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Title

Subliminal fatty acid-induced gut-brain signals attenuate sensitivity to exteroceptive rewards within but not across domains, in healthy men.

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Abstract

Background: Reward sensitivity can generalize across domains, but evidence for generalization of suppressive reward-related stimulation is sparse, especially in the context of interoceptive nutrient-related stimuli. We hypothesized that subliminal fatty acid-induced gut-brain signals could attenuate sensitivity to exteroceptive rewards, not only within the food domain but also across domains.

Method: Intragastric infusion of 2.5g lauric acid (fat condition) or saline (saline condition) was administered to 59 healthy heterosexual male volunteers in a blinded fashion. To assess whether the resulting interoceptive signals attenuate reward sensitivity within the food domain, participants rated the palatability of food images and performed a progressive ratio task. To assess whether such attenuation effect generalizes to the sexual and financial reward domains, participants rated attractiveness of female face images and performed an intertemporal monetary choice task.

Results: Participants' ratings of food images were lower ($F_{1,172}$ = 4.51, p=0.035, Cohen's *d*: -0.20) in the fat condition. The progressive ratio task terminated earlier in the fat condition compared to saline ($F_{1,52}$ = 4.17, p=0.046, odds ratio = 0.31, 95%CI [0.11, 0.98]). Participants' ratings of female face images did not differ between conditions ($F_{1,172}$ = 1.85, p = 0.19, Cohen's *d*: -0.15). Moreover, the monetary discounting rate did not differ significantly between conditions.

Conclusion: Overall, these findings suggest a domain-specific effect of subliminal fatty acid infusion on decreasing reward sensitivity.

Keywords

Homeostatic & hedonic, gut-brain axis, generalized reward sensitivity, food images, interoceptive gut signals

1.Introduction

The bidirectional communication system between the brain and the gastrointestinal (GI) tract is referred to as the 'brain-gut axis' (BGA). The afferent limb of the BGA is part of an integrated interoceptive system that continuously conveys homeostatic information about the physiological state of the body to the brain through neural and humoral pathways (1). This homeostatic interoceptive information, most of which is not consciously perceived, is integrated with exteroceptive sensory signals, input from the brain reward system, and signals from affective and cognitive brain circuits (1). However, the putative influence of such interoceptive signals on a variety of psychobiological functions and more specifically, the sensitivity to exteroceptive reward cues in different domains, remains poorly understood.

Prior behavioral research using exteroceptive stimuli has demonstrated that sensitivity to reward cues in one domain is enhanced by stimulation in another domain (2-4). For example, individual participants who were exposed to an attractive olfactory food cue contributed less money to a group-level resource pool (4). Moreover, Van den Bergh *et al*. (2) showed that exposure to sex cues leads to increased impatience in inter-temporal choices between monetary rewards. These results, therefore, supported the "spillover" effect on reward sensitivity across domains. In other words, exposure to a rewarding stimulus from one domain enhanced reward sensitivity in another domain. However, these studies exclusively used exteroceptive reward cues that enhance reward sensitivity. It remains unknown whether a stimulation in one domain (namely the food domain) would also influence participants' sensitivity to exteroceptive reward cues in other domains.

Previous studies have demonstrated that nutrient-related signals have potent effects on (dopaminergic) reward-related brain circuits. For example, intragastric fat infusion induced dopamine release in dorsal striatum, and enhanced self-stimulation behavior in mice (5). In humans, the pleasantness rating of certain types of food declined during the consumption of the food until satiation, and the pleasantness renewed with exposure to new food (6). Moreover, dopamine release in the dorsal striatum correlates positively with the perceived

pleasantness of a meal in humans, but not with the desire to eat (hunger) or with satiety after eating (7). Interestingly, de Araujo *et al*. suggested that interoceptive gastrointestinal nutrient-related dopamine release in the dorsal striatum may serve as a taste-independent calorie sensor in animal models (8). In humans, blunted striatal responses to food signaling is related to the development of obesity (9). In other words, intragastric nutrient signaling may affect dopaminergic neurotransmission in reward-related brain regions. However, it remains unclear whether the interoceptive nutrient-related dopamine release would enhance or attenuate sensitivity to food reward in humans.

