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Evaluation of Taste Aversion Learning Procedure for the Generalization of Aversion Using Palatable Foods

Evaluación del procedimiento aprendizaje aversivo gustativo para la generalización de la aversión con dos alimentos palatables

Antonio López-Espinoza[1*], Alma Gabriela Martínez Moreno[1], Virginia Gabriela Aguilera-Cervantes[1], Ana Patricia Zepeda-Salvador[1], Ana Cristina Espinoza-Gallardo[1], Fatima Ezahra Housni[1], Samantha Josefina Bernal-Gómez[1], Dalila Santillano-Herrera[1]

[1] Red Internacional de Investigaciones en Comportamiento Alimentario y Nutrición (RIICAN)

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Autor de correspondencia: antonio.lopez.espinoza@gmail.com (A. López-Espinoza)
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Abstract

An experiment to evaluate the effectiveness of the taste aversion conditioning paradigm in the generalization of aversion with palatable foods was performed. Ten experimentally naive females Wistar rats and three types of foods were used: a) standard purina rodent chow, b) vanilla flavor cookies and c) chocolate-flavored cookies. Subjects were divided into two groups, five subjects per group. Using the taste aversion conditioning paradigm protocol, subjects were exposed to the types of food keeping an intake record. The experiment demonstrated that rats can acquire an aversion to palatable foods accompanied by a tendency to generalize, when using elements that share characteristics such as nutritional content and texture. In addition, the intake of a food that does not share the characteristics of texture, taste, smell and energy content with the one used for conditioning was

not been affected. These findings indicate that the aversion generalization seems to depend on the properties of the food used.

Key words: feeding behavior, food aversion learning, palatable foods.

Resumen Se evaluó experimentalmente la efectividad del paradigma de aprendizaje aversivo gustativo en la generalización de la aversión utilizando alimentos palatables. Se utilizaron diez ratas Wistar hembras experimentalmente ingenuas y tres tipos de alimentos: a) purina comida estándar para roedores, b) galletas con sabor a vainilla y c) galletas con sabor a chocolate. Los sujetos fueron divididos en dos grupos, cinco sujetos en cada uno. Usando el paradigma de condicionamiento de aversión al sabor, los sujetos fueron expuestos a los tipos de alimentos manteniendo un registro de ingesta. El experimento demostró que las ratas pueden adquirir una aversión a los alimentos palatables acompañados por una tendencia a generalizarse, cuando se utilizan elementos que comparten características como el contenido nutricional y la textura. Además, la ingesta de un alimento que no comparte características como la textura, sabor, olor y contenido energético como el utilizado para el condicionamiento no se vio afectada. Estos hallazgos demuestran que la generalización de la aversión parece depender de las propiedades de los alimentos utilizados.

Palabras clave: comportamiento alimentario, aversion alimentaria, alimentos palatables

Introduction

Omnivores are animals that consume food of more than one trophic level. Every day they are presented with various food options and the selection of their diet depends on a large number of factors, including food search strategies, nutritional requirements and food availability (Agrawal & Klein, 2000). One of the important factors involved in the omnivore's daily diet selection are food preferences and aversions that the organism has acquired based on individual experiences (Vabo & Hansen, 2014). Diet selection should satisfy nutritional requirements, as well as avoid the ingestion of substances that endanger health and life.

Because of this, foods that cause gastrointestinal discomfort and nausea are quickly rejected. A neutral taste can become aversive when its consumption is followed by gastrointestinal discomfort (Deutsch, Molina & Puerto, 1976). This phenomenon can be explained through the taste aversion learning that is part of the classic conditioning paradigm (Staddon, 2016). In classic or Pavlovian conditioning, the flavor of the food is the conditioned stimulus and the subsequent gastrointestinal discomfort to the unconditioned stimulus, the aversion to the taste is the conditional response and is manifested by rejection or reduction on the food intake (conditioned stimulus) in subsequent encounters (Lin, Arthurs, Amodeo & Reilly, 2012; Roman, Lin & Reilly, 2010). This is considered a defense mechanism, since it prevents the ingestion of a poison or toxin a second occasion even though it's within a palatable food. The taste not only produces rejection or diminished intake, it also becomes an unpleasant taste in being associated with discomfort (Arthurs, Lin, Amodeo & Reilly, 2012).

In everyday life, foods that generate an aversion can be poisonous, therefore, conditioned aversion is a learning mechanism that ensures that food is not ingested again. In the laboratory, for the purpose of having an adequate experimental control, for the study of taste aversive learning, substances like lithium chloride are used, which administered through an intraperitoneal injection causes gastrointestinal discomfort, and when related to the consumption of a particular flavor generates a rejection of that associated flavor (Hishimura, 2000). This method has been used with rats

(Hishimura, 2001); goats (Oliveira et al., 2014); horses (Pfister, Stegelmeier, Cheney, Ralphs & Gardner, 2002) among others.

