

# Single-trial exercise induced taste and odour aversion learning

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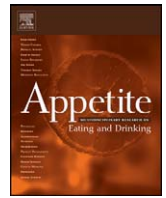
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## Short communication

## Single-trial exercise-induced taste and odor aversion learning in humans

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## ABSTRACT

In the present study, it was investigated whether humans acquire an aversion for a flavor paired with a single bout of exercise, and if so, to what degree this effect requires the experience of gastrointestinal distress. To this end, a total of 58 participants either consumed or merely tasted a specifically flavored solution directly prior to a 30 min running exercise. In both cases this led to a negative shift in subjective liking of the flavor (taste and odor) in comparison to the evaluation of another flavor not explicitly paired with exercise, indicative of a conditioned flavor aversion. The degree of subjectively experienced exercise-related gastrointestinal distress did not predict this negative hedonic shift for the flavor paired with the running exercise, implying that such distress may not be a prerequisite for exercise-induced flavor aversion learning in humans.

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## Introduction

In a classic paper, Garcia and Koelling (1966) demonstrated in rats that pairing consumption of a specific flavor plus an audiovisual cue with the administration of an illness inducing agent (i.e. lithium-chloride) conditioned a strong taste aversion, but not avoidance of the audiovisual stimulus. Considering the cue-consequence specificity that characteristically underlies taste aversion learning, Garcia, Lasiter, Bermudez-Rattoni, and Deems (1985) argue that organisms (both vertebrates and invertebrates) evolved this mechanism in face of the toxic defenses evolved earlier by plants. Selective pressure has shaped organisms to be extremely sensitive to foods that may cause illness. Other than cue-consequence specificity, this sensitivity is reflected by several peculiarities of the conditioning of taste aversions. Firstly, taste aversions are acquired effectively even with very long delays between consumption of a taste and illness (Garcia, Hankins, & Rusiniak, 1974). Secondly, it has been shown that cancer patients also readily acquire conditioned food aversions as a result of incidental pairings of foods with the nausea-inducing consequences of their disease (e.g. tumor-growth or treatment) (Bernstein & Borson, 1986). These patients are well aware that it is not the food that is making them feel ill, but they acquire a taste aversion nonetheless. This implicates a dissociation between belief and

conditioned response that has not been demonstrated in any other human conditioning preparation (Boakes, 2009).

More recently, Lett and Grant (1996) demonstrated in rats that wheel running also induces taste aversion learning. Rats developed an aversion for either salt or sour water depending on whether the specifically flavored water had been paired previously with 30 min confinement in a running wheel or not. This effect is not limited to wheel running as it extends to other forms of physical exercise as well. Rats also develop an aversion for tastes paired with running in a circular alley (Lett, Grant, Koh, & Parsons, 1999) or with forced swimming (Nakajima & Masaki, 2004; for an extensive review, see Boakes & Nakajima, 2009). Lett and Grant assumed that exercise induces some degree of sickness, but Nakajima, Hayashi, and Kato (2000), however, upon replicating taste aversion learning induced by confinement in a running wheel noted that their rats had acquired a taste aversion despite the fact that the running itself was spontaneous and the rats did not appear to show any overt behavioral signs of exercise-induced illness. Therefore, these researchers argued that perhaps exercise-induced taste aversions are the result of energy expenditure and, hence, does not require the experience of any exercise-induced discomfort. Corroborating this line of reasoning, more intense exercise has been found to induce stronger conditioned taste aversions (Hayashi, Nakajima, Urushihara, & Imada, 2002; Masaki & Nakajima, 2005). This does not preclude the possibility that exercise-induced taste aversion learning requires the experience of gastrointestinal distress, such as nausea. Even mildly nauseating events that are not reflected by any overt signs of illness in animals can induce taste aversion learning (Rusiniak, Hankins, & Garcia, 1976). Further, it is conceivable that increasingly intense exercise establishes more but still relatively mild gastrointestinal symptoms, thus leading to stronger taste aversions.

