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Article

Context Properties Modulate Flavor Neophobia Habituation

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Abstract

Background: One issue that has received relatively little attention in the analyses of the neophobic responses to a novel flavor is the influence of the context in which the flavor is first encountered, along with the effect of this context on the habituation of neophobia when the flavor is repeatedly consumed. Considering the predictions of the Contextual Safety Hypothesis, which proposes that the previous appetitive or aversive history of the context will modulate the intensity of neophobia and its habituation, we designed an experiment to evaluate the role played by the context in neophobia and its habituation to a new flavor. **Method:** Male Wistar rats had access to a new flavor solution (0.1% sacharin) in presence of a context previously submitted to a treatment intended to turn it into appetitive, aversive or merely familiar. An additional group of rats received the new flavor in their home cages. **Results:** After four days of saccharin exposure, those animals in the Appetitive and Home conditions showed significant faster neophobia habituation as compared to those in the Aversive and Familiar groups. **Conclusions:** These results revealed the potential applied value of using contextual manipulations to promote healthy eating behavior both in animals and humans.

Keywords: Neophobia; habituation; context; eating behavior.

Resumen

Las Propiedades del Contexto Modulan la Habituación de la Neofobia Gustativa. **Antecedentes:** la influencia del contexto en presencia del que aparece un sabor nuevo sobre la neofobia gustativa y su habituación es un aspecto que ha recibido relativamente poca atención en la investigación. En este artículo describimos un experimento diseñado para evaluar el papel que juega el contexto sobre la neofobia y su habituación partiendo de las predicciones de la Hipótesis de Seguridad Contextual, que propone que la historia previa apetitiva o aversiva del contexto modulará la intensidad de la neofobia y su habituación. **Método:** se expuso una muestra de ratas Wistar macho a una disolución de un sabor nuevo (sacarina al 0,1%) en presencia de un contexto previamente sometido a un tratamiento diseñado para convertirlo en apetitivo, aversivo o meramente familiar. Un grupo adicional de ratas recibió el nuevo sabor en sus jaulas hogar. **Resultados:** después de cuatro días de exposición a la sacarina, los animales en las condiciones Apetitivo y Hogar mostraron una habituación a la neofobia significativamente más rápida que los de los grupos Aversivo y Familiar. **Conclusiones:** estos resultados revelan el potencial valor aplicado del uso de manipulaciones contextuales para promover conductas alimentarias saludables tanto en animales como en humanos.

Palabras clave: neofobia; habituación; contexto; conducta alimentaria.

The considerable differences in food preferences observed between individuals are clearly indicative of the importance of experience and learning —along with genetic predisposition— in the development of food preferences (e.g., Birch, 1999). One mechanism that has evolved and is part of the foundation of food preferences and rejections is flavor neophobia, expressed behaviorally by the reduced consumption of a novel flavor in comparison with consumption of an already familiar flavor (see, for a review, Reilly, 2018). Neophobia tends to habituate progressively when the flavor is repeatedly consumed without being followed by aversive consequences, resulting in a progressive increase of consumption across successive encounters with the flavor (e.g., Domjan, 2018).

The neophobia habituation process has traditionally been considered an example of non-associative learning, but there is recent evidence indicating that neophobia habituation shares neural circuits with mechanisms of declarative memory (Grau-Perales et al., 2019; Morillas et al., 2017), thus representing a more complex form of flavor memory. In fact, neophobia habituation has been proposed, along with other classical conditioning processes, as a learning mechanism that determines the acquisition of preference or rejection to specific flavors (e.g., Reilly, & Schachtman, 2009; Sclafani, 1995).

Considering that the role of context in which the flavor is first encountered has received little attention in previous research on neophobia and neophobia habituation, we conducted an experiment analyzing the effects of such variable on consumption of a new flavor (saccharin) in laboratory rats. Previous results showed that presenting a new flavor in presence of a new context resulted in a reduction in consumption compared with the case in which the animals received the novel flavor in an already familiar context (e.g., De la Casa et al., 2003). These results were interpreted as the result of a non-associative process induced by the new context that increased the level of arousal of the animals, which consequently resulted in an increase in the exploratory responses that compete with taste consumption (Honey et al., 1992). More recently, some experimental results have revealed that the role of context in neophobia and its habituation goes beyond its mere novelty or familiarity. Thus, De la Casa (2018) have proposed the so-called

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contextual safety hypothesis, which proposes that the appetitive or aversive features of a specific context will serve to modulate consumption of the novel flavor. Therefore, when appetitive consequences consistently appear in a specific context, as occurs, for instance, in the animals' home cages, such a context will acquire safety properties that will be transferred to any novel stimuli that appear in its presence. Conversely, if aversive stimuli have appeared in the context, then the latter will become a predictor of aversive consequences, which will affect responding to any stimuli that are presented in this context in the future.

