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Overshadowing and latent inhibition in nausea-based context conditioning in humans: Theoretical and practical implications

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Volunteer participants underwent nausea-inducing body rotation in a distinctive context, and the acquired ability of the contextual cues to evoke nausea was subsequently assessed by a symptom rating scale. One group received prior exposure to the context (a latent inhibition procedure); a second consumed a novel flavour prior to rotation (an overshadowing procedure); a third group experienced both procedures; and a control group received neither. When tested in the context in the absence of rotation, all groups reported an increase in nausea-related symptoms at the time when rotation had previously occurred, an outcome consistent with the occurrence of conditioned nausea. The magnitude of this increase did not differ across the groups, but the overall level of responsiveness (the degree to which nausea-related symptoms were reported) was enhanced in the latent inhibition and reduced in the overshadowing condition. Cortisol levels showed the same pattern. The implications of these findings for the proposal that overshadowing and latent inhibition procedures might be used to control the development of anticipatory nausea in patients undergoing chemotherapy is considered.

Keywords: Nausea; Conditioning; Latent inhibition; Overshadowing; Cancer chemotherapy.

The state of nausea (as produced, e.g., by an injection of lithium chloride, LiCl) can serve as an effective unconditioned stimulus (US) for classical conditioning, as is evidenced by the phenomenon of flavour aversion learning. The early notion (e.g., Seligman, 1970) that this US might be effective only with conditioned stimuli (CSs) of a certain class (i.e., flavours) has not been supported by later work; there are now many experiments showing, for rats, that conditioning will occur with nausea as the US when the CS is a distinctive context (for a reviews see Hall & Symonds, 2006; Rock, Limebeer, & Parker, 2014). A context that has been paired with the effects of an injection of LiCl appears to be able to evoke a state of nausea (Limebeer, Hall, & Parker, 2006) as a conditioned

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response (CR). This observation constitutes a clear parallel with the phenomenon of anticipatory nausea (AN) in which chemotherapy patients, who experience the nausea-inducing effects of infusion of cytotoxic drugs in a given context (a clinic), develop a tendency to react with nausea to the clinic itself. It lends support to the interpretation (offered on other grounds; see, e.g., Stockhorst, Klosterhalfen, & Steingrüber, 1998) that AN is a consequence of classical conditioning (see also Stockhorst, Enck, & Klosterhalfen, 2007). Context-aversion conditioning in the rat can thus serve as an animal model in which we can assess the effectiveness of procedures that might be employed in the clinic to attenuate the degree to which AN occurs in patients. Rock et al. (2014) have reviewed studies of pharmacological treatments; here we focus on simple behavioural procedures (see also Quinn & Colagiuri, 2014).

Experiments with rats have shown that context aversion is sensitive both to *overshadowing* and to *latent inhibition*. Overshadowing occurs when the presence of a salient additional cue at the time of conditioning detracts from the acquisition of strength by the target cue. Thus, Symonds and Hall (1999) gave rats exposure to two distinctive contexts, each associated with an injection of LiCl. In one of the contexts the subjects were allowed to drink a novel-flavoured solution. On a subsequent test, the context in which the flavour had been present evoked a weaker conditioned response than the other context, indicating that the flavour had overshadowed context conditioning. Latent inhibition refers to the attenuation of conditioning produced by prior exposure to the event to be used as the CS. Hall, Symonds, and Rodriguez (2009) examined the effects of giving rats a series of preexposures to the context prior to the conditioning trial in which the context was paired with nausea. They found that in these circumstances, the size of the conditioned aversion to the context was reduced; that is, a latent inhibition effect was observed. These results encourage the conclusion that these procedures might be developed as clinical interventions for the alleviation of AN, and that a combination of both might be especially effective. But before attempting any direct study of a patient population, we thought it necessary (for reasons outlined below) to carry out further laboratory experiments with healthy volunteer participants to confirm that the effects of interest can be obtained in a nausea-based conditioning preparation with human subjects.

Previous work has made use of motion-induced sickness as the US. In this procedure, the participant is strapped into a specially constructed chair (the MARDER; see Probst, Dabrowski, Liebler, & Wist, 1993) capable of generating rotation in three dimensions, although only rotation around the vertical axis was used in these studies. Scores on a symptom rating scale show that this procedure results in a degree of nausea in susceptible participants, and the ability of this state to support conditioning was evidenced by the finding of Klosterhalfen et al. (2000) that subjects who had consumed a novel-flavoured drink prior to rotation developed an aversion to that flavour. We have used this procedure in separate studies intended to look at the effects of latent inhibition (Klosterhalfen et al., 2005) and overshadowing (Stockhorst, Hall, Enck, & Klosterhalfen, 2014) on conditioning to contextual cues. In the first, subjects in the critical condition received up to three sessions of exposure to the context to be used for rotation prior to experiencing the rotation procedure; they then received two sessions of rotation, followed by a test session consisting of exposure to the context, but with no rotation given. In the second study, the experimental subjects were given a novel and distinctively flavoured drink (a potential overshadowing stimulus) prior to each of three rotation sessions and were then tested in the absence of the flavoured drink (they were given plain water) on a fourth rotation session.

