Effects of exposure on Sensory-Specific Satiety: sensory variety and conditioned preferences

Efectos de exposición en la Saciedad Sensorial Específica: variedad sensorial y preferencias condicionadas

UNIVERSIDAD
DE GRANADA

Ph.D in Psychology

Department of Experimental Psychology
Centro de Investigación Mente Cerebro y Comportamiento (CIMCYC)

Ph.D candidate

Ana González Gómez

Advisor

Isabel de Brugada Sauras

April, 2022

Editor: Universidad de Granada. Tesis Doctorales
Autor: Ana González Gómez
ISBN: 978-84-1117-353-7
URI: http://hdl.handle.net/10481/75431

FINANCIACIÓN

El presente trabajo de investigación ha sido financiado por el siguiente proyecto de investigación:

This work was supported by PGC2018-095965-B-I00 funded by MCIN/ AEI /10.13039/501100011033/ FEDER “Una manera de hacer Europa”

A su vez, también ha sido financiado por el contrato predoctoral de formación al profesorado universitario denominado: FPU (FPU16/01767).

PUBLICACIONES DE LA TESIS

González, A., Recio, S. A., Sánchez, J., Gil, M., & de Brugada, I. (2018). Effect of exposure to similar flavours in sensory specific satiety: Implications for eating behaviour. *Appetite*, *127*, 289-295. https://doi.org/10.1016/j.appet.2018.05.015

González, A., Sánchez, J., & de Brugada, I. (2021). Habituation as an underlying mechanism for Sensory Specific Satiety: An assessment using flavor consumption and preference in rats. *Appetite*, 105821. https://doi.org/10.1016/j.appet.2021.105821

González, A., Sánchez, J., & de Brugada, I. (2022). The nature of training in flavor preference learning determines the underlying associative structure. [under review in *Journal of Experimental Psychology: Animal Learning and Cognition*]

AGRADECIMIENTOS

En primer lugar tengo que dar las gracias a Isa por confiar en mí. Siempre te estaré agradecida por todo lo que me has enseñado, fuese tanto en lo académico como en lo personal, y por todas las metas y oportunidades que me has ayudado a conseguir. Aunque no te gusta nada que te lo digan… ¡Qué suerte de "jefa" he tenido!.

A Marta y a Sergio por ayudarme a dar mis primeros pasos y por la paciencia con los despistes del principiante. Tengo que hacer especial mención a Sergio, a quien le doy las gracias por su supervisión en los experimentos de aprendizaje perceptivo, puesto que fue el quién empezó esa línea y sin él, honestamente, esos experimentos no estarían en este trabajo. A Jesús por hacer los findes en el laboratorio más entretenidos y menos solitarios. A Bea por su apoyo y amistad, pero también por su gran ayuda en el laboratorio.

Gracias a Michelle Symonds (Your English Lab) por sus correcciones de inglés en gran parte de este trabajo.

A Shauna y a Bob por su amabilidad y atención durante las estancias.

A Fer. Por todo el apoyo durante estos años, no se me ocurre un mejor compañero como tú. Es difícil encontrar a alguien que te anime con tanta fuerza y cariño a tomar decisiones que son difíciles, pero buenas para ti. Gracias por poner en palabras muchos de los pensamientos que tanto me costó organizar y convertir en valores tan importantes para mí.

Gracias a Jeane y Dani, quienes han sido mis compañeros más cercanos a lo largo de estos años. Por lo bien que lo hemos pasado juntos y por hacer los días de estrés un poco más llevaderos. Nunca olvidaré las tardes de infinitas bromas al salir de la universidad en el 3 jotas por calle Elvira. También a Antonio por las risas entre descansos en el CIMCYC y por el apoyo de estos últimos meses. A todos los compañeros fantásticos que he podido conocer durante todos estos años en Granada y de los que me llevo anécdotas muy divertidas.

A mi familia. Gracias a mis padres, por permitirme elegir libremente, siempre buscando lo que a mí me haría ser más feliz. Sé que no ha sido fácil, pero habéis tenido confianza plena en que con esfuerzo conseguiría las cosas y eso me ha dado fuerza para seguir peleando. A mamá, por sostenerme con firmeza cuando fue difícil. Aunque en algún momento no lo haya comprendido, hoy te doy las gracias. A papá, que aunque a veces nos llevemos como el perro y el gato, siempre ha sido y será un faro para mí. A mi hermana Lucía, por poner siempre una nota de humor a las situaciones más desagradables, haciendo que el mundo a veces no sea tan desesperante. A mis abuelas. A Lela de Noreña por su eterna mirada aceptación y comprensión. Siempre te recordaré con orgullo y admiración por tu capacidad de adaptarte a los nuevos tiempos. A Lela de Lugones, por su cariño, alegría y desvivir por los suyos. Aun repaso los veranos en Gijón con Charín y espero que esos recuerdos tan felices nunca se desvanezcan. A tío Carlos, gracias por tus mensajes durante todos estos años.

A Ana. Siempre he pensado que sin ella, nunca me hubiera atrevido a hacer gran parte de las cosas que me han llevado hasta aquí.

Finalmente a mis amigas, y especialmente a Andre, Meri (Asensio), María Ávila, Celia y a “las Amelies” (María Jaén y María Sevilla). Gracias por venir a verme a Granada durante todos estos años cuando habéis podido.

ABSTRACT

El objetivo de la presente tesis doctoral es estudiar los efectos de exposición a claves sensoriales en la Saciedad Sensorial Específica (SSE) a través de mecanismos de aprendizaje asociativo. Los fenómenos estudiados son contextualizados en las sociedades actuales, que se caracterizan por la omnipresencia de los denominados ambientes obesogénicos. Estos ambientes se distinguen por la masiva exposición a una gran variedad sensorial de alimentos altamente calóricos y la ubicuidad de claves señalizadoras de alimentos. En la mayor parte de los experimentos presentados en esta tesis se han empleado ratas como sujetos experimentales, salvo en el experimento del *Capítulo VII* en el que participaron sujetos humanos. En todos ellos se utilizaron estímulos sápidos (soluciones en el caso de los experimentos con ratas y comida en el caso de los humanos) y siendo el consumo la medida principal.

En el *Capítulo IV*, se exploró la habituación como posible mecanismo subyacente a la SSE . En estos experimentos se analizó el patrón de recuperación temporal de la SSE y el efecto de la presentación de un distractor durante la fase de saciación así como la de un deshabituador tras la misma. Estudiar si la SSE está sujeta a estas características es de gran relevancia ya que podría dar cuenta de algunos de los mecanismos por los que opera la SSE en el Efecto Bufet. Este efecto predice que cuanta mayor variedad alimentaria haya en una comida, mayor va a ser la ingesta total, en comparación con dietas más monótonas. Debido a la gran disponibilidad y oferta variada de alimentos altamente calóricos que existe en nuestra sociedad, el Efecto Bufet se ha propuesto como un factor potencialmente peligroso para la ingesta excesiva y el sobrepeso. Nuestros experimentos mostraron que ni la presentación del distractor ni del deshabituador tenían algún efecto en la expresión de la SSE. Concluimos que la

SSE no está sujeta a algunas de las características de la habituación, al menos cuando el consumo es la variable medida.

En el *Capítulo V*, estudiamos otro posible mecanismo que podría operar potenciando el efecto de la variedad a través de los ambientes obesogénicos. El mecanismo propuesto es el aprendizaje perceptivo que se refiere al incremento en la discriminación de estímulos similares tras la mera exposición. La exposición masiva a productos similares que varían solamente en algunos de sus atributos sensoriales, propiciará una mejor discriminación de los mismos, resultando en una ausencia de generalización de la SSE. Nuestros resultados apuntan a que la mera exposición a estímulos sápidos muy semejantes produce una mayor especificidad de este tipo de saciedad, anulando o debilitando su generalización entre estímulos semejantes. Proponemos este mecanismo como uno de los responsables de la ingesta excesiva en condiciones donde se presenta una gran variedad sensorial de alimentos similares.

En el *Capítulo VI* estudiamos mediante el uso de un paradigma de adquisición de preferencias, el efecto del grado de exposición a un compuesto aroma-sabor (EC-EI) en la expresión de la SSE. La mayor parte de la investigación en este paradigma ha mostrado que la asociación subyacente a las preferencias condicionadas son de tipo estímulo-estímulo (E-E). Sin embargo, tradicionalmente los procedimientos utilizados han sido de corta exposición; en escasos días, con una cantidad limitada a los compuestos y durante un intervalo temporal corto. En nuestros experimentos hipotetizamos que empleando procedimientos de condicionamiento extensos, donde la rata tiene más oportunidades de emparejamiento entre el aroma (EC+) y el sabor (EI), se desarrollará un aprendizaje más rígido dando lugar a una asociación de tipo estímulo-respuesta (E-R). Si esto ocurre, la devaluación del EI mediante un procedimiento de SSE, no afectará a la preferencia condicionada, siendo esta insensible a los procedimientos de devaluación. Nuestros resultados señalan que cuando las ratas tienen un condicionamiento extenso con el compuesto EC-EI, desarrollan una preferencia que no es sensible al procedimiento de la SSE, sugiriendo un aprendizaje E-R. Este efecto solo fue encontrado para un sabor, el azúcar. Dada la exposición constante a alimentos altamente hedónicos y calóricos característica de las sociedades actuales, se propone este aprendizaje como un mecanismo nocivo que podría provocar la ingesta excesiva de alimentos incluso cuando ya nos son deseables.

Por último, en el *Capítulo VII* estudiamos cómo la presentación de una clave externa previamente asociada con comida (anuncios de comida) afecta a la expresión de la SSE, empleando sujetos experimentales humanos y consumo y valoraciones subjetivas como medida principal. Así como las claves pavlovianas tienen un efecto apetitivo específico a la comida que señalan cuando los sujetos experimentales están en un estado de saciedad general, esperamos que tengan el mismo efecto cuando se trata de un proceso de saciedad específico como la SSE. Los resultados de esta investigación sugieren que las claves pavlovianas pueden alterar la expresión de este fenómeno, potenciando la ingesta de un alimento que ha sido ingerido previamente y por tanto, que ha perdido su valor temporalmente. De manera similar al *Capítulo VI* se discuten las potenciales consecuencias de estos resultados en las sociedades actuales donde existe una masiva exposición a claves de comida tales como anuncios, logos o contextos de comida.

TABLE OF CONTENTS

PART I

THEORETICAL INTRODUCTION

CHAPTER I

Contextualization and historical background

The obesogenic environment

Obesity has become one of the most alarming health problems in recent decades. According to WHO (2021), it has rapidly risen worldwide, increasing by almost three-fold since 1975, turning it into a public health priority of pandemic proportions. In 2016, 13% of the world's adult population was obese and 39% were considered overweight (WHO, 2021). Furthermore, obesity has been considered a risk factor for many other pathological conditions such as diabetes, cancer, or cardiovascular diseases (Blüher, 2019). Current treatments, which are mostly based on restrictive diets or food prohibition, do not achieve successful long-term results (Berthoud et al., 2020; Blüher, 2019). This lack of therapeutic success is not simply due to a lack of willingness to change, as many people with obesity express a need for treatment and a recognition of the detrimental health consequences of their condition (Ciria, et al., 2021).

This disease is characterized by a positive imbalance between the amount of energy consumed and expended, leading to an increase in body weight. The causes of this imbalance are now thought to be related to multiple agents including hormonal, nutritional, metabolic, or environmental (Blüher, 2019). One of the aspects that has been described and given most attention in the past decades as a major factor in the development and increase in obesity rates is the change in the “Global Food System” (Swinburn et al., 2011) which directly refers to the rising omnipresence of *obesogenic environments* (Blüher, 2019; Swinburn, et al., 1999). Swinburn et al. coined the term obesogenic environments in 1999, referring to what at the time they called "modern environments" which were characterized by encouraging individuals to overeat and lead sedentary lifestyles. The authors defined the obesogenicity of an environment as: "the sum of influences that the surroundings, opportunities or living conditions have on the promotion of obesity in individual populations"

(Swinburn et al., 1999, p.564). This concept — which has inspired much subsequent scientific literature — is also characterized by the constant exposure to food-related cues (for example advertisements or odors) and the massive variety of palatable, cheap, and highly caloric foods in our daily lives (Berthoud, 2012; Blüher, 2019; Martin, 2016; van den Akker et al., 2018).

This food paradigm shift has been spreading in society as a product of the globalization of these “toxic” environments and with it, the advent of sedentary lifestyles and the replacement of traditional foods with those that are cheap and processed. For all these reasons, it has been suggested that current environments are systematically encouraging intake habits that predispose the population to become overweight. And consequently, it has been emphasized that obesity is the natural response of some individuals to the unsafe and unhealthy environments to which they are exposed while protection from government policies remains absent or insufficient (Swinburn et al., 2011).

Models of intake regulation: from homeostatic setpoints to an integrative approach

Although the current dominant paradigm in research on overeating stresses the importance of the environment in which we live (Berthoud et al., 2020), historically, little attention has been paid to this aspect. Over the last century, a great deal of research on eating behavior has been carried out in multiple disciplines.

The dominant paradigm in early approaches on the theories of eating behavior focused on the concept of *homeostasis*. This idea was first raised by Claude Bernard (1879) who introduced the term “internal milieu”, which referred to the organism´s internal ambiance, composed of a set of biological parameters such as blood pressure or body temperature, which must be kept stable to ensure an individual´s wellbeing. Walter Cannon (1932) delved deeper into this idea, suggesting for the first time the term homeostasis, which was referred to as a compendium of physiological regulatory mechanisms that are responsible for keeping the internal milieu within its optimal values (Bellisle, 2020). According to this notion, the organism is composed of a series of sophisticated internal mechanisms, which, in the case of ingestive behavior, favor a balance between the individual's energy intake and expenditure. In this sense, hunger emerges as a response to the organism´s energy depletion and the cessation of intake or satiation are responses to energy repletion. Any deviation from this balance should be corrected accordingly by compensatory responses (Lowe & Butryn, 2007). The homeostatic models, often referred to as depletion/repletion models can be clearly recognized in this article published by Harvey P. Weingarten in the journal Appetite in 1985:

> “Current theories of hunger and meal initiation have their roots in a statement by Claude Bernard (1878)… Almost all current theories treat meal initiation as a necessary component of a homeostatic system that serves to maintain energy balance. Specifically, these theories suggest that feeding is a behavioral response activated by states of energy depletion, hence their description as depletion theories. Hunger is viewed as a behavioral state activated when energy levels of the organism are depleted; the net result of activation of the state of hunger is eating and the ingestion of nutrients that serve to redress the energy insufficiency.” (Weingarten, 1985, pp.387-388)

An illustrative example of homeostatic dogmas during the middle of the XX century can be seen in Mayer´s (1955) classic glucostatic/lipostatic hypothesis. Mayer stated that feeding behavior was monitored by the levels of blood glucose. From this perspective, glucose usage modulated states of short-term hunger (dropping levels of glucose) or satiety (increase in glucose levels). On the other hand, the lipostatic hypothesis suggested that the adipose tissue regulates body weight stability by increasing long-term appetite or satiety as a function of the fat stores. Thus, these notions often relied on the idea of the stability of setpoints, meaning that although the biological parameters may oscillate between different values, they always tend to return to the starting point. Thereby, homeostatic models relied on a negative feedback loop that continuously compares the state of the physiological parameters with the setpoint and are corrected if the values deviate.

From this approach, overeating was studied as an individual´s pathological condition in which these homeostatic mechanisms were abnormal or dysfunctional. In this vein, most of the research had long focused on studying physiological aspects involved in homeostatic regulation to explain the development and maintenance of obesity and overweight (Berthoud et al., 2020; Johnson, 2013). For example, during the 1960s, feeding behavior was believed to be governed by the hypothalamus, viewed as a homeostatic hub termed the hunger (lateral hypothalamus) and satiety (Ventromedial hypothalamus) center (Sclafani, 2018; Stellar, 1954). From this approach, obesity was often studied with animal models by ventromedial hypothalamic lesions causing hyperphagia/obesity syndrome.

The homeostatic approach based on the autonomy of the individual's internal mechanisms began to be questioned for being very simplistic or reductionist (Toates, 1981; Weingarten, 1985) and because it also failed to account for some of the results obtained through external and behavioral manipulations. An illustration of the latter can be seen in early animal studies concerning external influences on intake such as diet variety, exposure to palatable foods, or associative learning. For example, Sclafani & Springer (1976) conducted a study in which female rats were exposed to a “Supermarket diet”, offering a variety of palatable foods (such as chocolate, cheese, or sweetened condensed milk). Rats without any brain lesion or disruption in their physiological systems exhibited a pattern of behavior reminiscent of obesity, showing a pronounced weight gain compared to control rats. These authors, who had worked with the ventromedial hypothalamus lesion model to induce hyperphagia, observed that the pattern of behavior in supermarket diet rats was similar to that produced by the hypothalamic obesity syndrome. Consequently, they concluded that, although obesity had a very complex etiology, exposure to highly palatable, high-calorie diets provided a more optimal model to explain obesity than a lesion-based model. This study questioned the idea that eating behavior relied solely on a perfect homeostatic system by showing that animals do not react in a compensatory manner compared to a fixed and constant “set point” when the environment provides a wide variety of high palatable and energetic foods (Rogers, 1999).

As previously mentioned, the study by Sclafani & Springer (1976) was one of the first to challenge the homeostatic perspective by showing how external influences could trigger overconsumption in the absence of any physiological deficiency. While Sclafani and Springer's study focused on dietary factors, one of the first approaches focusing on the influence of food cues on intake began with the work of Harvey P. Weingarten (but see Schachter, 1971). This author reported a series of experiments in 1983 using rats as experimental subjects, demonstrating the relevance of external cues in meal initiation even when animals were satiated (Weingarten, 1983). In these experiments, food-deprived rats were subjected to multiple daily pairings of an initially neutral cue with a liquid food (CS+) and another cue without reinforcing consequences (CS-). Subsequently, the rats were given *ad libitum* access to the liquid food while exposed to single presentations of the CS+ or CS- each day. The author observed that CS+ presentation elicited an approach behavior to the food cup but not when the CS- cue was present. Furthermore, in a second study, these authors observed that when the CS+ cue was present, rats' consumption represented 20% of their total daily intake (in a later study in 1984, Weingarten found that this amount reached 50% of the total intake) (Weingarten, 1983; 1984).

However, this increase in intake was subsequently compensated. Thus, rats consumed in total no more than on days when the cue was absent. In 1984, after replicating his previous results, he observed, that: 1) rats do not only eat when they are hungry, as predicted by homeostatic models, but external cues can initiate the eating period, and 2) this effect does not completely override homeostatic mechanisms, since rats compensated for the cue-induced ingestion. Consequently, he proposed that both homeostatic and non-homeostatic mechanisms contribute to ingestion and called for an integrative approach to feeding behavior that considers both variables.

Interestingly, Weingarten published a review in 1985 in which he presented several limitations of homeostatic models (such as the fact that these were restricted to internal physiological factors of the organism) and instead highlighted the relevance of associative learning processes in eating behavior. In this paper, he proposed the theory called “the 2-factor hunger system”. He suggested that there were two distinct hunger control systems; one governed by energy depletion and guiding food intake in general (depletion-induced hunger), and the other governed by Pavlovian learning processes responsible for the association between external cues and food stimuli that promote stimulus-specific intake (incentive- or expectancy-induced hunger) (Weingarten, 1985). Weingarten recognized that although both feeding systems are different, they interact with each other, representing an integrative theory of both internal and external intake-related factors. This distinction, as discussed below, shares many similarities with currently proposed models of intake (hedonic vs homeostatic hunger).

Food-related cues, variety, palatability, and high caloric foods are core concepts of the obesogenic environment, as mentioned previously. Studies such as that of Sclafani & Springer (1976) and Weingarten (1983,1984) showed that the seemingly unshakable machinery of homeostasis could be overridden or at least, modulated by external influences such as food palatability or food paired cues. Thus, the concept of pleasure- driven eating or eating beyond our basic current physiological needs was established and conceived as an additional component of eating behavior at the same time that the scientific community became aware of the emergence of obesogenic environments (Swinburn, et al., 1999). For example, in 2004, Blundell & Finlayson proposed a different system other than homeostasis, known as the hedonic system. In this article they explored the role of both homeostatic feedback and hedonic input in overeating and suggested that obesity may emerge due to a failure of both the homeostatic and hedonic systems, with the latter associated with hyperreactivity to the sensory characteristics of food. In 2007, Lowe and Butryn coined the distinction between *homeostatic hunger* and *hedonic hunger*, arguing that:

> "For the vast majority of human history and prehistory, the primary objective of seeking food was survival through the maintenance of energy homeostasis and the avoidance of starvation. In modern times, among well-nourished populations, most food consumption occurs for reasons other than acute energy deprivation. As the growing prevalence of global obesity suggests, an increasing proportion of human food consumption appears to be driven by pleasure, not just by the need for calories." (Lowe & Butryn, 2007, p.432)

Thus, in contrast to homeostatic hunger, the term hedonic hunger was used to refer to eating without the need to make up an energy balance and driven by pleasure or reward, such as eating when we are already sated (Johnson, 2013). These distinctions that were raised at the beginning of the XXI century are reminiscent of those proposed in the 2-factor system put forward by Weingarten in 1985, and with the emergence of obesogenic environments and increasing rates of obesity over the decades, these ideas became predominant in the literature on feeding behavior. Although the distinction between two separate systems (homeostatic or reward-driven) has recently received some criticism in the literature since they seem to overlap in some of their associated brain circuit structures and are in continuous interplay (Berthoud et al., 2020; Rossi & Stuber, 2018), this distinction is useful for clarifying the different processes that come into play when studying eating behavior (Beaulieu & Blundell, 2021).

In this sense, one model that has been proposed to explain overeating emphasising the neural pathways of reward is the Incentive-Sensitazion Theory (Robinson & Berridge, 1993; For a recent review Morales & Berridge, 2020). Although this framework was originally suggested as an explanatory model for addictions, has recently extended to binge eating or obesity by possibly sharing some of their underlying mechanisms (e.g., Joyner et al., 2017). This model is based on the notion that reward experience is comprised of two main distinct psychological components: "Wanting" and "Liking". Both Wanting and Liking processes are understood interchangeably in everyday language, although they are responsible for completely different aspects of reward processing.

Wanting, also called incentive salience, refers to the motivational component of rewards that triggers craving and desire. This process causes individuals to be reactive to rewards and their associated cues, rendering them attractive and thus able to promote behavioral responses to obtain them. On the other hand, Liking concerns to the hedonic response or pleasure derived from consuming the reward (e.g., Berridge & Robinson 2003; Robinson & Berridge, 2008). While Wanting has usually been operationalized objectively through instrumental responses, Liking for a reward can be assessed with the Taste Reactivity Test. Both Liking and Wanting, have been well dissociated in the literature and are part of the reward/mesocorticolimbic system which is in continuous interplay with the homeostatic one to regulate feeding (e.g., Morales & Berridge, 2020). This model proposes that compulsive behaviors (e.g., addictions, overeating) can emerge as a consequence of a sensitization of the mesocorticolimbic circuit through repeated consumption. Contrary to tolerance, sensitization refers to the disproportionate increase in brain activity upon exposure to reward or its associated cues involving long-term changes. Specifically, this sensitization would occur in those neural structures associated to the Wanting component without necessarily affecting Liking. Thus, even if a food initially has a high hedonic (Liking) and motivational (Wanting) value, sensitization will cause Wanting to increase while Liking decreases or remains stable over time. Consequently, according to this model, the continuous experience of rewarding food stimuli will result in hyperactivity of Wanting processes to food-related cues that will ultimately lead to overeating. This is especially alarming in an environment characterized by the provision of highly caloric accessible foods (facilitating the sensitization process) and the constant exposure to food cues (triggering hyper-reactivity to cues) (e.g., Morales, Berridge, 2020).

Finally, another model that has integrated other factors apart from those related to homeostasis was the one suggested originally by Blundell et al. (1987), which has been continuously updated with advances in eating behavior research from a psychobiological perspective (e.g., Beaulieu & Blundell, 2021; Blundell & Bellisle 2013; Blundell, et al., 2010; Chambers et al., 2015). This model, known as the "Satiety Cascade Model", considers eating behavior related processes such as the start of the meal, food choice, or meal inhibition as a cascade of multiple signals (See Figure 1). Those signals extend from sensory and cognitive processes to pre-absorptive and post-absorptive (Chambers et al., 2015). Regarding meal inhibition, these signals will influence not only the amount of food eaten in a meal (Satiation) but also when the next meal occurs (Satiety). The satiety/satiation distinction stems from the original version of the model proposed by Blundell et al. (1987) in which it is argued that satiation is the process that determines the cessation of a meal, thus establishing the amount of food that is consumed in each eating period. On the other hand, satiety refers to the process of post-meal inhibition, and therefore determines when the next eating period will occur.

Although this model also considers that our body's homeostatic signals act by regulating our eating behavior, it also highlights that the latter can sometimes be overridden by other factors such as reward or pleasure. In this sense, hedonic appraisal can interact with homeostatic functioning, leading to overeating by delaying the end of a meal (satiation) or anticipating the period of the next meal course (satiety) (Beaulieu & Blundell, 2021). This approach is underpinned by the notion that hedonic aspects such as the pursuit of pleasure are just as influential (or more) than the fulfilment of homeostatic and energetic balance (Bellisle, 2013).

The Satiety Cascade framework also considers the role of associative learning in ingestion, especially the phenomenon of Conditioned Satiety; anticipation of satiety based on prior learned associations between sensory cues and their post-ingestive consequences. In this respect, in the present thesis we will focus on studying how other forms of associative learning can interact with sensory cues in modulating the satiation process in the current obesogenic environments.

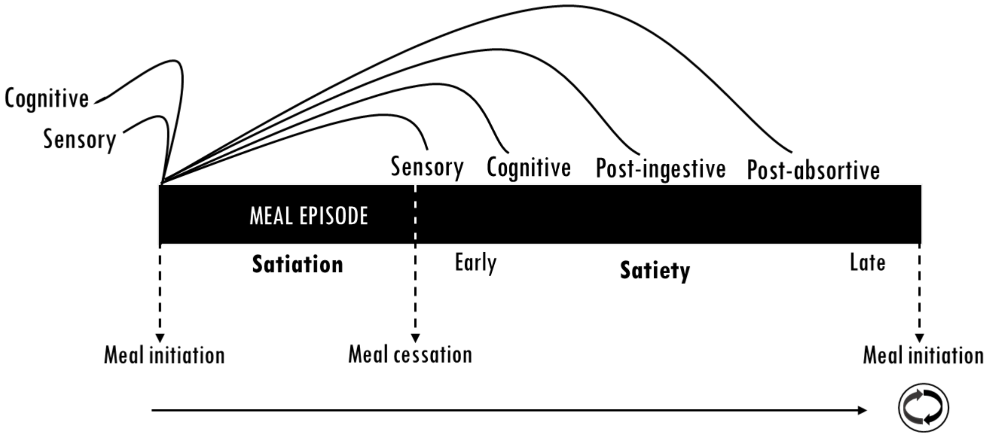


Fig 1. An adapted simplified version of the Satiety Cascade Model from Beaulieu & Blundell (2021).

The figure represents the cascade of signals that control the processes of meal initiation, food choice, satiety, and satiation.

CHAPTER II

Sensory and associative effects on food intake

Sensory influences on intake inhibition: Sensory-Specific Satiety

As mentioned above, factors other than those that are nutritional or metabolic-related may modulate the processes involved in feeding behavior, including sensory aspects of food. Sensory information contributes significantly to eating behavior by guiding us towards foods, driving serving size, influencing food choice, contributing to food preferences, or modulating satiation (McCrickerd & Forde, 2016). Regarding meal inhibition processes, at least three eating regulatory mechanisms mediated by the sensory properties of foods have been identified (Brondel et al., 2007). These are Conditioned satiety, Alliesthesia, and Sensory-Specific Satiety (SSS).

Conditioned satiety. This mechanism was proposed for the first time by David Booth in 1972 and will be discussed later in the associative learning section. Put simply, conditioned satiety helps to predict calories in anticipation of sensory cues such as taste, aroma, or texture, based on previous experience with the latter. Conditioned satiety has also been called "Expected satiety" or "Flavor-Nutrient Satiety learning" in the literature (Martin, 2016; Yeomans, 2012).

Alliesthesia. This concept was introduced in the 1970s by Michel Cabanac, who proposed a theory in which he defined the satisfaction or perceived pleasure obtained from stimuli (not only nutritional-related) as dependent on the physiological state of organisms. Thus, the value of stimuli depends on their current usefulness for an organism at a given moment and is therefore not static but state-dependent. Applied to eating behavior, the pleasantness experienced from consuming a food will depend on the homeostatic needs (depletion/repletion) of an organism in a given moment (Berridge, 1991; Rolls, 1986).

Sensory-Specific Satiety (SSS). This phenomenon was proposed by Barbara Rolls and Edmund Rolls, in the early 80s, and refers to the sensory devaluation of foods as they are consumed. This mechanism is different from alliesthesia, as it occurs only through sensory stimulation, independently of the nutritional status of the individual or the post-ingestive effects of nutrients on the organism. In turn, it is a much faster mechanism that starts at the very moment when the food is being ingested and is responsible for meal termination. According to the Satiety Cascade Model, while alliesthesia would occur during the satiation process inhibiting post-meal intake, SSS would cause the individual to stop eating a meal, and, therefore satiation.

Sensory-Specific Satiety/Satiation (SSS)

Historical background

The research on SSS has its roots in the observations of Katz who studied the eating pattern of chickens (Katz, 1935; see Rolls, 1986). Katz (1935) found how chickens enhanced their intake if they were offered multiple types of grain, but when offered just one type, eating cessation occurred very quickly. This author had already suggested the (now modern) notion that satiety could be specific to foods. In 1940, Young conducted a study with rats, in which he showed that the animals' food preferences could vary according to what they had consumed previously. He found that rats´ strong preference for sugar compared to wheat could be reversed when they had been pre-fed with sugar. The foods used had similar nutritional value (both were carbohydrates) and so again, this study revealed that satiation shows sensory specificity.

Later, Le Magnen (1956;1999) found similar results to those of Katz — this time with rats. Le Magnen offered rats different types of odor-flavored pellets with equal nutritional content and compared the intake pattern between animals given different or single flavored pellets. Animals had access to food during a 2-hour daily period in which they were exposed to their ordinary chow but flavored with the odors. In a first stage, rats were given their standard food flavored with a single odor per day, resulting in the same amount consumed across sessions. In a second stage, the animals were given the chow flavored with the 4 different odors in the same day, each presented in different sessions. Every half hour the chow was flavored with a different odor from the other 4, for up to 2 hours. Animals consumed considerably more when their diet was varied (only in their olfactory properties) compared to when were only given one flavor of pellet per day. In this respect, Le Magnen concluded that food has both sensory and nutritional components, both of which independently influence total food intake. Le Magnen's experiment was possibly the first demonstration of SSS, but despite being published in the 1950s (written in French and republished in English in 1999) it received little attention (Le Magnen, 2001). The study of this phenomenon would not be revisited again until 20 years later with the discoveries of Barbara and Edmund Rolls.

In the early 1970s, Cabanac and his collaborators developed the theory of alliesthesia, which, in contrast to SSS, emerged from the homeostatic tradition. As mentioned, alliesthesia was defined as a change in the hedonic sensation elicited by a stimulus as a function of its utility to the organism ("esthesia" denoting sensation and "allios" change) (Cabanac, 1971). Thus, Cabanac conceived that the pleasure obtained from food stimuli could be modulated by changes in the "internal milieu", so, for example, liking for energy-dense foods increases when an individual is hungry (positive alliesthesia) and decreases when it is satiated (negative alliesthesia) (Cabanac, 1971). During these years, negative alimentary alliesthesia was studied by measuring human participants' subjective evaluation of sweet solutions before and after having been administered glucose solutions intragastrically and orally. In these experiments, it was observed that the hedonic value of the sweet taste — but not the salty taste — decreased. Moreover, this glucose pre-loading decreased ratings for food related odors while leaving the ratings intact for non-food related odors. This specific change in hedonic evaluation occurred slowly over time after ingestion, with the maximum change occurring between 45 and 60 minutes. Due to the gradual pattern in the appearance of the hedonic change over time, the authors concluded that this effect stems from the change in the subjects' physiological state after ingestion of a certain nutrient. Subsequent studies suggested that this phenomenon was due to the absorption of nutrients in the gut (Rolls, 1986). As these investigations were focused on the role of nutrient absorption in the change in food palatability, the immediate effect on consumption was somehow ignored.

Almost in parallel to Cabanac's research, which demonstrated the modulation of sensory pleasure from food based on energy repletion-depletion states through long-term satiety, Barbara and Edmund Rolls began to study the sensory specificity of short-term satiety. In the late 1970s, Edmund Rolls & colleagues conducted several studies monitoring neuronal activity in the lateral hypothalamus while monkeys were feeding, uncovering a neurological correlate to sensory specific satiety (Burton et al., 1976; Rolls et al., 1976; Rolls et al., 1980). These studies showed how neurons that responded to the sight or taste of a particular food decreased their firing rate after its consumption. In contrast, these neurons continued to respond when another non-eaten food was presented or tasted. This pattern of results was accompanied by other intake measures; the primates rejected the eaten food but accepted a different non-eaten food.

In 1981, Barbara and Edmund Rolls and colleagues published an article demonstrating the sensory specificity of satiety in human participants just two minutes after pre-feeding (Rolls, Rolls et al., 1981). These authors showed how participants rated a food to be less pleasant immediately after having eaten it whereas the ratings for other non-ingested foods that did not share sensory attributes with the ingested food remained intact. Similar to their previous research in primates, these authors found that this reduction in the hedonic ratings was accompanied with a decline in later consumption of the eaten foods and a greater preference for other non-eaten foods. This demonstration led to the definition and naming for the first time of Sensory-Specific Satiety (SSS), a phenomenon that refers to the change in the hedonic value of specific sensory properties of food as a function of its previous intake. Unlike alliesthesia, these authors argued that this phenomenon occurs independently of absorptive factors, or the internal state of the organism as it takes place at a very early stage of intake. Moreover, the two phenomena show a different temporal course; while alliesthesia occurs gradually and slowly, reaching a peak with time, SSS shows the opposite pattern, peaking immediately after ingestion and decreasing over time. Furthermore, while alliesthesia occurs by inhibiting intake after a meal, SSS occurs during consumption, and has also been proposed as a food choice mechanism. In addition to these differences, later studies indicated that alliesthesia was not as specific as it first seemed, generalizing across different macronutrients, tastes, and smells. Thus, both alliesthesia and SSS are independent regulatory mechanisms of intake cessation; one affecting short-term (Satiation) and the other longer-term feeding inhibition (Satiety).

In this regard, although SSS has mostly been labelled in the literature as satiety, according to the two distinctions of satiety and satiation proposed by the Satiety Cascade Model (Blundell et al., 1987), the optimal term for SSS is better suited to a satiation process, as it facilitates finishing eating during the meal (Hetherington & Havermans, 2013). However, this discrepancy will not be considered in this thesis; we will refer to SSS as satiety in line with the majority of the literature and its original name so as not to create confusion.

Features of Sensory-Specific Satiety

SSS was defined as the phenomenon that promotes a temporary hedonic devaluation of the sensory properties of a specific food by the time it is eaten (Rolls, Rolls et al., 1981). While this was the original definition, it was later discovered that SSS also resulted in a specific devaluation of the motivational component of the food eaten (e.g., Ahn & Phillips, 1999; Havermans et a., 2009; Rogers et al., 2020). Although the SSS phenomenon is specific to the eaten food, it can also generalize to other foods that share similar sensory properties to the devalued food (e.g., Gonzalez et al., 2018; Griffioen-Roose et al., 2010). This hedonic and motivational change is not produced by a perceived change in the intensity of the food, suggesting that it is not due to a simpler mechanism such as sensory adaptation. (Rolls et al., 1983; Rolls & Rolls, 1997). SSS has been demonstrated in different animal species, including rodents (e.g., Myers, 2017), primates (Rolls et al., 1986), chickens (Katz, 1934) and humans (e.g., Havermans, 2012). Most animal species need to eat a varied diet to obtain all nutrients needed (Ahn & Philips, 2012). Hence, SSS has been proposed as an adaptive mechanism that ensures an adequate diet that contains a wide variety of nutrients needed by the organism. This way, animals do not eat just those foods that are more accessible or palatable in their natural environment (Reichelt et al., 2014, Rolls, Rolls et al., 1981).

The general SSS procedure varies depending on the species used as experimental subjects. In humans, this procedure consists of asking participants to initially provide a subjective rating of the target foods (usually with Liking and Wanting measures, that is, hedonic valuation and motivation to obtain a certain food, respectively), after which they consume one of the foods until satiety (pre-feeding). Finally, participants again complete the original scales (pre-fed vs non pre-fed foods). Subjective measures are usually assessed by presenting participants with Visual Analogue Scales (VAS) and on some occasions, this procedure is accompanied by a final choice test session in which participants are offered the pre-fed and non-pre-fed items to assess their intake patterns after pre-feeding.

In animal models based on rodents, the experimental procedure has been much more heterogeneous. The pre-feeding procedure varies from study to study. While some studies have used longer duration periods such as 1-hour (Parkes, et al., 2016) or 2-hour (Myers, 2017) exposure, others have used shorter periods such as 20 minutes (Reichelt et al., 2014) or even 10 minutes (Dwyer, 2005). The same is true for the amount offered and the stimulus used (LeMon et al., 2019; Myers, 2017; Parkes, et al., 2016; Reichelt et al., 2014). After pre-feeding, subjects are given a preference test between pre-fed and non-pre-fed stimuli, often with consumption or/and instrumental responses as principal measures. The duration between pre-feeding and the consumption test is also subject to variations, as the interval between phases is not always immediate (LeMon et al., 2019; Reichelt et al., 2014). All these procedural aspects usually depend on the phenomenon being studied and whether they are interested in studying SSS itself or other types of behavior such as instrumental responses. In addition, in animal models, it is extremely challenging to manage the initial animal's preferences or motivational state (finding an optimal motivational state that allows animals to consume enough to be sensitive to the effect being studied while avoiding floor or ceiling effects).

A main feature of this feeding-adaptive mechanism is its sensory nature. SSS had been shown to occur independently of absorptive or post-absorptive factors. Thus, several studies have shown how SSS is demonstrated by changing different sensory-related attributes such as the smell (Rolls & Rolls, 1997; Romer et al., 2006) the taste (Brondel, Romer et al., 2009; Rolls & Rolls, 1997), the texture (Guinard & Brun, 1998; Rolls & Rolls, 1997), the color (Rolls et al., 1982), or the sight or shape of foods, such as, for example, different kinds of pasta (Rolls et al., 1982). Moreover, SSS appears just two minutes after the end of a meal (Hetherington et al., 1989) which means that the change of pleasantness of the food appears before the meal has been absorbed. Other evidence that supports its sensory nature can be found in experiments using stimuli that are iso-caloric but have different sensory attributes (Reichelt et al., 2014) or non-caloric stimuli such as sweeteners (Rogers et al., 2020).

In addition, the macronutrient composition, energy content of the food, or participants knowledge of the nutrition composition of foods does not seem to affect the degree to which this effect is expressed, but other factors such as the volume ingested, the oro-sensory exposure rate, or initial preferences for food stimuli seem to be determinant (Hendriks-Hartensveld et al., 2022; Johnson & Vickers, 1992; Miller et al., 2000; Raynor & Epstein, 2001; Rolls et al., 1988). In addition, evidence of SSS has been reported in a study where the responses of pup rats were measured using sweet substances that these infant rats could not metabolize. This study showed that rats' mouthing responses decreased as they were exposed to these solutions (Swithers & Hall, 1994). In this sense, the orofacial responses of human and non-human animals have been taken as an index of palatability by measuring the hedonic reactions produced by exposure to sapid stimuli through the Taste Reactivity Test (Berridge, 2000). Finally, another argument for the sensory nature of SSS is based on the observation that the change in pleasantness can occur when foods are chewed but are not swallowed (Rolls & Rolls, 1982; Smeets & Westerterp-Plantenga, 2006).

Thus, in view of The Satiety Cascade Model (Blundell et al., 1987), sensory aspects can also modulate the amount of food eaten in a meal through SSS. This process occurs through the temporary devaluation of the sensory properties of food. In this respect, SSS is a regulatory process of intake that occurs during meal consumption and plays a role in the cessation of intake, and as such, understanding the mechanisms underlying this phenomenon has been the focus of much research, as we will see in the next section.

Mechanisms underlying SSS

SSS was defined as an intake regulation phenomenon, and most of the research aimed at understanding its characteristics has been conducted in humans. In this sense, there were two main approaches in the scientific literature that have been closely linked to the SSS phenomenon. One approach followed in the footsteps of Barbara and Edmund Rolls, studying its underlying mechanisms, characteristics, and its role in food variety and feeding behavior. This approach has been tested mainly using humans as experimental subjects (e.g., Havermans et al., 2009; Hendriks et al., 2021) although some works have used animal models (e.g., Berridge, 1991; Myers, 2017; Reichelt et al., 2014). Concerning the other approach, SSS has not been the subject of study but used as a method. This line of research has been carried out mainly with animal models (rodents), following the steps of Anthony Dickinson and Bernard Balleine and coming from an associative learning tradition (Balleine & Dickinson, 1998). In this sense, the associative learning tradition picked up this phenomenon to use it as a method of reinforcer devaluation, so that the focus of learning theorists was not the phenomenon itself, but how it might be used to dissociate S-S / S-R theories (goal directed/habit learning) of instrumental performance, along with its neural correlates. Thus, there is much research based on rat animal models that has made use of SSS, but from a different perspective to that of human research.

Following the theoretical approach arising from research in humans, many studies have focused on explaining the underlying mechanisms of the SSS phenomenon. The dominant hypothesis or the one that has received the most research attention comes from the non-associative learning process called habituation. Both phenomena share many similarities in their distinctive features, including decreased responsiveness, specificity, generalization, and temporality. Apart from these features, the habituation phenomenon, as described by Rankin et al. (2009; revised from Thompson & Spencer, 1966) has some other main properties such as potentiation, frequency and intensity effect, dishabituation, habituation of dishabituation (and distraction) and long-term habituation. A large body of research has focused on testing whether the properties of habituation are shared with SSS, which, as we will review in *Chapter IV*, has yielded mixed findings. These studies have focused particularly on characteristics such as dishabituation/distraction (e.g., Brunstrom & Mitchell, 2006; Romer et al., 2006), context dependency (e.g., Garcia-Burgos et al., 2015; Hendriks et al., 2021), long-term SSS (e.g., Myers, 2017; Raynor et al., 2006; Rolls & Waal, 1985) and spontaneous recovery (e.g., Hetherington et al., 1989; Weenen et al., 2005). As evidence seems to show that SSS do not completely have all the properties of habituation, other tentative explanation has emerged.

Another explanation of SSS, suggested by Hetherington & Havermans (2013), is based on stimulus satiation. This idea, which emerged from the research of Glanzer (1953), is that after SSS, subjects experience a state of boredom in response to an overexposed stimulus. This notion is based on the idea that living beings within a range of behavioral alternatives tend to switch between the possible options, to avoid continually repeating a chosen response. This idea is observed, for example, in multi-armed mazes where rodents do not always go to the same arm and instead tend to switch back and forth. While this stimulus satiation seems to overlap with habituation, Hetherington & Havermans (2013) suggest that the overexposed stimulus does not become irrelevant or lose effectiveness but becomes aversive in such a way that a stimulus changes from positive to negative valence. This notion is based on the possibility that SSS does not occur due to a decrease in responding but a qualitative change in responding from approach or appetitive responding to avoidance behavior. However, as we will see in *Chapter IV* of this thesis, there is solid evidence to oppose this hypothesis.

Obesity and Sensory-Specific Satiety

As mentioned above, SSS acts as a regulator of eating behavior by encouraging the end of a meal and, as such, its expression has been studied in different types of populations to assess its implications for intake. Regarding eating disorders, differences in the pattern of SSS expression have been found in populations with anorexia and bulimia (Hetherington & Rolls, 1989; Rolls et al., 1992) and in animal models of binge eating using consumption or instrumental responses (Ahn & Philips, 2012; Furlong et al., 2014; LeMon et al., 2019; Parkes et al., 2017) but not in restrain subjects (Tepper, 1992).

In relation to obesity, it has been suggested that those suffering from this disease may have difficulties in updating the hedonic value of food, having a lower or delayed decrease in pleasure derived from food consumption (Myers, 2017). Therefore, a large body of research has focused on studying whether SSS could play a relevant role in the development and maintenance of obesity by comparing its course in obese and healthy human or non-human animals. There is evidence that salivary responses, which tend to diminish or habituate as a food item is consumed, are reduced more slowly or to a lesser extent in obese or overweight subjects (e.g., Epstein et al., 1996; Bond et al., 2010). Salivation is not an index of palatability, but rather a measure of habituation to a food stimulus. However, there is evidence that both salivation and hedonic food ratings decrease in parallel as we eat and this has also been linked to hunger and the amount eaten in a meal (Epstein et al., 1992).

Nevertheless, other studies that have focused on directly examining how SSS is affected in obese people have yielded mixed results. Concerning pleasantness ratings, Pepino and Mennella (2012) reported that the hedonic decline produced by continuously tasting a sweet solution (24% sucrose solution) was slower for obese women than normal-weight women. However, other studies such as Brondel et al. (2007), Snoek et al. (2004), and Havermans et al. (2012) did not find differences in the pattern of SSS pleasantness ratings between both populations. Furthermore, in the latter experiment, which focused on the pattern of SSS recovery by using sweet drinks, the authors found no differences between groups for either the basic SSS effect or after 20 minutes of consumption.

Regarding animal models, some studies have employed diet-induced obesity (DIO) which can be achieved by using cafeteria diets (Reichelt et al., 2014), high-fat, or high-sugar diets (HF/HS diets) (Myers, 2017). Cafeteria diets, which appeared for the first time in the literature in Sclafani and Springer's (1976) study (already mentioned in the previous section), are based on exposing animals to "junk foods" that we might find in human obesogenic environments, such as biscuits, hamburgers, and cakes. Reichelt et al. (2014) applied a cafeteria diet methodology to assess possible differences in intake after a SSS procedure in both cafeteria and chow control rats. The results revealed that rats fed with a cafeteria diet regime did not show a SSS devaluation effect when they had been pre-fed with a cherry sucrose or grape maltodextrin solution. In contrast, chow-control rats expressed the SSS effect by showing a preference for the non-pre-fed solution. However, interestingly, cafeteria rats drank considerably less than the control rats in both the pre-feeding and choice test procedures. Thus, these results leave open the possibility that the rats expressed a negative contrast towards the target solutions used in the experiment after ingesting such palatable foods during the cafeteria diet. If this is the case, it is possible that the results do not reveal an absence of SSS expression, but rather a floor effect on consumption due to the absence of pleasure when consuming them. This effect of negative contrast within cafeteria diets is not a puzzling result as it has been previously found in various studies (for a review, see Lalanza & Snoeren, 2021).

Unlike cafeteria diets, HF/HS diets are based on exposing rats to pellets that are rich in fat, oil, sugar, or carbohydrates. Myers (2017) applied a HF/HS diet method and compared the SSS effect with a control group of rats fed with standard chow. The target pre-fed foods were two palatable snacks: cheese and cocoa. The results of this experiment challenge those of Reichelt et al. (2014) in finding no difference between obese and control rats in their expression of SSS. However, the use of these diets has been criticized when studying behavioral patterns since they do not emulate the real action of ultra-processed foods in the environment, such as the effects of palatability, sensory variety, and other organoleptic properties. Thus, it is possible that these null results were found because these diets are based on the energy content of the food but neglect the hedonic aspects. Moreover, in Myer's study, the animals were pre-fed with cheese or cocoa during a 2-hour period after which they were given *ad libitum* access. Thus, during those two hours, mechanisms other than strictly sensory processes could have come into play and possibly contaminated the final effect on the SSS process.

These results do not allow us to confirm that obese and healthy subjects differ in the expression of this mechanism of intake regulation. Nonetheless, it is possible that there are procedural aspects in the study of SSS that mask the possible differences between the two populations. Based on habituation theory, which, as we have seen, has been proposed as a mechanism underlying SSS, more intense or salient stimuli should require more exposure to habituate (See the fifth characteristic in the review by Rankin et al., 2009). In this sense, if we assume that hedonic value is a measure of food intensity or salience, there are related results that support this notion. For example, it has been found that initial preferences affect the expression of SSS; foods with higher initial hedonic ratings require more sensory stimulation to achieve the SSS effect than those foods with weaker initial ratings (Johnson & Vickers, 1992). Given that for obese people food stimuli are possibly very salient (As indicated by their enhanced food cue reactivity — see Van den Akker et al., 2018), their habituation process should be slower. Thus, obese people would need to be exposed to a larger amount of food than lean people to achieve this devaluation effect. It is therefore possible that by comparing the effects of limited and unlimited access during SSS procedures can provide a more precise way of detecting differences in the development of satiety. Consistent with this notion are previous findings mentioned in relation to the slower decline rate in the salivation pattern when food is consumed (Bond et al., 2010; Epstein et al., 2008; Epstein et al., 1996).

Associative learning and intake

As we have discussed in the previous sections, eating is a very intricate behavior in which multiple factors are involved. In this regard, the associative learning tradition has historically used eating behavior or food stimuli to study the mechanisms involved in the structures and nature of learned content. A clear example can be seen in the first study of classical conditioning, in which Ivan Pavlov used food as an unconditioned stimulus (US) and observed that by pairing the sound of a bell with the US, the dog subsequently salivated in the presence of the sound of the bell (Pavlov, 1927). However, for a long time, research did not focus on understanding feeding behavior itself, but rather on developing general principles of learning. Another example is the case of SSS mentioned above. Despite the regulatory action of SSS on intake, associative learning theorists since the late 1990s have focused on studying this feeding regulatory mechanism as an outcome-specific devaluation method, particularly to reveal the S-S/S-R structures of instrumental behavior (e.g., rodents: Balleine & Dickinson, 1998; Balleine & Dickinson; 2000; e.g., humans: Valentin et al., 2007; Watson et al., 2014).

Associative learning has been described in the literature as a very influential aspect in eating behavior from setting eating schedules, signaling the availability of food, the acquisition of food preferences or aversions, to affecting food choices. In addition, much of the literature examining the effects of the above-mentioned obesogenic environment has focused on associative learning as a method for partially revealing the causes and clinical solutions to this pandemic through, for example, the use of Exposure-Based Therapy (e.g., Jansen, 1998; Van den Akker et al., 2018). And in this context, multiple researchers have pointed out that associative learning directly shapes the way in which we interact with today´s environmental stimuli and make decisions regarding our eating behavior (Verhoeven et al., 2018). Among the multiple learning processes that are involved in feeding behavior, in the present section we will focus on two: the acquisition of flavor (or food) preferences and exposure to external cues associated with food.

Acquisition of food or flavor preferences

Most animals express innate hedonic reactions to two tastes that allow them to be protected or to cope successfully in their natural hazardous and food scarce environment. Animals, by instinct, tend to avoid or dislike bitter tastes, presumably because in nature these are often accompanied by toxic or poisonous consequences. The opposite is true for sweet tastes, which, perhaps due to their nutritive content, appear to be perceived as attractive from the very time many animals are born. In addition, these innate preferences appear to be changeable across the life span, as occurs in the case of coffee (which has a bitter taste but a positive energy consequence) or as can be seen in the wide variety of individual differences in adult humans towards preferences for sweet tastes (Yeomans, 2012). Furthermore, it is well known that not all likes and dislikes are given to us at birth, with experience and learning playing a crucial role in their acquisition. In this sense, it is believed that most flavor/taste preferences are developed through learning, and some authors have even argued that these presumed innate preferences could be learned by fetuses prenatally from the diet of the pregnant mother (Schaal et al., 2000).

Omnivore animals like humans require the intake of a wide variety of foods to obtain the necessary nutrients for survival. Most omnivore behavior must seek to achieve a balance between diversity and exploration of new foods (to ensure a varied diet) and caution due to the possible dangers of eating poisonous or harmful foods. Counter-intuitively, instead of being born with a pre-established compendium of taste preferences, we seem to show only two innate affective responses — those towards sweet and bitter tastes (Yeomans, 2012). This has been termed the Omnivore´s Paradox (Rozin, 1976) and reflects how our likes and dislikes seem not to be as rigid or solely dependent on inherited or instinctive reactions as one might assume. Instead, animals are prepared for a scarce food environment in which we can update our preferences by learning from previous experiences with particular tastes and their consequences (Gibson & Brunstrom, 2007). Thus, many likings for flavors are developed through various learning processes in either an associative (e.g., flavor-flavor/flavor-nutrient learning) or non-associative manner (e.g., mere exposure, learned safety). These learnedt preferences modulate food choice and portion size, and therefore, potentially help explain why we excessively eat when a set of highly liked foods are present (Yeomans, 2012).

