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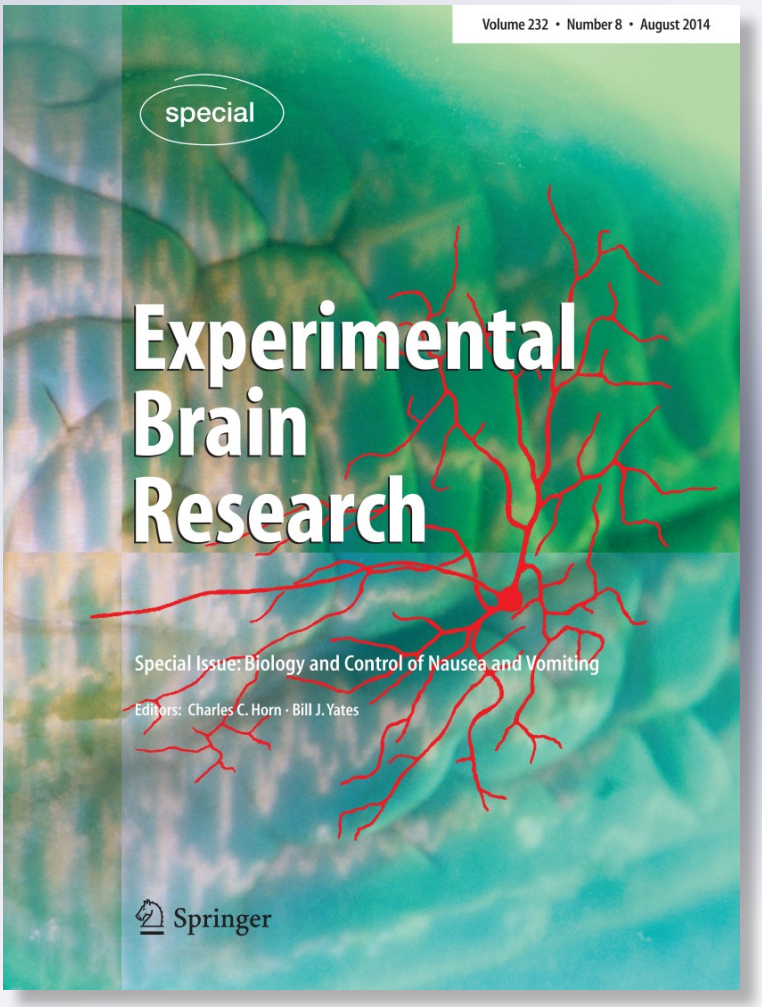

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RESEARCH ARTICLE

Effects of overshadowing on conditioned and unconditioned nausea in a rotation paradigm with humans

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Abstract We examine whether overshadowing by salient stimuli is effective in reducing the ability of a certain environment (the putative conditioned stimulus) to evoke conditioned nausea in healthy humans that experience nausea-evoking rotation (as the unconditioned stimulus, US) in that environment. Twenty-four rotation-susceptible subjects (12 males, 12 females) were randomly assigned to receive either overshadowing by salient tasting beverages (OS+), or a control treatment (a familiar beverage, water; OS−) prior to rotation on three consecutive days (acquisition). To control for taste experiences, the alternative beverage was consumed 12 h later in the home environment (OS+: water, OS−: salient beverage). At Day 4 (test), all subjects drank the familiar beverage (water) prior to rotation (US). Rotation was standardized as 2 × 1-min rotation/day. Nausea was determined by a 7-item symptom scale measuring symptom number (SN) prior to (anticipatory), immediately after, and 15 and 30 min after rotation and by the Nausea Profile (NP) questionnaire immediately after rotation. Cortisol and tumour necrosis factor (TNF)-α in saliva were sampled at the same time-points. SN and cortisol were also measured at home. Overshadowing reduced anticipatory (conditioned) SN. Post-rotation nausea (i.e. the unconditioned response) measured by the NP decreased within the OS+ group only. Anticipatory cortisol and TNF-α were not affected by overshadowing. Treatment × gender interactions manifested for post-rotation cortisol and TNF-α. Groups did not differ in SN and cortisol at home. Overshadowing is effective in reducing symptoms of anticipatory nausea and rotation-induced unconditioned nausea; its effect on endocrine and immunological parameters is gender specific. Its application in alleviation of anticipatory nausea in cancer patients is considered.

Keywords Rotation-induced nausea · Conditioned nausea · Overshadowing · Cortisol · Tumour necrosis factor alpha · Healthy humans · Cancer patients

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Introduction

Cancer patients undergoing cytotoxic drug treatment often experience side effects, the most distressing being nausea and vomiting (Jordan et al. 2005), associated with a significant negative impact on daily functioning (Haiderali et al. 2011). Side effects can occur not only in direct response to the treatment, but also prior to a subsequent drug treatment when being re-exposed to the stimuli that usually signal drug infusion treatment (e.g. the smell on

the ward, sight of the infusion, or tastes elicited by the drug). These symptoms are called anticipatory nausea (AN) and/or vomiting (ANV). Despite antiemetic drugs, 20–30 % of the chemotherapy patients report such anticipatory nausea and vomiting (Aapro et al. 1994, 2005; Jordan et al. 2005, Morrow et al. 1995; Kamen et al. 2014). We, among others, have provided empirical evidence for a classical conditioning interpretation of anticipatory side effects (Aapro et al. 1994; Alba et al. 1989; Andrykowski et al. 1988; Boakes et al. 1993; Stockhorst et al. 1993, 2000). In terms of classical conditioning, the cytotoxic drug [that is, its detection in the central nervous system (CNS)] functions as the unconditioned stimulus (US), and the stimuli contingently signalling the drug and prevailing in the infusion situation become conditioned stimuli (CS).

Analysis in terms of conditioning prompts consideration of two simple behavioural interventions, latent inhibition and overshadowing, which might be effective in the therapy of anticipatory side effects. In a latent inhibition procedure (Cannon et al. 1985; Lubow and Moore 1959), the events that will constitute the CS are pre-exposed prior to their first association with the US and the subsequent acquisition of a conditioned response (CR) is found to be attenuated. We have investigated the effectiveness of this procedure in limiting the development of conditioned nausea in healthy volunteer participants subjected to rotation-induced nausea (Klosterhalfen et al. 2005). The present report focuses on the overshadowing procedure.

Overshadowing, initially described by Pavlov (1927/1960), is obtained by pairing a compound of two potential CSs with the US, with one of the compound elements being more salient than the other. The more salient of the stimuli is found to override the effects of the less salient one, and the CR elicited by the less salient element will be weaker than if it had been paired with the US alone (Miller et al. 1990). According to standard associative theory, only minor associative strength will develop between the less salient CS and the US because the elements of the compound compete for the limited associative strength that is supported by the US (cf. Broberg and Bernstein 1987; Durlach and Rescorla 1980). The potential of overshadowing has been demonstrated by studies of an animal model of these effects in which rats received injections of a nausea-inducing drug in a distinctive context. Presenting a salient taste during the context-drug pairing reduced the extent to which the context acquired aversive properties (Symonds and Hall 1999); that is, the CR acquired by the context was attenuated. There was also an effect on the response evoked by injection of the drug (which constitutes the unconditioned response, the UR, according to our analysis). The magnitude of the observed UR was found to be enhanced when it was assessed in a conditioned context, suggesting that the CR evoked by the context will summate with the UR directly evoked by the drug. This enhancement was also reduced by the overshadowing treatment (Symonds and Hall 2002). These effects were confirmed in a pilot experiment conducted with a group of 16 adult cancer patients (Stockhorst et al. 1998). An experimental group (OS+) was given salient gustatory stimuli (beverages) prior to drug infusion, whereas patients in the control group (OS−) drank a non-salient beverage (water). We found attenuation of AN (of the CR in terms of conditioning theory) in the experimental group, and there was also a reduction in post-treatment nausea (PN), that is, the magnitude of the observed UR was also reduced.

