Behavioural mechanisms underlaying food aversion in guinea pigs

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Abstract. We analysed the mechanisms underlying behavioural food aversion of a plant defence (tannins) by guinea pigs (*Cavia aperea f. porcellus*) under the hypothesis that they can avoid ingesting these phenols by (a) directly monitoring their presence in their diet (unlearned aversion), by (b) using associative learning, or by (c) a combination of both mechanisms. Tannins were used as an aversive unconditional stimulus, and vanilla and wintergreen flavours were used as conditional stimuli. Tests consisted of simultaneous presentations of two bottles containing different water solutions. In the first test, subjects were offered a choice of the two flavoured water solutions in order to assess initial preferences. Unlearned avoidance was tested in two tests using different tannin concentrations. After a conditioning period in which subjects were offered their preferred flavour paired with tannins and their less preferred flavour in water solutions on alternate days, four tests were conducted using the two flavoured solutions without tannins in order to test for associative learning. Guinea pigs showed an unlearned avoidance towards tannins presenting a stronger response towards the solutions with the higher concentration. Flavours that had been paired with tannins during the conditioning period were avoided suggesting associative learning. We propose that the discriminatory abilities showed by *C. aperea* in the laboratory are involved in the development of simple 'rules of thumb' in the natural environment.
Key words: tannins, guinea pigs, unlearned avoidance, associative learning, diet selection

Resumen. *Mecanismos conductuales de elección de la dieta en Conejillos de Indias.* Todavía no se encuentran suficientemente dilucidados los mecanismos de elección de los componentes de la dieta de los herbívoros. Analizamos los mecanismos subyacentes a la aversión gustativa a defensas químicas vegetales (taninos) de los conejillos de Indias (*Cavia aperea f. porcellus*) bajo las hipótesis que pueden evitar el consumo de estos fenoles por (a) monitoreo directo de su presencia en la dieta (aversión no aprendida), (b) usando aprendizaje asociativo, y (c) una combinación de los dos. Como estímulo incondicionado aversivo fueron usados taninos, y sabores a vainilla y 'wintergreen', como estímulos condicionados. Los tests consistieron en presentaciones simultáneas de dos botellas conteniendo soluciones acuosas diferentes. In el primer test, los sujetos podían elegir entre dos soluciones acuosas saborizadas, con el objeto de estimar las preferencias iniciales. En dos tests que usaban soluciones de taninos como una de las opciones (los tests diferían en la concentración de taninos), los sujetos mostraron evitación no-aprendida hacia los taninos. Luego de un período de condicionamiento, en el que los sujetos podían elegir entre su sabor preferido apareado con taninos y su sabor menos preferido sin taninos, se realizaron cuatro tests usando la dos soluciones saborizadas sin los taninos. Los sujetos evitaron el sabor que había sido apareado con los taninos durante el período de condicionamiento. Se propone que las habilidades discriminativas mostradas por *C. aperea* en el laboratorio están involucradas en el desarrollo de 'reglas empíricas' de elección de dieta en condiciones naturales.

Introduction

Animals rely on their discriminatory abilities in order to choose what to eat. Several behavioral mechanisms are thought to be involved in the selection of diet composition (review by Barker et al., 1977), including specific hungers, neophobia, associative learning, social learning, and food imprinting. Most of these mechanisms were firstly described in omnivorous vertebrates such as rats, while evidence on their

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existence in herbivorous vertebrates has only recently been published, (Cassini, 1994; Provenza et al., 1998; Sih & Christensen, 2001).

Previous studies both in laboratory and field conditions, have shown that wild and domestic guinea pigs (*Cavia aperea*) use complex foraging decisions that depend on food and risk distribution (Cassini, 1991; Cassini & Galante, 1992; Cassini & Kacelnik 1994; Cassini et al., 1990; 1993). In this study, we investigated the food component discrimination abilities of domestic guinea pigs (*C. a. f. porcellus*) in relation to toxin avoidance. The first and apparently only study on food-aversion learning in guinea pigs was conducted by Braveman (1974). He demonstrated that these herbivores were able to learn the association between gustatory or visual cues and the aversive consequences of a poison (lithium chloride).