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Lett et al. (1999) speculated that exercise leads to gastrointestinal distress (henceforth termed GID) as the result of a delay of gastric emptying, thus inducing a conditioned taste aversion. Conceivably, the ingestion of food (or drink) as opposed to merely tasting the conditioned stimulus, would result in greater GID and hence stronger exercise-induced taste aversions. Indeed, Revusky, Parker, Coombes, and Coombes (1976) observed that flavor aversions are stronger if the substance is consumed rather than merely tasted. Similar findings have been observed in human participants; that is, Kondo et al. (2001) showed that exercise (i.e. 20 min or 60 min cycling on an ergometer) can induce mild nausea and this effect was more pronounced when participants had eaten something just prior to the exercise. This is not an isolated finding, gastrointestinal symptoms of the upper gastrointestinal tract such as nausea, belching and vomiting, and gastrointestinal symptoms of the lower tract such as side aches, and gastrointestinal cramps during exercise are particularly prevalent among endurance athletes such as cyclists and long-distance runners (Peters et al., 1999).

These findings suggest that humans, just as rats, might be sensitive to exercise-induced taste aversion learning. This effect may be stronger with actual consumption as opposed to merely tasting the flavored stimulus. The present study tests this hypothesis. More specifically, participants were given a distinctively flavored solution to either consume or taste-and-spit prior to a single running bout on a treadmill. Participants having to drink the solution were expected to experience abdominal bouncing and hence stronger exercise-related gastrointestinal discomfort. Such discomfort is generally thought to be the prerequisite for genuine taste aversion learning (Pelchat & Rozin, 1982) and it was thus hypothesized that the drinking group would show a stronger flavor aversion than the taste-and-spit group. Participants further received another flavor to either consume or taste-and-spit immediately after running. Animal studies suggest that such backward pairing will not establish a conditioned taste aversion, and in fact may even condition a taste preference (Hughes & Boakes, 2008; Salvy, Pierce, Heth, & Russell, 2004). A secondary aim of the present study therefore was to investigate whether backward pairing of a flavor with running in human subjects would also lead to a conditioned flavor aversion, or a flavor preference.

## Method

### Participants

A total of 58 healthy participants were recruited among the undergraduate psychology student population at Maastricht University ( $M$  age = 21.9,  $SD$  = 2.0;  $M$  BMI [ $\text{kg}/\text{m}^2$ ] = 22.3,  $SD$  = 2.9; 35 males). Participants were randomly divided into two groups of equal size: DRINK ( $n$  = 29) or TASTE ( $n$  = 29). The present study was approved by a local institutional review board with the restriction that only participants that were physically fit were included in the study. All participants provided informed consent prior to their participation.

### Procedure, materials and design

Participants were tested individually on a weekday in a continuously ventilated research laboratory equipped with a treadmill exerciser (SportsArt Fitness TR33, Taiwan). All participants were contacted by phone at least one day before their participation and were told that the main purpose of the experiment is to examine the effects of carbohydrate-rich drinks on both cognitive and physical performance and that in line with this aim they would have to abstain from consuming anything (including drinking water, and chewing gum) 2 h prior to participation in the experiment.

Upon entering the lab, each participant was given the opportunity to change clothing. Next, the participant was seated at a table and received three plastic cups each filled with approximately 25 ml of a distinctly flavored solution. The three distinct flavors were obtained by adding 1 part of mali-flavored lemonade syrup (providing a jasmine flavor; Hale's Blu Boy Brand, Thailand) or 1 part of sala (a typical South-East Asian fruit) flavored lemonade syrup (Hale's Blu Boy Brand, Thailand) or 1 part of cream soda flavored syrup (Hale's Blu Boy Brand, Thailand) to 10 parts of water. The participant was instructed to first smell, then taste and consume each drink and to mark on separate 200-mm line scales how much they liked (or disliked) its odor and taste at that moment. These hedonic ratings could range from  $-100$  "extremely disliked" to 0 "neutral" to  $+100$  "extremely liked".

After the evaluation of the three drinks, the participant was instructed to complete the first cognitive task which comprised solving as many sudoku-puzzles as possible in 15 min. Directly after this 15 min period, the participant received a set of 100-mm line scales (ranging from 0 "not at all" to 100 "very much") on which s/he had to mark to what degree during the preceding 15 min s/he had experienced: nausea, the need to belch, stomachache, the need to vomit, side aches, and gastrointestinal cramps. Next, the participant received 300 ml of one of the three drinks. This drink served as conditioned stimulus (CS henceforth) A, forwardly paired with the running exercise. Participants in group DRINK were instructed to consume CS A, whereas participants in group TASTE had to taste-and-spit this drink. The experimenter monitored adherence to the instruction and ensured that each participant completed drinking or tasting A within 5 min. Directly after the consumption or tasting of A, the participant was instructed to run for 30 min on the treadmill exerciser. Heart rate was continuously monitored by the experimenter during the exercise with the aid of a Polar RS400 heart rate monitor (Kempele, Finland).

