the rats learned that X was the best predictor of the delivery of the US despite its 15-s temporal distance from the US. X may act more as an occasion setter if it is further separated from the delivery of the sucrose. Another factor is the salience of the feature (Miller & Oberling, 1998). Typically, an experimental procedure is designed to enhance the salience of the feature to prevent configural learning of the feature and target from occurring. However, in our task the salience of the feature may have been so great as to overshadow learning of the value of the auditory stimulus. Lastly, the high levels of responding during A alone trials during FP Discrimination acquisition training resulted in the exclusion of five rats from the analysis. This may indicate either the difficulty of the task is too great, or the response cost of checking the magazine for food may be too low. It is also unlikely that the generally poor visual acuity of the rat is to blame for the poor discrimination performance during acquisition. The use of an instrumental procedure, in which the response is made at a location removed from the delivery of food may reduce unproductive responses and facilitate learning.

The current experiment did not find evidence for the facilitation of discrimination learning by a dynamic stimulus. The lack of facilitation is not indicative of poor occasion setting by a dynamic feature. Instead, it is likely the case that the motion of the dynamic feature was too weak to enhance the salience of the grating stimulus, and also did not provide the subject with more features to enhance the discrimination. These results also serve as another demonstration of the feasibility and utility of an iPad-equipped apparatus. This experiment adds to the body of research on the feature-positive procedure and raises a few interesting questions regarding the conditions which support occasion setting.

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## Abstract

### DO DYNAMIC STIMULI FACILITATE PERFORMANCE IN A PAVLOVIAN OCCASION SETTING PROCEDURE?

by Joshua Earl Wolf, M.S., 2014  
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In the domain of visual perception, motion cues are critical for the success of both predator and prey. To evaluate the superiority of motion cues in conditional relations, we trained rats on a feature-positive discrimination within a newly developed iPad-equipped apparatus. During feature-positive training animals must learn to respond to a target stimulus (e.g., A) in the presence of a feature (XA+) but not in its absence (A-). Two groups of rats were tested with a visual stimulus (feature) and an auditory stimulus (target). Group Dynamic received dynamic (left-to-right) presentations of the feature and Group Static received static presentations of the same feature. The dynamic visual feature did not facilitate FP discrimination learning during any portion of acquisition training. Responding on tests with combinations of X and C revealed that rats solved the feature-positive discrimination largely by learning the predictive value of X, rather than facilitation of A by X.