Order effects after blocked preexposure to two compound flavors

Alfredo Epinet[a], José M. Caramés[a], Victoria D. Chamizo[a]

[a]*Universidad de Málaga*
[b]*Universidat de Barcelona*

Abstract

Introduction: In the first three experiments two groups of rats received prolonged blocked preexposure to AX–BX or to BX–AX. Experiment 1 showed that conditioning of AX after preexposure resulted in less generalization to BX in the AX–BX group than in the BX–AX group. Experiments 2 and 3 constituted, respectively, retarda- tion and summation tests of the inhibitory properties acquired by B after preexposure and conditioning of A. Excitatory conditioning of B was retarded in the AX–BX group (Experiment 2) and only in this group B was able to alleviate the aversion conditioned to a new stimulus (Experiment 3). These results are explained as a consequence of the formation, in the AX–BX group, of inhibitory associations directed from B to A. A fourth experiment provided evidence that the A ↔ X association was preserved until the end of the preexposure phase in this group, which is a requisite for the formation of these inhibitory associations.

*Alfredo Espinet
Email address: espinet@uma.es

1. Introduction

Intermixed and blocked preexposure to two compound flavors, AX and BX, have been frequently used in studies on perceptual learning. In the intermixed preexposure (AX/BX) the two flavors are presented in alternation while in the blocked preexposure all the AX trials precede the BX trials (AX–BX) or vice versa (BX–AX).

McLaren and Mackintosh (2000) stated explicitly the associative mechanisms involved in each of these two preexposure schedules. In the intermixed preexposure mutual excitatory associations are formed between the elements preexposed together (X $\leftrightarrow$ A on AX trials and X $\leftrightarrow$ B on BX trials). These associations will produce, via X, the associative activation of B when AX is presented and the associative activation of A when BX is presented. Under these conditions inhibitory connections will be established between A and B insofar as the presence of A will become a signal for the absence of B and vice versa. There is now direct evidence showing that, indeed, mutual inhibitory associations are formed between A and B after prolonged intermixed preexposure to AX and BX (Dwyer et al., 2001; Dwyer and Mackintosh, 2002). McLaren and Mackintosh also suggested that weak unidirectional inhibitory associations may be formed after blocked preexposure. The direction of these associations would be determined by the order in which the com- pounds are presented: preexposure to AX–BX could give rise to the formation of inhibitory associations directed from B to A while pre exposure to BX–AX could result in inhibitory associations directed from A to B. Concretely they suggested that ". . . if all the AX trials precede BX trials, A will not be established as an inhibitor of B (since B is not yet predicted by the presence of X), and although, in principle, inhibitory associations from B to the now absent A might be formed on BX trials, evidence from other types of experiment suggest that they would be, at best, very weak" (McLaren and Mackintosh, 2000, p. 235). With these theoretical antecedents it is not surprising that few experiments have directly evaluated the existence of these unidirectional inhibitory

associations and the order effects that they could produce. Rather in the experiments on perceptual learning it has been usual to counterbalance the AX–BX and the BX–AX schedules into only one group of sub- jects, and as a consequence the small effects attributable to the order of presentation of the compounds in the blocked condition could have passed unnoticed. Nevertheless, some empirical data proceeding from recent experiments suggest the convenience of exploring more deeply the existence of these order effects.

For instance, in the studies on perceptual learning it has been usual to compare the effects of intermixed and blocked preexposure on generalization to BX of an aversion conditioned to AX. The common result of this test is a perceptual learning effect: less generalization is observed after alternated than after blocked pre- exposure (e.g., Symonds and Hall, 1995). Nevertheless, Sanjuan et al. (2004) found that blocked preexposure can be as effective as intermixed preexposure in reducing generalization.

More recently Hall and Rodríguez (2009, Exp. 2) have reported the existence of an order effect of stimulus presentation after blocked preexposure. In their experiment, rats received alternated preexposure to BX and X, but these BX/X trials were preceded or followed in different subgroups by a block of AX trials (the original nomenclature has been changed by convenience). Following Hall (2003) this preexposure schedule should contribute to maintain the salience of B, given that the representation of B is associatively activated on the X-alone trials. On the contrary the associative activation of A is not easy given that it is repeatedly presented in a block and therefore the salience of A should decline. As expected, subsequent conditioning of the stimuli A and B proceeded faster with B indicating that this stimulus was more salient than A. Nevertheless, separate analysis showed that this difference was evident only in the subgroup that had received the block of AX trials after the BX/X trials; when the AX trials preceded the BX/X trials there was no real difference between A and B during the conditioning phase. It is clear that in the BX/X–AX subgroup the associative activation of A becomes impossible and therefore this stimulus should lose salience. But also, the associative activation of A should be prevented in the AX–BX/X subgroup given that along the BX/X