In both these studies, the critical measure was the score on the symptom rating scale recorded at the very start of the test session, on the assumption that this initial response would reflect any anticipatory CR governed by the contextual cues. The first study found that subjects (especially females) given the preexposure procedure showed a smaller response to the context on the test session than those not given preexposure. This is consistent with the occurrence of latent inhibition. It should

be noted, however, that the groups differed in their response to the context at the start of the session in which the first rotation was given, which raises the possibility that habituation to the context rather than latent inhibition might be responsible for the test result. The second study found that the anticipatory response in the context increased slightly from the first session to the test session in control subjects, but that subjects given the overshadowing treatment showed a reduction, resulting in significant intergroup differences. This is consistent with the proposal that control subjects acquired a CR to the context, but that those given overshadowing did not. This study also found some evidence that the direct response to rotation itself (symptoms recorded immediately after experience of rotation) was reduced by the overshadowing treatment. Again, the effects were somewhat more marked in female than in male subjects.

Although these results are encouraging, we acknowledge that neither of these experiments included the control conditions necessary to confirm that the effects obtained were indeed a consequence of the modulation of a conditioned response. Accordingly, in the present study, we have investigated these procedures further, employing a more fine-grained analysis that should allow identification of any conditioned component of the symptoms reported. Specifically we made use of a test procedure that allowed assessment not just of the initial response to the context but also of the entire pattern of responsiveness over the course of the test session (further details are given below). To anticipate, this analysis provided evidence for the occurrence of conditioning, but not for the occurrence of latent inhibition and overshadowing, as they are usually understood.

In addition to studying the separate effects of latent inhibition and overshadowing, a central aim of the study was to examine a condition in which participants experienced both the latent inhibition (preexposure to the context) and the overshadowing (the novel flavour) procedures. If both are effective in attenuating context conditioning, it could be useful to combine them when it comes to devising an intervention regime for clinical use. According to many theories of conditioning, these two processes will operate independently, and their effect should summate, resulting in very poor learning about the target cue (the context in this case). According to the Pearce and Hall (1980) model, for instance, preexposure to the context will retard conditioning by reducing its associability, and the introduction of a novel cue, of high associability, on the conditioning trial, will further limit the acquisition of associative strength by the context. In contrast, however, the comparator theory developed by Miller and his colleagues (e.g., Denniston, Savastano, & Miller, 2001; Miller & Matzel, 1988) uniquely makes the prediction that latent inhibition and overshadowing should counteract each other, and that subjects experiencing both should condition more readily than subjects given just one or other of these treatments. Support for this prediction has come from experiments on fear conditioning in rats (e.g., Blaisdell, Bristol, Gunther, & Miller, 1998), but the counteraction effect has not been universally observed, and summation of latent inhibition and overshadowing has been the result found in flavour aversion learning procedures (e.g., Nagaishi & Nakajima, 2008). We may hope therefore, that counteraction will not occur in the present paradigm, but clearly it is necessary to resolve this issue prior to proposing a possible clinical intervention.

In outline, our experiment involved four treatment groups (see Table 1). All of them received two conditioning trials with rotation as the US, and their responding both to the rotation itself and the context in which it was given was assessed primarily by means of a self-report questionnaire. One treatment group (the overshadowing, OS, group) drank a novel-flavoured drink prior to each rotation session; a second (the latent inhibition, LI, group) was exposed to the rotation apparatus on three occasions prior to the first rotation; a third group (LI-OS) received both these treatments. The fourth group (the control, C, group) received neither the latent inhibition nor the overshadowing treatment. The critical results came from a test session, given after the final rotation session, in which response to the context in the absence of rotation was assessed. An elevated score for nausea-related symptoms

Table 1. *Experimental design*

			Day 3			
Group	*Day 1*	*Day 2*	*1st session*	*2nd session (+ 1 hour)*	*Day 4*	*Day 5*
LI-OS	Context	Context	Context	Rotation + flavour	Rotation + flavour	Context
LI	Context	Context	Context	Rotation	Rotation	Context
OS	Neutral context	Neutral context	Neutral context	Rotation + flavour	Rotation + flavour	Context
C	Neutral context	Neutral context	Neutral context	Rotation	Rotation	Context

Note: Context refers to the rotation chair; neutral context refers to an office environment. OS = overshadowing; LI = latent inhibition; C = control.

