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BRIEF REPORT

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The acquisition of instrumental responding can be supported by primary reinforcers or by conditional (also known as secondary) reinforcers that themselves have an association to a primary reinforcer. While primary reinforcement has been heavily studied for the past century, the associative basis of conditioned reinforcement has received comparatively little experimental examination. Yet conditioned reinforcement has been employed as an important behavioral assay in neuroscience studies, and thus an analysis of its associative basis is called for. We evaluated the extent to which an element from a previously trained compound would facilitate conditioned reinforcement. Three groups of rats received Pavlovian conditioning with a visual-auditory compound cue followed by food. After training, a lever was made available that, when pressed, produced the same trained compound (group compound), only the auditory cue (group element), or a novel auditory cue (group control). The rats in group compound pressed the lever at a higher rate than did rats in either group element or group control, demonstrating a strong conditioned reinforcement effect only in group compound. Interestingly, there was almost no difference in responding between group element and group control. The implications of this generalization decrement in conditioned reinforcement are discussed—particularly as they relate to research in behavioral neuroscience.

Keywords: conditioned reinforcement, secondary reinforcement, generalization decrement, elemental versus configural associations

Conditioned reinforcement (also known as secondary reinforcement) refers to the phenomenon in which a Pavlovian conditioned stimulus (CS) serves as a reinforcer in the acquisition of an instrumental action (Mackintosh, 1974; Parkinset al., 2005). This paradigm is often preferred for the study of instrumental acquisition because it avoids conflicts caused by the presentation of an appetitive outcome (e.g., food) that may interfere with operant responding (Parkinset al., 2005; Williams, 1994b). Conditioned reinforcement also provides an assessment of the value or motivational properties that a CS has potentially acquired as a result of having been paired with the unconditioned stimulus (US).

Conditioned reinforcement can be a useful tool to evaluate the role of motivational and associative processes in the acquisition and maintenance of instrumental actions; however, compared to what is known about primary reinforcement, there is little current research on the associative properties of conditioned reinforcers. Early research used the paradigm of secondary reinforcement to demonstrate the role of outcome components to maintain instrumental behavior. For instance, Bugelski (1938) trained rats to press a lever for the joint presentation of a food pellet and a click. After acquisition, lever pressing was followed by either no consequence or the click stimulus. The rats that received the click persisted in lever pressing compared to the group with no consequence. Although this serves as an early example of the reinforcing properties of the click, it is not clear if this was due to the click working as a conditioned reinforcer or a result of generalization decrement. That is, it is possible that the rats responded more during extinction with the click present because the extinction session was more comparable to training than the case when the lever was followed by neither outcome.

Given this problem in interpretation, researchers switched to using chain schedule procedures to study conditioned reinforcement. An early experiment by Zimmerman (1969) provided...
evidence of conditioned reinforcement using an instrumental chain. Rats first learned that pressing one lever produced a light under a fixed-interval (FI) schedule. After the FI was completed, a second lever was made available that, when pressed, resulted in the delivery of a reinforcer. During the test phase, the FI schedule was changed to a variable-interval schedule, in which the behavior shifted to a typical performance during a variable-interval schedule—a stable and steady rate of responding (as opposed to the scalloped response pattern produced by the FI schedule). While these results might be interpreted as evidence of conditioned reinforcement, the use of multiple components of a chain schedule makes it difficult to rule out alternative accounts for the responses to the first lever press (Williams, 1994a, 1994b).