trials X is not accompanied by A and the X → A association formed in the previous block of AX trials is expected to be extinguished. Hall and Rodríguez suggested that, when the block of AX trials pre- cedes the BX/X trials, both A and B can be associatively activated on the X-alone trials unless the X → A association is extinguished. The extinction of the X → A association is interesting with regard to the formation of inhibitory associations through blocked preexposure given that all that is required for the formation of these inhibitory associations is the associative activation of A when BX is presented along the second block. This activation could be possible if the X → A association formed during the first block of preexposure, instead of being extinguished, remained active throughout the second block. Nevertheless, the available experimental evidence does not favor the possibility that inhibitory associations are formed after blocked preexposure. Prados et al. (2004) explicitly compared three groups of subjects preexposed to AX and BX presented inter- mixed (Group I) or in the two possible blocked conditions: AX–BX (group B1) or BX–AX (group B2) and subsequently they tested the inhibitory properties acquired by B in each group. To detect these inhibitory properties, they used a procedure reported by Espinet et al. (1995) which found that conditioning of A after prolonged intermixed preexposure to AX and BX endowed B with the properties of a conditioned inhibitor. The inhibitory properties of B were showed by means of retardation tests in which conditioning of B was slower in the intermixed than in two adequate control groups (Experiments 1 and 3) and also by means of summation tests in which B was able to alleviate the aversion conditioned to another stimulus (Experiments 2 and 4). McLaren and Mackintosh (2000) attributed these results to the intervention of the inhibitory associations established between B and A during the preexposure phase suggesting that B was able to inhibit not only the activation of the representation of A but also that of the unconditioned stimulus associated to A. Using these retardation and summation tests Prados et al. (2004, Experiments 1 and 2, respectively) did not find significant differences in the inhibitory ability of B when they compared the intermixed and blocked groups. Nevertheless, if we compare the consumptions of the blocked groups in the tests it is striking that that the subjects in the AX–BX group always consumed more than the subjects in the BX–AX group. Although

these differences did not reach statistical significance this pattern of results was repeated in the tests of Experiments 1 and 2A showing a consistent tendency to differ. Given that the inhibitory associations are developed only after prolonged preexposure (McLaren and Mackintosh, 2000) it could be possible that the number of pre- exposure trials (six to each compound) was insufficient to establish inhibitory associations and this could be the reason why no reliable order effects appeared in their experiments.

The next experiments had as their starting point the idea that increasing the number of preexposure trials could facilitate the appearance of order effects after blocked preexposure. Therefore, the present experiments reproduced the two blocked conditions (AX–BX and BX–AX) employed by Prados et al. (2004), but the preexposure phase was prolonged. Experiment 1 tested the differential effects of these two preexposure schedules on the magnitude of generalization to BX of an aversion conditioned to AX. Experiments 2 and 3 constituted, respectively, retardation and summation tests of the inhibitory properties of B acquired after prolonged blocked preexposure and subsequent conditioning of A. Experiment 4 pro- vided additional data for an interpretation of the previous results based on the formation of inhibitory associations.

2. Experiment 1

Two groups of rats received blocked preexposure to two compound flavors AX and BX. The animals in the AX–BX group received first a block of AX trials followed by a block of BX trials while animals in the BX–AX group received first the BX trials and later the AX trials. Subsequently, the AX compound was aversively conditioned and generalization of this aversion was estimated in a test of consumption of BX. Bennett et al. (1999) suggested that generalization to BX of an aversion previously conditioned to AX could be attributed to two factors: the presence of the conditioned X in the BX test and the associative activation, via X, of the conditioned A when BX is tested. After conditioning to AX both X and A can elicit the conditioned response to a certain extent. After preexposure, inhibitory associations directed from B to A could have been formed in the AX–BX group where B could become a signal of the absence of A,

but not in the BX–AX group where B had not the opportunity of predict the absence of A. Therefore, when BX is presented in the test after conditioning of AX only in the AX–BX group B could be able to inhibit the associative activation of A and consequently the amount of conditioned response elicited by A. In such a case less generalization could be expected in the AX–BX group than in the BX–AX group.

2.1. Methods

2.1.1. Subjects and apparatus

Harlan Iberica supplied the animals for this and the next experiments. The subjects were 20 experimentally naïve male Wistar rats with a mean body weight of 363 g (range, 342–397 g) at the start of the experiment. During an adaptation period of two weeks, they were housed in groups of four with free access to food and water. Subsequently the animals were housed in individual cages and access to fluid was restricted as described below. In this and the next experiments, the colony room was artificially lit from 07:30 to 21:30 h.

In this and in the next experiments the flavor X was 0.3% (w/v) citric acid and the flavors A and B were 0.15% (w/v) saccharin and 0.5% (w/v) salt counterbalanced into each group. When the flavors were mixed the compound maintained the individual concentrations of each flavor. The flavored solutions were made with tap water and chemically pure products supplied by Probus or Merck laboratories. The fluids were presented in the home cages at room temperature in 100 ml plastic bottles equipped with metal spouts. Fluid consumption was measured by weighing the bottles before and after each drinking session. Injections of 10 ml per kg body weight of 0.3 M lithium chloride (LiCl) were used for the conditioning trials. These injections were administered in a room adjacent to the colony room

2.1.2. Procedure

The standard water bottles were removed overnight before the start of the experiment. In all the phases of this and the next experiments, fluid was only available for two 30 min sessions each day, starting at 8.00 and 20.00 h. The duration of these sessions was restricted to 15 min in the conditioning and test sessions. Through- out the next 14 days, rats were preexposed to the compound

solutions. The first preexposure week a half of the rats received the saccharine-acid compound in all the drinking sessions and the remaining received the salt-acid compound. Two groups of animals, group AX–BX and group BX–AX, were formed matched by their consumptions through the first week on condition that for a half of the subjects in each group saccharine was flavor A and salt was flavor B while for the other half flavor A was salt and flavor B was saccharine. During the second preexposure week each animal was preexposed to the compound that had not received on the first week. The six days following preexposure constituted a phase for the conditioning of AX. On days 1, 3 and 5 of this phase, all animals drank water in the morning session. In the evening session all animals had free access to the AX compound for 15 min and immediately they received an intraperitoneal injection of lithium chloride. On days 2, 4 and 6, all animals received water in the morning and evening sessions. The following two days all animals received water in the morning session and had free access to the BX compound in the evening session. The last day, the subjects drank water in the morning session and had free access to the X solution in the evening session

