



# Preservation of within-compound associations after blocked preexposure to two compound flavors<sup>☆</sup>

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## ABSTRACT

Three experiments investigated the extinction of the within-compound  $A \leftrightarrow X$  association established when two compound flavors, AX and BX, are preexposed in blocks (i.e., AX, AX, AX, ... BX, BX, BX). In Experiment 1, a group of rats received preexposure to a block of AX trials followed by a second block of BX trials (AX–BX), while a second group received blocked preexposure to the same stimuli in the opposite order (BX–AX). Subsequently, flavor A was paired with lithium chloride. This conditioning resulted in a similar reduction of consumption of flavor X in both groups. In Experiment 2 four groups of rats received blocked preexposure to AX–BX, AX–B, A–BX, or A–X. After aversive conditioning of X, consumption of A and B was significantly lower for the groups which received these flavors paired with X than for the groups for which these flavors were presented isolated. In Experiment 3 a group of rats was preexposed to a block of SaltX presentations followed by a block of BX presentations (SaltX–BX), and a second group received blocked preexposure to (BX–SaltX). After subsequent sodium depletion, consumption of X was high and similar for the SaltX–BX and for the BX–SaltX groups. These results indicate that the within-compound association established in the first block of a blocked preexposure is not extinguished when the preexposure phase is concluded.

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## 1. Introduction

Since the work of Honey et al. (1994) many experiments on perceptual learning have compared the effects of preexposure to two compound stimuli, AX and BX, presented in alternate trials (e.g., AX, BX, AX, BX, ...) or in two blocks of trials (e.g., AX, AX, AX ... , BX, BX, BX). Two sequences of preexposure are possible depending on which compound, AX or BX, is presented first and, although scarcely studied, the differential effects of these two sequences on a blocked preexposure have received some attention in the last years (Espinet et al., 2011; Rodríguez and Alonso, 2014). The results of these experiments have led us to investigate the extinction of the within-compound associations established in a blocked preexposure. It is well known that conjoint and repeated presentation of two flavors, A and X, gives rise to the formation of an  $A \leftrightarrow X$  within-compound association (e.g., McLaren and Mackintosh, 2000; Rescorla and Freberg, 1978). While there is no doubt that this association is preserved at the end of alternated preexposure (e.g., Rodríguez and

Alonso, 2014), it has been suggested that after an AX–BX blocked preexposure the  $A \leftrightarrow X$  association would be extinguished (Hall, 2003; Symonds and Hall, 1995). The purpose of the next experiments was to know if the  $A \leftrightarrow X$  association is extinguished at the end of preexposure to AX–BX, given that recent experiments have found some differential effects of the two possible orders of blocked preexposure, which challenge this suggestion.

Hall and Rodríguez (2009, Exp. 2) reported one of these order effects. Hall (2003) suggested that the direct activation produced by repeated presentations of a stimulus reduces its salience, and that this habituation process can be attenuated when the stimulus is associatively activated. Therefore it could be expected that during alternated preexposure to AX and BX, the salience of X (directly activated in each trial) declines, while the salience of the differential elements A and B is preserved given that B is associatively activated, via X, in the AX trials, and A is associatively activated, via X, in the BX trials. In contrast, in a blocked AX–BX preexposure the repeated presentations of AX in the first block of trials would produce a loss of the salience of both, A and X. The loss of salience should also affect B and X along the second block of preexposure trials. To test this possibility Hall and Rodríguez (2009) measured the salience by means of 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 proceeded faster

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than conditioning of C. Therefore, they concluded that after this sequence of preexposure the salience of B (preexposed in alternation with X) was greater than the salience 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 proceeded at a similar speed, and this was taken as evidence that C and B maintained a similar salience. Insofar as the salience is preserved by associative activation, they suggested that, perhaps, the CX–BX/X sequence of preexposure could allow the associative activation of both, C and B, in the X alone trials presented in the second block. This could happen if the C ↔ X association established in the first block of preexposure was not extinguished along the second block of the preexposure phase.