Departing from this hypothesis, the main purpose of the present experiment was to assess water-deprived male *Wistar* rats consumption of a novel saccharin solution for a 10-min period on four consecutive days as a function of context. Specifically, those animals in the Appetitive (App) Group were tested in a context that previously had been paired with food (half salty cracker) and subjected to an environmental enrichment treatment (e.g., Birch et al., 2013; Simpson & Kelly, 2011) for endowing the context with appetitive properties. It has been proven that pairing a context with food (Mustaca et al., 1991), or with new stimuli (e.g., Bevins et al., 2002) has rewarding properties as showed by place preference tests. The rats in the Aversive (Avers) Group were tested in presence of a context that had been paired with the presentation of mild shocks. In addition, two groups were included in the experimental design: A Familiar (Fam) Group, tested in presence of a context that was already familiar, and a Home Group for which the experimental context was the rats' home cages, since this context has already been shown to attenuate neophobia and expedite its habituation (e.g., De la Casa et al., 2013).

According to the predictions derived from the Contextual Safety Hypothesis, we expected to observe increased neophobia and slower habituation to neophobia when a novel flavor is presented in the aversive context, but reduced neophobia and faster habituation to neophobia in the appetitive context and in the rat´s home cage. Regarding the group tested in the familiar context, we expect an increase of neophobia and slower habituation as compared to Home and App groups, but a reduced effect of neophobia and faster habituation as compared to the Avers group, since in the Fam group the effect of neophobia would not be affected by novelty nor the appetitive or aversive properties of the context (De la Casa, 2018).

We registered two dependent variables: fluid consumption for all animals, that was considered as an index of neophobia and its habituation, and mean percentage of locomotor activity for those rats in App, Avers, and Fam groups, as an indirect way of evaluating the effectiveness of the experimental treatments in endowing the contexts with appetitive or aversive properties (e.g., Rescorla et al., 1985).

Method

Participants

32 male Wistar rats, (n=8 per group) with weights ranging from 290 to 455 g participated in this experiment. At the arrival to the laboratory, the animals were housed in groups of 2/3 (depending on the animals' weight) in type IIIH cages (820 cm^2), with wood shavings as bedding and other materials available in the cages (pieces of fabric, cardboard and wood, stones, etc.), except for the time they were submitted to the experimental procedure when they were individually housed to obtain a better control for water/food intake across the experimental procedure. Specifically, after a two weeks period of adaption to the vivarium, the animals were individually housed in 40 × 20 × 24 cm Plexiglas cages with wood shavings as bedding and maintained on a regular 12:12-h light/dark cycle (lights on at 07:00 A.M.) during the experimental period. All behavioral testing was conducted during the light period of the cycle, starting at 9:00 A.M. The vivarium was illuminated by four 100W bulbs. All animals were placed on a water deprivation schedule (30 min/day access to water) that was maintained across the entire duration of the experiment. All experimental procedures were approved by the Ethics Committee for Animal Research of University of Seville (code number CEEA-US2015-28/4), and conducted in agreement with the guidelines established by the EU Directive 2010/63/EU for animal experiments, and the Spanish R.D. 53/2013.

Instruments

All experimental sessions were conducted either in the home cages of the animals for Group Home, or in four experimental chambers (Panlab, model LE 111) designed to detect and record mean percentage activity for App, Avers, and Fam groups. Each chamber was enclosed in a soundproofed module (model LE 116), and the floor of the experimental chambers was composed of stainless steel rods, 2 mm in diameter, spaced 10 mm apart (center to center), resting on a platform that registered and recorded each animal's movements, which were then converted into analog signals by a piezoelectric unit attached to the platform. These signals were digitized and stored by a computer as a linear parameter. All fluids were provided at room temperature in 150 ml graduated plastic bottles, fitted with stainless steel spouts. The bottles were attached to the front wall of each cage during the experimental drinking sessions. The amount of fluid intake was measured by calculating the difference between the weight of the bottle before and after fluid presentation. The flavor was a 0.1 % sodium saccharin solution dissolved in tap water. A 0.5 mA, 1-sec unscrambled AC 50-Hz foot shock from a constant-current generator (Model LE100-26) was delivered to the floor of each chamber, as described in the Procedure section for the Avers Group. For the App Group, a 5 × 5 cm piece of fabric, a cardboard tube from a toilet paper roll, a 5 ×5 cm egg carton, and half of a salty cracker were introduced inside the experimental context for each trial.