2.2. Results and discussion

A significance level of $p < 0.05$ was adopted for the data analysis in this and the following experiments. The mean amounts of the AX compound consumed on each preexposure trial were 8.62 and 8.65 g and those of the BX compound were 9.51 and 8.91 g for the groups AX–BX and BX–AX, respectively. The groups did not differ significantly in their consumptions of AX or BX, $ts(1, 18) < 1$. The mean amounts of AX consumed by the groups AX–BX and BX–AX throughout the conditioning phase were, respectively, 9.3 and 8.9 g on the first trial; 1.9 and 3.5 g on the second trial; and 0.8 and 1.4 g on the third trial. Conditioning successfully produced an aversion to AX in both groups as indicated by a repeated measures analysis of variance (ANOVA) with group and trial as the factors which revealed only a significant effect of trial, $F(2, 36) = 164$. Neither the effect of group nor the group × trial interaction was significant, largest $F(2, 36) = 2.4$.

Fig. 1 shows the results of the tests. On the left hand appear the bars representing the group means for consumption of BX on each of the two

test trials with this compound. As can be appreciated in the figure the subjects in the AX–BX group consumed more than those in the BX–AX group in both trials. Also, both groups increased their consumptions in the second trial but this increase was more noticeable in the AX–BX group. A repeated measures ANOVA con- ducted on the data summarized in the figure for the consumptions of BX, with group and trial as the variables, confirmed these impres- sions showing a reliable main effect of group $F(1, 18) = 5.37$, a significant effect of trial $F(1, 18) = 30.83$ and a group × trial inter- action $F(1, 18) = 4.78$. An analysis of simple main effects revealed that the between-groups differences in consumption of BX felt near significance on the first trial, $t(1, 18) = 2.01$, $p = 0.059$, and were significant on the second trial, $t(1, 18) = 2.34$. On the right hand of Fig. 1 the bars represent the mean amounts of X consumed on the final test. These amounts were small and similar in both groups and in fact they did not differ significantly, $t(1, 18) = 1.02$.

These results show an order effect: after prolonged blocked pre-exposure generalization to BX from an aversion conditioned to AX was smaller when the compounds were preexposed in the AX–BX order than when they were preexposed in the BX–AX order. Given the results of the final test with X, the between-groups differences in consumption of BX observed in the test cannot be attributed to differences in the excitatory power of X and, therefore, they seem to be due to differences in the effect caused by B in each group at the very moment of the BX test.

The smaller generalization observed in the AX–BX group can be explained assuming that in this group, as suggested in the McLaren and Mackintosh model, inhibitory associations directed from B to A were formed during the preexposure phase. Later, when BX was presented on test, only in the AX–BX group B was able to inhibit the associative activation of A and the conditioned response controlled by this stimulus. The following experiments tested this possibility.

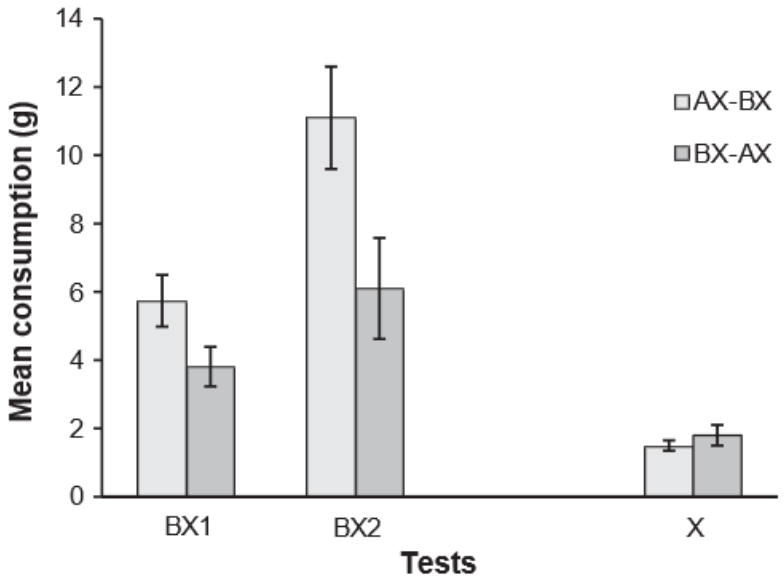

Fig. 1. Group mean consumption in the tests with BX (left-hand pair of columns) and in the final test with X (right-hand column). Group names refer to the order of presentation of the compounds. After blocked preexposure to AX followed by BX or vice versa the AX compound was aversively conditioned before the tests of generalization to BX. Vertical bars represent standard errors of the means.

3. Experiment 2

This experiment was designed to assess the inhibitory proper- ties of B after blocked preexposure to AX–BX or to BX–AX, using a retardation test similar to that employed by Prados et al. (2004) in their Experiment 1. After preexposure and aversive conditioning of A, stimulus B was also conditioned. If inhibitory associations directed from B to A are formed after prolonged blocked preexpo- sure to AX–BX, conditioning of B should be retarded in the AX–BX group (where B could inhibit the activation of the A-US representation) in comparison with the BX–AX group (where B could not be able to inhibit the activation of the A-US representation).

