

The highlight for February is by Dr. Alan Spector, Professor of Psychology and Neuroscience at Florida State University in Tallahassee. As Dr. Spector notes in his highlight, his association with aversion learning dates back to his early work with Jim Smith at Florida State on radiation-induced aversions in humans and the analysis within an animal model of the various factors that might impact such learning. His graduate work focused on a host of issues in aversion learning, two of which he describes as important in his training for his later work in animal psychophysics, i.e., the individual variation in behavioral output and the dissociation among multiple measures of a common phenomenon. Interestingly, neither of these issues has been resolved in taste aversion learning, although both issues remain important to understanding behavioral processes associated with conditioning and learning. From his graduate work with Smith, Dr. Spector joined Harvey Grill at the University of Pennsylvania where he utilized the taste reactivity assay developed by Grill and Norgren to examine specific mechanisms that might underlie the failure of animals with lesions of the parabrachial nucleus to display aversive reactions to intraorally-infused saccharin that was paired with LiCl. Dr. Spector notes the various interpretations associated with any failure to display such reactions and described his work that demonstrated that the failure in such lesioned animals was likely a function of the failure to acquire the taste-LiCl association. Dr. Spector's more recent work has focused on the use of the taste aversion design as a tool to examine issues related to taste psychophysics. He describes several findings from his laboratory in which the taste aversion procedure was used to carefully and systematically assay the taste characteristics of a number of stimuli, e.g., amiloride hydrochloride, l-serine and l-threonine. Dr. Spector's work has itself been characterized by thoughtful and methodologically sound science that integrates behavior and basic neurobiology, an approach nicely illustrated in his 2000 review "Linking gustatory neurobiology to behavior in vertebrates" in Neuroscience and Biobehavioral Reviews (24, 391-416).

The Lessons I Learned from Taste Aversion Conditioning Alan C. Spector

My phenomenological foray into the world of taste aversion learning began with a stomach virus when I was a child followed later in life by a salient taste-related visceral experience as a college student. My scientific introduction to the topic began when I met Dr. James C. Smith when he was a visiting Professor at Penn State University and with whom I eventually did my graduate training at Florida State University. At the time, Dr. Smith had a grant from the National Institutes of Health aimed at examining whether the decreases in food intake observed in cancer patients receiving radiotherapy could be partially explained by the formation of learned taste aversions occurring during treatment (see 14). My role was to work on a rodent model in which we would establish basic parameters of conditioning and explore how aversions could be systematically altered by manipulating various aspects of the conditioning trial. Although the animal work did not necessarily produce novel conceptual breakthroughs because many of the principles of taste aversion conditioning such as conditioned stimulus (CS) and unconditioned stimulus (US) pre-exposure effects, CS-US delays and US dose effects had already been established in general in the preceding decade, it did produce some interesting outcomes that have had a significant influence on my thinking about behavioral experiments throughout much of my professional life. One such finding was that after a given conditioning trial, the mean CS preference on the following days displayed an orderly monotonic increase representing the extinction of aversion, but this curve belied the actual trajectory of the individual animals among which there was tremendous variability (we had very large sample sizes of $n=32$). Some animals extinguished the aversion rapidly as if there was some type of extinction "epiphany" that occurred. Other animals never extinguished and then there were

many curves of different types in between these two extremes (18). Thus, extinction of an aversion was not a uniform process that could easily be explained as a gradual “unlearning” due to incremental post-conditioning exposure to the CS. As far as I know, the basis for such individual differences remains to be completely understood (but see 19), but observing this remarkable variability taught me that there is probably more to be learned in the variance than there is in the mean. It established a data analysis perspective which figured prominently in my animal psychophysical work later in my career.

The second finding that had an impact on my general scientific view on behavior was the fact that when the aversion was assessed through different means such as one-bottle tests, two-bottle tests, intake, preference, extinction rate, etc., the measures correlated only modestly (18). Thus, these measures were not necessarily assessing exactly the same thing, yet at the time they were all very common ways for quantifying the strength of a conditioned taste aversion (CTA) and remain so today. Since that time, I have seen many examples in my behavioral work in which different measures of an effect of a given manipulation do not necessarily correspond with each other. For some, this begs the question of which measure is best, but it is perhaps more useful to focus on why and how these measures differ, because addressing those questions has the potential to reveal some fundamental principles of the behavioral process of interest.