Procedure

The rats were randomly assigned to the Home, App, Avers, and Fam groups. On the first day of the experiment, a water deprivation schedule (with each animal having daily access to 30-min of fluid) was implemented and maintained for the entire duration of the experiment.

From Days 2 to 5, animals in the App, Avers, and Fam groups were introduced into the experimental cages where they remained for 60-min each day. The animals in the Home group were simply removed and immediately returned to the home cages. The rats in the Avers group received 3 shocks on each session (0.5 mA, 1 sec.) with a mean inter-shock interval of 15 min (+/- 5 min). The rats in the App group were allowed to eat the half salty cracker and to interact with the stimuli described in the Apparatus section (introduced into the experimental cages as a means of producing environmental enrichment). The animals in the Fam group remained for 60-min in the experimental cages without any additional stimulation. In order

to equate the experience with the salty cracker that was presented during the environmental enrichment trials for the App group, half of a salty cracker was placed in each home cage for the animals in the Fam, Avers, and Home groups on each day of this phase. After the experimental treatment, all animals had access to 30-min of water in their home cages in the same bottles that were used during the neophobia trials.

On Days 6 and 7 the animals remained undisturbed in their home cages and received water for the corresponding 30-min period.

From Days 8 to 11, all animals received 10-min access to the 0.1 % saccharin solution in the corresponding experimental (Fam, App, and Avers groups) or home cages (Home group) to evaluate the intensity of neophobia and subsequent habituation of this response. No additional stimuli were introduced at testing. The bottles were weighed before and after each session, and the mean percentage of motor activity was recorded for App, Avers, and Fam groups on each session.

To evaluate the effect of context on neophobia and its habituation we analyzed mean saccharin intake on each day. Neophobia would induce differences in consumption across groups on the first day, and neophobia habituation would result in differences in consumption across successive days. In addition to saccharin consumption, we recorded the percentage of locomotor activity for the rats in the App, Avers, and Fam groups on the neophobia tests in order to detect whether the appetitive or aversive properties of the context induce different levels of activity.

Data analysis

All statistical analyses were conducted with the version 24 of the statistical program SPSS. We explored our hypotheses using repeated measures mixed ANOVAs, with Trials (within-subject), and Groups (between-subject: Fam vs. Home vs. Avers vs. App) as main factors. LSD *post-hoc* comparisons between all groups, and analyses of simple effects using a α-level of 0.05 were conducted to identify the source of the significant main effects and interactions.

In order to discard any potential effect of differences in rats' weight at the start of the experiment on consumption, an ANOVA with main factor Groups was performed on such variable. The analysis revealed that there were no significant differences between groups, $F(3,21)<1$.

Figure 1 depicts mean saccharine consumption as a function of groups for the four test days. As can be seen in the Figure, there was a general effect of neophobia habituation across trials that was faster for the Home and App groups as compared to the Avers and Fam groups.

The ANOVA conducted on mean saccharine consumption revealed a significant main effect of Trials, $F(3,84)=10.88$; $p<.001$; $\eta^2=.28$, reflecting the overall effect of neophobia habituation across trials. The main effect of Groups was also significant, $F(3,26)=2.09$; $p<.05$; $\eta^2=.33$, revealing that mean consumption was different between groups. Post hoc comparisons between groups ($p<.05$) performed on such dependent variable revealed that the main effect of Groups was due to higher mean saccharin consumption for the Home and App groups (Mean = 8.65 ml., SEM = .58 and Mean = 9.15 ml., SEM = .75, respectively) as compared to the Fam and Avers groups (Mean = 6.76 ml., SEM = .38, and Mean = 6.39 ml., SEM = .77, respectively). Finally, the Trials × Groups interaction was also significant, $F(9,84)=3.03$; $p<.01$; $\eta^2=.18$. In order to explore the interaction we conducted separate repeated measures ANOVAs with Trials as the main factor on mean consumption for each group, and ANOVAs between groups for each trial. The repeated measures ANOVAs revealed a significant effect of Trials for the App, Home, and Avers groups, Fs$(3,21) > 4.1$; ps$<.05$; ηs$^2>.37$, due to an increase in consumption across trials indicating the habituation of neophobia to the new flavor. The effect of Trials was non-significant for the Fam group, $F(3,21)<1$.

