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## Effect of previous taste experiences on taste neophobia in young-adult and aged rats

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#### Abstract

Neophobia is an innate response that can be defined as the reluctance to consume novel-tasting substances. The differential effect of previous aversive and nonaversive taste memory on a subsequent neophobic response was studied in young-adult (Experiment 1) and aged rats (Experiment 2). Surprising, a previous nonaversive taste experience eliminated the subsequent neophobic response to a solution of 1% sodium chloride (NaCl) in young-adult and aged rats. This result is interpreted as a generalization of the previous safe taste memory and the emotional responses that might be induced when a new taste is presented. However, a differential effect associated with aging was found for a previous aversive taste memory induced with a low dose of lithium chloride (0.15 M; 1% b.w.). While in young-adult rats this aversive taste memory did not change the neophobic response to an NaCl solution, in aged rats this memory potentiated the subsequent neophobic response to NaCl. This result is interpreted as an increase in the generalization of aversive taste memory and the emotional responses associated with aging.

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Age-related learning and memory changes have been usefully studied with taste memory tasks, thanks to the weaker influence of noncognitive factors on other learning tasks such as visual acuity or motor function [1]. Taste memory tasks are based on the need to correctly select the diet for survival. When a novel taste is presented, it produces an innate response called neophobia. Taste neophobia can be defined as reluctance to consume novel-tasting food [2]. This innate behavior is probably useful in avoiding the ingestion of large amounts of a toxic substance [3]. This phenomenon is particularly adaptive for omnivorous species such as the rat, which is unable to reject dangerous substances by vomiting [4]. Traditionally, for palatable liquids, neophobia has been defined as the reduction in consumption compared with the previous day on which the animal drank water. However, the unequivocal demonstration of neophobia requires a minimum of two presentations of the novel taste [5]. According to earlier research, the increase in taste consumption induced by the successive presentation of a new taste without negative consequences (neophobia attenuation) is itself the best demonstration of the reluctance to consume that taste (neophobia), regardless of previous water consumption.

Depending on the consequences associated with the consumption of the novel taste, it will be classified as safe (safe taste memory) or as aversive (aversive taste memory) (for a review see Ref. [6]). Safe taste memories in rats are learned when a taste is presented without visceral malaise; this consequence increases consumption when the animals is re-exposed to the same taste. Aversive taste memories in rats are learned when a novel taste (conditioned stimulus) is followed by visceral malaise (unconditioned stimulus), usually induced by lithium chloride (LiCl) (for a review see Ref. [2]). This consequence will reduce consumption when the taste appears again.

Aging rats continue showing neophobia when a new taste is presented [7–14], and can even show enhanced neophobia [15]. Age-related enhancement is observed for aversive taste memory, with aged rats showing stronger aversions to the taste than young-adult rats [8,11,10,12–14]. On the other hand, for safe taste memory aged rats show a lower attenuation of

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neophobia [7,9]. In other words, aged rats need more exposures to the taste to consider the stimulus safe.

It is important to note that neophobia, as has been shown for safe and aversive taste memories, can be modulated in different ways. A good example is the type and concentration of taste. The neophobic response is reportedly enhanced by sour and bitter tastes and by high concentrations of the taste [3,16,17]. The neophobic response can also be modified by previous experience. Different studies have reported a decrease in neophobia by early handling [18] and anxiolytic drug treatment [19–22]. Other studies have reported enhancement of neophobia by previous aversive drug treatment [23–25] and previous taste aversion learning [26,25,27–29].

The effect of previous experience on taste memories has been studied in aged rats, mainly in association with aversive taste memories. It has been reported that whereas latent inhibition to a taste is preserved in aged rats [14], a blocking effect in taste aversion learning does not appear in aged rats [30]. It is important to note that, to our knowledge, no studies have been published on age-related changes in taste neophobia mediated by previous experience. In the present study we looked for evidence of how the neophobic response to a new taste might be modified depending on previous aversive or nonaversive experience with another taste in young-adult and aged rats. According to previous results, pervious taste aversion might be expected to increase the neophobic response to another new taste. This should be particularly true in aged rats, which develop stronger aversions to novel tastes than young-adult rats [11]. Regarding the nonaversive experience, it is important to note that the absence of previous studies makes it hard to predict whether rats will develop a neophobic response.

