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## Extinction of the initial within-compound association established in a blocked preexposure to two compound flavours

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Extinction of the  $A \leftrightarrow X$  association after blocked preexposure to AX-BX was studied in two experiments. In Experiment 1, two groups of rats received long (14 trials) or short (4 trials) blocked preexposure to AX-BX and subsequent conditioning of X. The results showed that the AX association was equally preserved after long and short preexposure. Experiment 2 studied the effect of blocked preexposure to 0, 1 or 2 ruptures of the AX association on extinction. In this experiment a "rupture" is produced when, in subsequent blocks, one element of the original compound is presented in compound with a different element. A significant extinction was observed only when the AX association was broken twice

Perceptual learning improves discrimination between two confusable stimuli AX and BX as a consequence of mere exposure to the stimuli. In the field of animal learning, the ability to discriminate between AX and BX is measured by means of generalization tests that require the use of conditioning. For instance, when AX and BX are two compound flavours, an aversion is conditioned to AX and the subsequent acceptance or reluctance to drink BX indicates the level of discrimination of the animal: The greater the acceptance of BX, the better the discrimination. In experiments on perceptual learning with animal subjects it is usual to compare the performance of two groups of subjects that receive the same amount of preexposure to AX and BX "intermixed", alternating presentations of the stimuli (AX, BX, AX, BX, ... AX, BX) or "blocked", in which the stimuli are presented in blocks (AX, AX, AX, ... BX, BX, BX). Subsequent conditioning of AX results in less generalization to BX in the group that received intermixed preexposure (i.e.,

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a perceptual learning effect). This phenomenon has come to be called the "intermixed-blocked effect" (Honey, Bateson, & Horn, 1994).

In the last years, some experiments have provided evidence suggesting that after blocked preexposure, the within-compound association established in the first block would not be extinguished in the second block. In part, this evidence comes from a group of experiments involving order effects, which compare presentations of two blocks of stimuli in two possible orders. Hall and Rodríguez (2009, Exp. 2) preexposed CX in a block before or after intermixed preexposure to BX/X and found that the salience of C was reduced in the subgroup of animals that received the CX trials after preexposure to BX/X (i.e., BX/X-CX). This result was expected, given that associative activation of a stimulus is the factor contributing to the preservation of its salience (Hall, 2003) and when the CX trials come last, associative activation of C is not possible. Most interesting was the finding that salience of C was maintained in the subgroup given CX first during preexposure (CX-BX/X) and this result was attributed to associative activation of C, via X, when X was presented in the second block of preexposure. This mechanism could not operate if the  $C \leftrightarrow X$  association established in the first block of preexposure had been extinguished during the second block.

Espinet, Caramés and Chamizo (2011, Exp. 4) compared two groups preexposed to AX-BX or to AX-BY. In the AX-BY group the  $A \leftrightarrow X$  association could not be extinguished. Consumption of A after conditioning of X was small and similar in both groups. This result led them to think that the  $A \leftrightarrow X$  association was not fully extinguished in the AX-BX group after preexposure. This conclusion does not fit with the salience mechanism proposed by Hall (2003) to explain the differences between intermixed and blocked preexposure to two compounds AX and BX. According to this mechanism, repeated presentations of a stimulus produce a loss of its salience. Associative activation of the stimuli can counteract this loss of salience. This associative activation is possible when the stimuli are preexposed in an intermixed schedule. Once the within-compound associations  $A \leftrightarrow X$  and  $B \leftrightarrow X$  have been established in the first preexposure trials, presentations of AX activate associatively, via X, the representation of B and presentations of BX activate associatively, via X, the representation of A and, hence, A and B both would maintain their salience. On the contrary, in a blocked preexposure to AX-BX, the associative activation of B is not possible and, following Hall (2003), the  $X \leftrightarrow A$  association should be extinguished given that AX is not presented any more. The experimental evidence supporting the basis for Hall's suggestion is unknown for us. Rather, some experiments that compared the strength of the within-compound

associations resulting from intermixed and blocked preexposure have provided evidence that these associations are stronger after blocked than intermixed preexposure (Rodríguez & Alonso 2014, 2015).

The possibility that the initial within-compound association is not extinguished after blocked preexposure has been recently tested by Espinet, Caramés and Cabo (2015) by comparing the strength of the  $A \leftrightarrow X$  and  $B \leftrightarrow X$  associations after blocked preexposure to the two possible sequences of presentation of two compound flavours: AX-BX or BX-AX. The logic of this comparison is based in the obvious assumption that the within-compound association established in the second block of preexposure is not extinguished. Therefore, in case that the association established in the first preexposure block were extinguished along the second block, the strength of the  $A \leftrightarrow X$  association should be smaller after preexposure to AX-BX than after preexposure to BX-AX. It does not matter whether the AX compound was presented in the first or in the second preexposure block, the results did not reveal significant differences in the strength of the  $A \leftrightarrow X$  association.