On the other hand, in a strict sense, all the situations an organism is faced with daily, are new, no environment is totally reproducible, even if it's only due to time as a variable, however, the organisms do not act in front of everyday situations as if they were facing them for the first time. This is possible thanks to the phenomenon of response generalization on classical conditioning, that is the propagation of a contingent behavior effects to other responses similar to the objective response (Phelps, 2011). No doubt, stimuli have different properties and organisms can respond to them separately, generalization refers to the control exerted by a stimulus shared by all its properties, so that if another stimulus has some of those properties it will also exert some control over the response. This control will depend on the properties in common with the objective stimulus and the learning history involved. The generalization allows, then, to emit responses to novel situations due to past experiences (Andreatta & Pauli, 2019; Pérez, 2007). From this, it is possible to hypothesize that in the case of taste aversive learning generalization with palatable foods, the aversion could then be generated by presenting other foods that share similar organoleptic characteristics with the food used to establishment the conditioning.

It is necessary to consider that palatability not only refers to the food 's flavor, is the relationship between the food's organoleptic characteristics and its nutrients and toxins content (Burrit, 2011). This relationship is determined by the organism physiological state in relation to food chemical characteristics. Fat and sweet taste are not only indicators of the energy content of a food, they are considered palatable and preferred flavors, given the existence of an innate preference for the sweet taste of food (Andreatta & Pauli, 2019; Drewnowski, Menella, Johnson & Bellisle, 2012; Rozin & Todd, 2016). However, despite the preference for palatable flavors, it has been described that taste aversive learning generates a reduction in palatability, also referred to as the hedonic value of the food. Palatability reduction can be verified in the laboratory by reducing the consumption of food, which is one of the measures used in the study of taste aversion learning (Lin, Arthurs & Reilly, 2014; Strickland, Austen, & Sanderson, 2018). However, in face of palatable foods preference, and the generation of conditioned aversion to palatable foods, the following question arises: is it possible to establish an aversion generalization towards palatable foods?

Method

Subjects. 10 females experimentally naive Wistar rats were used, within a body weight range of 225-275 gr, from the bioterium unit of University of Guadalajara. Subjects were divided into two groups, five subjects per group. They were housed in individual boxes and kept under a standard light cycle (12 hours light, 12 hours darkness, lights on at 08:00). The records and procedures were performed during the light period. All the procedures in the present study were performed in accor- dance to the principles outlined by the Mexican Official Norm (NOM-062-ZOO-1999), Technical Specifications for the Production, Care and Use of Laboratory Animals.

Apparatus. During the whole experiment the subjects were housed in acrylic room boxes (18 cm x 28 cm x 15 cm), all of them equipped with a metal cage lid top with two compartments for food and water. The boxes kept a wood shavings carpet that was removed every five days. A precision electronic scale was used to record each subject's food intake and body weight.

Foods. Three foods were used: (A) Standard Purina Rodent chow 5001 food containing 23.9% protein, 5% fat, 5.1% fiber and 48.7% nitrogen-free extract, with 3.02 kcal/g of feed. 28.507% of calories come from protein, 13.496% from fat and 57.996% from carbohydrates; (B) vanilla flavor cookies; and (C) chocolate-flavored cookies, both Emperor Gamesa® brand cookies, contain 4.7 kcal/g of food, in addition, 7% of calories come from protein, 38% from fat and 56% from carbohydrates. Foods B and C share characteristics of shape, texture, and nutritional content.

Experimental design. As shown in Table 1, the subjects were randomly divided into group 1 and 2. The experiment consisted of 3 phases, phase 1 was pre-exposure, both groups were exposed to food A, B and C during 15 days. Phase 2 was a conditioning period, group 1

was exposed to food B and group 2 to food C, being in both cases the discriminative stimulus (DS). During this period, they had a daily intraperitoneal injection of a lithium chloride solution (LiCl), as an unconditioned stimulus (US). The dosage depended on the weight of the animal (3.0 mEq/kg, just as it was done in the Martínez, et al 2014, study).

In phase 3, both groups were exposed again to foods A, B and C. Food A worked as a neutral stimulus (NS), while food that was not used during the conditioning process was a delta stimulus (ΔS), being food C for group 1 and food B for group 2. The foods were presented in the feeder contiguously, 24 hours a day during the indicated periods.

The data was processed in SPSS version 21 software and a non-parametric Mann-Whitney U test was performed.

Table 1: Experimental Design

Groups	Phase 1 Pre test	Phase 2 Conditioning	Phase 3 Test
1	A, B, C	CP: B	A, B, C
2	A, B, C	CP: C	A, B, C
Duration	15 d	3 d	15 d

Note. A: Standard food, B: vanilla flavor cookie, C: chocolate flavor cookie, CP: conditioning procedure.