Moreover, interoceptive fat-induced effects in the (dopaminergic) reward circuit may have generalizable effects on other brain functions. A meta-analysis of functional brain imaging studies in either food, sex or financial domains (10) found a 'common reward circuit', responding to all three types of rewards, which includes the bilateral (ventral) striatum, anterior insula, the mediodorsal thalamus, the bilateral amygdala and the ventromedial prefrontal cortex (vmPFC) extending into the pregenual anterior cingulate cortex (pgACC). A previous study by our group showed that intragastric fatty acid (2.5g lauric acid) inhibited the effect of negative emotion induction, both at the subjective and neural levels, among healthy volunteers (11). We also found that key regions in the aforementioned 'common reward circuit', including the anterior insula and striatum, are involved in the interaction between intragastric fatty acid and emotion induction. Therefore, it is possible that fat-induced responses in the abovementioned brain regions would influence other brain functions (reward sensitivity) regulated by these regions.

In the current study, we aim to test the hypothesis that a subliminal interoceptive nutrient-related signal induced by intragastric infusion of fatty acid would influence sensitivity to exteroceptive reward cues within the food domain, and that this effect would generalize to the sexual and financial reward domains.

2. Subjects and Methods

2.1 Subjects

Self-reported heterosexual Dutch-speaking male volunteers were invited for two sessions with a 2-week washout period in between to prevent carry-over effects. Before enrolment in the study, subjects filled in screening questionnaires for age, BMI (calculated from height and body weight), self-reported sexual orientation, food preference, and medical history. Exclusion criteria included any self-reported psychiatric disorder, history of abdominal or thoracic surgery (except appendectomy or cholecystectomy), and any neurological, endocrine or digestive diseases. The recruitment flowchart is presented in *Figure 1*. Participants who used any medication regularly must have had at least a 2-week washout period before they could participate in the first visit. The sample size was calculated *a priori* to detect a small-sized effect of fatty acid (Cohen's d = 0.35, alpha = 0.05, power = 85%) in a crossover design.

2.2 Procedure (Figure 2)

The study was conducted as a randomized, counterbalanced, single-blinded crossover design. Participants arrived at the lab after an overnight fast. First, an intragastric feeding tube was positioned with its tip in the stomach 60 cm from the incisors and fixed with adhesive tape to the subject's face. Participants then had a 10 min adaptation period during which they could read lifestyle magazines (in Dutch) provided by the researcher. Next, participants were seated in a cubicle where they completed a series of computer-based visual analogue scales (VAS) for appetite-related sensations. Specifically, these questions included hunger which we assessed via two questions, 'how much you think you will be able to eat' (prospective food consumption question1, PFC1), 'how much do you want to eat' (prospective food consumption question2, PFC2), satiety, and fullness, as well as nausea and the feeling of unpleasantness due to the presence of the nasogastric tube. The exact questions and their English translation are presented in *supplementary Table S1*. The questions were rated both before and 10 min after the infusion on a 100-point scales. The VAS scores at pre-infusion baseline and after

infusion were used to assess the influence of intragastric infusion on appetite-related sensations. The VAS rating of unpleasantness after the infusion was used as a covariate in all subsequent analyses and reported when its effect was significant.

After the first VAS measurement, a neutral movie showing natural landscapes was presented at t = 0–10 min. Simultaneously, 250 mL lauric acid (0.05 mol/L) was administered intragastrically (fat condition), or 250 ml saline as placebo control (saline condition), at t = 0–2 min, at body temperature (37°C). At t = 10 min, a post-infusion VAS for appetite-related sensations, nausea, and unpleasantness of the tube was collected. Subsequently, participants' momentary sensitivity to rewards was measured in three different reward domains. Specifically, participants completed the following tasks, presented in the following order: picture rating task in the sex and food domains, intertemporal monetary choice task, and progressive ratio food task. The first 19 participants had the nasogastric tube extubated after the progressive ratio task, but some participants reported that the presence of the tube was unpleasant during the tasks, and interfered with the tasks. Therefore, we chose to extubate right after the movie was finished for the remaining 40 participants, and the study protocol was kept constant over the two visits within the same participant. The difference in protocol was also added in the statistical model as a dummy variable, but it did not influence any of our outcomes.