The above-mentioned results support the conclusion that preexposure to AX-BX does not result in extinction of the  $A \leftrightarrow X$  association. An indirect test of this idea may be found in Rescorla and Freberg (1978) which studied different methods of extinguishing within-compound flavour associations and found that, after preexposure to a compound flavour AX, presenting A or X isolated or in compound with a new flavour resulted in attenuation of the strength of the  $A \leftrightarrow X$  within-compound association. Since in a blocked preexposure to AX-BX, X is separated of A and is presented in compound with a new stimulus B, perhaps some extinction of the  $A \leftrightarrow X$  association should be observed. But Espinet et al. (2015) found that the associations remained preserved. The differences between blocked preexposure to AX-BX and the exposure sequences used by Rescorla and Freberg in their experiments as well as the difficulty to establish a comparison between their procedures and those used in the more recent experiments have been highlighted previously (Espinete, et al. 2015). Nevertheless, it is possible to study the influence of some variables that could influence the level of extinction of the  $A \leftrightarrow X$  association established during preexposure to AX-BX. One of these variables is the amount of preexposure to the compounds. For instance, Espinet et al., (2011, 2015) gave their subjects long preexposure to AX (14 trials) while Rescorla and Freberg used a short preexposure (4 trials). The small extinction observed in the experiments by Espinet et al. (2015) in comparison with those of Rescorla and Freberg could be perhaps due to the different number of trials employed in the preexposure phase, to the extent that 14 presentations of AX could contribute to establish the  $A \leftrightarrow X$

association more firmly than only 4 presentations. Therefore, Experiment 1 explored this possibility. Subsequently, Experiment 2 explored whether the number of ruptures of the A-X association (by presenting each of these flavours in compound with another flavour, e.g., B-X and AY) could produce a significant extinction.

## **EXPERIMENT 1**

This experiment was designed to evaluate whether the amount of preexposure to AX-BX influences the level of extinction of the  $A \leftrightarrow X$  association established in the first block. Two groups received blocked preexposure to AX-BX. One of them received long preexposure (14 presentations) while the second group received short preexposure (4 presentations). A third group received long preexposure to AX-BY. After preexposure, flavour X was paired with nausea and subsequent tests evaluated the aversion to flavours A and B when they were associated to X. If long preexposure to AX contributed to make the  $A \leftrightarrow X$  association more resistant to extinction, it should be observed that consumption of A in the test should be less in the group that received 14 presentations of AX-BX than in the group that received only 4 presentations. On the other hand, the test with flavour B provides an indicator of the strength of the  $B \leftrightarrow X$  association after 14 or 4 presentations of the BX compound. Given that the BX compound is presented in the second block of preexposure, the  $B \leftrightarrow X$  association cannot be extinguished and no differences in consumption of B should be observed between the two groups that received the BX compound. In the group receiving extensive blocked preexposure to AX-BY, the  $A \leftrightarrow X$  association should be firmly established and should be very strong after the preexposure phase, given that the presentations of BY during the second block can hardly contribute to the extinction of the  $A \leftrightarrow X$  association. Therefore, in this group there is no chance for extinction of the  $A \leftrightarrow X$  association, and consumption of A in the test should be lower than in the other two groups, in which the presentations of BX during the second block (where X is separated of A) could produce extinction of the  $A \leftrightarrow X$  association. Besides, in the group preexposed to AX-BY, consumption of B in the test should be higher than in the other two groups where flavour B was associated to the conditioned flavour X.

## METHOD

**Subjects and apparatus.** Thirty experimentally naïve Wistar rats provided by Harlan Ibérica with an average weight of 339 g at the beginning of the experiment (range 281-432 g) were used for this experiment. A 2-week adaptation period was carried out in this and the next experiment. During the adaptation period the animals were housed in groups of four subjects in makrolon cages (60 x 37 x 19 cm). Following the adaptation period, rats were housed individually in 42 x 26 x 18 cm boxes with free access to food and water. In this and the next experiment, rats were housed in a colony room under constant temperature and humidity ( $22 \pm 1$  °C and 60% respectively) and a light-dark cycle (08:30-21:30).

