# Preservation of within-compound associations after blocked preexposure to two compound flavors

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**Abstract**

Three experiments examined the elimination of the within-compound A ↔ X link established when two compound flavors, AX and BX, are preexposed in blocks (i.e., AX, AX, AX, ... BX, BX, BX). In Experiment 1, one group of rats underwent preexposure to a block of AX trials followed by a second block of BX trials (AX–BX), while another group underwent blocked preexposure to the same stimuli in the reverse order (BX–AX). Subsequently, flavor A was associated with lithium chloride. This conditioning led to a comparable decrease in the intake of flavor X in both groups. In Experiment 2, four groups of rats underwent blocked preexposure to AX–BX, AX–B, A–BX, or A–X. After aversive conditioning of X, intake of A and B was markedly lower for the groups that received these flavors paired with X compared to the groups where these flavors were presented in isolation. In Experiment 3, one group of rats was preexposed to a block of SaltX presentations followed by a block of BX presentations (SaltX–BX), and another group received blocked preexposure to (BX–SaltX). After subsequent sodium depletion, intake of X was high and similar for both the SaltX–BX and the BX–SaltX groups. These findings suggest that the within-compound association established in the first block of a blocked preexposure is not extinguished when the preexposure phase is completed.

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## 1. Introduction

Since the work of Honey et al. (1994), many experiments on perceptual learning have examined the impact of preexposure to two compound stimuli, AX and BX, presented in alternating trials (e.g., AX, BX, AX, BX, . . .) or across two blocks of trials (e.g., AX, AX, AX . . ., BX, BX, BX). Two potential sequences of preexposure exist depending on which compound, AX or BX, is presented first, and, although scarcely studied, the distinct effects of these two sequences in a blocked preexposure have gained some attention in recent years (Espinet et al., 2011; Rodríguez and Alonso, 2014). The results of these experiments have prompted us to explore the extinction of the within-compound associations established in a blocked preexposure. It is well known that repeated conjoint presentations of two flavors, A and X, lead to the development of an A ↔ X within-compound association (e.g., McLaren and Mackintosh, 2000; Rescorla and Freberg, 1978). While there is no doubt that this association is maintained at the end of alternated preexposure (e.g., Rodríguez and Alonso, 2014), it has been proposed that after an AX–BX blocked preexposure, the A ↔ X association might be eliminated (Hall, 2003; Symonds and Hall, 1995). The aim of the following experiments was to determine if the A ↔ X association is extinguished at the end of preexposure to AX–BX, given that recent experiments have found distinct effects of the two possible orders of blocked preexposure, which call this suggestion into question.

Hall and Rodríguez (2009, Exp. 2) described one of these ordering effects. Hall (2003) proposed that the direct activation caused by repeated exposure to a stimulus diminishes its salience, and that this process of habituation can be attenuated when the stimulus is associatively activated. Therefore, one might expect that during alternated preexposure to AX and BX, the salience of X (directly activated in each trial) diminishes, while the salience of the differential elements A and B is retained, since B is associatively activated,

via X, in the AX trials, and A is associatively activated, via X, in the BX trials. By contrast, in a blocked AX–BX preexposure, the repeated presentations of AX in the first block of trials would produce a reduction in the salience of both A and X. The reduction in salience should also affect B and X during the second block of preexposure trials. To test this possibility, Hall and Rodríguez (2009) assessed salience using the speed of conditioning. After alternated preexposure to BX and X, followed by a block of CX trials (BX/X–CX), they observed that conditioning of B occurred more quickly than conditioning of C. Therefore, they determined that after this sequence of preexposure, the salience of B (preexposed in alternation with X) was greater than that of C (preexposed in a block). Nevertheless, when the block of CX trials preceded the alternated presentations of BX and X (CX–BX/X), the conditioning of C and B occurred at a comparable pace, and this was taken as evidence that C and B retained a similar salience. Insofar as salience is preserved by associative activation, they proposed that, perhaps, the CX–BX/X sequence of preexposure could enable the associative activation of both C and B during the X-alone trials presented in the second block. This could occur if the C $\leftrightarrow$ X association formed in the first block of preexposure was not eliminated during the second block of the preexposure phase.

More recently, Espinet et al. (2011) described certain order effects after blocked preexposure to AX–BX (Group AX–BX) in comparison with preexposure to BX-AX (Group BX–AX). They found that, after preexposure, training with AX led to reduced generalization to BX in the Group AX–BX compared to the Group BX–AX (Exp. 1). Additionally, conditioning of A after preexposure gave B the characteristics of a conditioned inhibitor in the Group AX–BX, as shown by retardation (Exp. 2) and summation tests (Exp. 3). All these order effects might be explained by assuming that the within-compound association established in the first block of preexposure was not extinguished. A fourth experiment was created to investigate this possibility. Three groups of rats received blocked preexposure to AX–BX, AX–BY, or AY–BX. After aversive conditioning of the X flavor, the consumption of flavor A was high in the Group AY–BX but low and similar in the groups AX–BX and AX–BY. Given that there are no grounds to expect the extinction of the A $\leftrightarrow$ X association in the Group AX–BY, where A and X were not separated in the

second block, the similar and low consumption of A observed in the groups AX–BX and AX–BY indicates that the A ↔ X association was not extinguished at the end of the preexposure phase in the Group AX–BX. However, this fourth experiment did not directly compare the two possible orders of a blocked preexposure (AX–BX and BX–AX) that were employed in the prior three experiments. To address this gap, the following experiments compared the strength of the within-compound A ↔ X association after blocked preexposure to AX–BX and BX–AX. Experiments 1 and 2 relied on a procedure based on sensory preconditioning and flavor aversion conditioning, which has offered a dependable indication of the permanence and strength of the within-compound associations established between two flavors when presented together (Rescorla and Freberg, 1978). The third experiment made use of a different procedure, sodium depletion, which provides a direct assessment of the strength of the within-compound association established between two flavors presented together.