(the score recorded on initial exposure compared to that on the apparatus) would provide evidence of conditioning. If the latent inhibition and overshadowing procedures are effective, we might expect lower scores on test in the LI and OS groups than in the C group, and if latent inhibition and overshadowing summate, the LI-OS group would show the lowest score of all. Given the susceptibility of females to the effects of interest, we carried out our study just with female participants. This will allow a possible "proof of principle"; further work will be needed to establish the generality of any effects obtained. As with previous studies from our group (Klosterhalfen et al., 2005; Meissner, Enck, Muth, Kellermann, & Klosterhalfen, 2009; Rohleder et al., 2006; Stockhorst et al., 2014), concentrations of salivary cortisol were assessed to explore whether symptomatic responses to the interventions were reflected in changes of this biological parameter of stress.

EXPERIMENTAL STUDY

Method

The study was conducted at the University of Düsseldorf. It consisted of two parts conducted in different contexts and using different rotation chairs. The first was a prestudy to screen for susceptibility to motion sickness; the main study was conducted on subjects identified as being susceptible. The protocol for the rotation-chair procedure was approved by the Ethical Board of the Medical Faculty of the Heinrich-Heine-University, Düsseldorf, and all subjects gave written consent prior to participation.

Apparatus

Screening was conducted using a simple chair (of the type known as an ENT or examination chair) that could rotate around its vertical axis. This was located in a daylit seminar room in the Department of Physiology. Experimental sessions were carried out in a novel and distinctive context —the artificially lit basement laboratory of the Institute of Psychology, which housed the MARDER apparatus. Subjects were strapped into a large, dark-coloured chair, positioned in a cage-like cabin at the centre of the apparatus.

Nausea was assessed by means of a German translation of the Motion Sickness Susceptibility Questionnaire of Golding (1998). This asked subjects to rate seven nausea-associated symptoms (vertigo, headache, nausea, urge to vomit, tiredness, sweating, stomach awareness) on a 6-point scale from 0 (not present) to 5 (very strong). A single summed score (with a maximum value of 35) was computed for each administration of the questionnaire. The flavours used in the groups given the overshadowing treatment (OS and LI-OS) were commercially available, but unfamiliar, juices (elderberry and sallow thorn) that in previous work (see Stockhorst et al., 2014; Stockhorst, Wiener, et al., 1998) had been judged to be salient and relatively neutral (being given a pleasantness ratings of 2, 3 on a 6-point scale).

Participants and screening

The volunteer participants were selected from a larger pool on the basis of the screening procedure. In this, subjects were seated in the ENT chair and rotated at a constant speed of 120° s^{-1}. With their eyes closed they were instructed, by audiotape, to move their heads up and down every 6 s, with a pitch of approximately 90°. They experienced up to five 1-min rotations, with an interval of 1 min between rotations. Subjects could terminate each rotation on request, but were asked to resume the next after the 1-min break. About 50% of those screened will tolerate less than the full length of 5 min of rotation and are regarded as "susceptible". Thirty-two of these (all female, mean age 25 years, range 20–41 years) were recruited for the present study. They were randomly assigned to one of the four experimental conditions, LI, OS, LI-OS, and C, with eight participants in each condition. Screening sessions were spread across several weeks; the minimum interval between screening and the first experimental session was two weeks.

Procedure: Main study

Participants were required to come to the laboratory on five consecutive days, at the same time each day. Investigations were performed between 8.00 and 16.00; the start-time was constant for each participant, but was balanced across the experimental groups. Each session (there were two on Day 3, see Table 1) lasted for about 60 min. Subjects were instructed to fast for 6 hours prior to arrival (blood glucose strips were used to confirm that this requirement had been complied with). All testing was conducted by one female investigator (S. Kellermann).

On all sessions, the participants completed the symptom-rating questionnaire four times. For sessions in which rotation occurred, these were when the subject was first seated in the chair (a baseline score, to be referred to as SR1), immediately after the rotation procedure had been completed (SR2), 15 min later (SR3), and 30 min later (SR4). Then test was administered at the equivalent times on sessions in which no rotation was given. At the same time as the SR was recorded, a saliva sample was taken, to allow subsequent analysis of stress-related hormonal responses.