Egger and Miller (1962) suggested that Pavlovian associations are not sufficient to support conditioned reinforcement. They proposed that the information value of a stimulus was more important than its excitatory properties for the acquisition of an instrumental response. To test this, they presented two CSs in compound, one of which onset earlier than the other, but both coterminated with the delivery of the US. Specifically, a tone-light compound was presented, with tone onset prior to light onset, but both terminated with the delivery of a food US. They found that only the cue with the earliest onset (the tone in this example) would subsequently reinforce an instrumental lever-press response when presented on its own. That is, the offset between cue onsets resulted in the cue with the earlier onset (tone) blocking the ability of the cue with the later onset (light) to serve as a conditioned reinforcer. This experiment suggests that only one stimulus acquired control over the behavior. Nevertheless, presenting the cues of the compound with sequential onsets results in the onset of each cue having a different temporal relationship with the food US—which has been shown to affect the formation of conditioned reinforcers (Mazur, 1997). Additionally, it is possible that the subjects learned the tone-light compound as a configural cue (e.g., Pearce, 1987). According to Pearce, responding to an element will generalize from the association acquired to the configural cue as a function of the similarity between the element and configural cue. Because tone onset was prior to light onset in the study by Egger and Miller, presentations of the tone alone were more similar to the tone-light compound than were presentations of the light alone, which may explain increased lever pressing supported by the tone as compared to the light.

There remain many open questions regarding the associative nature of conditioned reinforcement, despite its common use in behavioral neuroscience (e.g., Ostlund, 2019; Servonnet et al., 2020; Sharpe et al., 2017, 2019). The present experiment aimed to evaluate the associative structure of a compound cue serving as a conditioned reinforcer for the acquisition of an instrumental response by first pairing a tone-light compound CS with a food US, followed by using the tone-light compound to conditionally reinforce lever pressing. Unlike the study by Egger and Miller (1962), in our study, the tone-light compound shared a common termination. This design allowed us to directly investigate the extent to which stimulus generalization and discrimination transfers from initial Pavlovian training to conditioned reinforcement. The experiment consisted of three groups. During training, group compound and group element received presentations of compound AX+, a tone and a light respectively, followed by a sucrose pellet, whereas group control received presentations of BX+ (where B was a white noise—tone and white noise were counterbalanced in their roles as A and B, respectively). In the test phase, a lever that had not previously been available was inserted into the operant chamber. Each single lever press resulted in the delivery of the trained compound AX for group compound and only A for other elements and control (see Figure 1). We predicted group compound to show a high rate of lever pressing because it exactly matched the compound CS used in Pavlovian training. We predicted a low rate of lever pressing by group control, which had been trained on BX but tested on A, which was a novel cue for the rats in this group. This controls for possible sensory reinforcement effects of A on lever pressing (N. Winterbauer, personal communication, May 29, 2011). Finally, for group element, we predicted fewer lever presses than group compound given that this group was trained with AX+, but only one element was presented, thereby resulting in generalization decrement, but we had no a priori expectations of the magnitude of this decrement.

**Method**

**Subjects**

Twenty-four female Long-Evans rats (Rattus norvegicus) acquired from Envigo served as subjects. Subjects were approximately 90 days old at the start of the experiment. Subjects were pair housed in transparent plastic tubs with a wood shaving substrate in a vivarium maintained on a reverse 12-hr light cycle. Experiments were conducted during the dark portion of the cycle 7 days a week. A progressive food deprivation schedule was imposed prior to the beginning of the experiment to maintain rats at 85% of their initial free-feeding weights. Water was always available in their home cages. The procedures used in this experiment were conducted under approval and following the guidelines established by the Institutional Animal Care and Use Committee of University of California, Los Angeles.

**Apparatus**

This experiment was conducted using 16 experimental chambers, measuring 30 cm long × 25 cm wide × 20 cm high. Each chamber was housed in separate sound- and light-attenuating environmental isolation chests (ENV-008, Med Associates, Georgia, VT). The front and back walls and ceiling of the chambers were constructed of clear Plexiglas, the side walls were made of
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**CS A (groups element and control).** Resulted in a 10-s presentation of either AX (group compound) or just CS A (groups element and control). Note, group element received only the auditory cue compound onset together and coterminated.