## 2. Experiment 1

In the first experiment, we compared the effect caused by the conditioning of one of the differential elements on the two sequences of blocked preexposure. Two groups of rats were subjected to preexposure to two compound flavors (AX and BX) delivered in blocks. Subjects in Group AX–BX were exposed to AX in the first block and BX in the second block, while subjects in Group BX–AX were exposed to BX in the first block and to AX in the second block. With these different sequences of preexposure, the extinction of the A ↔ X association could be observed in Group AX–BX, where, during the second block, X is no longer paired with A, but not in Group BX–AX, where the last block consists specifically of A and X pairings. Following preexposure, consumption of X was assessed using a pretest. Subsequently, A was aversively conditioned in both groups, and the effect of this conditioning was examined in terms of consumption of X and B. After concluding the preexposure phase, the A ↔ X association would be expected to be strong in Group BX–AX, and a sensory preconditioning effect would likely be seen in this group (i.e., a decrease in consumption of X during the test compared to the pretest). Conversely, if the A ↔ X association had been extinguished during the second block of preexposure

in Group AX–BX, no significant differences would be expected in this group between X consumption in the pretest and the test. Alternatively, if the A ↔ X association had been preserved at the end of the preexposure phase, a sensory preconditioning effect similar to that expected in Group BX–AX should also be observed in Group AX–BX.

### *2.1. Subjects and apparatus*

The subjects were 20 experimentally inexperienced male Wistar rats, supplied by Harlan Ibérica, with an average free-feeding weight of 265 g (range: 240–320 g, SEM = 25.12). Subjects were housed in individual cages in a colony room lit from 8:30 to 21:45, and kept at 22 ± 1 °C.

The solutions serving as experimental stimuli were delivered in the home cages at room temperature, using 150 ml plastic bottles fitted with metal drinking spouts. Three flavored stimuli, the same as those used in our prior experiments (Espinet et al., 2011), were prepared with tap water and chemically pure substances provided by Probus or Merck laboratories: 0.3% (w/v) citric acid; 0.15% (w/v) saccharin, and 0.5% (w/v) sodium chloride. Two compound solutions, saccharin-acid and salt-acid, were made, preserving the aforementioned individual concentrations of each substance. Intake was measured by weighing the bottles before and after each session. Intraperitoneal injections of 0.3 M LiCl at 10 ml/kg of body weight were used for the conditioning trials. These injections were delivered in an experimental room adjacent to the housing area.

### *2.2. Procedure*

The night previous to the preexposure phase, the water bottles were removed from the cages at 21.00 h. Throughout all the phases of this and the next experiments, the rats had access to fluid for 15 min in four daily sessions starting at 9.00; 13.00; 17.00 and 21:00 h, during each session, the animals were permitted to drink freely from a bottle containing 100 ml of solution. Flavors A and B were counterbalanced so that half of the rats in each group received saccharin as flavor A and sodium chloride as flavor B. For the remaining half of the animals, this order was reversed. Flavor X was

citric acid for all the rats. The preexposure phase continued for one week, during which the rats received 28 preexposure sessions divided into two blocks. In the first 14 trials, half of the subjects were given the saccharin-acid compound, and the other half received the salt-acid compound. Subsequently, the animals were divided into two groups (Group AX–BX and Group BX–AX), matched based on their consumption in the first block of trials, counterbalancing the flavors so that half of the subjects in each group had received the saccharin-acid solution and the other half had received the salt-acid solution. In the second block, each animal had access to the compound that had not been preexposed in the first block. After preexposure, all the rats received the experimental treatments at 9:00 and drank water during the three remaining daily sessions. The first day after preexposure, all the animals received a pretest to assess consumption of X. The subsequent four days comprised the conditioning phase. On days 1 and 3 of this phase, each subject received flavor A (saccharin or salt) to which it had been preexposed, followed by an injection of LiCl. Days 2 and 4 of this phase served as recovery days, and all subjects drank water during the four daily sessions. The day after the conditioning phase, the experimental treatment consisted of a test of consumption of X. On the last day, a final test was conducted with the flavor designated as B during the preexposure phase.

*2.3. Results and discussion from experiment 1*

A significance level of $p < 0.05$ was adopted for the statistical tests in this and the next experiments. During the preexposure phase the groups AX–BX and BX–AX consumed similar amounts of AX: 58.4 g (SEM = 5.29) and 60.1 g (SEM = 4.22) respectively, $t(1,18) = -0.24$, $p = 0.62$. The intakes of BX were also similar in both groups: 58.6 g (SEM = 5.72) and 53.5 g (SEM = 3.44) respectively, $t(1, 18) = 0.75$, $p = 0.46$. The left part of Fig. 1 shows the amount of flavor A consumed by each group in the two trials of the conditioning phase. It seems evident that the conditioning procedure established an aversion to A in both groups. A mixed ANOVA performed on these data with group and trial as variables revealed only a significant effect of trial $F(1, 18) = 164.5$, $p = 0.000$, $h2 = 0.9$. Neither the main effect of group nor the interaction was significant [$F(1, 18) = 0.55$, $p = 0.46$, $h2 = 0.03$, and $F(1, 18) = 0.78$, $p = 0.38$, $h2 = 0.04$, respectively].