Three flavoured stimuli were prepared with tap water and chemically pure products provided by Probus or Merck laboratories: 0.3% (w/v) citric acid; 0.15% (w/v) saccharin, and 0.5% (w/v) sodium chloride. A fourth flavour was made from 9% of orange blossom essence (Vahiné-Ducros S. A). Two compound solutions, saccharin-acid and salt-acid, were made with these flavours maintaining the above-mentioned individual concentrations of each substance. Citric acid served as flavour X and orange-blossom served as flavour Y, while saccharin and salt were counterbalanced as flavours A and B. The compounds were presented to animals in 100 ml plastic bottles fitted with metal spouts. Consumption was measured by weighing the bottles before and after each session. At the beginning of each session, each bottle contained at least 70 ml of solution. Intraperitoneal injections of 0.3M LiCl at 10 ml/kg of body weight were used for the conditioning trials and were administered in an experimental room adjacent to the colony room.

**Procedure.** The day previous to the first session, water bottles were removed at 9:00 pm. Throughout all the phases of this and the next experiments the rats had access to fluid for 15 min in each of four daily sessions starting at 9.00; 13.00; 17.00 and 21.00 hours.

The design of the experiment is shown in Table 1. The first 14 sessions of preexposure constituted the first preexposure block. In these sessions 10 animals received the saccharin-acid compound and 10 received the salt-acid compound. Once concluded the first block, these rats were assigned to group 14AX-BX and group 14AX-BY, matched on their consumption during the first block, following the condition that for half of the subjects in each group the compound AX was saccharin-acid and for the other half AX was salt-acid. The following 14 sessions constituted the second block of preexposure. Each subject in the 14AX-BX group received the compound that had not received on the previous block, while subjects of the group 14AX-BY

received a compound made of orange blossom and the flavour (saccharin or salt) that they had not received on the previous block. The remaining 10 animals were assigned to group 4AX-BX and received water during the first 20 sessions of preexposure. In sessions 21-24 half of the animals in the group 4AX-BX received the saccharin-acid compound and the other half received the salt-acid compound. Subsequently, during sessions 25-28, each animal in the group 4AX-BX received the compound that was not presented in the previous sessions. After preexposure all the rats received the experimental treatments at 09:00 and drank water in the three remaining daily sessions. The four days after preexposure constituted the conditioning phase. On days 1 and 3 of this phase each rat received the flavour X (citric acid) followed by an injection of LiCl. The days 2 and 4 of this phase were recovery days and all the rats drank water in the four daily sessions. The last three days of the experiment constituted the test phase. The first test day, half of the animals in each group received flavour A and the other half received flavour B. The second day, each animal had access to the flavour (A or B) that was not presented the day before. The third day the animals received a test of consumption of flavour X.

**Table 1. Experimental Designs**

Experiment	Groups	Preexposure	Conditioning	Test
1	AX-BX	14 AX-BX	X +	A, B
	AX-BY	14 AX-BY	X +	A, B
	AX-BX	4 AX-BX	X +	A, B
2	AX-BX-AY	4 AX-BX-AY	X +	A, B
	AX-BX-Y	4 AX-BX-Y	X +	A, B
	AX-BY-Y	4 AX-BY-Y	X +	A, B

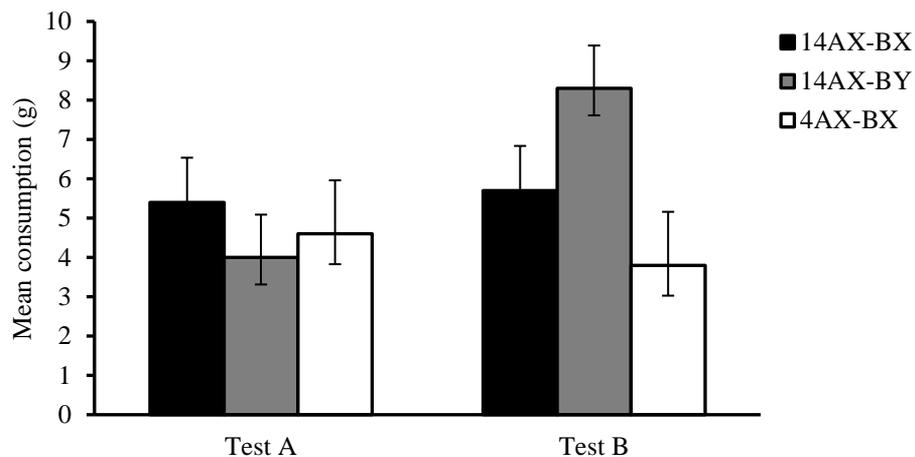
*Note.* A, B, X, and Y, refer to different flavour stimuli. A and B were saccharin and sodium chloride (counterbalanced in preexposure phase and tests), X and Y were citric acid and orange blossom respectively. The numbers refer to the amount of preexposure trials. Stimuli were presented in blocks, which appear separated by a dash (-). The + sign indicates conditioning with LiCl injections.