Research has shown that associative influences play a crucial role in the development of both flavor preferences and flavor rejection (Martin, 2016). Learning to reject initially neutral or positively valued tastes has been studied mainly from an associative learning perspective using the conditioned taste aversion (CTA) paradigm. In this procedure, a flavor is paired with negative consequences such as the gastric malaise produced by lithium chloride (LiCl) (e.g., Garcia & Koelling, 1966). Following this procedure, which has been demonstrated even with tastes that initially evoke a strong preference (e.g., sucrose), it is observed that experimental subjects develop a conditioned aversion to the taste paired with LiCl. Moreover, in the same way that animals can learn to dislike a flavor, they can acquire a conditioned preference for initially neutral flavors after these are paired with a palatable taste. These two Pavlovian learning processes are a highly adaptive tool for living beings to survive in a food-scarce and dangerous environment by avoiding poisonous foodstuffs or by rapidly selecting those with nutritional value (Harris et al., 2000).

Acquisition of flavor preferences has been widely studied in animal models by pairing a neutral taste or odor cue (CS) and a palatable flavor (US), either by direct ingestion of a compound of both elements or by administering an intragastric infusion of a nutrient (US) when a neutral cue is consumed (CS) (Sclafani, 1991). After this procedure, a relative preference for the paired flavor cue is observed (assessed by two-bottle tests; usually compared with another unpaired CS- or with water; e.g., Dwyer, 2005), along with an increase in acceptance (total amount ingested, as assessed by a one-bottle test; e.g., Pérez et al., 1998), a change in orofacial responses (e.g., Myers & Sclafani, 2001) and licking microstructure (e.g., Riordan & Dwyer, 2019).

The mechanism by which this process results in a conditioned preference has been primarily explained by three different types of associations acquired through Pavlovian learning (Delamater, 2012). These are i) Flavor-Flavor Learning (FFL), which is when the neutral cue is associated with the palatable taste of the US (e.g., the sweet taste of sucrose or saccharin or Stimulus-Stimulus association) (e.g., Gil et al., 2014), ii). Flavor nutrient Learning (FNL) where the CS is paired with access to the caloric properties of the US (e.g., caloric content of Sucrose or Stimulus-Stimulus) (e.g., Azzara & Sclafani, 1998); and iii). Flavor-Hedonic reaction where the CS is associated with an hedonic response resulting from consumption of the US (Stimulus-Response) (Harris et al., 2004).

In flavor-nutrient learning, it has been established that this not only has the capacity to increase preference and consumption for the conditioned taste cue, but paradoxically, it could also trigger another learning process that inhibits total consumption. This mechanism enables us to increase or reduce our intake of a given food based on prior learning between its sensory and nutritional properties and on our current physiological needs, enabling avoidance of over satiation. This eating regulatory mechanism, known as *Conditioned Satiety*, was initially proposed by Le Magnen (1955) but was later defined more precisely by Booth (1972), who demonstrated that rats can anticipate the satiating consequences of food through associative learning, and thus regulate the amount of intake that occurs in a meal (see also Warwick & Weingarten, 1996; Yeomans, 2012; Martin, 2016). It has been suggested that the mechanism by which this over satiation inhibition works is by modulating the sensory palatability of foods late in the meal (Booth, 1972) switching from initially pleasant at the beginning (through flavor-nutrient learning) to unpleasant (through conditioned satiety).

Flavor-nutrient associations have been dissociated from flavor-flavor associations on many occasions, and these have been the focus of much research. (e.g., Bonacchi et al., 2008; Gil et al., 2014; Harris et al., 2000). This has not been the case for the third possible association mentioned above. The latter refers to a link between the hedonic response evoked by consuming the US and the CS (Stimulus-Response type learning). S-R associations, such as the one mentioned above, are acquired when the presence of a particular stimulus — in this case the flavor CS+— produces an automatic response. Thus, the current association is not mediated by the sensory representation of the US (S-S learning) in memory and therefore, any change in the value of the US will not affect the individual's preference for the CS+. To verify this possible association in preference acquisition, a reinforcer devaluation procedure such as CTA or SSS should be applied. These two procedures make it possible to change the value of the US and thus observe whether the individual's preference for the CS changes accordingly. If the individual continues to maintain a preference for the CS+ even after the US has been devalued, the structure of the underlying association will not be mediated by the mental representation of the US (S-S type association) but will automatically elicit a response (S-R type association). Several studies have shown how appetitive flavor learning leads to the expression of a conditioned preference that can be reduced when the US is devalued (e.g., Delamater et al., 2006; Delamater, 2011; Dwyer, 2005; Harris et al., 2004), thus suggesting that this kind of association is not involved in acquired flavor preferences.

However, in these studies, the testing of this possible association in acquired taste preferences has scarcely been the subject of research in this area, with other phenomena being the true focus, such as extinction processes or studying the role of various motivational states. Thus, there is little or no evidence that supports S-R type learning in appetitive flavor learning. However, as this hypothesis has not been the real focus of the research, the procedures used in the literature have not been adapted to test this notion. In *Chapter VI*, some of these main procedural discrepancies will be discussed.

Obesity-induced models and Acquired flavor preferences

To date, there are only five studies that have focused on studying the differences in preference learning patterns based on flavor-nutrient associations. These studies have been carried out by using various diet induced-models in rats and the results have been mixed. On the one hand, Woods et al. (2016) showed that obese rats whose diet was composed of *ad libitum* milk chocolate Ensure and chow (10 days access) showed a deficit in flavor-nutrient learning compared to rats that were not obese. Similarly, Boakes et al. (1987) reported an impairment in flavor-nutrient learning in rats fed with a cafeteria diet compared to controls.

On the other hand, Wald & Myers (2015) showed the opposite pattern of results by employing a 36-day access High-Fat, High-Carbohydrate diet to induce obesity and a control group of chow-fed rats. In the obese induced rats, the authors examined individual differences by dividing them into obese-prone rats (those prone to being overweight) and those that were “resistant to obesity” (obesity-induced rats that do not gain excessive weight). After applying an intragastric-infusion conditioning procedure, the authors revealed that obese-prone rats were more sensitive to acquiring preference learning by showing a higher preference for a CS+ compared to a CS-.

Another study conducted by Palframan & Myers (2016) showed how a group of rats fed on a 3 month-cafeteria diet that contained ultra-processed foods like those in current human environments expressed a higher preference for a flavor cue that had been paired with a nutrient taste cue than rats that had not been exposed to a high caloric and palatable diet. Rats in the ultra-processed condition were more precise, being better able to discriminate between two flavored CSs paired with two USs with different nutritional content. Finally, another study carried out by Pérez et al., (1999) found that rats fed with a cafeteria diet expressed flavor-nutrient learning in the same way as a control group of rats fed with standard chow. In this latter case, flavor preference conditioning was established by pairing a flavor with an intragastric infusion of a polycose solution.

External food-related cues in the environment

One of the main features of obesogenic environments is the massive exposure to external food related cues. These food cues, learned through Pavlovian learning, can be noticed in many everyday situations, such as observing the golden arches of McDonald's, appetitive contexts, or watching food advertisements. There is evidence that these food-related cues, when present, affect many appetitive responses related to feeding behavior (For a review, see Belfort-DeAguiar & Seo, 2018; Johnson, 2013; van den Akker et al., 2018). It has been demonstrated that food cues can trigger not only conditioned intake responses (increased consumption; e.g., Harris et al., 2009) but also motivational appetitive responses (such as craving or the desire to eat; e.g., operationalized often in instrumental responses; e.g., Corbit & Balleine, 2005), allocation of attentional resources to food cues (e.g., Pool et al., 2014) or physiological responses (such as hormone release or salivation; e.g., Power & Schulkin, 2008).

Learning about predictive cues that signal the proximity of food is a useful tool for helping animals to survive under conditions of scarcity. However, conditions in today's human societies have changed. As mentioned previously, obesogenic environments provide full access to a wide range of food products, in which the vast majority are highly palatable, caloric, and cheap, potentially leading to overconsumption (Martin, 2014). Food cues can evoke increased consumption of nutrient-poor and unhealthy foods, causing health problems. For example, it has been shown that exposure to food advertisements can increase the intake of high-energy junk food in adults and children (e.g., Harris et al., 2009; Russell et al., 2019). Furthermore, it is of great concern that the effect of food cues on intake occurs even when the experimental subjects are not hungry (for an example in non-human animal subjects, see Reppucci & Petrovich, 2012; and human animals, see Emond et al., 2016) This effect within the associative learning framework has been termed Cue-Potentiated Feeding (CPF).

CPF began to be studied in the 80s with well controlled experiments such as those of Weingarten (e.g., 1983, 1984) mentioned previously, showing how discrete food cues could motivate food intake in rats even when these animals had not been food deprived. This effect was later replicated by demonstrating this increment in intake in a sample of children (Birch et al., 1989) and healthy adult humans (Cornell et al., 1989). A relevant issue in CPF is whether food-associated cues produce an effect specific to the food they signal or whether they can generate a general appetitive state towards other foods. In this sense, and contrary to other paradigms such as Pavlovian to Instrumental Transfer (PIT), most of the studies have shown that the presentation of the CS+ increases the intake of the food it signals but not others, even when foods are familiar and hedonic (Cornell et al., 1989; Galarce et al., 2007; Petrovich et al., 2007; Repucci & Petrovich, 2012, but see Boggiano et al., 2009; Kendig et al., 2018).

While CPF has been demonstrated using discrete stimuli such as lights or tones, it has also been shown that the context in which training takes place produces the same effect (e.g., Petrovich et al., 2007; Kending et al., 2016; Kendig et al., 2018). In this sense, Petrovich et al. (2007) pointed out the relevant implications of contextual conditioning in appetitive behavior within current obesogenic environments, noting that a large part of our intake is produced in restaurants or fast-food settings (especially in certain countries such as the USA). These authors emphasize that the very nature of some of the fast-food restaurant chains may be of particular concern, as they tend to be very similar in appearance, with a uniform setting, and with limited menu choices. These characteristics could be very relevant in providing multiple opportunities to pair contexts with the specific foods they offer and, therefore, in future encounters, promote overeating in their presence.

Furthermore, the same author, in contrast to Weingarten's seminal studies (1983, 1984), demonstrated that CPF not only produced an increase in intake but also showed that the rats did not compensate for their periods of increased intake when exposed to the cues, thus leading to overeating in the long-term (Reppucci & Petrovich, 2012). One key factor in understanding the differences between the pattern of results found by Weigmarten (1983, 1984) and Reppucci & Petrovich (2012) possibly lies in their experimental procedure. While in Weingarten´s study the only food offered during the training phase was the target food, Reppucci and Petrovich trained the animals with restricted access to the standard chow in their home cage. Therefore, from Weingarten's point of view, the intake occurred within the "homeostatic cycle", whereas in Reppucci & Petrovich’s procedure, rats received the target food during training as an additional supplement to their intake. Moreover, contrary to Weingarten, they also offered standard chow during the test in addition to the cued food. It is possible that Weingarten's procedure may have encouraged other processes such as SSS or alliesthesia by presenting a more monotonous food supply.

Another interesting issue is whether Pavlovian Cue-Potentiated intake is sensitive to other non-general types of satiety/satiation processes such as SSS.

Both processes have a specific effect and impact the hedonic component of food; for example, it has been shown that food cues can trigger positive responses in the Taste Reactivity Test (Johnson, 2013). While some studies in the literature have addressed this question, they have generally focused on other aspects rather than eating behavior in isolation. These studies have shown that contexts (rodents: Kendig et al., 2017) or initially neutral discrete cues (Humans: Watson et al., 2014) that had been paired with highly palatable foods, will still elicit instrumental responses towards the food (after satiation of the specific foods with which they had been paired) while the effect of SSS on intake remains intact. In this regard, most of the research that has studied this devaluation effect through SSS has focused on understanding the action systems that govern behavior depending on the training history (goal directed vs. habitual).

Under this framework, the employed procedure is as follows: after pre-feeding with a food used as a target outcome, an instrumental extinction test is then administered in which rats perform instrumental responses towards the devalued lever or the non-devalued lever. Finally, rats are given a choice test between the devalued and non-devalued outcome. This measure of consumption is often taken to assess whether the devaluation treatment has been effective. Nevertheless, in these studies — often carried out with rodents — the procedure is usually somewhat different to those employed in human SSS studies that are not concerned about the instrumental structure that governs performance but the eating regulatory mechanism itself. Rats are generally pre-fed with high quantities of food (e.g., 30 grams of pellets) for long periods (e.g., 1-hour), which may be producing effects on intake other than those exclusively sensory specific (Corbit & Balleine, 2005; Kendig et al., 2017; Parkes, et al., 2018). Therefore, from this approach, SSS is employed as a method of devaluation to study other psychological constructs rather than eating behavior *per se.*

Obesity and food cue reactivity

The appetitive responses that are triggered when we are exposed to food cues have been generally regarded as instances of food cue reactivity. Cue reactivity is recognized as a natural behavior that is present in healthy individuals (Jansen et al., 2016). Nevertheless, a great deal of work has shown that the pattern by which it is expressed differs between healthy populations and those with disordered eating behavior (For a review: Jansen et al., 2016; van den Akker et al., 2018). Obese or overweight people exhibit increased salivary responses (e.g., Epstein et al., 1996; Ferriday & Brunstrom, 2011; Jansen et al., 2003), increased motivational or craving responses (e.g., Boswell & Kober, 2016; Tetley et al., 2009), consume higher quantities of food when cues are present (e.g., Halford et al., 2004; Tetley et al., 2009) and express stronger attentional capture towards food cues (e.g., Castellanos et al., 2009; Hendrikse et al., 2015; Nummenmaa et al., 2011).

Increased food cue reactivity has been highlighted as a risk factor for overeating (Boswell & Kober, 2016) and “relapsing” in individuals, both during and after weight loss treatment. Although there is evidence for individual differences, there is clear evidence for the role of Pavlovian learning in the expression of food cue reactivity (Carnell et al., 2008; Jansen et al., 2016; Llewellyn & Wardle, 2015; van den Akker et al., 2018). In this regard, one therapeutic alternatives based on associative learning principles and suggested for reducing disproportionate food cue reactivity Exposure-Based Therapy. Exposure-Based Therapy is based on the extinction phenomenon; just as exposure to cues and their elicited reactivity makes healthy food selection challenging in today's environments, weakening the relationship between cues and palatable food could reduce reactivity to them. In this context, Jansen et al. (2016) argue that unless overweight or obese dieters are exposed to the reactive food cues or contexts without the consumption behavior occurring, reactivity will not diminish. If this does not occur, it is likely that the dieter's attempts will ultimately fail as a consequence of exaggerated cue reactivity. While some experiments have yielded promising results on this issue (van den Akker, Havermans, et al., 2014; van den Akker et al., 2015) other studies have produced mixed evidence regarding long-term outcomes (see van den Akker et al., 2018 for a review).

Access to variety in the obesogenic environment

As mentioned, access to an immense variety of food products is one of the main features of current environments. This variety is present in two different formats: sensory and nutritional. On the one hand, there is great diversity of food products in terms of their sensory aspects. Processed foods, unlike traditional food, are characterized by being very similar but varying only slightly in their taste, texture, and odor attributes. This example becomes obvious when thinking of the infinite number of potato crisps available in supermarkets, ranging from ham or mustard and honey to fried egg flavors. As another example, a study by Hardman et al. (2015) showed that the number of varieties of pizzas of a given flavor (pepperoni) available in UK supermarkets totaled 71 different units. Further, these authors revealed that among these 71 different pizzas brands, the taste of which are possibly very similar while differing subtly certain sensory attributes, the calorie variability ranged from 500 to 2000 kcals for a standard size pizza. This latter estimate indicates the second source of variability within the obesogenic environment. Variety is not only expressed along a sensory spectrum, but there are also very similar products with different caloric consequences (e.g., light products).

Regarding the first source of variability, sensory variety, it has been shown that adding different sensory components during a meal increases total consumption compared with a more monotonous diet. This has been called "the Variety effect" or "the Buffet effect" the robustness of which has been shown in human animals (e.g., Brondel et al., 2007; Hetherington, 1996; Hendriks et al., 2021; Norton et al., 2006; Snoek et al., 2004) and non-human animal studies (e.g., Ahn & Phillips, 2012; Le Magnen, 1999; Myers, 2017; Parkes et al., 2017; Reichelt et al., 2014; Roe et al., 2013). The variety effect has been shown to produce a 25% increase in total intake in animal models, while in studies with human subjects it appears to produce a 22% total increase compared to a one food-based meal (McCrory et al., 2012). A recent meta-analysis reported evidence for the variety-enhancing effect on intake by finding a statistically significant effect ranging in size from small to medium (Embling et al., 2021). While this effect has mostly been studied with high caloric and palatable foods it has also been found for low-caloric foods such as vegetables or fruits (McCrory et al., 1999; Meengs et al., 2012; Roe et al., 2013). It has been suggested that one potential mechanism underlying the Buffet effect could be SSS, due to its specificity along with a presumed dishabituation or distracting process (Raynor & Epstein, 2001, but see Hendriks et al., 2019). Thus, when the SSS phenomenon is occurring, the presence of other foods may cause the hedonic response of the ingested food to recover its initial value, promoting a higher total consumption of the food. Variety has been classified as a worrying factor promoting overeating and overweight when energy-dense foods are present in a diet. But, as mentioned, there is evidence that this effect is also expressed when presenting healthier food choices such as vegetables, thus presenting a possible therapeutic strategy that is worthy of consideration.

The second source of dietary variability in current environments is related to the energetic power of foodstuffs. In this sense, a single product can be provided by different brands and in each of these this food can have very different energy values. More striking is the fact that, within the same brand, several versions of a single product can coexist that contain completely different nutritional contributions. This is the case of light or zero products, which often contain multiple sweeteners that mimic the sweet taste of sugar. For example, a regular coke contains 139 calories per can (330 ml), while a zero coke has only 1 calorie for the same content. This access to a plethora of similar products with different nutritional consequences has been suggested to potentially disrupt associative learning processes that regulate intake in flavor-nutrient learning and, as a consequence, conditioned satiety. The idea behind this proposal, which has been termed "the flavor confusion hypothesis" (Palframan & Myers, 2016) is that if there are multiple variations of the nutritional input within the same sensory cue such as a taste, smell, or texture, these will no longer be predictive of a nutritional consequence. Support for this hypothesis comes from the observation that flavor learning has been robustly demonstrated in animal models (where the experimental subjects' prior experience with sensory cues is controlled) but in humans this effect appears to be diffuse and the literature has yielded mixed evidence.

In 2012, Yeomans published an article called *Flavor-nutrient learning in humans: an elusive phenomenon?* showing that this phenomenon was demonstrated on only 64% of the occasions on which it was studied. Yeomans also noted that this effect was likely to be actually lower due to publication bias, given that null results are scarcely published. Traditional Flavor-Nutrient paradigms using human participants make use of several paired presentations of "novel" flavored drink or food stimuli. One of these is usually characterized as low energetic (CS-) and the other high energetic (CS+). After this conditioning stage, the Liking or relative preference is measured for both CSs (Flavor-Nutrient learning) and the total intake of both CSs or a different meal

(Conditioned satiety). Although this absence of Flavor-Nutrient learning in humans could be taken as evidence of “The flavor confusion hypothesis”, research in humans brings with it many problems that could alter the results obtained. The most obvious and important is the fact that it is virtually impossible to eliminate the subjects' previous experience with novel food stimuli. If most of the stimuli are known or partially known (through generalization across stimuli), they will be familiar and learning about these will be difficult due to the phenomenon of latent inhibition.

Despite this and other important procedural problems in the methodology used in human studies (e.g., number of sessions or motivational state of participants; for a review, Yeomans, 2012) that preclude drawing firm conclusions, there is evidence from animal models suggesting this disruption in learning regulatory mechanisms after exposure to inconsistent sensory-nutrient diets. For instance, some studies show how animals that are exposed to sweet stimuli that differ in energy value such as sucrose or saccharin disrupt later Flavor-Nutrient learning and conditioned satiety, promoting excessive intake and increased bodyweight (e.g., Davidson et al., 2011; Davidson & Swithers, 2004; Roy et al., 2007; Swithers et al., 2009; Swithers et al., 2013; Swithers & Davidson, 2008).

The studies presented above manipulate the inconsistency between sweetness and calories usually by exposing animals to sweetness through sweeteners that are not followed by any energy boost. However, actual exposure to sensory variety in environments is not always like this, since we are exposed to different sensory cues that are sometimes followed by a specific caloric intake and on other occasions that same sensory cue is followed by a different caloric consequence. For example, drinking a zero cola would be an instance in which the sweetness is not followed by calories, but having a cake for dessert would be a rather different situation.

However, an elegant study based on animal models completely manipulated the consistency with which sensory aspects of a varied diet (mimicking a cafeteria diet) predict caloric intake and studied how this affected flavor-nutrient learning (Palframan & Myers, 2016). In this study, the authors employed three groups of rats; one group was exposed to a wide variety of sensory food cues that had inconsistent calorific consequences, another had a varied diet in which the sensory consistently predicted calories, and a control group were fed standard laboratory chow. An important aspect is that the diet of the varied-consistent group was based on processed foods (e.g., different kinds of flavored cheeses, crackers, and cereals of different brands that were followed by distinct caloric output) while that of the varied-consistent group consisted of low or non-processed foods (e.g., fruits, vegetables, and nuts). Thus, the diets were not exactly comparable in both groups in terms of calorie intake and hedonic value. After a three-month exposure to the diets, the authors employed a training procedure in which they paired a flavor with a 6.5% glucose solution (CS+) whereas another flavor was paired with a 0.1% glucose and 0.125% saccharin solution (CS-). A key point to note is that both solutions were equivalent in their hedonic value (Warwick & Weingarten, 1994) but differed in their caloric content. Thus, if the flavor confusion produced by the varied-inconsistent group disrupts later flavor-nutrient learning, this group would be less efficient at discriminate between CSs, but this would not occur for the chow and varied-consistent groups which had been exposed to consistent flavor cues that predict a specific caloric content. Surprisingly, these authors found the opposite pattern of results, that is, the varied-inconsistent group seemed to be more sensitive to the CSs. The authors discussed whether variety, rather than disrupting flavor-nutrient learning processes, favors greater precision in discrimination. They went on to propose this as a possibility but not an underlying mechanism that could explain their findings. In *Chapter V* we suggest a possible alternative mechanism: *perceptual learning*.

CHAPTER III

Goals of the present thesis

Goals of the present thesis

This thesis aims to study the mechanisms by which variety and SSS (*Chapter IV and V*) or food related cues and SSS (*Chapter VI and VII)* influence eating behavior in the current obesogenic environments. SSS is an intake regulatory mechanism that seems to remain intact across multiple experiential manipulations, conditions, or measures. However, in the present thesis we will try to look for exposure effects related to associative learning through which it could be disrupted, thus promoting overeating.

In *Chapter IV* we will focus on the underlying mechanism of SSS. Many human studies have focused on studying habituation as the underpinning mechanism of SSS. However, all of these studies have been conducted using humans as experimental subjects and with subjective ratings as the main measure, yielding mixed results. To overcome all the problems associated with subjective as opposed to objective measures, in this thesis we have studied this hypothesis using a rat-based animal model and tested whether some of the characteristics of habituation can be found in SSS. The study of the mechanisms involved in SSS is of great relevance as it could explain why we often overeat under certain situations, for example, when a variety of foods are offered in a meal (Buffet effect), in social appetitive contexts, or while being faced with distractors during eating (such as TV). These situations could, in part, be explained through the habituation model.

One of the features that characterizes today´s environment is the massive exposure to very similar products. There is evidence that rats exposed to a wide variety of similar foods (cafeteria diet) are more sensitive to discriminating sensory features that had been paired with two different caloric consequences compared to control rats. In *Chapter V* we will focus on studying a possible mechanism by which exposure to a wide variety of foods sharing sensory properties but differing subtly in others while having the same caloric content may promote a dysregulation of the SSS effect. We hypothesize that the mechanism operating during exposure is perceptual learning, which favors an increase in the discrimination of similar stimuli, reducing the typical generalization observed in SSS between foods that share similar properties. We expect that if the generalization between similar meals disappears or is attenuated, other meals that should express the devaluation effect will remain appetizing and therefore promote overeating. Therefore, this mechanism could operate by enhancing the Buffet Effect in today's obesogenic environments, thus promoting excessive intake.

Another feature of current environments is the massive exposure to highly palatable and caloric foods. In this regard, likes and dislikes for initially neutral flavors are mainly acquired through associative learning by pairing them with hedonic tastes or high-calorie foods. Most of the literature has shown that these acquired preferences are mediated by an S-S associative structure, either by flavor-flavor or flavor-nutrient learning. Nevertheless, most studies have employed short training procedures in which experimental subjects have limited access to the CS-US compounds. In *Chapter VI,* we studied the effect of pairing a neutral flavor cue (CS) with a palatable flavor (US) using different types of training procedures. We manipulated the amount of exposure given to the CS-US compound through the days or hours of access and the volume of the given solution (limited or unlimited). We hypothesize that the associative structure of the conditioned preference will change as a function of the type of exposure during conditioning. Thus, the more exposure to the CS-US compound, the greater the opportunity for a S-R association to develop, and, contrary to previous studies, this preference will not be sensitive to the SSS effect. The development of S-R associations in preference acquisition entails relevant implications, as it would lead to automatic behaviors such as eating foods when these are no longer desirable, for example, when we are satiated.

The omnipresence of external food cues is a core feature of our current obesogenic environments. The presence of these cues have shown to produce an increase in total intake even when individuals are under a general state of satiety. In *Chapter VII*, in addition to attempting to demonstrate the basic effect of SSS in a sample of lean humans, we focus on studying the role of food-related signals in a satiety state other than the general one that is specific to the previously ingested food (SSS). Just as we have seen that the CPF phenomenon is specific to the cued food, we wanted to determine whether, in a sample of subjects pre-fed with a food (sweet biscuits or chips), the presentation of a cue that signals the pre-fed food could reverse the SSS effect. To do so, we used a cue that is naturally present in our environment (e.g., a television commercial). One group of participants was exposed to an advertisement for the eaten food whereas the other group viewed an advertisement for non-food stimuli. If Pavlovian cues (advertisements) produce a potentiation effect on intake even after an SSS procedure, it is expected that those who have received the advertisement for the specific food that has been eaten will consume more in total of the pre-fed food on both a pre-fed and non-pre-fed food choice test.

PART II

EXPERIMENTAL SECTION

CHAPTER IV

Habituation and Sensory-Specific Satiety

Sensory-Specific Satiety refers to a decline in the hedonic value of the sensory properties of a particular food as it is consumed. This phenomenon is characterized by a decrement in responding as a consequence of repeated exposure, is stimulus specific, and recovers after time. All these characteristics are shared with the habituation phenomenon and for this reason, habituation has been proposed as the underlying mechanism that explains this eating regulatory system. However, several studies conducted with human models have yielded mixed results. Using rats as experimental subjects, the present study tested the following three characteristics of habituation within a Sensory-Specific Satiety framework: spontaneous recovery, dishabituation and the distractor ef-fect. Experiment 1 demonstrated the basic effect of SSS and its spontaneous recovery over time. In Experiment 2 we found that the presentation of a dishabituator after a pre-feeding procedure had no impact on the SSS effect. Finally, in Experiment 3 the presence of a distractor during a pre-feeding procedure did not alter the expression of SSS. These results challenge the idea that SSS constitutes a typical case of habituation, at least with the procedure used here.

Introduction

Sensory-Specific Satiety (SSS) is defined as the temporary devaluation of the sensory properties of foods as they are eaten (Rolls, 1986, but see Havermans et al., 2009). Although this phenomenon is specific to the consumed food, it can also generalize to other foods that share similar sensory properties to the devalued food. This phenomenon has been identified as a mechanism to stimulate animals to eat a varied, nutrient-rich diet in a food-scarce environment (Rolls, Rolls et al., 1981). In this respect, SSS has been proposed as the main phenomenon explaining the Buffet Effect, which predicts that the greater the variety of foods in a meal, the greater total intake (e.g., Raynor & Epstein, 2001; Rolls, Rowe al., 1981). While the Buffet Effect seems to be a useful tool for animals in their natural environment, this does not seem to be the case of humans in today's societies. The environments in which we live, so-called obesogenic environments, are characterized by a constant exposure to a wide range of highly caloric and palatable foods (e.g., Berthoud, 2012; Blüher, 2019; Swinburn, et al., 1999). Consequently, SSS has been identified as a possible potential driver of overeating (Hetherington, 2013; Hetherington & Rolls, 1989). It is therefore essential to understand the nature and underlying mechanisms through which SSS functions as an intake regulation mechanism.

In this regard, the mechanism that has been most widely used to explain the SSS effect is the habituation phenomenon. When considering the properties of SSS such as the decline in hedonic responding as a consequence of repeated exposure to food, stimulus specificity, and spontaneous recovery over time, it seems plausible to think that SSS could be a case of short-term habituation. In fact, it has even been proposed that the Buffet Effect could be explained in part on the basis of a dishabituation effect, with the different foods present in the meal acting as dishabituators of the rest (Raynor & Epstein, 2001). Thus, studying the characteristics of habituation in SSS could be a useful tool for developing strategies to cope with the excessive intake produced by the Buffet Effect in current environments.

However, the studies carried out to date (mainly with human subjects) with the intention of examining the different characteristics of habituation with an SSS paradigm have yielded contradictory results. Therefore, the purpose of the present study is to provide further evidence on this issue by using a rat model to demonstrate the spontaneous recovery of SSS and to test whether this phenomenon is sensitive to the effect of presenting a dishabituator and a distractor.

Habituation as an underlying mechanism of SSS

The habituation of responding to a stimulus is a non-associative learning process in which the repeated presentation of a target stimulus will produce a specific reduction in the original responses to that stimulus, which spontaneously recovers after time (Rankin et al., 2009). Due to its specificity, habituation differs from other basic changes in behavior such as muscular fatigue or sensory adaptation. Similar to habituation, SSS produces a decrement in hedonic responding as a consequence of repeated exposure to a particular food, which can recover after time and is specific to the eaten stimuli. Hence, the fact that the hedonic devaluation occurs specifically to the eaten stimuli and not to others can also be taken to indicate that SSS differs from other basic forms of behavior such as those mentioned above. In this respect, it has been shown that SSS is expressed within the secondary taste cortex by a specific reduction in neuronal activity in response to pre-feeding, and this activity recovers when other non-pre-fed foods are eaten (Rolls, 2005**)**. Moreover, both phenomena have been proposed as adaptive mechanisms of behavioral regulation. On the one hand, the habituation phenomenon has been proposed as a general adaptive mechanism for ignoring non relevant stimuli within the environment and thus, avoiding a depletion of attentional resources in order to focus on other important stimuli. On the other hand, and as mentioned previously, SSS has been proposed as an adaptive mechanism by which animals are able to obtain all the required dietary nutrients, ignoring those that are no longer necessary.

Therefore, within the definition of SSS we can also observe some of the main features of habituation. In fact, Epstein et al. (2009) in their review of the literature on habituation and food intake claim that both phenomena are sometimes used interchangeably in the literature. One way to test this hypothesis is to determine if SSS shows the same behavioral characteristics as habituation. In this regard, Epstein et al., (2009) proposed several paradigms for studying habituation, including dishabituation, stimulus specificity, distraction, variety, long-term habituation and sensitization. Some human studies have adapted the standard SSS procedure to some of these paradigms in order to explore the similarities between the features of habituation and SSS. The general SSS procedure in humans consists of asking participants to initially provide a subjective rating of the target foods (usually with Liking and Wanting measures), after which they consume one of the foods until satiety (pre-feeding). Finally, participants again complete the original scales (pre-fed vs non pre-fed foods). On some occasions, this procedure is accompanied by a final choice test session in which participants are offered the pre-fed and non-pre-fed items to assess their intake patterns after pre-feeding. In the following section we will describe the most relevant results from these studies.

Spontaneous recovery from the hedonic devaluation produced by the SSS

Some studies have found that human participants who had consumed a food to satiety still express SSS with subjective ratings after 2, 20, 40 and 60 minutes (Hetherington et al., 1989; Rolls et al., 1989), with the highest devaluation effect being observed two minutes after consumption (Hetherington et al.,1989). Regarding intake measures, Hetherington et al. (1989) showed that 1 hour after the pre-feeding session, human participants showed no differences between their intake of pre-fed and non-pre-fed foods, and therefore on this occasion the SSS effect had disappeared when using a consumption test. Another study carried out by Havermans et al. (2012) found no effect of SSS recovery when using pleasantness ratings after 20 minutes of consumption in a sample of obese and healthy-weight female participants. Finally, a study conducted by Weenen et al. (2005) found out that SSS was still expressed after more than 24hr of pre-feeding depending on the type of testing food. Therefore, at least within humans, it is not clear which are the variables that modulate the recovery of SSS´s hedonic decline over time (for example: individual differences, amount of orosensory exposure or food category).

Dishabituation in SSS

Dishabituation is the phenomenon in whereby the introduction of a different and salient stimulus will restore the original responding to the habituated stimulus (Epstein et al. 2009). Applying this paradigm to SSS, the introduction of a different stimulus after having eaten a food will reestablish the initial hedonic value of the latter through dishabituation. Some human studies have applied this paradigm to SSS by presenting a dishabituator after having eaten a meal to satiety, of either the same sensory modality (Havermans et al., 2010; Havermans, 2012; Meillon et al., 2013) or different (a computer game, Havermans, 2012), and have found no dishabituation effect. These results contrast with a study conducted by Romer et al. (2006; Experiment 2) in which the authors found that the decay in the olfactory hedonic value of an ingested meal (SSS effect) could be restored after eating a second course of a different food. Furthermore, in Experiment 3, Romer et al. (2006) showed that the typical SSS food devaluation could be reversed by presenting the same pre-fed food altered with seasoning. In a similar vein, Epstein et al. (1992) found that repeated presentation of a juice for a number of trials caused a reduction not only in hedonic but also in salivation measures, and in both cases this reduction was reversed when a dishabituator was presented (Experiment 1: different juice; Experiment 2: chocolate).

Distraction in SSS

The distraction procedure consists of presenting a different, novel stimulus while the habituation process is occurring. It is important to emphasize the differences between a dishabituator and a distractor, since sometimes these have been acknowledged to be the same. In particular, the differences between the two paradigms rely on the mechanism of action; while the dishabituator is presented at the end of the habituation procedure, the distractor interrupts the habituation process or in this case, the devaluation of the food stimulus through SSS. Distractors, as a regulatory mechanism, could be present in many everyday situations such as watching TV or being part of a social meeting whilst eating. Through a distraction process we would expect that these types of situations will promote a higher total intake due to the fact that the SSS process is being interrupted, thus, slowing down or preventing the decay of the hedonic value of the food. In fact, several studies have focused on how being distracted while eating can affect other general intake-satiety processes such as feelings of fullness, total intake or hunger, showing how distractors can promote an increase in total intake (Oldham-Cooper et al., 2011).

Whilst the results of human studies have shown that the use of a distractor such as a computer game can prevent Wanting responses towards a specific satiated food to decrease, such distractors do not affect the decline in the hedonic value of food (Brunstrom & Mitchell, Experiment 2, 2006). Hetherington, et al., (2006), however, found that distracting participants during the SSS process by allowing them to taste other different foods, promoted not only higher total intake but also led to a slowdown of the usual hedonic decline of pre-fed foods in comparison with a group that did not receive the distractor. Another study conducted by Brondel, Lauraine et al., (2009) assessed whether multiple or single alternations of foods within a meal could increase total intake in comparison with a meal with no repetitions. The results revealed that single repetitions of foods within a meal increased total consumption when compared with the other two conditions, presumably through the disruption of habituation. However, in this study the pleasantness ratings of foods did not reveal any groups difference in the pattern of hedonic decline of these foods. Finally, another study carried out by Epstein et al. (1992) showed how playing a computer game while tasting a juice on different trials slowed down the decrement in the salivation response to that juice, although in this study the hedonic value of the food was not measured.

Goals of the study

Thus, there appears to be mixed evidence for the notion that habituation is the underlying mechanism of SSS, at least with the methodology and procedures employed in existing studies in the literature. It is possible that the procedures employed within some human research could be critical when studying the mechanisms involved in SSS. For instance, human research has frequently made use of subjective ratings in order to assess the hedonic value (Liking) or motivation to consume a food (Wanting). Some authors, however, have pointed out that this type of measures can damage the validity of the constructs that are being assessed (Koranyi et al., 2020). In particular, ratings can be altered by participants´ supervision through the complete session such as the influence of recall in previous rating trials or perceiving food pleasure as a stable quality that does not change over time. For example, it is possible that even our own language can be a source of confusion with regard to what is really being assessed, since the concepts of Liking and Wanting are frequently used interchangeably in daily life situations regardless of their real meaning (Grigutsch et al., 2019). Even further, it has been argued that Liking and Wanting components are not always consciously perceived. Thus, modifying the hedonic value or the appetite for a particular food can alter the real perception of these foods when participants have to rate the foods by introspection (Berridge & Kringelbach, 2008). Furthermore, ecological validity issues can also affect the procedure since humans could feel uncomfortable when required to consume food in unfamiliar surroundings instead of their natural or usual feeding environment. Ultimately, with human studies it is not possible to control prior experiences — and thus familiarity — with the target foods. For these reasons, the present study made use of a rat model to determine whether three characteristics of habituation are also shared by the SSS phenomenon, that is, spontaneous recovery, dishabituation and distraction. We have chosen these three characteristics of all those outlined in the previous literature (see Rankin et al., 2009; Epstein et al., 2009) due to its controversial results within the human studies. To test this hypothesis, flavored fluids were used as stimuli in all the studies and SSS was assessed by total consumption of these flavors on a choice test.

Experiment 1: Spontaneous recovery

In this experiment we examined the temporal recovery of the hedonic value (spontaneous recovery of SSS effect) across time with a between-subject design. Animals were familiarized with two flavored solutions for two days, after which they were pre-fed with one of the two solutions to obtain the SSS effect.

Each group of rats was then tested for SSS after a specific time interval (0, 2, 5, 8 or 24 hr.) and by presenting a two-bottle test that contained both the pre-fed and non-pre-fed solution. If spontaneous recovery occurs, we should expect a direct relationship between the length of the time interval and recovery. A recovery of the SSS effect would be found when, after a certain amount of time has elapsed from the pre-feeding phase, total consumption of the pre-fed and non-pre-fed solutions do not differ.

Methods

Subjects and apparatus

A total of 30 male non-naïve Wistar rats with an average weight of 512 g (range: 420g -610 g) were used in the present experiment. Rats were between 14-16 weeks old. The animals had received previous experience with other solutions and different procedures to those used in the present experiment. The rats were supplied by the Animal Production Unit of the University of Granada. Animals were randomly assigned to one of five groups matched for body weight (0hr: 539 g, 2-hr: 540, 5hr: 496, 8hr: 486g, 24hr: 500g).

Animals were individually housed in translucent plastic cages (35 × 12 × 22 cm) with wood shavings as bedding. A 12-h light/dark cycle was maintained for the whole procedure, beginning the light cycle at 8:00 am. Solutions were prepared everyday with tap water, and given to animals in centrifuge tubes (50 ml capacity) with stainless steel, ball-bearing-tipped spouts. All solutions were placed in the middle of the front metal cover of the cages on all the sessions in order to avoid the emergence of position preferences during the two-bottle tests. Consumption was measured by weighing the tubes before and after each procedure. The flavored solutions were prepared by diluting 0.05% vanilla aroma (Manuel Riesgo, Madrid) with 0.3% saccharin

(Labortecnic) or 1% domestic soya sauce reduced in sucrose (Pearl River Bridge). All the procedures described in this paper were approved by the Comité de Ética en Experimentación Animal 06/06/2019/099 (Ethics Committee in Animal Research) at the University of Granada and were classified as low severity according to European guidelines. Animals were monitored daily by those responsible for animal welfare in the research center.

Procedure

The water bottles were removed one day before the beginning of the experiment, and access to water or experimental solutions was restricted to two daily sessions (10:00 am- 4:00 pm). On the first two days of the experimental procedure, the rats received 30 minutes ad libitum access to water in the morning and afternoon sessions. These sessions were carried out in order to habituate animals to the schedule of the sessions and the tubes used, as well as to record baseline water consumption. The animals then began the familiarization phase (days 3-4), which consisted of two daily 20-minute sessions (morning /afternoon) in which rats had access to 10ml of one solution. These sessions were carried out so that rats had two previous exposures to each solution before the pre-feeding treatment in order to avoid neophobia. The order of presentation of each solution across the 4 sessions of familiarization was also counterbalanced. On the morning of the 5th day, animals started the pre-feeding phase, which consisted of 20 minutes access to 15 ml of one solution. After this procedure, each group of animals was tested for SSS with the following timings: immediately after (n=6), 2h after (n=6), 5hr after (n=6), 8hr after (n=6) or 24hr after (n=6) presentation of the solution. The SSS test consisted of a 10-minute choice test in which animals were given free access to the pre-fed and non-pre-fed solution. The order and position in which the solutions were given on the test was counterbalanced to avoid any position preference. All rats received adlib access to water for 30 minutes in the afternoon sessions to rehydrate (4:00 pm). The rats from the 8hr group received this water session following the SSS test (6:00 pm). On the morning of Day 6, rats in the 24hr group received the SSS choice test while the rest of the groups had access to water. On the afternoon of Day 6 all rats again received 30 minutes access to water (4:00 pm). On Day 7-8 the same pre-feeding and SSS test cycles were carried out for all the groups but animals were pre-fed with the alternative solution to the one given on Day 5 (See Table 1).

Table 1. *Procedure of Experiment 1*

Group	Familiarization	Pre-feeding	Choice test	Time of testing
0’ 2hr 5hr 8hr 24hr	A / B	A / B	A vs B	Immediate 2hr interval 5hr interval 8hr interval 24hr interval

Note: A and B refer to the two solutions (vanilla + sucrose and soya, counterbalanced). “/” refers to alternate days of the counterbalancing, “vs” refers to the choice test between the two solutions (Pre-fed / Non-Pre-fed).

Data analysis

General linear model null hypothesis testing analyses were carried out, adopting a rejection level of $p < 0.05$, using Greenhouse–Geisser corrections for mixed factorial analysis of variance when needed. Partial eta squared and Cohen's d tests were used to measure effect sizes. The same statistical criteria were used for subsequent experiments.

Results and discussion

During the familiarization phase, consumption of the vanilla-saccharin solution was lower than that of the soya sauce solution, due to neophobia. However, two days of familiarization with the solutions was sufficient to produce an attenuation of neophobia. A repeated measures ANOVA was carried out to assess consumption of the groups during the pre-feeding phase with Day (Day1 / Day 2) as the within- subject factor and Group (0hr, 2hr, 5hr, 8hr, 24hr) as the between-subject factor. The analysis revealed no significant differences between days, groups or an interaction between these factors *Fs<1* (Mean consumption of both days of pre-feeding; 0hr: 11.41 SE= 0.91; 2hr: M=11.11 SE= 0.96, 5hr: M=11.74 SE: 0.40, 8hr: 11.43 SE=0.65, 24hr: M= 11.30 SE=0.76).

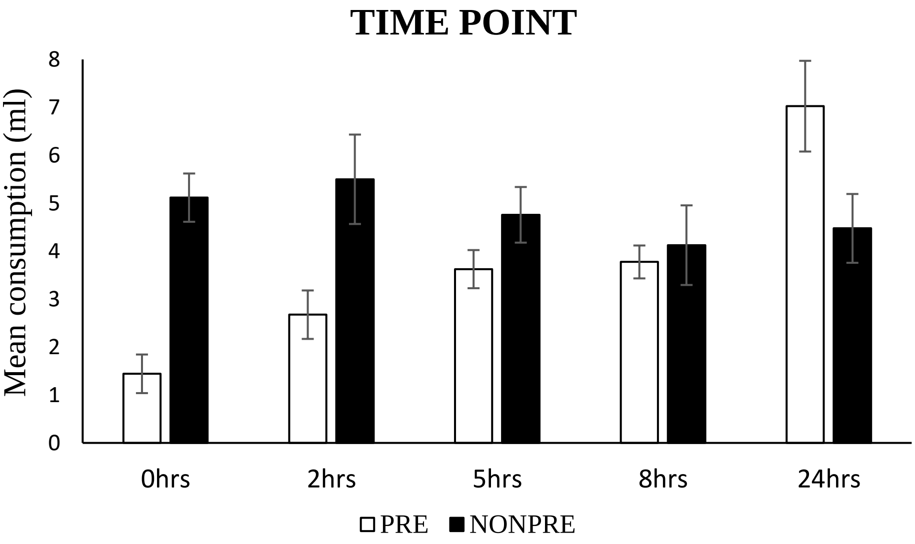


Figure 1. The effect of SSS across the different experimental groups. “PRE”: refers to the pre-fed solution whereas “NON-PRE” refers to the non-pre-fed solution.

Figure 1 shows the mean total consumption of the pre-fed and non-pre-fed solutions on the choice test across both days of testing. Inspection of this figure shows a clear SSS effect with the shorter time intervals, since subjects in these groups consumed less of the pre-fed solution than the non-pre-fed solution. This effect appears to be attenuated when animals are tested for SSS after a longer temporal interval. A repeated measures ANOVA conducted on these data with Pre-feeding as the within subject factor (Consumption of Pre-fed/ Consumption of Non- Pre-fed solution) and Group as the between-subject factor revealed a main effect of Pre-feeding $F(1,25)= 5.54$, $p=0.03$; $\eta^2_p= 0.18$. Moreover, there was an interaction between Group and Pre-feeding $F(4,25)= 5.51$, $p=0.003$; $\eta^2_p = 0.47$, and a significant effect of Group, $F(4,25)= 4.95$, $p=0.004$; $\eta^2_p = 0.44$.

As expected, the interaction between group and pre-feeding revealed that there were differences between the groups in the expression of the basic SSS effect. Thus, we conducted planned comparisons in order to observe the pattern of the SSS effect over time. A two tailed paired samples T-test was carried out on the consumption data for each group to compare direct consumption of the pre-fed solution with the non-pre-fed solutions between groups. Consumption of the solutions differed for the 0hr group $t(5)= -5.89$, $p=0.002$; $d= -2.40$; the 2hr group $t(5)= -2.57$, $p=0.05$; $d= -1.05$. No SSS effect was found for the 5hr group $t(5)= -2.10$, $p=0.09$; $d= -0.86$ the 8 hr group $t(5)= -0.34$, $p=0.75$, $d=-0.14$, or the 24hr group $t(5)= 1.65$ $p= 0.16$, $d =0.67$ (See Figure 1).

Therefore, and as expected, the present experiment provided evidence for an SSS effect immediately after the pre-feeding phase, since rats in the 0hr group showed significantly higher consumption of the non-pre-fed solutions than the pre-fed solutions. Moreover, rats tested within a 2-hour time interval also showed a SSS effect, a finding that is consistent with other previous studies conducted with rats and using direct consumption tests (Gonzalez et al, 2018; Parkes et al., 2016; Reichelt et al., 2014). In contrast, rats tested 5, 8 and 24 hr after the pre-feeding session did not express a preference for the non- pre-fed solution, thus, the hedonic value of pre-fed solution was recovered. Interestingly, rats in the 24 hr group showed a tendency to drink more of the pre-fed solution than the non-pre-fed solution. However, this effect was not significant and when this was tested again in our laboratory with a larger sample of experimental subjects (8 rats), this effect was not replicated.

Experiment 2: Dishabituation

The aim of Experiment 2 was to assess the effects of presenting a dishabituator following the pre-feeding procedure. As in Experiment 1, following familiarization with two different solutions, rats were pre-fed with one of the solutions. After this, the Dishabituation Group was exposed to a bottle with a different solution (dishabituator) while the Control Group was presented with a bottle that contained water. Immediately after this, rats received a two-bottle test with the pre-fed and non-pre-fed solutions. If SSS is sensitive to the effect of dishabituation, rats presented with a different solution after the pre-feeding phase would be expected to show an absence or attenuation of the preference for the non-pre-fed solution over the pre-fed solution.

Methods

Subjects and apparatus

16 male non-naïve Long-Evans rats with a mean weight of 262.5 g (240g -295 g) were used in this experiment. Rats were supplied by Janvier labs and were around 8-10 weeks old. Animals were randomly assigned to groups

matched for body weight (Dishabituation group: 263.1g; Control group: 262 g). All aspects of animal housing and the preparation and presentation of solutions were the same as Experiment 1. The flavored solutions were prepared by diluting 2% domestic vinegar with 10% domestic sucrose and 10% maltodextrin with 0.05% coffee aroma (Manuel Riesgo, Madrid). A solution of 0.9% domestic salt was used as the dishabituator for the Dishabituation group.

Procedure

The complete experimental procedure lasted for 6 days (See Figure 1b). As in Experiment 1, animals were water deprived two days before the experimental procedure and water access was restricted to two 30-minute sessions (10:00 am and16:00 pm). In this experiment animals received 30 minutes of access to water during all the afternoon sessions in order to rehydrate. On Days 1-2 the animals were familiarized with the experimental sessions as in Experiment 1 and baseline water consumption was recorded. On Days 3-4 animals were familiarized with one of each solution in the morning sessions, with the order of presentation of each solution being counterbalanced. In this experiment, the animals were familiarized with each solution only once since they showed very little neophobia to the solutions. Each familiarization session lasted for 20 minutes and the animals were given 10ml of each solution. The dishabituator was not familiarized in order to make this stimulus more salient and less familiar during the pre-feeding phase. On Days 5-6, the pre-feeding and test cycles took place; however, in this experiment, after the pre-feeding phase the animals in the Dishabituation group were given a salty solution (Dishabituator) and animals in the Control group were given plain water. The animals were given 20 minutes exposure to 12 ml of the solutions during pre-feeding and 5 minutes exposure to 4ml of the salt or water. As in Experiment 1, the flavor of the solutions given during the pre-feeding phase was counterbalanced across both days. Immediately after the presentation of the dishabituator or the water solution, the rats were tested for SSS, as in Experiment 1 (See Table 2).

Table 2. *Procedure of Experiment 2*

Group	Familiarization	Pre-feeding →	Dishabituation →	Choice test
CTRL	A / B	A / B	Water	A vs B
DISH			Y	

Note: A and B refer to the two solutions (Vinegar + sucrose and Coffee + Maltodextrin, counterbalanced). “Y” refers to the salt solution. “/” refers to alternate days of counterbalancing, “vs” refers to the choice test between the two solutions (Pre-fed / Non-Pre-fed). The arrow refers to different phases of a session.

Results and discussion

During familiarization, all animals drank almost all of the fluid presented on both morning sessions. Consumption during the pre-feeding phase was analyzed to assess any possible group differences in taste preference or quantity of fluid ingested. The data of one rat from the Dishabituation group were excluded from the analysis due to consuming only 1ml of the solution on the first day of pre-feeding. Furthermore, this rat was classified as an outlier in the choice test data by the JASP program. A repeated measures ANOVA was carried out with Day (Day1/Day2) as the within-subject factor and Group (Dishabituation or Control) as the between-subject factor. This analysis revealed no significant effect of Day, no effect of group, and no interaction between these factors, $F<1$ (Mean consumption (ml) across both days of pre-feeding; Control: M= 8.26 SE= 0.34; Dishabituation: M= 7.67, SE=0.75).

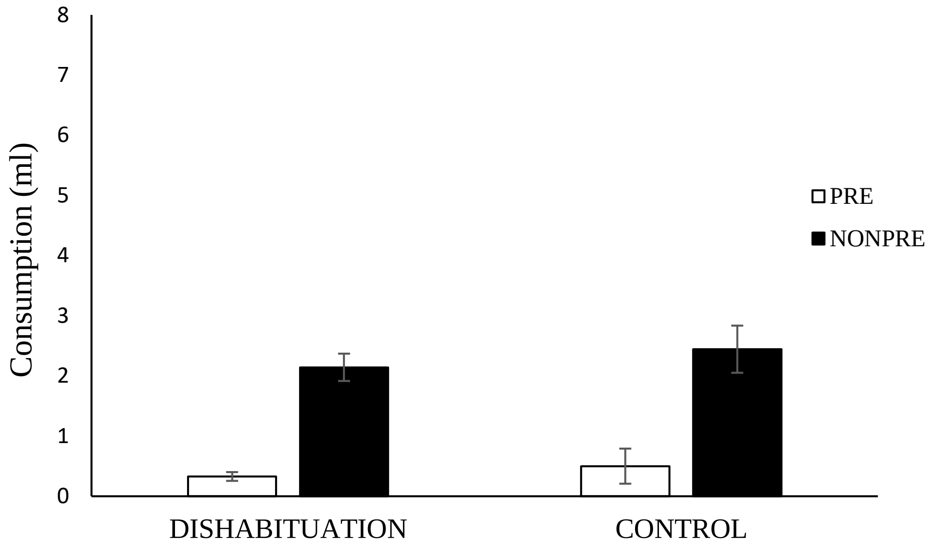


Figure 2. The SSS effect in the different experimental groups. "PRE": refers to the pre-fed solution whereas "NONPRE" refers to the non-pre-fed solution.