The central part of Fig. 1 shows the results of the pretest and the test with flavor X. It seems evident that conditioning of A produced a decrease in consumption of X that affected both groups similarly. A mixed ANOVA performed on the data summarized in Fig. 1 with group and trial (pretest vs. test) as variables, confirmed this impression showing a significant effect of trial, $F(1, 18) = 45$, $p = 0.000$, $h2 = 0.7$. Neither the effect of group nor the group x trial interaction was significant [$F(1, 18) = 0.88$, $p = 0.35$, $h2 = 0.04$, and $F(1, 18) = 0.007$, $p = 0.93$, $h2 = 0.00$, respectively]. To the extent that reduction in consumption of X could be attributed to a sensory preconditioning effect based on the A ↔ X association, it could be concluded that the initial A ↔ X association was not extinguished in Group AX–BX once the preexposure phase concluded.

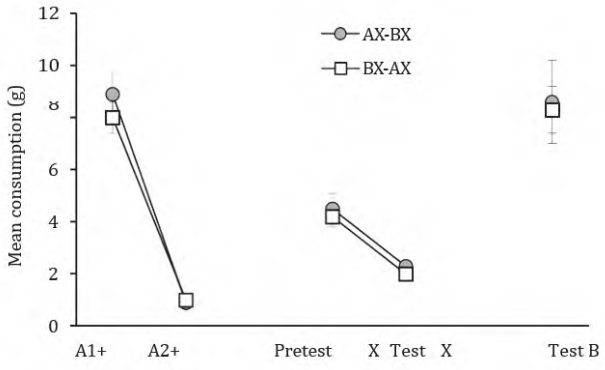


**Figure 1.** Experiment 1. Left part: Mean group consumption of the flavor A in the two conditioning trials. Central part: Mean group consumption of the flavor X before (Pretest X) and after (Test X) conditioning of A. Right part: Mean group consumption of flavor B in the test. Group AX–BX had received preexposure to a block of AX trials followed by a block of BX trials. Group BX–AX had received preexposure to a block of BX trials followed by a block of AX trials. Vertical bars represent the standard error of the means.

The purpose of the final test with B was to know if the aversion conditioned to A was generalized to B. Given that flavors A and B were counterbalanced it was expected that, in absence of conditioning, the consumptions of A and B were similar. Therefore, a lack of generalization could be apparent if consumption of A in the first conditioning trial (A1+) and consumption of B in the final test were similar. The amounts of A and B consumed in the A1+ trial and in the final test with B are respectively shown in the left and right parts of Fig. 1. It seems apparent that the two groups consumed similar amounts of A and B. This impression was confirmed by the results of a mixed ANOVA performed on these data with group and flavor (A or

B) as variables that revealed no effect of group, no effect of flavor, and no interaction between these variables [$F(1, 18) = 0.96$, $p = 0.33$, $h2 = 0.05$, $F(1, 18) = 0.08$, $p = 0.78$, $h2 = 0.004$, and $F(1, 18) = 0.086$, $p = 0.77$, $h2 = 0.005$, respectively]. This result shows that the aversion produced by the conditioning of A was not generalized to B. Given this lack of generalization between A and B there are no apparent reasons to expect that generalization could occur exclusively between A and X but, in the absence of a control group for sensory preconditioning, it cannot be unequivocally established that the reduction in consumption of X observed in both groups after conditioning of A is a result of a sensory preconditioning effect. The next experiment was designed to shed light on this doubt.

## 3. Experiment 2

In the second experiment we examined the formation and extinction of within-compound associations in a blocked preexposure, with explicit control for sensory preconditioning.

Four groups of rats underwent preexposure to flavor stimuli in two blocks. The subjects in Group AX–BX underwent preexposure to two compound flavors: AX in the first block and BX in the second block. The subjects in Group A-X received flavor A in the first block and flavor X in the second block. Subjects in Group AX–B were preexposed to the AX compound in the first block and flavor B in the second block. Group A–BX underwent preexposure to the single flavor A in the first block and to the BX compound in the second block. After preexposure, flavor X underwent aversive conditioning in all groups, and subsequently, the rats were tested for their consumption of A, B, and X. An A $\leftrightarrow$ X association could be formed in groups AX–BX and AX–B since they received flavors A and X in compound throughout the first block of preexposure. This association could be extinguished during the second block in Group AX–BX, because X was presented outside the AX compound, paired with a new stimulus B. On the contrary, once the preexposure phase was concluded, the A $\leftrightarrow$ X association should remain robust in Group AX–B, as the flavors A and X were never unpaired. In groups A–X and A–BX, where A and X were presented without pairing, the establishment of an A $\leftrightarrow$ X association was not feasible, and hence, their consumption of A acted as a control for the effects of sensory

preconditioning in groups AX–BX and AX–B..