A successful procedure for inducing nausea and related symptoms in healthy humans is body rotation. In terms of classical conditioning, the afferent signals from the vestibular system constitute the US. Body rotation results in a number of symptoms summarized as motion sickness. The rotation paradigm can be used in healthy subjects to examine the effectiveness of overshadowing more closely, and to refine parameters that might increase the effectiveness of overshadowing for clinical use. Thus, we examined healthy volunteers in the motion sickness paradigm employed in our previous studies of conditioning and latent inhibition (Klosterhalfen et al. 2000, 2005). To induce motion sickness, subjects are rotated around the vertical axis and instructed to bend their head with the eyes closed. This induces a reliable increase of symptoms in susceptible subjects, the most prominent being nausea and vomiting, pallor, cold sweating, and stomach awareness (Yates et al. 1998). Rotation is a stressor that is typically accompanied by an increase of cortisol (e.g. Rohleder et al. 2006); accordingly, we measured cortisol in saliva to confirm the stressor effect of rotation and its possible modification by the overshadowing intervention. The immunological correlates of nausea are also of interest here. Proinflammatory cytokines such as interleukin (IL) 1-β and tumour necrosis factor (TNF)-α are regarded as immunological correlates of sickness behaviour (Dantzer 2001). Sex-specific responses were shown in the adaptation of both endocrine and inflammatory responses in a rotation setting (Rohleder et al. 2006): A small sample of male and female subjects was exposed to repeated nauseogenic body rotation in a rotation drum on four consecutive days (with a maximum of five 1-min rotations per day). Free cortisol level increased after rotation, but these rotation-induced cortisol increases lowered from Day 2 to 4 in females, but not in males. Further, the in vitro production of the proinflammatory cytokines, IL-6 and TNF-α, did not habituate in men, but did so in women.

Under the assumption that TNF-α level is indicative of nausea and differentially sensitive to the effects of repeated rotation in men and women, we thus also examined

whether TNF-α levels are affected by overshadowing, and whether gender-specific responses occur.

Participants experienced a series of sessions in which rotation was given in a distinctive context. One group (OS+) was given a novel drink prior to rotation; the other (OS−) was not. Symptoms were scored, and salivary samples were taken at the start of each session and at intervals after rotation had been experienced. We focused on scores recorded at the start of the session, prior to rotation, in order to assess the primary hypothesis that subjects given the overshadowing stimulus would develop less anticipatory nausea in the rotation context than would the control (OS−) subjects. We also examined the post-rotation scores to explore the possibility that the direct response to the presumed US might be reduced by the overshadowing treatment. Finally, our previous work has revealed higher susceptibility to nausea induction (and to the effects of latent inhibition on anticipatory nausea) in females than in males (Klosterhalfen et al. 2005). Therefore, we included additional ad hoc, gender-specific, data analyses to explore the possibility that there might be a stronger overshadowing effect in females than in males.

Methods

The study consisted of two parts conducted in different rotation chairs: A pre-study to screen for susceptibility to motion sickness in 40 volunteer subjects and the main study, conducted with 24 subjects (identified as susceptible to motion sickness during screening) randomly assigned to overshadowing or a control treatment. The entire protocol was conducted with the approval of the Ethic Committee of the Medical Faculty of the Heinrich-Heine University of Düsseldorf, Germany. Subjects gave their written, informed consent. Subjects were recruited from the university campus. Exclusion criteria were smoking, a history of gastrointestinal diseases, and actual medication.

Pre-study: screening for susceptibility to motion sickness

A screening test to identify subjects susceptible to develop nausea during a body rotation procedure has been described previously (Klosterhalfen et al. 2000, 2005). In short, subjects are rotated around their vertical axis in a conventional 1-axial rotation chair (different from the rotation chair of the main study and presented in a different context) at a constant speed of 120 °/s with their eyes closed, for five 1-min rotations with 1-min intervals between single rotations. During rotations, subjects were instructed by audiotape to move their head up and down every 6 s. Forty healthy subjects, 20 males and 20 females, (26.2 ± 0.8 years; body mass index = 22.3 ± 0.8 kg/m^2) took part. They were allowed to stop the rotation if the symptoms became too strong. Based on rotation tolerance in the screening, subjects were ranked into 5 classes with the rotation tolerance of <1, 1–2, 2–3, 3–4.5, or 5 rotations. Subjects of classes 2–4 (i.e. subjects tolerating more than one single rotation and a maximum of 4.5, but not the complete 5 rotations) were considered for the main study.

Main study

Subjects

Subjects of the tolerance classes 2–4 were assigned to the experimental (OS+) and the control (OS−) groups at random, apart from the constraint that there are 6 males and 6 females in each group. Subjects had to fast for 12 h prior to each session, but were permitted to consume water and unsweetened herbal tea until the last hour preceding the experiment. On each day of the study, blood glucose concentration was tested to ensure compliance with the instructions. Blood glucose was in the fasting range in both groups and ranged between 84.5 ± 1.1 mg/dl (Day 3) and 83.0 ± 2.1 mg/dl (Day 4) in group OS+, and between 82.6 ± 1.8 mg/dl (Day 1) and 81.8 ± 1.9 mg/dl (Day 3) in group OS−. The groups were comparable in their mean ages [OS+: *M* (±SEM): 26.7 ± 1.1 years; OS−: *M* 25.8 ± 1.8 years] and mean body mass index (OS+: $M = 22.4 \pm 0.7$ kg/m^2; OS−: $M = 23.5 \pm 0.9$ kg/m^2).

Overshadowing procedure

The protocol is summarized in Table 1. Subjects in group OS+ were given 100 ml of a salient tasting beverage in the rotation environment immediately prior to rotation on Days 1–3 (the acquisition phase). Subjects in the control group (OS−) were instructed to drink an equal volume (100 ml) of (non-sparkling) water at the same time-point. Beverage consumption was standardized. Four minutes prior to the start of rotation, subjects were given a coloured plastic cup (with the colour changing from session to session) and were instructed by the experimenter to drink the contents in three portions over a period of 2 min, portion 1 immediately, portion 2 after 1 min, and portion 3 after 2 min (see also Fig. 1 for the timeline). The beverages used were selected on the basis of a separate pre-study with an independent student sample. Beverages identified as relatively unfamiliar, intense, and neutral (or not pleasant) were used for the main experiment. These were elderberry, hawthorn sallowthorn, sloe, and cranberry. The sequence of presentation of these flavours for group OS+ was counterbalanced across subjects. In order to equate their experience of the drinks, subjects in groups OS+ and OS− drank the complementary beverage type in their home environment 12 h after the

Table 1 Experimental design

	Acquisition (Days 1–3)			Test (Day 4)
	Day 1	Day 2	Day 3	Day 4
Group OS+				
Rotation environment	CS_1 sal + US	CS_2 sal + US	CS_3 sal + US	H_2O + US
Home (+12 h)	H_2O	H_2O	H_2O	CS_4 sal
Group OS−				
Rotation environment	H_2O + US	H_2O + US	H_2O + US	H_2O + US
Home (+12 h)	CS_1 sal	CS_2 sal	CS_3 sal	CS_4 sal

OS+: experimental group, exposed to overshadowing by salient tasting beverages in the rotation environment at Days 1–3. OS−: control group: receiving (non-salient) water (H_2O) in the rotation environment. $CS_{1–3}$: one of the salient tastes elderberry, hawthorn sallow-thorn, sloe (counterbalanced). The complementary beverage type was given at home at Days 1–3. At Day 4 (test), both groups received water plus US in the rotation environment, and the salient CS_4 (cranberry) in the home environment. Rotation (two 1-min rotations per day) as the unconditioned stimulus (US)