3.1. Methods

3.1.1 Subjects and apparatus

The subjects were 20 experimentally naïve female Wistar rats with a mean body weight of 214 g (range, 200–232 g) at the start of the experiment. The solutions were the same as described in Experiment 1. Injections of LiCl (10 ml/kg) were used for the conditioning trials in two different concentrations: 0.3

M for the conditioning of A, and 0.15 M for the conditioning of B.

3.1.2. Procedure

The adaptation period, schedule of water deprivation and the preexposure phase were identical to those described in Experi- ment 1. The flavors A and B were counterbalanced in each group as in the previous experiment and two groups of subjects were formed matched by their consumptions through the first week: group AX–BX and group BX–AX.

The 4 days following preexposure constituted a phase for the conditioning of A. On days 1 and 3 of this phase the animals received water in the 30 min morning session. In the evening session they had free access to the A solution for 15 min and were injected with lithium chloride 0.3 M. On days 2 and 4 all the animals received water in the two daily sessions.

The five days following the conditioning of A constituted a phase for the retardation test. On days 1 and 3 of this phase the animals drank water in the 30 min morning session. In the evening ses- sion the subjects had free access to the B solution for 15 min and immediately they received an intraperitoneal injection of lithium chloride 0.15 M. On days 2 and 4 all the animals received water in the two daily sessions. On day 5 the subjects received water in the morning session and a final 15 min test with B on the evening session.

3.2. Results and discussion

The mean amounts of the AX compound drunk on each preexposure trial were 7.1 and 6.9 g and those of the BX compound were 6.5 and 7.0 g for groups AX–BX and BX–AX, respectively. The groups did not differ significantly in their consumptions of AX or BX, $ts(1, 18) < 1$. The mean amounts of A consumed for the groups AX–BX and BX–AX over the conditioning phase were, respectively, 6.0 and 5.8 g on the first trial, and 0.7 and 0.9 g, respectively on the second trial. Conditioning produced a significant decrease in consumption of A between the first and second trial in both groups, as indicated by a repeated measures ANOVA with group and trial as the fac- tors that revealed a significant effect of trial, $F(1, 18) = 171$, but no significant effect of group nor interaction ($Fs < 1$).

Fig. 2 shows the mean consumption of B on each of the three trials of the retardation test. It is apparent from inspection of this figure that in the first and second trials animals in group AX–BX showed less aversion to B than those in group BX–AX, but finally both groups acquired a strong aversion to B. A repeated measures ANOVA conducted on the data presented in the figure with group and conditioning trial as the variables revealed that the main effect of group was significant $F(1, 18) = 7.96$ as was the main effect of trial $F(2, 36) = 21.05$. The interaction of these two factors was not significant $F(2, 36) = 1.58$.

The retardation in the conditioning of B observed in the AX–BX group of this experiment can be explained assuming that in this group, when presented in the test phase, B inhibits the A-US representation and this hinders the establishment of the B-US association. This interpretation receives support from previous results obtained in experiments specifically designed to produce unidirectional inhibitory associations directed from B to A. For instance, Bennett et al. (1999, Exp. 3B) found a similar retardation effect after preexposure to sequential presentations of AX followed by BX. They gave their subjects only one AX → BX trial each day. These trials should establish an inhibitory association directed from B to A, since B became a signal for the absence of A until the following day. After preexposure and subsequent conditioning of A, conditioning of B was retarded in the group that received preexposure to AX → BX trials in comparison with a group preexposed to BX → AX trials. The present results also closely parallel those found by Espinet et al. (2004, Exp. 1) in a retardation test with an appetitive procedure. In this experiment the preexposure phase was also specifically designed to produce unidirectional inhibitory associations directed from B to A: intermixed preexposure to X → A and XB trials. With this preexposure schedule, presentations of BX should activate associatively, via X, the representation of the absent A and give rise to the formation of inhibitory associations directed from B to A. The associative activation of A via X was prevented in a control group preexposed to separate presentations of X, A, and XB.

After preexposure and subsequent conditioning of A, conditioning of B was retarded in the group preexposed to the X → A/XB trials in comparison

with the group that received X/A/BX trials.

Although the results obtained in this retardation tests give support to the idea that preexposure to AX–BX and subsequent conditioning of A endow B with inhibitory properties, an adequate assessment of inhibition requires both retardation and summation tests. Therefore the next experiment was designed to assess the inhibitory properties of B by means of a summation test.

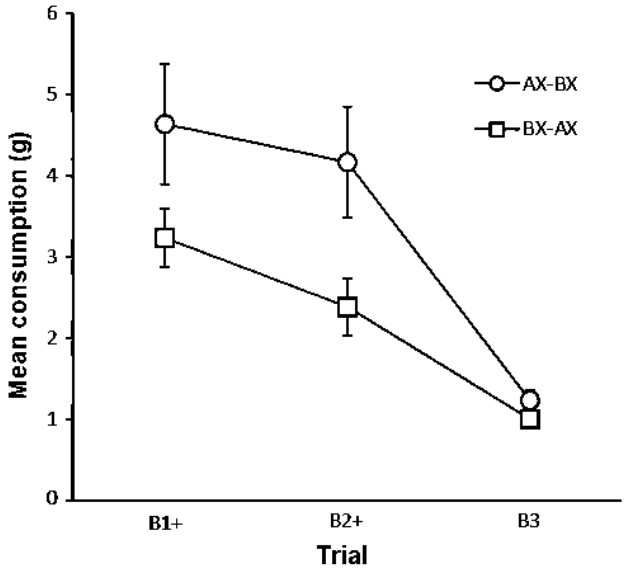

Fig. 2. Group mean consumption of flavor B in the retardation tests. After preexpo- sure, flavor A vas aversively conditioned in both groups before the retardation test. Injections of LiCl are represented by (+). Vertical bars represent standard errors of the means.