Figure 2 displays the mean total consumption of the pre-fed and non-pre-fed solutions during the two choice tests for both groups. This figure shows that both groups seem to express the SSS effect, that is, the total consumption of the non-pre-fed solution was higher than that of the pre-fed solution. These data were analyzed using a repeated measures ANOVA to assess the effect of the dishabituator on the expression of SSS, with Pre-feeding (pre-fed or no- pre-fed) as the within-subject factor, and Group (Dishabituation or Control) as the between-subject variable. This analysis revealed a significant effect of Pre-feeding, $F(1,13)= 28.15$, $p< 0.001$; $\eta^2_p = 0.68$, and no interaction Fs < 1 or effect of Group, $F(1, 13)= 1.51$, $p= 0.24$, $\eta^2_p = 0.10$.

To analyze consumption of the salty solution (dishabituator) and water (control) a repeated measures ANOVA was carried out with Day (1-2) as the within-subject factor and Solution (Salt/ Water) as the between-subject factor. The results revealed no significant effect of Solution $F(1,13)= 4.14$, p=0.06 η^2_p

=0.24, whilst no other effects or interactions reached significance *F*s < 1. These data suggest a higher total intake of the salty solution in comparison with water (mean consumption in ml across both days; Salt: M= 1.94, SE= 0.36, Water: M= 1.01, SE=0,28).

The present experiment investigated whether the presentation of a dishabituator following a pre-feeding procedure could restore the hedonic value diminished by the SSS effect when this is measured using a direct consumption test. The results of this experiment indicate that exposing rats to a dishabituator had no impact on the expression of SSS. Rats from both groups drank significantly less of the pre-fed solution than the non-pre-fed solution. If the dishabituator had disrupted the SSS effect, rats from the Dishabituator Group would have consumed more of the pre-fed solutions than those in the Control Group. However, in the present experiment, we this effect was not demonstrated, at least when using a dishabituator of the same sensory modality as the pre-fed solution (liquid) and when using a direct consumption test to evaluate the hedonic value of this solution.

Experiment 3: Distraction

In Experiment 3 we tested another property of habituation, that is, the effect of a distractor. Whilst the dishabituator is presented at the end of the habituation process, distractors act while the decrement in responding is occurring. To test this hypothesis, we used a within-subject design. The rats underwent different pre-feeding phases under two different conditions: distractor or control. The distractor condition involved the intermixed presentation of a different solution with the target solution, whilst in the control condition the subjects only received presentations of the target solution. After the pre-feeding phase, all rats received a two-bottle test containing the pre-fed and the non-pre-fed solution. If SSS is sensitive to the presentation of a distractor, we expect that the rats in the distractor condition will express a weaker (or absence of) SSS devaluation effect when consuming the pre-fed solution during the two-bottle test.

Methods

Subjects and apparatus

A total of 16 male naïve Wistar rats with a mean weight of 269 g (294g-230 g) were used in the present experiment. Rats were supplied by Janvier Labs and were about 8-10 weeks old. Animal housing was the same as described for Experiments 1 and 2. The flavored solutions were the same as those of Experiment 1 but in this experiment, we also used a distractor solution composed of 1% squeezed lemon and 1% maltodextrin.

Procedure

The experimental procedure lasted for nine days (see Table 1). One day before the beginning of the experimental procedure, the rats were water deprived and access to water was restricted to two experimental sessions: 9:00 am and 3:00 pm. On the first and second day, the same procedure was carried out for assessing the baseline consumption and to familiarize the subjects with the experimental procedure. On the third day, all rats were familiarized with 10ml of the distractor solution for 20 minutes in the morning session and received access to water for 30 minutes in the afternoon session. In this experiment we decided to provide the rats with an initial familiarization session with the lemon solution (distractor), given that the acidic properties of this flavor could elicit neophobia in the absence of previous exposure. Similarly, familiarization with the distractor was carried out on the first day of the procedure in order to maintain the salience or the novelty of the latter until the pre-feeding phase. On Days 4 and 5, all the rats were familiarized with the solutions in the morning and afternoon sessions, as in Experiment 1. From Day 6-9 the animals started the pre-feeding-choice test cycles in the morning and received 30 minutes access to water on all the afternoon sessions. During these four days, half of the animals received the Distractor treatment on the first two days (soya or saccharin counterbalanced) and the other half received this treatment on the last two sessions. In order to counterbalance the solution that they were given during pre-feeding (vanilla-saccharin /soya). The Distractor treatment lasted for 35 minutes. The animals were given full exposure to the target solutions for 20 minutes, whilst the distractor solution was presented for 15 minutes. The animals were presented with each of the stimuli for 5 minutes in an intermixed fashion (e.g., 5 minutes Soya/ 5 minutes distractor/ 5 minutes Soya… for 35 minutes). Animals in the Control condition received the target solution for 20 minutes without a distractor. During the pre-feeding phase, the animals were given 12 ml of the target solution and 6 ml of the distractor solution as appropriate. Given that the rats in the distractor condition will consume more than those in the control condition, both groups received the choice test one hour after the end of the pre-feeding phase. The procedural details of the choice tests were the same as those described for the previous experiments (See Table 3).

Table 3. *Procedure of Experiment 3*

Familiarization	Pre-feeding/Distraction →	Choice test
Y- A / B	A / B / A + Y / B + Y	A vs B

Note: A and B refer to the two solutions (vanilla + sucrose and soya, counterbalanced). "Y" refers to the lemon-maltodextrin solution. "/" refers to alternate days of counterbalancing, "-"refers to separate days. "vs" refers to the choice test between the two solutions (the Pre-fed and the non-Pre-fed). “+” denotes the presentation of two different solutions within the same session. The arrow refers to different phases of a session.

Results and discussion

During familiarization with the distractor, the animals consumed almost all of the lemon-maltodextrin, soya and vanilla-saccharin solution. Animals showed neophobia to the vanilla-saccharin solution, but this effect disappeared on the last day of the familiarization phase. Two animals were excluded from the analysis due to the fact that they showed higher levels of neophobia to the saccharin solution during the entire familiarization procedure (consuming less than 2.5 of the 10 ml available on both days).

Consumption of the target solutions during pre-feeding phase were analyzed with a repeated measures ANOVA with condition (Distractor/Control) and Day (Day 1 /Day 2) as within subject factors. This analysis revealed no significant effects of the Day factor $F<1$, the Condition factor $F(1,13)=2.13$, $p= 0.17$; $\eta^2_p= 0.14$ or the interaction Day*Condition $F<1$ (mean consumption of both days across both conditions; Distractor: M=10.64, SE=0.20/ Control: M=10.25, SE= 0.33).

Consumption of the distractor solution showed that all animals drank the lemon-maltodextrin solution during the pre-feeding phase in this condition (M=3.98, SE= 0.34; min=1.4, Max= 5.4).

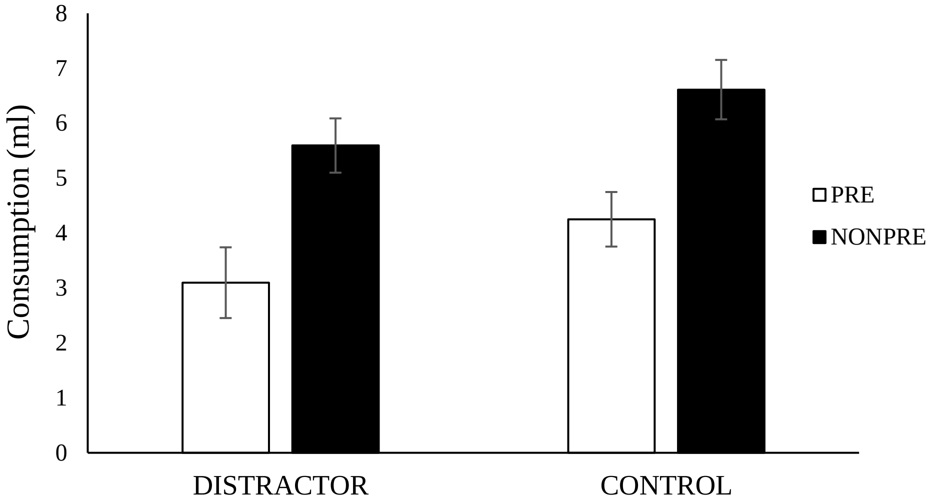


Figure 3. The SSS effect in the different experimental conditions. "PRE": refers to the pre-fed solution whereas "NON-PRE" refers to the non-pre-fed solution.

Figure 3 shows the mean total consumption of the pre-fed and non-pre-fed substances for the distractor and control conditions during the 4 days of testing. These results suggest no group differences in the expression of SSS, with the consumption of the pre-fed solution being lower than the non-pre-fed solution in both cases. These data were analyzed using a repeated measures ANOVA with Condition (Distractor/Control) and Pre-feeding (Pre-fed/Non-Pre-fed) as the within-subject factor. This analysis revealed a significant effect of Pre-feeding F (1,13)=20.43 p= <0.001, η^2_p = 0.61 and a significant effect of Condition F(1,13)= 7.91, p= 0.01, η^2_p= 0.38, but no significant interaction between these factors, F<1.

The results of this experiment have demonstrated that rats in both conditions show an effect of SSS by drinking less of the pre-fed solution than the non-pre-fed one. Animals in the distractor condition, however, consumed less overall during the two-bottle test. This might be expected, given the fact that animals in the distractor condition had an additional solution to drink before the choice test and therefore, were less thirsty. Thus, these results show that the presentation of a distractor of the same sensory modality did not impair the SSS effect.

General Discussion

Using a rat model, this study set out to assess whether habituation is the underlying mechanism of SSS by testing whether the latter phenomenon shares the following three characteristics of habituation: spontaneous recovery, dishabituation and distraction. The results of Experiment 1 confirmed the SSS effect when two flavored solutions were used as the target stimuli and the effect was measured using a direct consumption test (see also González et al., 2018). Moreover, these findings revealed a spontaneous recovery of the relative preference of the pre-fed solutions over time. Animals expressed a SSS effect both immediately and two hours after pre-feeding, consuming more of the non-pre-fed solution then the pre-fed solution on a choice test. After 5 hours, the recovery of the preference was apparent, with no differences in consumption between the two solutions. Interestingly, this result contrasts with that reported by Parkes et al. (2016) who measured the SSS effect in rats across various time intervals and by measuring both the patterns of intake and instrumental responses towards pre-fed and non-pre-fed solutions. In this study, after an interval of 5 hours, rats still consumed less of the pre-fed than the non-pre-fed solutions. However, there are two main procedural differences between our study and that of Parkes et al. (2016) that could possibly explain these discrepant findings. First, the different pattern of results could be explained by the type of pre-feeding procedure used, since in the present study, the pre-feeding session was shorter (20 minutes exposure compared with 60 minutes in Parkes et al., 2016). Second, in our study all the stimuli were liquid solutions as opposed to solid stimuli (flavored pellets). Some authors argue that satiety processes are weaker with liquid stimuli in comparison with solid stimuli since with the former sensory modality, oro-sensory exposure is weaker (Bilman et al., 2017). Thus, it is reasonable to suppose that with liquid stimuli the SSS processes could decay earlier in comparison with solid stimuli.

Experiment 2 showed that the SSS effect is not restored after the presentation of a dishabituator; on the choice test both groups consumed higher amounts of the non-pre-fed solution than the pre-fed solution. This finding is consistent with the results of many other human studies that tested this hypothesis although it contrasts with the results reported by Romer et al. (2006). In Experiment 2, these authors showed that after eating a first course, introducing a different meal in a second course reversed the devaluation effect when using measures of olfactory pleasure to evaluate the SSS effect. Moreover, Experiment 3 of the same study showed how presenting the same pre-fed food in a second course with seasoning could restore the decrement in the hedonic value of this food (see also Brondel, Romer et al., 2009 for a similar result). Whilst the results of this third experiment could be interpreted as evidence for dishabituation, these findings are open to an alternative interpretation in terms of positive hedonic contrast. In fact, Yeomans et al. (2020) have shown that the typical decrement in hedonic value that characterizes SSS can be reversed after presenting a more palatable version of the same meal (by adding more salt). Thus, comparison with a previous meal that is more or less valuable can, over time, modify the current value of the same food in a positive or negative way. Thus, if a food is eaten to satiety and is then presented in a more palatable format, any recovery in hedonic pleasure could be attributed to hedonic contrast rather than dishabituation.

Experiment 3 showed that the presence of a distractor during the SSS process did not alter the SSS effect since there were no significant group differences in consumption on the choice test. This finding contrasts with those of other studies conducted with human participants that found a disruption of the SSS effect as measured by Wanting (Brunstrom & Mitchell, Experiment 2, 2006), Liking (Hetherington et al., 2006) or intake patterns (Brondel, Lauraine, et al., 2009; Hetherington et al., 2006). A notable difference between the procedures conducted with human subjects and the one presented in this study is the way in which the distractor is presented during the procedure. In human studies, the distractor is presented simultaneously with the target stimuli, similar to the way it occurs in day-to-day life. On the present experiment, the distractor was not presented simultaneously with the target solution, but in an intermixed fashion. This procedure was carried out to ensure that consumption of the target and the distractor solution were equally distributed during pre-feeding, thus preventing rats from consuming their preferred substances first. In view of this situation, one could question whether this manipulation did not succeed in distracting the experimental subjects, as it should do in everyday life: talking with people, watching television etc… However, according to Epstein et al. (2009) for the distractor to be effective, it is sufficient that it interrupts the processing of the habituating stimulus. In this way, the intermixed presentation of the distractor would remove the pre-fed solution from working memory, disrupting its processing and thus, its habituation.

This study produced results which confirm the findings of many previous studies conducted in this field, ruling out the possibility that habituation is the only mechanism underlying SSS, at least with the experimental procedures used here. However, the study of the mechanisms involved in this SSS is of great interest for better understanding our eating behavior within so-called obesogenic environments. Such obesogenic environments are characterized by Cafeteria diets that include a wide variety of caloric foods within a meal, which have been proposed to increase total intake, at least in part due to the Buffet or Variety Effect. The Variety Effect predicts that the greater the variety of different foods within a meal, the higher the total intake within that meal (Brondel, Lauraine et al., 2009; Epstein et al., 2010; Raynor & Wing, 2006; Rolls, Rowe et al., 1981; Rolls et al., 1983). SSS has been proposed as the main explanatory phenomenon for the Variety Effect (Norton et al., 2006; Reichelt et al., 2014). One way in which SSS could promote this increase in consumption is through a dishabituation (when meals are presented sequentially) or distraction process (when meals are presented simultaneously).

However, there is also evidence to suggest that presenting different foods in a simultaneous or a sequential fashion during a meal does not reverse the hedonic devaluation that is characteristic of the SSS effect (e.g., Meillon et al., 2013). Thus, an alternative explanation of the Buffet effect involves the rate of exposure to the different foods within a meal. In contrast to the habituation approach, from this point of view, rather than variety, the amount of exposure to each food item during a meal is crucial. Thus, the SSS process occurs independently for each food stimulus and there are no interactions between food items. If we are given many food choices (high variety condition, Buffet Effect) oro-sensory exposure to each component of the meal is shared between many food items and the course of SSS is weaker for each stimulus. In a monotonous diet (low variety condition) there are relatively few options to choose from, so that exposure to each stimulus is higher, allowing for a rapid and strong sensory devaluation of all foods. Hendriks et al. (2019) found evidence for the latter proposal by manipulating the number of exposures to food stimuli as well as the level of variety within the presentations of the target foods. In this study they assessed SSS expression by measuring both hedonic and motivational responses. These authors found that whilst the most relevant factor for observing the SSS effect was the amount of exposure to the foods, alternation between different foods within a meal had no impact on SSS. Again, the latter study constitutes further evidence to suggest that SSS does not share all of the properties of the habituation phenomenon and does not help to explain the Buffet effect.

Havermans (2012) and Meillon et al. (2013) have argued that the fact that SSS does not share all of the properties of habituation does not necessarily mean that SSS does not reflect the expression of this phenomenon. In fact, Havermans (2012) argues that SSS is a special form of habituation in which stimuli specificity is expressed but dishabituation does not occur. Moreover, Meillon et al. (2013) suggested that SSS is composed of two main phases that are critical in determining whether or not the SSS effect can be disrupted. In the first stage, SSS is developing and can still be modified whilst in the second stage it is fully complete and this sensory devaluation cannot be disrupted or reversed. These authors argue that the very nature of SSS as an eating behavior mechanism is the property that distinguishes it from other types of behavior that can be habituated. As foods are ingested, it is not only the sensory input but also the feelings of fullness or satiety that make SSS impossible to dishabituate. Thus, according to this hypothesis, SSS can only be disrupted when it is in the development stage. However, in Experiment 3, animals were exposed to a distractor within five minutes, that is, when the SSS process was still developing, and no attenuation of the effect was found. On the other hand, SSS is defined as a specific devaluation of the sensory properties of food which occurs independently (or at least partly) of post-absorptive factors. Thus, if any physical input (feeling of fullness) affects the expression of SSS then this would operate by decreasing the total consumption of all the available foods (eaten and uneaten meals) leaving intact the preferences, or the value of the foods (Pre-fed: devalued vs Non-Pre-fed: non devalued). This pattern of results was found in Experiment 3 when rats in the distractor condition, which drank more in total during the pre-feeding phase due to the additional presence of the distractor, consumed less on the consumption test whilst still showing the SSS effect.

Another tentative explanation of the SSS suggested by Hetherington & Havermans (2013) has been in terms of stimulus satiation. What underlies this idea, which is based on the Glanzer´s (1953) proposals, is that after SSS a state of boredom to the exposed stimuli is experienced. So, in contrast to habituation, the eaten food does not become irrelevant but aversive in such a way that a stimulus changes from positive to negative valence. This notion is based on the idea that SSS would not occur due to a decrease in responding but a qualitative change in responding from approach to avoidance behavior. However, there is evidence that does not support this hypothesis. This idea is based on the assumption that the behavior of living beings always tends to alternate within the different existing possibilities. Just as, for example, rats in a maze with different arms do not always go to the same one, they always alternate. This happens even when one of the behavioral options is the preferred one. Apparently, this idea could be explained perfectly well by habituation, the only difference between them being the qualitative vs. quantitative change that each phenomenon would cause in behavior. Berridge (1991) found that after an SSS procedure the rats reduced their orofacial appetitive responses to the drank solution, however, in no case aversive responses increased. This evidence does not support an explanation in terms of a qualitative change of the stimuli valence. Also, stimulus satiation is perhaps not the most appropriate suggestion for the case of SSS, which does not require very extended exposure or even work in sham-feeding procedures. It seems more reasonable for other situations in which individuals ingest excessive amounts of food generating a state of aversion or even those that occur after a digestion process such as alliesthesia.

Finally, other attempts to explain SSS have been presented from a top-down process perspective. For instance, Wilkinson & Brunstrom (2016) hypothesized that SSS could be modulated by the perceived availability of food items. These authors argued that when a food stimulus is not available, its subjective value increases. Hence, when in some of the typical SSS experimental procedures, human participants are asked to rate the hedonic value of eaten and uneaten foods, the latter should be perceived as unavailable items, inaccessible during the whole procedure, and, as a consequence, more valuable. Furthermore, these authors suggest that eaten (available) foods would be perceived as less valuable due to negative contrast, when compared with uneaten (unavailable) foods. However, the results of their study did not support this interpretation of the SSS phenomenon (Wilkinson & Brunstrom, 2016). With regard to the Buffet effect, Havermans & Brondel (2013) suggested that this phenomenon occurs not as a consequence of eating different foods that could undermine SSS expression (presumed dishabituation/distraction or rate of exposure to foods), but is due to the mere perception of variety. This implies that variety itself has reinforcing properties and therefore can disrupt SSS, thereby preventing the normal course of food devaluation from occurring. Again, the results of their study did not support their hypothesis; mere perception of food variety did not disrupt the SSS effect. These suggestions, which are based on more complex cognitive processes, can be contrasted with the results of a study by Higgs et al. (2008) who showed how this eating regulatory mechanism was present in two amnesic patients when SSS was assessed by hedonic scales. These authors argue that the SSS effect is a basic process in which explicit memory for recent eaten meals is not necessary, suggesting that the mechanism responsible for this is habituation. Thus, the behavioral evidence seems to suggest that SSS is a phenomenon that operates through a basic mechanism in which the explicit memory of recently eaten meals is not critical (Higgs et al. 2008) and which is similar to habituation due to its stimulus specificity (Havermans, 2012).

A number of caveats need to be noted regarding the present study. First, although the present study did not show evidence for an habituation-based account of SSS, it should be noted that all the procedures carried out in this experiment measured preferences during consumption. This measure, however, fails to completely capture what was originally assumed to occur in SSS, that is, a change in the relative preference. Thus, we have to presume that any change in direct consumption of the target stimuli can be taken to indicate a change in the pleasantness of the solutions. Second, all the stimuli used in the three experiments were fluids. It is possible that using stimuli of the same sensory modality as the distractors and dishabituators could weaken the effects as a consequence of generalization to the target stimuli. Furthermore, we have no previous evidence that the distractor/dishabituator solutions have the capacity to dishabituate/distract the habituation process. Therefore, it remains the possibility that these processes are still occurring within this procedure but the stimuli used failed to detect them. Finally, on the present study we only tested 3 properties of habituation. Future research should be carried out in an animal model focusing on whether other characteristics are met (Rankin et al., 2009) such as long-term habituation, potentiation of habituation or measuring whether the intensity or the frequency of stimulation will affect the degree of SSS expression.

Conclusion

In summary, the presentation of a dishabituator after establishing SSS did not restore the value of the pre-fed solution. Furthermore, we found no evidence to suggest that the presence of a distractor during pre-feeding disrupts the SSS process and attenuates the hedonic devaluation of the pre-fed solution. These results suggest that with the paradigm used here, the SSS effect cannot be explained in terms of a typical case of response habituation, at least considering the idea that all habituated responses can be re-established after the presentation of a dishabituator or a distractor. Further studies should be conducted with other techniques such as the oro-facial reactivity test or an assessment of licking clusters to ascertain whether the use of dishabituator or distractor stimuli of sensory modalities different from the target stimuli could impair or attenuate the observed SSS effect.

CHAPTER V

Perceptual learning and Sensory-Specific Satiety

Sensory-specific satiety (SSS) refers to a decline in pleasantness of eaten foods in comparison to other non-ingested meals. Although SSS is specific to the eaten food, it can also generalize to other meals that share similar properties to the satiated food. It is possible that this phenomenon could become more specific after extensive experience, as repeated exposure to an assortment of similar food could cause perceptual learning (i.e. an increase in the distinctiveness of each specific item). This in turn would reduce generalization from the consumed food to other similar products, so they will be more readily consumed. To assess this hypothesis, two experiments were carried out using rats as experimental subjects and flavoured solutions as stimuli. In Experiment 1 our main goal was to find the basic SSS effect with two different solutions. As expected, the results showed that rats tended to consume a higher amount of non-sated solutions in comparison to sated ones. Experiment 2 evaluated how repeated exposure to two similar solutions affected generalization of the SSS. Results showed that rats that did not have extensive exposure to the flavoured solutions showed no preference for the non-sated drink. However, rats that had repeated exposure to the flavours showed SSS. The results suggest that easy and continuous access to a high variety of similar unhealthy foods might have long-term effects on food consumption, and highlight a potential mechanism linking obesogenic environments with dietary habits.

Introduction

Obesogenic environments are characterised by promoting sedentary lifestyles, constant exposure to food-related stimuli, and the massive variety of palatable and highly caloric foods in our daily lives (Berthoud, 2012; Martin, 2016). This leads to the fact that for a high proportion of the population from developed countries, their caloric intake exceeds that which is necessary for homeostasis (Reichelt et al., 2014). Hence, obesogenic environments promote excessive energy intake habits, disproportionate to the current sedentary lifestyles, that predispose individuals to becoming overweight due to the great accessibility and variety of highly caloric and cheap products (Berthoud, 2012; Martin, 2016; McCrory et al., 2012). Variety is directly related to intake by means of the so-called “Buffet Effect” (Reichelt et al., 2014; Rolls, Rowe et al., 1981). This phenomenon is characterised as an increase in the total intake as a consequence of access to a variety of foods in comparison with a monotonous diet (Brondel, Romer, et al., 2009; McCrory et al., 2012; Meengs et al., 2012; Raynor & Wing, 2006; Roe et al., 2013; Rolls, Rowe et al., 1981). The “Buffet Effect” is a robust phenomenon that has been found in several studies conducted in humans (e.g., Brondel et al., 2009; Epstein et al., 2010; Meengs et al., 2012; Rolls, Rowe et al., 1981), and also in animals (e.g., Ahn & Phillips, 2012; Treit et al., 1983; Le Magnen, 1999; Reichelt et al., 2014).

One possible mechanism underlying the “Buffet Effect” is the phenomenon known as Sensory-Specific Satiety (SSS). SSS is observed as a reduction in intake after consumption of a food compared to other unconsumed foods, presumably mediated by a reduction in the hedonic response to the former (Rolls, Rolls et al., 1981; but see Havermans et al., 2009). SSS promotes meal termination of a particular food as a consequence of its temporary devaluation. One important feature of SSS is the fact that it is specific to the food that has been eaten, in contrast to other non-eaten foods that keep intact their hedonic properties. For instance, eating a sweet meal will promote a greater preference for other salty foods (Raynor & Wing, 2006). Moreover, SSS has been shown with other kinds of sensory properties such as texture (Guinard & Brun, 1998), odors (Rolls & Rolls, 1996), colours (Rolls et al., 1982) and shapes (Rolls et al., 1982). However, despite being specific, this phenomenon can generalise or transfer to other foods that share similar sensory properties to the satiated food (Griffioen-Roose et al., 2010; Rolls et al., 1984). In fact, Rolls et al. (1984) found evidence of generalization by using a four-course meal in which participants ate a salty or sweet food. Participants showed a decreased Liking for salty foods after having eaten a savoury course, whereas the same generalization occurred for sweet meals after the consumption of a sweet course.

SSS has been proposed as a mechanism that could help organisms to actively seek dietary variety, thus ensuring access to all the nutrients they need (Reichelt et al., 2014; Rolls, Rolls et al., 1981). SSS may be altered in disordered eating, such as hyperphagia that contributes to obesity, but studies of this possibility in humans and animals have not produced consistent results. Some of the evidence suggests that obesity is associated with a reduction in SSS (Bond et al., 2010; Epstein et al., 1996; Reichelt et al., 2014). This may indicate that being subjected to highly caloric diets could impact on the expression of this mechanism, due to a lower devaluation of the consumed food. For instance, Reichelt et al (2014) found that rats put through a "cafeteria diet" expressed impaired SSS in comparison with another group that received standard lab chow. Furthermore, evidence from habituation research suggests lower food devaluation when comparing obese and lean subjects by using salivation measures (Bond et al., 2010; Epstein et al., 1996). However, other studies have found no differences in the expression of this phenomenon when comparing both populations (e.g., Brondel et al., 2007; Havermans et al., 2012; Myers, 2017;

Snoek et al., 2004). For instance, Snoek et al. (2004) found no difference between obese and normal weight women when comparing SSS expression by using food ratings before and after consumption. Therefore, further research needs to be conducted to address these discrepancies.

One possibility is that exposure to a considerable variety of slightly different foodstuffs, as occurs in an obesogenic environment, leads to better discrimination among them, and thus lower generalization of the SSS, as a consequence of perceptual learning. Perceptual learning can be defined as the learning process that results in an increased discrimination between two similar stimuli as a consequence of previous experience with them (for a recent review see Mitchell & Hall, 2014). Perceptual learning has proven to be a robust phenomenon, found in animals (e.g., Honey et al., 1994; Mondragón & Murphy, 2010; Prados et al., 2007; Recio, Iliescu, Honey & de Brugada, 2016; Symonds & Hall, 1995) and humans (e.g., Dwyer et al., 2011; Lavis et al., 2011; Mitchell et al., 2008; Mundy et al., 2006; Mundy et al., 2007; Recio, Iliescu, Mingorance, Bergés, Hall & de Brugada, 2016). In the typical perceptual learning procedure animals are repeatedly exposed to two similar flavored compound solutions, to later assess the discrimination between them. To achieve this effect, both appetitive (Recio et al., 2016) and aversive procedures (e.g., Symonds & Hall, 1995) have been used, so that after pre-exposure one of the solutions is paired with a motivationally relevant consequence (nutrients, toxins) and the subsequent generalization to the other is measured. Less generalization between stimuli means greater discrimination between them and vice versa. Note that the effect of preexposure on generalization will depend on the nature of the procedure. If a solution A is paired with a toxin that causes internal malaise, high generalization would cause a decrease in consumption of solution B, while a decrease in generalization would in turn cause higher consumption. The opposite would occur after appetitive conditioning.

Thus, we can predict that frequent consumption of a variety of items in a given food category will decrease generalization of SSS among them, which is observed among similar foods that share sensory characteristics. This will result in a lower decline in consumption for the similar foods that had not been eaten, and thus a total increase in intake. In order to test the hypothesis that exposure to variety modulates SSS expression, we carried out two experiments measuring generalization by using a modification of the typical perceptual learning procedure. Using rats as subjects, the main procedure consisted of satiating animals with one of two differently flavored sucrose solutions and later giving them the opportunity to choose between both. If SSS is expressed, there would be a lower consumption of the sated solution in comparison to the non-sated solution. However, if generalization is sufficiently high (i.e. the solutions are very similar), consumption could decrease for both. Thus, in the first experiment our main goal was to demonstrate the basic SSS effect by using two readily discriminable solutions. In the second experiment we used two more similar solutions and compared two groups, one that was briefly familiarised with the flavors and another one that received prolonged exposure to them. We predicted that the group with repeated exposure should show less generalization of SSS, and thus more consumption of the non-sated solution.

Experiment 1: Basic effect of Sensory-Specific Satiety

The main goal of this experiment was to demonstrate the basic SSS effect. To achieve this, we employed a procedure similar to that described by Reichelt et al. (2014, Experiment 1). In the present experiment, rats were offered two readily discriminable sucrose solutions, one flavored with vanilla and the other with almond. We used two discriminable sweet solutions rather than two highly distinctive flavors such as salt and sucrose to prevent differences in consumption based on baseline flavor preference. Given that rats show a much higher preference for sucrose than salt, it could be the case that they would always choose the sweet solution regardless of SSS. Each rat received access to one of those solutions, followed by a choice test in which both solutions were presented. According to our predictions SSS will be observed if the subjects show a preference for the non-sated solution over the sated solution.

Materials and methods

Participants and apparatus

Twelve Wistar male rats with an average weight of 300 g (range 285g-344g) were used in this experiment. The rats were supplied by the Animal Production Unit of the University of Granada. The rats were non-naïve, but had no previous experience with the solutions and procedures used in the present experiment.

All the animals were individually placed in translucent plastic cages (35 x 12 x 22 cm) using wood shavings as bedding. They were maintained on a 12-hour light / dark cycle, beginning at 8:00. All solutions were prepared every day with tap water, administered in 50 ml centrifuge tubes with stainless steel, ball-bearing-tipped spouts, being placed in the centre of the front metal cover of the cages at the beginning of the sessions. The consumptions were calculated by weighing the tubes before and after each session. The flavored solutions were prepared by diluting 0.05% v/v vanilla or almond flavoring (Manuel Riesgo, Madrid) with 100 g/L of domestic sucrose (granulated white sugar).

All the procedures described in this paper were approved by the Comité de Ética en Experimentación Animal (Ethics Committee in Animal Research) at the University of Granada and are classified as low severity according to European guidelines. Rats were monitored daily by the individual responsible for animal welfare in the research centre.

Procedure

The experimental procedure lasted a total of 6 days (see Table 1). The day before the beginning of the experiment the water bottles were removed, and access to water was restricted to two daily drinking sessions (11:00 and 16:00) of 30 minutes. On days 1 and 2 rats had ad libitum access to water during the drinking sessions, to habituate them to the experimental setting. The familiarisation phase occurred on Days 3 and 4, which consisted of 20 minutes of access to one of the flavored solutions during the morning session. On the first day half of the rats were exposed to 10 ml of the almond flavored solution and the other half to the vanilla solution, with the reverse arrangement being given on the following day. On Days 5 and 6 the sensory satiety-choice test was conducted. Each day began with the sensory satiation phase, which consisted of the presentation of 15 ml of one of the two solutions for 20 minutes at 11:00. The amount was limited to 15 ml to prevent considerable differences in consumption whilst being sufficient to generate SSS. The order of presentation of the two solutions was counterbalanced, so that half of the rats received the almond solution on the first day and the other half were sated with the vanilla solution, with the reverse arrangement on the following day. Two hours after the sensory satiation phase (13:00), a choice test was carried out in which the rats received 30 ml of each of the flavored solutions (sated and non-sated) for 10 minutes. The location of the tube containing each flavored solution (right/left) was counterbalanced. Throughout all the procedure rats received a daily session of ad libitum access to water for 30 minutes at 16:00 to keep them hydrated.

Table 1. *Procedure of Experiment 1*

Familiarisation	Sensory Satiation	→ 2 Hours Gap	Choice Test
Days 3 / 4	Days 5 / 6		
A / B	A / B	→	A vs B

Note: A and B refer to the two solutions (vanilla + sucrose and almond + sucrose, counterbalanced). “/” (slash) refers to alternate days, “vs” refers to the choice test between the two solutions.

Data analysis

General linear model null hypothesis testing analyses were conducted, adopting a rejection level of $p < 0.05$. Partial eta squared and Cohen’s d tests were used to measure effect sizes.

Data from the sensory satiation phase were analysed to find any possible flavor preference that could affect the choice test. We used paired samples t-test comparing consumptions of vanilla and almond flavored solutions. Consumption of sated and non-sated solutions during the choice test was analysed using a two-way repeated measures analysis of variance (ANOVA) with Flavor (almond-sucrose solution vs vanilla-sucrose solution) and Satiation (sated vs. non sated) as within-subjects factors. The decision was made to include the flavor as a factor in order to consider any possible interaction caused by differences in baseline preference.

Results and Discussion

During familiarisation, rats consumed almost all of the fluid available in the bottles, with a slightly lower consumption on the first day due to neophobia. Figure 1 (panel A) depicts data from the sensory satiation phase of the test, showing that animals consumed approximately the same amount of each solution, *t*(11)=-1.77, *p*= 0.11, *d*=-0. 51.

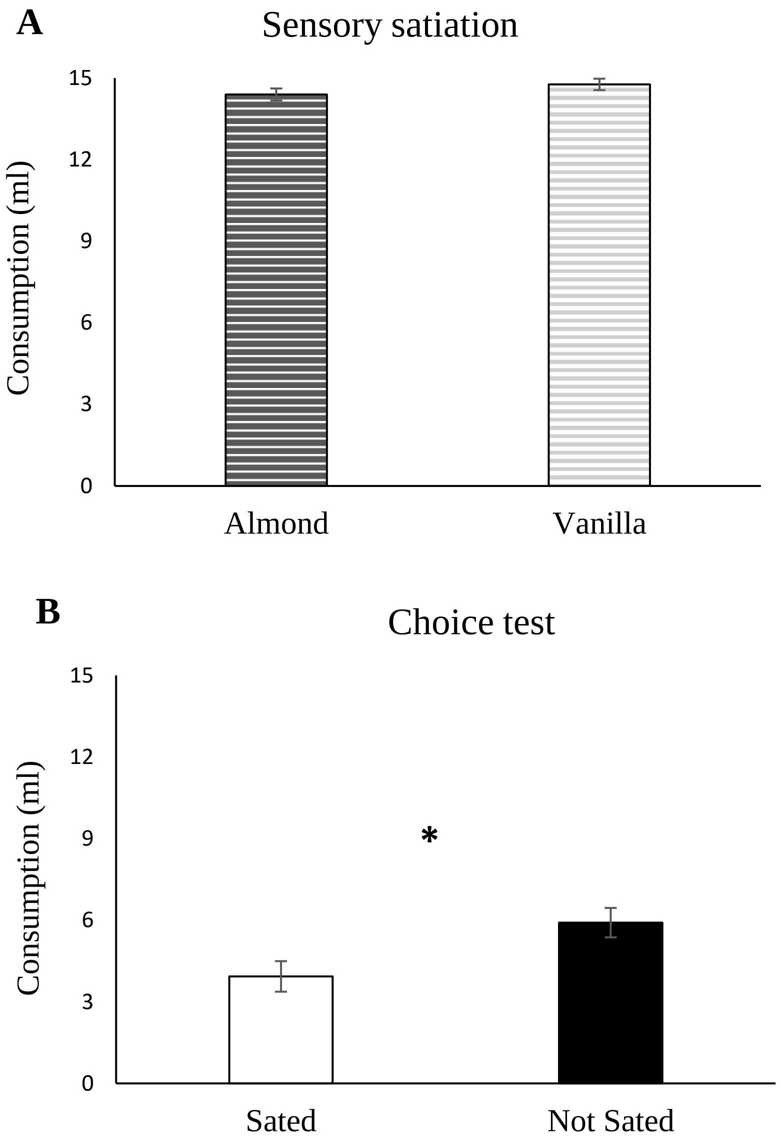


Figure 1. Panel A: Mean consumption for both solutions during sensory satiation phase. Panel B: Mean consumption of the sated and non-sated solutions during the choice test. Flavor data was collapsed across the Satiation factor and is not represented for clarity.

Figure 1 (Panel B) depicts the choice test data with Flavor data collapsed across the Satiation factor, showing that the animals consume less of the previously exposed flavor than the novel flavor; main effect of Satiation $F(1,11) = 5.90$, $p=0.03$; $\eta^2_p = 0.35$. Neither the main effect of flavor, $F(1,11)= 4.13$, $p= 0.07$, $\eta^2_p = 0.27$, nor the interaction effect, ($F < 1$), were significant.

The present results clearly show that our procedure elicits measurable SSS. In other words, a non-sated flavor is preferred over a sated flavor, thus suggesting a decline in pleasantness of the previously consumed solution. This also indicates that both solutions were clearly differentiable. If this were not the case, SSS would have generalized from the sated solution to the other solution, resulting in an equivalent consumption for sated and non-sated flavored solutions.

Having demonstrated the basic SSS effect, the purpose of the next experiment was to examine the effects of preexposure on the expression of SSS in two more similar substances. We expected that when two solutions share several sensory properties, the SSS effect could generalize readily from one to the other. However, if previous exposure results in perceptual learning, greater discrimination would be expected between them and thus generalization would be lower. For this reason, in Experiment 2 we added another common flavoring to both solutions in order to increase the similarity between them. Furthermore, we manipulated prior exposure to the solutions in order to affect generalization.

Experiment 2: Effects of exposure to similar flavors in Sensory-Specific Satiety

In this experiment we manipulated the solutions to make them more difficult to discriminate. There were two groups; one received simple familiarisation to prevent neophobia while the other received prolonged intermixed exposure to the flavored solutions. Intermixed pre-exposure has shown to be an effective way of enhancing discrimination in both animal (Honey et al., 1994; Mondragón & Murphy, 2010; Prados et al., 2007; Symonds & Hall, 1995), and human subjects (Dwyer et al., 2011; Lavis et al., 2011; Mitchell et al, 2008; Mundy et al., 2006; Mundy et al., 2007; Recio et al., 2016), and it reflects the way in which exposure is expected to take place in an obesogenic environment with easy access to a variety of similar foodstuff. According to our hypothesis, if perceptual learning results in better discrimination between the two similar solutions, then the intermixed group should show SSS. In contrast, because for the non preexposed group both flavored stimuli would be very similar, SSS of one should readily generalize to the other.

Materials and methods

Participants and apparatus

Twenty naïve Wistar male rats (supplied by Janvier Labs) with an average weight of 265 g (range 248 g – 285 g) were used in this experiment. Animal housing conditions were the same as those described for Experiment 1. Rats were randomly assigned to two groups of equivalent weight (Intermixed: 263.6 g / Non-Preexposed: 267.2 g). The flavored solutions were composed of 0.05% v/v vanilla or almond flavoring (Manuel Riesgo, Madrid), 1% v/v squeezed lemon and 100 g/L of domestic sucrose.

Procedure

The experimental procedure lasted for 10 days (see Table 2). As in the previous experiment, water was removed the day before, and on Days 1 and 2 the subjects were given two daily drinking sessions (10:00 and 16:00) of 30 minutes. On Days 3-8 rats in Group Intermixed (INT) received rapid alternated exposure to both solutions during the morning session. For 10 minutes, half of the rats received 6 ml of the almond solution while the other half received the vanilla solution. Immediately after this 10-minute period, the tubes were removed and replaced with tubes containing 4 ml of water for 5 minutes. The tubes were then removed again, and all animals received 6 ml of the remaining solution for another 10 minutes. Non-Preexposed Group (NOP) received ad libitum access to water on Days 3-6. On Days 7 and 8 they received the same schedule as animals in the INT group, but they had access to the same flavored solution for each of the 10-minute periods, with water in between. Half of the rats received the almond solution on Day 7 and the other half received the vanilla solution, with the reverse arrangement on Day 8. This familiarisation was modified from Experiment 1 to make it more similar to the INT group. The rapid alternation procedure with water presented in between presentations of the flavored solutions was chosen to emulate the perceptual learning procedure used by Recio et al., (2018), which resulted in a reliable perceptual learning effect. On Days 9 and 10 the sensory satiety-choice test was conducted for all groups. The procedure was the same as in Experiment 1 (See Table 2). On each day the rats received 30 minutes of ad libitum water at 16.00.

Table 2. *Procedure of Experiment 2*

	Preexposure		Sensory Satiation	→ 2 Hours Gap	Choice Test
	Days 3-6	Days 7-8	Days 9-10		
INT	A-B	A-B	A / B	→	A vs B
NOP	W	A-A / B-B			

Note: A and B refer to the two solutions (vanilla+lemon+sucrose and almond+lemon+sucrose, counterbalanced), W refers to water. "/" (slash) refers to alternate days, "vs" refers to the choice test between the two solutions; "-" (hyphen) represents rapid alternation. During preexposure, INT group received two different solutions per day every day while NOP group received one solution for each of the last two days.

Data analysis

Data from the sensory satiation phase was analysed with one way repeated-measures analysis of variance (ANOVA), with Flavor as within-subject factor (almond-lemon-sucrose vs vanilla-lemon-sucrose) and Group as between groups factor (intermixed vs non-preexposed). Choice tests were analysed using two-way repeated measures analysis of variance (ANOVA) with Flavor (almond-lemon-sucrose vs vanilla-lemon-sucrose) and Satiation (sated vs. non sated) as within-subject factors, and Group as between groups factor (intermixed vs non-preexposed).

Results and Discussion

As in Experiment 1, rats consumed virtually all of the available fluid in the bottles during the preexposure phase. We failed to find any differences in consumption between substances during the sensory satiation phase (Figure 2 - panel A). No main significant effects of Flavor ($F < 1$), Group, $F(1, 18)=2.16$, $p= 0.16$, $\eta^2_p = 0.11$ or the interaction were significant ($F<1$).

Figure 2 (panel B) shows the data from the choice test, with Flavor collapsed across levels. The ANOVA revealed a significant effect of Satiation $F(1,18)= 4.73$, $p=0.04$, $\eta^2_p = 0.21$. No other main effect was significant (Fs < 1). Moreover, there was a significant interaction between Group and Satiation, $F(1,18)=4.47$, $p=0.05$, $\eta^2_p = 0.20$. The interaction between Flavor and Group did not reach significance, $F(1,18)=4.04$, $p= 0.06$ $\eta^2_p = 0.18$ and neither did the triple interaction ($F < 1$). Planned comparisons were conducted by means of a t-test comparing mean consumption of satiated solutions vs. non-satiated solutions. There was a significant difference in consumption of these solutions in the INT group, $t(19)= -3.02$, $p<0.01$, $d= -0.68$, but not in the NOP group, t(19)= -0.06, $p=0.96$ $d=-0.01$.

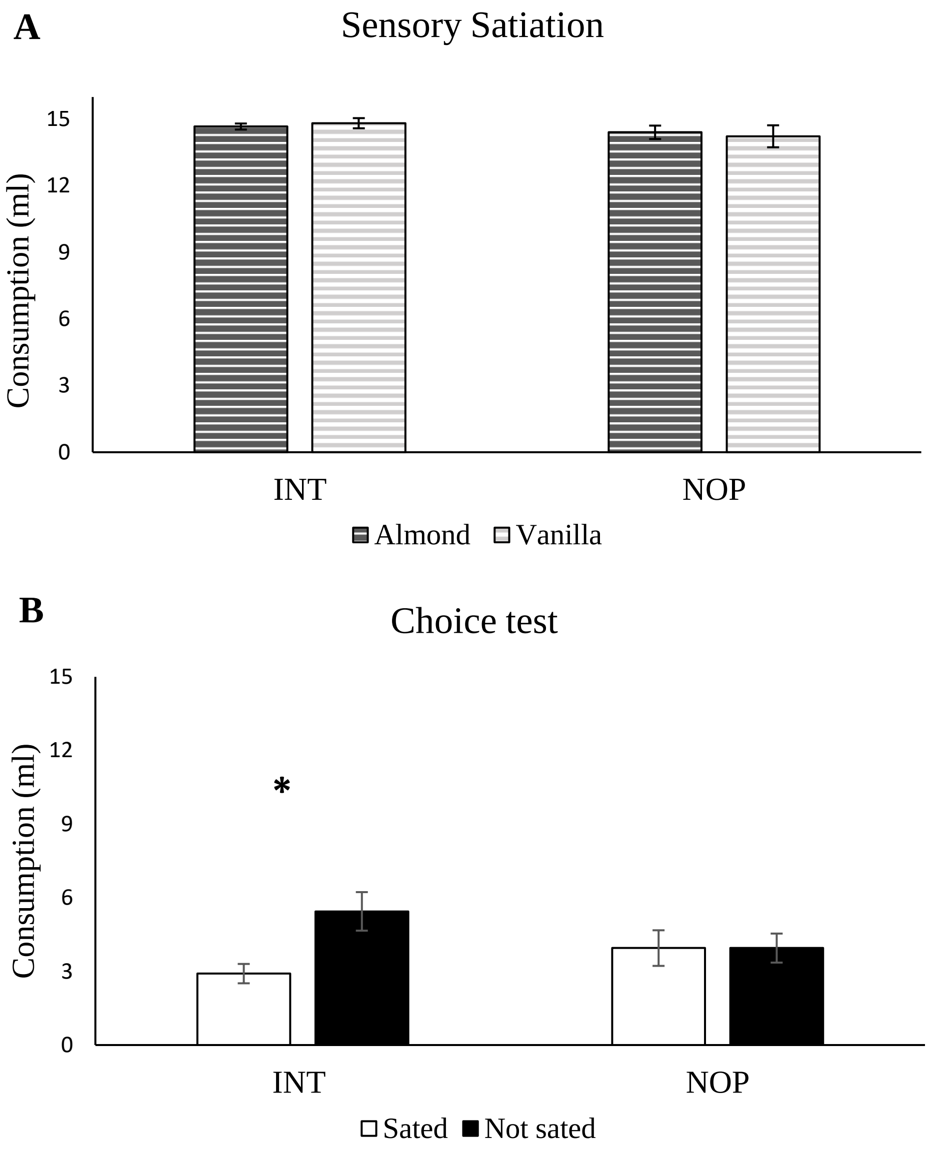


Figure 2. Panel A: Mean consumption for both solutions during the sensory satiation phase. Panel B: Mean consumption of the sated and non-sated solutions during the preference test. As in the previous experiment, data from both flavors were collapsed.

In line with our hypothesis, the data shows how SSS is only expressed in the INT group, and not in the NOP group. In fact, the NOP group exhibited similar mean consumptions for satiated and non-satiated solutions, showing a clear generalization effect. In contrast, intermixed exposure reduced generalization of SSS, resulting in higher consumption of the non-satiated solution. These results suggest that perceptual learning effects can make similar foods more distinctive so that the expression of SSS becomes even more specific. Thus, if SSS does not generalize between two similar foods, it would be reasonable to expect a higher total intake due to the lack of devaluation of the non-satiated meal.

General discussion

The results from Experiment 1 showed a clear SSS effect using two flavored solutions. After drinking a considerable volume of one flavored solution, animals tended to consume more of a different but familiar solution when both solutions were offered during a choice test. Experiment 2 showed that SSS generalized between two similar solutions in the absence of prolonged experience with them. In this case, animals showed similar consumptions of sated and non-sated solutions. However, SSS was found when those solutions had previously been exposed in an intermixed fashion for 6 sessions (Intermixed Group). Unlike the non-preexposed group, the intermixed group showed a significantly higher preference for non-sated solutions in comparison with those that had previously been sated. These results show how repeated exposure can enhance discrimination between similar solutions (perceptual learning). Furthermore, this enhancement of distinctiveness prevents the decline in consumption for satiated solutions to generalize to other similar non-sated solutions, resulting in a higher consumption of the latter.

Our results demonstrate that prolonged repeated exposure to flavored stimuli can modulate the expression of SSS. This result has several implications, since food diversity is an important factor for determining total food intake. Such experience with stimuli should result in a more detailed representation of them (Gibson, 1963). As a result, the greater the experience with a given food category, the more specific the SSS will be for each individual food in that category. This means that other foodstuffs very similar to the one recently consumed will maintain their hedonic value and lack the typical decline in pleasantness that is characteristic of SSS, which will result in an increase in total intake when there is availability of a variety of similar foods. Thus, exposure effects could have an impact on eating behavior, acting as a relevant factor in the development of obesity or metabolic disorders.

As mentioned previously, food diversity is an important factor for total food intake, that is, the greater the variety of food available, the higher the total intake in comparison with a more monotonous diet (Rolls, Rowe et al., 1981). However, in addition to food variety, similarity also plays an important role, since SSS is expected to generalize among foods that share sensory properties. There is a remarkable level of mass exposure to highly similar food products readily available within the obesogenic environments in many countries (Martin, 2016). In fact, many years ago it would have been inconceivable to find the vast array of brands that are currently producing similar highly palatable foods within the snack industry, such as crisps or chocolates. In this current climate, perceptual learning is a likely candidate for explaining how westernized societies are now developing these excessive intake habits. This learning mechanism can increase consumption given this huge variety of similar caloric products thanks to the differentiation process explained above. Without experience, after consumption of a particular food the typical loss of appetite for the latter will generalise to other similar products. This will restrict our food choices, rejecting those products that share similar sensory properties to the satiated meal. However, constant exposure to these products will lead to a better discrimination among them, making devaluation more specific to the unique sensory features of the consumed meals. This way, other similar food choices will still be perceived as pleasant and thus the appetite for these foods will remain intact.

It is possible that avoiding excessive consumption of unhealthy foods, especially during infancy and childhood when many dietary habits are formed, would prevent these changes in SSS. However, this can be difficult given the constant and intense exposure to food cues that surrounds people living in western societies. Cues such as food images, advertisements, or even smells can also promote appetite by acting as conditioned stimuli, initiating cephalic digestive responses and even promoting a higher total intake of the cued food (e.g., Nederkoorn et al., 2000; Ferriday & Brunstrom, 2011). This poses an even greater risk for overweight people, for whom such cues are particularly relevant (van den Akker, Stewart, et al., 2014). From this perspective, it is possible to understand how today's society is constantly being persuaded and tempted towards the consumption of a wide variety of palatable foods that attract the consumer. If the link between obesogenic environments and changes in satiety mechanisms we propose is further demonstrated, it could serve to justify policy changes to limit the amount of advertisements for unhealthy food, or to restrict the availability of certain products in spaces such as hospitals or schools.