2.3 Ethical approval and registration

The study was approved by the Medical Ethics Committee of the University Hospitals Leuven, Belgium (ML10475, 02 June 2014), registered at ClinicalTrials.gov (NCT02984150: https://clinicaltrials.gov/), and performed in accordance with the Declaration of Helsinki, including written informed consent.

2.4 Picture rating tasks

First, to assess reward sensitivity in the sexual domain, a block of pictures of female faces was presented. This was followed by a block of pictures of food items, to assess reward sensitivity

in the food domain. In each block, 10 rewarding and 10 neutral pictures were presented in random order.

Participants were asked to rate the pictures on 7-point scales. Specifically, for each image in the sex domain, participants were presented with the question: “To what extent do you feel attracted to this woman” with the anchors “totally not attracted” at 1, and “very strongly attracted” at 7. In the food domain, the question was “How much would you like to eat this dish now” with the anchors “totally not” at 1, and “certainly” at 7.

The pictures of female faces were chosen from hotornot.com (12). The following inclusion criteria were applied to all of the images: 1. Age within a range between 19 and 29 years; 2. At least 500 reviewers on the website; 3. Nose, mouth, and eyes clearly shown in the image; 4. One face in each image. Exclusion criteria included: 1. Blurry or small images; 2. Photos with animals; 3. Displays of wealth; 4. Photos in which emotionally salient objects such as guns, snakes, or motorcycles were visible; 5. Photos with subjects in provocative sexual positions or with nudity; 6. Photos in which the subjects appeared to be younger than 18 years. Eventually, we selected 10 highly attractive images, with average ratings of 7 to 8 /10 points on the website, and another 10 moderately attractive images with average ratings between 4 to 5.8 /10 points.

In the food domain, we selected 20 validated food images from Hou *et al*. (13) (10 high-caloric images, and 10 low-caloric images).

To evaluate the consistency of participants’ picture ratings, a single linear regression is performed on average picture ratings in each category (highly- and moderately-attractive female face pictures, and high- and low- caloric pictures) between the nutrient conditions.

2.5 Intertemporal choice task

Participants indicated the amount of money they would expect to receive after a waiting period of 1 week, 1 month, 3 months, 6 months or 1 year to be equivalent to receiving 15 euros immediately (2). Based on the amount of money participants indicated, we calculated the area

under the discounting curve (AUC) of each individual, as a measure of their intertemporal discounting (14). The AUC provides a single statistic that does not depend on theoretical assumptions regarding the form of the discounting function and represents how impulsive a person is. Specifically, the AUC can vary between 0 (steepest possible discounting) and 1 (no discounting), where a larger number indicates a higher preference for larger-later payoffs (i.e., less impulsivity).

2.6 Progressive ratio task

Participants were presented with a bowl containing 20 pieces of food with 30-40 kcal per piece (either chocolate candies, cheese or cookies, depending on participants' previously indicated favorite choices)(15). Participants were allowed to take only one piece of the food after clicking the computer mouse for a number of times, with a progressively increasing set ratio. The starting ratio was 10 clicks, and doubled for each additional piece of food wanted to obtain (for example, 10, 20, 40, 80, *etc*.). Every time the participants received a piece of food, they were required to finish the piece of food before they could proceed. The participants terminated the task *ad libitum*. The number of clicks when particpants terminated the progressive ratio task were recorded as an indicator of participants' sensitivity to food reward. Earlier termination of the task indicated that participants were less sensitive to the food reward and therefore performed less number of clicks during the task.