An ANOVA with Groups as main factor conducted on mean consumption for each trial revealed significant differences between groups for the second, third, and fourth trial, Fs$(3,28) > 3.48$; ps$<.05$; ηs$^2>.27$. Post hoc comparisons between groups ($p<.05$) revealed a significant increase in consumption in the App and Home groups as compared to the Fam and Avers groups for the second, third and fourth test trials, except for the third trial when the difference between Fam and Home groups was non-significant.

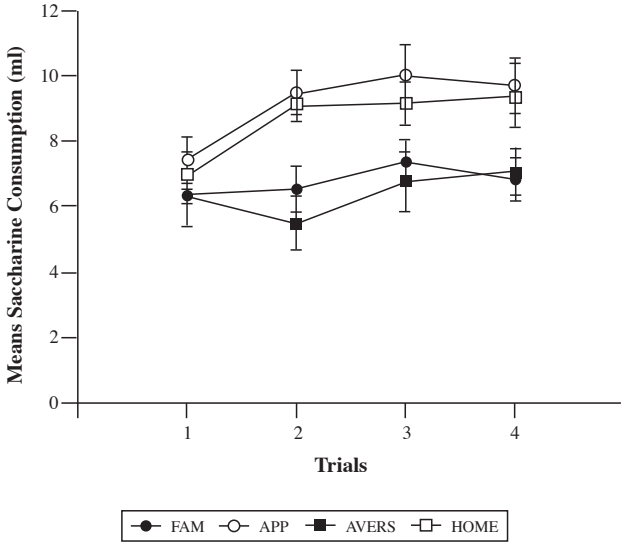

Figure 1. Mean saccharine consumption as a function of groups (Familiar, Home, Aversive, and Appetitive) at testing. Error bars represent SEMs

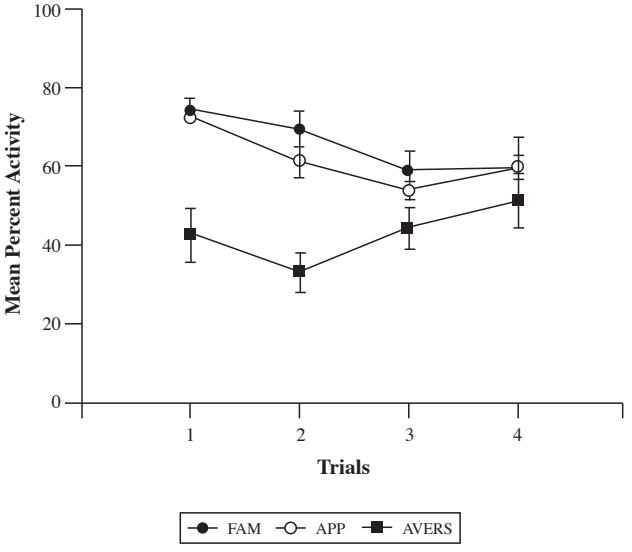

Figure 2. Mean percent activity as a function of groups (Familiar, Home, Aversive, and Appetitive) at testing. Error bars represent SEMs

Figure 2 depicts mean percent activity as a function of groups for the four test days. As can be seen in the Figure, mean percent activity was lower in the Avers as compared to the App and Fam groups during the first two test trials.