In the present study, tannins were used as the aversive unconditional stimulus. Tannins are plant defences present in 80% of woody perennial dicotyledons (Rhoades, 1979). The deleterious effects of this group of phenolics are the erosion of gut epithelia and the toxicity from the breakdown products of hydrolyzable tannins (Bernays et al.,1989). Investigations on food choice by mammalian herbivores in the wild including colobus monkeys (*Colobus satanas*), vervet monkeys (*Cercopithecus aethiops*), gorillas (*Gorilla gorilla gorilla*), kudus (*Tragelaphus strepsiceros*), impalas (*Aepyceros melampus*), grey squirrels (*Sciurus carolinensis*), and goats, indicate an avoidance of plants with high tannin contents (Calvert, 1985; Cooper & Owen-Smith, 1985; Swain, 1979; Wrangham & Waterman, 1981; Smallwood & Peters, 1986). However, little is known about the behavioural mechanisms which cause this rejection. In this paper we analize the mechanisms underlying food aversion of guinea pigs under the hypothesis that they can avoid ingesting tannins by (a) directly monitoring the presence of tannins in their diet (unlearned aversion), by (b) using associative learning to relate a taste with the post-ingestional consequences of consuming tannins, or by (c) a combination of both mechanisms. We predict that if guinea pigs have an unlearned aversion toward tannins, a decrease in the consumption of a preferred flavoured solution in the first exposure to tannins would be seen. If learning was involved we should find a decrease in the intake after a conditioning period.

Methods

Subjects

Ten three month old male guinea pigs were used. The subjects were obtained from a commercial supplier who had bred guinea pigs in captivity for many generations. From birth they were fed with commercial guinea pig feed and a daily supplement of lettuce. In the laboratory they were kept at 23º C on a natural light-dark cycle of approximately 12h L, 12h D. Subjects were weighed daily to control for their physical condition, and fed

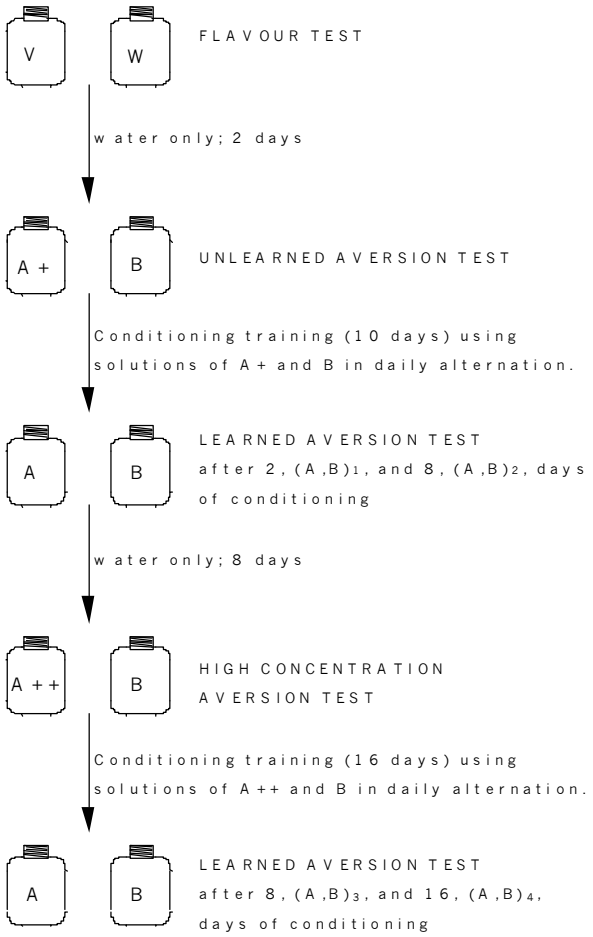

Figure 1. Experimental design, see text for details. V: vanilla, W: wintergreen. A indicates the flavour solution to which the tannin was added (vanilla for the vanilla-reinforced group, and wintergreen for the wintergreen-reinforced group), B indicates the other conditioning solution (wintergreen and vanilla respectively, for the two groups). A+ is the solution with low concentration of tannins, and A++ the solution with the higher concentration of tannins. The two first learned aversion tests are labelled $(A,B)_1$ and $(A,B)_2$ respectively; the two learned aversion tests after a training with a high tannin concentration were labelled $(A,B)_3$ and $(A,B)_4$

ad libitum with commercial guinea pig food. Subjects remained in individual cages (measuring 50 x 30 x 25 cm) throughout the experiment, including the test periods.

Conditioning solutions

The conditioning solutions consisted of flavoured tannin and flavoured water. These were prepared by diluting 0.115 ml of a 23.4% (v/v) wintergreen or 0.2 ml of a 5% (v/v) vanilla solution into 200 ml of 0.5% or 2% tannin solutions (w/v) or plain distilled water. These flavours were chosen because the animals had not experienced them before so they could be considered as neutral stimuli. Flavours for the experiment were provided by Frietsche Saica, Argentina, and Quebracho tannins by Ciba-Geigy, Argentina.

