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Augmentation's boundary conditions? Investigation of spatial contiguity, temporal contiguity, and target flavor familiarity

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ABSTRACT

When a preconditioned flavor (A) is conditioned in compound with a novel target flavor (X), the aversion to the target X is increased; this enhanced aversion to X is called *augmentation*. In 6 experiments with rat subjects, we manipulated the spatial contiguity of cues during compound conditioning (AX+), the temporal contiguity of cues during compound conditioning (AX+), and the familiarity of the target. In all 6 studies, augmentation was recorded with spatially separated flavors. In Experiments 2–4, augmentation was not detected if the two flavors were temporally discontiguous, but augmentation still occurred if the cues were partially contiguous (i.e., the flavors co-occurred for 2 min of a 4-min exposure). Even though stimulus preexposure can often weaken subsequent conditioning, augmentation was observed following 1 or 4 preexposures to the target taste (Experiment 5A) or target odor (Experiment 5B). In sum, manipulations that should weaken, but not eliminate, the within-compound association formed during AX+ conditioning did not prevent augmentation, suggesting the robustness of the within-compound association when one of the elements is a preconditioned flavor.

1. Introduction

Taste-aversion learning is a form of classical conditioning in which a flavor conditioned stimulus (CS) is followed by an illness-inducing unconditioned stimulus (US). Subsequent presentations of this flavor CS may elicit a conditioned response (CR) of nausea or an avoidance of consumption of that flavor. This type of learning has obvious adaptive value for foraging animals that must avoid consumption of toxic meals, and indeed, taste-aversion learning has been demonstrated across the animal kingdom.

In classical conditioning, when two CSs are presented in compound before a US, it is commonly thought that these CSs will compete with each other for associative strength and thus yield a weaker conditioned response (CR). One notable example of this type of cue competition is the blocking effect introduced by Kamin (1969). In Kamin's blocking design (henceforth, the A+/AX+ design), CS A (e.g., tone) was paired extensively with a shock US in phase 1 before being conditioned in compound with a novel CS X (e.g., light) during phase 2. During testing, the CR to CS X was significantly reduced, and Kamin proposed that preconditioning of CS A 'blocked' subsequent learning to CS X because it provided no new information about the US. Subsequent work has demonstrated blocking across a range of classical conditioning preparations and species (e.g., Prados et al., 2013). Indeed, all current models of associative learning (e.g., Miller & Matzel, 1988; Pearce & Hall, 1980; Rescorla & Wagner, 1972) have incorporated an explanation for blocking.

Although the inclusion of explanations of blocking within these associative learning models might suggest it is a ubiquitous phenomenon, recent reports suggest this is not the case. For example, Maes et al. (2016) published a report containing 15 unsuccessful attempts to replicate blocking experiments. Moreover, in other situations, conditioning within the A+/AX+ design does

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not yield competitive conditioning, but instead, it produces synergistic or facilitative conditioning. Using flavor-conditioning methodology, our lab has shown that when flavor A (taste or odor) is preconditioned, and then conditioned in compound with a novel flavor X, the subsequent aversion to X is significantly stronger than the aversion to X of rats that only experienced AX+ conditioning (e.g., Batsell & Batson, 1999; Batsell, Paschall, Gleason, & Batson, 2001; Batson & Batsell, 2000). We have used the term *augmentation* to describe the facilitative learning to X produced by A+/AX+ conditioning.