## RESULTS AND DISCUSSION

In this and in the next experiment, a signification level of  $p < .05$  was adopted. Mean consumptions of the AX flavour in each session of the

preexposure phase were 5.06 g (*SEM* 0.56), 5.15 g (*SEM* 0.65) and 4.93 g (*SEM* 0.48) for groups 14AX-BX, 14AX-BY and 4AX-BX, respectively. An one-way ANOVA revealed that the differences among groups were not statistically significant [ $F(2, 29) = 0.038, p > 0.05$ ]. Mean consumption per session of the BX flavour for groups 14AX-BX and 4AX-BX were 5.8 g (*SEM* 0.29) and 5.21 g (*SEM* 0.24) while group 14AX-BY consumed 6.05 g of the BY flavour (*SEM* 0.25). An one-way ANOVA did not reveal statistically significant differences among-groups [ $F(2, 29) = 2.65, p > 0.05$ ].

Conditioning successfully established an aversion to X in the three groups. Throughout the conditioning phase the mean amount of flavour X consumed by rats of groups 14AX-BX, 14AX-BY and 4AX-BX were, respectively, 4.3 g (*SEM* = 0.66), 4.4 g (*SEM* = 0.71) and 3.9 g (*SEM* = 0.22) in the first conditioning trial; 1.5 g (*SEM* = 0.49), 2.8 g (*SEM* = 0.65) and 1.8 g (*SEM* = 0.57) in the second conditioning trial; and 1.0 g (*SEM* = 0.43), 0.7 g (*SEM* = 0.08), and 0.5 g (*SEM* = 0.15) in the final test. A mixed 3 (Group) x 3 (Trial) ANOVA found a significant main effect of trial,  $F(2, 54) = 49.2, p = 0.000, \eta_p^2 = 0.64$ . The main effect of group was not significant  $F(2, 27) = 1.2, p = 0.29, \eta_p^2 = 0.08$ , neither were significant the group x trial interaction  $F(4, 54) = 2.1, p = 0.09, \eta_p^2 = 0.13$ .



**Figure 1.** Mean consumption of flavours A and B in the test phase of Experiment 1. After the different types of blocked preexposure to the compounds represented in the group names, flavour X was paired with nausea before this test. Vertical bars represent standard error of the means.

Figure 1 shows the mean amounts of A (left) and B (right) consumed by each group in the test phase. As it can be seen on the left part of the figure, consumption of A was low and similar across groups. The right part of the

figure shows that the highest consumption of B corresponded to group 14AX-BY and that the intake of B was similarly lower in groups 14AX-BX and 4AX-BX.

A mixed 3 (Group) x 2 (Test, A or B) ANOVA did not reveal significant main effects of group or test ( $F_{max} = 3.29$ ) but the interaction of group x test was statistically significant [ $F(2, 27) = 5.05, p < 0.05, \eta_p^2 = 0.27$ ]. Further analyses of this interaction by means of analysing the simple effect of Group at each level of test found that the groups did not differ significantly in their consumption of flavour A [ $F(2, 27) = 0.34, p = 0.71$ ] but they did differ significantly in their consumption of flavour B [ $F(2, 27) = 8.076, p < 0.05$ ]. Duncan's post-hoc test confirmed that group 14AX-BY consumed an amount of B significantly higher than that consumed by group 14AX-BX (Cohen's  $d = 1.1$ ) and the group 4AX-BX (Cohen's  $d = 1.8$ ). These last two groups did not differ significantly from each other (Cohen's  $d = 0.7$ ).

The results of this experiment are quite clear. The  $A \leftrightarrow X$  association established in the first block remains equally preserved after long or short preexposure to AX-BX. The comparison of the amounts of flavour A consumed by these groups did not differ significantly from the amount of A consumed by the group 14AX-BY where the initial  $A \leftrightarrow X$  association should be very strong, given that presentations of BY could not contribute to the extinction of this association. The lack of differences between the groups 14AX-BX and 4AX-BX not only in consumption of A but also in consumption of B is not surprising given that the most of the experiments on perceptual learning using rats and flavoured stimuli have found that four presentations of the compound are enough to establish a strong within-compound association. More interesting, these results show that presenting BX 14 times in the second block was not enough to attenuate significantly the strength of the initial  $A \leftrightarrow X$  association.