In relation to these food cues, another effect has recently attracted interest, known as Sensory Specific Appetite (SSA) (Ramaekers et al., 2014). SSA refers to the greater appetite for a particular food that occurs after exposure to the sight or smell with which it is associated. Therefore, SSA has been proposed to be the opposite phenomenon to SSS (Ramaekers et al., 2014). This phenomenon has been studied primarily using odors as stimuli (Ramaekers et al., 2014; Zoon et al., 2016). Like SSS, in SSA the specific appetite can generalize to other meals that share similar sensory properties to the cued food, although this generalization effect has only been found for sweet/savoury food categories. In fact, it has been proposed that this effect of generalization could be specific to food categories that predict different kinds of macronutrients (sweet/savoury), with sweet being a major predictor for carbohydrates and savoury food the predictor for proteins (Ramaekers et al., 2016). These authors have argued that food cues prepare the body to process specific types of macronutrients and this causes the organism to have a major appetite for meals that are congruent with the cued food. However, as we have shown in this study, it seems plausible that this phenomenon could also gain specificity beyond the sweet/savoury category, since odors are also continuously exposed. If exposure effects are able to make those odors (or the foods they cue) more discriminable, then SSA could promote specific appetites for foods with similar smells to previously consumed food.

On a positive note, perceptual learning could also potentially increase the consumption of healthy foods such as fruits and vegetables. This has particularly important implications for young children who tend to prefer eating highly palatable and caloric foods instead of vegetables. Hence, repeated exposure to a wide variety of vegetables could increase their total consumption, and possibly a meal composed of small amounts of different vegetables could help prevent food ‘boredom’, which is more likely with less palatable products. There is some evidence that mere exposure to a non-preferred food increases its preference to levels of an initially preferred food, eliminating the initial neophobia (Birch & Marlin, 1982; Dovey et al., 2008). In addition, children initially perceive all vegetables as a single type of food, encompassing them within a category that they reject (Birch et al., 1998). Exposure to a variety of vegetables in each meal could help to break this categorization and promote their differentiation. Thus, SSS would be exclusively expressed for each eaten vegetable and the decline in pleasantness should not be expressed for other varieties. Furthermore, with experience, they could develop preferences and, consequently, increase consumption of different types of existing vegetables. Following this process, the children should no longer perceive vegetables as an aversive variety of food and, therefore, the process of familiarisation and the onset of voluntary consumption could proceed more rapidly. In sum, it is worth considering the implications of perceptual learning in the formation of healthy eating habits.

The present research is a first approach towards investigating the effects of exposure to a variety of foods on SSS. However, we acknowledge that our study has certain limitations. For instance, while animal models are a fundamental part of research, it would be useful to replicate the same effects in humans to demonstrate their general validity. The stimuli we used were also quite simple and limited in number, and it would be necessary to replicate the results with more complex and varied foodstuffs. Furthermore, this study was rather short-term, spanning a period of less than two weeks; further research should focus on studying whether these exposure effects are maintained over time. Finally, and more importantly, even though our results show differences in relative preference, our design was not appropriate for measuring differences in total intake between the group with extensive exposure and the non-preexposed group. We propose two possibilities to explain this result: 1) it is possible that the duration of the consumption test (10 minutes), is too short a duration to find differences in total consumption. Thus, during this interval time the animals consume as much as they can consume, making impossible to find differences between groups (ceiling effect). 2) The pre-exposed rats had access to the target substances for 6 days, it is likely that this long previous experience may have produced a long-term SSS effect to both substances, inhibiting total consumption in the test. This pattern of results would not be found in the non-pre-exposed group, as they had only received one exposure to each substance prior to testing. So, future research should elucidate whether, controlling these procedural aspects of the experimental design, the consequences of this SSS modulation can lead to an increase in total intake. To convincingly demonstrate a causal link between repeated exposure and excessive food intake, a clear demonstration of differences in total intake is necessary. So, whilst our research is suggestive of a model of overeating based on exposure to food variety, further research is needed to resolve these issues.

To conclude, there are multiple factors that are involved in eating behavior, SSS being one of them. Research on this phenomenon, along with its possible implications for eating disorders, has potential practical relevance and deserves further study. The results obtained in this study have revealed, for the first time, a link between SSS and exposure effects that could help to explain overeating in obesogenic environments. Future research should extend this knowledge to develop interventions for reducing and preventing excessive food consumption, and to promote the intake of healthy foods.

CHAPTER VI:

The hedonic response as a component of flavor learnt preferences

Pairing a palatable flavor (US) with an initially neutral flavor cue (CS) results in an acquired conditioned preference for the latter. Two main associations have been proposed to explain the acquisition of flavor preferences: flavor-flavor and flavor-nutrient learning. Although the hedonic reaction triggered by US consumption has also been suggested as a possible additional component underlying acquired flavor preference, this issue has received little attention. Here we explored whether the amount of training to the CS-US compound can favor the formation of a flavor-hedonic reaction association using rats as subjects and sucrose as the US. We expected that the more exposure to the CS-US compound, the stronger the S-R type association. Since S-R associations are not sensitive to devaluation procedures, we used a Sensory-Specific Satiety procedure to devalue the US after conditioning and then measured preferences for the CS. This effect was studied also by using different tastes as USs with different hedonic and nutritive properties (sucrose, saccharin, maltodextrin and saccharin+maltodextrin). These results show that, as with an instrumental paradigm, extensive training in classical conditioning promotes the formation of S-R associations. However, this effect seems to be exclusive for sucrose, at least with the procedures used here.

Introduction

Associative learning has been shown to play a very important role in acquiring flavor preferences (Martin, 2016). In the same way that animals learn to avoid a food after having suffered from aversive consequences post-ingestion (conditioned taste aversion), they can acquire a conditioned preference to initially neutral flavors after pairing with a palatable taste (flavor preference conditioning). Both learning processes have an adaptive utility so that animals can avoid harmful or poisoned foods or learn which flavors could provide them with energy sustenance in a food-scarce environment (Harris et al., 2000).

Acquisition of flavor preferences has been traditionally studied by pairing an appetitive taste (US) with a neutral flavor cue (CS), a case of Pavlovian conditioning. However, in the literature, it has been proposed that such conditioning may represent a special or unique form of Pavlovian learning, mainly due to the difficulties in dealing with the extinction phenomenon (Delamater, 2012). From a Pavlovian perspective, an organism can learn simultaneously from multiple components of the US rather than this being a unitary process (e.g., Delamater, 2012; Delamater & Oakeshott, 2007; Hall, 2002; Konorski, 1967). In this sense, the acquired flavor preference has been explained in terms of three possible associations that can sometimes be learned in an overlapping way (Badolato et al., 2021; Delamater, 2012). Flavor-flavor Learning refers to the learning that occurs when a neutral cue is associated with the palatable taste of the US (e.g., sweet taste of sucrose or saccharin; Stimulus-Stimulus association) (e.g., Fanselow & Birk, 1982; Gil et al., 2021). Flavor-nutrient Learning is demonstrated when the CS is paired with the caloric properties of the US (e.g., the caloric content of Sucrose; Stimulus-Stimulus association) (e.g., Azzara & Sclafani, 1998; Palframan & Myers, 2016). Finally, Flavor-hedonic Reaction learning occurs when the CS is associated with a hedonic response resulting from consuming the US (Stimuli-Response) (Harris et al., 2004).

Both Flavor-Flavor and Flavor-Nutrient associations have been well dissociated in the literature and have been the focus of many types of research. These two associations have been examined by using non-caloric tastes (e.g., Fanselow & Birk, 1989; Gil et al., 2014; Gil et al., 2021), changing motivational states during training, testing, or extinction (e.g., Capaldi et al., 1994; Harris et al., 2000; Harris et al., 2004), using sham feeding procedures (Bonacchi et al., 2008) or intragastric infusions (e.g., Ackroff et al., 2012; Myers & Sclafani, 2001). Nevertheless, the third possible association mentioned above (flavor-hedonic reaction) has been rather less studied in the literature on flavor preferences, and there is no objective evidence of its existence.

This third association involves a link between a stimulus and a hedonic response elicited by the US (Stimulus-Response). Thus, it represents a scenario in which the conditioned flavor is preferred not because it activates the sensory representation of the US or because it predicts its caloric content but because it automatically triggers the hedonic response evoked by the US. Thus, since it is not governed by a specific US representation, this acquired preference should be insensitive to changes in the current value of the US. Therefore, if the US loses its reinforcing value (for example, through Conditioned Taste Aversion or Sensory-Specific Satiety), the CS will continue to elicit hedonic responses rather than reduce them as a consequence of devaluation. Indeed, several studies have shown how the expression of a conditioned preference resulting from an appetitive flavor learning procedure can be reduced when the US had been devalued through LiCl injections (e.g., Delamater et al., 2006; Delamater, 2007; 2011; Harris et al., 2004) or Sensory-Specific Satiety (Dwyer, 2005). Consequently, these results rule out the possibility that hedonic responding is a major component of acquired preferences, at least with the procedures used in these studies.

However, Harris et al. (2004; Experiment 3) trained thirsty rats by pairing an odor with a sucrose solution. After this procedure, a group of rats underwent several extinction trials in which they were presented with the odor cue alone, while another group did not receive the extinction treatment. Next, half of each group of rats was injected with LiCl after drinking sucrose, while the remainder did not receive the devaluation treatment. The results revealed that the rats did not only show resistance to extinction of their conditioned preference (extinguished and not conditioned group) but also kept intact their preference after the devaluation procedure (extinguished and devalued group). However, the rats that did receive extinction trials and had undergone the conditioned aversion procedure (non-extinguished and devalued group) expressed a devaluation effect for the CS+ by reducing their preference for this flavor. The authors argued that extinction may have weakened the flavor-flavor association, while the remaining preference that was still expressed (extinguished and devalued group) was the result of an association between the odor cue and the US-hedonic reaction. Thus, these authors provided a tentative interpretation of their results in terms of the expression of a possible S-R association.

Delamater (2007; Experiment1 and 2) also reported a similar pattern of results but suggested an alternative explanation. In Experiment 1, thirsty rats underwent a training procedure in which two different flavored cues were paired with the same US. After this, all rats were presented with one of the flavors on several extinction trials (without the US). Half of the rats were then subjected to a conditioned taste aversion to the US (Devalued Group) while the other half of the rats received unpaired presentations of the US and the LiCl injections (Non-devalued Group). Rats in the non-devalued group preferred the non-extinguished flavor over the extinguished flavor, showing evidence of extinction, unlike the study by Harris et al. (2004). However, similar to Harris et al. (2004), rats in the devalued Group showed the opposite pattern of results, preferring the extinguished over the non-extinguished flavor. In Experiment 2, these results were replicated by employing a full within-subjects design. Delamater (2007), in line with Harris et al. (2004), argued that when thirsty rats undergo an extinction procedure, the flavor-flavor association is weakened, as seen in the non-devalued group. But, unlike Harris et al. (2004), he proposes that after US devaluation, the preference for the extinguished over the non-extinguished CS+ is due to the latter activates the sensory representation of the US to a greater extent. Likewise, a previously extinguished CS+ is preferred after US-devaluation not because it automatically elicits the hedonic response of the US but because it weakly retrieves its sensory attributes.

Another study that challenges Harris et al.'s (2004) hypothesis is that of Dwyer et al. (2009). These authors initially paired a neutral flavor cue (CS+) with 16% sucrose and another (CS-) with 2 % sucrose. After this procedure, the authors assessed the preference for both CSs across consumption tests and assessed the hedonic reaction to the latter with a microstructural analysis of licking behavior. The rats showed a preference for the CS+ over the CS- that persisted across several consumption tests. However, these results were not accompanied by a maintenance of the hedonic response, which was extinguished after the first test. Thus, this dissociation also runs counter to the findings of Harris et al. (2004) by showing how the hedonic response to the CS+ disappears even though the rats still prefer the latter.

Although a large body of research has shown that acquired preferences are largely determined by the representation of the US and that the preference is maintained after the hedonic CS responses extinguish, this does not detract from the fact that one can learn from the hedonic US attributes and its associated responses. In this regard, Holmes et al. (2016) showed how conditioned preferences are sensitive to positive and negative hedonic contrasts when conducting a micro-analysis of licking patterns. According to these authors, this implies that the CS preference is also governed by the hedonic characteristics of the US. In a similar vein, it has been observed that rats may learn more than one association during training, such as flavor-flavor and flavor-nutrient associations, and the conditions under which the preference is tested subsequently favor the expression of one or the other. Moreover, the motivational state of the animals during training may encourage learning of one association over another; for example, states of hunger amplify learning about nutritional content, whereas when animals are not hungry, they learn more about the sensory aspects of the US (e.g., Delamater, 2007; Harris et al., 2000). Thus, just as the motivational state of the animals during the various experimental stages is crucial for encouraging the expression of the flavor-flavor or the flavor-nutrient association, under certain conditions, some procedures may support the formation of a flavor-hedonic reaction association. Therefore, the hedonic reaction elicited by consuming the US may be a preference-sustaining component during training. However, the training conditions established in most experiments do not favor the formation of such an association. Thus, the acquisition of flavor preferences has traditionally been studied using short training procedures and restricted access to the target solutions. In parallel, within associative learning framework, it has been suggested that during the initial stages of acquisition, the learned content is flexible and plastic and guided by the anticipation of obtaining a certain outcome. As learning is consolidated, it becomes much more rigid, automatic, or stimulus-driven (e.g., Adams & Dickinson, 1981). And along these lines, the traditional view of associative learning regarding instrumental behavior is the belief that S-R associations develop over lengthy training procedures because of Thorndike's (1911) Law of Effect (e.g., Adams, 1982; Dickinson, 1985; Tricomi et al., 2009; Killcross & Coutureau, 2003).

Thus, when the S-R-O chain is reinforced multiple times, the S-R association is progressively strengthened (but see Bouton, 2021). Although there is considerable evidence of the relationship between S-R learning and overtraining in instrumental training, this has not been the case in Pavlovian learning (e.g., Holland, 2005; Holland et al., 2008; but see Pool et al. 2019). However, there is evidence that the representation of learned content in Pavlovian learning may change as training is extended; in the initial stages of learning the CS activates a more complete and perceptual representation of the US while with overtraining a less detailed representation is activated (Holland, 2005; Holland et al., 2008). In the case of flavor preference learning, it is still possible that traditional procedures in this paradigm reduce the likelihood of demonstrating a potential underlying S-R association. In this sense, drawing a parallel with instrumental learning, the underlying associative structure will change depending on the total amount of training received during acquisition. For example, after a short period of training with animals having restricted access to the CS-US compound, the CS+ preference will be mediated by the memory representation of the US, which can be updated after revaluation processes. On the other hand, after a long and continuous access training procedure, with animals having free access to a CS-US solution for several hours per day, the S-R association will be strengthened and predominate over the taste or caloric consequences. Therefore, in this situation, the CS will not update the current value of the US and will be insensitive to a devaluation treatment.

Given this hypothesis, in the present study we test the effect of US devaluation on the expression of a conditioned preference after manipulating the regime of exposure to the CS-US compound during training. We manipulated both the type of exposure (hours vs. minutes and restricted vs. unrestricted access to the compound) and the length of training (number of days). For the general training procedure, we used a within-subject design in which rats had access to a neutral flavor (CS+) that was paired with a highly rewarding taste (US) and a different solution that was comprised only a neutral flavor alone (CS-). The USs were sucrose (Experiment 1, 2, 3, 4), saccharin (Eperiment 5, 6), maltodextrin (Experiment 7) or mixture of saccharin and maltodextrin (Experiment 8). Thus, these tastes differed in their reinforcing properties, some being characterized by having a highly hedonic taste (saccharin), others by only having a high nutritional content (maltodextrin) or by having both properties (sucrose and saccharin + maltodextrin). We expect this effect to be more likely for USs that possess at least a highly hedonic taste, since preferences acquired through a flavor-nutrient association do not always involve the development of conditioned hedonic responses (Myers, 2018; Myers & Sclafani, 2003). To produce devaluation, we used a Sensory-Specific Satiety procedure. Sensory-Specific Satiety is defined as the temporary loss of the specific motivational and hedonic value of the sensory properties of food after its ingestion (e.g., Rolls, Rolls et al., 1981; Havermans et al., 2009). This effect is defined as sensory-specific, occurring without the need for metabolic feedback and specifically affecting the sensory properties of the ingested food. It is, therefore, a very useful tool to temporarily devalue the specific sensory and motivational properties of a US. Concerning the experimental series, we demonstrate the basic US devaluation effect by training rats with a conventional procedure in which they have restricted access (in both quantity and time) to the target compounds (Experiment 1, 5). On the rest of the experiments we study the effect of a short (Experiment 2) and long unrestricted training procedure (Experiment 3, 4, 6 ,7, 8) in which animals can drink the target compounds for six hours per day (See Table 1 and 2).

Table 1. *General experimental procedure used in Experiments 1, 2, 3, 5, 6, 7, 8.*

Exp.	Training	2-bottle training	Choice test	Pre-feeding	Choice test
	CS+US/CS-	W vs W	CS+ vs CS-	US/W →	CS+ vs CS-
Exp.1 (Suc)	10ml, 20 min 10 days	2 days,adlib, 20 min	1 day,adlib, 20 min	2 days 10ml, 10 min →	adlib, 20 min
Exp.2 (Suc)	adlib, 6h, 6 days				
Exp.3 (Suc)	adlib, 6h, 12 days				
Exp.5 (Sac)	10ml, 20 min 8 days				
Exp.6 (Sac)	adlib, 6h, 12 days				
Exp.7 (M)	adlib, 6h, 12 days				
Exp.8 (Sac+M)	adlib, 6h, 12 days				

Table 2. *Experimental procedure used in Experiment 4.*

Group	Training adlib, 6h	Familiarization adlib, 6h	2-bottle tr. adlib, 20min	Choice test adlib, 20 min	Pre-feeding 10ml, 10 min	Choice test adlib, 20 min
	12 days	1 day	2 days	1 day	2 days	
Ctrl	CS+US/CS-	Ø	W vs. W	CS+vsCS-	US/W →	CS+vsCS-
Malto.		M			US/M	

Note. "CS+" denotes the flavor cue paired with a palatable taste during training. "CS" reers to the flavor cue that was presented alone during training. "US" refers to the palatable taste paired with the flavor cue CS+ during training. “W” denotes water, “Suc” sucrose, “Sac” saccharin and “M” maltodextrin. "/" indicates counterbalanced presentations across different sessions, and "vs." refers to the simultaneous presentation of different substances. “Ø"denotes that a group of rats was not subjected to any manipulation during this phase. The black arrow presented in the table indicates a series of sessions conducted on the same day (with a gap of 30 minutes).

Experiment 1: Sucrose restricted

Experiment 1 was conducted to show the US-devaluation effect after a typical training procedure (limited amount and time period during CS-US exposure). This experiment employed a within-subject training procedure. Rats were trained for 10 days, 20 minutes each, and were given a limited amount of the solutions. The training procedure consisted of pairing an odor cue (CS+) with a sucrose solution (US) and by presenting another odor cue (CS-) with plain water. After that, rats started the pre-feeding-choice test cycles that lasted 2 days, being pre-fed with sucrose (Pre-fed condition) or a water solution (Control Condition), after which the preference for the two odors was assessed (CS+ vs. CS-). Pre-feeding cycles were implemented with a within-subject design so that half of the rats received the sweet solution on the first day of testing and water on the next, while the other half of the rats received the opposite arrangement. We expected a devaluation effect to occur if rats reduced their preference for the CS+ solution when they had been pre-fed with a sucrose solution compared to the case in when they had received only water.

Methods

Subjects and apparatus

Sixteen naïve male Wistar rats with a mean weight of 294 g (max: 340gr – min: 263g) and supplied by Janvier Labs were used in the present experiment. Rats were individually housed in translucent plastic cages (35 × 12 × 22 cm) with wood shavings as bedding. They were maintained on a 12-h light/dark cycle for the whole procedure, starting the light cycle at 8:00 am. The experimental solutions were prepared every day with tap water and presented to animals in centrifuge tubes (50 ml capacity) with stainless steel, ball-bearing-tipped spouts. All tubes were placed in the middle of the front metal cover of the cages in the sessions in which just a single bottle was presented to avoid the effects of any side preferences during the choice tests. Consumption was measured by weighing tubes before and after each procedure. The flavored solutions were composed of 0.05% Vanilla or Almond aroma (CS+) (Manuel Riesgo, Madrid) and 10% domestic sucrose (US) or 0.05 Vanilla or Almond aroma (CS-) (Manuel Riesgo, Madrid) diluted with water. The odor paired with the sucrose was counterbalanced across rats, with half receiving vanilla paired with sucrose and the other half receiving almond paired with sucrose. The Ethics Committee for Animal Research at the University of Granada (06/06/2019/099) approved all the procedures described in this paper. These procedures were classified as low severity according to European guidelines. Animals were monitored daily by those responsible for animal welfare in the research center.

Procedure

One day before the beginning of the experiment, the water bottles were removed at 4:00 pm, and access to water or experimental solutions was restricted to two daily sessions (10:00 am- 4:00 pm). On the first two days of the experimental procedure, rats had access in the morning to 30 minutes of unrestricted water in the drinking tubes to habituate them to the experimental schedule and tubes. On the afternoon sessions of the first two days, the rats had unrestricted access to water for 30 minutes.

From day 3rd to 15th, rats underwent the training procedure. This procedure consisted of 20 minutes of access to 10 ml of the sweet solution (CS+) or the odor alone (CS-) in both the morning and afternoon sessions. The presentation of both solutions was counterbalanced across morning and afternoon sessions. Half of the animals received the CS+ or CS- solutions with the following order across sessions: ABBA, and the other half received the opposite pattern: BAAB. On Day 7, the training procedure was interrupted to assess whether the rats had acquired a preference (baseline), and the rats received two-tube water training to habituate them to the test procedure. This habituation procedure consisted of two 20-min sessions (7th and 8th days) in which rats were offered two water bottles on both sides of the cage. After 10 minutes, the experimenter changed the position of the tubes to acclimatize animals to this procedure on the final test (the position of the tubes was switched on the tests since it helps to abolish any effects of position bias). On the 9th day, animals were given both CSs presented in water in the same fashion as the habituation procedure described previously. After this test, the rats underwent four more training sessions to establish the CS+ preference. These four training sessions were divided into two (Days10-11 and 13-14), followed by a preference test (Days 12 and 15). At the end of the training procedure, the rats had 10 days of training in which they were exposed 10 times to each solution (CS+ and CS-) and were tested for their preference over 3 days.

Once this procedure ended, rats underwent the Pre-feeding cycles for 2 days with one day of rest in between in which they were given just plain water. This pre-feeding consisted of presenting 10ml of a 10% sucrose (Pre-fed condition) or water (Control condition) solution for 10 minutes. After 30 minutes from the beginning of this Pre-feeding phase, all rats received a two-bottle test in which they were exposed to the CSs in the same manner as described previously. The order in which the rats received both conditions (Pre-feeding/Control) was counterbalanced across the two days. Half received the sucrose solution on the first day, and the other half received the water solution.

Data analysis

General linear model null hypothesis testing analyses were conducted, assuming a rejection level of $p < 0.05$, using Greenhouse–Geisser corrections for mixed factorial analysis of variance when needed. Partial eta squared, and Cohen's d tests were used to measure effect sizes. To assess the conditioned preference in all the experiments, both direct consumption and preference ratios were calculated. Preference ratios were calculated by the following formula: (Consumption of the CS+) / (Consumption of the CS- + Consumption of the CS+). The resulting score ranges from 0 to 1, with values below 0.5 indicating a preference for the CS- and those above 0.5 indicating a preference for the CS+. All ratios were then analyzed using a one-sample t-test to assess whether these differed from chance level (0.5). These statistical criteria were adopted for all the experiments.

Transparency and Openness

This study have not been preregistered. The data of all the experiments are available in the APA´s repository on the Open Science Framework(OSF): https://mfr.osf.io/render?url=https%3A%2F%2Fosf.io%2F6n7f8%2Fdownload

Results and Discussion

Figure 1 (panel-A) shows rats' consumption across the 10 days of training for both CS+US and CS- solutions. This figure shows how consumption of the CS+US solution is higher than for the CS-, except for the first day of training. A repeated-measures ANOVA with Day (1-10) and CS solution (CS+US vs. CS-) as within-subject factors was conducted to analyze consumption during training. This analysis revealed a main effect of CS $F(1,15)= 120.59$, $p<0.001$; $\eta^2_p = 0.88$ reflecting higher total consumption of the

CS+US over the CS-. A Greenhouse-Geisser correction was applied to the factor Day, and for the interaction, Day*CS since Mauchly Tests revealed that the assumption of sphericity was violated. After applying Greenhouse-Geisser correction, this analysis revealed no significant effect of either Day $F(1.77, 23.43)= 2.57$, $p= 0.10$, $\eta^2_p = 0.14$ or the interaction CS*Day $F(1,56, 23,43)= 2.69$, $p= 0.09$, $\eta^2_p = 0.15$.

The Preference of the CS+ over the CS- across the three initial repeated preference baseline tests was measured through a preference ratio. The three tests were analyzed with a repeated-measures ANOVA with Day as a within-subject measure (1-2-3). This analysis revealed no significant differences across the three tests $F<1$. Thus, an average of the three preference ratios was calculated for each rat, and this mean was compared with 0.5 using a One sample t-test. The results revealed that these differed significantly from 0.5 $t(15)=2.39$, $p=0.03$, $d= 3.36$ ($M= 0.60$, $SE= 0.04$).

The pre-feeding phase was analyzed to assess differences in the total consumption of water and sucrose. A Paired samples t-test revealed that consumption of water was significantly lower than that of sucrose $t(15) =3.44$, $p=0.004$, $d=0.86$ (M Sucrose= 9.62, $SE= 0.09$; M Water = 7.96, $SE= 0.48$). Water consumption during pre-feeding in the control condition is used to balance the total consumption of the two conditions on testing. A lower total intake during this phase and condition is not expected to impact preference testing due to its familiarity, lack of taste, and calories. Similarly, a Pre-feeding phase without water in the control condition could have been carried out, but the test intakes may have been lower in the pre-feeding condition. If this situation had occurred, it is possible that a drop in preference was due to a floor effect or a reduction in the motivational state of the animals, by, for example, not being thirsty on the test.

Preference tests across the pre-feeding/control conditions were assessed with preference ratios and direct consumption. The data from this stage are displayed in Figure 1 (panel-B and C. Inspection of this figure suggests that the preference for the CS+ was modified after rats had been pre-fed with sucrose. A paired samples t-test was carried out to compare the CS+ preference ratio during both conditions (pre-fed vs. control). This analysis revealed a significant difference between the measures $t(15)= -2.84$, $p= 0.01$, $d=- 0.71$. Both preference ratios were compared with the 0.5 chance level using a One-sample t-test. Only those rats that had not been pre-fed with sucrose (control condition) showed significant differences t(15)= 4.37, $p<0.001$ $d= 3.38$, whereas the pre-fed condition did not differ from chance: t(15)=0.23, p=0.81, d= 1.80 (see Figure 1, panel-B). Direct consumption during testing was analyzed with a repeated-measures ANOVA with CS (CS+ or CS-) and Pre-feeding (pre-fed or control) as the within-subject factors. The results revealed that the factors CS $F(1,15)= 2.88$, $p=0.11$, $\eta^2_p = 0.16$ and Pre-feeding $F(1,15)= 1.81$, $p=0.19$, $\eta^2_p = 0.10$ did not reach significance. However, the interaction CS*Pre-feeding was significant $F(1,15)= 6.42$, $p=0.02$, $\eta^2_p = 0.30$. This interaction was analyzed using a simple main effects analysis revealing that in the Pre-fed condition, there were no differences in total consumption for both CSs ($F<1$), but for the control condition, there were significant differences $F(1,15)= 13.24$, $p=0.002$, $\eta^2_p = 0.46$, with consumption of the CS+ being higher than that of the CS- (See Figure 1, panel-C).

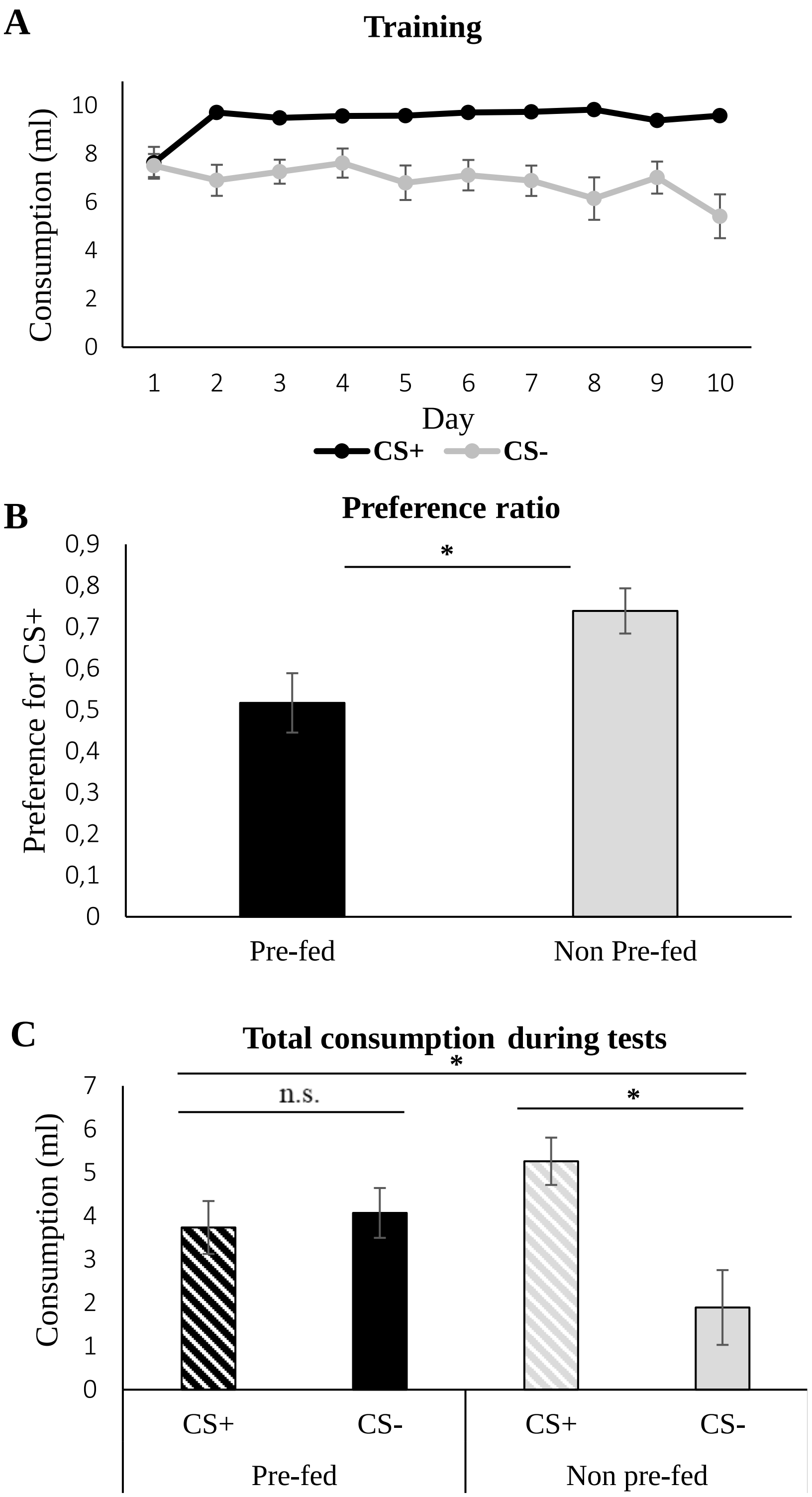

A
Training
Consumption (ml)
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
Day
CS+
CS-
B
Preference ratio
Preference for CS+
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
Pre-fed
Non Pre-fed
C
Total consumption during tests
Consumption (ml)
7
6
5
4
3
2
1
0
*
n.s.
*
CS+
CS-
CS+
CS-
Pre-fed
Non pre-fed

Figure 1. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Preference ratio for CS+ when animals were pre-fed with sucrose (Pre-fed) or were just given water (Non-pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

Therefore, the US-devaluation effect was observed in the present experiment when using a typical training procedure with a limited quantity and time of exposure to the target compounds. When rats were pre-fed with a sucrose solution, this decreased their preference for its associate CS+ over the CS-. Thus, in line with previous results in the literature, rats' preferences for the CS+ appear to be governed by the US representation, updating its value on tests. In the next experiment, we will increase the amount of time and access to the CS-US compound during training to assess whether, under these conditions, rats still express the US-devaluation effect.

Experiment 2: Sucrose unrestricted short

In Experiment 1, we found an effect of devaluation by using a pre-feeding procedure. In the present experiment, we wanted to analyze the effects of overexposure to the CS-US compound during training. Rats were trained for six days (3 days per CS) with unrestricted access to the solution for 6 hours. We expected that continuous access to a palatable solution would result in an acquired flavor preference for the CS+ and strengthen the association between the CS+ and the hedonic response to the US. In contrast to Experiment 1, under these circumstances we expected to find no effect of US devaluation on the CS+ preference.

Methods

Subjects and apparatus

Sixteen male, naïve Wistar rats with an average weight of 273 g (max: 290 – min: 250) supplied by Janvier Labs were used as experimental subjects. Animals were kept under the same healthcare conditions as in Experiment 1. The flavored solutions were also the same as those described in Experiment 1.

Procedure

As in Experiment 1, rats were water-deprived at 4:00 pm the day before the experimental procedure began. One day after water deprivation, rats had access to water from 10:00 am to 4:00 pm (6 hours). This session was carried out to assess baseline consumption and habituate rats to the schedule of the training sessions.

On day 2, animals started the training procedure that lasted 6 days. The training procedure consisted of a daily 6-hour exposure to the CS+US or the CS- solution (3 days each). The order in which animals received the CS+ and the CS- solution was kept as in Experiment 1, starting with half of the animals receiving the CS+ and the other half the CS-. During this procedure, animals had 15 minutes of access to water at 4:00 pm.

From the afternoon of day 8, the experimental sessions were divided into two as in Experiment 1 (10:00 am-16:00 pm). To habituate the animals to the new schedule, they received two water two-tube training sessions on the morning of days 9 and 10, as explained in Experiment 1. After this procedure, on Day 11, animals were tested for the CS+ preference in the same way as explained in Experiment 1. Finally, on days 12 and 14, animals started the pre-feeding-choice test cycles. These cycles were also identical to those in Experiment 1.

Results and Discussion

To analyze consumption during the training phase, a repeated measures ANOVA was conducted with Day (1-3) and CS solution (CS+US/CS-) as within-subject factors. As in Experiment 1, this analysis revealed a significant effect of CS $F(1,15) = 48.31$, $p < 0.001$, $\eta^2_p = 0.76$, with a higher total consumption of the CS+US (total mean across the 3 days: $M=76.50$, $SE=3.6$) than the CS- (total mean across the 3 days: $M=51.73$, $SE=2.28$). The factor Day $F(2,30)=2.12$, $p=0.13$, $\eta^2_p = 0.12$ and the Day*CS interaction $F(2,30) = 2.85$, $p = 0.07$, $\eta^2_p = 0.16$ were not significant.

The initial preference for the CS+ over the CS- after training was measured through a preference ratio, as in Experiment 1. A one sample t-test revealed that the ratios differed significantly from 0.5, $t(15)=5.03$, $p < 0.001$, $d = 3.90$ ($M = 0.74$, $SE = 0.04$).

Data from the Pre-feeding phase were analyzed to assess differences in total consumption for both water and sucrose solution. As on Experiment 1, a paired samples t-test revealed that consumption of water was significantly lower than sucrose $t(14) = 3.89$, $p = 0.002$, $d=1.04$ (Mean Sucrose $M = 9.52$, $SE = 0.23$; Mean Water $M = 8.00$, $SE = 0.30$).

During the pre-feeding-choice test cycles, two rats had to be removed from the analysis since on one of the days of testing, one tube of each rat was spilled, making it impossible to determine the amount consumed. The direct consumption data and preference ratios are illustrated in Figure 2 (panel-A and B). The results seem to show an attenuation of the CS+ preference across conditions. First, both preference ratios were submitted to a paired samples t-test comparing the pre-fed and control conditions. This analysis revealed significant differences across conditions $t(13)=-4.28$, $p<0.001$, $d = -1.15$. Both ratios were tested against the chance level of 0.5 with a one sample t-test, revealing significant differences for the control condition ratio $t(13)=3.43$, $p=0.004$, $d=2.96$, and non-significant differences for the pre-fed ratio $t(13)=0.42$, $p=0.967$, $d=1.71$ (See Figure 2, panel-A). Further, a repeated measures ANOVA was carried out to assess direct consumption during pre-feeding/control conditions with CS (+/-) and Pre-feeding (control/Pre-fed) as within-subject factors. This analysis revealed non-significant differences according to the main factors CS $F(1,13)=1.54$, $p=0.23$, $\eta^2_p = 0.11$ and Pre-feeding $F<1$. However, the interaction Pre-feeding*CS $F(1,13)=15.34$, $p=0.002$, $\eta^2_p = 0.54$ reached significance. A simple main effects analysis was conducted to explore the source of this interaction, revealing that only rats in the control condition showed a preference for the CS+ $F(1,13)=10.88$, $p=0.006$, $\eta^2_p = 0.45$; whereas those in the Pre-fed condition did not $F<1$.

Again, we found the same pattern of results as Experiment 1 with a short unrestricted access procedure (3 days exposure to each solution in total). Despite having continuous access for 6 hours to the target solutions, rats still expressed the US-devaluation effect. Thus, we can confirm that with this procedure, rats still expressed a preference mediated by the US representation. However, although the rats had continuous access to the target solutions in this experiment, they were only exposed to the CSs for 3 days. For this reason, we decided to extend the training procedure by doubling the number of days of exposure for both solutions before testing.

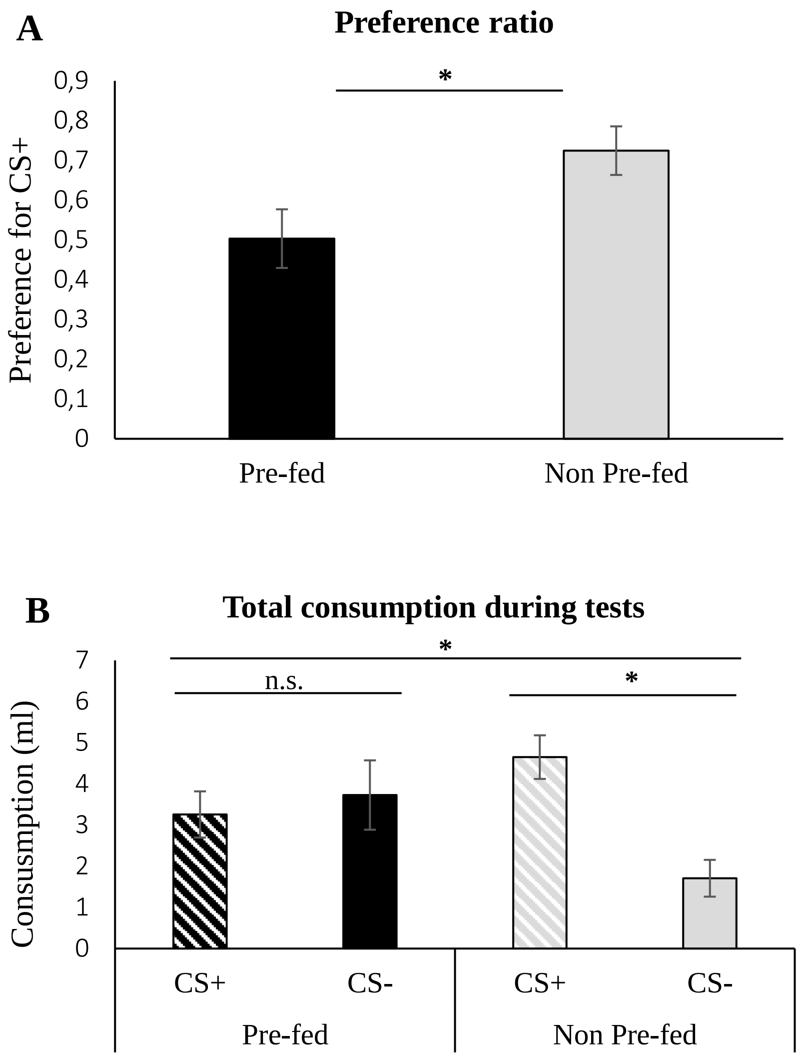


Figure 2. Panel A: Preference ratio for CS+ when animals were pre-fed with sucrose (Pre-fed) or were just given water (Non-Pre-fed). Panel B: Direct consumption (ml) of both CSs across conditions.

Experiment 3: Sucrose unrestricted long

In Experiment 3, we doubled the total training days from 6 to 12 days to increase the amount of exposure to the CS-US compound. We expect that by giving animals more opportunities to pair an odor cue (CS+) with sucrose (US), learning will become much more rigid and automatic, leading to an association that is insensitive to devaluation methods.

Methods

Subjects and apparatus

Sixteen non-naïve male Wistar rats with an average weight of 472 g (max: 529 – min: 397) supplied by Janvier Labs took part in the present experiment. The rats were naïve to the stimuli used in the present experiment. Animals were kept under the same conditions as in Experiment 1, and the flavored solutions were the same as those used in Experiments 1 and 2. In the present experiment, bottles with metal stoppers were used instead of tubes to prevent the rats from nibbling on the rubber stoppers of the tubes during the 6-hour training sessions. Once this phase was finished, we used the same tubes as in Experiments 1 and 2 for the remaining experimental sessions.

Procedure

As in Experiments 1 and 2, rats were water-deprived at 4:00 pm the day before the experimental procedure, with a baseline session given on Day 1, as in Experiment 2.

On Day 2, animals started the training procedure that lasted 12 days. This procedure consisted of a daily 6-hour exposure to the CS+US or the CS- solution (6 days each) as explained in Experiment 2. The order in which animals received the CS+ and the CS- solution was the same as in Experiments 1 and 2. Once the bottles were removed, the rats also had access to 15 minutes of water (4.00pm) throughout the entire experimental procedure.

After training, on Day 13, the rats' access to fluid was restricted to two daily sessions as in Experiments 1 and 2. The rest of the procedure was the same as the previous experiments.

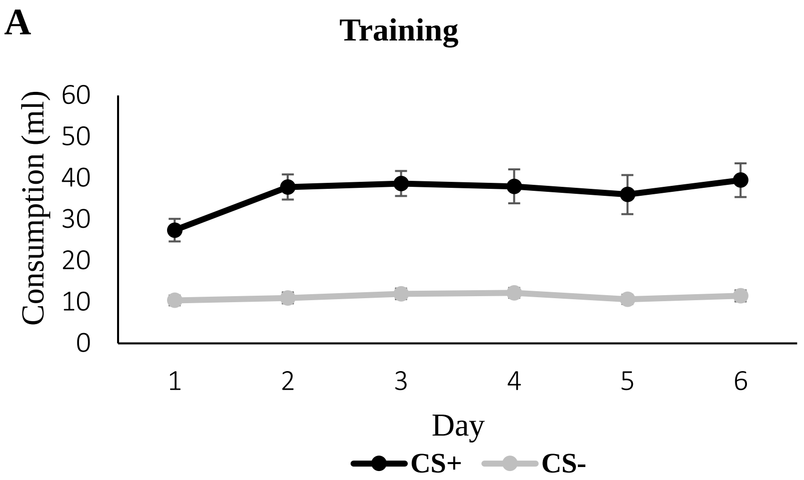
Results and Discussion

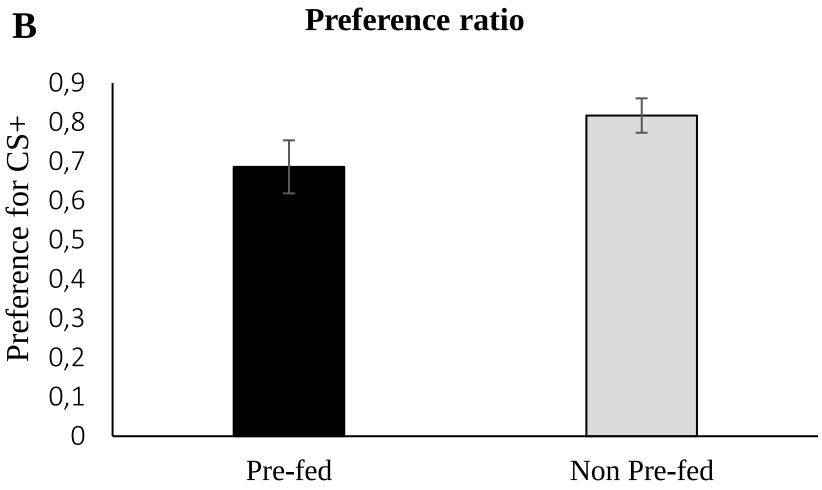
As Figure 3 (panel-A) shows, animals consumed higher amounts of the CS+US solution than the CS- throughout the whole training procedure. A repeated-measures ANOVA was conducted with Day (1-6) and CS (CS+US/CS-) as within-subject factors to analyze these data. This analysis revealed a significant effect of CS $F(1,15)= 109.89$, $p< 0.001$, $\eta^2_p= 0.88$ and Day $F(5,75)=2.38$, $p=0.04$, $\eta^2_p =0.13$ although the Day*CS interaction did not reach significance $F(5,75)= 1.26$, $p= 0.29$, $\eta^2_p =0.07$. These results confirm that the rats consumed more of the CS+US solution during training, as in previous experiments. Moreover, consumption increased across days, possibly due to the habituation of a neophobic response that was present on the first day of training.

A one-sample t-test was conducted to assess whether the initial preference for the CS+ over the CS- was significant compared to chance level. This analysis revealed significant differences from the 0.5 value, $t(15)=9.50$, $p< 0.001$, $d= 5.92$ ($M= 0.83$, $SE= 0.03$), thus indicating that the training procedure had been effective.

Consumption during the Pre-feeding phase was analyzed to assess differences in total consumption of the water and sucrose solution. A paired samples t-test revealed again that consumption of water was significantly lower than sucrose $t(15) = 2.98$, $p = 0.009$, $d = 0.74$ (*M* Sucrose= 9.32, *SE*= 0.10; *M* Water = 8.05, *SE*= 0.40).

Preference ratios after pre-feeding are shown in Figure 3 (panel-B). This figure suggests that rats expressed a preference for the CS+ in both the pre-fed and control conditions. A Paired samples t-test revealed non-significant differences between both preference ratios $t(15) = -1.54$, $p = 0.14$, $d = -0.38$. As the two ratios did not differ, the mean consumption of the CS+ of rats in both conditions was analyzed using a one-sample t-test to reveal whether the CS+ preference was not due to chance. This analysis revealed significant differences from the chance level $t(15) = 6.63$, $p < 0.001$, $d = 4.95$. Direct consumption data on the tests are displayed in Figure 3 (panel-C). These data were analyzed with a repeated measures ANOVA with CS and Pre-feeding as the within-subject factors. This analysis revealed only a main effect of CS $F(1,15) = 145.96$, $p < 0.001$, $\eta^2_p = 0.75$ whereas Pre-feeding $F(1,15) = 2.49$, $p = 0.14$, $\eta^2_p = 0.14$ and the CS*Pre-feeding interaction did not reach significance $F(1,15) = 3.29$, $p = 0.09$, $\eta^2_p = 0.18$. As seen in Figure 3 (panel-C), these results indicate, in general, that rats drank more of the CS+ than the CS- regardless of condition.


A
Training
Consumption (ml)
60
50
40
30
20
10
0
1
2
3
4
5
6
Day
CS+
CS-


B
Preference ratio
Preference for CS+
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Pre-fed
Non Pre-fed

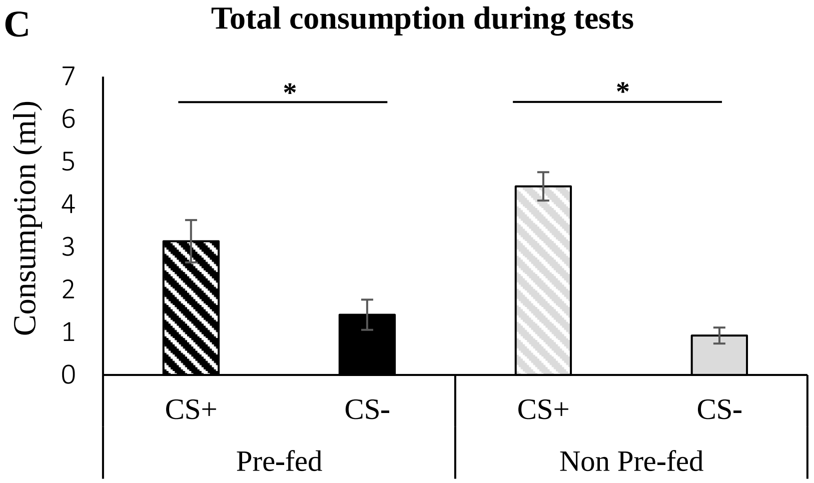

C
Total consumption during tests
Consumption (ml)
7
6
5
4
3
2
1
0
*
*
CS+
CS-
CS+
CS-
Pre-fed
Non Pre-fed

Figure 3. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Preference ratio for CS+ when animals were pre-fed with sucrose (Pre-fed) or in the control condition (Non-Pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

The results of Experiment 3 show that after extended training in which rats had access to the CS-US compound for several hours, they expressed a conditioned preference that was resistant to the devaluation procedure. These results suggest that the CS+ preference was not mediated by the US representation, with rats expressing a conditioned preference for the CS+ that was persistent when rats had been pre-fed with sucrose. However, an apparent decrease in total CS+ consumption was observed when animals were under the pre-fed condition. Although the rats in the pre-fed condition showed this tendency to consume less (albeit not significant), the preference was still intact. Thus, we suggest two main accounts to explain this finding. First, during the pre-feeding cycles, animals consume more in a total of the US solution than water in the control condition. Therefore, it is possible that the animals were less thirsty and consumed less overall. However, this pattern of results was not found in either Experiment 1 or 2, and this explanation therefore seems less plausible. Second, it is possible that this apparent reduction in consumption was observed because the CS+ had been associated with the general motivational properties of the US during training. Thus, the CS+ could have the ability to prompt an individual's general state of arousal that favors consumption of the US or other stimuli with similar motivational valence. Thus, changing the motivational state of rats through satiation could reduce the preference for the CS+ by diminishing or abolishing its appetitive motivational properties (Corbit et al., 2007; Watson et al., 2014). To determine whether this effect on total consumption was due to an artifact, or to some of the reasons mentioned above, for the next experiment, we compared a group of rats that underwent the same experimental procedure as the one carried out here and another that underwent the pre-feeding/control cycles by substituting water for maltodextrin in the control conditions. We first aimed to replicate the effect found in Experiment 3, demonstrating a lack of devaluation effect after pre-feeding. Regarding total consumption, as maltodextrin has a similar caloric value to sucrose, we expect the former to produce a similar motivational change after it has been consumed. Therefore, if the previous results are due to the motivational shift produced by general satiety, these differences in total consumption across conditions will disappear in the group of rats that consume maltodextrin instead of water in the control phase. In contrast, the same trend in total intake reduction will occur in the pre-feeding condition compared to the control condition in which the rats consume water.

Experiment 4: Replication of sucrose unrestricted

Experiment 4 had two aims. First, we wanted to replicate the lack of the US-devaluation effect found in Experiment 3. Second, to equate the total consumption during testing in both conditions (Pre-fed vs. Control), we added a further control group who received a maltodextrin solution in during the pre-feeding cycles. Maltodextrin does not have a sweet taste but has caloric properties similar to sucrose. Thus, the devaluation procedure is not expected to modify the specific sensory component of the US but should instead alter the motivational state of the animals. Unlike Experiment 3, we expect that the Maltodextrin-Group will not show differences in total consumption across conditions. Finally, we anticipated that neither of the groups would show the US-Devaluation effect, thus expressing a persistent CS+ preference on all the tests. The testing of both groups was carried out in two batches of experiments.

Methods

Subjects and apparatus

Thirty-two naïve male Wistar rats with an average weight of 352 g (max: 450gr – min: 308g) supplied by Janvier Labs took part in the present experiment. Animals were kept under the same conditions as all the previous experiments. The flavored solutions were the same as in previous experiments, and 10% maltodextrin.

Procedure

In the present experiment, a group of rats underwent the same experimental procedure as in Experiment 3, while the other half was treated identically except that after training, they received 6 hours of access to maltodextrin and during pre-feeding cycles, water was substituted for a maltodextrin solution in the control condition.