4. Experiment 3

The results of the previous experiment indicate that stimulus B could have acquired inhibitory properties in the AX–BX group. To test this possibility the same preexposure sequences of Experiment 2 were used in Experiment 3 but, after conditioning of A, a new stimulus Y was aversively conditioned and tested subsequently in compound with B. If B acquires inhibitory properties in the AX–BX group the conditioned response to Y should be reduced by the addition of B in this group but not in the BX–AX group.

4.1. Methods

4.1.1. Subjects and apparatus

The subjects were 20 experimentally naïve female Wistar rats with a mean body weight of 199 g (range, 188–215 g) at the start of the experiment. A new flavor Y, a solution of 9 ml/l of orange- blossom essence (Vahiné, Ducros S. A., Spain) was used in addition to the solutions used in Experiments 1 and 2. Injections of 10 ml per kg body weight of 0.3 M lithium chloride (LiCl) were used for all the conditioning trials.

4.1.2. Procedure

The adaptation period, maintenance and deprivation conditions were the same as those described in the previous experiments. Flavors A and B were counterbalanced and two groups of subjects, AX–BX and BX–AX were formed following the same procedure described in the previous experiments.

Preexposure and conditioning of A proceeded exactly as described in Experiment 2. The four days following the conditioning of A constituted a phase for the conditioning of Y and proceeded exactly as the previous phase except that all animals had free access to the Y solution instead of the A solution. In each of the next two days all animals received water in the morning session and summation tests with the BY compound in the evening session. On the last day a consumption test of the Y solution was per- formed in the evening session.

4.2. Results and discussion

The mean amounts of the AX compound drunk on each preexposure trial for the groups AX–BX and BX–AX, respectively, were 6.9 and 6.6 g and those of the BX compound were 6.77 and 7.03 g. The groups did not differ significantly in their consumptions of AX or BX, $ts(1, 18) < 1$. On the first conditioning trial to A, the subjects in the AX–BX and BX–AX groups drank 6.6 and 7.4 g, respectively. On the second conditioning trial, consumption declined in both groups: 1.1 and 1.3 g for groups AX–BX and BX–AX, respectively. A repeated measures ANOVA with group and trial as the variables revealed a significant effect of trial, $F(1, 18) = 144$. Neither the

effect of group nor the group × trial interaction was significant, largest $F(1, 18) = 1.58$. The mean amounts of the Y solution drunk for the AX–BX and BX–AX groups were, respectively, 5.7 and 6.3 g on the first conditioning trial and 4.1 and 3.3 g on the second conditioning trial. A repeated measures ANOVA conducted on these data with group and trial as the variables revealed a significant effect of trial, $F(1, 18) = 10.61$. Neither the effect of group nor the group × trial interaction was significant, largest $F(1, 18) = 1.11$.

Fig. 3 shows the mean consumptions of the BY compound on the two trials of the summation test, and the consumption of the Y solution on the final test trial. The right part of the figure shows that both groups drank small and similar amounts of Y alone in the final test, indicating that conditioning was successful and that both groups acquired a strong and similar aversion to Y. The left part of the figure shows that in the first trial both groups drank small amounts of the BY compound and that only the animals in the AX–BX group increased their consumption in the second trial. A repeated measures ANOVA performed on the difference scores for consumption of BY minus consumption of Y alone with group and trial as the factors confirmed this observation. There was a significant effect of group, $F(1, 18) = 6.28$, and a significant effect of trial, $F(1, 18) = 16.62$. The group × trial interaction was also significant, $F(1, 18) = 8.95$. An analysis of simple main effects revealed that the consumptions of the groups did not differ in the first trial, $t < 1$, but in the second trial the subjects in the AX–BX group drank significantly more BY than those in the BX–AX group, $t(1, 18) = 3.03$. The groups did not differ in their consumptions of Y in the last test trial, $t < 1$.

Therefore, only in the AX–BX group the addition of B was able to alleviate the aversion caused by Y. In other words, B showed the properties of a conditioned inhibitor only in the AX–BX group. Parallel results have been found in the summation tests of previous experiments in which the preexposure phase was designed to pro- duce mutual inhibition between A and B (Artigas et al., 2001, Exps. 1A and 1B; Espinet et al., 1995, Exps. 2 and 4) or in experiments were the preexposure phase was designed to establish unidirectional inhibitory associations directed from B to A (Bennett et al., 1999, Exp. 3C).

The problem to accept the parallelism with these previous experiments relies on the maintenance of the A ↔ X association formed during the first block of preexposure to AX–BX. This association could be extinguished through the second block of preexposure given that X is no longer paired with A, but with B. If the A ↔ X association was extinguished, the presentations of BX would be unable to activate the representation of A and the mechanism promoting the formation of inhibitory associations (i.e., the associative activation of the absent A on the BX trials) would be unable to operate. By contrast, it is obvious that the A ↔ X association is not extinguished after intermixed preexposure to AX and BX or after repeated presentations of AX followed by BX (the preexposure conditions used in the previous experiments) given that the AX trials are presented until the end of the preexposure phase.