Perhaps this should not be surprising taking into account that Rescorla and Freberg (Exp. 1) did not observe extinction of the initial  $A \leftrightarrow X$  association after only four AX presentations followed by six presentations of X or A isolated. In fact, Rescorla and Freberg (1978) only observed extinction of the initial  $A \leftrightarrow X$  association when in their Experiment 2 a three-stage preexposure sequence to AX-BX-AY was used. Note that with this sequence, the  $A \leftrightarrow X$  association established in the first block is broken twice; in the second block, when X is presented separated from A (in compound with B) and in the third block, when A is presented separated from X (in compound with Y). In a blocked preexposure to AX-BX the  $A \leftrightarrow X$  association established in the first block is broken only once: when in the second block X is presented separated from A (in compound with B). Given that, as

Rescorla and Freberg (1978) concluded, presentations of A or X isolated or in compound with another flavour attenuated the initial  $A \leftrightarrow X$  association, two ruptures of the  $A \leftrightarrow X$  association should produce more extinction of this association than only one rupture.

## EXPERIMENT 2

Rescorla and Freberg (1978, Exp. 2) exposed their subjects to AX-BX-AY. In this experiment, two "ruptures" of the  $A \leftrightarrow X$  association were produced when in subsequent blocks, the flavours A or X were presented in compound with a different flavour (for instance BX or AY). After these two ruptures, the  $A \leftrightarrow X$  association was clearly extinguished.

To test if the number of ruptures of the initial  $A \leftrightarrow X$  association was a factor influencing the level of extinction of this association, our second experiment compared three groups of rats that received three blocks of preexposure. In the first block, all the animals received AX presentations. In the following blocks the  $A \leftrightarrow X$  association was broken twice (group AX-BX-AY), only once (group AX-BX-Y) or never (group AX-BY-Y). After preexposure, an aversion was conditioned to X and subsequent tests measured the reluctance to drink flavours A or B. If the number of ruptures of the initial  $A \leftrightarrow X$  association determines the amount of extinction, consumption of A should be the lowest in the AX-BY-Y group where the initial  $A \leftrightarrow X$  association was not broken, greater in the AX-BX-AY group where the initial association was broken once, and should be the largest in the AX-BX-AY group where the initial association was broken twice.

## METHOD

**Subjects and apparatus.** Thirty experimentally naïve Wistar rats provided by Harlan Ibérica were used for this experiment, with an average weight of 271 g at the beginning of the experiment (range 224-330 g). The individual flavours, compound solutions, and the concentration and doses of LiCl were identical to those used in Experiment 1.

**Procedure.** The design of the experiment is shown in Table 1. The adaptation period and the schedules for the drinking sessions were the same as those described in the previous experiment. The solutions were presented in three blocks of four sessions each. Preexposure lasted three days. Each block consisted of four 15-min sessions and took place in one day. In the first block half of the animals received the saccharin-acid compound and the other half received the salt-acid compound. They were then orthogonally assigned

to three groups AX-BX-AY, AX-BX-Y and AX-BY-Y matched on the amount of liquid consumed during the first block. Flavours A and B were counterbalanced in such a way that half of the subjects in each group received saccharin as flavour A and the other half received saccharin as flavour B. During the second block each animal in the groups AX-BX-AY and AX-BX-Y received the compound (saccharin-acid or salt-acid) that was not presented in the previous block while half of the animals in group AX-BY-Y received a compound of saccharin and orange blossom and the other half received a compound of salt and orange blossom. During the third block animals in groups AX-BX-Y and AX-BY-Y received the orange-blossom flavour while half of the animals in group AX-BX-AY received the saccharin-orange blossom compound and the other half received the salt-orange blossom compound, following the condition that saccharin or salt had been presented as A on the first block. Conditioning of X and the test phase followed the same procedure described in the previous experiment.