Results and Discussion

During the training procedure, animals consumed more of the CS+US solution than the CS-, as in the previous experiments. Figure 4 (panel-A) shows that consumption of the CS+ solution increased across days until reaching asymptotic levels, whereas consumption of the CS- solution remained stable. To analyze the consumption data during this phase, a repeated measures ANOVA was conducted with Day (1-6) and CS (CS+US/CS-) as within-subjects factors. This analysis revealed a significant effect of CS $F(1,31)=159.01$, $p<0.001$; $\eta^2_p=0.83$ and Day $F(5,155)= 5.59$, $p<0.001$; $\eta^2_p=0.15$. Greenhouse-Geisser corrections were applied to the CS*Day interaction, resulting in a significant effect, $F(3.57, 110.81)= 5.41$, $p<0.001$; $\eta^2_p=0.14$. Analysis of simple main effects revealed that consumption of the CS+US solution differed over the training days, resulting in an increase in total consumption from Test 1 to 6; $F(3.53, 109.48)=6.49$, $p<0.001$ $\eta^2_p=0.17$ (again Greenhouse-Geisser corrections were applied). However, consumption of the CS- solution did not differ between from Test 1 to 6, showing a stable pattern of consumption across sessions F<1 (Greenhouse-Geisser corrections were also applied).

The mean total Maltodextrin consumption after training in the Maltodextrin-Group was: $M= 31.6$; $SE= 4.0$.

Data from the initial preference test were analyzed with an ANOVA using the preference ratio for the CS+ over the CS- with Group (Sucrose or Maltodextrin) as the between-subject factor. The results revealed no significant differences between groups $F<1$. Analysis of the global CS+ preference ratio (including both groups) was compared to 0.5 with a One-Sample t-test, revealing significant differences $t(31)= 3.99$, $p<0.001$, $d=2.55$ ($M=0.69$, $SE=0.04$).

Data from the pre-feeding phase were analyzed using a repeated measures ANOVA with Pre-feeding (Sweet; sucrose solution/Non-sweet; water or maltodextrin solution) as a within-subject factor and Group (Water or Maltodextrin) as a between-subject factor. This analysis revealed a significant effect of Pre-feeding $F(1,30)= 5.73$, $p=0.02$, $\eta^2_p=0.16$ but no significant effect of Group $F(1,30)= 1.69$, $p= 0.20$, $\eta^2_p=0.05$ or an interaction between both factors $F<1$. Both groups (maltodextrin or water pre-fed in the control condition) consumed less overall in the Control condition ($M=8.61$, $SE= 0.28$) than in the Pre-feeding condition ($M=9.33$, $SE= 0.07$)

The CS+ preference ratio across conditions was analyzed with a repeated measures ANOVA with Pre-feeding (pre-fed or non-pre-fed) as the within-subject factor and Group as the between-subject factor. This analysis revealed no significant effect of Pre-feeding or Group and no interaction between these variables *Fs*<1. The mean preference ratios of the groups was compared to 0.5 using a One samples t-test, showing significant differences from the chance level *t*(31)= 2.21, *p*=0.03, *d*=2.65 (*M*= 0.58, *SE*= 0.03). To assess the global pattern of consumption across tests and groups, the data were submitted to a repeated measures ANOVA with CS (CS+ or CS-) and Pre-feeding (Pre-fed or Control conditions) as the within-subject factors and Group (Sucrose or Maltodextrin during Pre-feeding conditions) as a between-subject factor. This analysis revealed only a significant effect of CS *F*(1,30)= 6.38, *p*= 0.017, η^2_p =0.17. The factor Pre-feeding *F*(1,30) = 1.57, *p*=0.21, η^2_p =0.05, Group *F*(1, 30)= 1.75, *p*=0.19, η^2_p =0.05 and the interactions *Fs*<1 did not reach significance (See Figure 4 panel-B).

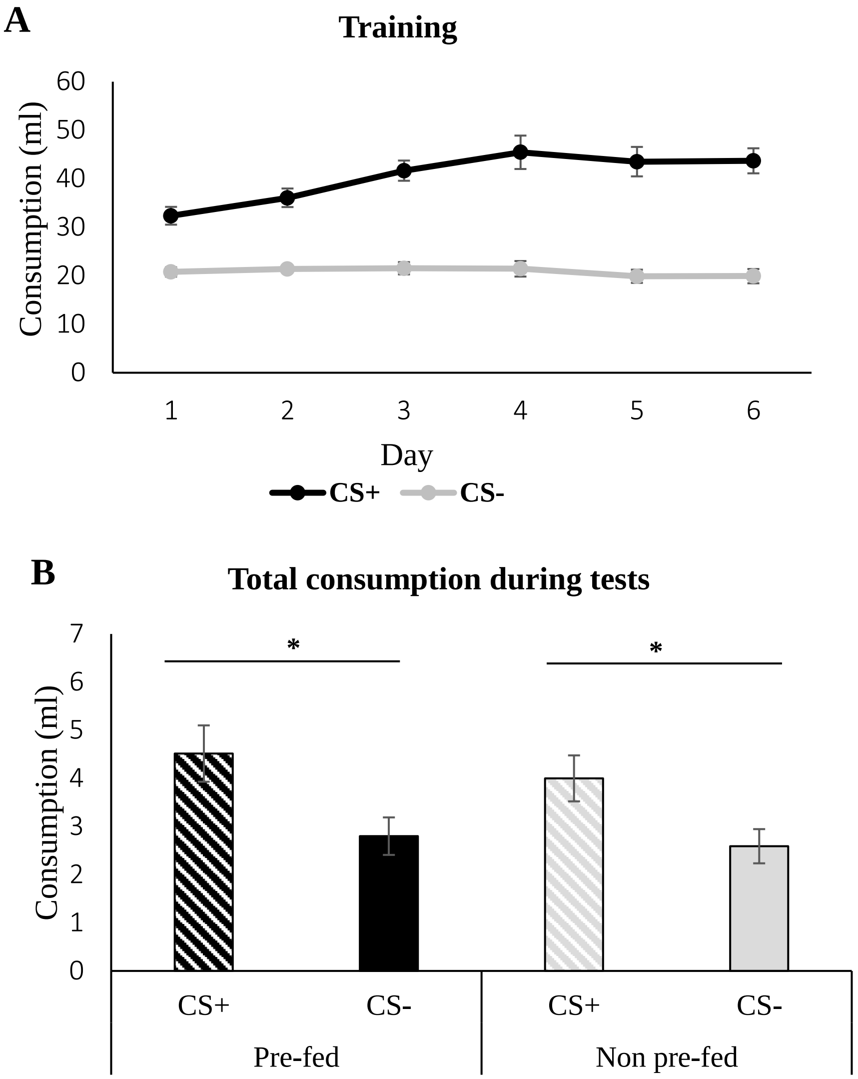


Figure 4. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Direct consumption (ml) of both CSs across conditions.

The results of Experiment 4 replicated those of Experiment 3 by demonstrating that rats still prefer the CS+ when the US had been devalued through Sensory-Specific Satiety. The rats showed a conditioned preference for the CS+ regardless of whether they had been pre-fed with the US or if they had received just water. Furthermore, in this experiment, there were no differences in the pattern of consumption during preference testing between experimental groups, suggesting that the tendency of the pre-fed condition to consume less on the tests in Experiment 3 could be due to an artifact. These results, again, suggest that a US representation did not mediate the CS+ preference following an unrestricted access training procedure.

Sucrose is a US that has a high hedonic value but is also a source of calories, which makes it a highly rewarding stimulus. In the following experiments we are trying to replicate those results found with sucrose in a non-caloric US: saccharin. Saccharin is characterised by having only a sweet hedonic taste (at low concentrations) but has no calories. As nutrient-acquired preferences do not always generate strong hedonic responses like taste-acquired ones, we expect its taste component sufficient to produce the S-R learning found in sucrose during unrestricted-access training. To replicate this effect, we first demonstrate the basic effect of US devaluation with short, limited-access training and saccharin as US like in Experiment 1.

Experiment 5: Saccharin restricted

After having found how the type of training procedure modulated the expression of the devaluation effect to the CS+ with sucrose as US, we proceed to analyse this effect with a non-caloric and sweet US. On Experiment 5 we carried out a limited access training procedure in order to show the basic devaluation effect as on Experiment 1.

Methods

Subjects and apparatus:

16 naïve male Wistar rats with an average weight of 295,3 g (max: 316gr – min: 261g) and supplied by Janvier Labs, took part on the present

experiment. Animals were kept under the same conditions as in Experiment 1. The flavored solutions were composed by saccharin 0.3% and almond and vanilla aroma 0.05% (Aromas Riesgo).

Procedure:

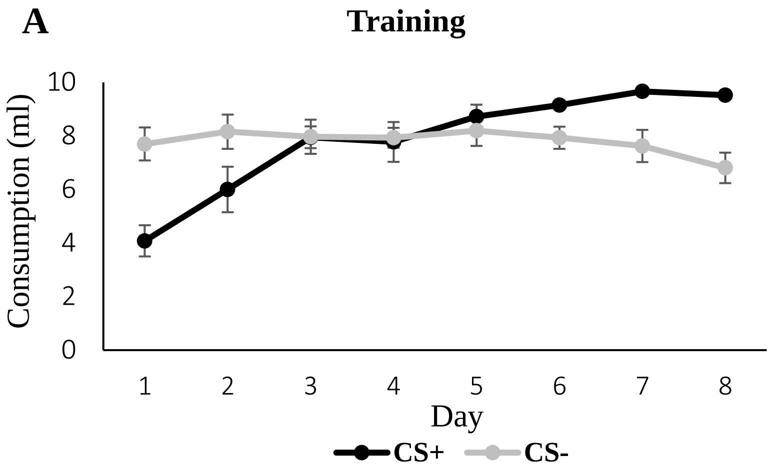
The procedure of this experiment was the same as in Experiment 1 with the exception of some details of the training procedure. Exposure to the CS+ and CS- compound in this experiment lasted a total of 8 days. At the end of the sixth day of training, the two-tube training procedure was performed for two days. On the ninth day a preference test between the CS+ and CS- substances was performed. On day 10 and 11 the rats were re-trained and on day 12 the choice preference was measured again. After this procedure, the pre-feeding cycles were carried out as mentioned before.

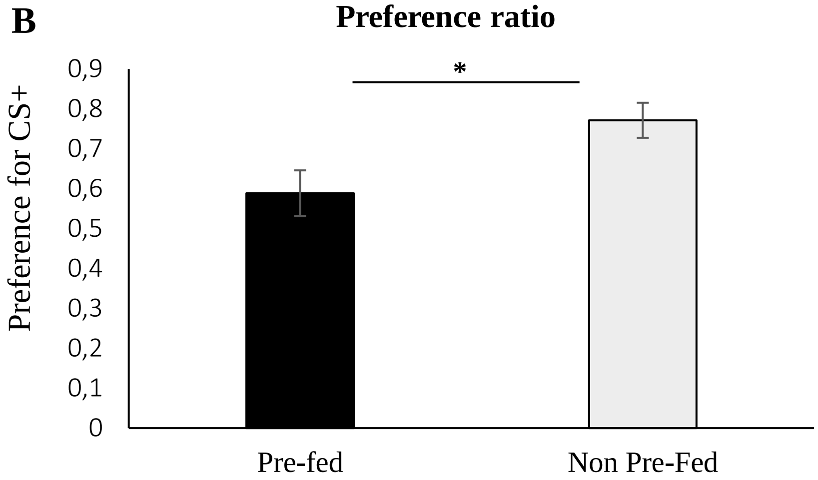
Results and discussion

An Inspection of Figure 5, panel-A shows total consumption of CS solutions across the 8 days of training. It shows how on the first and second day of training the CS+ consumption was lower than the CS- consumption due to neophobia. On the following days this pattern of consumption was reversed. a repeated measures ANOVA with Day (1-8) and CS (CS+ vs CS-) as within subject measures was carried out to analyse consumption during training. A Greenhouse-Geisser correction was applied to the factor Day and for the interaction Day*CS, since Mauchly Tests revealed that the assumption of sphericity was violatied. After applying Greenhouse-Geisser correction, analysis revealed a significant effect for the factor Day $F(3.37, 50.64)= 8.72$, $p< 0.001$, $\eta^2_p = 0.36$ and for the interaction Day*CS $F(2.95 , 44.3)= 8.35$, $p= 0.001$, $\eta^2_p = 0.35$. The CS factor did was insignificant $F<1$. An analysis for simple main effects was carried out to analyse the interaction between Day*CS. Results showed how for the CS+ there were significant differences of total consumption across days *F*(3.18, 47.71)= 18.07, $p < 0.001$, $\eta^2_p = 0.54$ whereas these differences were not present for the CS- $F<1$. This pattern of results probably reflects the initial effects of neophobia for the bitter taste of the saccharin solution.

The Preference of CS+ over the CS- across the two initial tests was measured through preference ratios. Analysis of the two tests were carried out with a paired samples *t* test (comparing both tests 1st and 2nd). Analysis revealed no significant difference across the two tests *t*(15)= -0.68, *p*= 0.50, *d*=-0.17. Thus, an average of both preference ratios was made for each rat, and the total average was compared to 0.5 in order to assess any difference with the chance limit. A one samples t-test was carried out with 0.5 as test value. Results revealed significant differences compared to the chance level 0.5 *t*(15)= 3.9, *p*= 0.001, *d*= 3.39 (*M*= 0.67, *SE*= 0.04).

Pre-feeding phase was then analysed to assess differences in total consumption of water and sucrose solution. Paired samples t-test revealed that consumption of water and saccharin didn´t differ across both days of the pre-feeding phase *t(*15)= -1.17, *p*=0.12, *d*= 0.29 (*M* Saccharin=9.12, *SE*=0.09; *M* Water=8.75, *SE*=0.27).


A
Training
Consumption (ml)
10
8
6
4
2
0
1
2
3
4
5
6
7
8
Day
CS+
CS-


B
Preference ratio
Preference for CS+
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
Pre-fed
Non Pre-Fed

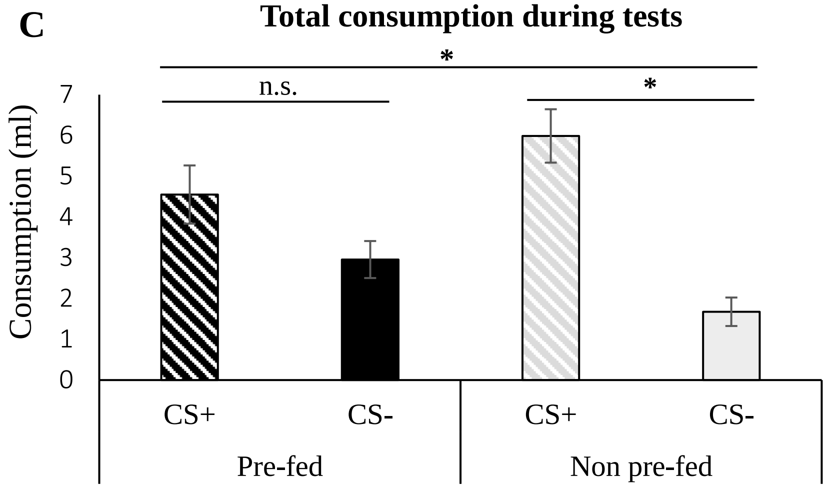

C
Total consumption during tests
*
n.s.
*
Consumption (ml)
7
6
5
4
3
2
1
0
CS+
CS-
CS+
CS-
Pre-fed
Non pre-fed

Figure 5. Panel A: Total consumption (ml) of both CSs across the training days. Panel B:Preference ratio for CS+ when animals were pre-fed with saccharin (Pre-fed) or in the control condition (Non-Pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

Choice tests were first analysed through preference ratios as in the previous experiments Analysis reflected a significant difference between both conditions $t(15)=-2.5$, $p=0.02$, $d= -0.63$. Both preference ratios were compared with the 0.5 chance level with a one sample t-test. Only the non pre-fed outcome reflected significant differences $t(15)= 6.18$, $p<0.001$ $d= 4.39$, whereas the pre-fed outcome didn´t differ with this level $t(15)=1.54$, $p=0.14$, $d= 2.56$ (see Figure 5, panel-B).

Again, consumption during the choice test were also analysed with a repeated measures ANOVA with CS (consumption for CS+ or CS-) and Pre-feeding (Pre-fed or Non Pre-fed) as within-subject measure. Analysis reflected a significant effect of CS $F(1,15)= 16.22$, $p= 0.001$, $\eta^2_p =0.52$ and an interaction between CS*Pre-feeding $F(1,15)= 6.72$, $p= 0.02$, $\eta^2_p =0.31$. The factor Pre-feeding $F<1$ did not reach significance. A simple main analysis was done to interpret the interaction showing that only the control condition showed preference for the CS+ $F(1,15)= 26.18$, $p< 0.001$, $\eta^2_p = 0.64$, whereas the Pre-feeding condition did not reach significance $F(1,15)=2.77$, $p= 0.11$, $\eta^2_p =0.15$ (See Figure 5, panel-C).

The present results replicate the same pattern of result as in Experiment 1 with saccharin as US. Rats expressed the US-devaluation effect by reducing their preference when had been pre-fed with saccharin after having underwent a limited access training procedure. On the next experiment we assessed the effect of a massive training procedure like that carried out on Experiments 3 and 4 using saccharin as US.

Experiment 6: Saccharin unrestricted long

Experiment 6 aimed to study the effect of an unlimited access training procedure using saccharin as US on the devaluation effect as done in Experiment 3 and 4.

Methods

Subjects and apparatus

16 non naïve male Wistar rats with an average weight of 499,3 g (max: 550gr – min: 473g) supplied by Janvier Labs, took part on the present experiment. Animals were kept under the same conditions as all the experiments carried out on this study. The flavored solutions were the same as those in Experiment 4.

Procedure

The exact same procedure of the Experiment 3 was carried out in this experiment.

Results and discussion

Figure 6, panel-A depicts total consumption of animals during training. Again, this figure illustrates that rats consumed higher amounts of the CS+ in contrast to the CS- solution during training. Interestingly, the figure also shows an increasing pattern of consumption of the CS+ solution until day 4th and an apparent decreasing pattern of consumption from day 4th to 6th. Otherwise, consumption for the CS- solution remains constant across days. To analyse these data, we performed a repeated measures ANOVA with Day (1-6) and CS (CS+/CS-) as within-subject measures. A Greenhouse-Geisser correction had to be applied to the factor Day. Analysis revealed a significant main effect of Day $F(2.57, 38.54)= 10.63$, $p<0.001$, $\eta^2_p = 0.41$, of CS $F(1,15)=121.77$, $p<0.001$, $\eta^2_p = 0.89$ and a significant interaction Day*CS $F(3.87, 58.1)= 8.20$, $p<0.001$, $\eta^2_p = 0.35$. A simple main effects analysis revealed that consumption of CS+ differed across days of training $F(3.08 , 46.30)=11.68$, $p<0.001$, $\eta^2_p = 0.43$ whereas CS- did not $F(2.83, 42.54)=2.03$, $p= 0.12$, $\eta^2_p = 0.12$.

The base line preference for the CS+ over the CS- prior the Pre-feeding/Control cycles was analysed. A one samples t-test reflected that the preference ratio differed significantly with 0.5 $t(15)= 2.84$, $p= 0.01$, $d= 2.49$ ($M=0.69$, $SE=0.07$).

Consumption for water and saccharin during pre-feeding was analasyed. Results reflected significant differences between both solutions $t(15)= -2.11$, $p=0.05$, $d=-0.52$, being higher for Saccharin ($M=8.93$, $SE=0.12$) than for Water ($M=8.18$, $SE=0.32$).

The preference ratios after the pre-feeding phase were analysed with a paired sample t-test. Results revealed significant differences when rats had been pre-fed with saccharin compared to water $t(15)=-2.32$, $p= 0.03$, $d=-0.58$. A One sample t-test reflected that only the non pre-fed preference ratio showed significant differences when compared to the chance level (0.5): $t(15)=9.79$, $p<0.001$, $d=6.75$. The pre-fed ratio failed to reach significant differences with 0.5 $t(15)=1.79$, $p= 0.09$, $d=2.264$ (See Figure 6, pane-b).

Again, to test for any differences in direct consumption after the pre-feeding phase, a repeated measures ANOVA was performed with CS and Pre-feeding as within-subject measures as in previous experiments. Analysis showed a main significant effect for CS $F(1,15)= 16.35$, $p=0.001$, $\eta^2_p = 0.52$ and for the CS*Pre-feeding interaction $F(1,15)= 6.13$, $p=0.03$, $\eta^2_p = 0.29$ whereas the factor Pre-feeding $F(1,15)= 1.33$, $p=0.27$, $\eta^2_p = 0.08$ did not reach significant differences. A simple main effect analysis was carried out which yielded significant differences in consumption for the CSs in the control condition $F(1,15) = 52.02$, $p<0.001$, $\eta^2_p = 0.78$ whereas there were non-significant differences in the pre-feeding condition $F<1$ (See Figure 6, panel-C).

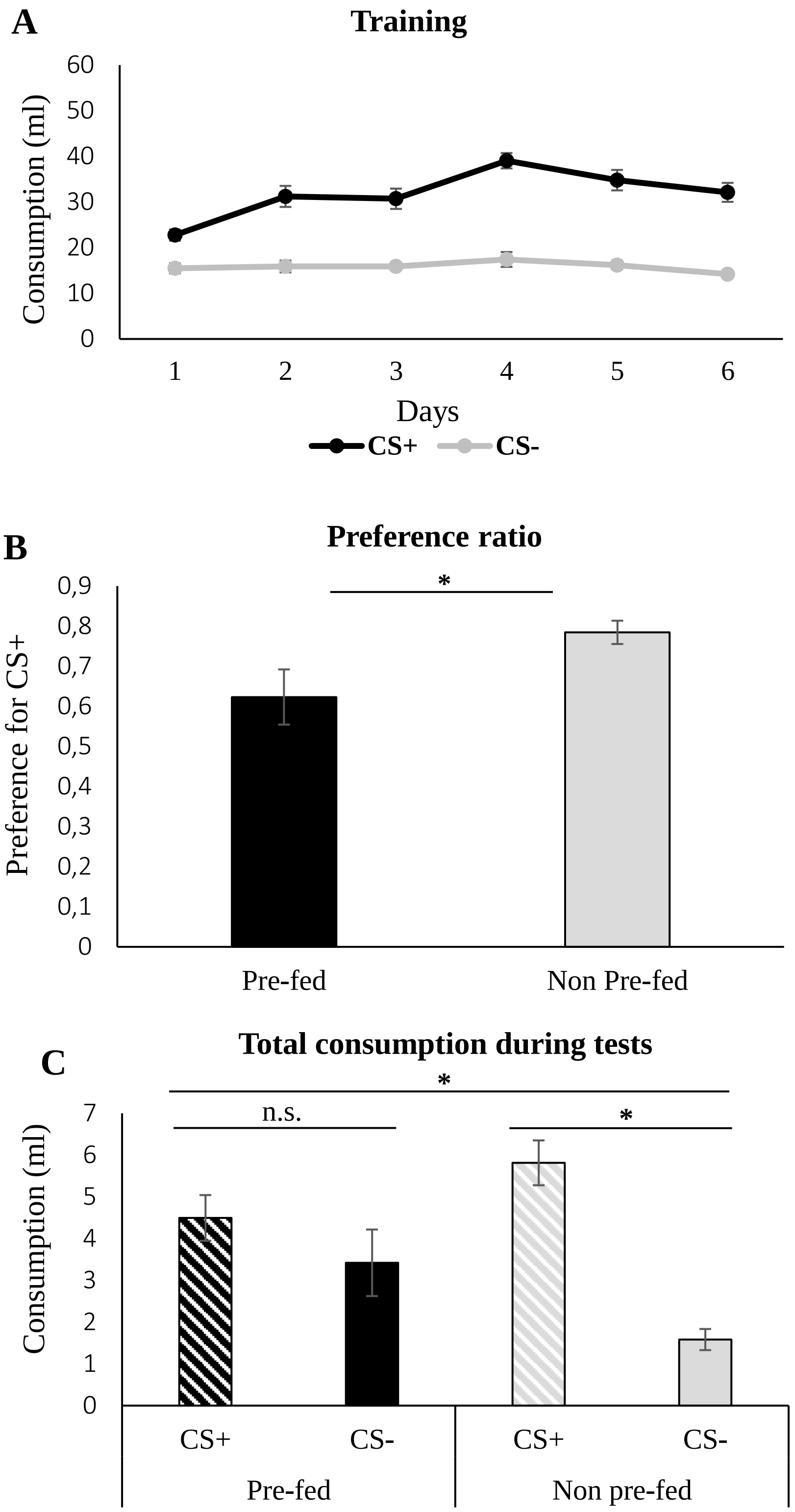

A
Training
Consumption (ml)
60
50
40
30
20
10
0
1
2
3
4
5
6
Days
CS+
CS-
B
Preference ratio
Preference for CS+
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
Pre-fed
Non Pre-fed
C
Total consumption during tests
*
n.s.
*
Consumption (ml)
7
6
5
4
3
2
1
0
CS+
CS-
CS+
CS-
Pre-fed
Non pre-fed

Figure 6. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Preference ratio for CS+ when animals were pre-fed with saccharin (Pre-fed) or in the control condition (Non-Pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

Experiment 6 showed that rats exhibited a reduced preference for the CS+ after having been pre-fed with the saccharin. Thus, this pattern of result did not replicate those of Experiment 3 and 4, in which the same training procedure was carried out but a sweet caloric US was employed. One possibility to explain these differences, is the fact that saccharin and sucrose at the concentrations used during these experiments do not have the same hedonic value, with sucrose having a higher hedonic value than saccharin (Harris et al., 2000). It is therefore possible that the hedonic response of saccharin as US is insufficient to be directly associated with CS, at least with the amount of exposure used here.

This hypothesis is based on the notion that sweetness and calories form a pre-established association. Therefore, the subsequent repeated presentation of saccharin (a sweet taste that is not followed by calories) could trigger a process of extinction of the sweet-calorie presumed innate association. This process could favour the loss of the hedonic value of saccharin and, therefore, of its reinforcing properties as US (Davidson et al., 2013; Gil et al., 2021). If the sweet-calorie extinction is taking place during training, the US would lose its value and therefore its elicited hedonic responses would be weaker. This idea seems plausible when looking at the total training consumption across days, which from day 4 onwards show an apparent decreasing trend in the case of the saccharin-containing solution.

To determine whether the results of Experiment 6 indicate a weakening of the sweet-calorie association, we decided to carry out another experiment in which saccharin was combined with maltodextrin (Experiment 8). Maltodextrin has a similar energy boost to that of sucrose but has a weak non-sweet taste. So, by combining it with saccharin we could interrupt the presumed extinction process of the sweet-calories pre-stablished association, and to mimic the effect of sucrose (a US rich in sweet taste and nutritive properties). Before testing this hypothesis, we decided to analyse the individual effect of maltodextrin as a US in this unrestricted access training procedure (Experiment 7). Maltodextrin does not have a highly hedonic taste; it is its nutritive properties that support its strength as a US (Bonacchi et al., 2008). Also, as calorie-based preferences are not good triggers of hedonic reactions when the CS+ is consumed (Myers & Sclafani, 2003; Myers, 2018), we hypothesise that for maltodextrin the unrestricted access procedure will not be able to develop S-R learning.

Experiment 7: Maltodextrin unrestricted long

Experiment 7 was carried out in order to assess the effects of an unlimited access training procedure using a solution of maltodextrin as US.

Methods

Subjects and apparatus

16 non naïve male Wistar rats with an average weight of 492 g (max: 451gr – min: 345g) supplied by Janvier Labs, took part on the present experiment. Animals were kept under the same conditions as all the experiments carried out on this study. The flavored solutions were composed by maltodextrin 10% and almond and vanilla aromas 0.05% (Aromas Riesgo).

Procedure

The exact same procedure of the Experiment 3 and 6 was carried out on this experiment but employing a different palatable solution as US.

Results and discussion

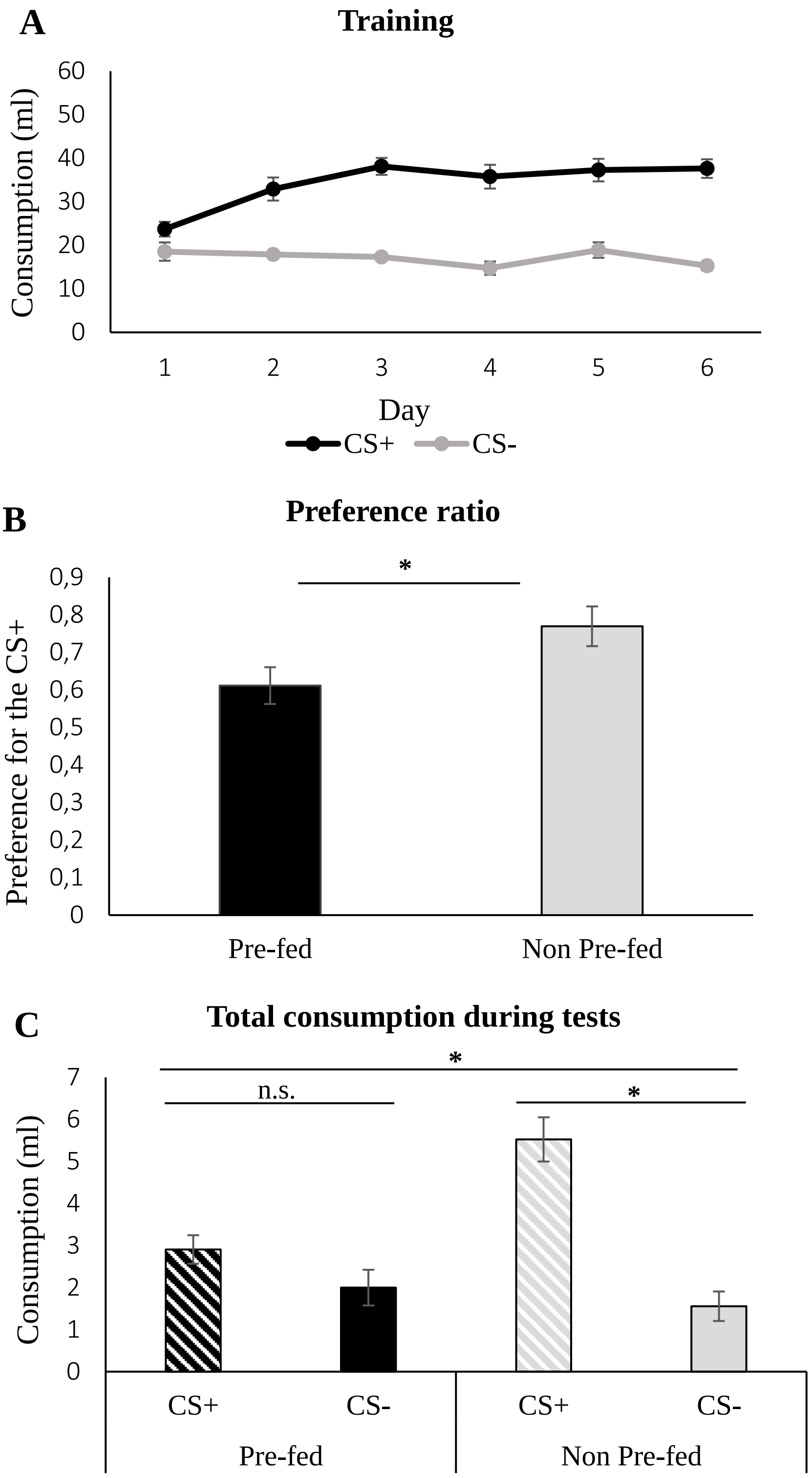
Figure 7, panel-A depicts total consumption of CS+ and CS- solutions during training. Again, the rats apparently consumed greater amounts of the CS+ solution throughout the training procedure. A repeated measures ANOVA was carried out with Day (1-6) and CS (CS+/CS-) as within-subject measure. Analysis yielded to a significant effect of CS $F(1,15)=104.11$, $p < 0.001$, $\eta^2_p = 0.87$, Day $F(5,75)=5.07$, $p < 0.001$, $\eta^2_p = 0.25$, and a significant interaction CS*Day $F(5,75)=8.20$, $p < 0.001$, $\eta^2_p = 0.35$. A simple main effects analysis showed that the factor CS yielded significant differences in consumption across days $F(5)= 9.90$, $p < 0.001$, $\eta^2_p =0.39$ whereas the factor CS- did not $F(2.9, 43.80)=1.53$, $p = 0.21$, $\eta^2_p =0.09$ (Greenhose-Geisser corrections applied). These results indicate an increasing pattern of consumption for the CS+ solution over the days, probably due to attenuation of neophobia and calorie learning.

Preference for the CS+ solution during the first test without pre-feeding was analysed with a one samples t-test. Analysis reflected that the preference ratio differed significantly with 0.5 $t(15)= 6.62$, $p< 0.001$, $d=4.71$ ($M=0.77$, $SE=0.04$).

Analysis of total consumption of water and maltodextrin during pre-feeding was analyzed with a paired samples t-test. Results showed a significant higher total consumption $t(15)= 2.67$, $p=0.01$, $d=-0.66$ for the maltodextrin solution (Maltodextrin: $M=8.99$, $SE=0.26$) over water ($M=7.76$, $SE=0.33$).

The preference ratio after the pre-feeding phase were analysed with a paired sample t-test. Results revealed significant differences between both ratios $t(15)=-2.39$, $p= 0.03$, $d=-0.59$, being higher in the control condition compared to the pre-feeding condition. A One sample t-test reflected that both preference ratios differed with 0.5 significantly: Pre-fed $t(15)= 2.276$, $p= 0.038$, $d=3.121$; Non pre-fed $t(15)=-5.093$, $p<0.001$, $d=3.636$ (See Figure 7, panel-B).

The analysis for total consumption during testing was again analysed with a repeated measures ANOVA with CS and Pre-feeding as within subject measure. Results reflected a main effect of Pre-feeding $F(1,15)= 12.84$, $p =0.003$, $\eta^2_p = 0.46$ and CS $F(1,15)= 31.89$, $p < 0.001$, $\eta^2_p = 0.68$. The interaction between both factors also was significant $F(1,15)= 9.01$, $p =0.009$, $\eta_p^2 = 0.38$. Analysis of simple main effects showed a significant effect of CS preference in the control condition $F(1,15)= 23.05$, $p < 0.001$, $\eta^2_p = 0.60$ but not in the pre-feeding condition $F(1,15)= 3.92$, $p = 0.07$, $\eta^2_p = 0.20$ (see Figure 7, panel-C).



A Training

B Preference ratio

C Total consumption during tests

Figure 7. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Preference ratio for CS+ when animals were pre-fed with saccharin (Pre-fed) or in the control condition (Non-Pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

The present experiment has shown that after an unlimited access training procedure under the same parameters as in Experiments 3, 4 and 6 and using maltodextrin as US, the preference for the CS+ was sensitive to a US-devaluation procedure. This can be interpreted as the current CS+ preference is being mediated by the representation of the US. These results are not surprising as maltodextrin do not have an hedonic taste. In this sense, its caloric supply is the main reinforcing source as US, and caloric based preferences are not well predictors of conditioned hedonic responses (Myers & Sclafani, 2003).

Experiment 8: Maltodextrin + Saccharin unrestricted long

Experiment 8 was carried out in order to assess the effects of an unlimited access training procedure using a mixture of saccharin and maltodextrin as US. The purpose of this experiment was to assess whether another US different to sucrose, with similar nutritional properties but also with an hedonic taste, could mimic it and thus trigger an S-R association after a massive training procedure such as in Experiment 3 and 4.

Methods

Subjects and apparatus

16 naïve male Wistar rats with an average weight of 403 g (max: 461gr – min: 329g) supplied by Janvier Labs, took part on the present experiment. Animals were kept under the same conditions as all the experiments carried out on this study. The flavored solutions were composed by a mixture of 0.3%

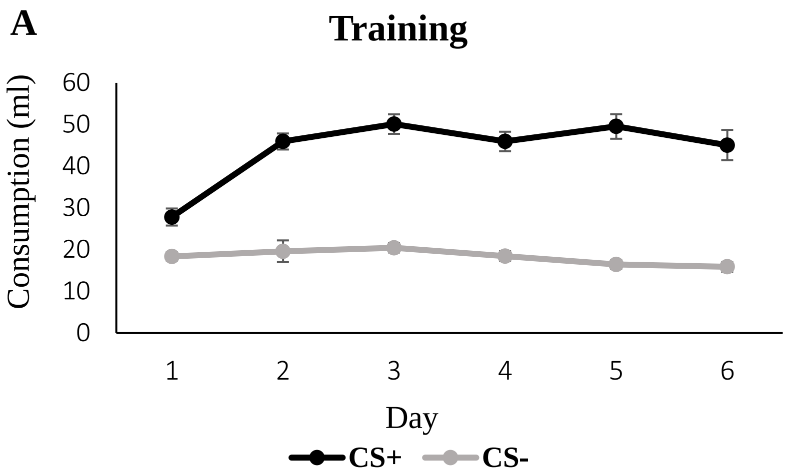
saccharin and maltodextrin 10% as USs and almond and vanilla aromas 0.05% as CSs (Aromas Riesgo).

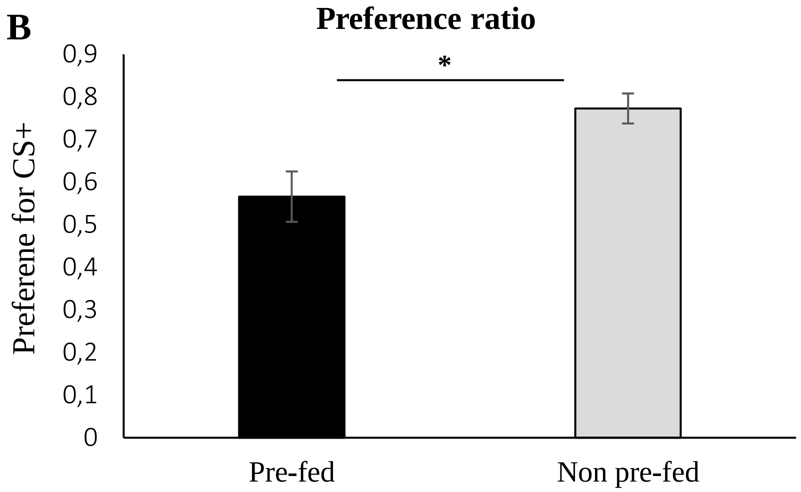
Procedure

The exact same procedure of the Experiment 3 was carried out on this experiment.

Results and discussion

Figure 8, panel-A shows consumption for both CSs across training. The graph shows how the consumption of CS+ follow an increasing pattern across days while the consumption of CS- remains stable at a lower level of consumption. A Repeated measures ANOVA reflected a significant main effect of CS $F(1,15)=208.96$, $p<0.001$ $\eta^2_p=0.93$, of Day $F(7,75)=11.96$, $p<0.001$ $\eta^2_p=0.44$ and an interaction between CS*Day $F(5,75)=9.00$, $p<0.001$ $\eta^2_p=0.37$. A simple effects analysis revealed that only the CS+ reflected significant differences in consumption across the 6 days, showing an increasing pattern of consumption across days $F(5,75)=14.03$, $p<0.001$ $\eta^2_p = 0.48$, whereas this change in the pattern of consumption did not occur for the CS- $F(2.52, 37.91)=1.50$, $p=0.23$, $\eta^2_p = 0.09$ (Greenhouse-Geisser applied) (see Figure 8).


A
Training
Consumption (ml)
60
50
40
30
20
10
0
1
2
3
4
5
6
Day
CS+
CS-


B
Preference ratio
Preferene for CS+
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
Pre-fed
Non pre-fed

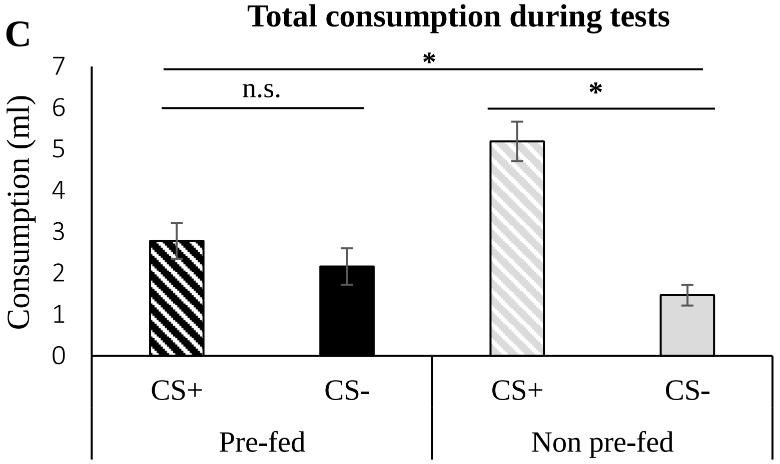

C
Total consumption during tests
Consumption (ml)
7
6
5
4
3
2
1
0
*
n.s.
*
CS+
CS-
CS+
CS-
Pre-fed
Non pre-fed

Figure 8. Panel A: Total consumption (ml) of both CSs across the training days. Panel B: Preference ratio for CS+ when animals were pre-fed with saccharin and maltodextrin (Pre-fed) or in the control condition (Non-Pre-fed). Panel C: Direct consumption (ml) of both CSs across conditions.

Preference for the CS+ solution during the first preference test was analysed with a one samples t-test. Analysis reflected that the preference ratio differed significantly with 0.5 *t*(15)= 3.32, *p*=0.005, *d*=2.89 (*M*=0.70, *SE*=0.06*)*.

Analysis of total consumption of water and maltodextrin during pre-feeding was analysed with a paired samples t-test. Results showed a higher total consumption for the saccharin + maltodextrin solution *t*(15)= 3.88, *p*=0.001, *d*=-0.970 (Saccharin + Maltodextrin: *M*=9.2, *SE*=0.075) than for Water (*M*=7.74, *SE*=0.38*)*.

The preference ratio after the pre-feeding phase were analysed with a paired sample t-test. Results reflected significant differences when rats had been pre-fed with saccharin + maltodextrin than water *t*(15)=3.38, *p*=0.004, *d*=-0.84. A One sample t-test reflected that only the non pre-fed ratio differed to 0.5 level *t*(15)= 7.73, *p*<0.001, *d*=5.48. The pre-fed ratio did not differ to 0.5 *t*(15)= 1.11, *p*=0.28, *d*=2.39 (See Figure 8, panel-B).

Finally, an analysis for total consumption of both CSs during testing, was carried out to measure the preference in both conditions. CS and Pre-feeding were both within-subjects factors in the repeated measures ANOVA. Results showed a main effect of Pre-feeding *F*(1,15)=5.05, *p* =0.04, η^2_p = 0.25 and CS *F*(1,15)=17.21, *p* < 0.001, η^2_p = 0.53. The interaction of both factors also reach significance *F*(1,15)=12.30, *p*=0.003, η^2_p = 0.45. Analysis by means of the interaction reflected significant differences for both CSs in the control condition *F*(1,15)=34.83 *p* < 0.001, η^2_p = 0.70 while the pre-feeding condition did not reach significance *F*<1 (See Figure 8, panel-C).

The results of this experiment again failed to replicate those found with sucrose in the long unrestricted access procedure (Experiments 3 and 4). These results do not show support for the hypothesis suggested in Experiment 5, which held that massive saccharin exposure could triggered an extinction process by disrupting the sweet-calorie association. If this had been the case, in this experiment the caloric contribution of maltodextrin would have prevented this process and therefore the saccharin-maltodextrin compound would act mimicking sucrose as in Experiment 3 and 4. In the final section, alternative interpretations will be given in order to explain why the effect found in Experiments 3 and 4 with sucrose were not replicated with other USs.

General discussion

Experiment 1 demonstrated the US devaluation effect with a restricted access training procedure (10 days per CS solution). Rats showed a reduction in the sucrose paired CS+ preference over the unpaired CS- when they had been previously pre-fed with a sucrose solution. Experiment 2 replicated these findings with a short unrestricted access training procedure (3 days per CS solution). In Experiment 3, we extended the length of the training procedure by doubling the number of sessions given in Experiment 2. We found that the US-devaluation effect disappeared, since the rats still preferred the CS+ over the CS- after pre-feeding with sucrose. However, the rats in the pre-feeding condition showed an apparent trend to drink less overall than those in the control condition. To avoid possible influences of the change in motivational state after pre-feeding, in Experiment 4 we examined how using a non-sweet caloric solution in the pre-feeding control condition affected total consumption during the preference tests. This experiment yielded no such differences in global consumption across conditions and replicated the pattern of results found in Experiment 3.

The results from Experiment 1 and 2 appear to be consistent with those of previous studies, which found that acquisition of conditioned preferences is mediated by the representation of the US (Stimulus-Stimulus). On the other hand, when animals were trained with sucrose and an extended unrestricted access procedure (Experiment 3 and 4), the pattern of intake during the test changed; rats still expressed a conditioned preference even though sucrose had been devalued, suggesting that the CS+ preference was not sensitive to a US-devaluation procedure. Thus, the representation of the US does not govern the association that mediates the acquired preference, since presentation of the CS automatically elicits a conditioned response (Stimulus-Response). Sucrose has a highly hedonic taste but is also a source of calories. To elucidate the necessary characteristics or conditions for insensitivity to the US devaluation procedure to occur, we used saccharin as US, which has a palatable taste but does not contain calories. It has been suggested that preferences based on caloric consequences do not always result in hedonic responses. Therefore, we expected that this phenomenon should occur also in hedonic non-nutritive USs.

So, in Experiments 5 and 6 we attempted to replicate those found with sucrose by using saccharin as US. However, although Experiment 5 replicated the the US-devaluation effect found with a restricted access training procedure in Experiment 1, on Experiment 6 we did not find the same pattern of results in Experiments 3 and 4. One hypothesis to explain these results was that saccharin, by being sweet but not nutritive, might undergo an extinction process by breaking the sweet-calories presumed innate association. Through this process, saccharin would be devalued in the course of the training procedure, and therefore the hedonic reactions elicited by it would be diminished and hinder the development of a S-R association.

In this regard, to test this hypothesis we conducted Experiments 7 and 8 using maltodextrin and sasccharin+maltodextrin as US respectively. In both experiments, we reported a US-devaluation effect. In this sense, the results obtained in Experiment 7 do not seem to be surprising, as maltodextrin does not own an attractive taste. More surprisingly, the compound saccharin + maltodextrin, which contains a hedonic taste and a source of calories roughly comparable to sucrose, did not show an insensitivity to devaluation. To explain this pattern of results we suggest that 1) The compound saccharin + maltodextrin instead of being perceived as one configuration is perceived as two distinct units, preventing the compound from mimicking the effects of sucrose 2) Although the saccharin + maltodextrin compound is likely to be hedonically stronger than each taste separately, it is still unable to equate the high hedonic value of sucrose. Under this possibility we hypothesise that the greater the hedonic response produced by the consumption of the US, the greater the possibility that the S-R associative structure will occur. 3) Assuming that sucrose has an innate preferred taste, it may have unique or distinctive properties that make it different as a reward from other USs preventing the replication of this effect.

The dissociation of stimulus-stimulus (S-S) and stimulus-response (S-R) theories of behavior has mostly focused on instrumental, rather than Pavlovian, learning. Furthermore, approaches to studying S-R learning with Pavlovian cues have rarely succeeded with the exception of second-order conditioning (Holland, 1981; Holland & Rescorla, 1975) or Pavlovian to Instrumental Transfer (PIT; e.g., de Tommaso et al., 2018; Watson et al., 2014; But also see Pool et al., 2019). Moreover, evidence has shown that associative learning is not based solely on an association between a neutral stimulus and a unique component of the US — its specific response. Instead, the nature of the learned content is highly complex and polyhedric, arising from multiple possible associations, such as a link between two neutral stimuli (CS-CS), between a response and its consequence (R-O), between stimuli that are not physically present but are activated associatively or between representations of a neutral stimulus and an unconditioned stimulus (CS-US) (Rescorla, 1988). In this regard, multi-component approaches to Pavlovian conditioning argue that learning is based not only on the relationship between single event representations of the CS and the US, but also on the possibility that multiple overlapping representations of a diverse nature can enter into an association with the CS. Thus, the sensory (or perceptual), motivational, hedonic, temporal, and responsive properties of the US can be viewed as different components that can be associated with the CS (Delamater, 2012; Delamater & Oakeshott, 2007). In this sense, Delamater (2012) questions the use of devaluation treatments to study possible stimulus-response relationships in Pavlovian learning. According to this author, we cannot directly rule out the possibility that the learned content may be driven by an association between the CS and another component of the US that is insensitive to devaluation, such as its motivational (appetitive or aversive) or hedonic (positive or negative value) properties.

The hedonic component of the US represents a universally positive or negative quality shared between appetitive or aversive USs that evokes a response (Hedonic/Aversive reactions). Delamater (2012) argues that the application of devaluation methods might not reveal the exact underlying structure of the association (CS-US Hedonic properties; S-S or CS-Hedonic Reaction; S-R). From this perspective, the possibility remains that the results obtained cannot be fully explained by the S-R association but by a direct link between the CS and another hedonic component of the US (not necessarily the response it evokes).

However, Holmes et al. (2016) suggest that as flavor conditioned preferences can be modulated by either positive or negative incentive contrasts, this means that the hedonic properties of the US mediate at least some part of the acquired preference. Their study demonstrated that immediately after training when the CS+ is presented in the absence of the US, rats show an initially reduced palatability response that increases when the test is performed after 7 days of training. The authors explain that this initial reduction in CS+ palatability occurs due to a comparison of the experience of the CS+ value during the test and the experienced value of the CS+ during training (presented with 0.4% saccharin in this study). As time passes, the memory trace of the CS-US hedonic experience during training decays. Therefore, the comparison or contrast between the two experiences is reduced, increasing the perceived palatability of the CS+ when it is presented alone. In addition, re-exposure to a highly concentrated (0.1%) or mild (0.05%) saccharin solution one day before the 7th day of testing restores the memory representation of the perceived palatability during training, again reducing the hedonic value experienced when the CS+ is presented in water. In contrast, when a weak sweet solution (0.025%) is re-exposed, the opposite pattern of results is found. A positive contrast results from comparing a weak sweet solution with the CS+, increasing its palatability. In this sense, hedonic contrast phenomena could be understood as a way of updating the hedonic component of the US.

Regarding our methodology, the results from Experiment 1 and 2 could reflect a devaluation effect of the specific sensory and motivational properties of the US and the hedonic aspect of the latter due to prior exposure of the US without the CS+ before the choice test. However, this explanation of the results carries the same implications. The fact that after the long, unlimited access procedure used in Experiments 3 and 4, rats still expressed a preference for CS+ indicates that neither the hedonic nor the specific sensory or motivational component is being affected by devaluation, again providing evidence in favor of an association between the odor cue and the US response. If the underlying association was linked to the hedonic component of the US and not the response it elicits, the CS+ preference would be sensitive to hedonic contrast effects.

Regarding the general motivational component, we argue that a strong association with the motivational properties of the US seems a less likely candidate for explaining the present results. First, the animals were not food restricted throughout the experiments, making it implausible to suppose that sucrose consumption would be associated with a boost of energy or a general arousal effect. In Experiments 2-4 in particular, animals had adlib access to fluid throughout the whole CS-US exposure, thus avoiding thirst-residual hunger. Second, we argue that any association between the CS+ and the general arousing properties of the US is likely to be devalued through Sensory-Specific-Satiety. If the preference had been based on the general motivational properties of the US, changing the motivational state of the animals, now pre-fed, should eliminate the appetitive or general arousal-increasing effect by reducing preference or total CS+ consumption. Similar results have been reported using the PIT paradigm, showing that the effect of the general arousing properties of a food-associated (Pavlovian) cue on subsequent instrumental responding can be abolished after a change in the experimental subject's motivational state (Corbit et al., 2007; Watson et al., 2014).

To our knowledge, this is the first attempt to study the effect of different extended training procedures to assess the role of S-R learning in the acquisition of flavor preferences. The notion that Pavlovian learning might be subject to the effects of an S-R link has been largely abandoned since the advent of the more cognitive models of associative learning and the realization that associative learning arises from complex associations rather than solely between stimuli and responses. However, recently, Thrailkill et al. 2018 (see also Bouton et al., 2020; Bouton, 2021) applied Pearce & Hall's (1980) model (which was developed in a Pavlovian learning context) to instrumental learning and habit formation (S-R). According to this theory, as the CS becomes a good predictor of a US, attention to the CS declines, along with its salience and associability. This is because our ability to process stimuli is limited, and therefore, when a CS is no longer surprising, and we are fully aware of its consequences, we process it automatically. Thraikill et al. (2018) propose that through this process, the conditioned response may also be elicited in an automated fashion when the CS is present. These authors draw parallels with operant learning, establishing that during training, when a particular discrete stimulus and instrumental response becomes highly predictable from the reinforcer (S-R-O), a similar process can occur, encouraging the development of a behavioral habit (S-R without O processing). From this perspective, if an individuals' attention to the predictive stimuli of an outcome and their associated instrumental responses decreases, the behavior will be automatically triggered when a stimulus sets the occasion. Thus, extending this interpretation to the present results, a tentative explanation based on Pearce and Hall's model (1980) emerges. It is possible that during the general training procedure, when rats are exposed for long periods to the CS and the US, the CS reaches an asymptotic level of learning. Therefore, no more can be learned about it, leading to a decrease in attention to this cue. Thus, the CS might be expected to produce an automatic conditioned response irrespective of the representation of the US. Future studies should aim to manipulate the magnitude of the predictive relationship between the CS and US by partially pairing the two events during training. If the preference produced by this training procedure is sensitive to the devaluation procedure, this would constitute further evidence for this hypothesis.