I received my postdoctoral training in Harvey Grill’s lab at the University of Pennsylvania. When Harvey was a postdoc with Ralph Norgren at Rockefeller, he had developed the taste reactivity paradigm in which intraoral cannulae are chronically implanted in rats and taste solutions are infused under experimenter control (7; 8). Rats and other mammals will display stereotypical reflex-like oral motor responses that depend on the chemical solution and concentration infused. Two classes of these oral motor responses have been defined: ingestive and aversive. Ingestive responses are elicited by normally preferred solutions such as sucrose in a concentration-dependent manner and include behaviors such as tongue protrusions, lateral tongue protrusions and mouth movements. Aversive responses are elicited by normally rejected solutions such as quinine also in a concentration-dependent manner and include behaviors such as gapes, chin rubs, head shakes and forelimb flails. The taste reactivity paradigm was developed as a means to assess taste function in severely compromised neural preparations. For example, Harvey and Ralph showed that although the supracollicular decerebrate rat, which has the forebrain neurally isolated from the rest of the nervous system, could not spontaneously eat and drink (these animals must be nourished by gavage), they will still display relatively normal taste reactivity behavior (8).

Interestingly, if an aversion to sucrose is conditioned in intact animals, the taste reactivity profile changes from ingestive to aversive. Although intact rats will reliably exhibit this switch, Harvey and Ralph showed that decerebrate rats will not, even after multiple conditioning trials, nor will they express a presurgically conditioned aversion (6). Following up on prior work conducted by Pat DiLorenzo at SUNY Binghamton (1), Bill Flynn, a fellow postdoc in Harvey’s lab, in collaboration with Harvey, Ralph and Jay Schulkin, demonstrated that lesions in the gustatory zone of the parabrachial nucleus (PBN), the second central relay in the ascending gustatory system, similarly eliminated acquisition of a CTA as assessed by taste reactivity (5).

I mention this because it set the stage for some of the taste aversion experiments that I conducted with Harvey. Any student of the taste aversion literature understands that there are several different reasons why a given neural manipulation could disrupt taste aversion acquisition. First, the animal might be unable to process the conditioned stimulus. In other

words, the animal cannot detect or recognize the taste stimulus. Second, the animal might be unable to process the visceral signals associated with the delivery of the unconditioned stimulus. Put simply, the animal might not “feel sick”. Third, the animal might be unable to display a conditioned response. For example, perhaps an animal is unable to gape. Fourth, the animal might have disrupted memory and be unable to recall the aversion when subsequently tested. Finally, if the other four possibilities can be dismissed, then it is likely that the animal is unable to integrate taste with visceral signals to form an association. I, along with Paul Breslin and Harvey, became interested in using the taste reactivity paradigm to help dissociate these possible explanations for lesion-induced deficits in taste aversion acquisition.

We reasoned that if we inject rats with LiCl, a commonly used unconditioned stimulus, and then probe them with very brief periods of intraorally delivered sucrose infusion (30 s) on some periodic schedule, we might be able to see changes in taste reactivity as the LiCl is taking effect and that this could potentially serve as a reliable and quantifiable assay of the animal’s ability to “feel sick”. Indeed, rats injected with LiCl at time 0 and then given 30-s sucrose infusions every 5 min thereafter for 30 min displayed clearly ingestive taste reactivity behavior for the first 10 min, but then at 15 min ingestive behavior started to drop and aversive behavior started to increase. At 20 min, ingestive behavior had dropped precipitously and the response to sucrose was primarily characterized as aversive. Control rats injected with NaCl maintained a highly ingestive profile throughout the 30-min session. It was an amazing thing to behold to see the changes in the LiCl-injected rats (15).

However, as is often the case in science, especially behavioral science, things aren’t always as they first appear. It turned out that if rats were injected with LiCl at time 0 and then given the 30-s sucrose infusion starting 20 min after the injection, the animals displayed a normal ingestive behavior profile with virtually no aversive responses. This is true despite the fact that they could often be observed to have signs of gastrointestinal distress such as diarrhea. Thus, this observation suggested that the sucrose infusions that occurred in the original experiment before the 20-min mark were critical to the generation of the aversive profile observed at 15-20 min post-LiCl and beyond. This outcome provided strong evidence that the development of an aversive response profile to sucrose at 15-20 min after LiCl injection when the 30-s taste stimulus infusions started at time 0 and continued every 5 min thereafter was due to a rapid conditioning process. Consequently, the paradigm was not particularly useful for assessing the ability of the animal to *unconditionally* process visceral signals associated with visceral malaise. However, it was useful for measuring the rapid acquisition of a CTA and, therefore, had potential utility in the assessment of whether any lesion-induced impairment in taste aversion expression was due to the animal “forgetting” the aversion when tested the next day (or longer).