Each participant was instructed to run at approximately 80% of his/her maximal heart rate ( $\text{HR}_{\text{max}} = 207 - 0.7 \times \text{age}$ ; Gellish et al., 2007), providing a moderately intense exercise. The experimenter increased the speed of the treadmill exerciser if heart rate remained below the 80%  $\text{HR}_{\text{max}}$  for 2 min. Conversely, the experimenter decreased the speed of the treadmill exerciser if heart rate exceeded the 80%  $\text{HR}_{\text{max}}$  for 2 min.

Immediately after the 30 min running bout, the participant was again seated at the table and rated his/her degree of gastrointestinal symptoms experienced during the exercise on separate 100-mm line scales. Next, s/he received another 300-ml drink (CS B) to either consume (group DRINK) or to taste-and-spit (group TASTE). Participants from group DRINK thus drank all the beverages, whereas participants from group TASTE had to sip and spit these beverages.

After the consumption or tasting of CS B, the participant received 15 min to recover from the exertion. The participant remained within the laboratory and was instructed to solve another sudoku during this time period. Immediately after the 15 min rest, the participant again received 25 ml of each of the three drinks to taste and evaluate. These drinks included CS A, CS B and CS C (C referring to the drink that had not explicitly been paired with the running exercise). Which drink served as CS A, or B, or C was determined randomly for each separate participant. Table 1 displays the experimental design.

At the end of the experiment, each participant was asked to indicate what s/he thought had been the purpose of the experiment, to check that participants were unaware of the specific hypothesis of the study. After full debriefing, the participant received a course credit as a compensation for their participation. The total duration of the experimental procedure was approximately 1 h.

**Table 1**

The experimental design, displaying each phase of the experiment for each separate group (DRINK and TASTE).

Group	Phase				
	Pre-test	Rest	Conditioning	Rest	Post-test
DRINK	Evaluation of CS A, B and C	15 min sudoku	Consume CS A → 30-min run → consume CS B	15 min sudoku	Evaluation of CS A, B and C
TASTE	Evaluation of CS A, B and C	15 min sudoku	Taste-and-spit CS A → 30-min run → taste-and-spit CS B	15 min sudoku	Evaluation of CS A, B and C

### Data analysis

As noted above, all participants were asked at the end of the experiment to indicate what they believed had been the true aim of the experiment. Most participants claimed they had no idea, but still a substantial proportion of participants ( $n = 10$ ; 8 from group DRINK and 2 from group TASTE) correctly identified that we were interested in examining shifts in the flavor preference as a result of the running exercise. As the dependent variable comprises a subjective measure (i.e. subjective hedonic ratings of taste and odor) the results of these 10 participants were excluded from the analyses.

The degree of the hedonic shift from pre-test to post-test was first calculated for each CS by subtracting the hedonic ratings at post-test with the ratings at pre-test. These difference scores were calculated separately for the hedonic ratings of taste and odor, and served as the primary dependent variable in the subsequent analyses. Note that the difference scores for both the taste and odor ratings were only calculated for those participants who at pre-test evaluated the taste/odor of all three drinks between  $-70$  and  $+70$  as ratings within these limits still allows the observation of either a positive or negative shift at post-test. For the taste ratings this resulted in the further exclusion of 14 participants and for the odor ratings the results of 15 participants were excluded from the analyses.

## Results

### Exercise-induced taste aversion learning

Fig. 1 displays the mean shift in ratings of taste (panel A) and odor (panel B) of each CS for each separate group (DRINK or TASTE). To determine taste aversion learning a  $2 \times 3$  ANOVA was conducted with Group (DRINK vs. TASTE) as between-subjects variable and CS (A, B, or C) as the within-subject variable with shift in hedonic ratings of taste as the dependent variable.