**US.** A 2-min variable ITI separated trials. The two elements of the compound were presented, it was perceived as being different enough from the background to serve as CS A and CS B, counterbalanced within groups. Two white LED lights were located on the left chamber wall, 6 cm from the ceiling. These lights were located above and to either side of the pellet dispenser. The light was counterbalanced across subjects. One of the lights was flashed at a rate of 2 Hz and served as CS X for each subject. The other light was visible but off during the sessions. A 62-dB background noise was produced from the ventilation fans.

**Procedure**

On Day 1, rats were trained to eat pellets from the cup by delivering one pellet every 20 ± 15 s. Actual intertrial interval (ITI) values = 5, 10, 15, 20, 25, 30, and 35 s.

On Day 2, all rats received four presentations each of CS A, CS X, and CS B without the US to habituate any unconditional orienting behaviors. The duration of each stimulus presentation was 10 s with a 2-min variable ITI.

On each of Days 3–6, all rats received daily conditioning sessions lasting 76 min. In each session, rats received 32 AX+ (groups element and compound) or BX+ (control) presentations, with each presentation being preceded by a 10-s pre-CS period. Each compound CS was 10 s in duration and was followed immediately upon termination with the delivery of one sucrose pellet US. A 2-min variable ITI separated trials. The two elements of the compound onset together and coterminated.

On Days 7–8, rats received 30-min training sessions to press a lever for conditioned reinforcement by the presentation of compound CS AX (group compound) or just CS A (groups element and control). Note, group element received only the auditory cue from the audio-visual compound on which it had been trained. In this phase, the novel lever was always available. Each lever press resulted in a 10-s presentation of either AX (group compound) or CS A (groups element and control).

**Results**

Acquisition to the compound cues proceeded normally (see Figure 2a). An elevation score was calculated by subtracting the number of magazine entries made during the 10-s CS period from the 10-s pre-CS period on each trial. A mixed-measures analysis of variance (ANOVA) conducted on mean elevation scores with group as the between-group factor and session as the repeated measure revealed a main effect of session, F(3, 63) = 16.803, MSE = 20.829, p < .001, η² = .176, but no main effect of group, F(2, 21) < 1.0, and no group by session interaction, F(6, 63) = 1.537, MSE = 1.905, p = .181, η² = .032. There was no effect of session, group, or session by group interaction for entries made during the pre-CS period (lowest p = .199). Of most interest to the aims of this study was lever responding to eliciting cue presentation during lever press training. The sum of lever pressing during both 30-min test sessions is presented in Figure 2b. A one-way ANOVA conducted on total lever presses with group as a factor revealed a main effect of group, F(2, 21) = 8.406, MSE = 3327.167, p = .002, η² = .445. Planned comparisons showed a significant increase in lever pressing in group compound relative to group element, t(21) = 3.242, p = .010, Cohen’s d = 1.346, and to group control, t(21) = 3.795, p = .003, Cohen’s d = 1.656. Interestingly, there was no difference in total responses between group element and group control, t(21) < 1.0. Finally, we compared lever pressing during the first and last 5 min of the conditioned reinforcement procedure because it has been suggested that conditioned reinforcement can extinguish quickly (Winterbauer, personal communication). These data are depicted in Figure 2c. A mixed-measures ANOVA revealed a main effect of time (first 5 min vs. last 5 min), F(1, 21) = 5.708, MSE = 157.687, p = .026, η² = .056, and a main effect of group, F(2, 21) = 10.352, MSE = 504.521, p < .001, η² = .359, but no interaction between time and group, F(2, 21) < 1.0. Lever pressing was significantly higher for group compound relative to group element and group control in the first and last 5 min (all ps < .05). There was no difference in responding between group element or group control during either time period (lowest p = .721). Thus, conditioned reinforcement was specific to the compound cue that was used in Pavlovian training. Instrumental responding was not supported by an element of the Pavlovian compound nor by an entirely novel cue.