2.7 Statistical analysis

All statistical analysis were performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA). Values were reported as mean ± SEM when the variable was normally distributed, and median [IQR] when the distribution was not normal. Differences were considered significant with $p < 0.05$.

VAS ratings of appetite-related sensations and unpleasantness were analyzed using a mixed-effect model with nutrient (fat or saline condition) and time (before or after infusion) as within-subject factors. The mean picture ratings in each category (food images & female face images)

were boxcox-transformed to normalize the distribution and analyzed using linear mixed models with nutrient and type of images (highly attractive versus moderately attractive in the sexual domain, high-calorie versus low-calorie in the food domain) as within-subject factors, and visit order, VAS unpleasantness and BMI as covariates (for the analysis of both domains). The AUC of the intertemporal choice task was calculated per nutrient condition, and analyzed in the linear mixed model, with nutrient as within-subject factor, and visit order, VAS unpleasantness and BMI as covariates. The variance-covariance structure providing the best fit was chosen based on the minimum value of Akaike's Information Criterion (AIC). The effects of visit order and BMI were reported in the results when significant.

The number of clicks when participants terminated the progressive ratio task (Median 7 [IQR 2, 39]) was not normally distributed (Shapiro-Wilk normality test, $W = 0.77$, $p < 0.0001$). Therefore, this variable was grouped into 4 categories (1st, 2nd, 3rd, and 4th quartiles), and analyzed using generalized linear mixed models (glimmix) with a cumulative logit link function for ordinal response variables, with nutrient as a within-subject factor, and visit order, VAS unpleasantness and BMI as covariates. In other words, the frequency of the participants who dropped in one of the quartiles was compared between nutrient conditions. The effects of visit order, VAS unpleasantness and BMI were reported in the results when significant.

Moreover, we performed additional analysis on the abovementioned mixed effect models on picture ratings and AUC, and glimmix models on progressive ratio task with the hunger rating as an extra between-subject covariate.

3. Results

3.1 Recruitment of participants

Seventy-seven participants were recruited in the study between 10 Nov 2014 and 21 Dec 2015. Two participants were excluded because they could not tolerate the nasogastric tube. Fifteen participants dropped out after the first visit and were therefore also excluded. One participant was excluded upon arrival because he did not fast prior to the experiment. Fifty-nine participants (age: 21±0.4 years, BMI: 22.7±0.2 [range: 19-26] kg/m^2) successfully completed both visits and were included in the analysis. The baseline hunger and satiety ratings were not different between nutrient conditions. Minimal nausea scores (a strongly zero-inflated distribution with minimal variability between nutrient conditions, time points, and participants, therefore not permitting formal statistical analysis) were reported. No other adverse events occurred during the study. *Figure 2* shows the recruitment flowchart of the current study.

Tabel 1. Numeric values of the major outcomes in each condition. Data are presented with mean±SD if data were analyzed in parametric models, and median [IQR] otherwise. Food and female face ratings are presented with sums of all 10 pictures in each catalog.

		fat condition (mean±SD)	saline condition (mean±SD)	effect size	test statistics	p value
food domain						
	high caloric food	4.05±1.36	4.28±1.14	Cohen's d -0.20	$F_{1,172}$ = 4.51	0.035
	low caloric food	3.28±1.19	3.49±1.16			
	progressive ratio task	OR 0.31, 95%CI [0.11, 0.98]			$F_{1,52}$ = 4.17	0.046
sex domain						
	highly-attractive female face	4.74±0.92	4.85±0.80	Cohen's d -0.15	$F_{1,172}$ = 1.85	0.19
	moderately-attractive female face	2.19±0.86	2.27±0.77			
financial domain						
	delay discounting task	0.46±0.25	0.46±0.24	-	$F_{1,56}$ = 0.02	0.87

3.2 Unpleasantness

The unpleasant feeling of the tube was significantly stronger at the participants' first visit compared to their second visit (42.6±3.1 vs. 34.4±3.1 mm, $F_{1,56}$ = 6.83, p = 0.012). The unpleasantness was not significantly different between nutrients (fat vs. saline, 36.4±3.1 vs. 40.6±3.2 mm respectively, $F_{1,57}$ = 1.75, p = 0.19). To control for this novelty/order effect, unpleasantness ratings were added as a covariate to all analyses and reported if significant ($p < 0.05$).