A marginally significant effect of CS was found,  $F(2, 64) = 2.86$ ,  $p = .06$ ,  $\eta^2_{\text{partial}} = .08$ . Simple contrasts with CS C as reference revealed a significant effect of CS when comparing CS A with CS C,  $F(1, 32) = 4.21$ ,  $p < .05$ ,  $\eta^2_{\text{partial}} = .12$ , and when comparing B with C, no effect was found,  $F < 1$ . Further, no effects of Group ( $F[1, 32] = 2.62$ ,  $p = .12$ ,  $\eta^2_{\text{partial}} = .03$ ) and CS  $\times$  Group ( $F < 1$ ) were found.

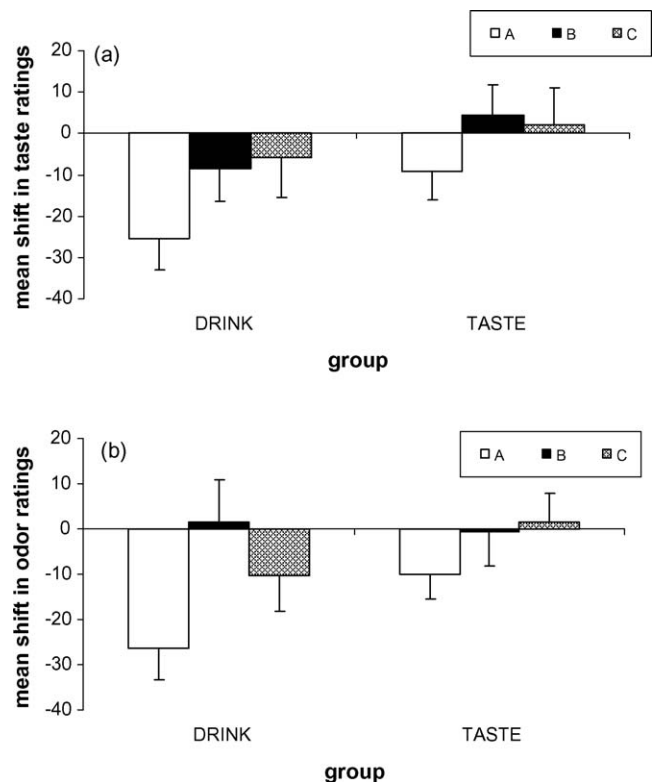
It was further tested whether the effects were related to the exercise-induced GID. To test this hypothesis, we first calculated difference scores by subtracting ratings of gastrointestinal symptoms for the 15 min pre-exercise resting period from the ratings of the gastrointestinal symptoms for the running exercise. As the shift in ratings for the symptoms of the upper tract (viz., nausea, belching, vomiting, and stomachache) all positively correlated with one another (smallest  $r = .38$ ,  $p = .025$ , between the shift in stomachache and belching; largest  $r = .76$ ,  $p < .001$ , between the shift in stomachache and nausea), these difference scores were averaged into a single score representing symptoms of the upper tract. Three separate t-tests were then conducted for exercise-induced symptoms of the upper tract, side aches and gastrointestinal cramps, comparing group DRINK with TASTE. None of these tests revealed a significant difference between groups, all  $ts < 1$ .

The absence of a group difference in exercise-induced GID does not preclude the possibility that the extent of exercise-induced GID

determined the degree of the observed negative shift in hedonic ratings of the taste of CS A. To test this possibility, a backward regression analysis was conducted with exercise-induced symptoms of the upper tract, gastrointestinal cramps and side aches as predictors and the mean shift in hedonic ratings of the taste of CS A as the dependent variable. In the first step, all predictors were entered into the model and in the following steps non-significant predictors were removed with the standard removal criterion of  $p > .10$ . This led to the removal of all predictors, meaning that none of the exercise-induced gastrointestinal symptoms significantly contributed to the observed negative shift in hedonic ratings of the taste of CS A, with the final predictor removed from the model being exercise-induced side aches ( $\beta = .22$ ,  $p = .21$ ).

### Exercise-induced odor aversion learning

As for the taste ratings, the shift in hedonic ratings of the odor of the CSs served as the dependent variable in a  $2$  (Group: DRINK vs. TASTE)  $\times$   $3$  (CS: A, B, or C) ANOVA. This analysis revealed a main effect of CS,  $F(2, 62) = 3.76$ ,  $p < .05$ ,  $\eta^2_{\text{partial}} = .11$ . Simple contrasts with CS C as reference further revealed a significant effect of CS when comparing A with C ( $F[1, 31] = 6.00$ ,  $p < .05$ ,  $\eta^2_{\text{partial}} = .16$ ), but not when comparing B with C,  $F < 1$ . Further, no effects of Group ( $F[1, 31] = 1.77$ ,  $p = .18$ ,  $\eta^2_{\text{partial}} = .14$ ) and CS  $\times$  Group ( $F < 1$ ) were found.