The interpretation of the results obtained in the three experiments presented here is based on the inhibitory mechanism suggested by McLaren and Mackintosh (2000). Therefore it requires that the A ↔ X association formed during the AX presentations would be maintained in some degree throughout the second block of trials (presentations of BX). The next experiment was designed with the intention to explore this possibility.

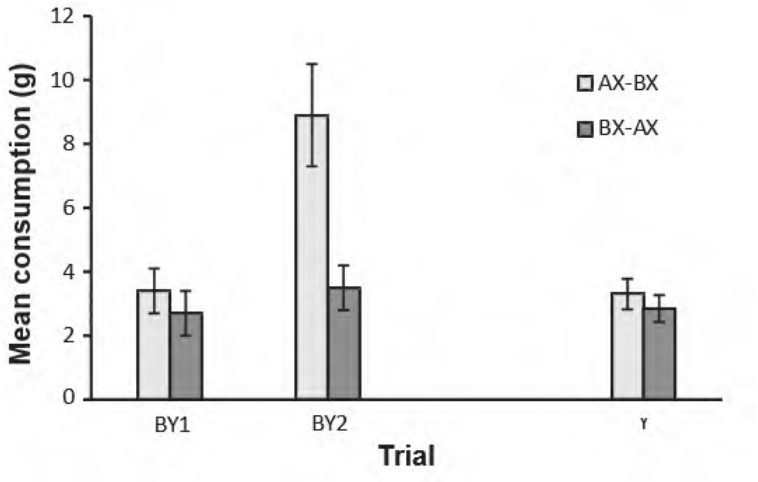

Fig. 3. Group mean consumption in the two summation test trials with BY (left- hand) and in the test with Y (right-hand). After preexposure, flavor A vas aversively conditioned in both groups. Subsequently, a new flavor Y was aversively conditioned before the summation tests. Vertical bars represent standard errors of the means.

5. Experiment 4

One group of rats received preexposure to AX–BX while two groups received preexposure either to AX–BY or to AY–BX. After preexposure flavor X was aversively conditioned and the effect of this conditioning on consumption of A and B was tested. It was expected that conditioning of X reduced the consumption of their associates in the tests (i.e., a sensory preconditioning effect). Concretely, the consumption of flavor A was expected to be high in the AY–BX group, where A was not associated with X. On the contrary, consumption of A should be low in the AX–BY group given that the initial A ↔ X association could not be extinguished in this group. In the AX–BX group, if the initial A ↔ X association had not been extinguished at the end of the preexposure phase, consumption of A should be similar to that of the AX–BY group. On the contrary, if this association had been extinguished, the amount of A consumed by the subjects in the AX–BX group should be greater than that consumed by the subjects in the AX–BY group and nearest to the amount of A consumed by the subjects in the AY–BX group. On the other hand, consumption of B should be high in the AX–BY group and low in the AX–BX group and in the AY–BX group, providing thus an additional measure of the magnitude of sensory preconditioning.

5.1. Methods

5.1.1. Subjects and apparatus

The subjects were 24 male Wistar rats with a mean ad lib weight of 384 g (range, 368–415 g). They had been previously used in an experiment with sounds and lights as stimuli and reinforced with food pellets. The solutions were identical to those described in Experiment 3. Injections of lithium chloride 0.3 M were used in the conditioning sessions.

5.1.2. Procedure

The experiment started with a deprivation schedule. The water bottles were removed from the cages overnight. On the next three days the subjects had access to water in two daily 30 min sessions as described in Experiment 1. Three

groups of animals were formed matched by their water consumptions during the deprivation schedule. Throughout the next 14 days the subjects were preexposed to two flavored solutions presented in two blocks. The first week groups AX–BX and AX–BY received a block of 14 trials with the compound AX while subjects in the AY–BX group received the compound AY. On the second week the groups AX–BX and AY–BX received 14 presentations of BX while subjects in the AX–BY group received the compound BY.

The four days following preexposure constituted a phase for the conditioning of X. On days 1 and 3 of this phase the animals received water in the 30 min morning session. In the evening session they received 15 min of free access to X followed by an injection of LiCl. On days 2 and 4 all animals received water in the two daily sessions. Following the conditioning phase there were two test days. On the first test day a half of the animals in each group were tested with the flavor A and the remaining with the flavor B. The second test day each animal received the flavor that had not been presented the first test day.

5.2. Results and discussion

The mean amounts of fluid consumed during the first block by the groups AX–BX, AX–BY and AY–BX were, respectively, 9.0, 9.3 and 10.5 g and 10.3, 11.1 and 11.4 g during the second block. The groups did not differ significantly in their consumptions in neither of the blocks, largest $F(2, 21) = 2.54$. The conditioning procedure established an aversion to X in the three groups. The mean amounts of X consumed by the groups AX–BX, AX–BY and AY–BX were, respectively, 2.5, 2.6 and 3.0 g on the first conditioning trial, 2.1, 2.1 and 1.3 g on the second conditioning trial and 0.4, 0.7 and 0.3 g on the test trial. A repeated measures ANOVA performed on these data with group and trial as the variables revealed a significant effect of trial, $F(2, 42) = 22.9$. The effect of group and the interaction between the variables were not significant ($Fs < 1$).