Therefore, after conditioning of X, the existence of the A ↔ X association in groups AX–BX and AX–B could be detected by means of a sensory preconditioning effect: lower consumption of A in these groups than in groups A–BX or A–X. Additionally a test with flavor B would provide additional information to measure the strength of the A ↔ X association. Given that the B ↔ X association was established in the second block it could not be extinguished. Therefore, and given that the flavors A and B were counterbalanced, consumption of B corresponding to the groups AX–BX and A–BX provided a measure of a non-extinguished within-compound association.

*3.1. Subjects and apparatus.*

The subjects were 40 naïve male Wistar rats with a mean ad lib weight of 311 g (range 255–384 g). They were maintained in the conditions described in Experiment 1. The solutions used as experimental stimuli were the same as described in Experiment 1.

*3.2. Procedure*

T The experimental sessions adhered to a schedule identical to that outlined in Experiment 1. Prior to the commencement of the first block of preexposure, the animals were allocated into two groups matched based on their body weights. In each of the first 14 trials of preexposure, one of these groups was administered the AX compound, whereas the second group received flavor A. Following the first block of preexposure, each group was further divided into two groups matched based on their consumption during the first block: the group exposed to AX was subdivided into groups AX–BX and AX–B, whereas the group exposed to A was divided into the groups A–BX and A–X. During the second block of preexposure, the subjects in groups AX–BX and A–BX were administered the BX compound, while the subjects in groups AX–B and A–X received, respectively, flavors B and X.

The four days following the preexposure phase constituted the conditioning phase. In days 1 and 3 of this phase all subjects received flavor X followed by an injection of LiCl. The days 2 and 4 of this phase were recovery days and all the subjects drank water in the four daily sessions. The first day after the

conditioning phase half of the animals in each group received a test of consumption of the flavor used as A while the other half of animals received the flavor B. The next day each animal received the flavor (A or B) that had not been tested the previous day. The last day all the rats received a final test of consumption of flavor X.

### *3.3. Results and discussion*

Throughout the first block of the preexposure phase the groups AX–BX and AX–B consumed respectively 76 g (SEM = 2.2) and 79.2 g (SEM = 1.8) of the AX compound. These amounts did not differ significantly: $t(1,18) = -1.1$, $p = 0.28$. The intake of A correspond- ing to groups A–BX and A–X were, respectively, 90.3 g and 98.2 g and did not differ significantly $t(1, 18) = -1.2$, $p = 0.24$. Through- out the second block of preexposure the subjects in Group AX–BX drank 84.2 g of BX (SEM = 2.09) while the subjects in Group A–BX drank 79.6 g of BX (SEM = 2.3). These amounts did not differ significantly $t(1, 18) = 1.4$, $p = 0.16$. Conditioning successfully established an aversion to X in the three groups. Throughout the conditioning phase the mean amounts of the flavor X consumed by the sub- jects of the groups AX–BX, AX–B, A–BX and A–X were, respectively, 4.3 g (SEM = 0.28), 4.4 g (SEM = 0.33), 4.1 g (SEM = 0.28) and 4.6 g (SEM = 0.18) in the first conditioning trial; 2.3 g (SEM = 0.51), 1.9 g (SEM = 0.31), 1.4 g (SEM = 0.14) and 2.3 g (SEM = 0.41) in the second conditioning trial; and 1 g (SEM = 0.52), 1.1 g (SEM = 0.28), 0.78 g (SEM = 0.14), and 1.3 g (SEM = 0.40) in the final test. An ANOVA con- ducted on these data with group and trial as variables revealed a significant effect of trial, $F(2, 72) = 173.3$, $p = 0.000$, $h2 = 0.82$. The effect of group was not significant $F(3, 36) = 1.06$, $p = 0.37$, $h2 = 0.08$, as well as the group x trial interaction $F(6, 72) = 0.42$, $p = 0.86$, $h2 = 0.034$.

Fig. 2 shows the mean amount of flavors A and B consumed by the four groups in the test phase. It is clear from inspection of the left-hand side of the figure that consumption of A was smaller in groups AX–BX and AX–B than in groups A–BX and A–X. On the right- hand side of the figure it can be appreciated that the biggest intake of B corresponded to the Group AX-B while groups AX–BX and A–BX consumed small and similar amounts of B and consumption by Group A–X was situated between these two extremes. A mixed ANOVA with group and test type (A or B) as the factors, revealed a significant effect of group