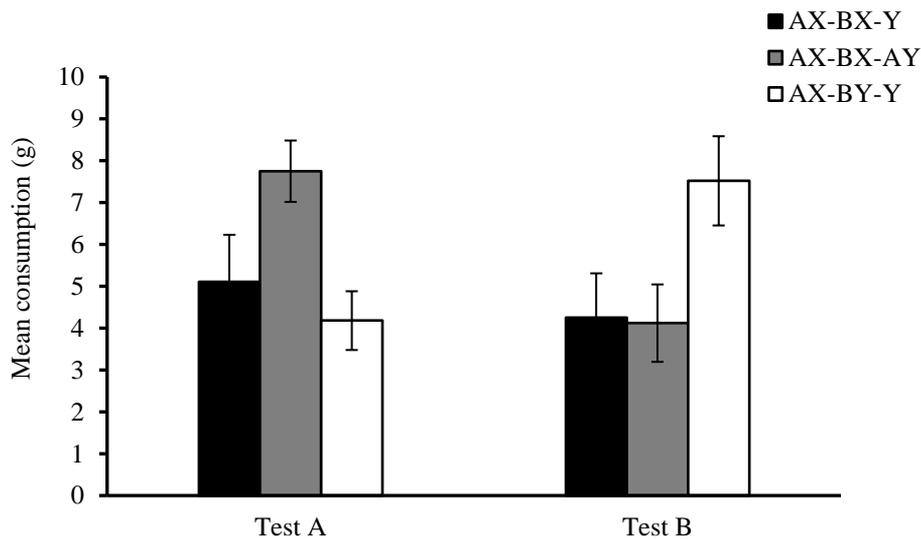
## RESULTS AND DISCUSSION

Mean consumptions per session of the AX flavour during the preexposure phase were 5.4 g (*SEM* 0.40), 5.5 g (*SEM* 0.46) and 5.4 g (*SEM* 0.41) for groups AX-BX-AY, AX-BX-Y and AX-BY-Y, respectively. These amounts did not differ significantly:  $F(2, 29) = 0.004$ ,  $p = 0.99$ . The mean consumed amount of the BX compound during each session of the second block by groups AX-BX-AY and AX-BX-Y was, respectively, 5.6 g (*SEM* = 0.43) and 5.0 g (*SEM* = 0.46) and did not differ significantly  $t(18) = 0.94$ ,  $p = 0.36$ . The mean amounts per session of Y consumed by the groups AX-BX-Y and AX-BY-Y during the third block of preexposure were 6.9 g (*SEM* = 0.56) and 7.3 g (*SEM* = 0.60) respectively. These differences were not significant  $t(18) = 0.56$ ,  $p = 0.57$ .

Conditioning successfully established an aversion to X in the three groups. Throughout the conditioning phase the mean amount of X consumed by subjects in groups AX-BX-AY, AX-BX-Y and AX-BY-Y was, respectively, 4.5 g (*SEM* = 0.82), 4.6 g (*SEM* = 0.58) and 4.9 g (*SEM* = 0.53) in the first conditioning trial; 1.6 g (*SEM* = 0.34), 1.7 g (*SEM* = 0.42) and 2.3 g (*SEM* = 0.42) in the second conditioning trial; and 0.9 g (*SEM* = 0.20), 0.8 g (*SEM* = 0.10), and 1.1 g (*SEM* = 0.18) in the final test. A mixed 3 (Group) x 3 (Trial) ANOVA revealed a significant main effect of trial,  $F(2, 54) = 62.2$ ,

$p = 0.000$ ,  $\eta_p^2 = 0.69$ . The main effect of group was not significant  $F(2, 27) = 0.81$ ,  $p = 0.45$ ,  $\eta_p^2 = 0.09$ , neither it was the group x trial interaction  $F(4, 54) = 0.07$ ,  $p = 0.98$ ,  $\eta_p^2 = 0.05$ .

**Figure 2. Mean consumption of flavours A and B in the test phase of**



**Experiment 2. After the different types blocked preexposure to the compounds represented in the group names, flavour X was paired with nausea before the test. Vertical bars represent standard error of the means.**

Figure 2 shows the amount of A (left) and B (right) consumed by each group of rats during the tests. As it can be appreciated, consumption of A was high in group AX-BX-AY while the other groups consumed similar and low amounts of this flavour. The highest consumption of B corresponded to the group AX-BY-Y while the other groups consumed small and almost identical amounts of this flavour. A mixed 3 (Group) x 2 (Test A or B) ANOVA revealed a significant group x test interaction [ $F(2, 27) = 6.162$ ,  $p < 0.01$ ,  $\eta_p^2 = 0.31$ ]. No main effect was significant ( $F_{max} = 1.227$ ). Separate ANOVAs explored the group x test interaction revealing that groups differed significantly in their consumptions of A [ $F(2, 29) = 4.49$ ,  $p < 0.05$ ] and B [ $F(2, 29) = 8.05$ ,  $p < 0.01$ ]. Duncan's post-hoc tests revealed that consumption

of A was significantly higher in the AX-BX-AY group than in groups AX-BX-Y (Cohen's  $d = .94$ ) and AX-BY-Y (Cohen's  $d = 1.7$ ) and that these last two groups did not differ significantly from each other. Duncan's post-hoc test also confirmed that group AX-BY-Y consumed an amount of B significantly higher than that consumed by groups AX-BX-AY (Cohen's  $d = 1.4$ ) and AX-BX-Y (Cohen's  $d = 2.0$ ) which did not differ significantly from each other (Cohen's  $d = 0.2$ ).