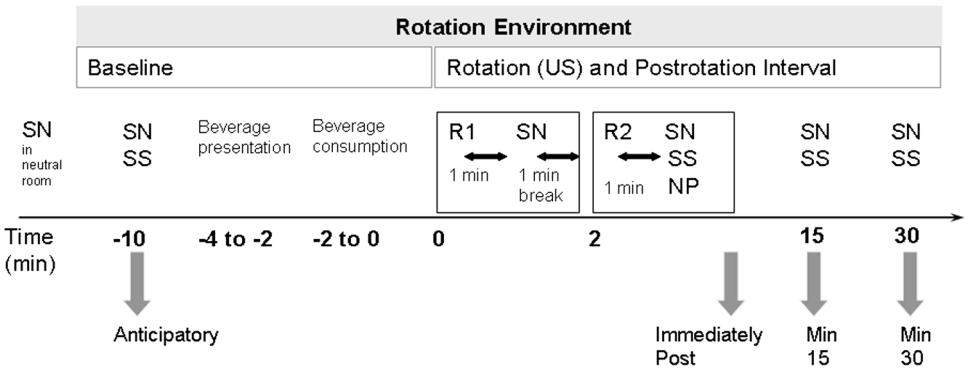

Fig. 1 Sequence of events and measurements in the rotation environment. Timeline is defined relative to the start of rotation 1 (as min 0). Two rotations, 1-min each, separated by a 1-min break were administered. Symptom measurement (SN), cortisol via saliva sampling (SS), and TNF-α were measured anticipatorily, directly after rotation 2 = immediately post, in min 15 and 30. The Nausea Profile (NP) was filled in immediately post only. After beverage consumption, a questionnaire was filled in

rotation session (i.e. group OS+ consumed 100 ml water, whereas group OS− consumed one of the salient tastes). On the test day, Day 4, neither group was given a salient taste but water in the rotation environment, and both were given cranberry as the salient beverage in their home environment. Sessions were scheduled at 8:00, 9:15 and 10:30 with starting times balanced between groups, but kept constant from Day 1 to Day 4 for each subject. Each session lasted about 60 min. Subjects had to refrain from eating in the 12 h preceding each rotation session, i.e. the fasting period started immediately after the last fluid intake in the home environment 12 h prior to the main session.

Rotation procedure

Compared with the screening, rotation was performed in a different chair and different room. Subjects were given two periods of rotation, each lasting 1 min with a 1-min break between them. As in the screening, subjects were rotated around their vertical axis at a speed of 120 °/s while being instructed to bend their head up and down every 6 s with their eyes closed. Subjects knew from the informed consent that they were allowed to stop rotation if symptoms were too strong. Of the 24 subjects, only two terminated the rotation prior to the maximum 120 s, and the minimum period of rotation experienced by an individual was 92 s.

Dependent variables

Nausea-associated symptoms were assessed on a 7-item symptom scale (vertigo, headache, nausea, urge to vomit, sweating, general discomfort, stomach awareness). Subjects were instructed to rate these symptoms from 0 (symptom not present) to 5 (strong). To simplify analysis, responses were coded into a 1/0 score to indicate that a symptom was present or not, producing a score indicative of the number of symptoms (SN). SN was recorded six times in each session (see Fig. 1). It was recorded on arrival in the department in a neutral experimental room (prior to entering the rotation laboratory) and five times in the rotation environment:

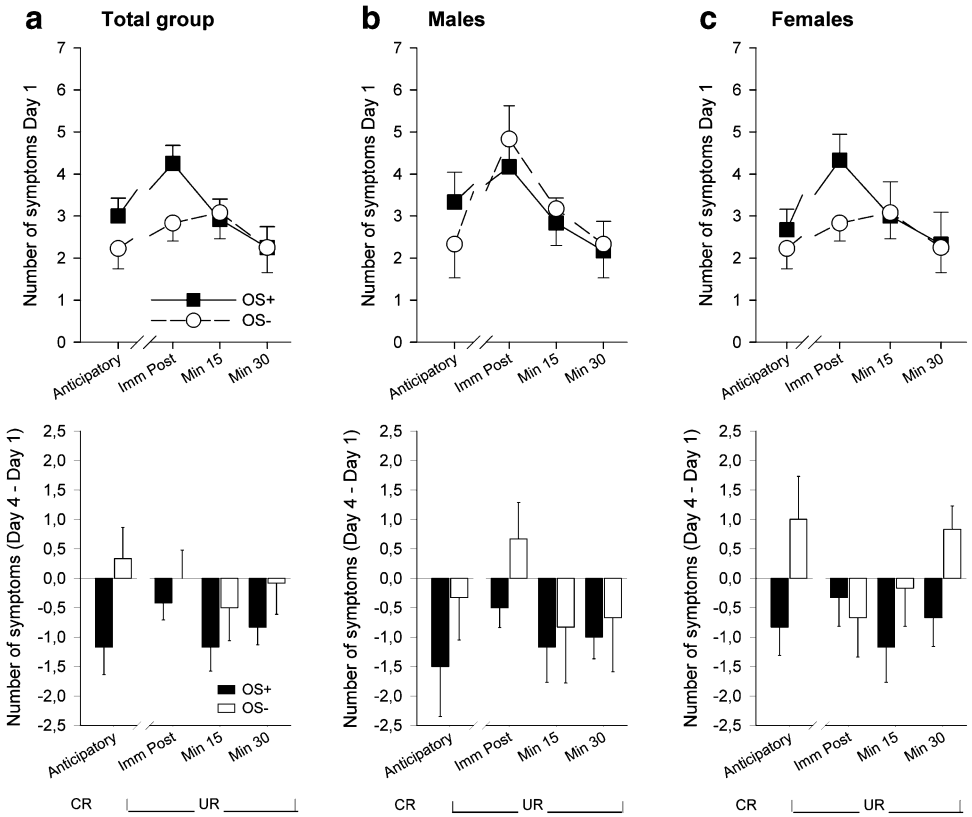

Fig. 2 Absolute levels at Day 1 and change score (Day 4 minus Day 1): symptom rating in the rotation environment. Number of symptoms at the four measurement points: anticipatory, immediately post (imm post), min 15, min 30. **a** Total sample (group), **b** males, **c** females. Overshadowing+ (OS+), with *solid lines* and *squares*, or *dark bars*, respectively, and overshadowing− (OS−) with *dashed lines* and *circles*, or *white bars,* respectively. *CR* conditioned response, *UR* unconditioned response

10 min (min −10 in the figure) prior to the first rotation, constituting the anticipatory assessment and then four times in the post-rotation phase: immediately after rotation 1, immediately after rotation 2 (= immediately post), and 15 min and 30 min after rotation 2. Subjects were also given the German version of the Nausea Profile (NP) immediately after the second rotation. The NP (Muth et al. 1996) assesses the presence and intensity (0–9) of 17 physiological and psychological nausea-related symptoms, grouped into 3 dimensions somatic, gastrointestinal, and emotional distress and quantified as the percentage of total points possible per dimension as described by Muth et al. (1996).

Immediately after beverage consumption (min −2), subjects were asked to rate the pleasantness, familiarity, and intensity of the beverages on 6-point verbally anchored bipolar rating scales.