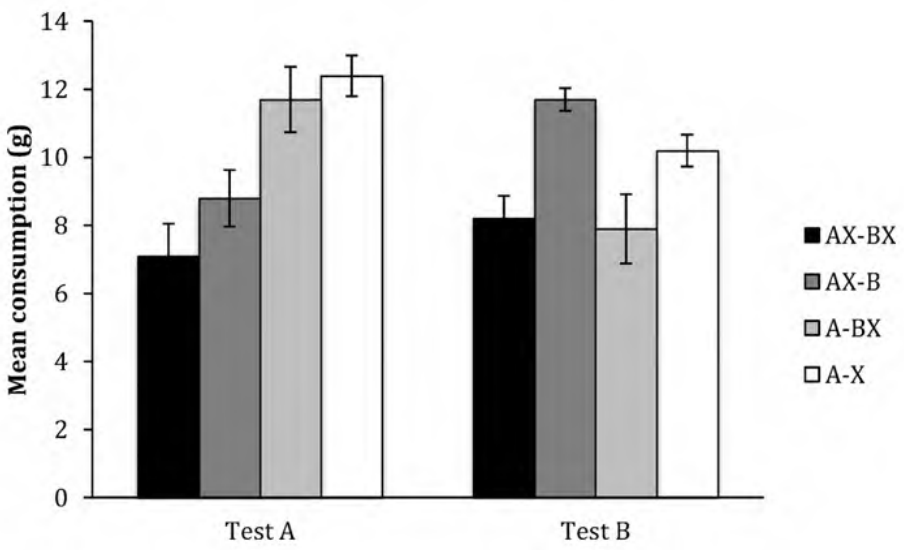


**Figure 2.** Experiment 2. Left part: Mean group consumption of the flavor A in the test phase. Right part: Mean group consumption of the flavor B in the test phase. The names of the groups indicate the flavors that each group received during the first preexposure block (before the hyphen) and during the second block (after the hyphen). Preexposure was followed in all the groups by aversive conditioning of X. Vertical bars represent the standard error of the means.

$F(3, 36) = 5.6$, $p = 0.003$, $h2 = 0.31$ and a significant interaction $F(3, 36) = 10.1$, $p = 0.000$, $h2 = 0.45$ while the effect of test type was not significant $F(1, 36) = 0.56$, $p = 0.45$, $h2 = 0.015$. Subsequent comparisons confirmed that the groups differed significantly in their intakes of A, $F(3, 39) = 7.26$, $p = 0.001$ and B $F(3, 39) = 7.37$, $p = 0.001$. The intakes of A corresponding to groups A–BX and A–X did not differ significantly each other and were significantly larger than those of the other two groups. The intakes of B corresponding to groups AX–BX and A–BX did not differ significantly each other and were significantly smaller than those of groups AX–B and A–X.

These results indicate that the preexposure procedures used in this experiment allowed the establishment of within-compound associations between the flavors preexposed together. Regarding the A ↔ X association, conditioning of X resulted in significantly lower consumption of A in the groups where A was preexposed in compound with X, than in the groups where the A ↔ X association could not be formed. Given that the consumption of A was significantly high in groups A–X and A–BX, the two groups in which an A ↔ X association could not be formed, the low consumption of A in groups AX–BX and AX–B appears to be the result of a sensory preconditioning effect which

depends on the A ↔ X association. Much the same can be said regarding the B ↔ X association, given that consumption of B after conditioning of X was significantly lower in groups AX–BX and A–BX than in Group AX–B in which the B ↔ X association could not be formed. The fact that consumption of B was lower in Group A–X than in Group AX–B probably reflects neo- phobia to the flavor B which was presented in this group for the first time in the test trial. On the other hand, the large intake of A in groups A–X and A–BX together with the high consumption of B in Group AX–B indicate that the aversion conditioned to X was not generalized to the flavors A or B when they were preexposed isolated and, hence, the low intake of the flavors paired with X during the preexposure reveals a genuine sensory preconditioning effect. Therefore these results lead to conclude that blocked preexposure to AX–BX did not result in the extinction of the A ↔ X association established in the first block of the preexposure phase. This conclusion is mainly supported by the fact that the intake of A in Group AX–BX was similar to the intake of A in Group AX–B where the A ↔ X association could not be extinguished. In other words, the intake of A corresponding to Group AX–B reveals the magnitude of the sensory preconditioning effect resulting from a non-extinguished A ↔ X association, and this intake did not differ significantly from the intake corresponding to Group AX–BX where the A ↔ X association could have been extinguished. A second argument supporting the idea that the A ↔ X association was not extinguished is that the intake of A was low and similar to the intake of B in Group AX–BX, where the B ↔ X association could not be extinguished given that the BX compound was presented in the second block of the preexposure phase.

Due to the fact that the compounds and procedures used in this experiment were the same as those employed in Experiment 1, the present results lend support to the interpretation of the results of Experiment 1 in terms of sensory preconditioning rather than in terms of generalization between A and X exclusively. Despite this reasoning, we designed a third experiment using a procedure that does not require the use of conditioning and, hence, makes unfeasible any interpretation in terms of generalization of the conditioned response.

## 4. Experiment 3

This experiment assessed the strength of the A ↔ X association after blocked preexposure to AX–BX using sodium depletion. Two groups of rats received blocked preexposure to SaltX–BX (Group SaltX–BX) or to BX–SaltX (Group BX–SaltX). Two additional groups were included; one (Group Salt/X) received unpaired presentations of Salt and X, and the second (Group NoD) received preexposure to SaltX and BX but not sodium depletion. After preexposure, a need for salt was induced in the first three groups by sodium depletion, and subsequently, appetite for flavor X was tested. Sodium depletion increases consumption of a flavor previously associated with salt (Rescorla and Durlach, 1981), so consumption of X was expected to be high in Group BX–SaltX, as the SaltX compound was presented in the last block of preexposure trials, suggesting a strong Salt ↔ X association. Consumption of X in Group SaltX–BX should be low if the initial Salt ↔ X association was extinguished; however, if preserved, intake should be similar to Group BX–SaltX. Conversely, consumption of salt was expected to be low in Group Salt/X, where salt was not paired with X, and also in Group NoD, where the need for salt was not induced.