Procedure

The experimental procedure is summarised in Fig. 1. The experiment had an intra-subject design with three

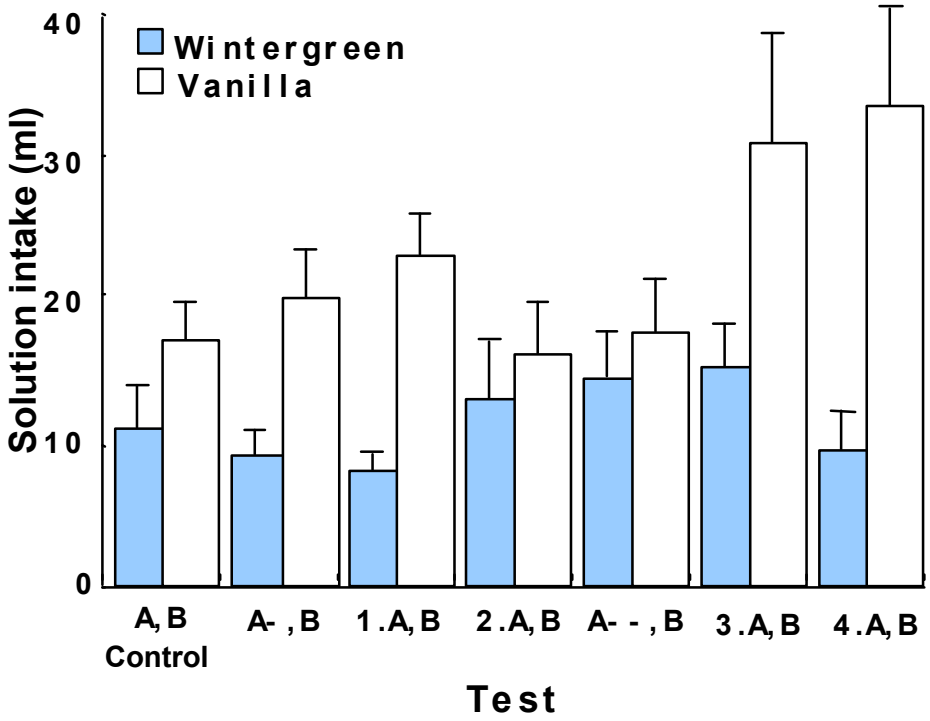

Figure 2. Each subject (N=10 guinea pigs) was presented with a choice of two solutions per test. Mean intake (ml) of each flavoured solution is represented. V,W is the control (flavour) test; A+,B is the unlearned aversion test, A++,B is the second aversion test, $(A,B)_{1\text{-}4}$ are the four learned aversion tests. See text for details about the tests. Highly significant differences were found between flavours (F(1,8)=12.01, p=0.008), and among tests (F(6,48)=2.53, p=0.033), but there was not significant interaction between treatments (F(6,48),1.88, p=0.202).

types of pairwise choice tests: 'flavour', 'unlearned-aversion', and 'learned-aversion.' All tests were conducted during the morning, starting at 9:00 h, and were preceded by 15 h of water deprivation. The duration of the tests was 0.5 h. During the tests two bottles containing different solutions were simultaneously presented. Their initial positions were randomly selected and swapped in the middle of the test, in order to control for spatial preference. All bottles used during tests and conditioning periods were identical.

The experiment began with a two bottle 'flavour test' (in Fig. 1: V, W) in which the initial preferences for vanilla and wintergreen flavours were assessed using flavoured water solutions. As a result of this test, subjects were divided into two groups, assigning the five animals with greatest preferences for vanilla to the first group, and the rest to the second. In subsequent tests, A (in Fig. 1) indicates the flavour solution to which the tannin was added (*i.e.*, vanilla for the vanilla-reinforced group, and wintergreen for the wintergreen-reinforced group), while B indicates the other conditioning solution (wintergreen and vanilla respectively for the two groups).

The unlearned aversion test with low tanin concentration was conducted two days after the flavour test and involved presenting the animals with a 0.5% tannin solution paired with their preferred choice (A+, B). During the subsequent 10 day conditioning period guinea pigs were offered, in alternation on a daily basis, two conditioning solutions: their preferred flavour paired with tannins (A+), or their less preferred flavour (B). Two learned-aversion tests were intercalated, using the conditioning solutions without tannins. The learned aversion tests involved presenting the animals with the flavoured solutions free from the unconditional stimulus (tannins). The first test, $(A,B)_1$, took place two days after the conditioning training had started, and the second $(A,B)_2$, took place eight days later. After eight days without training, a test using the high concentration of tannin: 2% (w/v), (A++,B) was conducted, followed by a conditioning period of 16 days with daily alternation of the solution containing the higher tannin concentration and the less preferred solution, (A++) and (B). Two learned-aversion tests, $(A,B)_3$ and $(A,B)_4$, were conducted after 8 and 16 days respectively.