At four time-points in the session (anticipatory, immediately post, and 15 and 30 min after the end of rotation; see Fig. 2), saliva was collected in a plastic beaker (Sarstedt, Nümbrecht, Germany) over a 4-min period. For later analysis of salivary free cortisol, 1,000 µl was pipetted into a salivette (Sarstedt, Nümbrecht, Germany) and stored at −20° C. Prior to analysis, the salivettes were centrifuged at 2,000 rpm for 5 min. Salivary free cortisol concentrations were determined using a commercial time-resolved immunoassay with fluorometric detection as described in detail by Dressendörfer et al. (1992). For determination of TNF-α concentration, aliquots (100 µl) of the centrifuged saliva were pipetted into Eppendorf (2,000 µl) caps, centrifuged for 5 min at 3,000 rpm, stored on liquid ice during the session, and frozen at −80° C. Immediately prior to analysis, samples were centrifuged again for 5 min at 16.000 rpm. The concentration of TNF-α in saliva was measured using enzyme-linked immunosorbent assay set specific for human TNF-α (BD, Pharmigen, Heidelberg, Germany) (OptEIA Set 555212). Plates were read by a microplate reader (Model 2010, Anthos Microsystems, Krefeld, Germany), and absorbance was transformed to cytokine concentration (pg/ml) using a standard curve.

Twelve hours after the start of each rotation session, subjects had to drink beverages and fill in questionnaires in their home environment. The symptom scale was filled in 10 min before the subjects drank the beverage. They then rated the beverage (min 0) and filled in the symptom scale immediately, 15, and 30 min later. Saliva for the measurement of cortisol was sampled at min −10, 0, 15, and 30. Since saliva for cytokine analyses has to be stored at –80° C immediately, TNF-α was not measured in the home environment.

Data analysis

Kolmogorov–Smirnov goodness of fit test revealed no significant deviations from the normal distribution (all *p*s > .20) at the first measurement point of all dependent

variables (i.e. SN, NP, salivary cortisol, and salivary TNF-α) at Day 1, also when analysing the subgroups of men and women separately.

Means (M) ± SEM are reported for each dependent variable, SN, NP (per single dimension), salivary cortisol, and TNF-α. To evaluate the change in each response (SN, NP, cortisol, TNF-α) with treatment, the difference between the measures at Day 4 and Day 1 was calculated for each variable and each measurement point. To assess comparability of the groups, all baseline measures on Day 1 were first compared by one-way analyses of variance (ANOVAs).

Addressing the main hypothesis, concerning the effect of overshadowing on the conditioned (anticipatory) response, an ANOVA with the variable group (OS+ vs. OS−) was conducted for the anticipatory SN with the p level set at .05. The effects on the other dependent variables (cortisol, TNF-α) are reported for descriptive purpose only and thus not adjusted for multiple testing. For descriptive purpose, effect sizes are reported. With regard to the two-group design of our study, we report the effect size Cohen´s d of the group mean differences (not part η^2 of the ANOVA statistics) when expressing the pairwise intergroup differences, taking $d = 0.2$, $d = 0.5$, and $d = 0.8$ indicating small, medium, and large effects, respectively (Cohen 1977, 1992). To address the secondary hypothesis of an overshadowing effect on the UR, the same statistical analyses were conducted analysing the subjective responses (SN and NP), as well as cortisol and TNF-α for the post-rotation measurement points. The time-points with the expected maximum effects were used for analysis, i.e. directly post-rotation for symptoms and TNF-α, and min +30 for cortisol, thereby referring to the time course of the peak of responses to nauseogenic body rotation found in our screening pre-study. Where appropriate, post hoc intragroup comparisons were conducted for explorative purpose, using paired t tests (two-sided). All analyses were done using SPSS 21 (SPSS Inc. Chicago, IL, USA).

For exploratory purposes, gender-specific manifestations of the overshadowing treatment were examined by calculating effect sizes for the comparison of OS+ versus OS− means separately in males and females, and by analysing treatment × gender interactions. For quantifying the effect size of the interactions, part η^2 values are also reported. These analyses allowed us to assess whether females are especially responsive to the protective effect of overshadowing in conditioned and unconditioned nausea, and whether gender-specific treatment effects are detected in the endocrine and immune parameters.

Results

The results for the principal analyses (*difference scores* for SN, cortisol, and TNF-α) are presented in Figs. 2, 3, 4 and 5. The total sample (OS+ vs. OS−) is always illustrated in part a of each figure; the males (OS+ vs. OS−) are separately illustrated in b, the females in c. In the upper part of each figure, the (absolute) values of the Day 1 are presented in order to provide the reference for interpreting the difference scores, whereas the difference scores (Day 4 minus Day 1) are presented in the lower part. In Figs. 2, 4 and 5, the anticipatory measurement point is illustrated as the potential CR; the three post-rotation measurement points are labelled as the UR. Figure 3 (Nausea Profile) refers to post-rotation nausea only (a measure of the UR), since the NP was only administered immediately after rotation.

Rotation environment

Baseline levels at Day 1

The groups (OS+ vs. OS−) did not differ in the number of symptoms reported at min −10 on Day 1 (OS+: $M = 3.0 \pm 0.4$; OS−: $M = 2.3 \pm 0.5$; $F[1, 22] = 1.369$, $p = 0.255$). Further, OS+ and OS− had comparable baseline cortisol levels (OS+: $M = 20.3 \pm 3.1$ nmol/L, OS−: $M = 28.1 \pm 4.4$ mmol/L; $F[1, 22] = 2.110$, $p = .160$). A full set of TNF-α values (i.e. all four measurement points on all four days) was available for 19 of the 24 subjects: 8 in group OS+ (5 males and 3 females) and 11 in group OS− (6 males and 5 females). TNF-α baseline levels did not differ between groups (OS+: $M = 8.9 \pm 2.4$; OS−: $M = 15.2 \pm 3.5$; $F[1, 17] = 1.936$, $p = .182$).

Symptoms

Anticipatory symptoms As shown in Fig. 2a, the number of anticipatory nausea-related symptoms was reduced from Day 1 to 4 by $M = -1.2 \pm 0.5$ in group OS+ ; in contrast, group OS− showed a small increase of $M = 0.3 \pm 0.5$, resulting in a significant group difference ($F[1, 22] = 4.477$, $p = .046$) with a large effect size ($d = 0.86$).

Post-rotation symptoms Post-rotation nausea symptoms scores (SN) were reduced in group OS+ at all three time-points, but the differences between the groups were small and were not significant for the largest difference between groups ($d = 0.51$) at min 30 (Fig. 2a), $F[1, 22] = 2.283$, $p = .145$.

Nausea Profile Difference scores for the separate dimensions of the Nausea Profile are shown in Fig. 3. As shown in Fig. 3a, the groups differed most obviously in the somatic score, which was reduced in group OS+ (−10.5 ± 4.2 %), but scarcely changed in group OS− (0.8 ± 5.4 %). The difference did not reach significance ($F[1, 22] = 2.714$, $p = .114$), but a medium effect size was obtained ($d = 0.67$).