The first three sessions consisted of preexposure to the experimental context for the LI and LI-OS groups. These participants were seated in the rotation chair and remained there until a set of response measures had been taken. Participants in the OS and C groups spent an equivalent period of time in what was assumed to be a neutral context (an office). The second session on Day 3 (see Table 1) took place one hour after the first. For this, all subjects were seated in the rotation chair and completed SR1. They then experienced the rotation procedure in the experimental context. As in screening, the rotation speed was 120° s^{-1}, with the subjects instructed to bend their heads every 6 s, with their eyes closed. Subjects were given two periods of rotation, each lasting 1 min, with a 1-min break between them. Participants could terminate each rotation sequence on request, but were asked to continue with it after a break of 1 min. Immediately after the rotation procedure had been completed, the participants completed SR2, with SR3 and SR4 following after 15 and 30 min. Subjects in the overshadowing conditions were given 100 ml of one of the flavoured drinks prior to rotation; those in the other groups were given 100 ml of water. The procedure for Day 4 was identical to that for Session 2 of Day 3, except that the subjects in the overshadowing groups were given the flavour not used in the previous session. The 2nd session of Day 3 and the Day 4 session constituted the conditioning phase. The procedure for the test session on Day 5 was the same except that all subjects were given water at the appropriate time point, and no rotation followed.

Saliva samples. At four time points in each session (immediately following completion of the symptom-rating scale), saliva was collected in a plastic beaker (Sarstedt, Nuembrecht, Germany) over a 4-min period. For later analysis of salivary free cortisol, 1000 μl was pipetted into a salivette (Sarstedt, Nuembrecht, Germany) and stored at –20°C. Prior to analysis the salivettes were centrifuged at 2000 rpm for 5 min. Salivary free

cortisol concentrations were determined using a commercial time-resolved immunoassay with fluorometric detection as described by Dressendörfer and coworkers (for details see Rohleder et al., 2006).

Analysis of results. A single summed score (with a maximum value of 35) was computed for each administration of the questionnaire. For the critical sessions (Days 3 to 5), group differences were assessed by analysis of variance (ANOVA) with group and trial (i.e., SR1–SR4) as the variables; the same analyses were performed for saliva cortisol levels. For analyses of the preexposure phase, data were collapsed across the OS condition (producing two groups, LI and no LI), as the OS factor was introduced only at the start of conditioning. Comparisons between individual means were made using Tukey's test. A significance level of $p < .05$ was used throughout.

Results and discussion

We focus here on the results from our primary measure of nausea, the symptom rating scale; results from assays of salivary cortisol can be found in the Appendix.

Preexposure

The rating scale was administered four times per session throughout the preexposure phase (i.e., on Days 1 and 2, and the first session of Day 3). The results (group means) for all four tests on the very first session are presented in Figure 1. Unsurprisingly, given the absence of any nausea-inducing intervention, scores were in general rather low and declined somewhat over the course of the session. The scores for the subjects given the latent inhibition treatment (i.e., scores recorded in the chair in the experimental room) were higher than those for the control subjects. This may mean simply that the experimental context was intrinsically slightly aversive; alternatively, although the contexts and chairs were rather different, it could indicate generalization of a response acquired to the rotation chair as a consequence of the screening procedure. An ANOVA was conducted on the data

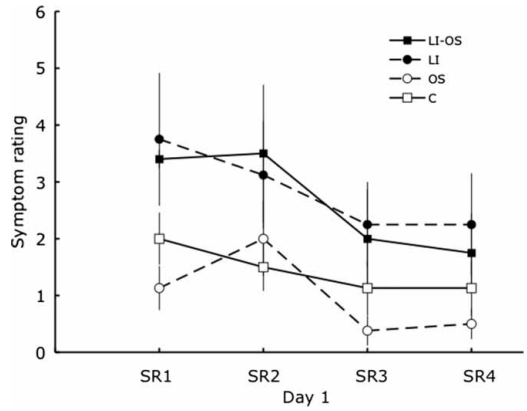

Figure 1. *Group mean symptom rating scores for each of the four administrations of the symptom rating (SR) scale on Day 1. LI = latent inhibition; OS = overshadowing; C = control. Vertical bars represent standard errors of the mean.*

summarized in the figure, the variables being group (LI or no LI) and trials. This revealed significant effects of group, $F(1, 30) = 5.29$, $MSE = 14.18$, $\eta_p^2 = .15$, $p = .029$, and of trial, $F(3, 30) = 10.48$, $MSE = 1.29$, $\eta_p^2 = .26$, $p < .001$. The interaction was not significant ($F < 1$). Tukey's tests found no significant differences between the scores for SR1 and SR2 and the scores for SR3 and SR4; each of the first two scores differed ($p < .01$) from each the latter scores.

This difference between the latent inhibition and control conditions was maintained throughout preexposure. The group means, over all 12 scores for each individual in this stage, were 3.13 for the LI group, 2.34 for the LI-OS group, 0.98 for the OS group, and 1.22 for the control group. An ANOVA revealed a significant effect of the LI variable (i.e., of the place where the measure was taken), $F(1, 30) = 5.74$, $MSE = 3.57$, $\eta_p^2 = .16$, $p = .023$.