**Discussion**

We found that a previously trained compound cue would support acquisition of an instrumental lever press, while one of its elements would not. Moreover, an element of a previously trained compound was not any more effective than a novel cue in supporting the acquisition of instrumental lever pressing. One explanation of these results is that rats learned the compound as one configurual cue; consequently, when just one element of the compound was presented, it was perceived as being different enough from the configurual cue to produce substantial generalization decrement (Pearce, 1987). Another explanation is that rats had learned elemental associations to food, with one element potentially overshadowing behavioral control by the other. In the present experiment, it is possible that the light overshadowed conditioning to the tone and therefore attenuated the tone’s effectiveness in supporting conditioned reinforcement. There is some evidence of visual cues dominating over auditory cues in appetitive learning (Foree & LoLordo, 1973; Schindler & Weiss, 1982; Weiss et al., 1993; but see Sanderson et al., 2016). Thus, it may be necessary to control for overshadowing when compound cues consisting of elements from different modalities (e.g., visual and auditory) are used in studies of conditioned reinforcement.

Most elemental accounts of Pavlovian conditioning (e.g., Rescorla & Wagner, 1972) predict the total amount of associative strength to be shared across the individual elements of a compound, and thus these accounts cannot easily explain why group element showed no greater conditioned reinforcement than the control group. Unique-cue versions of elemental theories of Pavlovian conditioning have been developed to account for some
learning phenomena claimed to be unique to configural theory (see Ghirlanda, 2015). According to a unique-cue model, it is possible that associations may have formed to three unique components during compound conditioning of the audiovisual: the tone, the light, and the combination of the tone and light. Assuming associative strength is equally distributed across each of these three components, group element is expected to show relatively little conditioned reinforcement relative to group compound. Further studies are needed to dissociate these accounts, but to our knowledge, this is the first attempt to use a multisensory simultaneous Pavlovian compound as a conditioned reinforcer, instead of sequential Pavlovian cues as have been used previously (e.g., Egger & Miller, 1962).

While there is still debate (see Alvarado & Rudy, 1992; Williams et al., 1994) about the conditions that promote learning about elements versus forming configural representations when multiple cues are presented in compound during Pavlovian conditioning, Rescorla (1981, 1982) argued that in some circumstances, simultaneous presentation of two cues may allow the formation of a unitary representation of both cues as a configural whole (see also Pearce, 1987). It is possible that when cues are presented simultaneously, there is no opportunity for the subject to learn about the stimuli as being independent of each other. This explanation seems unlikely in our present study, however, because all the stimuli were presented a few times individually during the habituation phase. If a unique configural representation was formed, it was after having had the opportunity to experience each cue as a separate unit from the compound.

Investigations of associative structure are relevant not only for learning theory but also for understanding how nervous systems represent stimuli and their role in neural mechanisms of learning. Configural and elemental frameworks of associative learning are still relevant today because we still do not have a principled understanding of stimulus generalization. Furthermore, even though configural and elemental models have their theoretical value, simulations of these models can mimic each other. For this reason, it

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**Figure 2**

*Experimental Results*

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**Note.** Shaded error bars (Panel a) and column error bars (Panels b, c) are measured in standard error of the mean. Panel a: Pavlovian conditioning was observed and did not differ between groups. Panel b: Total lever presses over two 30-min sessions of conditioned reinforcement. Panel c: Total lever presses during the first and last 5 min of conditioned reinforcement. CS = conditioned stimulus. *p < .05. **p < .01. ***p < .001. See the online article for the color version of this figure.
is difficult to find evidence that unambiguously supports a configurational versus an elemental account (Ghirlanda, 2015). Nevertheless, in the present study, we chose a compound formed by two cues of different sensory modalities in an effort to make their integration as one element of an auditory and visual characteristic more difficult. Finally, there has been little study of how conditioned reinforcers are encoded and how they support instrumental responding. For example, is the rate of learning (e.g., alpha in associative models) the same for conditioned reinforcement as it is for responding. For example, is the rate of learning (e.g., alpha in associative models) the same for conditioned reinforcement as it is for associative learning, and is the associative strength (i.e., \( V \)) gained from conditioning proportional or equivalent to a cue’s ability to facilitate conditioned reinforcement?