Results

Consumption of the solutions in the seven choice tests are shown in Fig. 2. Mean intakes were examined using a two-way analysis of variance for repeated measures, with flavours and tests as treatments. Highly significant differences were found between flavours ($F_{1,8}$=12.01, p=0.008). Significant differences existed among tests ($F_{6,48}$=2.53, p=0.033), but there was no significant interaction between treatments ($F_{6,48}$=1.88, p=0.202). This analysis indicates that guinea pigs preferred solutions with vanilla over solutions with wintergreen in all choice tests, and that they showed variability in consumption among tests.

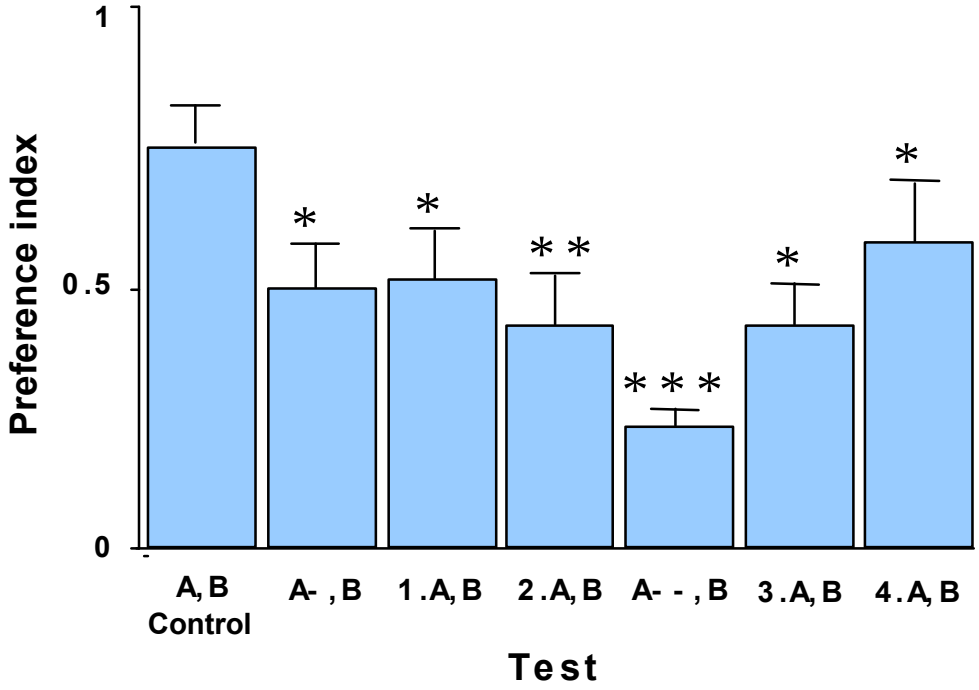

Figure 3. Intersubject mean preference index defined as the intake of solution A as a proportion of the total intake (V/(V+W) for the vanilla group, and W/(V+W) for the wintergreen group) along the different treatments. The preference index of the individuals of the two groups (N=10) have been pooled together. Highly significant differences were found among tests ($F(8,54)=5.17$, $p=0.001$). Dunnett tests were conducted to compare the first test with each of the other tests. All comparisons showed significant differences; * represents: $p<0.05$; ** represents $p< 0.01$; and *** represents $p<0.001$.

A preference index, which is defined as the intake of solution A as a proportion of a total intake in a test, was calculated for each subject and each test (Fig. 3). The arcsines of the square root of these indices were subjected to a one-way analysis of variance for repeated measures, which indicated highly significant differences among tests ($F_{8,54}=5.17$, $p=0.001$). Dunnett tests were conducted to compare the first flavour-test with each of the other tests. All comparisons showed significant differences ($p<0.05$).

Our prediction was that if guinea pigs had an unlearned mechanism of recognising tannins, then a reduction of the intake of the reinforced solution in its first presentation would be found. Accordingly, when subjects were exposed to 0.5% (w/v) tannin solutions for the first time (unlearned avoidance test), there was a significant decrease (Dunnett test, $P<0.05$) in the consumption of their preferred solution indicating a non-conditioned avoidance of the toxin. The most significant difference between a control and an aversion test was found in the test with the first presentation of the higher concentration of tannin (A++, B) (Dunnett test, $p<0.001$). This result suggests that the unlearned avoidance was directly related to the concentration of tannins.