Conditioning

Figure 2 shows group mean symptom rating scores for each administration of the scale on the conditioning sessions (i.e., for the second session of Day 3 and that for Day 4). When first seated in the chair on Day 3, all groups showed a similar and low baseline score (SR1). The rotation

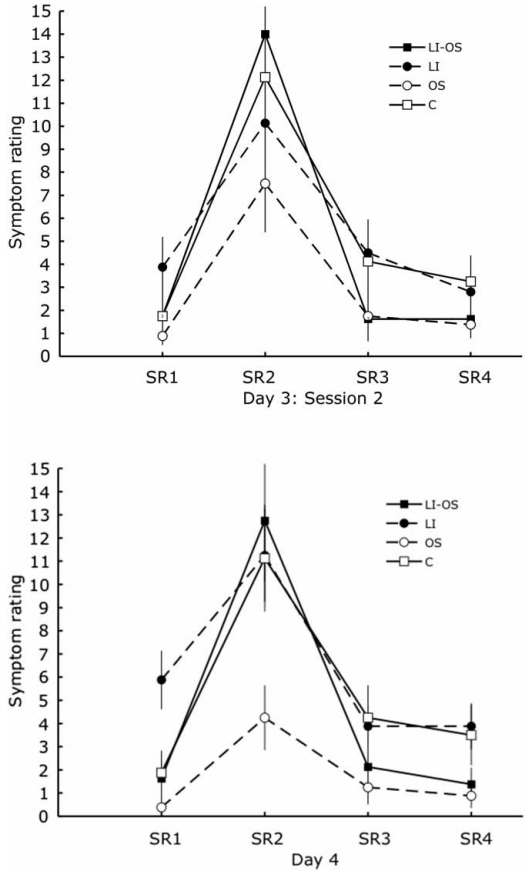

Figure 2. *Group mean symptom rating scores for each of the four administrations of the symptom rating (SR) scale on the conditioning sessions. LI = latent inhibition; OS = overshadowing; C = control. Vertical bars represent standard errors of the mean.*

procedure was clearly effective, as evidenced by the increased scores for SR2, given immediately after the treatment (note the change of scale of the *y* axis from Figure 1 to Figure 2). The increase was most marked in the LI-OS group, and least in the OS group, but was present in all. Scores returned to baseline levels for the final two tests, SR3 and SR4. An ANOVA was conducted on these data, the variables being group and test trial. There was no significant main effect of group, $F(3, 28) = 1.15$, but there was a significant effect of trial, $F(3, 84) = 69.90$, $MSE = 8.32$, $\eta_p^2 = .71$, $p < .001$, and a significant interaction between the variables, $F(9, 84) = 2.29$, $MSE = 8.32$, $\eta_p^2 = .19$, $p = .024$. Analysis of simple main effects showed that the groups differed only on SR2, $F(3, 112) = 3.96$, $p = .01$ (for other trials the largest value of F was 1.18); pairwise comparison using Tukey's test showed that on this trial the LI-OS group differed significantly ($p < .05$) from the OS group. Tukey's tests also confirmed that for all of the groups the score for SR2 was significantly different from the scores recorded for the other three test trials ($ps < .05$).

The pattern of results for Day 4 was similar to that just described, the only apparent difference being that the OS group showed a lower level of responsiveness (see Figure 2). An ANOVA paralleling that just reported revealed significant effects of group, $F(3, 28) = 3.62$, $MSE = 33.29$, $\eta_p^2 = .28$, $p = .025$, of trial, $F(3, 84) = 54.09$, $MSE = 7.85$, $\eta_p^2 = .66$, $p < .001$, and of the interaction, $F(9, 84) = 3.18$, $MSE = 7.85$, $\eta_p^2 = .25$, $p = .002$. Simple main effects analysis showed there to be differences among the groups on SR1, $F(3, 112) = 3.20$, $p = .026$, and on SR2, $F(3, 112) = 8.14$, $p = .001$. Tukey's test showed that the OS group differed from the LI group on SR1 and from each of the other groups on SR2 ($ps < .05$). Tukey's test showed that the increase from SR1 to SR2 was significant ($ps < .05$) for all of the groups, even for the OS group.

This last finding shows that the OS group was not totally immune to the effects of the rotation procedure, but the magnitude of their reaction to it was clearly substantially less than that shown by all the other groups. This result confirms those of Stockhorst et al. (2014) who found that symptom scores of the Nausea Profile (Muth, Stern, Thayer, & Koch, 1996, which measures somatic, gastrointestinal, and emotional distress), recorded immediately after rotation, were reduced in subjects given the overshadowing treatment. The implication is that consuming a flavoured substance (as opposed to plain water) prior to treatment can reduce the immediate effectiveness of a nausea-inducing procedure. We may note that a reduction in the measured response to rotation in these circumstances might be expected on the basis of the notion that consuming a novel flavour might

reduce conditioning. The measured response might reflect not only the direct response to rotation (the UR) but also a contribution from a CR that is controlled by the contextual cues. If so, then the measured postrotation response will be enhanced when the CR is strong. Overshadowing, by limiting the development of the CR, would reduce its contribution to the overall response to rotation. Such an effect would take at least one conditioning trial to develop, and indeed the OS group differed significantly from the control group only on Day 4. This account provides no explanation, however, as to why the effect should be absent in the subjects given context preexposure prior to the overshadowing treatment.