### *4.1 Subject and apparatus.*

The subjects were 40 naïve Wistar rats (20 male and 20 female) with a mean ad-lib weight of 334 g (range: 220–385 g). In this experiment the flavors were not counterbalanced; for all the animals flavor X was saccharin 0.15% (w/v) and flavor B was citric acid 0.3% (w/v). This was made with the intention of facilitating the consumption of the flavor used in the test (X), given that the animals drink only small amounts of citric acid and this could hamper the detection of differences between the groups. The salt solution was prepared with a concentration of 1% (w/v). The compound solutions, salt-saccharin and acid-saccharin, maintained the above-mentioned individual concentrations of each substance. Injections of 0.5 ml of a mixture of 200 mg furosemide and 100 mg deoxycorticosterone acetate dispersed in 10 ml of distilled water with 1 drop of the dispersant Tween 80 were used to induce sodium appetite.

*4.2. Procedure.*

Five females and five males matched according to their body weights were assigned to each of the following four groups: SaltX–BX, BX–SaltX, Salt/X and Control. The distribution of trials during the preexposure phase was identical to that described in Experiment 1. The animals in Group SaltX–BX received a first block of 14 SaltX trials followed by a second block of 14 BX trials. This order was reversed for the subjects in Group BX–SaltX. In Group Salt/X the subjects received 14 presentations of salt and 14 presentations of saccharine. These presentations were alternated and counterbalanced (half of the animals starting with salt and the other half starting with saccharine). In Group NoD the subjects received blocked preexposure to the SaltX and BX compounds (half of the animals starting by 14 presentations of the SaltX compound and the other half starting by 14 presentations of BX). In the day following the preexposure phase the animals received tap water in the first three sessions. At 18.00 h the subjects in groups SaltX–BX, BX–SaltX and Salt/X received a subcutaneous injection containing the furosemide preparation while the animals in Group NoD remained in their home cages. The food was removed from all the home cages and all the subjects had access to distilled water overnight. The next day the distilled water was removed from the cages 3 h prior to the test trials. The test trials started at 14.00 and they were separated by intervals of 30 min. The first and second tests consisted of 15 min of free access to a bottle containing the flavor X (saccharin). The third test consisted of 15 min of free access to a bottle containing salt.

*4.3. Results and discussion*

The total amounts of the salt-saccharin solution consumed by groups SaltX–BX, BX–SaltX and NoD during the preexposure phase were, respectively, 105.11 g (SEM = 3.91), 104.08 g (SEM = 7.44), and 105.88 g (SEM = 4.87). There were no group differences in the consumption of salt-saccharin, $F(2, 27) = 0.26$. The total amounts of the acid-saccharin solution consumed by the same groups were, respectively, 53.20 g (SEM = 1.66), 58.11 g (SEM = 2.23), and 60.19 g (SEM = 3.01) and did not differ significantly $F(2, 27) = 2.30$. The sub- jects in Group Salt/X consumed in the preexposure phase a total of 98.04 g of salt (SEM = 7.42) and 76.05 g of saccharin (SEM = 3.12).

Fig. 3 shows the mean amounts of saccharin (X) consumed by each group in the two tests with this substance (left hand) and the mean amount of salt consumed in the final test (right hand). It appears from inspection of the left part of this figure that groups SaltX–BX and BX–SaltX drank more saccharin than groups Salt/X and NoD. A mixed ANOVA performed on these data with group and trial as the factors showed significant effects of group $F(3, 36) = 6.7$, $p = 0.002$, $h2 = 0.33$ and trial $F(1, 36) = 31.9$, $p = 0.00$, $h2 = 0.47$ and no interaction between these factors $F(3, 36) = 0.48$, $p = 0.69$, $h2 = 0.03$. Post hoc tests revealed that groups SaltX–BX and BX–SaltX did not differ significantly from each other in their consumptions, but consumed an amount of X significantly greater than groups Salt/X and NoD, which did not differ significantly from each other.

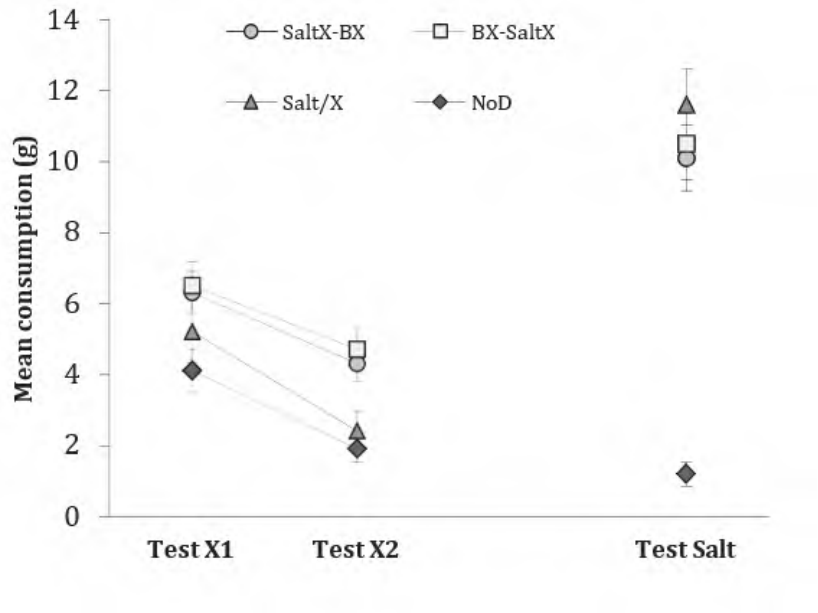


**Figure 3.** Experiment 3. Left part: Mean group consumption of the flavor X in the test phase. Right part: Mean group consumption of salt in the test phase. Group SaltX–BX and BX–SaltX received blocked preexposure to the compounds SaltX and BX. Group Salt/X received alternated preexposure the flavors Salt and X. Group NoD received blocked preexposure to SalX and BX in a counterbalanced order. After preexposure, sodium depletion was induced in all the groups except in Group NoD. Vertical bars represent the standard error of the means.