In the literature, flavor preference learning has been suggested as one of the factors involved in overeating by determining food likes and dislikes or food choices in today's environments (Yeomans, 2012). Most today's societies are featured by the omnipresence of the obesogenic environments that expose us to a wide variety of foods that share very similar sensory properties but have different calorific content. Extensive exposure to a wide variety of highly caloric and palatable foods and food-related cues could affect flavor-nutrient learning, a process that regulates food intake, for example, through conditioned satiety. For example, a study by Hardman et al. (2015) showed that the number of varieties of pizzas of a given flavor (pepperoni) available in UK supermarkets totaled 71 different units. Further, in this study, the authors revealed that among these 71 different pizzas brands, which probably taste very similar but differ in certain sensory attributes, the variability in calories ranged from 500 to 2000 kcals on a standard size pizza, depending on the brand. Concerning this, it has been argued that individuals may lose the ability to anticipate the ideal portions of each food based on its nutritional properties (conditioned satiety) due to a continuous inconsistency between sensory cues and caloric load. This massive exposure to flavor-nutrient inconsistency has been suggested as a major problem for intake regulation by hindering flavor-nutrient learning and leading to overconsumption (For a review, see Martin, 2016; Yeomans, 2012). On the other hand, others have argued that this exposure to sensory variety could generate an increase in discrimination between sensory cues, leading to more effective flavor-nutrient learning (Palframan & Myers, 2016) that may finally result in overeating, for example, through the disruption of Sensory-Specific Satiety generalization (Gonzalez et al., 2018). Another consequence of massive exposure to high-palatable foods could be the formation of S-R associations between flavor and hedonic reactions to food such as those observed in our study. In this sense, a preference for a CS+ insensitive to a US revaluation process could encourage excessive eating such as that shown in hedonic hunger (eating without hunger). In this regard, the present study has explored whether it is possible for S-R learning to occur in flavor preference learning by manipulating different parameters of the experimental procedure, such as the amount of access to the CS-US compound or the length of training. And we have observed that the length of training and the amount of exposure to the CS-US compound is important. It should be noted that the type of exposure that has been found to trigger S-R learning (Experiment 3-4) is characterized by unrestricted access for many hours to the CS-US compound, which could hinder its translation to real-life settings—particularly if we consider that humans, as omnivores, have limited eating or drinking periods throughout the day. Nevertheless, it remains a possibility that with an extended but not massive training, in a way that is more comparable to real life, S-R learning may eventually occur. Although this possibility has not been addressed in these experiments, it could be explored in the future, given its implications.

Finally, we should consider several limitations of this study. First, only relative preference and direct consumption were analysed as a principal measure. Assessing the pattern of consumption of animals gives an indirect assessment of hedonics, and can be inexact being altered by many factors (Berridge, 1996; Riordan & Dwyer, 2019). To assess hedonic reactions, more precise measures should be employed such as analysing the pattern of hedonic reactions or licking microstructure analysis. In this sense, we have assumed that the response that supports the preference is hedonic, as this is the one that has been suggested in the literature. But it is conceivable that the effect found in the present study may be governed by other types of responses that can be elicited by the US.

CHAPTER VII:

Food cue exposure in Sensory-Specific Satiety

Obesity has become a very worryingly problem in the last decades. One of the reasons that can explain the increasing rates of obesity is the omnipresence of the obesogenic environments, which are featured by a huge variety of highly caloric foods, sedentary lifestyles or constant exposure to cues associated with food. These food-associated cues have been shown to increase the total intake of the cued foods even when individuals are in a general state of satiety. This phenomenon has been called Cue Potentiated Feeding (CPF) and has been demonstrated in both humans and rodents. On the other hand, Sensory-Specific Satiety (SSS) is the phenomenon by which sensory properties of a specific food are devaluated by the time this food is eaten. Hence, CPF and SSS seem to act in an opposite manner. Accordingly, on the present experiment we tested in a sample of humans whether the presence of a cue (food advertisements) associated with a specific snack (biscuits or crisps) could abolish or weaken the effect of SSS. SSS effect was measured by means of total consumption and subjective measures of wanting and liking for pre-fed and non pre-fed snacks.

Introduction

The pervasiveness of obesogenic environments has been the subject of much research on eating behavior, given the dramatic increase in obesity rates in the world's population (Blüher, 2019; Swinburn, et al., 1999). Among the characteristics of these environments is the massive exposure to food-related cues, such as junk food advertisements, logos and contexts related to feeding (Jansen et al., 2016; van den Akker et al., 2018). These cues are of particular concern because they are often associated with highly palatable and caloric foods. Palatability is a highly rewarding property that can intensify the effects of Pavlovian conditioning and as much literature has pointed out promote hedonic hunger, (eating without hunger) as opposed to homeostatic one (Leigh et al., 2018). Moreover, these cues have been shown to affect multiple intake-related responses, ranging from motivation to eat, attentional capture, cephalic responses and meal initiation (for reviews: Jansen et al., 2016; Johnson, 2013).

As a result, the omnipresence of food cues in current environment, by being associated with the sensory, hedonic or motivational properties of food, has been suggested as a potential mechanism for overeating. Therefore, many researchers have developed therapeutic and intervention strategies derived from the associative principles of learning to help understand why many diet-based interventions fail in their long-term outcomes (Boutelle & Bouton, 2015; Bouton, 2011; Jansen et al., 2016; van den Akker et al., 2018). These conditioned food cues have been shown to stimulate intake even when individuals are in a general state of satiety. From an associative learning perspective, this phenomenon has been called Cue-Potentiated Feeding (CPF) and has been demonstrated in both rodents (e.g., Boggiano et al., 2009; Galarce et al., 2007; Petrovich et al., 2007; Kendig et al., 2018; Kendig et al., 2016; Reppucci & Petrovich, 2012; Weingarten, 1983;1984) and humans (Birch et al., 1989; Cornell et al., 1989; Emond et al., 2016; Halford et al., 2004). This effect has been demonstrated with discrete cues (e.g., Weingarten, 1984; Repucci & Petrovich, 2012) such as lights or tones but also using physical contexts (e.g., Boggiano et al., 2009; Kendig et al., 2018).

In other paradigms different than CPF, food cues have been shown to produce both a specific effect towards the food reward they signal and a general effect towards other stimuli of similar motivational significance. This is the case of the Pavlovian to Instrumental Transfer (PIT), in which a phase of Pavlovian learning takes place, followed by an instrumental and finally a transfer phase (phases 1 and 2 can be in reverse order). In the transfer phase, the individual's instrumental responses to the presence of different Pavlovian cues are measured. In this paradigm it is observed that cues related to a reward, including food, can increase instrumental responses to the specific food that the cue signals (specific transfer) or to other foods with similar valence (general transfer) when the cued food is not available (e.g., Cartoni et al., 2016). One way of dissociating the two components of the cues has been by manipulating the motivational state of the subjects; the general transfer seems to disappear when individuals are in a state of general satiety, while the specific transfer remains intact (Corbit et al., 2007; Watson et al., 2014).

Conversely, one of the CPF´s characteristics is its specificity, referring to the increase in intake specific to the food that is associated to the cue when animals are under a non-deprived state. Most research has shown that cues, when animals are satiated, act exclusively for the targeted foods and not for others, even when their motivational value is similar. In this respect, there are only two experiments that have shown a general effect of food cues and both were conducted in animal models. Firstly, Boggiano et al., (2009) which paired a context other than homecage with access to a palatable food (oreos) but also had access to water and chow. Subsequently, it was observed that when in test rats were exposed to the “palatable context” increased total intake of chow in comparison to the homecage in a 4-hour period. However, one can not clearly speak of a general appetitive effect in this case because the chow, although familiar and less palatable, was also present during the training sessions. Other evidence comes from Kendig et al. (2018) also using again rats as experimental subjects. It should be noted that on this experiment, the procedure was different to that typically used on CPF´s experiments. On Kendig et al., (2018) experiments studied the influence of pairing multiple rewarding food stimuli to a context instead of a single food. They found that the specificity of the CPF could be overcome when multiple foods are paired with the contextual cue, promoting intake of other different palatable foods (novel or familiar) that had not been paired with the context. Thus, regarding the specific nature of the CPF only these two exceptions have been found, evidencing that food cues when sated induce a specific appetite rather than a general hunger state (Petrovich, 2013).

As mentioned, CPF is characterized by an increase in intake after cue presentation that is not sensitive to a general state of satiety. However, this satiation state has been manipulated in a general manner, usually by giving rats ad libitum chow pellets prior to the test. However, the effect of CPF in other different satiety processes such as Sensory-Specific Satiety (SSS) process has not been clearly elucidated. SSS is a specific satiation process by which as we consume foods, the value of sensory properties of foods is decreased (Rolls, Rolls et al., 1981). SSS has a specific nature because affects only those foods that had been eaten or other which share similar sensory properties, leaving intact other non-eaten foods (e.g., Gonzalez et al., 2018; Griffioen-Roose et al., 2010). As CPF has a specific effect on consumption, the interaction of both processes which act in an antagonistic manner would be an interesting phenomenon of study.

Research carried out by Kendig et al., (2016) assessed the effect of SSS on CPF in the pattern of instrumental responses and consumption in rats. Authors found that the CPF altered the pattern of SSS expression on instrumental responses but not in total consumption. Nevertheless, as the focus of this experiment was the instrumental performance in order to distinguish between habitual and goal-directed behavior (habitual or goal directed) the experimental procedure was quite different as such used usually in human´s SSS paradigms. Authors pre-fed the rats ad libitum during a one-hour interval. This procedure was followed by an extinction test in which the rats could press two levers each associated to the devalued or non-devalued outcome. After the extinction test a 10-minute consumption test of the pre-fed and non pre-fed outcome was carried out. As mentioned above, SSS procedures do not usually overfeed experimental subjects to the point that they become stuffy. Otherwise, it is a sensory devaluation, which according to the SSS definition, should not require an exaggerated intake-amount, as other metabolic or post-ingestive processes such as alliesthesia could come into play.

In this sense, from the associative framework, the influence of Pavlovian cues on individuals' instrumental response has been often investigated using the PIT paradigms or in free operant procedures where the context is the cue (e.g., Cartoni et al., 2016; Holmes et al., 2010; Thrailkill et al., 2018), and often SSS has been used as a method of reinforcer devaluation. So, the effects of these cues on eating behavior under this approach have not been the real focus of the study (but see Colagiuri & Lovibond, 2015) and consumption is measured as a method of ascertaining whether devaluation has been effective (e.g., Corbit & Balleine, 2005; Kendig et al., 2018; Watson et al., 2014). For example, Watson et al. (2014) found that in a sample of human participants a set of conditioned food cues, which had been learned in the experiment itself, subsequently abolished the effect of SSS on instrumental responses. Participants continued to perform instrumental responses to the pre-fed food when exposed to its specific cue. However, in this case, participants did not ultimately perform a consumption choice test between the pre-fed and non pre-fed food.

Therefore, the aim of the present experiment was to assess the effect of food cues on the pattern of expression on SSS. To study this hypothesis, we investigated whether presenting food cues to a sample of human subjects could alter the normal pattern of intake and related subjective measures (Liking and Wanting) after a devaluation procedure of SSS. For this purpose, we tested in a sample of humans whether two food cues associated with two different kind of snacks each prompted an automatic intake of the signaled food that had previously been sensory specific satiated. To assess this hypothesis, we used advertisements as food related stimuli due to the similarity that they share with the natural cue exposure in current obesogenic environments. Previous studies have shown that food advertisements can trigger an increase in total intake of the highly palatable food they signal (e.g., Halford et al., 2004; Harris, et al., 2009; Russell et al., 2019). Thus, we expected that the food cue-exposure could override the SSS effect which acts as regulatory system of short-term eating inhibition. So, we expect that intakes and ratings of the pre-fed foods would be higher when a specific food cue had been presented previously.

Experiment 1: Cue-Potentiated feeding in Sensory-Specific Satiety

Methods

Experimental subjects

A total of 20 students of the University of Granada (11 women and 9 men) were recruited to do the experiment. Participants had an average age of 24.8 years and a body mass index of 21.9. All the participants signed an informed consent at the beginning of the experiment and were rewarded with academic credits at the end. Exclusion criteria for the experiment were: suffering eating disorders, having any diet restriction or any allergy to the food stimuli used on the present experiment. Participants were not allowed to eat or drink anything other than water in a period two hours period before the experiment. They were also not allowed to engage in high performance physical activities before the beginning of the experiment. One of the control participants was excluded since was classified as outlier due to the lack of consumption during the consumption test.

Materials

The snacks provided were 52 gr of Mini Chips-Ahoy cookies (504 kcal per 100gr) and 42 gr of Lays crisps (522 kcal per 100 gr). This difference in quantity was necessary to offset the volume occupied by both foods on the bowl. All the snacks were weighted before and after each presentation with an ordinary food scale. A glass of spring water was given to participants during all the phases in which snacks were offered, and was filled whenever it was finished.

A brief initial questionnaire about dietary habits and possible diet-related pathologies was used. Also, two different kind of rating scales were used in the present experiment, both were 100 mm Visual Analogue Scales (VAS) (0 = Nothing; 100 = Much). One of them was a general scale to assess the current general motivation for eating sweet or savory stimuli and hunger with questions like “how hungry are you?” and was only presented at the beginning of the experiment. The other scale was presented three times across the experiment and was specific for the two target food snacks of the experiment, this scale was designed for assessing both hedonic value (Liking) and desire to eat a particular stimulus (Wanting). Questions in the scale were as follows: “How much do you like this food”? or measures of Wanting “How much do you want to eat this food?”.

Finally, we used 5 different videos during the whole procedure for two different purposes. One of the videos was presented during the pre-feeding and consumption test phase in order to avoid any artifacts that could affect the experimental procedure because of the awkward situation of eating in a laboratory. This way, the procedure could be more ecological and comfortable for participants. This video was a short Spanish fiction film (14 minutes video), and was displayed until minute 8 during the pre-feeding phase, the rest of the video was visualized during the consumption test. The other four videos were advertisements of cookies or crisps (Experimental Group) and shampoo or detergent (Control condition). These advertisements were presented during the Cue Presentation phase and lasted 1:20 minutes. All videos were displayed with a computer and headphones. The procedure was approved by the Comité de Ética de la Universidad de Granada 694/CEIH/2018 (Ethics Committee of the University of Granada).

Procedure and design

All the sessions were carried out between 12:00 and 14:00 and lasted 25 minutes each. Upon arrival, participants had to sign the inform consent form and were randomly assigned to a group (experimental/control) and a snack condition (savory/sweet). Immediately after, they had to fill the questionnaire about food habits and a scale about their current motivational state and appetite for sweet or salad food (general Scale). Then, participants had to eat a bite size of a cookie and crisps and fill a Liking and Wanting scale for each snack as a base line measure (specific Scale). The order in which participants rated and ate both foods was counterbalanced.

After that, participants started the pre-feeding phase, in which were offered a bowl containing cookies or fried chips, a glass of water and were displayed a short film. Participants were told that they were going to watch a short film and they could eat everything they wanted until it the video finished. On this stage, the short film was watched until minute 8, so that the short film was not totally displayed. Once that the video stopped, participants had to rate for a second time Liking and Wanting scales for both snacks.

Until now, both Control and Experimental groups had undergone the same procedure, however, the next phase was the one that makes the difference between the two groups. On the Cue Presentation phase, the Experimental group was displayed a series of advertisements of the food that they had eaten (cookies or crisps) whereas the Control Group was displayed a non-food related advertisement (shampoo or detergent). When the videos were finished, both groups had to rate again both snacks with the Wanting and Liking scales.

Finally, all participants were told that the experiment was finished but as a compensation, they could see the end of the short film and were offered two different bowls containing the pre-fed or the non pre-fed food. Participants were told that they could eat whatever they wanted until the video finished and then, the experimental procedure was finished (See Table 1).

Table 1. *Experimental design*

Group	Pre-Feeding	Cue Presentation	Consumption Test
Food-Cue	Cookies or Fried chips	Advertisement A or B (Food)	Pre vs Non-Pre
Neutral-Cue		Advertisement C or D (Neutral)	
	GSR t1	SR t2	SR t3

Note: “t” denotes the different assessment of the specific and general ratings across the experimental procedure. “GSR” denotes rating of specific and general scales and “SR” means specific ratings.

Analysis of the data

General linear model null hypothesis testing analyses were conducted, adopting a rejection level of $p < 0.05$. Partial eta squared and Cohen's d tests were used to measure effect sizes. Data from the rating scales was analyzed separating Wanting and Liking scales for each snack since both scales measure two different processes. Both scales were analyzed with a repeated measures ANOVA which compared ratings across the three times. Analysis for the consumption test was were analyzed by comparing a total intake of pre-fed vs non pre-fed food with a repeated measures ANOVA.

Results

Initial general ratings

A One-way ANOVA was carried out for assessing initial hunger ratings hunger in both groups. These results revealed that there were not significant differences in their initial motivational state $F<1$. Also, a repeated measures ANOVA was carried out to assess initial ratings of appetite for sweet and savory foods. Taste (savory and sweet) was used as within-subject measure and Group as between subject's variable. Results showed significant differences for the factor Taste $F(1,17)= 18.67$, $p< 0.001$, $\eta^2_p= 0.52$ but neither the interaction Taste*Group nor the factor Group was significant $Fs<1$.

Differences in the factor Taste revealed an overall higher average score for savory snacks over the sweet ones (Savory: M=68.57, SE= 4.64; Sweet: M=42.63, SE= 5.41). A one sample t-test showed that only the savory´s scores differed to the value 50 ($t(18)= 4.00$, $p< 0.001$ d=3.38), thus, participants not showing initial appetite for the sweet foods ($t(18)= -1.36$, p=0.19 d=0.18).

Pre-Feeding Phase

A 2x2 ANOVA was carried out with Group (Experimental vs Control) and Flavor (Sweet vs Savory) as between-subjects. No significant effects were found in Group $F<1$, Flavor $F(1,15)= 1.72$, $p= 0.21$ $\eta^2_p= 0.10$, or the interaction between them $F<1$ (Experimental savory M= 24,75, SE= 4.01, Experimental sweet M= 31.16, SE= 5.82; Control savory M= 22.87, SE= 2.27, Control sweet M= 27.00, SE= 6.08).

Wanting and Liking ratings

Data from the Liking scales was submitted to a repeated measures ANOVA with Pre-feding (Pre-fed food vs non Pre-fed snack), Time (Base line, post-intake and post-cue) as within-subject measures and Group (Experimental vs Control) as between subject measure. An effect of Pre-feeding*Time will be expected if participants express a SSS effect (being lower only in the pre-fed condition those scores in the t2 and t3 time point compared to t1). According to our main hypothesis, we expect a triple interaction of Pre-feeding*Time*Group reflecting a different pattern of expression of SSS for both groups after the cue exposure (t3). We expect that those in the Experimental Group will not express significant differences from the t1 and the t3 time point (cue exposure effect) but significant differences between the t1 and the t2 time point (SSS effect).

Results showed that there was no significant effect of Pre-feeding $F(1,34)= 1.54$, $p=0.23$, $\eta^2_p = 0{,}08$, Time $F<1$ or Group $F(1,17)= 1.17$, $p=0.29$, $\eta^2_p = 0.06$. Surprisingly, neither of the interactions between the main factors were significative; Pre-feeding*Group $F<1$, Time*Group $F(2,34)= 1.04$, $p=0.25$, $\eta^2_p = 0.07$, Pre-feeding*Time $F(2,34)= 1.81$, $p=0.17$, $\eta^2_p = 0.09$, Pre-feeding*Time*Group $F(2,34)= 1.23$, $p=0.30$, $\eta^2_p = 0.06$. Thus, on the Liking scales we weren´t able to find an effect of SSS (See Figure 1).

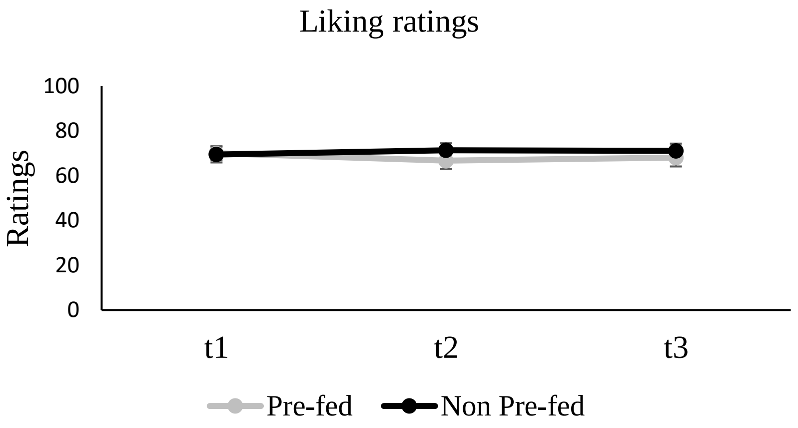


Figure 1. Participants´ Liking ratings - groups collapsed.

For the Wanting scale, a repeated measures ANOVA was carried out with Pre-feding (Pre-fed food vs non Pre-fed food) and Time (Base line, post-intake and post-cue) as within-subject measures and finally Group (Experimental vs Control) and Flavor (sweet vs savory) as between subject measure. As in the initial rating scales participants showed a general reduced appetite (Wanting) for sweet foods in contrast to savory ones, we decided to introduce this factor in the analysis. We added this factor to ensure that there were no differences in the pattern of SSS expression as a matter of flavor or as a matter of group and flavor.

Results revealed no significant differences for Pre-feeding $F(1,15)= 2.95$, $p=0.23$, $\eta^2_p = 0.16$, Flavor or Group $Fs<1$. Otherwise, the main factor Time was significant $F(1,30)= 8.18$, $p=0.001$, $\eta^2_p = 0.35$ Regarding to the interactions only the interaction Pre-feeding*Flavor $F(1,15)= 8.85$, $p=0.009$, $\eta^2_p = 0.37$ and Pre-feeding*Time $F(1,30)= 9.44$, $p<0.001$, $\eta^2_p = 0.38$ were significant. The rest of the interactions were not significant Pre-feeding*Group, Pre-feeding*Time*Group, Pre-feeding*Time*Flavor $Fs<1$, Time*Group $F(1,30)= 3.10$, $p= 0.06$, $\eta^2_p = 0.13$, Time*Flavor $F(1,30)= 1.07$, $p= 0.35$, $\eta^2_p = 0.06$, Group*Flavor $F(1,15)= 3.03$, $p=0.10$, $\eta^2_p = 0.16$, Pre-feeding*Group*Flavor, $F(1,15)= 2.40$, $p= 0.14$, $\eta^2_p = 0.13$, Pre-feeding*Time*Group*Flavor $F(1,30)= 1.59$, $p=0.22$, $\eta^2_p = 0.09$.

Planned comparisons were also carried out to study the Pre-Feeding*Flavor interaction with a paired samples t test. The results showed that in the sweet pre-fed condition, the scores of the pre-fed (cookies) and non pre-fed (crisps) snack were significantly different $t(8)=-9.20$ $p< 0.001$ $d=2.91$ while this difference was not significant in the savory pre-fed snack condition $t(8)=-1.27$ $p=0.23$, $d=0.42$. As Figure 2 shows, this interaction may show that compared to the savory pre-feeding condition, in the sweet pre-feeding condition scores for the non pre-fed snack (crisps) were higher but constant throughout the procedure (Non pre-fed scores in the sweet pre-fed condition: $M= 71.20$ $SE= 4.32$; Non pre-fed scores in the savory pre-fed condition: $M= 55.185$, SE=7.84). This higher score leads to differences in the sweet condition between the total pre-fed and non-pre-fed snack scores. However, this does not affect the effect of pre-feeding, as for both conditions the pre-fed snack scores decrease as a consequence of satiation (see Figure 2- panel A and B).

According to our prior hypothesis planned comparison were carried out to analyze the interaction Time*Pre-feeding. A repeated measures ANOVA with Time as within-subject measure (t1, t2, t3) was done for the pre-fed and non pre-fed snack independently. Analysis revealed that there were not significant differences in the factor Time for non pre-fed snacks $F>1$. Regarding to the Pre-fed snacks, Greenhouse-Geisser corrections had to be applied, these corrections showed a significant effect of Time $F(1.31, 23.50)= 10.50$, $p= 0.002$ $\eta^2_p=0.16$. A simple main effects analysis was performed with a paired samples t test, comparing the different time points (t1, t2, t3) in the pre-fed condition. This analysis showed that there were significant differences between t1 and t2 $t(18)=3.19$, $p= 0.005$ $d=7.33$, and between t1 and t3 $t(18)=3.64$ $p=0.002$ $d=0.83$, but not between t2 and t3, $t(18)=0.25$, $p=0.81$, $d=0.05$ (See Figure 2- panel C). Hence, for the Wanting ratings we found an effect of SSS by specifically decreasing the motivation to eat the consumed snack after Pre-feeding. However, in contrast to our prior hypothesis, this effect was not different across groups.

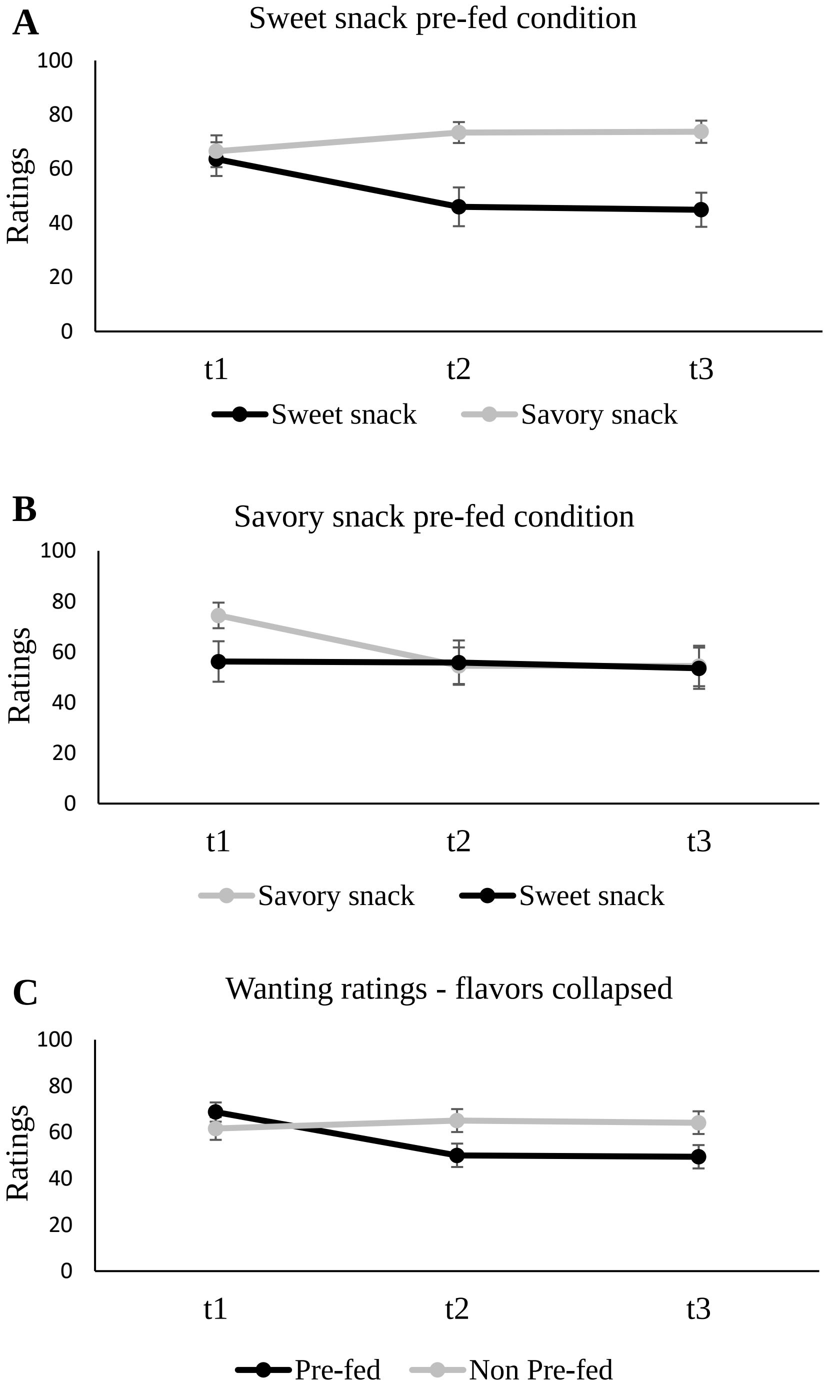


Figure 2. Wanting ratings- groups collapsed. Panel A: shows Wanting ratings in the sweet pre-fed condition. Panel B: depicts Wanting ratings in the savory pre-fed condition. Panel C: depicts general Wanting ratings - flavors collapsed.

Consumption test

A 2x2x2 repeated measures ANOVA was carried out with Pre-Feeding (Pre-fed food vs non Pre-fed food) as within-subject measure and Flavor (Sweet vs Savory) and Group (Experimental vs Control) as between-subject measure. Results showed that there were significant differences for Pre-feeding *F*(1,15)= 58.29, *p*=0.001, η^2_p= 0.79, Pre-feeding*Group *F*(1,15)=5.21, *p*=0.03, η^2_p= 0.25, and Pre-feeding*Flavor*Group *F*(1,15)= 5.92, *p*= 0.02, η^2_p= 0.28. There were no significant differences for the Pre-feeding*Flavor interaction *F*>1 and no significant effects in Group *F*(1,15)= 1.31 *p*=0.27, η^2_p=0.08, Flavor *F*(1,15)= 2.55, *p*=0.13, η^2_p=0.14 or Flavor*Group *F*>1.

Planned comparison were carried out to study the significant triple interaction. A paired samples t test was performed for each pre-fed and no pre-fed food condition in each group. Analysis of comparisons of pre-fed and not pre-fed foods by Flavor and Group showed that for the Experimental Group there were not significant differences in pre-fed and not pre-fed crisps condition *t*(4) = -1.11, *p*=0.33, *d*=-0.50 but significant differences were found for pre-fed and not pre-fed cookies condition *t*(4)= -4.17, *p*= 0.01, *d*=-1.87. For the Control Group, significant differences were found for both the Pre-fed and not Pre-fed crisps condition *t*(3)= -5,09, *p*=0,01 *d*= -2,55 and for pre-fed and not pre-fed cookies condition *t*(4)= -5,96, *p*= 0,004 *d*= -2,67 (See Figure 2).

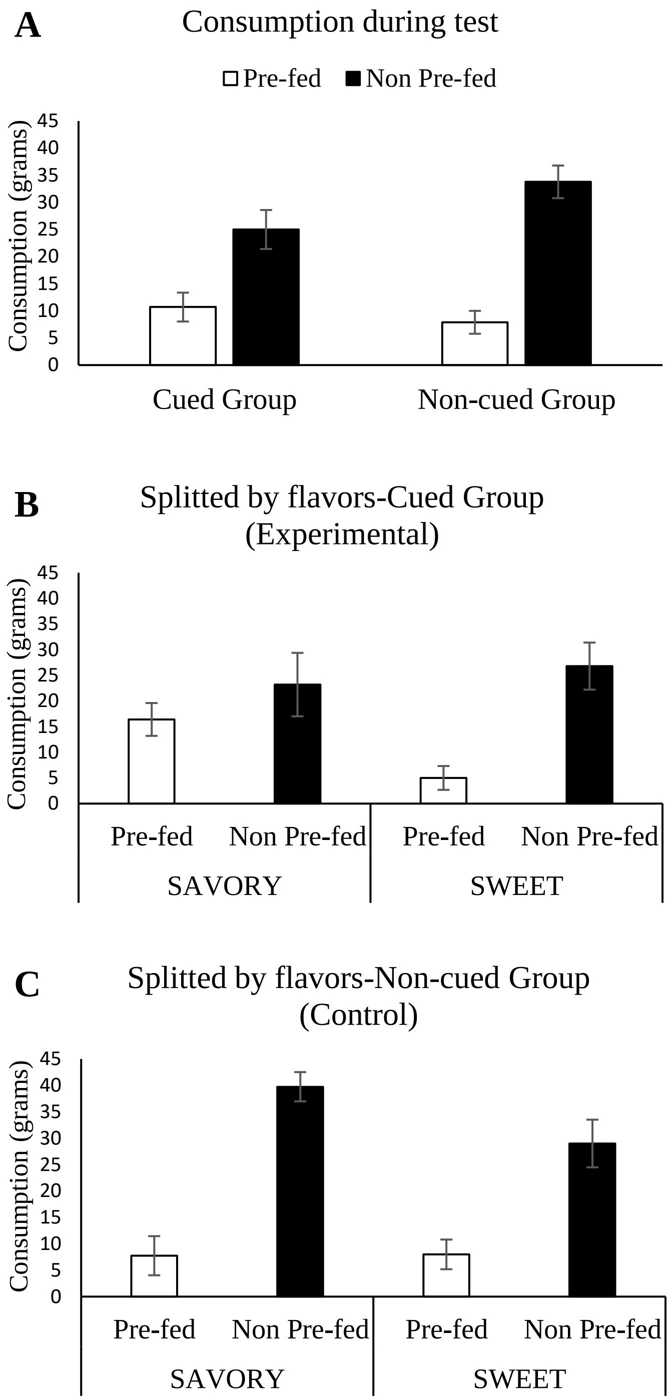


Figure 2. Total Consumption of participants during test. Panel A: represents results of both groups for the pre-fed and non pre-fed snack. Panel B: represents results of the Cued group (Experimental group) of both pre-fed and non pre-fed snacks and splitted by flavors. Panel C:

represents results of the Non-cued group (Control group) of both pre-fed and non pre-fed snacks and splitted by flavors.

Discussion

The main aim of the present experiment was to assess the interaction between a daily life food cue such as food commercials and an eating regulatory mechanism: SSS. Beyond this idea, we wanted to test whether the sight of a cue related to a specific food that had been previously devalued by a SSS procedure, could prompt increased later consumption, Liking and Wanting ratings for the ingested food.

Regarding to Liking Ratings results didn´t show any effect of SSS in both groups. The fact that participants rated the same value for pre-fed outcomes over the whole procedure could be explained as a matter of subjective measurements (Koranyi et al., 2020; Grigutsch et al., 2019). Multiple problems with subjective measures have been reported in the literature, such as the influence of participants' own monitoring throughout the study. For example, in everyday life Liking is conceived as a stable dimension; when we think of the food we like, we do not usually conceive that this property can be modified in a short period of time. Otherwise, the Liking and Wanting dimensions may be expressed in an implicit way, which would mean that subjective measures are not completely reliable, with other measures such as instrumental responses or the measurement of orofacial responses being more sensitive assessments. On the other hand, another aspect that may have interfered with the expression of SSS in the Liking scales is the fact that in the first assessment of the scale participants were allowed to taste a bite size of the target foods. In the following ratings, this procedure was not carried out in order not to affect the final consumption test. It is possible that this may have affected the sharpness of the snacks assessments in the different time points; being the rating in the first time point a direct experience and in the next two a recalling memory experience.

This lack of effect in relation to the subjective Liking scales was not observed for the Wanting assessment. When participants had to rate the Wanting scale did show an effect of SSS reflecting that both groups rated less motivation to eat the pre-fed outcome after the pre-feeding phase. This pattern of results did not occur for the non pre-fed snacks. The SSS effect could be explained in this case by the fact that unlike the Liking dimension, participants might perceive food desires as a time-varying dimension. While our likes and dislikes are conceived as static, although actually they are not, the willingness or desire to consume foods is likely to be perceived as fluctuating aspect of eating behavior. For example, a recurring question in our daily lives is “Do you want to eat X?” as opposed to “What is your favorite food?”. This common conceptualization may have favored less monitoring along the different time points of the task in which participants had to do the ratings. Finally, although SSS was only expressed in the Wanting scales, no interaction with cue-presentation was observed; both groups equally expressed a decrease in the subjective desire to consumed the previously eaten food.

Regarding consumption measures we observed an effect of SSS effect by participants consuming less amounts in total of the pre-fed snacks in contrast to the non pre-fed ones. Furthermore, results revealed a weaker SSS expression for the participants that were cued with the eaten food (Experimental Group) in comparison to the group that was exposed to a non-food advertisement (Control Group). The full analysis showed that these differences were only reflected in one of the two foods eaten. While in the case of pre-feeding with the sweet snack, both groups expressed the effect of SSS on the test by consuming less cookies than crisps, we did not find the same pattern of results for the crips-fed condition. When crisps were pre-fed, the cued group did not express the effect of SSS, eating in the consumption test comparable amounts to cookies.

We suggest that two main factors may have influenced this pattern of results. Firstly, the experiment was conducted in the 12:00 pm to 2:00 pm time slot, which in Spain is mostly associated with lunch consumption, that generally tends to be savory. In fact, initial taste appetite ratings reflected that both groups scored the savory foods higher than sweet ones, being sweet scores lower than 50. So, by not having initial appetite for sweet foods, it is possible that in this case, the effect of the food cues could not modulate the expression of SSS. Therefore, the temporal context also could have played an appetitive role in favoring the consumption of savory snacks rather than sweet ones. Furthermore, in contrast to rodents and infant humans, among adult humans there is a great variability in sweet taste preference (Yeomans, 2012). It is plausible that strong initial preferences for the snacks offered in the experiment may narrow the strength with which food cues act; if the cued food is not perceived as palatable or appetizing, the effect of Pavlovian cues will be null. Therefore, future studies should consider subjects' initial taste preference to control which target food is presented during the pre-feeding phase.

Another potential phenomenon that might have affected the different expression of SSS in the different snacks for both groups is conditioned satiety (Booth, 1972; Martin, 2016). Conditioned satiety is an inhibitory mechanism of intake that acts on the basis of a prior association between a sensory cue of a particular food and the caloric load that owns the latter. These associations are formed through Pavlovian conditioning as organoleptic properties of the food such as aroma, taste or texture are experienced with the energy content of foods through ingestion. Thus, in future encounters with the food, individuals regulate the total amount ingested during a meal, inhibiting excessive consumption to avoid subsequent gastric malaise. Conditioned satiety is not specific, but affects other foods if a high-energy meal has been ingested (Swithers & Davidson, 2008). In our experiment we used savory and sweet snacks. Sweet taste is often linked to sugary and unhealthy high caloric foods, and therefore, it is possible that participants perceived the sweet snack as more caloric than the savory one. This may have caused participants to adjust their intake in the final test to compensate for the energy intake consumed. In this line, during the choice test of our experiment, those participants in the Experimental group who had been pre-fed with the sweet snack, may have inhibited further consumption of sweet snacks anticipating the caloric boost, and thus, interfering with the effect of food cues. Actually, a recent study has shown that adding nutritional labels to foods do not affect the expression of SSS but modulate the total consumption during the pre-feeding meal; eating less in total those who received high-filling labels on the target food, and the opposite pattern found in the light-label condition (Hendriks-Hartensveld et al., 2022).

The study of the effects of conditioned cues on intake is of great relevance, as they promote overconsumption even when individuals are in a state of satiety. In this sense, cues promote the homeostatic machinery to be overcome by the hedonic and motivational aspects of food, and thus, promote eating in the absence of hunger (hedonic hunger). As a consequence, food cue reactivity has been identified as a model for studying obesity and overeating. Cue reactivity refers to how the presence of food cues can trigger a range of food-related consummatory responses. These responses, as noted in the introduction, can affect many levels, from physiological (e.g: salivation or hormone segregation) to cognitive (e.g., attentional patterns) or behavioral (e.g., instrumental responses or intake). Although food cue reactivity is a normal response, it has been shown to be expressed differently in overweight or obese people compared to healthy ones, exhibiting stronger reactivity responses in the former (e.g.; Epstein et al., 1996; Ferriday & Brunstrom, 2011; Jansen et al., 2003, For reviews: Boswell & Kober, 2016; Hendrikse et al., 2015; Jansen et al., 2016; van den Akker et al., 2018).

From a theoretical approach, it has been proposed that when a Pavlovian cue (CS) becomes associated with a palatable food (US), a link is established between the representations of both (S-S) that prompts consumption via two mechanisms (Marshall et al., 2018). On the first glance, it has been proposed that such cues, when present, can activate a central motivational process that is responsible for invigorating food-seeking or craving behaviors. This motivational approach is based on the observations from the PIT studies in which it is demonstrated how cues can lead to instrumental behaviors for achieving the cued food or others similar (for a review: Cartoni, 2016). On the other hand, the hedonic approach suggests that food cues can acquire the hedonic properties of the food they signal or even potentiate them when present (Johnson, 2013, but see Marshall et al., 2018). This perspective comes from the studies that have shown that food cues are capable of evoking hedonic responses in the absence of the target food (e.g., Holland et al., 2008; Kerfoot et al., 2007). Both approaches, that remind to the Liking (hedonic) / Wanting (motivation) dimensions of reward proposed by Robinson & Berridge (1993), are not incompatible and may reflect different properties of the CPF (Johnson, 2013; Marshall et al., 2018). However, according to the Incentive Sensitization Theory, the main component that would ultimately lead to overeating would be that related to motivation (Morales & Berridge, 2020).

From a therapeutic point of view, it has been proposed that as long as all these food cues remain ubiquitously in our environment, therapies based on strict diets and physical exercise will tend to fail. Since individuals are continuously exposed to these Pavlovian cues, they will trigger an excessive cascade of appetitive responses that will ultimately lead to overeating. In this sense, under this S-S approach, the Exposure-Based Therapy has been proposed as an additional therapeutic tool, according to which patients should not only modify their eating habits, but also be exposed to the food related cues without acquiring the expected reinforcing food. Thus, just as reactivity to cues is a learned response based on repeated pairing of the CS with the US, exposure to the CS without the US on multiple occasions should reduce reactivity responses through the extinction process. In fact, according to this hypothesis, there is evidence that successful dieters show similar patterns of reactivity to cues as healthy ones (Jansen et al., 2010).

Hence, Exposure-Based Therapy seems to be an interesting therapeutic alternative to confront the current "toxic" environments. And indeed, there are some promising experiments on the matter (van den Akker, Havermans et al., 2014; van den Akker et al., 2015). However, some studies that have focused on its long-term results have produced mixed evidence (see van den Akker et al., 2018 for a review). It has been proposed that this lack of long-term effectiveness may be due to the very characteristics of the extinction phenomenon and its implementation in obesogenic environments (e.g., Bouton, 2011).

In this sense, the generalization of learning is very plastic, occurring very quickly between multiple contexts or stimuli. Otherwise, extinction is much rigid and context dependent (e.g., Bouton, 2004; Steinfield & Bouton, 2021). In fact, it has been suggested that extinction does not erase the initial learning (unlearning process) but rather that new learning takes place (Bouton, 2004). This new association, as we have mentioned, depends on the context where it has taken place, and therefore, is subject to renewal. So, one may have extinguished the relationship of McDonalds´golden arches and the availability of rewarding food but this association can be preserved for another fast-food chain cue (e.g., Burguer King). Therefore, given that today´s societies offer multiple opportunities to associate external cues with highly palatable food allowing this learning to be fruitfully overgeneralized, the Exposure-Based Therapy seems unlikely to be an effective strategy. In this respect, it is of particular concern the massive omnipresence of similar appetitive contexts (especially in the case of fast-food chains, characterised by a uniform appearance), and thus allowing learning to be easily generalized (Petrovich et al., 2007). Similarly, other properties of extinction that could hinder the effectiveness of Exposure-Based Therapy are reinstatement and the rapid reacquisition. Reinstatement means that if after the CS-US extinction process the US is offered again (e.g., a palatable food like pizzas), it is possible that the extinguished response will be expressed again upon future presentations of the Pavlovian cue. This feature also constitutes a real obstacle in addressing food cue reactivity given the accessibility and overexposure of highly hedonic food products. Finally, other crucial property would be the rapid reacquisition; quickly relearning the extinguished association when it is experienced again (CS-US presentations after extinction). Thus, for those patients who have achieved therapeutic success, exceptional laxity in their intake habits may be risky, as it may reinforce the previously learned association that has now been extinguished. Consequently, from this perspective, obesogenic environments would not only promote overconsumption through exposure to intake-related cues, but also difficult the implementation of behavioral therapeutic strategies (e.g,, Bouton, 2011; van den Akker et al., 2018).

On the other hand, another possibility is that the content of the association formed between these cues present in our environment and the food is not formed on the basis of the US-representation (S-S) but on a response that the US produces (S-R association) (Marshall et al., 2018). Such associations are insensitive to devaluation procedures such as the SSS, since occur without processing the US. Thus, individuals when faced with food cues would elicit automatic appetitive responses regardless of the motivational state or value of the food at the time. Furthermore, it is believed that these associations arise through extensive training following the Thorndike´s Law of Effect (e.g., Dickinson, 1985). In this way, learned content changes from being controlled and mediated by the expectancy of obtaining a particular reward to being automatically driven when a predictive stimulus is present (Thrailkill & Bouton, 2015).

In the present experiment, we found that the group pre-exposed to the food advertisement (CS+) expressed a lower SSS effect than the group that had been cued with non-food advertisements (CS-). In addition, food advertisements are food cues that are commonly present in our daily lives and thus, have a long history of prior learning. So, based on our results, it seems that the potentiating effect of Pavlovian cues could be operating through an automatic mechanism characteristic of S-R associations. In this sense, S-R associations by not being sustained by the activation of the US in memory, repeatedly presenting the CS without the US would not alter the S-R connection. Therefore, such associations are believed to be persistent, thus being resistant to extinction. Unlike S-S associations, which are much more flexible and generalizable across contexts, S-R associations would be much more rigid and context-dependent (e.g., Steinfeld & Bouton, 2021; Thrailkill & Bouton, 2015). So, more pessimistically, Exposure-Based Therapy would not be an effective alternative, as extinction may not alter S-R learning. In this regard, given that the obesogenic environment offers multiple opportunities to learn from these cues, whether discrete or contextual, to overcome this learning would require a global change in today´s societies.

To sum up, the results of this research suggest that everyday food cues, which, compared to those learned in a laboratory environment, have a long history of learning with multiple pairings, could undermine a regulatory mechanism of intake such as the SSS. However, there are a number of important caveats that should be noted. First of all, the sample size used. This research was conducted in a pre-pandemic context, and, to continue this research after the lock-down was a challenging task due to the hygienic and preventive measures. A study with a larger sample size would be needed to replicate this effect and confirm its validity. Second, the effect of SSS was observed for consumption and Wanting assessments, but was not found for Liking ones. As mentioned, it is possible that the very nature of the subjective rating scales has affected the results obtained. Future studies should make use of different methodologies that, through objective assessment, can detect changes in these reward processing components.

PART III:

GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER VIII

General discussion and conclusions

Discusión general y conclusiones

Summary of the results

The current environments in which we live, given their characteristics, offer multiple opportunities for overeating. Among these characteristics, in the present Doctoral Dissertation we have studied how exposure to sensory variety, food cues or exposure to highly palatable flavours affects the expression of an eating regulatory mechanism: the Sensory-Specific Satiety (SSS).

One of the peculiarities of our food environment is the constant access to a wide variety of different foods. In this respect, research has proposed that having a wide variety of foods during a meal leads to an increase in total intake compared to a more monotonous one, the so-called Buffet Effect. In this context, the effect of variety in intake has been identified as a potentially dangerous factor for overeating and some of the research has sought to uncover the underlying mechanisms by which this effect operates. The major explanation for this effect is the SSS phenomenon. In the literature, SSS has been taken as an example of a case of habituation and therefore one way in which SSS could intervene in the Buffet effect is via dishabituation/distraction effects. When we are exposed to different foods on the same plate or within several courses, the phenomenon of dishabituation and distraction could act by reversing or slowing down the normal hedonic decrement to the eaten food. Most of the research that has addressed this question, mostly carried out with human experimental subjects, has led to results that refute it. Thus, these previous results point out that SSS do not dishabituate, nor do the effect of distractors interrupt its process by causing a slowing of the decay of the hedonic or the motivational response.

However, the majority of this research has focused on studying the pattern of the participants' subjective Liking or Wanting ratings towards the experimental foods. These measures are not always very reliable since are subjected to participant´s monitoring during the tasks. And indeed, research that has used more objective measures such as salivation or instrumental responses, has shown that SSS can be altered when implementing dishabituation/distraction paradigms. To overcome the potential problems arising from human experimentation methodology, we decided to study this hypothesis using rats as experimental subjects and using consumption and relative preference as the main measure of SSS (*Chapter IV*). Thus, although consumption is not a strictly direct measure of the hedonic and motivational components of food, the use of animal models eliminates the top-down modulation to which subjective measures may be constrained, and also allows to study the effect of the distractor/dishabituation paradigm on immediate eating behavior. Our results confirmed the same as those found in humans, the effect of SSS dissipated after time but was not altered by the presentation of a distractor during the pre-feeding phase nor after the exposure to a dishabituator. These results seem to support the view that SSS is not a classic case of short-term habituation, or at least does not completely behave as such when assessed with the measures used here.

In this sense, the experiments in *Chapter IV* do not seem to support the idea that the variety effect is enhanced through the disruption of the hedonic and motivational decrement on the basis of a distractor or dishabituator. However, in the present thesis we also provided another tentative mechanism that can underlie the Buffet Effect along current environments. We argue that previous exposure to similar foods that only vary in some of their sensory attributes causes the Buffet Effect to be amplified (*Chapter V*). The suggested mechanism for this phenomenon is perceptual learning, characterised by increasing discrimination between similar stimuli on the basis mere exposure. So, when very similar foods are consumed frequently, a better discrimination between them will occur and, as a consequence, there will be a decrease in the generalizability of SSS among these similar foods. If the generalization of the

SSS is attenuated, total consumption when we have a similar food supply would be higher since other similar foods will be still perceived as palatable instead of expressing the SSS.

In our experiment, we showed how mere exposure to similar sapid stimuli disrupts the normal pattern of the generalization of SSS. Rats in the pre-exposed group consumed less of the pre-fed solution compared to the non-pre-fed one, whereas those rats that had not been pre-exposed did consume the same amount of both solutions, reflecting a lack of preference between the two. Consequently, we interpret that the non-pre-exposed rats, by not discriminating the two highly similar solutions (differing only in their aroma), generalize the devaluation from the pre-fed to the non-pre-fed solution. In contrast, when the pre-exposed rats are pre-fed with one solution, the other one is perceived as different, the degree of generalization decreases, and therefore, it is still perceived as palatable. Therefore, perceptual learning is proposed as a regulatory mechanism of the SSS effect across the wide food sensory variety of current societies.

Just as food variety is a characteristic of current environments, exposure to cues related to high-calorie and high-palatable foods is also a hallmark of obesogenic environments. In this sense, these cues can be found in the food itself in the form of smells, flavours and textures or in environment like food advertisements, brand logos or contexts. In both cases, sensory cues (CS) become associated with a palatable taste or a nutrient (US) after being paired as a result of Pavlovian learning. On the former case mentioned above, this learning process results in the acquisition of a preference for the flavored CS that persists even if the CS continues to be presented without the US (flavor preference learning). In this way, individuals learn to like the sensory cues present in food, such as smells, tastes or textures, which may ultimately influence meal initiation, food choices or the amount of food consumed in a meal.

A relevant aspect of this learning is the associative structure that is developed through conditioning depending on the type of exposure given to the CS-US compound. So far, in most studies using the preference acquisition paradigm, it has been observed that the stablished association between the CS and the US´s properties during training corresponds to a Stimulus-Stimulus type (S-S). This CS´s preference, being sustained by the US representation (that is, activation of the US on mind or memory), is characterized by being sensitive to devaluation procedures such as SSS or conditioning taste aversion (CTA). That is, when a US such as food has been pre-fed by a SSS procedure and has therefore temporarily lost its value, the CS also expresses this devaluation by reducing its preference. In contrast, Stimulus-Response associations (S-R), unlike S-S associations, are much more rigid and automatic and by not being mediated by the US representation, are not sensitive to devaluation. S-R associations in the instrumental paradigm are believed to emerge through extended training, as a response occurs in a particular context and is followed by a concrete reinforcer repeatedly. In this sense, flavor preference learning has been traditionally studied with short procedures in which animals have limited access to the CS-US compound during a short-limited time period, which according to this notion, limits the chances of S-R development.