Test

The scores for the test session (Day 5) are presented in Figure 3. Although the levels were lower, the general pattern was the same as that shown on the conditioning days, with scores being elevated on SR2 and falling away thereafter. That is, in spite of the fact that no rotation was imposed, the subjects showed elevated symptom-rating scores on the test that immediately followed the period when rotation had been given on the preceding sessions. This pattern, which contrasts with that shown in the preexposure phase (Figure 1), is consistent with the occurrence of conditioning, with the conditioned response being controlled not simply by contextual cues but also by the time at which the US had been presented in the context. There were clear differences among the groups, with the LI group showing the highest scores and the OS group the lowest; the other two groups, which did not differ from one another, fell between these extremes. An ANOVA, with group and trial as the variables, showed there to be significant main effects of group, $F(3, 28) = 3.84$, $MSE = 17.25$, $\eta_p^2 = .29$, $p = .020$, and of trials, $F(3, 84) = 8.76$, $MSE = 2.67$, $\eta_p^2 = .24$, $p < .001$; the interaction between the variables was not significant ($F < 1$). Tukey's tests showed that the overall score for the LI group differed significantly from that of the OS group ($p < .05$); neither differed significantly from either of the two groups, which did not differ from each other. Importantly, Tukey's tests also showed that score for SR2 differed significantly ($ps < .05$) from those recorded on each of the other three trials.

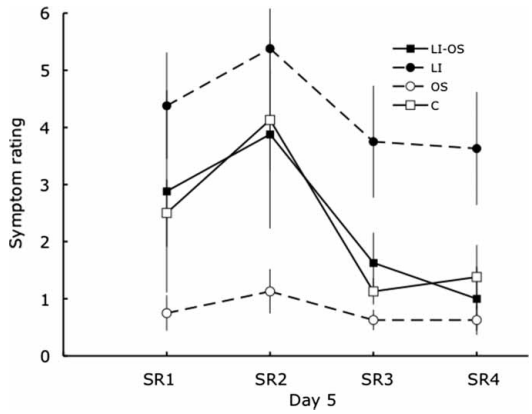

Figure 3. *Group mean symptom rating scores for each of the four administrations of the symptom rating (SR) scale on the test session (Day 5). LI = latent inhibition; OS = overshadowing; C = control. Vertical bars represent standard errors of the mean.*

Although the overall scores may seem to suggest this conclusion, it would be premature to conclude on the basis of these data that our latent inhibition procedure enhances conditioning and that our overshadowing procedure reduces it. Certainly the score for the LI group is high, and that for the OS group is low, but the groups started from different baselines; that is, the groups differed in the levels shown on SR1 (and on the return to baseline after SR2). The evidence for a conditioning effect comes from the elevation in scores on SR2. Thus, when it comes to assessing the effect of conditioning, the important observation is the degree to which the critical (temporal) cue elevates the score above the initial baseline. In an attempt to accommodate this, we calculated a difference score for each subject (SR2 score − SR1 score). Group mean difference scores were: 1.00 for the LI group, 0.50 for the LI-OS group, 0.39 for the OS group, and 1.63 for the control group. By this measure, all three treatment groups show a lesser effect than the control group, but a one-way ANOVA showed there to be no significant difference among these scores ($F < 1$).

GENERAL DISCUSSION

Participants asked to report nausea-related symptoms, immediately after experiencing a period of whole-body rotation, produced scores that were significantly elevated above baseline conditions; we take this to be an index of the unconditioned response of nausea elicited by the rotation. When subsequently tested in the context, in the absence of rotation, the participants showed elevated scores for the interval in which the rotation had previously been experienced. This outcome is consistent with the proposal that nausea can occur as a conditioned response, controlled, in this case, both by the context and by the specific timing of occurrence of the unconditioned stimulus. This finding confirms and extends our previous results (e.g., Klosterhalfen et al., 2000), demonstrating the effectiveness of this procedure in generating a conditioning effect.