#### 1. Experiment 1

The aim of Experiment 1 was to study the effect of an aversive taste memory or a safe taste memory on a subsequent neophobia test and on attenuation of the neophobia (studied with two successive presentations of the taste) in young-adult rats. Previous studies have reported enhanced neophobia after taste aversion learning [26,25,27-29]. However, some of these studies did not control appropriately for hedonic (preference or avoidance) characteristics of the taste and its concentration. In these situations it is difficult to determinate how much of the decrease in consumption associated with the novel taste was produced by the neophobia per se, and how much of the decrease reflected its hedonic characteristics [18]. In this connection it has been reported that sour or bitter tastes decreased consumption [17]. This fact is related with the similar sour and/or bitter tastes that are present in toxic or spoiled foods. In addition, the possible sensitization associated with lithium chloride (LiCl) should be controlled for [26,23,4,28]. Without appropriate control, LiCl might decrease consumption of the novel taste, but the decrease would not be distinguishable from that caused by neophobia.

In the present experiment, hedonic characteristics of the tastes and LiCl sensitization were controlled. In the taste aversion phase we used a solution of 0.1% sodium saccharin (Sac). This solution is preferred to water [3,17]. For the neophobia test, a solution of 1% sodium chloride (NaCl) was used. Again, this solution is

preferred to water [31–33,17,34]. With these preferred tastes, the neophobic response to the taste should be explainable only by the innate response of avoiding the ingestion of potentially lethal substances, in accordance with the definition of the neophobia. Because of possible sensitization, a period of 5 days with water *ad libitum* was used between the taste aversion phase and the neophobia test. Confirmation of the neophobic response was determined not only by comparisons between taste consumption and previous-day water consumption, but also by the increase in taste consumption on second presentation (attenuation of the neophobia), as proposed by Reilly and Bornovaloya [5].

Initially, Experiment 1 was to be conducted with two groups: one group with previous taste aversion learning, other with a previous safe taste memory in which saccharin was present without negative consequences. However, earlier results suggested that three additional groups should be included. A group with a noncontingent association between Sac and LiCl was included to study the specific effect of the Sac-LiCl association on neophobia. Another group injected with LiCl only, without previous taste experience, was included to study possible LiCl sensitization. A fifth group without any learning experience was also included.

#### 2. Method

#### 2.1. Subjects and apparatus

Forty naïve male Wistar rats 3 months old, weighing 220–260 g, were assigned to one of 5 groups:  $Sac \rightarrow LiCl (n=8)$ , Sac (n=8), Sac/LiCl 1% (n=8), LiCl 1% (n=8) and Baseline (n=8). Rats were housed in individual home cages with continuous access to food throughout the experiment, and were maintained on a 23 h 30 min water deprivation schedule. The procedures were approved by the University of Granada Ethics Committee for Animal Research, and were in concordance with both the NIH (United States of America) guidelines for the ethical treatment of animals and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

The home cages measured  $50 \times 26 \times 14.5$  (long × wide × high) and were kept in a large colony room under a 12:12 h light:dark illumination cycle, with lights on at 08:00 a.m. All the experimental treatments were given during the light period of the illumination cycle. The walls and the floors of the cages were made of translucent plastic, and the roof was made of wire mesh; a layer of wood shavings covered the floor. Fluids were administered at room temperature in 50-ml plastic centrifuge tubes with a rubber stopper fitted with stainless steel ball bearing-tipped

Table 1
Design of Experiment 1

Groups	Conditioning phase	Two saccharin tests	Neophobia test
Sac→LiCl 1%	Sac→LiCl 1%	Saccharin	NaCl
Sac	Saccharin	Saccharin	NaCl
Sac/LiCl 1%	Sac/LiCl 1%	Saccharin	NaCl
LiCl 1%	Water	Water	NaCl
Baseline	Water	Water	NaCl

Sac, Saccharin; LiCl, Lithium chloride; NaCl, Sodium chloride.

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