So, in *Chapter VI* we studied the effect of the extension and amount of exposure to the CS-US compound during training and by using several USs with different sensory, hedonic and nutritional properties (Sucrose, Saccharin, Maltodextrin, Saccharin + Maltodextrin). Throughout several experiments we observed that when rats were exposed to extended training with unlimited access to the CS-US compound, exhibited an insensitivity to the SSS effect only when sucrose was used as US. This pattern of results suggests that in flavor preference learning it is possible for S-R associations to develop if the learning conditions are favourable. The fact that this phenomenon is only expressed when sucrose is used as a US suggests that the latter may possess unique characteristics that distinguish it from other tastes that function as US.

Finally, in the same way that one can learn about sensory cues that are present in foods, one can also stablish relationships between external cues and foods. Translating it to the environments in which we live, these cues can be contexts related to food, commercials, logos or even the time point of the day. These cues, when present, not only initiate consumption of the food they signal, but also initiate other intake-related responses. The specific increase in consumption produced by exposure to food cues has been observed even when individuals are in a state of general satiety, this effect being called Cue-Potentiated Feeding (CPF).

Although CPF has been clearly shown with motivational states of general satiety, its effect on SSS seems less clear. Food cues have been shown to increase instrumental responses to a food that signal when it has been previously consumed, but their effect on consumption is unclear or appears to be unaffected. However, many of these studies have been carried out with rodents and with pre-feeding procedures where very large quantities are offered to the animals for a long time. In this sense, other processes different than those sensory-specific could be playing a role.

So, in *Chapter VII*, the effect of food cues on the expression of SSS in a sample of human subjects was studied. The results of this study showed a weaker effect of SSS in consumption for the group of participants who viewed the snack advertisement compared to the group who viewed advertisements of non-food related products. This pattern was only found for the experimental subjects pre-fed with the savory snack, while in the category of sweet snacks participants expressed the SSS effect. In relation to the subjective measures, an SSS effect was observed only on the “Wanting” scales; observing a specific worse valuation towards the pre-fed snack after satiation. This effect was found in both groups of participants, so, we did not detect any effect of food cues in subjective measures. Whereas for the “Liking” scales, subjects maintained the same ratings across the different time points of the experiment in both the pre-fed and non pre-fed snacks, reflecting a lack of SSS effect in this scale.

Implications of the results and future research

Variety effect and overeating: Habituation and Perceptual learning

While for our ancestors or for most of the omnivore animals in their natural habitat the Buffet Effect might have an adaptive sense, for humans in today's societies this intake-enhancing mechanism might result in overconsumption. In this vein, the environments that surround us are also characterised by the constant accessibility of highly palatable, calorie-dense and low-cost food, which is likely to enhance the Buffet Effect. Therefore, studying the underlying mechanisms that operates through it is crucial to develop therapeutic tools or dietary recommendations to counteract it.

The results found in this thesis do not support the notion that SSS is a case of habituation, at least when considering all the characteristics of habituation on direct consumption. In this regard, the results also do not support the view that the Buffet Effect occurs partly due to a dishabituation of the SSS delaying the cessation of intake. Moreover, the introduction of a distraction, at least of the same sensory modality, does not interrupt the SSS process delaying the decay in the hedonic and motivational response to the consumed food.

Some caveats have to be acknowledged, first these studies have been carried out with liquid stimuli, both for target stimuli and for distractors or dishabituators. It is likely that by being stimuli of the same sensory modality, the distractor or dishabituating solutions do not reach to disrupt the SSS effect due to generalization between stimuli. For possible future studies, other distractors or dishabituators should be chosen, which, being of different sensory modality than the target stimuli, could eliminate the generalization between stimuli. Similarly, our main measure for assessing hedonic and motivational value during testing has been the animals' direct consumption and their relative preference between pre-fed and non pre-fed substances. Consumption is a very informative measure about motivational or hedonic value but this does not mean that always operate in parallel. For example, pairing a taste with an electric shock causes animals to reject its consumption, but presenting the taste involuntarily in a taste reactivity test does not result in aversive responses, so its hedonic aspect remains intact (Pelchat et al., 1983). The same is true for the instrumental response, which may reflect a motivational component of food reward (wanting), which in this sense, there are countless examples in the literature of results showing how, under certain conditions, intake is suppressed but the motivational response to the target food is unaffected (e.g., Holland & Gallagher, 2003; LeMon et al., 2019; Parkes et al., 2016; Kendig et al., 2016).

Thus, our results can not be interpreted as conclusive, although they are in line with previous results in human research. But, the fact that most of the literature seems support that SSS does not meet all the characteristics of habituation, does not mean that the basic phenomenon is not a case of the latter. Given the complex nature of eating behaviour, it would not be surprising that dishabituation and distraction are occurring in the hedonic and motivational component of the specific eaten food but, other silent or uncovered feeding processes (e.g., alliesthesia, conditioned satiety) inhibit its expression on direct consumption or subjective measures. Thus, given the problems involved in the methodologies employed within human subjects, and the measures used in this study, it is possible that the procedures used so far are not sensitive to detecting any changes in subjects' appetitive or affective responses to ingested food. In this sense, to exhaustively assess this hypothesis, the use of more reliable measures would be required, such as analysis of the licking microstructure, taste reactivity tests or instrumental responses. In any case, even if Dishabituation/Distraction paradigms affect the expression of SSS on a different aspect than consumption, we conclude that habituation does not modulate the Buffet Effect.

The fact that SSS is not dishabituated or affected by distractors on intake, does not mean that is responsible for the Buffet Effect. The Buffet Effect could be explained simply by the specific nature of SSS, and therefore, acting precisely on the eaten foods while others that had not been consumed remain unaffected. As a consequence, when we have a wide range of foods within a meal, we eat more because a large supply of food items is available that still did not reach the SSS effect and are therefore still palatable. However, given the immense variety of foods present in obesogenic environments, which are very similar to each other but vary subtly in some of their sensory attributes (e.g different varieties of crisps, savoury sandwiches or pizzas), this regulatory mechanism of intake should generalize across many food choices. So, considering the very nature of current environments, it is possible that other mechanisms may contribute to enhance the effect of food variety.

In this sense, in *Chapter V* we demonstrated how pre-exposure to sweet substances that only differed in their aroma, altered the expression pattern of the SSS generalization by means of perceptual learning. In view of the present results, we propose that massive exposure to similar foods regulates the expression of the SSS, making it more effective and thus, reducing its generalization. This effect is therefore suggested as an eating regulatory mechanism through the current obesogenic environments that would encourage overconsumption. Regular consumption of a varied food offer that is very similar but subtly differs in its sensory features, could activate this mechanism and abolish or weaken the normal SSS generalization across them. Thus, when we have a variety of foods in a meal, instead of generalizing the SSS effect across similar foods, other non-eaten similar foods will still remain appetizing. The prediction for this situation is that the Buffet Effect will be amplified; with a higher total intake will occur. Otherwise, if we are not massively exposed to a high food sensory variety, when eating a particular food, the SSS will generalize to other similar foods and thus, other alternatives will not be appetizing, reducing our intake.

These results highlight the need for public policies to reduce the level of exposure to this enormous sensory unhealthy variety. For example, typically in places such as hospitals, schools or offices, we are exposed to a wide variety of similar sweet or savoury processed snacks and beverages in vending machines. The same would be true for the endless corridors of supermarkets with food supplies of crisps, chocolates or pizzas. This calls for an intervention, at the very least, to boost the intake of a variety of healthier food choices as opposed to processed ones. In this sense, although the consequences of these results point to the fact that perceptual learning would act in a damaging and hazardous manner for the development of overweight and obesity, this mechanism could be used to generate better eating habits, and especially for children. Children are a particularly delicate population in terms of food likes and dislikes and rarely enjoy eating healthy choices such as fruits or vegetables. Exposing them to a varied offer of these healthy foods could attenuate neophobia and also encourage a higher intake of the latter through the decrease of SSS generalization, improving their health and preventing from overeating.

Conditioned preferences

The majority of food likes and dislikes are believed to be learned through the lifespan. This learnt preferences can be in part acquired through appetitive or aversive conditioning. From an approach of the obesogenic environments, it is necessary to understand the mechanisms by which preferences are learned from very highly palatable tastes such as sucrose. In this sense, the way we behave through learned flavor preferences can condition eating processes such as meal initiation, food choices or meal cessation.

We found that massive exposure to an aroma-sucrose compound led to a preference for the aroma that was not sensitive to SSS. These results were interpreted by means of a S-R association, which are featured by not being affected by devaluation treatments. This implies another mechanism by which SSS is disrupted, and may be promoting intake under situations where eating should be ceased. In this regard, this S-R learning was found only for sucrose, whereas did not occur neither for saccharin, nor for maltodextrin or saccharin + maltodextrin compounds. One possibility is the fact that sucrose has unique properties that cause it to behave differently from the other USs, for example because its taste seems to be innately preferred. Moreover, if we assume the hedonic reaction as the US-component responsible for the S-R equation, a US with a higher hedonic value will be more conducive to the development of this association. Indeed, sucrose at the concentrations used in the present experiments has a higher hedonic value compared to maltodextrin and saccharin separately, thus probably favouring the development of this learning. On the other hand, we have no evidence that sucrose has a higher hedonic value than the saccharin and maltodextrin mixture; one possibility is that the compound Maltodextrin + Saccharin, while having a highly hedonic taste and powerful nutritional consequences, still does not lead to a comparable hedonic reaction to that produced by sucrose consumption, preventing the formation of an S-R association. Furthermore, it is possible that when both tastes are presented together in a compound, animals process them elementally rather than as a configuration, impeding mimicking of sucrose. However, the possibility remains open that this effect could be also be expressed in other different USs with more exposure to that used here.

Taking these results to the practical domain, the establishment of this S-R learning mechanism could be of particular concern given the huge variety of sweetened food products (such as soft drinks, snacks or desserts) offered by the big industries. So, the presence of sensory cues in those foods that have been subjected to this learning could trigger automatic hedonic responses, promoting overconsumption even in physiological states of satiety. On the other hand, S-R type associations are very difficult to weaken, being highly resistant to extinction and also context-specific. This last consideration is of great relevance as given the pervasiveness of obesogenic environments, coping with this type of learning through therapeutic strategies would be challenging. Changing the current global food context, would require a general effort on the part of governments to implement public policies that control the food and consumer markets; reducing the supply of highly palatable foods that can lead to this type of learning.

On the theoretical domain, one model that could fit with these results could be the Pearce & Hall (1980) model of CS´s attention decrement and its application for response automatization proposed by Bouton and colleagues (Bouton, 2021; Bouton et al., 2020; Thraikill et al., 2015; Thraikill et al., 2018; Thraikill et al., 2021). To test this, future studies should manipulate the CS+ predictiveness by partially pairing it with the US. An inconsistently paired CS+ during training will maintain its surprisingness and effectiveness, preventing the automatization of its processing in memory and therefore the automatic execution of the conditioned response. Thus, if this explanation is valid for the present results, rats that are given both CS+US and CS exposures during extensive training, should not express an insensitivity to devaluation treatments compared to those given consistent pairings.

To conclude, we have measured preference for the CS+ over the CS- to determine the hedonic response of the former. As mentioned, preference assessed by consumption is a well predictor of hedonics but, can also be affected by other factors. In this sense, to elucidate the exact pattern of rats towards the CS+ after pre-feeding, it would be necessary to study the facial hedonic responses towards a taste-reactivity test or an assessment of the licking cluster size. Furthermore, we have assumed the hedonic response as the main component of the S-R equation that underpins preference. This assumption is based on the fact that it is the only one that has been proposed in the literature of flavor preference acquisition (Badolato et al., 2021; Harris et al., 2004). However, there remains the possibility that the response underpinning preference has a different nature to that developed from the hedonic component.

External food cues

Food cues are not only present in the form of tastes or smells in foods, but also in the constant exposure to environmental cues that indicate the availability of certain foods. These types of cues such us logos, advertisements, or intake related contexts, encourage consumption even when our physiological state is not suitable for eating. This effect has been demonstrated in general motivational states of satiety, whereas in the case of SSS it is less clear, as the methodologies used in the research may had constrained its expression.

Our results showed that food cues modulated the expression of SSS by potentiating the consumption of the pre-fed foods, but this effect was only observed for the savory snack (crisps). We mainly attribute this taste-dissociation to a possible different initial reinforcing value for the two snacks. While at the beginning of the experiment participants expressed explicit motivation for eating savory foods, participants did not show appetite for eating sweet ones. This result could be explained by the temporal context in which the experiment was conducted, being a time of the day that is generally associated with the consumption of savory foods. In this sense, by participants not having an initial appetite for sweet foods, the specific effect of food cues could be non-substantial for this snack. Also, this dissociation could be explained by individual taste preferences. In contrast to rats or human infants, preferences for sweetness vary greatly in adulthood and may bias food choices on test. If a food is not perceived as reinforcing, perhaps because it is disliked, the cues associated with it will not have the capacity to trigger appetitive responses toward the signalled food.

Thus, the results observed in the present experiment seem to show that Pavlovian cues associated with food, when present, are able to override not only the general motivational effect of satiety, but also the effect of the SSS. As mentioned previously, food cues have been proposed as a potential factor in overeating, and indeed it has been shown how overweight or obese subjects express greater reactivity toward them. On the contrary, healthy and obese subjects do not seem to differ in their pattern of expression of SSS. So, something to consider, would be to study how both populations behave in their interaction with food-related cues and SSS expression. As obese subjects are more reactive to food cues, it is possible that their effect on SSS is more pronounced, weakening the impact of specific hedonic and motivational decline and therefore, delaying the cessation of eating at a meal. These studies would be of great relevance as their understanding allows the development of therapeutic strategies that do not rely exclusively on dietary treatments and incorporate notions of associative learning as proposed by Exposure-Based Therapy.

In this regard, Exposure-Based Therapy has been the main approach from the associative framework to address the food cue-reactivity in obesity. However, this therapy is based on the assumption that the underlying association between the cue and the food is S-S type, in such a way that when the CS is present, activates the representation of the US that depending on its current value triggers or not appetitive responses towards it. However, in view of the results found, with the cues and foods used here, this association does not seem to be sensitive to the US-devaluation procedure. Therefore, these results suggest the formation of an S-R link that sustain responding for the US even when the latter is no longer desirable. This learning would be subjected to different characteristics to that proposed for the S-S association. As we have mentioned previously, unlike S-S associations, S-R learning would be automatic and rigid, and thus, by not being mediated by the representation of the US would be protected from extinction. Therefore, under this other possibility, Exposure-Based Therapy may not be as effective as it is believed to be, not because of the peculiarities of the extinction phenomenon, but because of the characteristics of S-R learning.

For example, although S-R associations are resistant to extinction, are also less generalizable than S-S ones, and therefore being specific to the context in which they have been learned. In this sense, these characteristics could be particularly concerning in the case of fast-food chains, which always preserve in each of their restaurants a very similar aesthetic and atmosphere (being easy for this learning to be expressed between the different restaurants). More worrying is the case of the omnipresence of the cues associated with these fast-food chains, such as the sight of McDonald's golden arches from any road, urging to stop for a fast, cheap and caloric meal at any time of the day. Thus, providing the global food context in which we live multiple opportunities for this type of rigid learning to take place. In order to address this automatic context-dependent learning, it would be required a change in the global food context being necessary public policies to protect individuals from the pervasiveness of these appetitive contexts or cues.

To sum up, the present results need to be interpreted with caution; the sample used was very small, making it difficult to draw firm conclusions. On the other hand, in terms of subjective measures, an effect of SSS was only observed in the measures of "Wanting", while in the assessment of "Liking" no decrease in responses was observed. Future research will have to consider larger experimental samples and develop better tools for measuring hedonic and motivational responses that are not subject to subjectivity.

Final comments and conclusions

The results of this thesis, once again, highlight the relevance of the study of associative learning processes in eating behaviour. We not only propose intake regulatory mechanisms on the basis of exposure effects, but also their translation to the obesogenic environments and possible solutions to reverse them.

In relation to the relationship between habituation and SSS, we found no evidence that dishabituation or distraction produces a disruption of the pattern of SSS expression in consumption. Therefore, these data, in line with previous studies, suggest that dishabituation/distraction is not involved in the intake-enhancing effect of the Buffet effect. However, we acknowledge that these results are not conclusive; even if the effect of distraction and dishabituation is not expressed in consumption, this does not mean that SSS is not a case of habituation. We therefore leave the possibility that future research, using more sensitive techniques that may isolate the SSS phenomenon from other uncovered mechanisms of feeding, may demonstrate an effect of dishabituation and distraction.

We claim that the Buffet Effect is indeed due to the sensory-specific character of SSS, however, this does not mean that there are no other additional mechanisms mediating this effect, especially in today's environments. In view of our results, we propose perceptual learning as a tentative mechanism to amplify the Buffet Effect and thus, encouraging excessively unhealthy eating habits. To balance this mechanism, we also propose that this mechanism could be used for promoting consumption of healthier foods, especially in populations with a more delicate intake pattern such as children.

In relation to the acquisition of preferences, we have found that the type of exposure to the compounds during training modulates the content that is being learning. In the same way that the motivational state modulates the predominant learned association (flavor-flavor/flavor-nutrient), the degree of exposure to the stimuli generates a much more rigid and automatic associative structure such as S-R learning. Finally, in view of the abundance of highly palatable foods such as snacks and sugary drinks, this type of learning could generate automatic and rigid behaviours that are insensitive to motivational states of satiety, leading to overeating.

Finally, exposure to cues related to food availability has also been studied. The results of this experiment seem to show that, just as cues increase consumption when individuals are in a state of general satiety, they can also disrupt the SSS process. Therefore, this type of food cues present in our environment, through massive exposure could lead to overeating, even under conditions in which food are no longer desirable disrupting the homeostatic control of intake. Given the increasing prevalence of obesity, the study of the mechanisms by which these ubiquitous cues act is of great relevance to develop therapeutic strategies to combat their harmful effects on eating behavior.

CHAPTER IX

Discusión general y conclusiones

Resumen, discusión y conclusiones

El paradigma de investigación en el comportamiento alimentario ha cambiado su foco de estudio en las pasadas décadas con la aparición y posterior propagación de los ambientes obesogénicos en las sociedades actuales. La llegada de estos entornos a nuestra sociedad, ha supuesto en primer lugar la transición de la comida tradicional a la comida ultraprocesada, caracterizada por ofrecer una amplia variedad sensorial de alimentos que generalmente son altamente hedónicos y calóricos. Por otro lado, estos entornos también se caracterizan por la omnipresencia de claves señalizadoras de la disponibilidad de alimentos y por promover el sedentarismo.

Así, de manera paralela a estos cambios ambientales, diversos estudios fueron evidenciando la relevancia de los factores externos en la conducta alimentaria mostrando el efecto potenciador de la ingesta de las claves relacionadas con la comida, los aspectos hedónicos de la misma o la variedad sensorial. Todos estos estudios sugirieron la necesidad de una conceptualización más amplia del comportamiento alimentario, proponiendo otros mecanismos, además de aquellos homeostáticos, que fomentan el desarrollo de hábitos alimentarios nocivos y la ingesta excesiva. En este sentido, hoy en día existe una comprensión mucho más amplia de la ingesta, en la que múltiples factores tienen cabida, desde aspectos sensoriales, ambientales y psicológicos hasta genéticos. Dentro de los factores sensoriales, la Saciedad Sensorial Específica ha sido propuesta como un mecanismo que promueve el cese específico de la ingesta de alimentos, potenciando la variedad nutricional. Este fenómeno, se caracteriza por la disminución de la valoración hedónica y motivacional de las propiedades sensoriales de los alimentos a medida que son consumidos. Este mecanismo regulador de la ingesta, es específico a los alimentos ingeridos, pero puede generalizarse hacia otros alimentos que compartan propiedades sensoriales al alimento consumido. Dadas las características de este fenómeno, la Saciedad Sensorial Específica se concibe como el principal mecanismo responsable del Efecto Bufet, caracterizado por incrementar el consumo total de alimentos cuando la oferta alimentaria en una comida es variada. Por tanto, se ha señalado que dada la alta disponibilidad de alimentos altamente calóricos y hedónicos que están presentes en los entornos en los que vivimos, la Saciedad Sensorial Específica podría estar potenciando una ingesta excesiva a través del Efecto Bufet.

Por otra parte, dentro de los factores psicológicos que intervienen en la conducta alimentaria, uno de los procesos que en las pasadas décadas ha recibido gran atención a la hora de explicar posibles mecanismos no homeostáticos de regulación de la ingesta ha sido el aprendizaje asociativo. Se ha constatado que el aprendizaje asociativo puede afectar a distintas etapas de la conducta alimentaria tales como la selección de alimentos, el inicio de la ingesta o la modulación de los procesos de saciedad. Por ejemplo, el aprendizaje pavloviano juega un papel imprescindible en la adquisición de preferencias gustativas. Gran parte de las preferencias que adquirimos a lo largo de nuestra vida son aprendidas, a través del emparejamiento de claves sensoriales inicialmente neutras (aromas, sabores o texturas) con otras ya apetitivas, ya sea por su sabor o por sus consecuencias energéticas. Así, mediante este aprendizaje se modulan las preferencias, la elección de los alimentos o la cantidad de comida que consumimos. Por tanto, dada la masiva exposición a productos altamente calóricos y hedónicos en las sociedades actuales, estudiar la estructura de asociación que se genera a través de este aprendizaje resulta de gran relevancia para entender cómo interactuamos con la comida.

Del mismo modo que las claves sensoriales presentes en la comida pueden resultar en preferencias gustativas, otro mecanismo por el que el aprendizaje asociativo puede modular los procesos de ingesta es a través del aprendizaje de claves externas señalizadoras de la comida. Estas claves están presentes en nuestro ambiente en forma de anuncios, logotipos o contextos apetitivos. En este sentido, se ha demostrado que en presencia de estas claves de comida, se produce un aumento de la ingesta incluso en estados de saciedad. Por lo tanto, este aprendizaje podría ser potencialmente peligroso; promoviendo la ingesta excesiva independientemente de nuestras necesidades fisiológicas. Este fenómeno se ha denominado “alimentación potenciada por las claves” y se caracteriza por actuar de manera específica al alimento señalado.

En la presente tesis doctoral, analizamos efectos de exposición en la expresión de la Saciedad Sensorial Específica desde una aproximación del aprendizaje asociativo y sus implicaciones para los entornos actuales. La mayor parte de los experimentos realizados en este trabajo han sido llevados a cabo con modelos animales, utilizando ratas como sujetos experimentales, con la excepción del *Capítulo VII*, cuya muestra la conformaron sujetos humanos. Todos los experimentos han sido llevados a cabo empleando estímulos sápidos (soluciones o comida), siendo la medida principal el consumo de los sujetos experimentales.

En la primera parte de la tesis, nos centraremos en elucidar los posibles mecanismos de aprendizaje que modulan la Saciedad Sensorial Específica y el Efecto Bufet. En concreto, estudiamos la habituación como posible mecanismo subyacente a la Saciedad Sensorial Específica, pudiendo dar cuenta este fenómeno al Efecto Bufet a través de la deshabituación o la distracción. Por otro lado, también estudiamos el aprendizaje perceptivo como posible mecanismo adicional al Efecto Bufet. Este fenómeno de aprendizaje podría operar favoreciendo un incremento en la discriminación de alimentos semejantes, y por tanto reducir la generalización de la Saciedad Sensorial Específica entre alimentos similares.

En la segunda parte de la tesis doctoral estudiamos en el efecto de la exposición masiva a distintas claves sensoriales relacionadas con la comida en la Saciedad Sensorial Específica. Este efecto se mide en primer lugar mediante el empleo de un paradigma de adquisición de preferencias condicionadas, emparejando aromas neutros con sabores de un alto valor hedónico o motivacional. En estos experimentos se manipula el grado de exposición a los compuestos durante el condicionamiento y se mide el grado de expresión del efecto de la Saciedad Sensorial Específica del sabor en el aroma. Por otra parte, el efecto de las claves externas de comida en la Saciedad Sensorial Específica también fue estudiado. Dado que las claves de comida tienen un efecto específico cuando los sujetos experimentales están en un estado de saciedad general, esperábamos que de la misma manera fuesen capaces de eliminar el efecto de la Saciedad Sensorial Específica, incrementando el consumo un alimento saciado cuando ha sido señalizado por su clave específica.

Los resultados de la primera parte de la tesis sugieren que, al menos con los procedimientos empleados, la habituación no es el mecanismo subyacente a la Saciedad Sensorial Específica. Ni la presentación de un distractor durante el proceso de saciación, ni la presentación de un estímulo deshabituador tras el procedimiento de saciación, produjo un incremento en el consumo de la sustancia consumida previamente. A pesar de la gran similitud que presentan ambos fenómenos, la Saciedad Sensorial Específica no parece compartir todas las características de la habituación, al menos cuando el consumo es la medida utilizada.

Por un lado, el hecho de que no hayamos encontrado una alteración en el patrón de la Saciedad Sensorial Específica tras la presentación del distractor o la deshabituación en el consumo, no descarta completamente la idea de que el aspecto hedónico o motivacional de la comida ingerida si esté siendo afectado. En este sentido, el consumo es un buen índice del valor hedónico y motivacional de la comida, pero eso no quiere decir que exista una relación directa entre ambos, no siendo esta una medida completamente sensible. El consumo total podría verse afectado por otras variables de distinta índole, como factores homeostáticos que intervengan en la alimentación, previniendo la expresión de los efectos estudiados en el consumo. Sin embargo, estos resultados están en sintonía con aquellos reportados en la investigación con sujetos humanos, cuya evidencia parece apuntar que la Saciedad Sensorial Específica ni se deshabitua ni se ve interrumpida por la presentación de un distractor. Del mismo modo que nuestra metodología podría tener limitaciones a la hora de esclarecer interpretaciones concluyentes, gran parte de la investigación con humanos ha empleado metodologías basadas en medidas subjetivas. Estos procedimientos están sujetos a grandes limitaciones, debido a que los constructos que se miden pueden estar siendo afectados por la propia monitorización de los participantes durante la tarea o a una mala interpretación de los constructos que se están midiendo.

En definitiva, parecen necesarias futuras investigaciones con medidas objetivas, que examinen el patrón exacto de aspectos hedónicos (Liking: e.g., test de reactividad al sabor) y motivacionales (Wanting: e.g., respuestas instrumentales) tras la saciación. En todo caso, podemos concluir que todas estas investigaciones parecen demostrar que el mecanismo subyacente al Efecto Bufet no es la interrupción o restauración de la Saciedad Sensorial Específica a través de las características de la habituación. El Efecto Bufet podría explicarse simplemente por el hecho de que la Saciedad Sensorial es específica, y por tanto nos saciamos de los alimentos que consumimos mientras otros no ingeridos se mantienen intactos en este proceso de devaluación.

Sin embargo, dada la inmensa variedad de alimentos en los ambientes obesogénicos, que son muy semejantes entre ellos, pero varían sutilmente en algunos de sus atributos sensoriales, este mecanismo regulador de la ingesta debería generalizarse, y por tanto reducir el consumo entre alimentos semejantes. Esta idea encaja con el *Capítulo V* en el que estudiamos otro posible mecanismo subyacente al Efecto Bufet que podría incrementar la ingesta cuando tenemos una oferta variada de alimentos similares en una comida.

En el *Capítulo V* demostramos cómo la exposición repetida a estímulos sápidos muy semejantes (soluciones que varían únicamente en su aroma) produce un incremento en la discriminación de los mismos en base al aprendizaje perceptivo. En estos experimentos observamos cómo la mejor discriminación de estas soluciones producía mayor efectividad de la Saciedad Sensorial Específica al incrementarse su especificidad. Así, a través de este mecanismo, la Saciedad Sensorial Específica podría dejar de actuar ante alimentos similares al que se ha saciado, y por tanto, anular el efecto de la generalización. Al permanecer estos alimentos exentos de la generalización, seguirán siendo percibidos como apetecibles y por tanto, la ingesta total se incrementaría. En consecuencia, este mecanismo podría amplificar el efecto de la variedad, mediante la disminución de la generalización entre alimentos consumidos y otros similares que estén presentes en el menú de comida. Además, dada la masiva exposición a productos alimentarios variados a la que estamos expuestos, en la que un mismo alimento varía de propiedades sensoriales en una misma marca (por ejemplo las patatas fritas de bolsa o las chocolatinas), los ambientes actuales estarían potenciando la puesta en marcha de este mecanismo y por tanto, desencadenando en hábitos nocivos para la ingesta. En la presente tesis no sólo destacamos las consecuencias negativas de este mecanismo, sino que también proponemos su utilización de forma beneficiosa para incrementar el consumo de alimentos saludables, como es el caso de las frutas y las verduras. Así, este mecanismo podría ser de gran interés para poblaciones concretas como es el caso de los niños, quienes tienden a ser muy reacios a la ingesta de alimentos sanos como frutas y verduras.

Además de la variedad sensorial que presentan los alimentos en las sociedades actuales, estas comidas suelen estar caracterizadas por poseer un alto valor hedónico y energético. Se ha observado que claves sensoriales cuyo sabor o aporte nutritivo es elevado (EI), pueden generar preferencias hacia otras claves sensoriales inicialmente neutras (EC) cuando ambas son emparejadas (EC-EI). El resultado de este aprendizaje pavloviano da lugar a una preferencia del EC sobre otras claves sensoriales neutras y un incremento en su consumo total. La mayoría de las investigaciones que han estudiado este fenómeno han apuntado a que las asociaciones que sustentan estas preferencias adquiridas pueden desarrollarse a través del sabor (asociación sabor-sabor) o través de las propiedades nutricionales de un alimento (asociación sabor-calorías). Ambas asociaciones hacen referencia a una estructura de asociación de tipo Estímulo-Estímulo (E-E), y por tanto la preferencia adquirida se sustenta en base a la activación de la representación en memoria del EI (ya sea de sus propiedades sensoriales o motivacionales). Las asociaciones de estas características son sensibles a los procedimientos de devaluación, es decir, cuando el EI ha perdido su valor, el EC, también refleja esta devaluación. La alternativa a las asociaciones E-E, son las asociaciones de tipo Estímulo-Respuesta (E-R), caracterizadas por una mayor rigidez y automatismo. Al contrario que las asociaciones de tipo E-E, las asociaciones de tipo E-R no son sensibles a la devaluación del EI, puesto que una vez que se presenta el EC, este elicita una respuesta de manera automática sin que el EI se procese.

La disociación de este tipo de estructuras asociativas generalmente se ha estudiado en el paradigma instrumental mediante los procedimientos de aversión del sabor o la Saciedad Sensorial Específica. Tradicionalmente, el punto de vista general acerca de cómo emergen los hábitos de conducta o las asociaciones de tipo E-R, es la repetición conductual de acuerdo a la Ley del Efecto propuesta por Thorndike. De tal manera que con un entrenamiento extendido, la conducta deja de ser flexible y se vuelve dependiente de los estímulos, fortaleciendo la asociación entre las claves ambientales y las repuestas sin procesar la consecuencia. En el caso del aprendizaje pavloviano, el estudio de las asociaciones E-R se abandonó con la llegada de modelos de aprendizaje de naturaleza más cognitiva y con la falta de evidencias que apoyaran que tales mecanismos pudieran darse en este paradigma de aprendizaje. En el caso concreto del aprendizaje de preferencias, la mayoría de las investigaciones han mostrado cómo las preferencias sobre los ECs pueden modularse de acuerdo al estado del EI. De este modo, estas investigaciones sugieren que las preferencias adquiridas se sustentan un aprendizaje de tipo E-E. Sin embargo, tradicionalmente la metodología empleada en el aprendizaje de preferencias condicionadas ha utilizado procedimientos de condicionamiento de corta duración y exposición a las sustancias (pocos días de condicionamiento, acceso limitado a las sustancias y sesiones breves).

En el *Capítulo VI* estudiamos la hipótesis de que, de la misma manera que en el paradigma instrumental la repetición de la cadena Estímulo-Respuesta-Consecuencia puede dar lugar al fortalecimiento de la asociación E-R, en el paradigma de adquisición de preferencias, la exposición masiva al compuesto EC-EI pueda dar lugar a la emisión automática de la respuesta condicionada del EC (asociaciones E-R). Esta hipótesis ha sido contrastada a través de distintos experimentos en los que comparamos los procedimientos tradicionales con los procedimientos extensivos, empleando distintos EIs (Azúcar, sacarina, maltodextrina y sacarina+maltodextrina) y aplicando como método de devaluación la Saciedad Sensorial Específica.

Los resultados de esta serie experimental sugieren que el contenido aprendido a través de los distintos procedimientos cambia, pudiendo darse una asociación de tipo E-R. Este efecto solo fue encontrado cuando se utilizó un procedimiento de condicionamiento extendido, con acceso ilimitado al compuesto EC-EI durante largos periodos y usando azúcar como EI. Por tanto, los resultados obtenidos sugieren que este efecto es exclusivo del sabor dulce y más concretamente del azúcar. En este sentido, es posible que las características del azúcar, un sabor preferido consistentemente entre especies de manera aparentemente innata, le conviertan en un EI diferente, con propiedades distintivas o únicas frente a otros EIs. Sin embargo, queda abierta la posibilidad de que con otros EIs, diferentes al azúcar, se puedan establecer preferencias condicionadas basadas en asociaciones E-R, con un entrenamiento más extenso que el utilizado en esta serie experimental.

Trasladando esta idea a un contexto real, los resultados obtenidos podrían tener implicaciones relevantes para nuestro día a día. La forma en que nos exponemos a estímulos alimentarios determina el contenido aprendido y modula la expresión de los procesos reguladores de la ingesta. Dados los ambientes en los que vivimos, donde se nos ofrecen múltiples oportunidades para consumir alimentos altamente hedónicos y calóricos, las sociedades actuales se convierten en el escenario perfecto para que se aprendan este tipo de comportamientos rígidos y automatizados. Como consecuencia, este aprendizaje E-R podría llegar a alterar el patrón habitual de mecanismos reguladores de la ingesta tales como la Saciedad Sensorial Específica; resultando en hábitos alimentarios nocivos que promueven el consumo de comida incluso cuando esta ha perdido su valor.

Por último, de la misma manera que se puede adquirir preferencias gustativas a través de claves sensoriales presentes en los alimentos, podemos aprender de claves externas que señalicen la disponibilidad de alimentos. En este sentido, existen innumerables ejemplos en nuestro día a día de estas claves, tales como anuncios relacionados con comida, contextos apetitivos y logotipos de marcas de comida rápida. Estas claves señalizadoras de comida cuando están presentes son capaces de activar múltiples respuestas relacionadas con la conducta alimentaria. Estas respuestas van desde la captura atencional, el incremento de las respuestas motivacionales o de craving, la activación de las respuestas cefálicas hasta la propia iniciación de la ingesta. En relación a la ingesta, la investigación ha demostrado que las claves son capaces de incrementar el consumo de la comida que señalan incluso cuando los sujetos experimentales, ya sean humanos o roedores, están en un estado de saciedad general.

Este fenómeno se ha denominado en la tradición asociativa “ingesta potenciada por claves” (Cue-Potentiated Feeding) y se caracteriza por ser específico a la comida que señala, no afectando a otros alimentos incluso cuando su valor hedónico es similar al del alimento señalado. Este efecto, ha sido mayoritariamente demostrado en estados de saciedad general, cuando por ejemplo en el caso de los roedores no tienen restricciones a sus alimentos habituales. En el caso de la Saciedad Sensorial Específica, se han llevado a cabo experimentos (mayoritariamente empleando roedores como sujetos experimentales) con el fin de estudiar la interacción de este proceso de saciedad y las claves, pero el objetivo de estudio mayoritariamente ha sido otro que el de analizar la conducta alimentaria. Generalmente el foco de estudio ha sido desvelar el patrón de las respuestas instrumentales ante la presentación de claves para disociar los sistemas de acción subyacentes a la conducta (acciones dirigidas a metas/hábitos). En este sentido, el patrón de consumo tras el procedimiento de Saciedad Sensorial Específica y su interacción con la presentación de claves pavlovianas, ha sido estudiado como método para constatar si el procedimiento de devaluación ha sido efectivo, más que como objeto de análisis per se. En estas investigaciones generalmente encuentran que la presentación de claves pavlovianas no afecta al patrón de consumo habitual de la Saciedad Sensorial Específica. Sin embargo, los procedimientos típicos de estos estudios emplean fases de saciación largas (aproximadamente 1 hora) y ofrecen a los animales grandes cantidades de los alimentos a saciar, quizás produciendo algo similar a un "empacho", y por tanto otros mecanismos reguladores de la ingesta más que los sensoriales podrían estar involucrados. Esta metodología es eficaz para producir un efecto de devaluación, pero al no estar diseñada para estudiar el propio proceso de Saciedad Sensorial Específica en sí, quizá no sea la adecuada para aislar el efecto sensorial de otros procesos reguladores de la ingesta.

Con este objetivo, en el *Capítulo VII* realizamos un experimento con una muestra de sujetos humanos sanos, en el que estudiamos el efecto de la presentación de una clave de comida muy habitual en nuestro entorno (anuncios de comida) en la Saciedad Sensorial Específica. Los resultados del experimento mostraron que el grupo pre-expuesto a los anuncios no relacionados con comida expresaban el efecto de la Saciedad Sensorial Específica para ambos sabores; ingiriendo menor cantidad del alimento saciado que del no saciado. Por otro lado, el grupo pre-expuesto al anuncio alimentario expresó un efecto de Saciación Sensorial Específica más débil que el grupo no pre-expuesto. Este menor efecto en el grupo pre-expuesto al anuncio de comida se vio reflejado en el grupo de participantes que había sido saciado con los snacks salados; los participantes consumieron cantidades comparables tanto de las patatas fritas como de las galletas de chocolate, no expresando Saciedad Sensorial Específica a la comida saciada. Sin embargo, en el caso de los participantes que habían sido saciados con el snack dulce, los participantes expresaron el efecto de la Saciedad Sensorial Especifica; ingiriendo menos cantidad del alimento ingerido (dulce) que el no saciado (salado). Esta disociación del efecto por sabores, se propone que podría estar influenciada por el contexto temporal del experimento o con las preferencias individuales de cada participante.

En relación con las medidas subjetivas, se observó un efecto de SSS sólo en las escalas de "Wanting"; observando una peor valoración específica hacia el snack saciado después de la saciedad. Este efecto se encontró en ambos grupos de participantes, por lo que no se detectó ningún efecto de las señales alimentarias en las medidas subjetivas. Mientras que para las escalas de "Liking", los sujetos mantuvieron las mismas valoraciones a lo largo de los distintos momentos del experimento tanto en los snacks saciados como en los no saciados, lo que refleja la ausencia del efecto Saciedad Sensorial Específica.

Estos resultados parecen mostrar que las claves pavlovianas asociadas a la comida, cuando están presentes, podrían ser capaces de anular no sólo el efecto motivacional general de la saciedad, sino también el efecto específico de la SSS. Como se ha mencionado anteriormente, las claves de comida se han propuesto como un factor potencial para la ingesta excesiva, y de hecho se ha demostrado cómo los sujetos con sobrepeso u obesidad expresan una mayor reactividad hacia ellas. En este sentido, los resultados encontrados en el *Capítulo VI* complementan aquellos del *Capítulo VII*, observando que las claves de comida son capaces de desencadenar respuestas apetitivas hacia un alimento incluso cuando este ha perdido su valor. Este paralelismo parece sugerir que la asociación que sustenta este aprendizaje no está mediada por el procesamiento de la comida (EI), y por tanto, cuando las claves están presentes elicitan respuestas automáticas de ingesta hacia la misma. De nuevo, estos resultados parecen señalar las consecuencias nocivas de la exposición masiva a claves sensoriales predictivas de comida en los ambientes obesogénicos, pudiendo resultar en hábitos de alimentación rígidos e indeseables que promueven el hambre hedónica y por tanto, el comer en exceso.

Por último los resultados de este capítulo deben interpretarse con cautela; la muestra utilizada fue muy pequeña, lo que dificulta la obtención de conclusiones firmes. Por otra parte, en cuanto a las medidas subjetivas, sólo se observó un efecto del SSS en las medidas de "Wanting", mientras que en la evaluación de "Liking" no se observó ninguna disminución de las respuestas. Las investigaciones futuras tendrán que considerar muestras experimentales más amplias y desarrollar mejores herramientas para medir las respuestas hedónicas y motivacionales que no estén sujetas a la subjetividad.

References

Ackroff, K., Drucker, D. B., & Sclafani, A. (2012). The CS-US delay gradient in flavor preference conditioning with intragastric carbohydrate infusions. *Physiology & behavior*, *105*(2), 168-174. https://doi.org/10.1016/j.physbeh.2011.07.030

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *The Quarterly Journal of Experimental Psychology Section B*, *34*(2b), 77-98. https://doi.org/10.1080%2F14640748208400878

Adams, C. D., & Dickinson, A. (1981). Instrumental responding following reinforcer devaluation. *The Quarterly Journal of Experimental Psychology Section B*, *33*(2b), 109-121. https://doi.org/10.1080%2F14640748108400816

Ahn, S., & Phillips, A. G. (1999). Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. *Journal of Neuroscience*, *19*(19), https://doi.org/10.1523/JNEUROSCI.19-19-j0003.1999

Ahn, S., & Phillips, A. G. (2012). Repeated cycles of restricted food intake and binge feeding disrupt sensory-specific satiety in the rat. *Behavioral brain research*, *231*(2), 279-285. https://doi.org/10.1016/j.bbr.2012.02.017

Azzara, A. V., & Sclafani, A. (1998). Flavor preferences conditioned by intragastric sugar infusions in rats: maltose is more reinforcing than sucrose. *Physiology & Behavior*, *64*(4), 535-541. https://doi.org/10.1016/S0031-9384(98)00113-9

Badolato, C., Hall, G., & Boakes, R. A. (2021). Sucrose-based flavor preferences in rats: Factors affecting detection of extinction. *Journal of Experimental Psychology: Animal Learning and Cognition*, *47*(2), 120-136. https://psycnet.apa.org/doi/10.1037/xan0000253

Balleine, B. W., & Dickinson, A. (1998). The role of incentive learning in instrumental outcome revaluation by sensory-specific satiety. *Animal Learning & Behavior*, *26*(1), 46-59. https://doi.org/10.3758/BF03199161

Balleine, B. W., & Dickinson, A. (2000). The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. *Journal of Neuroscience*, *20*(23), 8954-8964. https://doi.org/10.1523/JNEUROSCI.20-23-08954.2000

Beaulieu, K., & Blundell, J. (2021). The psychobiology of hunger–a scientific perspective. *Topoi*, *40*(3), 565-574. https://doi.org/10.1007/s11245-020-09731-0

Belfort-DeAguiar, R., & Seo, D. (2018). Food cues and obesity: overpowering hormones and energy balance regulation. *Current obesity reports*, *7*(2), 122-129. https://doi.org/10.1007/s13679-018-0303-1

Bellisle, F. (2020). Food intake and physiological regulation: the means and the end. *Handbook of Eating and Drinking: Interdisciplinary Perspectives*, 113-129.

Bernard, C. (1879). *Leçons sur les phénomènes de la vie commune aux animaux et aux végétaux* (Vol. 2). Baillière.

Berridge, K. C. (1991). Modulation of taste affect by hunger, caloric satiety, and sensory-specific satiety in the rat. *Appetite*, *16*(2), 103-120. https://doi.org/10.1016/0195-6663(91)90036-r

Berridge, K. C. (1996). Food reward: brain substrates of Wanting and Liking. *Neuroscience & Biobehavioral Reviews*, *20*(1), 1-25. https://doi.org/10.1016/0149-7634(95)00033-B

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience & Biobehavioral Reviews*, *24*(2), 173-198. https://doi.org/10.1016/S0149-7634(99)00072-X

Berridge, K. C., & Kringelbach, M. L. (2008). Affective neuroscience of pleasure: reward in humans and animals. *Psychopharmacology*, *199*(3), 457-480. https://doi.org/10.1007/s00213-008-1099-6

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in neurosciences*, *26*(9), 507-513. https://doi.org/10.1016/S0166-2236(03)00233-9

Berthoud, H. R. (2012). The neurobiology of food intake in an obesogenic environment. *Proceedings of the Nutrition Society*, *71*(4), 478-487. https://doi.org/10.1017/S0029665112000602

Berthoud, H. R., Morrison, C. D., & Münzberg, H. (2020). The obesity epidemic in the face of homeostatic body weight regulation: What went wrong and how can it be fixed? *Physiology & behavior*, *222*, 112959. https://doi.org/10.1016/j.physbeh.2020.112959

Bilman, E., van Kleef, E., & van Trijp, H. (2017). External cues challenging the internal appetite control system—Overview and practical implications. *Critical reviews in food science and nutrition*, *57*(13), 2825-2834. https://doi.org/10.1080/10408398.2015.1073140

Birch, L. L., & Marlin, D. W. (1982). I don't like it; I never tried it: effects of exposure on two-year-old children's food preferences. *Appetite*, *3*(4), 353-360. https://doi.org/10.1016/S0195-6663(82)80053-6

Birch, L. L., Gunder, L., Grimm-Thomas, K., & Laing, D. G. (1998). Infants' consumption of a new food enhances acceptance of similar foods. *Appetite*, *30*(3), 283-295. https://doi.org/10.1006/appe.1997.0146

Birch, L. L., McPhee, L., Sullivan, S., & Johnson, S. (1989). Conditioned meal initiation in young children. *Appetite*, *13*(2), 105-113. https://doi.org/10.1016/0195-6663(89)90108-6

Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. *Nature Reviews Endocrinology*, *15*(5), 288-298. https://doi.org/10.1038/s41574-019-0176-8

Blundell J.E., Rogers P.J., & Hill A.J. (1987). Evaluating the satiating power of foods: implications for acceptance and consumption. In: Solms J., Booth D.A., Pangbourne R.M. & Raunhardt O. (eds). *Food Acceptance and Nutrition*. p. 205–219, Academic Press: London.

Blundell, J. E., & Finlayson, G. (2004). Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption?. *Physiology & behavior*, *82*(1), 21-25. https://doi.org/10.1016/j.physbeh.2004.04.021

Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., Van Der Knaap, H., & Westerterp, M (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews*, *11*(3), 251-270. https://doi.org/10.1111/j.1467-789X.2010.00714.x

Blundell, J.E., & Bellisle F. (2013). Satiation, satiety: Concepts and organisation of behaviour. In: *Satiation, Satiety and the Control of Food Intake* (Blundell, J.E. & Bellisle, F. eds), pp. 3–11. Wood-head Publishing Limited: UK

Boakes, R. A., Rossi-Arnaud, C., & Garcia-Hoz, V. (1987). Early experience and reinforcer quality in delayed flavour-food learning in the rat. *Appetite*, *9*(3), 191-206. https://doi.org/10.1016/S0195-6663(87)80013-2

Boggiano, M. M., Dorsey, J. R., Thomas, J. M., & Murdaugh, D. L. (2009). The Pavlovian power of palatable food: lessons for weight-loss adherence from a new rodent model of cue-induced overeating. *International Journal of Obesity*, *33*(6), 693-701. https://doi.org/10.1038/ijo.2009.57

Bonacchi, K. B., Ackroff, K., & Sclafani, A. (2008). Sucrose taste but not Polycose taste conditions flavor preferences in rats. *Physiology & behavior*, *95*(1-2), 235-244. https://doi.org/10.1016/j.physbeh.2008.06.006

Bond, D. S., Raynor, H. A., McCaffery, J. M., & Wing, R. R. (2010). Salivary habituation to food stimuli in successful weight loss maintainers, obese and normal-weight adults. *International Journal of Obesity*, *34*(3), 593-596. https://doi.org/10.1038/ijo.2009.267

Booth, D. A. (1972). Conditioned satiety in the rat. *Journal of Comparative and Physiological Psychology*, *81*(3), 457–471. https://doi.org/10.1037/h0033692

Boswell, R. G., & Kober, H. (2016). Food cue reactivity and craving predict eating and weight gain: a meta-analytic review. *Obesity Reviews*, *17*(2), 159-177. https://doi.org/10.1111/obr.12354

Boutelle, K. N., & Bouton, M. E. (2015). Implications of learning theory for developing programs to decrease overeating. *Appetite*, *93*, 62-74. https://doi.org/10.1016/j.appet.2015.05.013

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory*, *11*(5), 485-494. https://doi.org/10.1101/lm.78804

Bouton, M. E. (2011). Learning and the persistence of appetite: Extinction and the motivation to eat and overeat. *Physiology & behavior*, *103*(1), 51-58. https://doi.org/10.1016/j.physbeh.2010.11.025

Bouton, M. E. (2021). Context, attention, and the switch between habit and goal-direction in behavior. *Learning & Behavior*, *49*(4), 349-362. https://doi.org/doi/10.3758/s13420-021-00488-z

Bouton, M. E., Broomer, M. C., Rey, C. N., & Thrailkill, E. A. (2020). Unexpected food outcomes can return a habit to goal-directed action. *Neurobiology of learning and memory*, *169*, 107163. https://doi.org/10.1016/j.nlm.2020.107163

Brondel, L., Lauraine, G., Van Wymelbeke, V., Romer, M., & Schaal, B. (2009). Alternation between foods within a meal. Influence on satiation and consumption in humans. *Appetite*, *53*(2), 203-209. https://doi.org/10.1016/j.appet.2009.06.009

Brondel, L., Romer, M., Van Wymelbeke, V., Pineau, N., Jiang, T., Hanus, C., & Rigaud, D. (2009). Variety enhances food intake in humans: Role of sensory-specific satiety. *Physiology & Behavior*, *97*(1), 44-51. https://doi.org/10.1016/j.physbeh.2009.01.019

Brondel, L., Romer, M., Van Wymelbeke, V., Walla, P., Jiang, T., Deecke, L., & Rigaud, D. (2007). Sensory-specific satiety with simple foods in humans: no influence of BMI?. *International Journal of Obesity*, *31*(6), 987-995. https://doi.org/10.1038/sj.ijo.0803504

Brunstrom, J. M., & Mitchell, G. L. (2006). Effects of distraction on the development of satiety. *British Journal of Nutrition*, *96*(4), 761-769. https://doi.org/10.1079/BJN20061880

Burton, M. J., Rolls, E. T., & Mora, F. (1976). Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. *Experimental neurology*, *51*(3), 668-677. https://doi.org/10.1016/0014-4886(76)90189-8

Cabanac M. (1971) Physiological role of pleasure. *Science*. *173*, 1103–107.

Cannon, W. (1932). *Wisdom of the body*. New York WW Norton & Company. *Inc*, 1, 52.

Capaldi, E. D., Owens, J., & Palmer, K. A. (1994). Effects of food deprivation on learning and expression of flavor preferences conditioned by saccharin or sucrose. *Animal Learning & Behavior*, *22*(2), 173–180. https://doi.org/10.3758/BF03199917

Carnell, S., Haworth, C. M., Plomin, R., & Wardle, J. (2008). Genetic influence on appetite in children. *International journal of obesity*, *32*(10), 1468-1473. https://doi.org/10.1038/ijo.2008.127

Cartoni, E., Balleine, B., & Baldassarre, G. (2016). Appetitive Pavlovian-instrumental transfer: a review. *Neuroscience & Biobehavioral Reviews*, *71*, 829-848. https://doi.org/10.1016/j.neubiorev.2016.09.020

Castellanos, E. H., Charboneau, E., Dietrich, M. S., Park, S., Bradley, B. P., Mogg, K., & Cowan, R. L. (2009). Obese adults have visual attention bias for food cue images: evidence for altered reward system function. *International journal of obesity*, *33*(9), 1063-1073. https://doi.org/10.1038/ijo.2009.138.