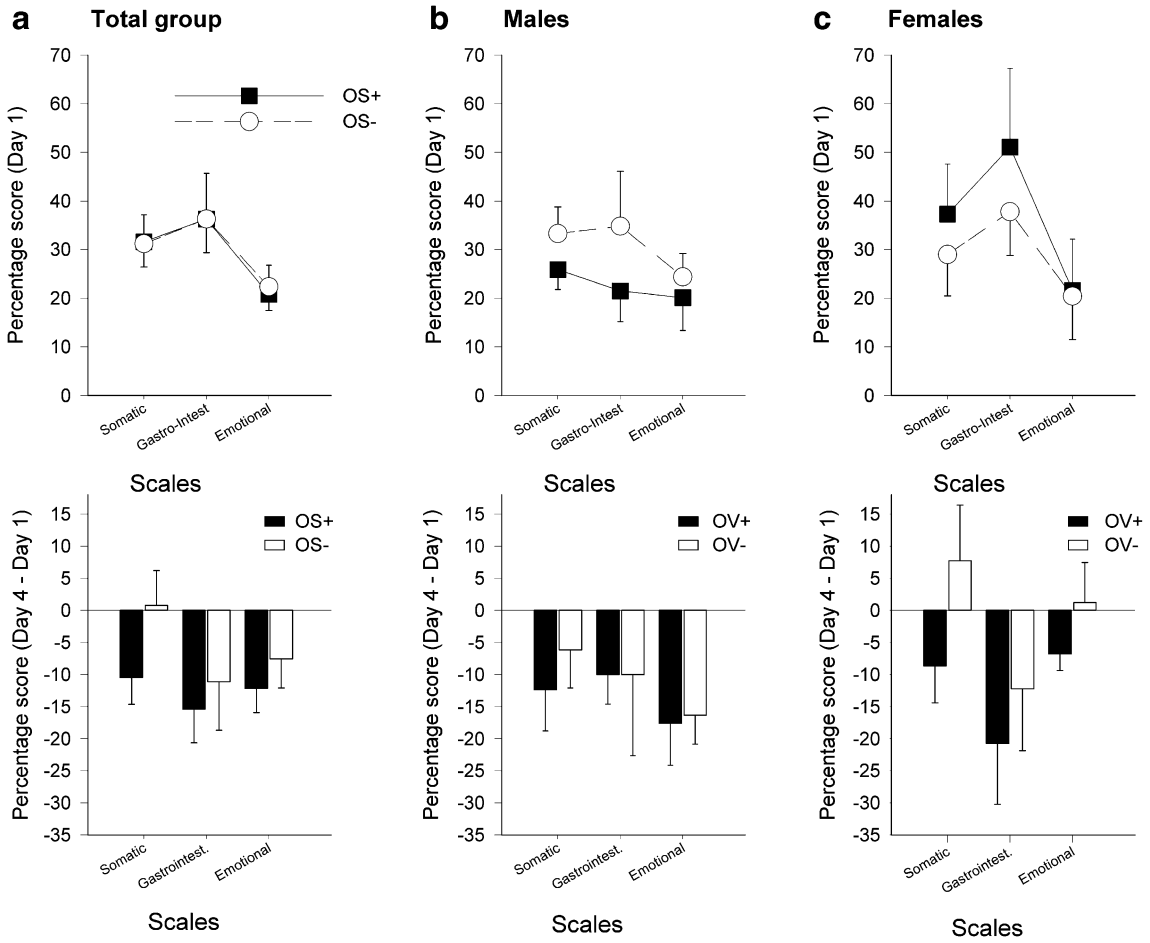

Fig. 3 Absolute levels at Day 1 and change score (Day 4 minus Day 1): Nausea Profile (percentage of total points possible) for the three dimensions somatic, gastrointestinal and emotional distress at the measurement point immediately post. **a** Total sample (group), **b** males, **c** females. Overshadowing+ (OS+), with *solid lines* and *squares*, or *dark bars*, respectively, and overshadowing− (OS−) with *dashed lines* and *circles*, or *white bars,* respectively

For the gastrointestinal and emotional scores, both groups exhibited a decrease from Day 1 to Day 4 (gastrointestinal: OS+: −15.4 ± 5.3 %, OS−: −11.1 ± 7.6 %; emotional: OS+: −12.2 ± 3.7 %, OS−: −7.6 ± 4.5 %); but for neither measure was the treatment effect statistically reliable (both Fs < 1).

Although there was no between-group difference in the NP, the ability of overshadowing to reduce unconditioned nausea is suggested by post hoc intragroup comparisons. Group OS+ exhibited a significant reduction in all three dimensions from Day 1 to 4, i.e. for the somatic, $t(11) = 2.522$, $p = .028$, for the gastrointestinal, $t(11) = 2.919$, $p = .014$, and for the emotional scale, $t(11) = 3.270$, $p = .007$ (two-sided). In contrast, in group OS−, neither of the single intragroup comparisons reached a significant p level (somatic, $t(11) = 0.142$, $p = .889$, gastrointestinal, $t(11) = 1.466$, $p = .171$, and emotional, $t(11) = 1.679$, $p = .121$).

Cortisol

Anticipatory cortisol Subjects of both groups exhibited a lower cortisol level on Day 4 than on Day 1 in the anticipatory phase (Fig. 4a). Thus, the main effect of group did not achieve significance ($F[1, 22] = 1.407$, $p = .248$).

Post-rotation cortisol Relative to Day 1, subjects of both groups manifested a cortisol decrease also after rotation (indicative of the UR). Thus, there was also no main effect of treatment on post-rotation cortisol, neither in min 15 post, nor in min 30 (both Fs < 1).

TNF-α

Anticipatory TNF-α Group OS− showed a reduction in TNF-α, whereas in group OS+, Day-4 levels exceeded those of Day 1 (Fig. 5a) (medium effect size, $d = 0.63$), but the