The acquisition of a conditioned response can be retarded by prior exposure to the event to be used as the conditioned stimulus (the latent inhibition phenomenon), also by presenting another salient cue along with the target stimulus at the time of conditioning (overshadowing). We looked for these effects by giving some subjects preexposure to the context and giving others a salient novel drink prior to rotation. The possibility of interaction between the latent inhibition and overshadowing procedures was investigated by giving a third group of subjects both treatments. Control subjects received neither. No obvious effects on conditioning were found, in that the size of the elevation in reported nausea symptoms at the critical time period during the test did not differ among the groups. By this measure, our present results failed to confirm previous results suggestive of latent inhibition (Klosterhalfen et al., 2005) and overshadowing (Stockhorst et al., 2014) in conditioned nausea. The reasons for these apparent discrepancies are discussed below.

Although an elevation in responding at the critical time period (SR2) was present in all groups in this experiment, this is not to say that our experimental treatments were without effect. There were marked differences among the groups in the scores recorded across the entire test session, with scores being elevated by the latent inhibition treatment and reduced by the overshadowing treatment. Salivary cortisol levels showed parallel effects with (on the trial associated with SR2) the LI group showing the highest level and the OS group the lowest. We cannot specify the source of these effects with certainty, but the following observations may be made.

First we may note that the SR scores for subjects given the LI treatment were higher than those of controls throughout preexposure—that is, the experimental context was, in itself, somewhat unsettling (either intrinsically, or as a consequence of generalization from the screening procedure). Whatever its source, this response did not habituate (or extinguish) over the course of preexposure and was still evident at the start of conditioning, with the LI groups showing the highest scores on SR1, before rotation (see Figure 2). Thus, rather than eliminating any negative response, exposure to the context appeared to maintain the response to it. In contrast, subjects who had been introduced to the laboratory and general procedure but not to the conditioning context showed lower scores when they experienced the context for the first time. The pattern of results seen in the test phase (Figure 3) is consistent with the suggestion that the baseline response governed by the context sums with effects produced by conditioning, with the consequence that the preexposed and nonpreexposed groups show the same pattern across trials, but with the former being elevated throughout.

To an extent, this pattern of results accords with that reported by Klosterhalfen et al. (2005) from their study of latent inhibition, in that performance on the test session in that experiment too appears to reflect a general change in level of responsiveness. In that experiment, the measure used was the symptom rating score at the start of the session (equivalent to our SR1), comparison being made between the baseline scores recorded prior to rotation and those recorded on the test session. All subjects showed increased scores after training, and control subjects had higher scores than

preexposed subjects. As we have noted, however, there was a difference between the preexposed and control groups even on the baseline scores recorded prior to the first rotation. These results are thus consistent with the possibility that preexposure to the context allowed habituation of the initial negative response to it, producing a sustained difference between the groups during the conditioning procedure. The difference between that experiment and the results reported here is that preexposure did not produce a reduction in the general level of responsiveness in this case; rather the level was lower in the control groups. We have no ready explanation for this discrepancy, although the fact that the subject populations differed (half of the participants in the earlier study were male) may contribute.

The effects generated by the overshadowing treatment require a different explanation. There was, of course, no difference between the OS and control groups during preexposure (they received the same treatment). There was, however, a difference between the groups during the conditioning sessions, with subjects in the OS group showing a much-reduced response to the immediate effects of rotation (SR2 score, Figure 2). This outcome accords with the results of Stockhorst et al. (2014) who suggested that the overshadowing procedure (consumption of a novel drink prior to rotation) reduces the effectiveness of the unconditioned stimulus. It is a problem for this proposal that subjects in the LI-OS group, who also had the novel drink, showed sizeable SR2 scores on the rotation trial. It is possible that the LI treatment counteracts the effects of the OS treatment; however, before accepting this conclusion it should be noted that by a different measure (salivary cortisol, see Table A1) both OS and LI-OS groups showed low levels of response to rotation.

Stockhorst et al. (2014) also found, as we did, that the initial response to the context on test was lowered in the overshadowing condition. The comparison between our OS and control groups for SR1 on Day 5 (Figure 3) parallels that made by Stockhorst et al., and the same effect is seen in the comparison of our LI and LI-OS groups for SR1, Day 5. It is debatable whether or not these effects should be labelled overshadowing. Overshadowing is usually interpreted in terms of competition between CSs for association with an effective unconditioned stimulus. In our experiments, the failure of the context to acquire strength appears to reflect a reduction in the effectiveness of the US in the OS groups. But whatever the label, the results none the less indicate that the overshadowing procedure might be useful in restricting the development of a conditioned aversion.