These results show that only one rupture of the  $A \leftrightarrow X$  association does not produce a significant extinction of the association while two ruptures did produce the extinction of this association. The  $A \leftrightarrow X$  association was not broken in the AX-BY-Y group and, hence, could not be extinguished. This explains the low consumption of A in this group. Consumption of A was slightly higher in the AX-BX-Y group where the  $A \leftrightarrow X$  association was broken once. Nevertheless, the groups AX-BX-Y and AX-BY-Y did not differ significantly in their consumptions of A and, hence, it must be concluded that the  $A \leftrightarrow X$  association was not extinguished in the AX-BX-Y group. Given that presentations of Y in the third block are unlikely to produce extinction of the  $A \leftrightarrow X$  association, the lack of extinction found in group AX-BX-Y can be considered comparable to that found by Espinet et al. after blocked preexposure to AX-BX and, hence, is not surprising. The absence of extinction in group AX-BX-Y is also supported by the fact that consumption of A in this group does not significantly differ from consumption of B in the groups that received BX in the second block. In these groups the  $B \leftrightarrow X$  association was not extinguished and consumption of B in these groups may be used as an indicator of the amount of fluid drunk by the animals when the association of this fluid with X was not extinguished. Consumption of A in the AX-BX-AY group reveals an extinction of the  $A \leftrightarrow X$  association after two ruptures. This affirmation is based in the fact that consumption of A in group AX-BX-AY is almost identical to consumption of B in group AX-BY-Y. Given that in this last group B was not associated to X, the large consumption of B is not surprising and this consumption can be taken as an indicator of the amount of fluid that the animals drank when they were not reluctant to drink. The fact that the amount of B consumed by the AX-BY-Y group was similar to the amount of A consumed by the AX-BX-AY group indicates that animals in this last group were not reluctant to drink A and, therefore, it can be concluded that the  $A \leftrightarrow X$  association was fully extinguished in this group, as well as it happened in the AX-BX-AY group of Rescorla and Freberg (1978).

## DISCUSSION

The results of the two experiments presented here show that blocked preexposure to AX-BX, does not produce a significant extinction of the initial  $A \leftrightarrow X$  association, regardless of the length of the exposure (Exp. 1), and also that the two ruptures of this association produced by the addition of a third block of exposure (i.e., AX-BX-AY) result in extinction of the  $A \leftrightarrow X$  association (Exp.2).

In spite of some procedural differences, the latest result coincides with those obtained by Rescorla and Freberg (1978, Exp. 2). The absence of a significant extinction of the  $A \leftrightarrow X$  association observed after preexposure to AX-BX in the two experiments presented here does not fit with the salience modulation account proposed by Hall (2003) which predicts that, in a blocked preexposure to AX-BX, the absence of presentations of the AX compound during the second block will produce the extinction of the  $A \leftrightarrow X$  association. Whatever the basis for this proposal, one might wonder if the results of Rescorla and Freberg (1978) constitute a basis for expecting the extinction of the  $A \leftrightarrow X$  association when blocked preexposure is limited to two blocks (i.e., AX-BX). We suggest that the answer to this question is no. Rather, the evidence provided by some recent experiments and the experiments reported here, leads to two conclusions: First, that preexposure to AX-X or to AX-A does not produce a significant extinction of the  $A \leftrightarrow X$  association and, second, that blocked preexposure to AX-BX does not result in a extinction of the  $A \leftrightarrow X$  association.

The first conclusion is based on the results obtained by Rescorla and Freberg (1978) and, those recently reported by Rodríguez and Alonso (2014, 2015). Rescorla and Freberg (1978, Exp. 1) found that isolated presentations of A or X in the second block of preexposure (i.e., preexposure to AX-A or AX-X) produced only a certain amount of extinction after the addition of an extra-phase of X or A alone presentations. Therefore, preexposure to AX-A or to AX-X (i.e., only one rupture of the  $A \leftrightarrow X$  association) does not seem to be enough as to produce extinction. This statement receives recent support from the experiments of Rodríguez and Alonso (2014) who preexposed AX and X intermixed (AX/X) or in the two possible orders of blocked exposure (AX-X or X-AX). The results showed that the  $A \leftrightarrow X$  association was better preserved in the blocked than in the intermixed group and, what is more important, the strength of the  $A \leftrightarrow X$  association in the AX-X group did not differ significantly from that corresponding to the X-AX group, in which the  $A \leftrightarrow X$  association could not be extinguished, given that AX presentations took place in the last preexposure block.