Fig. 4 shows the mean amounts of A and B consumed by each of the groups in the tests. As illustrated in the figure, the smallest consumption of A and B corresponded to the groups in which these flavors had been preexposed in compound with X. It is also apparent from inspection of the

figure that subjects in the AX–BX group consumed small and similar amounts of both flavors, A and B. An ANOVA performed on the data summarized in the figure with group and test (A vs. B) as the variables revealed a significant interaction between these variables, $F(2, 21) = 7.5$. Neither the effect of group nor the effect of test was significant (largest $F = 2.6$). Separate ANOVAs conducted to explore the source of the interaction revealed the existence of significant differences between the groups in consumption of A, $F(2, 21) = 4.4$, and in consumption of B, $F(2, 21) = 3.7$. Post hoc Duncan tests revealed that the group AY–BX consumed an amount of A significantly greater than that consumed by the groups AX–BX and AX–BY which did not differ each other. On the other hand, the group AX–BY consumed significantly more B than the groups AX–BX and AY–BX which did not differ each other. Therefore, conditioning of X produced, in the three groups, a similar reduction in the consumption of the flavors associated with X (i.e., a sensory preconditioning effect). The most important results are those concerning the AX–BX group. On one hand, if the A ↔ X association had been extinguished in this group, it could be expected that their subjects consumed an amount of A similar to that consumed by the subjects in the AY–BX group. However, the consumption of flavor A in the AX–BX group was smaller and similar to that consumed in the AX–BY group where there is no reason to expect the extinction of the A ↔ X association. On the other hand the consumptions of B were significantly smaller in the groups AY–BX and AX–BX than in the AX–BY group. This is not surprising since there is no reason to expect a weakening of the B ↔ X association. But the fact that the subjects in the AX–BX group consumed similar amounts of the flavors A and B indicates that the A ↔ X association and the B ↔ X association had a similar strength in this group. These considerations leave to conclude that the A ↔ X association was not extinguished in the AX–BX group after preexposure.

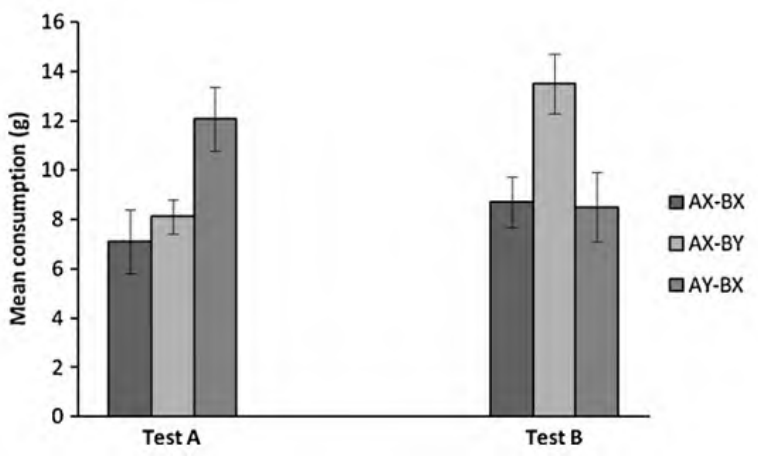

Fig. 4. Group mean consumption of the flavors A and B in the tests of Experiment 4. After preexposure, flavor X vas aversively conditioned. Vertical bars represent standard errors of the means.

6. General discussion

The results of the present experiments appear to provide evidence that prolonged blocked preexposure to AX–BX can give rise to the formation of inhibitory associations directed from B to A as suggested by McLaren and Mackintosh (2000). Two groups of rats received preexposure to either AX–BX or BX–AX. Experiment 1 revealed an order effect: after preexposure and subsequent conditioning of AX, generalization of this conditioning to BX was less in the AX–BX group than in the BX–AX group. The explanation for this result was found in the proposals of Bennett et al. (1999). They suggested that generalization to BX of an aversion conditioned to AX depends on two factors: the amount of conditioned response caused by the presence of X and the amount of conditioned response caused by the associative activation of A. The results of Experiment 1 could be explained admitting that preexposure to AX–BX gave rise to the formation of inhibitory associations directed from B to A, so that only in the AX–BX group B was able to inhibit the associative activation of A, thus reducing this source of generalization. Therefore the purpose of Experiments 2 and 3 was to test the inhibitory properties of B. The procedure to detect inhibition was the same used by Prados et al. (2004). Flavor A was conditioned after preexposure and the inhibitory ability of B was tested first by

means of a retardation test in which B was paired with the unconditioned stimulus used for the conditioning of A. The results (Experiment 2) showed that the acquisition of aversive properties by B was slower in the AX–BX group than in the BX–AX group. In Experiment 3 summation tests revealed that the aversion conditioned to a new flavor Y was alleviated in the AX–BX group by the addition of flavor B, but the same was not true for the BX–AX group.

Therefore the results of Experiments 2 and 3 lend support to the initial interpretation suggested for Experiment 1 and lead to the conclusion that prolonged blocked preexposure to AX–BX gives rise to the formation of inhibitory associations directed from B to A. Additional support for this interpretation comes from the results of previous experiments that used the same procedures here employed to assess inhibition (i.e., retardation and summation tests with B after conditioning of A). With these procedures mutual inhibition between A and B has been found after intermixed preexposure to AX/BX (Artigas et al., 2001; Espinet et al., 1995). More interesting, inhibitory associations directed from B to A have been detected with preexposure procedures specially designed to produce these unidirectional inhibitory associations. For instance, after preexposure to AX → BX trials (Bennett et al., 1999), or after intermixed preexposure to X → A/XB trials (Espinet et al., 2004). All those experiments have in common that preexposure was prolonged (12 trials to each stimulus). Given that the formation of inhibitory associations requires a prolonged preexposure (e.g., Espinet et al., 1995, Experiment 4) the results of Experiments 2 and 3 suggest that in the experiments by Prados et al. (2004) between-groups differences could have appeared if their subjects had received more preexposure trials.