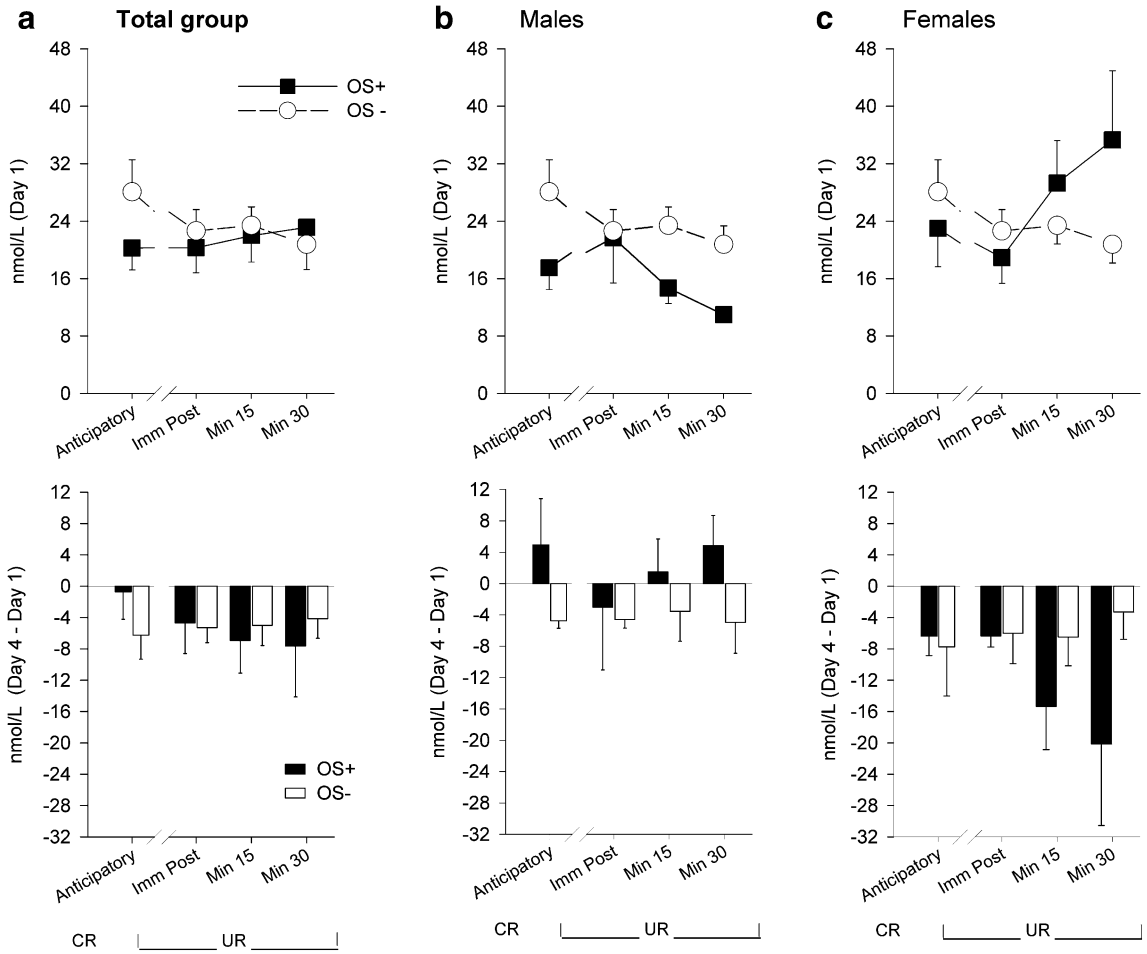

Fig. 4 Absolute levels at Day 1 and change score (Day 4 minus Day 1): cortisol in saliva (nmol/L) at the four measurement points: anticipatory, immediately post (imm post), min 15, min 30 in groups OS+ and OS−. **a** Total sample (group), **b** males, **c** females. Overshadowing+ (OS+), with *solid lines* and *squares*, or *dark bars*, respectively, and Overshadowing− (OS−) with *dashed lines* and *circles*, or *white bars,* respectively. *CR* conditioned response. *UR* unconditioned response

difference was not statistically reliable ($F[1, 17] = 1.721$, $p = .207$).

Post-rotation TNF-α At Day 4, the post-rotation TNF-α levels exceeded those of Day 1 at all time-points for the OS+ group, whereas there was a decrease in group OS−. As expected, the difference was greatest immediately after rotation (see Fig. 5a) where the effect size was large ($d = 0.91$). A main effect of treatment fell short of the conventional level of significance ($F[1, 17] = 3.555$, $p = .077$).

Gender-specific analyses

Gender-specific analyses were conducted for exploratory purpose only. Gender per se did not affect baseline values of either dependent variable at Day 1 (SN: men: $M = 2.8 \pm 0.5$; women: $M = 2.4 \pm 0.4$; $F[1, 22] = 0.405$, $p = .531$; cortisol: men: $M = 20.4 \pm 2.8$ nmol/L; women: $M = 27.9 \pm 4.6$ nmol/L; $F[1, 22] = 1.942$, $p = .177$; TNF-α: men: $M = 15.1 \pm 3.5$ pg/ml; women: $M = 9.0 \pm 2.2$ pg/ml; $F[1, 17] = 1.814$, $p = .196$).

In order to *describe* a putative differential impact of overshadowing in men and women, effect sizes of the OS+ versus OS− differences were determined separately for men and women. Due to the small sample size, we did not calculate inferential intergroup comparisons of OS+ versus OS− separately for males and females. But treatment × gender interactions were calculated.

As summarized in Table 2, for most of the nausea measures, effect sizes were greater in women than in men. For clarity, we include the sign of the effect sizes within Table 2, with a positive sign indicating that the OS+ condition showed the higher value, and a negative sign when the OS+ condition had a lower value than the OS− condition. For cortisol (in the post-rotation phase) and TNF-α (anticipatory and post-rotation phase), the responses differed in

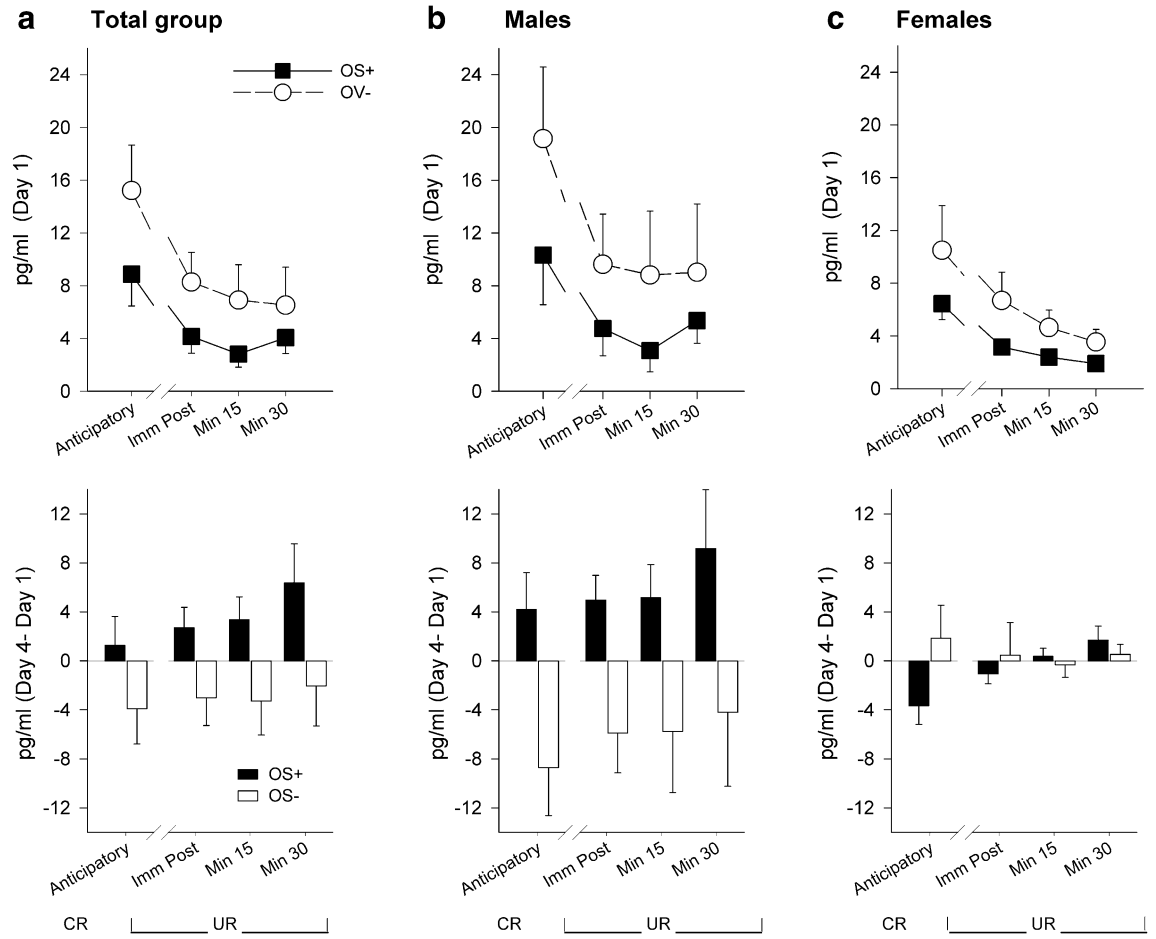

Fig. 5 Absolute levels at Day 1 and change score (Day 4 minus Day 1): TNF-α in saliva (pg/ml) at the four measurement points: anticipatory, immediately post (imm post), min 15, min 30 in groups OS+ and OS−. **a** Total sample (group), **b** males, **c** females. Overshadowing+ (OS+), with *solid lines* and *squares*, or *dark bars*, respectively, and Overshadowing− (OS−) with *dashed lines* and *circles*, or *white bars,* respectively. *CR* conditioned response, *UR* unconditioned response

direction, referring to putative treatment × gender interactions; these are detailed below.

Symptoms

Anticipatory responses Descriptively (see Fig. 2b, c), the difference between the OS+ and OS− groups in their symptom scores was greater in females (effects size $d = 1.21$) than in males ($d = 0.61$). But the responses did not differ in direction, and a treatment × gender interaction was not found $F = [1, 20] = 0.223$, $p = .642$, part $\eta^2 = 0.011$).