The right part of Fig. 3 shows that in the last test the three depleted groups consumed high and similar amounts of salt while the subjects in Group NoD consumed only a small amount of this flavor. An ANOVA revealed the existence

of significant differences between the four groups $F(3, 39) = 30.01$, $p = 0.00$. Post hoc Duncan tests confirmed that the three depleted groups did not significantly differ from each other in their consumption of salt, which were significantly greater than the consumption of Group NoD. This result indicates that the treatment used to induce a sodium appetite was effective and affected similarly to the three depleted groups. Under sodium depletion the largest appetite for flavor X associated with salt corresponded to groups SaltX-BX and BX–SaltX which consumed similar amounts of X. Given that the Salt ↔ X association should not have been extinguished in the group BX–SaltX this result indicates that the Salt ↔ X association was also preserved in Group SaltX–BX after preexposure.

Other aspects of the present procedure and results deserve further attention. One of them refers to the convenience of using the two test trials with X and the two control groups: Group Salt/X which controlled for the influence of the Salt ↔ X association on consumption of X during the test, and Group NoD which controlled for the effects of sodium depletion. Although these two groups consumed similar and smaller amounts of X than those consumed by groups SaltX–BX and BX–SaltX, the differences were more evident in the second than in the first trial. It is interesting that both, the Salt/X and the NoD groups, drank in the first test a sensible amount of X nearest to that consumed by the SaltX–BX and BX–SaltX groups, although their subjects did not have a special motivation to drink this flavor, except for the three hours of fluid deprivation before this first test. It does not seem likely that the rats were thirsty but, if this were the case, the differences between the groups in the second test trial, when thirst was an improbable motivational factor, should reflect more appropriately the between-group differences in the motivation to consume specifically flavor X. In absence of an increased appetite for salt, the high consumption of X of the Control group in the first test indicates, probably, the tendency to drink saccharin, a palatable taste. The consumption of X in Group NoD did not significantly differ from the consumption of X in Group Salt/X and was lower than that of groups SaltX–BX and BX–SaltX. This result leads to conclude that in these last two groups the flavor X remained associated to the salt once concluded the preexposure phase and, hence, that the Salt ↔ X association was not extinguished in Group SaltX–BX.

## 5. General discussion

Taken together, the results of the three experiments presented here using two different procedures provide converging evidence that the within-compound association formed during the first block of blocked preexposure to two compound flavors is not fully extinguished once the preexposure is concluded. This conclusion was proposed by Espinet et al. (2011) as a requisite to explain some differential effects observed after blocked preexposure to the AX-BX and BX-AX sequences, and, given that the results presented here have been obtained using identical compounds and preexposure procedures to those employed by them, the present results provide support for their previous proposal.

The results presented here should be compared with those by Rescorla and Freberg (1978), who specifically studied the extinction of the within-compound associations. They reported that after preexposure to AX, presenting A or X separately (Exp. 1) or in compound with B (Exp.2) resulted in "attenuation" (p. 416) or "disruption" (p.424) of the aversion preconditioned to A as a consequence of the conditioning of X and concluded that "within-compound associations may be extinguished in a manner analogous to other Pavlovian associations" (p.424). It should be noted that the A ↔ X association was not fully extinguished in their experiments (except for the "Group Q" of their Experiment 3). Although it is true that the groups that received separate presentations of A or X following the AX presentations drank more A than a control group in which A and X were not presented separately, it is also true that the extinguished groups drank amounts of A significantly lower than those of a control group for which A and X were never presented together (Exp. 1). This indicates that the extinction of the A ↔ X association was not complete despite the fact that A and X were presented separately after preexposure to AX. On the other hand, none of the preexposure procedures used by Rescorla and Freberg was identical to that of a blocked preexposure to AX-BX. Table 1 outlines the experimental designs used by Rescorla and Freberg (1978), Rodríguez and Alonso (2014) and the experiments reported here and facilitates a comparison of the similarities and differences between these experiments. As it can be appreciated, the Experiment 1 of Rescorla and Freberg and the Experiment 2 of

Rodríguez and Alonso are very similar, while the experiments here reported resemble only the first two preexposure phases of the Experiment 2 of Rescorla and Freberg, given that the latter includes a third stage (Pre 3) where A is again separated from X and paired with a new flavor Y, and this could contribute sharply to a complete extinction of the A ↔ X association.