The second conclusion, that there is no significant extinction of the  $A \leftrightarrow X$  association after blocked preexposure to AX-BX comes from Rodríguez and Alonso (2015, Exp. 1). They preexposed two groups of rats to AX, BX, and X. One of the groups received first intermixed presentations of AX and X, followed by a block of presentations of BX (i.e., AX/X-BX). The second group received first the presentations of BX and later the presentations of AX and X (i.e., BX-AX/X). X was highly concentrated sucrose solution, and the tests consisted of consumption of A and B under a hunger state. The greater the consumption of A or B, stronger should be the association of A or B with X. The results suggested that the BX association was better maintained than the AX association in both groups. The  $B \leftrightarrow X$  association could not be extinguished in the A/X-BX group, where the BX compound was presented in the last block. Some extinction of the  $B \leftrightarrow X$  association should be observed after preexposure to BX-AX/X given that preexposure in this group combines preexposure to BX-AX and preexposure to BX-X, and these are precisely the two ways used by Rescorla and Freberg (1978) to produce extinction of the within-compound associations. However, contrarily to what it might be expected, the results showed that consumption of B, although lower in the BX-AX/X group, did not differ significantly from that of the AX/A-BX group in which there is no reason to expect the extinction of the  $B \leftrightarrow X$  association. It can be argued that in Rescorla and Freberg (1978, Exp. 1) the isolated presentations of A or X in the second block (i.e., AX-A or AX-X) produced some amount of extinction of the  $A \leftrightarrow X$  association. The question, then, is whether presentations of AX-X and presentations of AX-BX produce comparable amounts of extinction of such association. It is not possible to answer this question with our data, but evidence proceeding from Rodríguez and Alonso (2015, Exp. 1) suggests that the presentations of AX-X undermine the strength of the  $A \leftrightarrow X$  association more than presentations of AX-BX do. As the authors noted, their results contradict Hall's proposal and, concerning the question we presented above, these results support the idea that extinction of the  $A \leftrightarrow X$  association should not be expected after blocked preexposure to AX-BX.

The results of the experiments reported here give new support to this suggestion. They are particularly clear in showing this by comparing the groups AX-BY-Y, AX-BX-Y and AX-BX-AY. Given that there are no reasons to expect the extinction of the  $A \leftrightarrow X$  association in the AX-BY-Y group, the amount of A consumed by this group in the test, after conditioning of X, can be used as a reference to evaluate the level of extinction of the other groups. Inasmuch as the AX-BY-Y and the AX-BX-Y groups drank similar amounts of A in the test it seems necessary to conclude that blocked

preexposure to AX-BX-Y did not produce a significant extinction of the  $A \leftrightarrow X$  association in these experiments. On the contrary, in consonance with the results obtained by Rescorla and Freberg (1978, Exp. 2), the extinction of this association was observed in the AX-BX-AY group. Having into account the precedent considerations, a simple explanation for the results presented here is that in a blocked preexposure, one rupture of the  $A \leftrightarrow X$  association is insufficient to produce significant extinction but two ruptures are enough to produce extinction.

It is clear that the significant extinction of the initial  $A \leftrightarrow X$  association observed in the present Experiment 2 is dependent of the third block, but there is no clear whether extinction is produced by presentations of A alone or in compound with Y. Following Rescorla and Freberg (1978) any presentation of A outside the AX compound should produce extinction of this initial association. Therefore, it could be thought that presentations of A (alone or in compound with Y) in the third block effectively produce a second rupture, which causes the extinction of the  $A \leftrightarrow X$  association. Moreover, one might expect that comparing the results of a test with A after preexposure to AX-BX-AY and AX-BX-A could resolve this answer. These two preexposure sequences include two ruptures of the initial  $A \leftrightarrow X$  association but differ in important aspects. Clearly, extensive research is needed to address these issues. Their resolution will contribute to a deeper understanding of the processes involved in blocked preexposure.

## RESUMEN

**Extinción de la asociación intracompuesto inicial establecida en una preexposición por bloques a dos compuestos de sabores.** Se estudió la extinción de la asociación  $A \leftrightarrow X$  después de una preexposición por bloques a AX-BX en dos experimentos. En el Experimento 1, dos grupos de ratas recibieron una preexposición en bloques larga (14 ensayos) o corta (4 ensayos) a AX-BX y posteriormente se condicionó X. Los resultados mostraron que la asociación AX se preservó igualmente tanto después de la preexposición larga como de la corta. En el Experimento 2 se estudió el efecto de la preexposición por bloques a 0, 1 o 2 rupturas en la extinción de la asociación AX. En este experimento una “ruptura” se produce cuando, en los posteriores bloques, uno de los sabores que forman el compuesto inicial es presentado formando un compuesto con otro sabor diferente. Solo se observó una extinción significativa cuando la asociación AX se rompió dos veces.

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