The ANOVA conducted on mean percent activity collapsed across each 60 min session revealed significant main effects of Trials and Groups, $F(3,63)=5.00$; $p<.01$; $\eta^2=.19$, and $F(2,21)=10.01$; $p<.05$; $\eta^2=.49$, respectively. The main effect of Trials was due to a progressive reduction of activity across trials. The main effect of Groups reflects the lower mean percent of activity collapsed across trials for the Avers (Mean = 42.77 %, SEM = 5.3) as compared to Fam and App groups (Mean = 65.57 %, SEM = 3.62, and Mean = 61.9 %, SEM = 1.92, respectively), as revealed by post hoc comparisons ($p<.05$) between groups. The 2-way interaction was also significant, $F(6,63)=4.11$; $p<.01$; $\eta^2=.39$. Separate repeated measures ANOVAs with Trials as main factor for each group, and ANOVAs between groups for each trial were conducted on mean percent activity to identify the source of the interaction. The analyses revealed a significant effect of Trials for the Avers and the App groups, $F(3,21)=4.68$; $p<.05$; $\eta^2=.40$, and $F(3,21)=10.85$; $p<.001$; $\eta^2=.61$, respectively. The effect of Trials for the Avers group reflects the progressive increase of activity across trials, probably due to extinction of the context's aversive properties. The main effect of Trials for the App group reflects a decrease of activity across trials that could be reflecting context habituation. The effect of Trials for the Fam group was non-significant, $F(3,21)=2.55$; $p>.08$; $\eta^2=.27$.

An ANOVA with Groups as main factor conducted on mean percent activity for each trial revealed significant differences between groups for the first and the second trial, $F(2,21)=16.35$; $p<.001$; $\eta^2=.61$, and $F(2,21)=17.02$; $p<.001$; $\eta^2=.62$, respectively. Post hoc comparisons between groups ($p<.05$) revealed lower percent activity in the Avers as compared to the Fam and App groups for the first and second trials.

Discussion

The main objective of the experiment reported in this paper was to analyze neophobia and its habituation to a novel flavor (a saccharin solution) when it was presented in the presence of familiar, appetitive/home, or aversive contexts. We observed that the context did not affect neophobia on the first day of consumption, contrary to what has been observed in previous experiments in which consumption of saccharin, when presented for the first time in the home cage, was significantly higher in comparison with the case in which this flavor was presented in either a new or familiar context (De la Casa & Díaz, 2013, Exp. 2). However, in the mentioned experiment the saccharin concentration used was 0.04%, which is notably weaker than the concentration used in the present experiment (0.1%). It is likely that the effect of context on neophobia was weaker when the neophobic response was more intense.

The results related to habituation of neophobia were in general consistent with the hypotheses proposed according to the contextual safety theory (De la Casa, 2018), since habituation of neophobia was significantly faster when the test was conducted in the rats' home cages and in an appetitive context in comparison with merely familiar or aversive contexts. The reduction in consumption when the test is conducted in the presence of the new context has previously been interpreted in the literature as being the result of an increase in the arousal level of the animals, which would lead to an increase in orientation responses to the context that are incompatible with the response of drinking (e.g., Honey et al., 1992). In fact, such non-associative effect could be responsible of the absence of neophobia habituation observed in the Fam group, since the levels of activity for such group did not decrease across trials, indicating that four days of exposure to the experimental context was not effective enough to successfully reduce orientation/exploratory responses. However, such non-associative interpretation is unable to explain the changes in habituation of neophobia observed in the Avers group, since the reduced consumption observed in such group was not due to an increase in exploration of the context that is incompatible with the drinking response (in fact, there were a significant decrement in activity for the animals tested in the context associated with the electric shocks). Also, the reduced consumption in the Avers as compared to the App and Home groups can be interpreted as the result of freezing induced by a process of fear conditioning to the context in the Avers group, but the lack of differences when comparing locomotor activity between the groups for trials 3 and 4 make such possibility unlikely.

The absence of differences in neophobia habituation between the Fam and the Avers groups was unexpected, since we predicted higher levels of saccharine consumption in the former as compared to the latter. A possible explanation of this result could come from two sources: First, the lack of effectiveness of the context exposure to reduce the rats' exploratory activity that could have compete with the drinking response in the Fam group; second, the use of mild shocks in the Avers group could have reduced the aversive properties of the context. Both alternatives merit additional experimental analysis (for instance, by incrementing the number of context familiarization sessions, and/or increasing the intensity of the aversive stimuli).

The observed increase in the speed of habituation to neophobia (manifested as an increase in consumption of the novel flavor) in the presence of an appetitive context is a finding that is somewhat difficult to interpret from a non-associative perspective. This result was observed both when the flavor was presented in the home cage, and when it appeared in a context that had received a treatment designed to turn it into appetitive. One possible explanation for the effect of the appetitive context on consumption comes from the idea of energization proposed by Konorski (1967), which implies that an appetitive Conditioned Stimulus (CS) increases the activity of the positive motivational system, whilst aversive CSs increase activity of the negative motivational system. Thus, similar to what occurs in a Pavlovian-instrumental transfer experiment when the presence of an appetitive CS increases the rate of responding maintained by an appetitive reinforcer (e.g., Lovibond, 1983) whilst an aversive CS increases an avoidance response (e.g., Rescorla y Lolordo, 1965), the presence of an appetitive context in a neophobia situation would increase activity of the positive motivational system, favoring the consumption of the novel flavor, whilst the opposite would occur in the aversive context.