Post-rotation nausea responses Similarly, for females, treatment effects are obvious in the symptom scores (Fig. 2b, c), especially 30 min after the end of rotation, reaching a large effect size ($d = 1.36$), while the group difference reaches an effect size only below 0.2 ($d = 0.19$) in males. In correspondence with this response pattern, a treatment × gender interaction was not found ($F[1, 20] = 0.984$, $p = 0.333$, part $\eta^2 = 0.047$). The pattern is qualitatively the same for the NP scores (Fig. 3b, c), where larger effect sizes, indicating a larger symptom reduction after overshadowing compared with the control condition, are mainly observable in females in the somatic ($d = 0.91$) and the emotional ($d = 0.69$) score, but treatment × gender interactions were not found (all Fs < 1.0).

Cortisol

Anticipatory cortisol For cortisol, on the other hand, males showed an anticipatory increase after OS+ compared with OS− (Fig. 4b) with a large effect size ($d = 0.93$), whereas in females (Fig. 4c), there was little difference between the treatment groups, both showing a decrease ($d = 0.12$). But a significant treatment × gender interaction was not evident in the anticipatory cortisol response ($F[1, 20] = 0.844$, $p = .369$, part $\eta^2 = 0.040$).

Table 2 Effect sizes *d* of the groups' mean differences (OS+ vs. OS−) separately for males and females and for the total group

Measures	*d* Males[a]	*d* Females[a]	*d* Total Group[b]
Anticipatory SN	−0.61	−1.21	−0.86
Anticipatory cortisol	0.93	0.12	0.48
Anticipatory TNF-α	1.56	−1.19	0.63
Post-rotation SN (min 30)	−0.19	−1.36	−0.51
Post-rotation Nausea Profile (imm post)			
Somatic	−0.41	−0.91	−0.67
Gastrointestinal	0.00	−0.36	−0.19
Emotional	−0.09	−0.69	−0.32
Post-rotation cortisol (min 30)	1.04	−0.88	−0.21
Post-rotation TNF-α (imm post)	1.67	−0.35	0.91

SN, cortisol, TNF-α, and the NP-questionnaire, measuring post-rotation somatic, gastrointestinal, and emotional distress. The sign of the effect size is included: positive value if OS+ > OS−, negative value if OS+ < OS−

Imm immediately, *OS* overshadowing, *NP* Nausea Profile, *Post* post-rotation, *SN* number of symptoms, *TNF-α* tumour necrosis factor-α

[a] Number of males and females is $n = 12$, with 6 participants in the OS+ and in the OS− group for all dependent measures except TNF-α. For TNF-α, complete data (all measurement points, all days) are available from $n = 5$ males in the OS+, and $n = 6$ males in the OS− group, and $n = 3$ females in the OS+ and $n = 5$ females in the OS− group

[b] Accordingly, in the total sample ($n = 24$, 12 OS+, 12 OS−), complete TNF-α data are available from $n = 8$ participants in the OS+, and $n = 11$ participants in the OS− group

Post-rotation cortisol The overshadowing treatment produced a marked decrease in cortisol in females and an increase in males (Fig. 4b, c). In both, the effect size at min 30 was large ($d = 1.04$ for males and $d = 0.88$ for females. A significant treatment × gender interaction was obtained ($F[1, 20] = 4.719$, $p = 0.042$, part $\eta^2 = 0.191$).

TNF-α

Anticipatory TNF-α The scores for TNF-α (Fig. 5b, c) also show a gender-specific pattern: In females, we found an anticipatory decrease of TNF-α after overshadowing as compared to the control condition, with a large effect size ($d = 1.19$) in contrast to males with an increase at a large effect size ($d = 1.56$). This manifests in a significant treatment × gender interaction ($F[1,15 = 7.085$, $p = .018$, part $\eta^2 = 0.321$).

Post-rotation TNF-α As with cortisol, a treatment × gender interaction was found in the post-rotation TNF-α levels: In females, overshadowing led to a reduction of TNF-α, whereas there was an increase in males immediately after the end of rotation ($F[1, 15] = 4.690$, $p = .047$, part $\eta^2 = 0.238$) (Fig. 5b, c). Accordingly, the effect sizes of the OS+ versus OS− mean differences revealed a large effect ($d = 1.67$) in males (with OS+ showing higher levels than OS−) and only a small effect ($d = 0.35$) in females (with OS+ showing lower levels than OS−).

Home environment

There were no differences between groups OS+ and OS− in the symptom scores recorded in the home environment (difference scores Day 4 minus Day 1: all $Fs < 1.3$). Cortisol levels were lower in the home assessment than in the test environment (as would be expected given the effect of time of day on cortisol) at Day 1. There were no group differences in the cortisol change scores (Day 4 – Day 1), neither prior to beverage consumption (which constituted the anticipatory measurement point) nor in the later measurements.

Discussion

This experiment examined whether presenting a salient event (a novel flavour) prior to nausea-inducing rotation could influence any acquired ability of the rotation context to evoke nausea-related symptoms and responses. To the extent that the context comes to function as a CS with nausea as the US, an overshadowing effect might be expected; that is, conditioning to the flavour might restrict the acquisition of strength by the contextual cues. To assess this, we focused on the responses evoked by the training context at the beginning of each training session (i.e. anticipatory responses). Subjects in the control (OS−) condition reported a small increase in symptoms of nausea from Day 1 (prior to any rotation) to Day 4 (a test given after three rotation sessions). Consistent with the occurrence of overshadowing, subjects given the novel flavour prior to each rotation (the OS+ condition) showed no such increase (in fact, showed a decrease). Interestingly, the groups were also different in the symptoms they reported after the rotation had been given. These declined in all subjects from Day 1 to Day 4, perhaps indicating a degree of habituation to the procedure, but the reduction was more marked in group OS+ than in group OS−. Although this did not become statistically significant in the intergroup comparisons of the 7-item nausea symptom scale (SN), we found significant reductions (i.e. effects in intragroup comparisons) of acute post-rotation nausea in all three dimensions of the Nausea Profile (somatic, gastrointestinal, and emotional distress) after overshadowing only, whereas the attenuation of post-rotation nausea was not significant in the OS− controls. Since the difference between the groups was only evident

in the intragroup comparisons, this result has to be interpreted with caution, but we may note that symptom reduction in post-rotation nausea is consistent with the occurrence of overshadowing: If the direct response to rotation (the UR) summates with a CR that is controlled by the contextual cues, then the measured post-rotation response will be enhanced when the CR is strong. Overshadowing, by limiting the development of the CR, will reduce its contribution to the overall response to rotation, allowing habituation (or adaptation) to repeated nauseogenic stimulation to become evident in the OS+ condition. Such adaptation might also occur in the controls (OS−) but could be obscured by the development of a CR (anticipatory nausea) that summated with the UR (post-rotation nausea, resulting in no overall change.

It must be acknowledged, however, that the procedures used in our study were not optimal for producing adaptation effects. Our rotation sessions were separated by 24 h, and only two 1-min rotations occurred in each session. Adaptation (as a decreased response of a receptor system) is more probable after continuous stimulation (Schmäl 2013), and rotation experiments that have used a comparable spacing of rotations over consecutive days have also not found habituation: Subjective nausea either did not change over time (Meissner et al. 2009: 5 days with a maximum of five rotations in a vection drum per day), or even increased (Klosterhalfen et al. 2005). Moreover, rotation tolerance (as a behavioural indicator of nausea) has been shown to be subject to sensitization rather than habituation (Meissner et al. found for male subjects, that rotation tolerance reached its minimum on the third of 5 days). From this perspective, the absence of a habituation effect in the OS− group is to be expected; it remains to explain, however, why a significant reduction in the post-rotation response was found in the OS+ group.