Accordingly, in collaboration with Ralph Norgren, Harvey and I sought to examine whether the deficit in CTA acquisition caused by PBN lesions could be explained by some type of memory impairment by which animals with such brain damage could acquire a CTA but such associations decayed by the time the animals were tested 24 hours (or more) later. The results were very clear. Rats with PBN lesions did not display the change in taste reactivity to either sucrose or NaCl over a 30-min test period that could be seen in sham-operated rats when injected with LiCl at time 0. This finding, coupled with others, strongly suggested that the lesions centered in the gustatory zone of the PBN were interfering with the animals’ ability to actually form an association between the taste CS and the visceral malaise US (17). I went on to examine the ability of rats with PBN lesions to perform competently in several other taste-related tasks with Ralph, Harvey and Sue Grigson. Ralph and his colleagues, including Sue and Steve Reilly, have continued studying the effect of PBN lesions on CTA as well as on other

behavioral tasks such as stimulus contrast and have extended their analysis to include lesions in other loci along the gustatory neuraxis (see 10).

All the studies that I have mentioned up to this point were focused on understanding something about the conditioning process. In other words, they were conducted in the service of understanding something about CTA itself. However, CTA has been successfully exploited by researchers as a tool to make inferences about taste perception in animal models (e.g., (11; 12; 21)). My laboratory has found the CTA procedure very useful in this regard (3; 4; 9; 16; 20). For example, Stacy Markison importantly demonstrated that amiloride hydrochloride, an epithelial sodium channel blocker known to suppress neural responses to sodium salts, was an ineffective CS in a CTA paradigm in rats (9). Shachar Eylam replicated that result in both C57BL/6J and DBA/2J mice (4). Those findings strongly suggested that amiloride was tasteless to these animals (or at least it was very weak) and this conclusion tremendously simplified the interpretation of behavioral effects caused by the addition of the drug to taste solutions.

Shawn Dotson used the CTA procedure to examine generalization profiles in mice that were conditioned to avoid either sucrose, L-threonine or L-serine (2). The latter two compounds are considered to be sweet-tasting amino acids to mice, but using an operant taste discrimination procedure, Shawn demonstrated that mice (C57BL/6J) can distinguish sucrose from both of those L-amino acids. Thus, Shawn wanted to examine whether groups of mice that had aversions separately conditioned to one of those compounds would cross-generalize their avoidance to the other two compounds and whether any “side-band” generalization would occur to NaCl (“salty”), citric acid (“sour”) or quinine (“bitter”). Aversions conditioned to L-threonine and L-serine generalized to sucrose. L-serine aversions also generalized somewhat to quinine suggesting that the amino acid has a “bitter” component in addition to its “sweetness” (incidentally, I use the scare quotes because I am reluctant to ascribe human adjectives to the immeasurable perceptual experience of the animal, but do so here out of convenience). Interestingly, sucrose aversions did not cross-generalize to the L-amino acids. The asymmetrical aversions could not easily be explained by intensity generalization decrements because both a high and a low concentration of each test compound were included. Shawn concluded that although L-threonine and L-serine do share some perceptual similarity with sucrose, they appear to possess additional orosensory properties that allow mice to distinguish them from the prototypical “sweetener”. These data provide a context for evaluating molecular and neurobiological findings associated with gustatory function in the mouse model.

As a methodological side note, Shawn conditioned the aversions in 15-min single bottle presentations in the home cage, but the generalization test was conducted in a lickometer referred to as the Davis Rig (13). This device permits the presentation of up to 16 stimuli in very brief trials. The trials are generally delivered in randomized blocks (although a variety of stimulus presentation orders are programmable). The fact that trial duration is on the order of seconds (for Shawn’s experiment it was 5 s) and immediate responses are measured, increases the confidence that the response is driven by the orosensory properties of the stimulus. In Shawn’s experiment he was able to test 13 taste stimuli plus water and, as such, he was able to obtain a complete generalization profile from a mouse in a single 25-min session.

In retrospect, the CTA paradigm was a wonderful platform on which to learn about experimental design and the analysis of animal behavior. Although I have benefited from the collective wisdom and training from many teachers and colleagues (and continue to) over my years as an academic scientist, three individuals stand out in this regard. Jim Smith taught me to let the data

lead the way. His guidance through the very formative years of graduate school left me with a strong understanding of experimental design and an appreciation of maintaining an objective eye when interpreting behavioral outcomes. Harvey Grill provided significant polish and helped me relate my behavioral training to neural analyses. Ralph Norgren taught me the nuances of the gustatory system as well as how to appreciate the concept of motivation from a neural systems perspective. These three outstanding scientists define the template I continue to strive to emulate. Finally, I would be remiss if I did not acknowledge the wonderful contributions of my students, postdocs and technicians, past and current, to my research program. They have been the lifeblood of the lab, full of fresh ideas and energy. They help make the long hours worth it.

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