3.3 Appetite-related sensations

As shown in Figure 3, none of these parameters was significantly different between nutrient conditions (fat vs. saline), confirming that the manipulation did not affect conscious perception of appetite-related sensations (time by nutrient interaction effects: hunger $F_{1,174}$ = 0.87 p = 0.35, PFC1 $F_{1,174}$ = 0.09 p = 0.77, PFC2 $F_{1,174}$ = 1.72 p = 0.19, satiety $F_{1,174}$ = 0.89 p = 0.35, fullness $F_{1,174}$ = 0.12 p = 0.73).

3.4 Food domain

3.4.1 Picture rating task

Averaged ratings on high- and low-caloric food images in fat condition were significantly correlated with the respective ratings in saline condition (r = 0.44 & 0.71, p = 0.0004 & <0.0001, high- and low-caloric food images, Figure 4A&B, respectively).

Participants rated high-caloric food images significantly higher than low caloric-food images ($F_{1,172}$ = 28.69, p<0.0001). Consistent with our hypothesis, ratings of food images were significantly lower in the fat condition compared to saline condition (the main effect of nutrient, $F_{1,172}$ = 4.51, p=0.035, Cohen's *d*: -0.20, see Figure 5A). However, there was no interaction between nutrient and high-caloric/low-caloric images ($F_{1,172}$=0.17, p=0.68).

Additional analysis indicated that hunger significantly increased food picture ratings ($F_{1,169}$=16.94, p<0.0001). Moreover, the main effect of condition was significant after hunger was added as a covariate ($F_{1,169}$=4.38, p=0.038).

3.4.2 Progressive ratio task (Table 2.)

Participants' progressive ratio terminated significantly earlier in the fat condition compared to saline (fat vs. saline, odds ratio = 0.31, 95%CI [0.11, 0.98], $F_{1,52}$ = 4.17, p = 0.046). Furthermore, the unpleasantness rating of the feeding tube was also associated with earlier termination of the progressive ratio task (main effect of unpleasantness, $F_{1,52}$ = 10.36 p = 0.0022).

Additional analysis indicated that hunger significantly influenced progressive ratio ($F_{1,51}$=5.47, p=0.023). Moreover, the main effect of condition was significant after hunger was added as a covariate ($F_{1,51}$=4.59, p=0.037).

Table 2. Frequency distribution of participants' performance in the progressive ratio task in each quartile by condition.

	number of clicks [range]	frequency	
		fat condition	saline condition
1st quartile	[0 2]	16 (27.1%)	16 (27.1%)
2nd quartile	[3 7]	18 (30.5%)	14 (23.7%)
3rd quartile	[8 38]	12 (20.3%)	9 (15.2%)
4th quartile	[39 45]	13 (22.0%)	20 (33.9%)
sum		59	59

% are column percentages

3.5 Sex domain

3.5.1 Picture rating task

Most participants (57/59) rated the attractiveness of female faces higher than average (>3.5/7) in saline condition. We did not exclude the participants who did not rate highly attractive female above average in saline condition, and the distribution of the data was normal after boxcox-transformation. Moreover, averaged ratings on highly- and moderately-attractive female face pictures in fat condition were significantly correlated with the respective ratings in saline condition (r = 0.63 & 0.77, both p<0.0001, highly- and moderately-attractive face pictures, Figure 4C&D, respectively).

Participants rated highly-attractive female face pictures significantly more attractive than moderately-attractive female face pictures ($F_{1,172}$ = 671.55, p<0.0001). The ratings of female face images did not differ between conditions (main effect of nutrient, $F_{1,172}$ = 1.85, p = 0.18, Cohen's *d*: -0.15, Figure 5B). There was no interaction between nutrient and *a priori* attractiveness of the faces ($F_{1,172}$ = 0.04, p = 0.84).