**Fig. 1.** Mean shift in hedonic ratings of taste (panel a) and odor (panel b) (+SEM) for each CS (A, B, and C) and for each separate group (DRINK and TASTE). Note that A, B, and C refer to different flavors.

Next, we tested for group differences in exercise-induced gastrointestinal symptoms of the upper tract, side aches and gastrointestinal cramps, and found no significant differences on these variables between the two groups, largest  $t(31) = 1.60$ ,  $p = .13$  for exercise-induced gastrointestinal symptoms of the upper tract.

Further, we assessed whether exercise-induced GID predicts the degree of the observed negative shift in hedonic ratings of the odor of CS A by conducting a backward linear regression analysis. As for the hedonic shift for taste, this analysis failed to reveal a significant contribution of the different exercise-induced gastrointestinal symptoms, with the final predictor removed from the model being symptoms of the upper tract,  $\beta = -.28$ ,  $p = .12$ .

## Discussion

The primary aim of this study was to examine whether exercise-induced flavor aversion learning observed in rats could also be found in human participants. The present results of interest indicate that this is the case. A taste and odor aversion was found for the flavor CS A forwardly paired with a single running exercise.

Further, we hypothesized that in the case of consumption of CS A as compared with the mere tasting of this drink, subjectively experienced GID would be stronger during exercise, hence promoting taste aversion learning. Although some indication for a group effect was found, this effect (insofar one is allowed to speak of any effect) was weak and this absence of a strong and clear group difference in degree of conditioned flavor aversion might indicate that the manipulation of GID had failed. In that case an equal degree of exercise-induced GID should be observed when comparing the groups DRINK and TASTE. This indeed appeared to be the case, but note that this does not preclude the possibility of GID affecting the degree of the observed negative hedonic shift. Subsequent regression analyses, however, demonstrated that the exercise-induced hedonic shift of both taste and odor ratings of CS A is not significantly predicted by the extent of exercise-induced GID.

The secondary aim of the present study was to examine whether backward pairing between a flavor and exercise would induce flavor preference learning as it appears to be the case in rats. The hedonic shift in both taste and odor ratings for CS B though did not differ when compared to CS C, indicating that no learning had occurred to CS B. It is possible that backward conditioning of a flavor preference, as opposed to forward conditioning of a flavor aversion, requires multiple learning trials. Another possibility is that the participants were largely unfamiliar with running on a treadmill exerciser. Recently, Hughes and Boakes (2008) demonstrated that familiarity with the unconditioned stimulus (US) can promote flavor preference learning, while attenuating flavor aversion learning (see also Salvy, Pierce, Heth, & Russell, 2002). Without prior exposure to running inside a running wheel, rats did not demonstrate the development of a preference for tastes that were backwardly paired with running. Although we only recruited and selected participants who on average engaged in a sports-related activity at least once per week, it is possible that almost none of the participants had any experience with treadmill running. Unfortunately, we did not assess participants' prior degree of experience with running on an exerciser, so further research is required to determine to what degree such US familiarity affects both exercise-induced aversion and preference learning in humans.

It should be noted here that the post-test closely followed conditioning, whereas in animal research, the final test of a flavor aversion is usually conducted a day or even longer after the last conditioning trial. The present results thus show that exercise can induce a near immediate conditioned flavor aversion (i.e. a conditioned flavor aversion), but it says very little about how long such an effect will hold. To determine the complete relevance of the

present finding that exercise can induce flavor aversion learning in humans, this remaining question requires further investigation.

To recapitulate, the present study demonstrates single-trial exercise-induced flavor aversion learning in humans, which appears independent of amount of intake and of the degree of subjectively experienced activity-related GID. Note that the absence of a contribution of GID to the present exercise-induced conditioned flavor aversion does not preclude the possibility that perhaps with other modes or more intense exercise GID may still affect the degree of exercise-induced flavor aversion learning. Further research is required to determine the generality, robustness, exact cause and parameters of exercise-induced flavor aversion learning.

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