If learning was involved in diet selection, a significant difference would be found between the initial 'flavour test' and the 'learning' tests. After a training period of one week we tested the animals presenting them with the flavoured solutions without tannins. This and the other three learned-aversion tests $(A, B)_{1-4}$ showed significant reductions in the preference indices in relation to the initial test (Fig. 3), and provided evidence that guinea pigs learned the relationship between the reinforced flavour and the aversive consequences of ingesting tannins.

Discussion

Guinea pigs were found to have an unlearned avoidance toward tannins, and to reduce their consumption through learning. The unlearned avoidance was probably due to a mechanism of censoring the immediate unpleasant consequences of this toxin, *i.e.*, to the unpalatable astringent taste properties of the tannins (Swain, 1978). The avoidance to a 2% tannin solution being significantly greater than that to the 0.5% tannin solution, that is the rejection was found to be a function of the concentration of tannins. Studies with sheep, goats and cattle (Provenza & Cincotta, 1993) have shown a similar trend.

There was a significant difference between the initial 'flavour test' and the 'learning' tests providing evidence that guinea pigs learned the relationship between the reinforced flavour and the aversive consequences of ingesting tannins. Guinea pigs were able to learn after two days of conditioning training, and further training did not alter significantly their response to the conditional stimulus. The conditioned aversion that was found after a training period could be produced because guinea pigs learned the contingency between the conditional stimulus and either the 'bad taste' (flavour-flavour relationship) or the aversive post-ingestional consequence (flavour-

gastrointestinal illness relationship).. We conclude supporting our hypothesis (c) enunciated in the Introduction that guinea pigs are able to avoid dietary tannins by directly monitoring their presence and by using associative learning to relate a taste to the post-ingestional consequences of consuming tannins.

Ruminants are the main group of herbivorous mammals for which food aversion learning has been demonstrated (Olsen & Ralph, 1986; Provenza et al., 1990; Thorhalldottir et al., 1990; Zahorik et al., 1990). Provenza et al. (1990) conducted an experiment in which goats were fed with blackbrush pellets with or without the addition of tannins. Although goats did not show an unlearned tannin aversion they reduced the consumption of the aversively reinforced pellets after one day of trial, expressing a learned response due to post-ingestive feedback. However the consumption of pellets coated with tannins increased by the end of the experiment in accordance with our results. Goats were found to have a detoxification mechanism, and morphological and physiological adaptations to tannin consumption (Distel & Provenza, 1991). These mechanisms were also found in deer (Austin et. al., 1989), mule deer (*Odicoileus hemoinus hemoinus*), black bears (*Ursus americanus*) (Robbins et al., 1987, 1991), rats and mice (Mehansho et al., 1983), and were attributed to the production of tannin-binding proteins. We believe that the increase in the intake of tannins that we observed in the two learned aversion tests, although statistically non-significant, may indicate that similar mechanisms allowing adaptation to tannins are functioning in guinea pigs. The discriminatory capacities showed by guinea pigs in the laboratory are probably involved in the development of their diet choice decision rules in nature. This does not imply the existence of complex mechanisms for detection and selection of plant chemical components ('nutritional wisdom'). Instead, 'unlearned' palatabilities and associative learning are thought to be involved in the development of simple 'rules of thumb' that could provide mammalian herbivores with a balanced diet without the need for nutritional wisdom (Cassini, 1994). Rules such as 'eat young growing shoots' (Zahorik & Houpt, 1977) for forb-eaters or 'choose tall green grass' (Illius & Gordon, 1990) for large grass-eaters can increase the efficiency of food selection without the need to identify many specific chemical components of the plants. Simple rules of thumb may be able to provide herbivores with diets low in tannins and rich in nitrogen and energy, because the amount of tannins and the proportion of fibre versus protein increase with the age of leaves (Zahorik & Houpt, 1977). These rules could be achieved by a combination of generalized 'non-learned' palatabilities, such as astringency, with learning processes, which modulate these original preferences as the result of forming associations between initially neutral flavours and the consequences of the ingestion of the different plant types or plant parts. Future empirical research is needed to evaluate the relevance of the 'rule of thumb' hypothesis, and more generally, to test the implicit and explicit assumptions about the psychological abilities of herbivores which constrain their food selection decisions. The validation of hypotheses on the evolution and ecology of foraging strategies and plant-herbivore coevolution will depend on the results of this future research.

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