Chambers, L., McCrickerd, K., & Yeomans, M. R. (2015). Optimising foods for satiety. *Trends in Food Science & Technology*, *41*(2), 149-160. https://doi.org/10.1016/j.tifs.2014.10.007

Ciria, L. F., Watson, P., Vadillo, M. A., & Luque, D. (2021). Is the habit system altered in individuals with obesity? A systematic review. *Neuroscience & Biobehavioral Reviews. 128*, 621-632. https://doi.org/10.1016/j.neubiorev.2021.07.006

Colagiuri, B., & Lovibond, P. F. (2015). How food cues can enhance and inhibit motivation to obtain and consume food. *Appetite*, *84*, 79-87. https://doi.org/10.1016/j.appet.2014.09.023

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *Journal of Neuroscience*, *25*(4), 962-970. https://doi.org/10.1523/JNEUROSCI.4507-04.2005

Corbit, L. H., Janak, P. H., & Balleine, B. W. (2007). General and outcome-specific forms of Pavlovian-instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. *European Journal of Neuroscience*, *26*(11), 3141-3149. https://doi.org/10.1111/j.1460-9568.2007.05934.x

Cornell, C. E., Rodin, J., & Weingarten, H. (1989). Stimulus-induced eating when satiated. *Physiology & behavior*, *45*(4), 695-704. https://doi.org/10.1016/0031-9384(89)90281-3

Davidson, T. L., & Swithers, S. E. (2004). A Pavlovian approach to the problem of obesity. *International journal of obesity*, *28*(7), 933-935. https://doi.org/10.1038/sj.ijo.0802660

Davidson, T. L., Martin, A. A., Clark, K., & Swithers, S. E. (2011). Intake of high-intensity sweeteners alters the ability of sweet taste to signal caloric consequences: implications for the learned control of energy and body weight regulation. *Quarterly journal of experimental psychology*, *64*(7), 1430-1441. https://doi.org/10.1080%2F17470218.2011.552729

De Tommaso, M., Mastropasqua, T., & Turatto, M. (2018). Working for beverages without being thirsty: Human Pavlovian-instrumental transfer despite outcome devaluation. *Learning and Motivation*, *63*, 37–48. https://doi.org/10.1016/j.lmot.2018.01.001

Delamater, A. R. (2007). Extinction of conditioned flavor preferences. *Journal of Experimental Psychology: Animal Behavior Processes*, *33*(2), 160–171. https://doi.org/10.1037/0097-7403.33.2.160

Delamater, A. R. (2011). Partial reinforcement and latent inhibition effects on stimulus–outcome associations in flavor preference conditioning. *Learning & Behavior*, *39*(3), 259–270. https://doi.org/10.3758/s13420-011-0026-6

Delamater, A. R. (2012). Issues in the extinction of specific stimulus-outcome associations in Pavlovian conditioning. *Behavioural processes*, *90*(1), 9-19. https://doi.org/10.1016/j.beproc.2012.03.006

Delamater, A. R., & Oakeshott, S. (2007). Learning about multiple attributes of reward in Pavlovian conditioning. *Annals of the New York Academy of Sciences*, *1104*(1), 1-20. https://doi.org/10.1196/annals.1390.008

Delamater, A. R., Campese, V., LoLordo, V. M., & Sclafani, A. (2006). Unconditioned stimulus devaluation effects in nutrient-conditioned flavor preferences. *Journal of Experimental Psychology: Animal Behavior Processes*, *32*(3), 295–306. https://doi.org/10.1037/0097-7403.32.3.295

Dickinson, A. (1985). Actions and habits: The development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *308*, 67–78. http://dx.doi.org/10.1098/rstb.1985.0010

Dovey, T. M., Staples, P. A., Gibson, E. L., & Halford, J. C. (2008). Food neophobia and 'picky/fussy' eating in children: a review. *Appetite*, *50*(2-3), 181-193. https://doi.org/10.1016/j.appet.2007.09.009

Dwyer, D. M. (2005). Reinforcer devaluation in palatability-based learned flavor preferences. *Journal of Experimental Psychology: Animal Behavior Processes*, *31*(4), 487. https://doi.org/10.1037/0097-7403.31.4.487

Dwyer, D. M., Mundy, M. E., & Honey, R. C. (2011). The role of stimulus comparison in human perceptual learning: effects of distractor placement. *Journal of Experimental Psychology. Animal Behavior Processes*, *37*(3), 300–307. https://psycnet.apa.org/doi/10.1037/a0023078

Dwyer, D. M., Pincham, H. L., Thein, T., & Harris, J. A. (2009). A learned flavor preference persists despite the extinction of conditioned hedonic reactions to the cue flavors. *Learning & Behavio*r, *37*(4), 305-310. https://doi.org/10.3758/LB.37.4.305

Embling, R., Pink, A. E., Gatzemeier, J., Price, M., Lee, M. D., & Wilkinson, L. L. (2021). Effect of food variety on intake of a meal: A systematic review and meta-analysis. *American Journal of Clinical Nutrition*, *113*(3), 716-741. https://doi.org/10.1093/ajcn/nqaa352

Emond, J. A., Lansigan, R. K., Ramanujam, A., & Gilbert-Diamond, D. (2016). Randomized exposure to food advertisements and eating in the absence of hunger among preschoolers. *Pediatrics*, *138*(6). https://doi.org/10.1542/peds.2016-2361

Epstein, L. H., Paluch, R., & Coleman, K. J. (1996). Differences in salivation to repeated food cues in obese and nonobese women. *Psychosomatic medicine*, *58*(2), 160-164. https://doi/10.1097/00006842-199603000-00011

Epstein, L. H., Robinson, J. L., Roemmich, J. N., Marusewski, A. L., & Roba, L. G. (2010). What constitutes food variety? Stimulus specificity of food. *Appetite*, *54*(1), 23-29. https://doi.org/10.1016/j.appet.2009.09.001

Epstein, L. H., Robinson, J. L., Temple, J. L., Roemmich, J. N., Marusewski, A., & Nadbrzuch, R. (2008). Sensitization and habituation of motivated behavior in overweight and non-overweight children. *Learning and Motivation*, *39*(3), 243–255. https://doi.org/10.1016/j.lmot.2008.03.001

Epstein, L. H., Rodefer, J. S., Wisniewski, L., & Caggiula, A. R. (1992). Habituation and dishabituation of human salivary response. *Physiology & Behavior*, *51*(5), 945–950. https://doi.org/10.1016/0031-9384(92)90075-D

Epstein, L. H., Temple, J. L., Roemmich, J. N., & Bouton, M. E. (2009). Habituation as a determinant of human food intake. *Psychological review*, *116*(2), 384. https://doi.org/10.1037/a0015074

Fanselow, M. S., & Birk, J. (1982). Flavor–flavor associations induce hedonic shifts in taste preference. *Animal Learning & Behavior*, *10*, 223–228. https://doi.org/10.3758/BF03212274

Ferriday, D., & Brunstrom, J. M. (2011). 'I just can't help myself': effects of food-cue exposure in overweight and lean individuals. *International journal of obesity*, *35*(1), 142-149. https://doi.org/10.1038/ijo.2010.117

Furlong, T. M., Jayaweera, H. K., Balleine, B. W., & Corbit, L. H. (2014). Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *Journal of Neuroscience*, *34*(14), 5012-5022. https://doi.org/10.1523/JNEUROSCI.3707-13.2014

Galarce, E. M., Crombag, H. S., & Holland, P. C. (2007). Reinforcer-specificity of appetitive and consummatory behavior of rats after Pavlovian conditioning with food reinforcers. *Physiology & behavior*, *91*(1), 95-105. https://doi.org/10.1016/j.physbeh.2007.01.021

Garcia, J., & Koelling, R.A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic Science 4*, 123–124 https://doi.org/10.3758/BF03342209

Garcia-Burgos, D., Secchiari, F., & Calviño, A. (2015). Is sensory-specific satiety for a bitter-sweet infusion modulated by context?. *Physiology & Behavior*, *140*, 180-187. http://dx.doi.org/10.1016/j.physbeh.2014.12.035

Gibson, E. J. (1963). Perceptual Learning. *Annual Review of Psychology*, *14*(1), 29–56. https://doi.org/10.1146/annurev.ps.14.020163.000333

Gibson, E. L., & Brunstrom, J. M. (2007). Learned influences on appetite, food choice, and intake: Evidence in human beings. *In Appetite and body weight* (pp. 271-300). Academic Press. https://doi.org/10.1016/B978-012370633-1/50011-6

Gil, M., de Brugada, I., & Hall, G. (2021). Motivational factors controlling flavor preference learning and performance: Effects of preexposure with nutritive and nonnutritive sweeteners. *Behavioural Processes*, *191*, 104462. https://doi.org/10.1016/j.beproc.2021.104462

Gil, M., Recio, S. A., de Brugada, I., Symonds, M., & Hall, G. (2014). US-preexposure effects in flavor-preference and flavor-aversion learning with nonnutritive USs. *Behavioural processes*, *106*, 67-73. https://doi.org/10.1016/j.beproc.2014.04.015

Glanzer, M. (1953). Stimulus satiation: an explanation of spontaneous alternation and phenomena. *Psychological Review*, *60* (4), 257–268. https://doi.org/10.1037/h0062718

González, A., Recio, S. A., Sánchez, J., Gil, M., & de Brugada, I. (2018). Effect of exposure to similar flavours in Sensory-Specific Satiety: Implications for eating behaviour. *Appetite*, *127*, 289-295. https://doi.org/10.1016/j.appet.2018.05.015

Griffioen-Roose, S., Finlayson, G., Mars, M., Blundell, J. E., & de Graaf, C. (2010). Measuring food reward and the transfer effect of Sensory-Specific Satiety. *Appetite*, *55*(3), 648-655. https://doi.org/10.1016/j.appet.2010.09.018

Grigutsch, L. A., Lewe, G., Rothermund, K., & Koranyi, N. (2019). Implicit ‘Wanting’ without implicit ‘Liking’: a test of incentive-sensitization-theory in the context of smoking addiction using the Wanting-implicit-association-test (W-IAT). *Journal of Behavior Therapy and Experimental Psychiatry*, *64*, 9-14. https://doi.org/10.1016/j.jbtep.2019.01.002

Guinard, J. X., & Brun, P. (1998). Sensory-specific satiety: comparison of taste and texture effects. *Appetite*, *31*(2), 141-157. https://doi.org/10.1006/appe.1998.0159

Halford, J.C., Gillespie, J., Brown, V., Pontin, E. E., & Dovey, T. M. (2004). Effect of television advertisements for foods on food consumption in children. *Appetite*. *42*(2), 221-5. https://doi.org/10.1016/j.appet.2003.11.006.

Hall, G. (2002). Associative structures in Pavlovian and instrumental conditioning. In R. Gallistel (Ed.), *Stevens' handbook of experimental psychology*: Vol. 3. Learning, motivation, and emotion (pp. 1–45). New York: Wiley.

Hardman, C. A., Ferriday, D., Kyle, L., Rogers, P. J., & Brunstrom, J. M. (2015). So many brands and varieties to choose from: does this compromise the control of food intake in humans?. *PLoS One*, *10*(4), e0125869. https://doi.org/10.1371/journal.pone.0125869

Harris, J. A., Gorissen, M. C., Bailey, G. K., & Westbrook, R. F. (2000). Motivational state regulates the content of learned flavor preferences. *Journal of Experimental Psychology: Animal Behavior Processes*, *26*(1), 15-30. https://psycnet.apa.org/doi/10.1037/0097-7403.26.1.15

Harris, J. A., Shand, F. L., Carroll, L. Q., & Westbrook, R. F. (2004). Persistence of preference for a flavor presented in simultaneous compound with sucrose. *Journal of Experimental Psychology: Animal Behavior Processes*, *30*(3), 177–189. https://doi.org/10.1037/0097-7403.30.3.177

Harris, J. L., Bargh, J. A., & Brownell, K. D. (2009). Priming effects of television food advertising on eating behavior. *Health psychology*, *28*(4), 404. https://doi.org/10.1037/a0014399

Havermans, R. C. (2012). Stimulus specificity but no dishabituation of sensory-specific satiety. *Appetite*, *58*(3), 852-855. https://doi.org/10.1016/j.appet.2012.02.009

Havermans, R. C., & Brondel, L. (2013). Satiety in face of variety: On sensory-specific satiety and perceived food variety. *Food quality and Preference*, *28*(1), 161-163. https://doi.org/10.1016/j.foodqual.2012.07.009

Havermans, R. C., Janssen, T., Giesen, J. C., Roefs, A., & Jansen, A. (2009). Food Liking, food Wanting, and sensory-specific satiety. *Appetite*, *52*(1), 222-225. https://doi.org/10.1016/j.appet.2008.09.020

Havermans, R. C., Roefs, A., Nederkoorn, C., & Jansen, A. (2012). No rapid recovery of sensory-specific satiety in obese women. *Flavor*, *1*(1), 5. https://doi.org/10.1186/2044-7248-1-5

Hendriks, A. E., Havermans, R. C., Nederkoorn, C., & Bast, A. (2019). Exploring the mechanism of within-meal variety and sensory-specific satiation. *Food Quality and Preference*, *78*, 103740. https://doi.org/10.1016/j.foodqual.2019.103740

Hendriks, A. E., Nederkoorn, C., van Lier, I. M., van Belkom, B., Bast, A., & Havermans, R. C. (2021). Sensory-specific satiety, the variety effect and physical context: Does change of context during a meal enhance food intake?. *Appetite, 163*, 105179. https://doi.org/10.1016/j.appet.2021.105179

Hendrikse, J. J., Cachia, R. L., Kothe, E. J., McPhie, S., Skouteris, H., & Hayden, M. J. (2015). Attentional biases for food cues in overweight and individuals with obesity: a systematic review of the literature. *Obesity reviews*, *16*(5), 424-432. https://doi.org/10.1111/obr.12265

Hendriks-Hartensveld, A. E., Brodock, J. L., Hayes, J. E., Rolls, B. J., Keller, K. L., & Havermans, R. C. (2022). The relative importance of complexity, variety, and portion size in ice cream preference in Dutch and American participants. *Food Quality and Preference*, *99* 104523. https://doi.org/10.1016/j.foodqual.2021.104523

Hetherington, M. (1996). Sensory-specific satiety and its importance in meal termination. *Neuroscience & Biobehavioral Reviews*. *20*(1), 113–117. https://doi.org/10.1016/0149-7634(95)00048-J

Hetherington, M. M., & Rolls, B. J. (1996). Sensory-specific satiety: Theoretical frameworks and central characteristics. In E. D. Capaldi (Ed.), *Why we eat what we eat: The psychology of eating* (pp. 267–290). American Psychological Association. https://doi.org/10.1037/10291-010

Hetherington, M. M., Foster, R., Newman, T., Anderson, A. S., & Norton, G. (2006). Understanding variety: tasting different foods delays satiation. *Physiology & behavior*, *87*(2), 263-271. https://doi.org/10.1016/j.physbeh.2005.10.012

Hetherington, M., & Havermans, R. C. (2013). Sensory-specific satiation and satiety. *In Satiation, satiety and the control of food intake* (pp. 253-269). Woodhead Publishing. https://doi.org/10.1533/9780857098719.4.253

Hetherington, M., & Rolls, B. J. (1989). Sensory-specific satiety in anorexia and bulimia nervosa. *Annals of the New York Academy of Sciences*, *575*(1), 387-398. https://doi.org/10.1111/j.1749-6632.1989.tb53259.x

Hetherington, M., Rolls, B. J., & Burley, V. J. (1989). The time course of sensory-specific satiety. *Appetite*, 12(1), 57-68. https://doi.org/10.1016/0195-6663(89)90068-8

Higgs, S., Williamson, A. C., Rotshtein, P., & Humphreys, G. W. (2008). Sensory-specific satiety is intact in amnesics who eat multiple meals. *Psychological Science*, *19*(7), 623-628. https://doi.org/10.1111/j.1467-9280.2008.02132.x

Holland, P. C. (1981). The effects of satiation after first—and second-order appetitive conditioning in rats. *The Pavlovian journal of biological science: official journal of the Pavlovian*, *16*(1), 18-24. https://doi.org/10.1007/BF03001266

Holland, P. C. (2005). Amount of training effects in representation mediated food aversion learning: No evidence of a role for associability changes. *Learning & Behavior*, *33*, 464-478. https://doi.org/10.3758/BF03193185

Holland, P. C., & Gallagher, M. (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *European Journal of Neuroscience*, *17*(8), 1680-1694. https://doi.org/10.1046/j.1460-9568.2003.02585.x

Holland, P. C., & Rescorla, R. A. (1975). The effect of two ways of devaluing the unconditioned stimulus after first- and second-order appetitive conditioning. J*ournal of Experimental Psychology: Animal Behavior Processes*, *1*(4), 355–363. https://doi.org/10.1037/0097-7403.1.4.355

Holland, P. C., Lasseter, H. & Agarwal, I. (2008) Amount of training and cue-evoked taste-reactivity responding in reinforcer devaluation. *Jornal of Experimental Psychology. Animal Behavior Process. 34*, 119–132. https://doi.org/10.1037/0097-7403.34.1.119

Holmes, N. M., Hutton-Bedbrook, K., Fam, J., & Westbrook, R. F. (2016). Incentive contrast effects regulate responding to a flavor presented in compound with a saccharin unconditioned stimulus in rats. *Journal of Experimental Psychology: Animal Learning and Cognition*, *42*(3), 233. https://doi.org/10.1037/xan0000101

Holmes, N. M., Marchand, A. R., & Coutureau, E. (2010). Pavlovian to instrumental transfer: a neurobehavioral perspective. *Neuroscience & Biobehavioral Reviews*, *34*(8), 1277-1295. https://doi.org/10.1016/j.neubiorev.2010.03.007

Honey, R. C., Bateson, P., & Horn, G. (1994). The role of stimulus comparison in perceptual learning: an investigation with the domestic chick. *The Quarterly Journal of Experimental Psychology. B, Comparative and Physiological Psychology*, *47*(1), 83–103. https://doi.org/10.1080/14640749408401349

Jansen, A. (1998). A learning model of binge eating: cue reactivity and cue exposure. *Behaviour research and therapy*, *36*(3), 257-272. https://doi.org/10.1016/S0005-7967(98)00055-2

Jansen, A., Schyns, G., Bongers, P., & van den Akker, K. (2016). From lab to clinic: extinction of cued cravings to reduce overeating. *Physiology & Behavior*, *162*, 174-180. https://doi.org/10.1016/j.physbeh.2016.03.018

Jansen, A., Stegerman, S., Roefs, A., Nederkoorn, C., & Havermans, R. (2010). Decreased salivation to food cues in formerly obese successful dieters. *Psychotherapy and psychosomatics*, *79*(4), 257. https://doi.org/10.1159/000315131

Jansen, A., Theunissen, N., Slechten, K., Nederkoorn, C., Boon, B., Mulkens, S., & Roefs, A. (2003). Overweight children overeat after exposure to food cues. *Eating behaviors*, *4*(2), 197-209. https://doi.org/10.1016/S1471-0153(03)00011-4

Johnson, A. W. (2013). Eating beyond metabolic need: how environmental cues influence feeding behavior. *Trends in neurosciences*, *36*(2), 101-109. https://doi.org/10.1016/j.tins.2013.01.002

Johnson, J., & Vickers, Z. (1992). Factors influencing sensory-specific satiety. *Appetite*, *19*(1), 15-31. https://doi.org/10.1016/0195-6663(92)90233-V

Joyner, M. A., Kim, S., & Gearhardt, A. N. (2017). Investigating an incentive-sensitization model of eating behavior: impact of a simulated fast-food laboratory. *Clinical Psychological Science, 5*(6), 1014-1026. https://doi.org/10.1177%2F2167702617718828

Katz D. (1935). Some fundamental laws of the psychology of needs: hunger. *Journal of Personality*. *3*, 312–26.

Kendig, M. D., Boakes, R. A., & Corbit, L. H. (2018). Variety overcomes the specificity of cue-potentiated feeding in rats. *Journal of Experimental Psychology: Animal Learning and Cognition, 44*(1), 56. https://doi.org/10.1037/xan0000159

Kendig, M. D., Cheung, A. M., Raymond, J. S., & Corbit, L. H. (2016). Contexts paired with junk food impair goal-directed behavior in rats: implications for decision making in obesogenic environments. *Frontiers in behavioral neuroscience*, *10*, 216. https://doi.org/10.3389/fnbeh.2016.00216

Kerfoot, E. C., Agarwal, I., Lee, H. J., & Holland, P. C. (2007). Control of appetitive and aversive taste-reactivity responses by an auditory conditioned stimulus in a devaluation task: a FOS and behavioral analysis. *Learning & memory, 14*(9), 581-589. https://doi.org/10.1101/lm.627007

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex*, *13*(4), 400-408. https://doi.org/10.1093/cercor/13.4.400

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.

Koranyi, N., Brückner, E., Jäckel, A., Grigutsch, L. A., & Rothermund, K. (2020). Dissociation between Wanting and Liking for coffee in heavy drinkers. *Journal of Psychopharmacology*, *34*(12), 1350-1356. https://doi.org/10.1177/0269881120922960

Lalanza, J. F., & Snoeren, E. M. (2021). The cafeteria diet: a standardized protocol and its effects on behavior. *Neuroscience & Biobehavioral Reviews*, *122*, 92-119. https://doi.org/10.1016/j.neubiorev.2020.11.003

Lavis, Y., Kadib, R., Mitchell, C., & Hall, G. (2011). Memory for, and salience of, the unique features of similar stimuli in perceptual learning. *Journal of Experimental Psychology. Animal Behavior Processes*, *37*(2), 211–9. https://doi.org/10.1037/a0021888

Le Magnen, J. (1955). Sur le mecanisme detablissement des appetits caloriques. *Comptes rendus hebdomadaires des seances de l 'academie des sciences, 240*(25), 2436-2438.

Le Magnen, J. (1956). Le rôle des stimulations olfacto-gustatives dans les mécanismes de régulation de la prise alimentaire. *In Annales de la nutrition et de l'alimentation* (pp. 153-188). Centre national de la recherche scientifique.

Le Magnen, J. (1999). Role of dietary odour in the short-term control of intake in the white rat (first published in French in 1956). *Appetite*, *33*(1), 30-32 https://doi.org/10.1006/appe.1999.0256

Le Magnen, J. (2001). My scientific life: 40 years at the College de France. *Neuroscience & Biobehavioral Reviews*, *25*(5), 375-394. https://doi.org/10.1016/S0149-7634(01)00020-3

Leigh, S. J., Lee, F., & Morris, M. J. (2018). Hyperpalatability and the generation of obesity: roles of environment, stress exposure and individual difference. *Current obesity reports*, *7*(1), 6-18. https://doi.org/10.1007/s13679-018-0292-0

LeMon, J. V., Sisk, C. L., Klump, K. L., & Johnson, A. W. (2019). Reduced sensitivity to devaluation for instrumental but not consummatory behaviors in binge eating prone rats. *Physiology & behavior*, *206*, 13-21. https://doi.org/10.1016/j.physbeh.2019.03.005

Llewellyn, C., & Wardle, J. (2015). Behavioral susceptibility to obesity: Gene-environment interplay in the development of weight. *Physiology & Behavior*, *152*, 494-501. http://dx.doi.org/10.1016/j.physbeh.2015.07.006

Lowe, M. R., & Butryn, M. L. (2007). Hedonic hunger: a new dimension of appetite? *Physiology & behavior*, *91*(4), 432-439. https://doi.org/10.1016/j.physbeh.2007.04.006

Marshall, A. T., Halbout, B., Liu, A. T., & Ostlund, S. B. (2018). Contributions of Pavlovian incentive motivation to cue-potentiated feeding. *Scientific reports*, *8*(1), 1-13. https://doi.org/10.1038/s41598-018-21046-0

Martin, A. A. (2016). Why can't we control our food intake? The downside of dietary variety on learned satiety responses. *Physiology & behavior*, *162*, 120-129. https://doi.org/10.1016/j.physbeh.2016.04.010

Mayer, J. (1955). Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. *Annals of the New York Academy of sciences*, *63*(1), 15-43. https://doi.org/10.1111/j.1749-6632.1955.tb36543.x

McCrickerd, K., & Forde, C. G. (2016). Sensory influences on food intake control: Moving beyond palatability. *Obesity Reviews*, *17*(1), 18-29. https://doi.org/10.1111/obr.12340

McCrory, M. A., Fuss, P. J., McCallum, J. E., Yao, M., Vinken, A. G., Hays, N. P., & Roberts, S. B. (1999). Dietary variety within food groups: association with energy intake and body fatness in men and women. *The American journal of clinical nutrition*, *69*(3), 440-447. https://doi.org/10.1093/ajcn/69.3.440

McCrory, M.A, Burke, A. & Roberts, S.B, (2012). Dietary (sensory) variety and energy balance. *Physiology & Behaviour*. *107*(4), 576-83. https://doi.org/10.1016/j.physbeh.2012.06.012

Meengs, J., Roe, L. and Rolls, B. (2012). Vegetable Variety: An Effective Strategy to Increase Vegetable Intake in Adults. *Journal of the Academy of Nutrition and Dietetics*, *112*(8), 1211-1215. https://doi.org/10.1016/j.jand.2012.05.013

Meillon, S., Thomas, A., Havermans, R., Pénicaud, L., & Brondel, L. (2013). Sensory-specific satiety for a food is unaffected by the ad libitum intake of other foods during a meal. Is SSS subject to dishabituation? *Appetite*, *63*, 112-118. https://doi.org/10.1016/j.appet.2012.12.004

Miller, D. L., Bell, E. A., Pelkman, C. L., Peters, J. C., & Rolls, B. J. (2000). Effects of dietary fat, nutrition labels, and repeated consumption on sensory-specific satiety. *Physiology & behavior*, *71*(1-2), 153-158. https://doi.org/10.1016/S0031-9384(00)00319-X

Mitchell, C., & Hall, G. (2014). Can theories of animal discrimination explain perceptual learning in humans? *Psychological Bulletin*, *140*(1), 283-307. https://doi.org/10.1037/a0032765

Mitchell, C., Kadib, R., Nash, S., Lavis, Y., & Hall, G. (2008). Analysis of the role of associative inhibition in perceptual learning by means of the same-different task. *Journal of Experimental Psychology. Animal Behavior Processes*, *34*(4), 475–85. https://doi.org/10.1037/0097-7403.34.4.475

Mondragón, E., & Murphy, R. A. (2010). Perceptual learning in an appetitive Pavlovian procedure: Analysis of the effectiveness of the common element. *Behavioural Processes*, *83*, 247–256. https://doi.org/10.1016/j.beproc.2009.12.007

Morales, I., & Berridge, K. C. (2020). 'Liking'and 'wanting'in eating and food reward: Brain mechanisms and clinical implications. *Physiology & behavior*, *227,* 113152. https://doi.org/10.1016/j.physbeh.2020.113152

Mundy, M. E., Dwyer, D. M., & Honey, R. C. (2006). Inhibitory associations contribute to perceptual learning in humans. *Journal of Experimental Psychology. Animal Behavior Processes*, *32*(2), 178–84. https://doi.org/10.1037/0097-7403.32.2.178

Mundy, M. E., Honey, R. C., & Dwyer, D. M. (2007). Simultaneous presentation of similar stimuli produces perceptual learning in human picture processing. *Journal of Experimental Psychology. Animal Behavior Processes*, *33*(2), 124–38. https://doi.org/10.1037/0097-7403.33.2.124

Myers, K. P. (2017). Sensory-specific satiety is intact in rats made obese on a high-fat high-sugar choice diet. *Appetite*, *112*, 196-200. https://doi.org/10.1016/j.appet.2017.01.013

Myers, K. P. (2018). The convergence of psychology and neurobiology in flavor-nutrient learning. *Appetite*, *122*, 36-43. https://doi.org/10.1016/j.appet.2017.03.048

Myers, K. P., & Sclafani, A. (2001). Conditioned enhancement of flavor evaluation reinforced by intragastric glucose: I. Intake acceptance and preference analysis. *Physiology & Behavior*, *74*, 481-493. https://doi.org/10.1016/S0031-9384(01)00595-9

Myers, K. P., & Sclafani, A. (2003). Conditioned acceptance and preference but not altered taste reactivity responses to bitter and sour flavors paired with intragastric glucose infusion. *Physiology & behavior*, *78(*2), 173-183. https://doi.org/10.1016/S0031-9384(02)00890-9

Nederkoorn, C., Smulders, F. T. Y., & Jansen, A. (2000). Cephalic phase responses, craving and food intake in normal subjects. *Appetite*, *35*(1), 45-55. https://doi.org/10.1006/appe.2000.0328

Nolan, L. J., & Hetherington, M. M. (2009). The effects of sham feeding-induced sensory specific satiation and food variety on subsequent food intake in humans. *Appetite*, *52*(3), 720-725. https://doi.org/10.1016/j.appet.2009.03.012

Norton, G. N.M., Anderson, A. S. & Hetherington, M. M. (2006) Volume and variety: relative effects on food intake. *Physiology & Behavior*, *87*, 714–722 https://doi.org/10.1016/j.physbeh.2006.01.010

Nummenmaa, L., Hietanen, J. K., Calvo, M. G., & Hyönä, J. (2011). Food catches the eye but not for everyone: A BMI–contingent attentional bias in rapid detection of nutriments. *PLoS One*, *6*(5), e19215. https://doi.org/10.1371/journal.pone.0019215

Oldham-Cooper, R. E., Hardman, C. A., Nicoll, C. E., Rogers, P. J., & Brunstrom, J. M. (2011). Playing a computer game during lunch affects fullness, memory for lunch, and later snack intake. *The American journal of clinical nutrition*, *93*(2), 308-313. https://doi.org/10.3945/ajcn.110.004580

Palframan, K. M., & Myers, K. P. (2016). Modern 'junk food' and minimally-processed 'natural food' cafeteria diets alter the response to sweet taste but do not impair flavor-nutrient learning in rats. *Physiology & behavior*, *157*, 146-157. https://doi.org/10.1016/j.physbeh.2016.01.010

Parkes, S. L., Furlong, T. M., Black, A. D., & Balleine, B. W. (2017). Intermittent feeding alters sensitivity to changes in reward value. *Appetite*, *113*, 1-6. https://doi.org/10.1016/j.appet.2017.02.009

Parkes, S. L., Marchand, A. R., Ferreira, G., & Coutureau, E. (2016). A time course analysis of satiety-induced instrumental outcome devaluation. *Learning & behavior*, *44*(4), 347-355. https://doi.org/10.3758/s13420-016-0226-1

Parkes, S. L., Ravassard, P. M., Cerpa, J. C., Wolff, M., Ferreira, G., & Coutureau, E. (2018). Insular and ventrolateral orbitofrontal cortices differentially contribute to goal-directed behavior in rodents. *Cerebral Cortex*, *28*(7), 2313-2325. https://doi.org/10.1093/cercor/bhx132

Pavlov, I. P. (1927). *Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex*. Oxford Univ. Press.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological review*, *87*(6), 532. https://doi.org/10.1037/0033-295X.87.6.532

Pelchat, M. L., Grill, H. J., Rozin, P., & Jacobs, J. (1983). Quality of acquired responses to tastes by Rattus norvegicus depends on type of associated discomfort. *Journal of Comparative Psychology*, *97*(2), 140-153. https://doi.org/10.1037/0735-7036.97.2.140

Pepino M.Y., & Mennella J.A. (2012). Habituation to the pleasure elicited by sweetness in lean and obese women. *Appetite*. *58*(3), 800-805. https://doi.org/10.1016/j.appet.2012.01.026

Pérez, C., Fanizza, L. J., & Sclafani, A. (1999). Flavor preferences conditioned by intragastric nutrient infusions in rats fed chow or a cafeteria diet. *Appetite*, *32*(1), 155-170. https://doi.org/10.1006/appe.1998.0182

Pérez, C., Lucas, F., & Sclafani, A. (1998). Increased flavor acceptance and preference conditioned by the postingestive actions of glucose. *Physiology & behavior*, *64*(4), 483-492. https://doi.org/10.1016/S0031-9384(98)00104-8

Petrovich, G. D. (2013). Forebrain networks and the control of feeding by environmental learned cues. *Physiology & behavior*, *121*, 10-18. https://doi.org/10.1016/j.physbeh.2013.03.024

Petrovich, G. D., Ross, C. A., Gallagher, M., & Holland, P. C. (2007). Learned contextual cue potentiates eating in rats. *Physiology & behavior*, *90*(2-3), 362-367. https://doi.org/10.1016/j.physbeh.2006.09.031

Pool, E. R., Pauli, W. M., Kress, C. S., & O'Doherty, J. P. (2019). Behavioural evidence for parallel outcome-sensitive and outcome-insensitive Pavlovian learning systems in humans. *Nature human behaviour*, *3*(3), 284-296. https://doi.org/10.1038/s41562-018-0527-9

Pool, E., Brosch, T., Delplanque, S., & Sander, D. (2014). Where is the chocolate? Rapid spatial orienting toward stimuli associated with primary rewards. *Cognition*, *130*(3), 348-359. https://doi.org/10.1016/j.cognition.2013.12.002

Power, M. L., & Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: cephalic phase responses. *Appetite*, *50*(2-3), 194-206. https://doi.org/10.1016/j.appet.2007.10.006

Prados, J., Artigas, A. A., & Sansa, J. (2007). Preexposure effects in the spatial domain: Dissociation between latent inhibition and perceptual learning. *Journal of Experimental Psychology: Animal Behavior Processes*, *33* (2), 115–123. https://doi.org/10.1037/0097-7403.33.2.115

Ramaekers, M., Boesveldt, S., Gort, G., Lakemond, C., van Boekel, M. & Luning, P. (2014). Sensory-specific appetite is affected by actively smelled food odors and remains stable over time in normal-weight women. *The Journal of Nutrition*, *144*(8), 1314-1319. https://doi.org/10.3945/jn.114.192567

Ramaekers, M.G., Luning, P.A., Lakemond, C.M., van Boekel1, M.A., Gort, G. & Boesveldt, S. (2016). Food preference and appetite after switching between sweet and savoury odours in women. *PLoS One, 11*(1), e0146652 https://doi.org/10.1371/journal.pone.0146652

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C., & McSweeney, F. K. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiology of learning and memory*, *92*(2), 135-138. https://doi.org/10.1016/j.nlm.2008.09.012

Raynor, H. A., & Epstein, L. H. (2001). Dietary variety, energy regulation, and obesity. *Psychological Bulletin*, *127*(3), 325–341. https://doi.org/10.1037/0033-2909.127.3.325

Raynor, H. A., Niemeier, H. M., & Wing, R. R. (2006). Effect of limiting snack food variety on long-term sensory-specific satiety and monotony during obesity treatment. *Eating behaviors*, *7*(1), 1-14. https://doi.org/10.1016/j.eatbeh.2005.05.005

Raynor, H., & Wing, R. (2006). Effect of limiting snack food variety across days on hedonics and consumption. *Appetite*, *46*(2), 168–176. https://doi.org/10.1016/j.appet.2005.12.001

Recio, S. A., Iliescu, A. F., & de Brugada, I. (2018). The role of stimulus comparison in animal perceptual learning: Effects of distractor placement. *The Quarterly Journal of Experimental Psychology. 71*(12), 2488-2496. https://doi.org/10.1177/1747021818757101

Recio, S. A., Iliescu, A. F., Honey, R. C., & de Brugada, I. (2016). Perceptual learning in flavor preference conditioning: Restricting generalization of acquired preferences between flavors. *Learning and Motivation*, *56*, 31–37. https://doi.org/10.1016/j.lmot.2016.09.001

Recio, S. A., Iliescu, A. F., Mingorance, S. P., Bergés, G. D., Hall, G., & de Brugada, I. (2016). The role of instructions in perceptual learning using complex visual stimuli. *Journal of Experimental Psychology: Animal Learning and Cognition*, *42*(4), 359–365. https://doi.org/10.1037/xan0000113

Reichelt, A. C., Morris, M. J., & Westbrook, R. F. (2014). Cafeteria diet impairs expression of sensory-specific satiety and stimulus-outcome learning. *Frontiers in psychology*, *5*, 852. https://doi.org/10.3389/fpsyg.2014.00852

Reppucci, C. J., & Petrovich, G. D. (2012). Learned food-cue stimulates persistent feeding in sated rats. *Appetite, 59*(2), 437-447. https://doi.org/10.1016/j.appet.2012.06.007

Rescorla, R. A. (1988). Pavlovian conditioning: It's not what you think it is. *American Psychologist*, *43*(3), 151–160. https://doi.org/10.1037/0003-066X.43.3.151

Riordan, J. E., & Dwyer, D. M. (2019). Licking microstructure and hedonic changes after flavour preference learning in rats. *Quarterly Journal of Experimental Psychology*, *72*(12), 2717-2725. https://doi.org/10.1177%2F1747021819857052

Robinson, T. E., & Berridge, K. C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research. Reviews*. *18*, 247–291. https://doi.org/10.1016/0165-0173(93)90013-P

Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1507), 3137-3146. https://doi.org/10.1098/rstb.2008.0093

Roe, L. S., Meengs, J. S., Birch, L. L., & Rolls, B. J. (2013). Serving a variety of vegetables and fruit as a snack increased intake in preschool children. *The American journal of clinical nutrition*, *98*(3), 693-699. https://doi.org/10.3945/ajcn.113.062901

Rogers, P. J. (1999). Eating habits and appetite control: a psychobiological perspective. *Proceedings of the Nutrition Society*, *58*(1), 59-67. https://doi.org/10.1079/PNS19990009

Rogers, P. J., Ferriday, D., Irani, B., Hoi, J. K. H., England, C. Y., Bajwa, K. K., & Gough, T. (2020). Sweet satiation: Acute effects of consumption of sweet drinks on appetite for and intake of sweet and non-sweet foods. *Appetite*, *149*, 104631. https://doi.org/10.1016/j.appet.2020.104631

Rolls B. J. (1986). Sensory-specific satiety. *Nutrition reviews*, *44*(3), 93–101. https://doi.org/10.1111/j.1753-4887.1986.tb07593.x

Rolls, B. J., Andersen, A. E., Moran, T. H., McNelis, A. L., Baier, H. C., & Fedoroff, I. C. (1992). Food intake, hunger, and satiety after preloads in women with eating disorders. *The American journal of clinical nutrition*, *55*(6), 1093-1103. https://doi.org/10.1093/ajcn/55.6.1093

Rolls, B. J., Hetherington, M., & Burley, V. J. (1988). Sensory stimulation and energy density in the development of satiety. *Physiology & behavior*, *44*(6), 727-733. https://doi.org/10.1016/0031-9384(88)90053-4

Rolls, B. J., Rolls, E. T., Rowe, E. A., & Sweeney, K. (1981). Sensory-Specific Satiety in man. *Physiology & behavior, 27*(1), 137-142. https://doi.org/10.1016/0031-9384(81)90310-3

Rolls, B. J., Rowe, E. A., & Rolls, E. T. (1982). How sensory properties of foods affect human feeding behavior. *Physiology & Behavior*, *29*(3), 409-417. https://doi.org/10.1016/0031-9384(82)90259-1

Rolls, B. J., Rowe, E. A., Rolls, E. T., Kingston, B., Megson, A., & Gunary, R. (1981). Variety in a meal enhances food intake in man. *Physiology & behavior*, *26*(2), 215-221. https://doi.org/10.1016/0031-9384(81)90014-7

Rolls, B. J., Van Duijvenvoorde, P. M., & Rolls, E. T. (1984). Pleasantness changes and food intake in a varied four-course meal. *Appetite*, *5*(4), 337–348. http://dx.doi.org/10.1016/S0195-6663(84)80006-9.

Rolls, B. J., Van Duijvenvoorde, P. M., & Rowe, E. A. (1983). Variety in the diet enhances intake in a meal and contributes to the development of obesity in the rat. *Physiology & Behavior*, *31*(1), 21-27. https://doi.org/10.1016/0031-9384(83)90091-4

Rolls, E. T. (2005). Taste, olfactory, and food texture processing in the brain, and the control of food intake. *Physiology & behavior*, *85*(1), 45-56. https://doi.org/10.1016/j.physbeh.2005.04.012

Rolls, E. T., & De Waal, A. W. L. (1985). Long-term sensory-specific satiety: evidence from an Ethiopian refugee camp. *Physiology & Behavior*, *34*(6), 1017-1020. https://doi.org/10.1016/0031-9384(85)90032-0

Rolls, E. T., & Rolls, J. H. (1997). Olfactory sensory-specific satiety in humans. *Physiology & Behavior*, *61*(3), 461-473. https://doi.org/10.1016/S0031-9384(96)00464-7

Rolls, E. T., Burton, M. J., & Mora, F. (1976). Hypothalamic neuronal responses associated with the sight of food. *Brain research, 111*(1), 53-66. https://doi.org/10.1016/0006-8993(76)91048-9

Rolls, E. T., Burton, M. J., & Mora, F. (1980). Neurophysiological analysis of brain-stimulation reward in the monkey. *Brain research, 194*(2), 339-357. https://doi.org/10.1016/0006-8993(80)91216-0

Rolls, E. T., Murzi, E., Yaxley, S., Thorpe, S. J., & Simpson, S. J. (1986). Sensory-specific satiety: food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. *Brain research, 368*(1), 79-86. https://doi.org/10.1016/0006-8993(86)91044-9

Romer, M., Lehrner, J., Van Wymelbeke, V., Jiang, T., Deecke, L., & Brondel, L. (2006). Does modification of olfacto-gustatory stimulation diminish sensory-specific satiety in humans?. *Physiology & behavior*, *87*(3), 469-477. https://doi.org/10.1016/j.physbeh.2005.11.015

Rossi, M. A., & Stuber, G. D. (2018). Overlapping brain circuits for homeostatic and hedonic feeding. *Cell metabolism*, *27*(1), 42-56. https://doi.org/10.1016/j.cmet.2017.09.021

Roy, S. L., Davidson, T. L., & Swithers, S. E. (2007). The effects of high-intensity sweeteners (saccharin and acesulfame potassium) on food intake and body weight regulation in rats. *Appetite*, *49*(1), 325. https://doi.org/10.1016/j.appet.2007.03.171

Rozin, P. (1976). Psychobiological and cultural determinants of food choice. In T. Silverstone (Ed.), *Appetite and food intake*: *Report of the Dahlem Workshop*. Abakon Verlagsgesellschaft.

Russell, S. J., Croker, H., & Viner, R. M. (2019). The effect of screen advertising on children's dietary intake: A systematic review and meta-analysis. *Obesity reviews*, *20*(4), 554-568. https://doi.org/10.1111/obr.12812

Schaal, B., Marlier, L., & Soussignan, R. (2000). Human foetuses learn odours from their pregnant mother’s diet. *Chemical senses*, *25*(6), 729-737. https://doi.org/10.1093/chemse/25.6.729

Schachter, S. (1971). Some extraordinary facts about obese humans and rats. *American Psychologist*, *26*(2), 129. https://doi.org/10.1037/h0030817

Sclafani, A. (1991). Conditioned food preferences. *Bulletin of the Psychonomic Society*, *29*(2), 256-260. https://doi.org/10.3758/BF03335250

Sclafani, A. (2018). From appetite setpoint to appetition: 50 years of ingestive behavior research. *Physiology & behavior*, *192*, 210-217. https://doi.org/10.1016/j.physbeh.2018.01.001

Sclafani, A., & Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiology & behavior*, *17*(3), 461-471. https://doi.org/10.1016/0031-9384(76)90109-8

Smeets, A. J., & Westerterp-Plantenga, M. S. (2006). Oral exposure and sensory-specific satiety. *Physiology & behavior*, *89*(2), 281-286. https://doi.org/10.1016/j.physbeh.2006.06.011

Snoek, H. M., Huntjens, L., Van Gemert, L. J., De Graaf, C. & Weenen, H. (2004). Sensory-specific satiety in obese and normal-weight women. *The American Journal of Clinical Nutrition*, *80*(4), 823-831. https://doi.org/10.1016/j.appet.2016.08.123

Steinfeld, M. R., & Bouton, M. E. (2021). Renewal of goal direction with a context change after habit learning. *Behavioral Neuroscience*, *135*(1), 79-87. https://doi.org/10.1037/bne0000422

Stellar, E. (1954). The physiology of motivation. *Psychological review*, *61*(1), 5. https://doi.org/10.1037/h0060347

Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., Moodie, M. L., & Gortmaker, S. L. (2011). The global obesity pandemic: shaped by global drivers and local environments. *The Lancet*, *378*(9793), 804-814. https://doi.org/10.1016/S0140-6736(11)60813-1

Swinburn, B., Egger, G., & Raza, F. (1999). Dissecting obesogenic environments: the development and application of a framework for identifying and prioritizing environmental interventions for obesity. *Preventive medicine*, *29*(6), 563-570. https://doi.org/10.1006/pmed.1999.0585

Swithers, S. E. & Hall, W. G. (1994) Does oral experience terminate ingestion? *Appetite*, *23*(2),113-138. https://doi.org/10.1006/appe.1994.1041

Swithers, S. E., & Davidson, T. L. (2008). A role for sweet taste: Calorie predictive relations in energy regulation by rats. *Behavioral Neuroscience*, *122*(1), 161–173. https://doi.org/10.1037/0735-7044.122.1.161

Swithers, S. E., Baker, C. R., & Davidson, T. L. (2009). General and persistent effects of high-intensity sweeteners on body weight gain and caloric compensation in rats. *Behavioral Neuroscience*, *123*(4), 772. https://doi.org/10.1037/a0016139

Swithers, S. E., Sample, C. H., & Davidson, T. L. (2013). Adverse effects of high-intensity sweeteners on energy intake and weight control in male and obesity-prone female rats. *Behavioral Neuroscience*, *127*(2), 262-274. https://doi.org/10.1037/a0031717

Symonds, M., & Hall, G. (1995). Perceptual learning in flavor aversion conditioning: Roles of stimulus comparison and latent inhibition of common stimulus elements. *Learning and Motivation*, *26*, 203–219. https://doi.org/10.1016/0023-9690(95)90005-5

Tepper, B. J. (1992). Dietary restraint and responsiveness to sensory-based food cues as measured by cephalic phase salivation and Sensory-Specific Satiety. *Physiology & Behavior*, *52*(2), 305-311. https://doi.org/10.1016/0031-9384(92)90276-8

Tetley, A., Brunstrom, J., & Griffiths, P. (2009). Individual differences in food-cue reactivity. The role of BMI and everyday portion-size selections. *Appetite*, *52*(3), 614-620. https://doi.org/10.1016/j.appet.2009.02.005

Thompson, R. F., & Spencer, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychological review*, *73*(1), 16. https://doi/10.1037/h0022681

Thorndike, E.L. (1911) *Animal intelligence: Experimental studies.* New York: Macmilan.

Thrailkill, E. A., & Bouton, M. E. (2015). Contextual control of instrumental actions and habits. *Journal of Experimental Psychology: Animal Learning and Cognition*, *41*(1), 69-80. https://doi.org/10.1037/xan0000045

Thrailkill, E. A., Michaud, N. L., & Bouton, M. E. (2021). Reinforcer predictability and stimulus salience promote discriminated habit learning. Journal of Experimental Psychology: *Animal Learning and Cognition*, *47*(2), 183–199. https://doi.org/10.1037/xan0000285

Thrailkill, E. A., Trask, S., Vidal, P., Alcalá, J. A., & Bouton, M. E. (2018). Stimulus control of actions and habits: A role for reinforcer predictability and attention in the development of habitual behavior.

Journal of Experimental Psychology: Animal Learning and Cognition, *44*(4), 370. https://doi.org/10.1037/xan0000188

Toates, F. M. (1981). The control of ingestive behavior by internal and external stimuli—A theoretical review. *Appetite*, *2*(1), 35-50. https://doi.org/10.1016/S0195-6663(81)80035-9

Treit, D., Spetch, M. L. & Deutsch, J. A. (1983). Variety in the flavor of food enhances eating in the rat: a controlled demonstration. *Physiology & Behavior*, *30*(2), 207-211 http://doi.org/10.1016/0031-9384(83)90007-0.

Tricomi, E., Balleine, B. W., & O'Doherty, J. P. (2009). A specific role for posterior dorsolateral striatum in human habit learning. *European Journal of Neuroscience*, *29*(11), 2225-2232. https://doi.org/10.1111/j.1460-9568.2009.06796.x

Valentin, V. V., Dickinson, A., & O'Doherty, J. P. (2007). Determining the neural substrates of goal-directed learning in the human brain. *Journal of Neuroscience*, *27*(15), 4019-4026. https://doi.org/10.1523/JNEUROSCI.0564-07.2007

van den Akker, K., Havermans, R. C., & Jansen, A. (2015). Effects of occasional reinforced trials during extinction on the reacquisition of conditioned responses to food cues. *Journal of Behavior Therapy and Experimental Psychiatry*, *48*, 50-58. https://doi.org/10.1016/j.jbtep.2015.02.001

van den Akker, K., Havermans, R. C., Bouton, M. E., & Jansen, A. (2014). How partial reinforcement of food cues affects the extinction and reacquisition of appetitive responses. A new model for dieting success?. *Appetite*, *81*, 242-252. https://doi.org/10.1016/j.appet.2014.06.024

van den Akker, K., Schyns, G., & Jansen, A. (2018). Learned overeating: applying principles of pavlovian conditioning to explain and treat overeating. *Current addiction reports*, *5*(2), 223-231. https://doi.org/10.1007/s40429-018-0207-x

van den Akker, K., Stewart, K., Antoniou, E. E., Palmberg, A., & Jansen, A. (2014). Food cue reactivity, obesity, and impulsivity: are they associated?. *Current Addiction Reports*, *1*(4), 301-308. https://doi.org/10.1007/s40429-014-0038-3

Verhoeven, A. A., Watson, P., & de Wit, S. (2018). Failing to pay heed to health warnings in a food-associated environment. *Appetite*, *120*, 616-626. https://doi.org/10.1016/j.appet.2017.10.020

Wald, H. S., & Myers, K. P. (2015). Enhanced flavor–nutrient conditioning in obese rats on a high-fat, high-carbohydrate choice diet. *Physiology & behavior*, *151*, 102-110. https://doi.org/10.1016/j.physbeh.2015.07.002

Warwick, Z. S., & Weingarten, H. P. (1994). Dissociation of palatability and calorie effects in learned flavor preferences. *Physiology & behavior*, *55*(3), 501-504. https://doi.org/10.1016/0031-9384(94)90107-4

Warwick, Z. S., & Weingarten, H. P. (1996). Flavor–postingestive consequence associations incorporate the behaviorally opposing effects of positive reinforcement and anticipated satiety: Implications for interpreting two-bottle tests. *Physiology & Behavior*, *60*(3), 711–715. https://doi.org/10.1016/S0031-9384(96)00087-X

Watson, P., Wiers, R. W., Hommel, B., & De Wit, S. (2014). Working for food you don't desire. Cues interfere with goal-directed food-seeking. *Appetite*, *79*, 139-148. https://doi.org/10.1016/j.appet.2014.04.005

Weenen, H., Stafleu, A., & De Graaf, C. (2005). Dynamic aspects of Liking: post-prandial persistence of Sensory-Specific Satiety. *Food quality and preference*, *16*(6), 528-535. https://doi.org/10.1016/j.foodqual.2004.11.002

Weingarten, H. P. (1983). Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science*, *220*(4595), 431-433. https://doi.org/10.1126/science.6836286

Weingarten, H. P. (1984). Meal initiation controlled by learned cues: basic behavioral properties. *Appetite*, *5*(2), 147-158. https://doi.org/10.1016/S0195-6663(84)80035-5

Weingarten, H. P. (1985). Stimulus control of eating: Implications for a two-factor theory of hunger. *Appetite*, *6*(4), 387-401. https://doi.org/10.1016/S0195-6663(85)80006-4

Wilkinson, L. L., & Brunstrom, J. M. (2016). Sensory-Specific Satiety: More than 'just'habituation? *Appetite*, *103*, 221-228. https://doi.org/10.1016/j.appet.2016.04.019

Woods, C. A., Guttman, Z. R., Huang, D., Kolaric, R. A., Rabinowitsch, A. I., Jones, K. T., Cabeza de Vaca, S., Sclafani, A., & Carr, K. D. (2016). Insulin receptor activation in the nucleus accumbens reflects nutritive value of a recently ingested meal. *Physiology & behavior*, *159*, 52-63. https://doi.org/10.1016/j.physbeh.2016.03.013

World Health Organization (2021). Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Yeomans, M. R. (2012). Flavor–nutrient learning in humans: an elusive phenomenon? *Physiology & behavior*, *106*(3), 345-355. https://doi.org/10.1016/j.physbeh.2012.03.013

Yeomans, M. R., Morris, J., & Armitage, R. M. (2020). Hedonic contrast and the short-term stimulation of appetite. *Appetite*, *155*, 104849. https://doi.org/10.1016/j.appet.2020.104849

Young, P. T. (1940). Reversal of food preferences of the white rat through controlled pre-feeding. *The Journal of General Psychology*, *22*(1), 33-66. https://doi.org/10.1080/00221309.1940.10544318

Zoon, J., van Genderen, L., de Graaf, C. & Boesveldt, S. (2016). Food odours direct specific appetite. *Foods*, *5*(1), 12. https://doi.org/10.3390/foods5010012