In recent years, the conditioned reinforcement procedure has been extensively used in behavioral neuroscience research to assess whether various behavioral, surgical, or optogenetic manipulations have endowed cues with value and/or reward (e.g., Ostlund, 2019; Servonnet et al., 2020; Sharpe et al., 2017, 2019). Optogenetics is a method used to temporarily increase or decrease the activity of neurons in freely moving animals (Deisseroth et al., 2006). Optogenetic manipulation uses light to activate or deactivate neurons that have been tagged by specific viruses. One of the main advantages of this technique is the flexibility to administer the light stimulation without damaging the brain and to activate or deactivate targeted brain regions with rigorous temporal specificity. Nevertheless, unless strict precautions are taken, the laser can be visible from outside the head and thus perceived as a stimulus by the animal. Indeed, there is evidence that the administration of the light can be perceived internally by the animal (for an example on optogenetic self-administration, see Cole et al., 2018). As a result, the presence (or absence) of visible laser light may contribute to the behavioral responses recorded to study the functional role of the neural target of laser stimulation.

For example, if a rat receives Pavlovian conditioning and the laser is then used to activate or deactivate a target population of neurons, the presence or absence of the light might become part of the stimulus configuration to which the animal has learned to respond. Let us say a rat receives laser stimulation during the presentation of an auditory CS on trials in which it is paired with a food US. Then, the auditory CS is tested alone without the US, and without laser stimulation, to test if optogenetic activation or deactivation during training affected conditioning to the auditory CS. If the rat was able to see the laser light during the initial conditioning, then a light-auditory compound cue may have been conditioned, rather than an auditory cue alone. Testing on the auditory cue alone, which is only one element of the compound cue, could potentially result in generalization decrement. Indeed, audiovisual compounds have been shown to result in generalization decrement when only the auditory element is presented at test (e.g., Bouton et al., 2012; Fast et al., 2016). Thus, rather than some brain region having been manipulated during training, responses might be altered at test. For instance, the auditory cue in the absence of the light cue provided by laser stimulation could result in generalization decrement, thereby attenuating the auditory cue’s ability to serve as a conditioned reinforcer to support acquisition of a new instrumental response. A similar argument could be made in terms of the laser light overshadowing the auditory cue if both are present during conditioning. Fortunately, these confounding effects can be circumvented by including a control group in which animals receive laser activation at the same time as the experimental group (i.e., during cue or reward presentation), but in animals injected with a virus lacking the genetic material activated by light. This is not currently standard practice as it is also common for a control group to receive laser stimulation during the ITI instead of during cue or reward presentation. The results reported here advocate for the former method and caution against the latter.

Overall, conditioned reinforcement has been central to learning theory, and a useful tool for neuroscience research, despite the relatively limited knowledge about its mechanisms. Here, we found that animals responded more to a trained compound cue when both elements were presented during conditioned reinforcement training than when only one cue was presented. It is currently unresolved whether animals had learned a configurational or an elemental association.

One way to explore cue-competition effects in conditioned reinforcement would be to include a group in which the compound cue is formed by elements of the same sensory modality, such as two auditory cues or two visual cues. It could also be the case that our results were due to the simultaneous presentation of the compound cue, making the discrimination between the two elements harder, in which case a sequential presentation, as in the studies by Egger and Miller (1962), could lead to different results.

We also suggest that optogenetic research take care to include proper controls for the potential of generalization decrement or overshadowing as training and testing situations may differ perceptually—for instance, by including a control group that maintains the same schedule of laser activation as the experimental group. Taken together, the results of this simple experiment emphasize that there is still much to be learned about the basic mechanisms of learning and behavior and that further understanding of these mechanisms is critical to the continuation and expansion of behavioral neuroscience research.

References


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