However there is a problem drawing a parallel between such previous experiments and those presented here. While in the previous experiments the X → A association was preserved given that the AX compound or the X → A trials were presented from the beginning to the end of the preexposure phase, it has been suggested that in a blocked preexposure to AX–BX the X → A association is extinguished throughout the second block of trials. Once extinguished the X → A association the mechanism promoting inhibition (i.e., the associative activation of the absent A when BX is presented) would remain inactive. Therefore only the first presentations of BX in the second block of trials could be able to

activate the representation of the absent A and this would probably be insufficient to establish strong inhibitory associations between B and A.

The precedent considerations lead us to wonder whether the results here obtained can be explained without appealing to the maintenance of the X → A association through the second block of preexposure. If the X → A association had been extinguished in the AX–BX group at the end of the preexposure phase it could have been restored in Experiment 1 at the moment of the conditioning trials with AX. But the results of Experiments 2 and 3 have been obtained with procedures that prevent the re-establishment of the X → A association. Is it possible that the X → A association was pre- served throughout the second block of preexposure allowing B to become an inhibitor of A? The results of Experiment 4 show that this association was, in fact, preserved after 14 preexposure trials to AX–BX.

The results of Experiment 4 seem to contradict those reported in previous studies on the extinction of within-compound flavor associations (Rescorla and Freberg, 1978). They found that, after preexposure to a compound, separate presentations of either element outside this compound disrupted the within-compound associations. It is not easy to apply the Rescorla and Freberg's findings to the present experiments given the procedural differences concerning the preexposure schedule, the amount of preexposure to the compounds before the extinction phase and also the procedures addressed to produce extinction. In any case the extinction obtained in their experiments was not complete. On the other hand, some general phenomena related with the extinction process indicate that an original association can be preserved after extinction (e.g., spontaneous recovery). Also the results obtained by Alonso and Hall (1999) indicate that the within-compound associations established between two flavors when they are presented concurrently are not easily extinguished.

The results reported here provide evidence compatible with the idea that the prolonged AX–BX preexposure used in the present experiments allowed the A ↔ X association to be maintained enough as to turn B in an inhibitor of A throughout the block of BX presentations. These results suggest the convenience of developing further thorough studies about the associative processes involved in a prolonged blocked preexposure.

Acknowledges

This research was supported by grants from the Spanish “Min- isterio de Educación y Ciencia” (Ref. SEJ2007-67409-C02-02 and SEJ2007-67409-CO2-01) to A. Espinet and V.D. Chamizo.

References

Alonso, G., Hall, G., 1999. Stimulus comparison and stimulus association processes in the perceptual learning effect. Behavioural Processes 48, 11–23.

Artigas, A.A., Chamizo, V.D., Peris, J.M., 2001. Inhibitory associations between neutral stimuli: a comparative approach. Animal Learning & Behavior 29, 46–65.

Bennett, C.H., Scahill, V.L., Griffiths, D.P., Mackintosh, N.J., 1999. The role of inhibitory associations in perceptual learning. Animal Learning & Behavior 27, 333–345.

Dwyer, D.M., Bennett, C.H., Mackintosh, N.J., 2001. Evidence for inhibitory associa- tions between the unique elements of two compound flavors. Quarterly Journal of Experimental Psychology 54B, 97–109.

Dwyer, D.M., Mackintosh, N.J., 2002. Alternating exposure to two compound flavors creates inhibitory associations between their unique features. Animal Learning & Behavior 30, 201–207.

Espinet, A., González, F., Balleine, W.B., 2004. Inhibitory sensory preconditioning. Quarterly Journal of Experimental Psychology 57B, 261–272.

Espinet, A., Iraola, J.A., Bennett, C.H., Mackintosh, N.J., 1995. Inhibitory associations between neutral stimuli in flavor-aversion conditioning. Animal Learning & Behavior 23, 361–368.

Hall, G., 2003. Learned changes in the sensitivity of stimulus representations: associative and nonassociative mechanisms. Quarterly

Journal of Experimental Psychology 56B, 43–55.

Hall, G., Rodríguez, G., 2009. Factors determining the effects of associative activation on habituation. Journal of Experimental Psychology: Animal Behavior Processes 35, 266–270.

McLaren, I.P.L., Mackintosh, N.J., 2000. An elemental model of associative learning:

I. Latent inhibition and perceptual learning. Animal Learning & Behavior 28, 211–246.

Prados, J., Hall, G., Leonard, S., 2004. Dissociation between the Espinet and percep- tual learning effects in flavour aversion conditioning. Behavioural Processes 65, 221–229.

Rescorla, R.A., Freberg, L., 1978. The extinction of the within-compound flavor asso- ciations. Learning & Motivation 9, 411–427.

Sanjuan, M.C., Alonso, G., Nelson, J.B., 2004. Blocked and test-stimulus exposure effects in perceptual learning re-examined. Behavioural Processes 66, 23–33.

Symonds, M., Hall, G., 1995. Perceptual learning in flavor aversion conditioning: roles of stimulus comparison and latent inhibition of common elements. Learning & Motivation 26, 203–219.