A possible shortcoming of this study is related to recent evidence revealing that neophobia in rats differs as a function of sex, in such way that female rats display stronger neophobia than male rats (Angulo & Arévalo-Romero, 2021). Therefore, the results of our experiment should be taken cautiously since our sample only included males. Future research on this domain should incorporate both males and females rats in order to check for possible sex

differences in neophobia and its habituation, and in the effect of context on neophobia.

We will conclude with a proposal regarding possible applications of our results to the field of nutrition and food preferences, both for animals and humans. Specifically, since animals in the wild inhabit a complex and changing environment with high pressure for survival and continuous exposure to new and varied stimuli, some studies have indicated that these circumstances are associated with less food neophobia (Modlinska et al., 2015, 2020; Modlinska & Stryjek, 2016). Conversely, farm and domestic animals, with stable housing conditions, have less variability in their environment, including a low exposure to different foods. The food neophobia that usually appears when we try to implement changes in the diet of these animals can result in growth problems or decrease in weight, especially in animals more reactive and fearful (Neave et al., 2018). In this vein, several studies have proposed methods intended to decrease food neophobia, such as masking the new food with a familiar flavor (Launchbaugh et al., 1997) or a familiar smell (van den Berg et al., 2016), presenting the new flavor in presence of other members of the group (Arrazola et al., 2020; Modlinska & Pisula, 2018), or using taste preferences conditioning procedures (Clouard et al., 2012). Considering our results, we can propose effective strategies to facilitate the introduction of new types of food in the diet of animals based on the manipulation of the context where they are presented, since a new food in presence of a context previously associated with aversive consequences will increase neophobia and, therefore, hinder the eating process. Conversely, presenting the new food in the presence of a familiar of appetitive context will enhance the consumption of new foods.

Also, the notion of contextual control could have relevant implications for neophobia modulation in children, since childhood is one of the most critical periods for the formation of dietary preferences and aversions (e.g., Cashdan, 1994; Cooke et al., 2003). Experiences with different foods during the first year of life can result in the development of dietary habits that will last for the entire lifespan (e.g., Nicklaus & Monnery-Patris, 2018). In addition, this is the period in which flavor neophobia is more intense, particularly in children between 2 and 5 years old (Addessi et al., 2005). It has been observed that those children with intense neophobia eat less fruits, vegetables, and proteins (Nicklaus & Monnery-Patris, 2018). Considering that neophobia affects between 40% and 60% of children (Brown et al., 2016), procedures that can help to attenuate the neophobia response will be of great relevance for the prevention of possible health problems derived from inadequate feeding.

Whilst the present results have directly demonstrated the effect of context on the habituation of neophobia, there is also converging evidence from experiments with humans that have shown the relevance of social facilitation (which can be considered to be a more general type of context) on eating behavior (see, for a review, Herman, 2015). Thus, for instance, people eating in a group consume more food than when eating alone (e.g., de Castro & de Castro, 1989; Herman, 2015), and children are more willing to eat a particular food when they observe their caregivers or other children tasting the same food (e.g., Addessi et al., 2005; Hendy & Raudenbush, 2000).

Given that there is now sufficient evidence to indicate the influence of context on neophobia and its habituation, we can take advantage of such knowledge to promote the consumption of a healthy and varied diet during childhood. The principles derived from the contextual safety hypothesis suggest that when introducing a new food into the diet it is important to consider the context in which it is presented, since this can play an important role in determining whether it is accepted or rejected. Thus, if the new food is presented in a context that has previously been associated with an unpleasant or aversive event, then neophobia will be more intense and its habituation slower. For instance, to pressure or threaten children to try a new food creates an emotionally negative context that results in an increase in the rejection of such food (Kaar et al., 2016). According to the results obtained in our experiments, if we wish to increase the acceptance of a new type of food, it should be presented in an environment that has previously been associated with pleasant experiences.

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