Results

The consumption of food A, B and C was similar in the groups during phase 1 (P <0.974, 0.541, 0.708, respectively). So it is assumed the groups were similar at the beginning of the experiment (see figure 1).

In the case of group 1, during phase 3, an aversion to food B was presented, in addition, this was generalized partially towards food C, since its consumption also decreased during phase 3 although without statistical significance. Something similar happened in group 2, which showed a decrease in the consumption of foods B and C, without statistical significance in the consumption of food B. In both groups the DS was associated with gastrointestinal discomfort caused by lithium chloride. Also, although ΔS shared sensory characteristics with DS, including palatability, aversion was not generalized. NS was not associated with gastrointestinal distress, in both groups the consumption of food A remained similar (P 0.752).

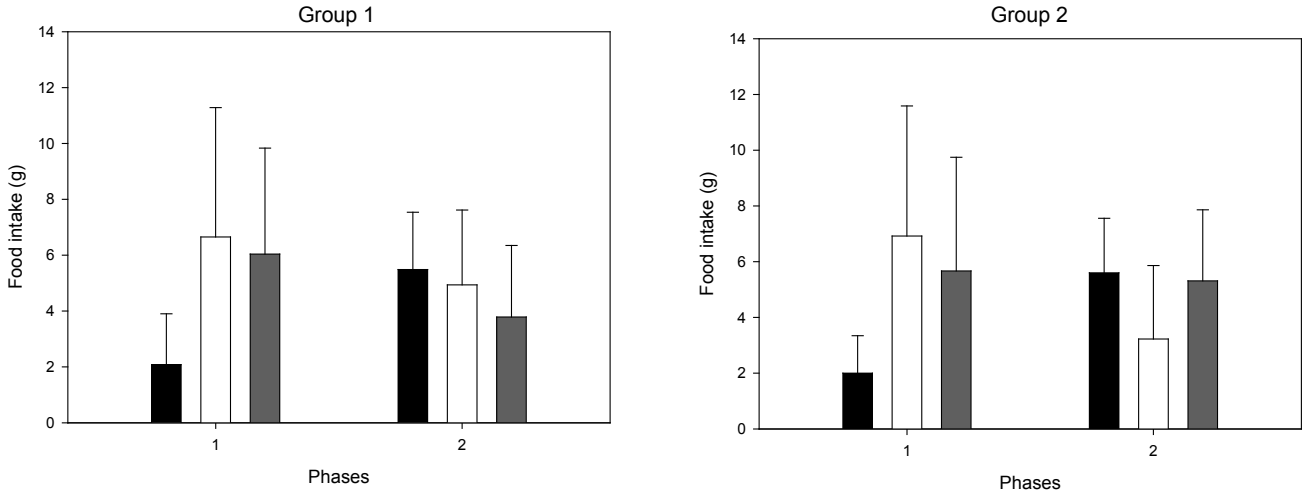
Discussion

The results of the present experiment demonstrated the following: a) rats can acquire a conditioned aversion to palatable foods, b) under the presented experiment conditions the aversion generalization did not occur with palatable foods; c) the consumption of a food that does not share texture, taste, smell and energy content characteristics with the food used for the conditioning is not modified.

The literature indicates like presence of food aversion a significant decrease in its consumption, therefore, it is considered that there was an aversion to the associated palatable food under the conditioning procedure (Burrit & Provenza, 1990). Scientific evidence shows that aversion can be established with palatable foods. The hedonic value of a flavor even with innate preference as the sweet flavor, is potentially modified by post-ingestive factors (Saper, Chou & Elmquist, 2002).

Liang, Smith and Moran (2013) established a food aversion to butter cream in rats through 4 conditioning cycles consisting of a conditioning day followed by two days of standard feed intake, on the conditioning day the rats received access during 15 minutes to a container with butter cream, once the rat consumption was confirmed after 10 minutes the rats received an injection of lithium chloride. Food aversion was confirmed by a significant decrease in the consumption of butter cream. On the other hand, Pfister et al. (2002) generated a food aversion to *Oxytropis sericea* in horses, which is considered a palatable food for horses, however, its consumption is harmful in the long term because it is toxic. Although the horses were previously exposed to the plant, aversion was generated through a conditioning procedure, since they significantly decreased their consumption compared to a control group. In the present experiment there was no generalization of the aversion, since there was a tendency to decrease the consumption of both palatable foods, but the decrease was not significant. Probably the procedure used and

Figure 1
Mean and standard deviation of group food intake. The graphic on the left represents group 1, while the one on the right represents group 2. The black bar represents food A intake, food B is represented by the gray one, and the white one corresponds to food C.



fact of having few subjects had an effect on this result. In contrast, Burrit and Provenza (1990) conducted a study in which an aversion generalization for a palatable food was present. In the study by Burrit and Provenza (1990) lambs were used producing an aversion to the *Cercocarpus montanus* shrub through its association with lithium chloride, and when exposed to the *Amelanchier alnifolia* shrub, they consumed significantly less than the control subjects, showing a generalization of aversion. Both shrubs are considered palatables for lambs.