Turning to the practical implications of these findings, an immediate conclusion is that one should be cautious about the use of the latent inhibition procedure as a strategy for the alleviation of AN. The effect is normally powerful and is well attested in studies with animal subjects using a range of conditioning procedures; for human subjects, however, the latent inhibition is obtained only in a more restricted range of conditions (see Lubow, 2010), and no clear effect was evident with the present procedures. Preexposure to the context might be useful if it allows habituation of the general level of negative responsiveness to it (as in Klosterhalfen et al., 2005). But such habituation cannot be guaranteed. In this experiment, subjects given preexposure to the context tended to give higher subjective ratings of nausea-related symptoms when subsequently trained and tested in that context. If preexposure sensitizes the subject to unpleasant aspects of the context then such a treatment would be counterproductive. On the other hand, the outcome of the overshadowing procedure is more hopeful. Consumption of a novel flavour immediately prior to a nausea-inducing treatment attenuated the impact of that treatment; the direct response to rotation was reduced, as was the response to the context on a subsequent test. The source of this effect needs to be examined further and its reliability confirmed, but it shows promise as possible intervention strategy and merits further investigation.

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APPENDIX

Cortisol levels

As noted above, a saliva sample was taken in conjunction with each administration of the rating scale. Group mean cortisol levels are presented in Table A1 for the four trials of Day 1 (prior to rotation), Day 4 (the second rotation session), and Day 5 (the test session). (Cortisol levels are sensitive to time of day, and accordingly the results for the first rotation session, which was given as the second session of the day, are not comparable with those presented in the table and are excluded from the analysis.)

Although within-group variability on this measure was high (see table), some differences emerged with conditioning, which are potentially of interest. Thus an ANOVA conducted on the Day 1 scores, with group (LI or no LI) and trial as the variables, showed no significant effects [effect of group, $F(1, 30) = 1.52$; other Fs < 1]. However, analysis of the data for the four groups on the conditioning trial (Day 4) yielded a marginally significant effect of group, $F(3, 28) = 2.82$, $MSE = 54.35$, $\eta_p^2 = .23$, $p = .057$, a significant effect of trial, $F(3, 84) = 5.89$, $MSE = 11.93$, $\eta_p^2 = .17$, $p = .001$, and a significant interaction between the variables, $F(9, 84) = 2.66$, $MSE = 11.93$, $\eta_p^2 = .22$, $p = .009$. Simple main effects analysis showed there to be differences among the groups on Trial 2, immediately following rotation, $F(3, 112) = 4.89$, $p = .003$, and on Trial 3, $F(3, 112) = 4.37$, $p = .006$. Tukey's test showed that the LI group differed from the OS group on both these trials, and from the LI-OS group on Trial 2 (ps $< .05$).

The results for the test session showed a similar pattern to those of Day 4. An ANOVA revealed no significant effect of group, $F(3, 28) = 1.69$, but there was a significant effect of trial, $F(3, 84) = 3.11$, $MSE = 15.38$, $\eta_p^2 = .19$, $p = .03$, and a significant interaction, $F(9, 84) = 2.38$, $MSE = 15.38$, $\eta_p^2 = .20$, $p = .01$. Analysis of simple main effects showed there to be a significant difference among the groups only on Trial 2, the trial associated with the period at which rotation had been given on previous sessions; $F(3, 112) = 4.75$, $p = .004$. Tukey's test showed that on this trial the LI group differed significantly ($p < .01$) from the OS group. Thus, in accord with the results generated by the symptom-rating scale, the response on the test was greatest for the LI group, and least for the OS group, with the other groups falling in between.

Table A1. *Cortisol levels*

	After SR1	*After SR2*	*After SR3*	*After SR4*
Day 1				
LI	8.06 (2.32)	8.98 (2.75)	9.59 (3.03)	7.32 (3.74)
OS	1.46 (0.92)	2.90 (0.99)	2.07 (1.07)	2.53 (1.04)
LI-OS	4.18 (1.45)	4.40 (1.22)	3.26 (1.00)	4.09 (1.67)
Control	5.55 (2.61)	7.09 (2.28)	5.28 (2.00)	5.15 (2.67)
Day 4				
LI	2.86 (0.99)	10.70 (3.44)	10.84 (2.65)	6.16 (1.97)
OS	0.60 (0.19)	2.99 (1.17)	2.48 (1.11)	3.67 (1.02)
LI-OS	4.91 (1.69)	2.92 (1.46)	5.30 (1.57)	6.22 (1.57)
Control	3.95 (1.17)	6.89 (1.49)	7.17 (1.55)	5.93 (1.52)
Day 5				
LI	4.26 (1.55)	13.64 (3.74)	10.67 (3.18)	6.59 (3.78)
OS	2.88 (1.40)	3.23 (1.03)	4.50 (0.79)	4.29 (1.01)
LI-OS	5.81 (3.92)	5.47 (1.46)	4.80 (1.53)	4.11 (1.85)
Control	5.07 (0.93)	7.36 (1.08)	4.64 (1.07)	6.32 (1.49)

Note: Group means (nmol/L; standard error of the mean in parentheses) for Day 1, Day 4 (rotation session), and Day 5 (test). Samples were taken immediately after each completion of the symptom rating (SR) scale. OS = overshadowing; LI = latent inhibition.