**Table 1**

Design of the experiments. (R & F): Rescorla and Freberg, 1978; (R & A): Rodríguez and Alonso, 2014; (E, C, & C): Espinet et al. in this article. Only the comparable groups are represented here. The initials naming the stimuli in the experiments of Rescorla and Freberg have been changed for a better comparison.

| Experiment | Pre 1 | Pre 2 | Pre 3 | Conditioning | Test 1 | Extinction | Test 2 | Test 3 |
|---|---|---|---|---|---|---|---|---|
| R & F Exp.1 | 4 AX | 6 X | | 3 X -> LiCl | A vs W | 4 X | X vs W | X vs AX |
| R & F Exp. 2 | 4 AX | 4 BX | 4 AY | 3 X -> LiCl | A vs W | | A | |
| R & A Exp. 2 | 4 AX | 4 X | | 3 A -> LiCl | 6 X | | | |
| E, C, & CExp. 2 | 14 AX | 14 BX | | 2 X -> LiCl | A | | B | X |

The suggestion that the A ↔ X association is not entirely extinguished after blocked preexposure receives support from the findings reported by Rodríguez and Alonso (2014, Exp. 2). They compared the strength of the within-compound A ↔ X association after preexposure in three groups of rats. One of these groups received alternated preexposure to AX/X while the other two groups underwent blocked preexposure (AX–X or X–AX). The results obtained by Rodríguez and Alonso indicated that, after aversive conditioning of flavor A, consumption of X was higher in the group that received alternated preexposure to AX and X than in the group that underwent AX–X blocked preexposure. Given that there is no reason to anticipate extinction of the A ↔ X association after alternated preexposure, this finding suggests that, instead of being extinguished, the A ↔ X association was stronger after blocked preexposure to AX–X than following alternated preexposure. In conclusion, blocked preexposure to AX–X did not result in extinction of the A↔X association. This outcome differs from the findings obtained by Rescorla and Freberg (Exp. 1), despite employing a very similar procedure (see Table 1). Both Rescorla and Freberg (Exp. 1) and Rodríguez and Alonso (Exp. 2) provided the animals with preexposure to AX–X followed by aversive conditioning of A and subsequent tests of consumption of flavor X. Both studies included 4 AX preexposure trials but differed in the number of subsequent X alone trials (initially 6 in the experiment of Rescorla and Freberg and 4 in the experiment of Rodríguez and Alonso). Again, it should be noted that Rescorla and Freberg did not find a

complete extinction of the A ↔ X association in the Test 1 and they had to give four additional presentations of X. The largest number of extinction trials (i.e., presentations of X alone) in the experiment of Rescorla and Freberg, could perhaps explain the discrepancy with the results obtained by Rodríguez and Alonso. Nevertheless, not always separate presentations of two flavors presented previously in compound leads to a complete extinction of the within-compound associations. For instance, Alonso and Hall (1999) reported that these associations survived after separate presentations of the two flavors.

Having discussed the discrepancies between some results, the next paragraphs will concentrate on the convergences. The results obtained by Rodríguez and Alonso using blocked preexposure to AX-X are in line with the results we have obtained using blocked preexposure to AX-BX and both lead to conclude that the A ↔ X association formed in the first block is not fully extinguished after blocked preexposure. Furthermore, Rodríguez and Alonso preexposed their subjects to the two possible orders of blocked preexposure (AX–X and X–AX) and this allows a second comparison with the experiments presented here; their results and those presented here are coincident in showing that the strength of the A ↔ X association after blocked preexposure is the same, whatever of the order of the AX compound.

One implication of these results is that they question one of the mechanisms suggested to explain why discrimination between two similar stimuli is better after intermixed than after blocked pre- exposure (the so-called "intermixed/blocked effect"). Hall (2003) suggested that the salience of the differential elements A and B is greater after intermixed preexposure to AX and BX. Although the amount of preexposure to A and B is the same in the intermixed and blocked preexposure schedules, the salience of A and B does not decline during intermixed preexposure given that A and B are associatively activated via X. In contrast, during blocked preexposure to AX-BX, the associative activation of B via X is not possible and the associative activation of A will be very limited since the AX compound is no longer presented in the second block and, as Hall suggested, this will lead to the extinction of the initial A ↔ X association. However, to our knowledge, with the exception of the report of Espinet et al. (2011, Exp. 4) this assumption had never been explicitly tested. Their results and those presented here together with those of Rodríguez and Alonso lead to conclude that, whatever the mechanism responsible for the

intermixed-blocked effect, such a mechanism cannot rely on the extinction of the within-compound association formed in the first block of a blocked preexposure. The latest research on the intermixed-blocked effect (e.g., Artigas and Prados, 2014) gives support to a peculiarity of the blocked preexposure reported by Mondragón and Hall (2002) and Mondragón and Murphy (2010): the fact that the salience of the common element X is greater after blocked than intermixed preexposure. This fact is compatible with Hall's suggestion that the differential elements will be more salient after alternated preexposure. In the intermixed preexposure X is alternatively accompanied by A or B while in the blocked preexposure X is consistently accompanied by A or B in each trial of the same block. Therefore X becomes a better predictor of A or B in the blocked than in the intermixed preexposure. Assuming that the animals learn to ignore the stimuli which are bad predictors of the outcome (Mackintosh, 1975), the attention paid to X should decline more in the intermixed than in the blocked preexposure, rendering A and B more salient in the intermixed preexposure. Whatever the plausibility of this suggestion it is evident that there is not a conclusive explanation of the mechanisms underlying the intermixed/blocked effect. Twenty years after the pioneer work of Honey et al. (1994) the study of the peculiarities of the intermixed and blocked preexposure schedules continues being necessary for the improvement of the perceptual learning models.

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