More recently, Espinet et al. (2011) reported some 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, conditioning of AX resulted in less generalization to BX in the Group AX–BX than in the Group BX–AX (Exp. 1). Also, conditioning of A after preexposure endowed B with the properties of a conditioned inhibitor in the Group AX–BX as indicated by retardation (Exp. 2) and summation tests (Exp.3). All these order effects could be explained assuming that the within-compound association established in the first block of preexposure was not extinguished. A fourth experiment was designed to explore this possibility. Three groups of rats received blocked preexposure to AX–BX, to AX–BY, or to AY–BX. After aversive conditioning of the flavor X, 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 reasons to expect the extinction of the A ↔ X association in the Group AX–BY in which 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 explicitly compare the two possible orders of a blocked preexposure (AX–BX and BX–AX) that were used in the three previous experiments. To fill this gap, the following experiments compared the strength of the within-compound A ↔ X association after blocked preexposure to AX–BX and to BX–AX. Experiments 1 and 2 made use of a procedure based on sensory preconditioning and flavor aversion conditioning, which has provided a reliable indication of the permanence and strength of the within-compound associations established between two flavors when they are presented together (Rescorla and Freberg, 1978). The third experiment made use of a different procedure, sodium depletion, which provides a direct measure 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 produced by the conditioning of one of the differential elements on the two sequences of blocked preexposure. Two groups of rats were preexposed to two compound flavors (AX and BX) presented in blocks. Subjects in Group AX–BX received presentations of AX in the first block and presentations of 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 the Group AX–BX, where along the second block X is no longer paired with A, but not in the BX–AX group where the last block consists, precisely, of A and X pairings. After preexposure, consumption of X was evaluated by means of a pretest. Subsequently, A was aversively conditioned in both groups and the impact of this measure was tested on consumption of X and on consumption of B. Once

concluded the preexposure phase, the A ↔ X association should be very strong in Group BX–AX and a sensory preconditioning effect should be observed in this group (i.e., a decrease in consumption of X on the test in comparison with the amount of X consumed on the pretest). On the other hand, if the A ↔ X association had been extinguished along the second block of preexposure in Group AX–BX, no substantial differences should be observed in this group between the consumptions of X in the pretest and test. On the contrary, if the A ↔ X association had been preserved once concluded 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. Method

#### 2.1.1. Subjects and apparatus

The subjects were 20 experimentally naïve male Wistar rats provided by Harlan Ibérica with a mean ad lib weight of 265 g (range: 240–320 g, SEM = 25.12). They were housed in individual cages in a colony room that was lit from 8.30 to 21.45 and maintained at  $22 \pm 1$  °C.

The solutions used as experimental stimuli were dispensed in the home cages, at room temperature, in 150 ml plastic bottles fitted with stainless steel drinking spouts. Three flavored stimuli, identical to those employed in our previous experiments (Espinete et al., 2011), were prepared with tap water and chemically pure products provided by Probus or Merck laboratories: 0.3% (w/v) citric acid; 0.15% (w/v) saccharin, and 0.5% (w/v) sodium chloride. Two compound solutions, saccharin-acid and salt-acid, were made maintaining the above-mentioned individual concentrations of each substance. Consumption 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 administered in an experimental room adjacent to the colony room.

#### 2.1.2. 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. In each session the animals were allowed to drink freely from a bottle containing 100 ml of fluid. Flavors A and B were counterbalanced in such a way that half of the rats in each group received saccharin as flavor A and salt as flavor B. For the other half of animals this order was reversed. Flavor X was citric acid for all the rats. The preexposure phase lasted one week, during which the rats received 28 preexposure sessions distributed in two blocks. In the first 14 trials, half of the subjects received the saccharine-acid compound and the remaining ones the salt-acid compound. Subsequently, the animals were divided in two groups (Group AX–BX and Group BX–AX) matched according to their consumptions in the first block of trials and counterbalancing the flavors in such a way that half of the subjects in each group had received the saccharine-acid solution and the other half had received the salt-acid solution. In the second block, each animal had access to the compound that was not preexposed in the first block. After preexposure all the rats received the experimental treatments at 9.00 and drank water in the three remaining daily sessions. The first day after preexposure all the animals received a pretest of consumption of X. The next four days constituted the conditioning phase. In days 1 and 3 of this phase each subject received the flavor A (saccharine or salt) to which it had been preexposed, 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 day after the conditioning phase the experimental treatment consisted of a test

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