Although our primary concern was the individual's subjective experience of nausea, we also monitored endocrine and immunological responses, again comparing Day 1 with Day 4. These proved less sensitive to the overshadowing intervention when considering the total sample: There were no reliable differences between the groups in the salivary cortisol response or levels of TNF-α for the anticipatory time period. The post-rotation cortisol response was declined from Day 1 to Day 4. This might be indicative of habituation and did so in both groups. Only in the post-rotation TNF-α levels did the groups tend to differ, with the OS+ groups showing an increase and the OS− group showing a decrease. There were, however, some differences between the genders on these measures, and these are discussed below. In future studies it would be worthwhile to extend the measures of nausea. Specifically, electrogastrography (EGG) could be included in order to quantify the effect of the overshadowing intervention on typical electrophysiological indicators of nausea such as gastric dysrhythmia. EGG-recording would also allow comparing the validity of EGG with that of the endocrine (cortisol) and immunological (TNF-α) parameters that we measured in our experiment.

There has been some interest recently in gender-specific effects in conditioning. Gender-specific responses in eyeblink-conditioning and fear conditioning, interacting with the preconditioning stress, have been reported for animals (see Dalla and Shors 2009, and Shors 2004 for reviews). In male rats stress often enhanced conditioned responses, whereas stress attenuated conditioned responses in females (Shors 2004). Preliminary studies are also available in humans (see Dalla and Shors). Given the relevance of fear conditioning for the aetiology and treatment of anxiety disorders, human studies of sex differences in conditioning have focused mainly on the acquisition and extinction of fear. Here, fear extinction and extinction memory have been shown to benefit from high estradiol levels in women in their luteal phase as compared to women in their follicular phase (e. g., Zeidan et al. 2011).

For the present study, concerned with learning about nausea, possible gender differences in learning in the taste aversion paradigm are particularly relevant. Conditioned taste aversion is the acquired aversion to a taste that may initially be highly preferred (e.g. a saccharin solution) after pairing (even on only a single trial) with a nausea-inducing drug (e.g. cyclophosphamide). In this procedure, female rats have been shown to extinguish an aversion more readily than male rats, an effect mediated by testosterone (Chambers 1976). Some potentially interesting gender effects were obtained in the present experiment. For the symptom scores, effects were generally more marked in females than in males; the difference between the OS+ and OS− groups in their anticipatory scores was greater in females, as was the reduction in post-rotation nausea when referring to the effect sizes. Thus, there is some evidence that women received more benefit from the behavioural intervention of overshadowing than did men, when compared to the respective "untreated" subjects of the corresponding control group (OS−). This differential sensitivity to the conditioning intervention is in line with data from Klosterhalfen et al. (2009) which showed that females were more responsive to a conditioning procedure in which a salient tasting beverage preceded the nauseogenic rotations, whereas men were more responsive to an instruction inducing the expectancy that the beverage would increase symptoms of nausea. We might also expect that women would be more sensitive to a conditioning intervention that involves learning a new contingency, i.e. forming an association between a salient CS (the overshadowing stimulus) and the US. The neurobiological correlates of this gender difference need to be examined.

The attenuating effects of overshadowing on nausea symptoms in females are paralleled, to some extent, by the salivary cortisol measure. For females the anticipatory cortisol response declined numerically from Day 1 to Day 4 in both treatment groups, whereas in males, the response increased in the overshadowing condition. For the post-rotation scores, males in both groups showed little change, but females showed a reduction, particularly in group OS+. This result latter result is consistent with data from Rohleder et al. (2006) showing that men did not habituate from Days 2 to 4 of a rotation protocol, whereas women did so. Our results also suggest that females are more able than males to profit from the overshadowing intervention and that several responses are affected—overshadowing in females not only attenuated the reported symptoms, but also produced a reduction in an endocrine correlate of the rotation stress in females; males, on the other hand, demonstrated a smaller symptom reduction, and a higher cortisol level after overshadowing, compared with the control condition. As to cortisol, one needs to take into account that the rotation-induced cortisol response at Day 1 reflected a gender difference with a trend for a higher rotation-induced cortisol level in women as compared to men (min 15 and min 30), and a treatment gender × interaction (min 30). This suggests a stronger initial stress response to rotation in females than men which was successfully modified under the influence of overshadowing.

The most striking difference between the genders was seen in the anticipatory TNF-α response: females responded to the overshadowing intervention with a decrease of TNF-α (compared with the controls), whereas the opposite pattern (i.e. an increase after overshadowing and a decrease in the control group) was shown in males. Given that the reduction in anticipatory symptoms occurred primarily in females, this response pattern corresponds to results published by Lekander et al. (2004) showing that in women, but not men, subjective health parameters were inversely correlated with circulating TNF-α levels (i.e. poor subjective health was associated with a higher TNF-α level). Extrapolating these findings would support the hypothesis that an improvement in a subjective well-being—as indicated by symptom reduction after overshadowing—is found more in females than males, and only in females this also manifests in a concordant TNF-α difference. Further investigation of correlations of this sort (and the same applies to the results reported for cortisol) requires studies involving experimental manipulation of the relevant factors. In this context, it might be also interesting to explore the putative sexual dimorphism in the pituitary-adrenal response to TNF-α, as reported in rats (Watanobe 2002). Moreover, TNF-α was shown to act as a neuromodulator at the central processes of the vagus nerve to produce malaise by potentiating visceral afferent signalling (Rogers et al. 2006). Thus, a nausea protection via overshadowing might also affect TNF-α levels.

Finally, we should note some important limitations of the present study. First, the sample size was only 12 participants per group, and when subdividing by gender, we obtain a sample size of 6 in the OS+ versus OS− subsamples (and even smaller for the TNF-α data). We conducted post hoc power (1-β) calculations using G*Power 3 (Faul et al. 2007). For the one-way ANOVAs, the analyses revealed power values between 1-β = 0.076 (NP gastrointestinal score) and 0.561 (anticipatory SN); for the 2 × 2 ANOVA (main effects and interactions), the levels varied between 0.078 (anticipatory SN) and 0.799 (anticipatory TNF-α). Thus, the probability of obtaining significance for a valid alternative hypothesis was very low for several of our dependent variables. Second, the procedure of giving some experience of rotation in the pre-study might have allowed some conditioning to occur at that stage. Although the rotation environment and the rotation chair in screening differed from those used in the main study in a number of features, any prior learning experience might still have transferred to the main study and reduced the effectiveness of the overshadowing manipulation. Third, with regard to the changes in cortisol, we have to take into account that sessions were conducted in the morning (sessions starting between 8:00 and 10:30 h) and thus at time-points in the circadian cycle where cortisol levels decline after the post-awakening peak. (This timing was necessitated in order to submit subjects to rotation after a 12-h fasting phase.) We note, however, that an equal number of subjects per group (OS+ and OS−) were scheduled at 8:00, 9:15, and 10:30 and that the timing of the session cannot explain the gender-specific effects. Fourth, the examination of gender-specific responses was done for exploratory purpose only, and for firmer conclusions to be drawn, we need to examine larger samples and assess sex hormone levels (estradiol and testosterone).

To return to the general picture, the results presented here indicate that people given a series of nausea-inducing rotations show a reduced tendency to report nausea-related symptoms (both prior to and immediately after rotation) when each rotation has been preceded by consumption of a novel and distinctively flavoured beverage. This outcome (most clearly shown in females) is consistent with the notion that conditioning to the contextual cues can contribute to nausea and that the presence of the novel flavour on conditioning trials has generated an overshadowing effect. This outcome encourages further consideration of the possibility that an overshadowing procedure might have application in the clinic, where anticipatory nausea can be an unfortunate and distressing side effect of chemotherapy procedures. As a first step, however, it would be also useful to confirm that intervention that applies to rotation-induced nausea is effective for nausea produced by chemical means.

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Conflict of interest The authors declare that they have no conflict of interest.

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