It is not presently known why the A+/AX+ design sometimes yields competitive effects, no effects, or facilitative effects, but the presence of a within-compound association appears necessary to produce augmentation. Through experiments that preexposed Flavor A before A+ conditioning, extinguished Flavor A between A+ and AX+ conditioning, or extinguished Flavor A after A+/AX+ conditioning (Allswede, Curley, Cullen, & Batsell, 2014; Batson & Batsell, 1999; Batsell et al., 2001), we have shown that augmentation requires an intact aversion to Flavor A at the time of AX+ conditioning and testing, and it appears dependent on the existence of an A-X within-compound association. Yet, the mere *presence* of a within-compound association is not responsible for producing augmentation because within-compound associations have also been reported between Stimuli A and X after A+/AX+ training that should yield blocking (e.g., Esber, Pearce, & Haselgrove, 2009; Rescorla & Durlach, 1981; Speers, Gillan, & Rescorla, 1980). Similar to an argument proposed by Pearce, Nicholas, and Dickinson (1981), Allswede et al. speculated the augmented aversion is due to the *strength* of the A–X within-compound association counteracting the expression of the competitive A −US and X – US associations at the time of testing. Therefore, the strength of the within-compound association may be the important contributing factor if procedures that foster formation of a strong A–X within-compound association are the only situations that produce augmentation. To date, examinations of flavor-mediated augmentation have employed procedures to maximize formation of a strong within-compound association, and thus, the detection of augmentation (Allswede et al., 2014; Batsell & Batson, 1999; Batsell et al., 2001; Batson & Batsell, 2000; Good, Allswede, Curley, & Batsell, 2015). Specifically, some of these procedures include: 1) mixing the flavors together in a single bottle (spatial contiguity), 2) simultaneously presenting both flavors for the entire duration (temporal contiguity), and 3) testing a novel target flavor (familiarity). Conceivably, manipulation of each of these factors should weaken the A–X within-compound association, and thus, may eliminate augmentation. Therefore, the purpose of the present series of six experiments was to manipulate the three variables listed above to explore the boundary conditions of augmentation.

2. Experiment 1: spatial contiguity

We have shown that separate, sequential flavor presentations during the AX+ phase do not yield augmentation (cf., Experiment 1, Batsell & Batson, 1999; Experiment 3, Batsell et al., 2001), but in those studies, these groups differed in both spatial contiguity and temporal contiguity of the flavors. Therefore, the purpose of Experiment 1 was to determine if a contiguous, but separate, presentation of the preconditioned flavor and the target flavor during compound conditioning eliminates augmentation. Experiment 1 used a 2 × 3 design with the factors of conditioning [control conditioning (AX+) vs. augmentation training (A+/AX+)] and mode of stimulus presentation during AX+ conditioning [spatially and temporally discontiguous, mixed together, or separate-simultaneous presentations].[1] The key group simultaneously received the two flavors in separate tubes (SEP), and it was compared to a group that had the flavors mixed in a single tube of solution (MIX) and a control group that experienced sequential presentation (SEQ) of the flavors. Based on previous augmentation work, we expected that the MIX group would show a strong, augmented flavor aversion compared to non-preconditioned controls, but no differences would be seen across the SEQ groups. The question of interest was whether the preconditioned Group SEP would also demonstrate augmentation.

2.1. Method

2.1.1. Subjects

Subjects were 60 naïve male rats of the Holtzman breed (*Rattus norvegicus*). All rats were purchased from Harlan Spraque-Dawley Corp. in Indianapolis, Indiana when they were approximately 100–125 g of weight. After arrival at the laboratory, rats were housed in groups of three until they had all reached a weight of 250 g or higher (weight range at start of study was 299–385 g). At that time, they were moved to single cages for the entirety of the experiment. Rats were maintained on a diet of ad lib. access to LabDiet 5001 rat chow (TestDiet, Brentwood, MO). Room temperature tap water was provided ad lib. until rats were moved to single cages. After being moved to single cages, the rats were switched to a daily 20-min drinking period during which they were provided access to 40 ml of water for 20 min per day at 1000 h. A light:dark cycle of 12:12 h was implemented beginning daily at 700 h. All procedures were approved by the Kalamazoo College Institutional Animal Care and Use Committee (IACUC), and all rats were cared for following guidelines of the American Psychological Association.

2.1.2. Materials

Target stimuli used for this experiment were presented in the form of fluid solutions with a distinguishable taste or odor characteristic. The taste stimulus was sodium saccharin mixed with water for a 0.15% concentrated solution (SAC) [Sigma Chemical Co. (St. Louis, MO)]. The odor stimulus was Adams almond odor extract (Austin, TX) mixed with water for a 0.01% concentrated solution

[1] In flavor experiments in which the organism chooses when and how long to interact with the stimuli, it is particularly difficult to insure simultaneous and separate presentations of the flavors. Nonetheless, for the purposes of these experiments, we are using the term "temporal contiguity" in situations when both flavor tubes were affixed to the cage at the same time, regardless of the animal's behavior.