Although there was a significant decrease in the consumption of palatable foods, there was no total avoidance of consumption, a possible explanation is based in the subject's previous exposure to the palatable foods, so it is assumed that they were familiar foods and it has been documented that a familiar food is less likely to generate a learned food aversion compared to novel foods (Scott, 2011). This has been called latent inhibition, a repeated exposure to a stimulus usually delays the subsequent conditioning and it has been shown that the more immediate the pre-exposure to the stimulus and the conditioning procedure, the greater the degree of latent inhibition (Ishii, Yamada & Hishimura, 2002). Therefore, probably if palatable foods had been novel, the generalization would have presented itself in a more forceful way.

On the other hand, it is likely that the palatability of the foods used influenced the result. Massey and Calhoun (1977) pointed out that palatability is a very important factor for an aversion generalization conditioned by flavor. The less palatable flavors are more susceptible to taste conditioned aversion compared to high palatability flavors. Houpt, Zahorik and Swartzman-Ander (1990) reported that it is more difficult to establish an aversion to highly palatable foods compared to other foods. They established food aversion to various foods such as alfalfa pellets, corn and sweet food on horses. Their results showed that, despite having established food aversion, subjects consumed more of the sweet food compared to the rest. This could be due a greater palatability of the sweet food. In the same way, Martínez, López-Espinoza, de León, Solano and Hernández-Leonardo (2014) reported that after the generation of food aversion through the injection of lithium chloride in 3 occasions, the subjects presented a decrease in the consumption of a sugar solution, however, procedure used is expected to stop the intake completely. The authors argued that this could be due to the characteristics of the food used, which in addition to having a sweet taste, and being palatable, contained energy, so establishing a food aversion had a greater difficulty.

Likewise, another probable explanation to the result obtained in the present experiment is that not only flavor influences food intake, the nutrients contained in food also play a fundamental role in a food acceptance or rejection. In the same way, the differences or similarities of the foods used can facilitate or interfere with the results obtained in this study. However, a relevant fact is that foods that generate satiety will be preferred (Provenza, 1995). In this case the food used was had high energy density and high fat content, and it has been shown that the most palatable foods have this type of characteristics, given that they provide flavor, smell, mouth sensations and hedonic properties (Drewnowsky & Almiron-Roig, 2010).

With respect to the increase in the consumption of standard food that functioned as NS during the post-conditioning phase, it is likely to be due to stimulus discrimination. Discrimination is an important adaptive tool, allowing animals to avoid dangerous or potentially dangerous foods, while allowing them to continue consuming foods they have consumed before without harmful post-ingestive effects (Arriola, Alonso, Vázquez & Rodríguez, 2014). The stimuli are not equally associable, they have different properties and the organisms respond to these properties separately. Thus, stimuli that have different properties can be discriminated. It has been proven in the laboratory that taste, more than any other sensory quality of food, has a significant influence on the generation of aversion (Nachman, Rauschenberger & Ashe, 1977). Therefore, food stimuli, which have a different flavor, will be discriminated and their consumption will not be reduced in the case of conditioning for the generation of a food aversion.

A phenomenon similar to that presented in the present experiment occurs in humans in response to the consumption of the drug Orlistat, that is a gastrointestinal lipase inhibitor has as secondary effect the excretion of dietary fat in the feces. This effect can cause aversion to high fat foods and decrease their hedonic value (Yanovski, 2003).

Several studies have shown that, presented with an increase on a food palatability, humans increase their consumption, that is, the food characteristics like appearance, smell and taste can promote its overconsumption, which has been associated with an increase on body weight (Appelhans et al., 2011; McCrickerd & Forde, 2016). Given the increase in overweight and obesity figures worldwide, the generation of experimental procedures that allow the decrease consumption of palatable foods is considered of great importance. Likewise, the generation of experimental procedures that analyze the generalization of food aversion towards palatable foods is of great importance. Therefore, we recommended to continue experimenting and using foods that are novel for rats, decreasing or eliminating pre-exposure to them, using other palatable food options and varying the lithium chloride dose. With this, we will continue with the study of gustatory aversive learning with palatable foods.

Conclusion

In this study it was shown that it is possible to create a food aversion to palatable foods, confirmed through a significant intake reduction associated with the gastrointestinal discomfort caused by the injection of lithium chloride. However, the aversion generalization was not present, although a food with similar organoleptic characteristics was offered, there was only a tendency to decrease the consumption of the food not associated with gastrointestinal discomfort. Continuing with the search for an experimental procedure that achieves the generalization of food aversions to palatable foods is suggested.

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