Additional analysis indicated that hunger did not significantly influence face picture ratings ($F_{1,169}$=0.21, p=0.64). Moreover, the main effect of condition was not significant after hunger was added as a covariate ($F_{1,169}$=1.70, p=0.19).

3.6 Financial domain

3.6.1 Intertemporal choice task

The AUC was not different in the fat condition compared to the saline condition (fat 0.456±0.018 vs. saline 0.463±0.017, main effect of conditoin, $F_{1,56}$ = 0.02, p = 0.87). Moreover, participants showed a higher preference of larger-later reward at their second visit ($F_{1,56}$=4.55, p=0.037).

Additional analysis indicated that participants with higher hunger had lower preference of larger-later reward ($F_{1,54}$=4.41, p=0.040). Moreover, the main effect of condition was not significant after hunger was added as a covariate ($F_{1,54}$=0.01, p=0.91).

4. Discussion

In this study, we tested whether subliminal infusion of fatty acid attenuates sensitivity to exteroceptive food rewards and whether this effect generalizes across reward domains (i.e., sex and money). Concerning the food domain, we found that subliminal intragastric fatty acid infusion significantly reduced participants' ratings of food images, as well as participants' efforts to obtain more food in a progressive ratio task. However, we found that infusion of fatty acid did not significantly influence ratings of images of young females' faces, nor did the fatty acid influence intertemporal choices in the financial domain. Although the effect sizes were small, these findings suggest that the subliminal fatty acid attenuates reward sensitivity within the food domain, but not generalizable to the sexual and the financial domains.

To our knowledge, our findings are the first to provide evidence that an interoceptive subliminal nutrient signal attenuates the reward value of exteroceptive food stimuli, even if only in a very subtle manner. Moreover, the effect was significant even after subjective hunger was added as a covariant. Compared to a neutral saline solution, it lowers the evaluations of food pictures and reduces participants' effort to acquire and consume attractive foods. A previous study indicated that exteroceptive nutrient cues, such as palatable food odor decreased food reward value (16). However, food odor is a consciously perceived exteroceptive food reward cue known to have the capability to evoke memories (17). Therefore, prior personal experiences and learning/anticipation effects may interfere with the effect of food odor on participants' reward sensitivity. The current study shows that a subliminal interoceptive food cue could also modulate reward responses to food, despite the fact that it was not consciously perceived.

We found that subliminal fatty acid infusion decreased the reward value of food images regardless of the caloric content of the foods. Frank *et al*. (18) found that both high- and low-caloric food images could activate reward-related brain regions, such as the OFC, insular cortex, and anterior cingulate cortex (ACC). However, these regions responded more strongly to high-caloric food images. Interestingly, they found significant differential activation between high- and low- caloric foods in the OFC in healthy females but not in healthy male volunteers.

Frank *et al.* (18) suggested that women probably have lower ability to inhibit food-induced brain activities, and hence appeared to have stronger brain response to high caloric foods compared to low caloric foods. We, on the other hand, limited our participants to healthy male volunteers. This may explain why we did not find an interaction between calorie content and the effect of fatty acid infusion on the food image ratings. It would, therefore, be interesting to replicate the study in female volunteers.

In addition to the significant findings in the food domain, we observed that fatty acid infusion did not influence picture rating in the sex domain. Further, fatty acid did not affect performance on the intertemporal choice task in the financial domain, albeit food and monetary reward processing possibly share common neural pathways. We based our hypothesis on a 'common reward circuit' which responds to all three types of rewards (10). However, there are also 'reward type-specific' brain regions that are only responsible for each reward domain. For example, the anterior OFC was more frequently activated in response to monetary rewards, whereas the the amygdala were related to rewards in the sex domain (10). The regions specific to the food rewards (e.g. dorsal anterior insula & somatosensory cortex) may have modulated the domain-specific effects we found in the food domain. In addition, the effect size we found in the food domain was rather small, probably due to a low dose of fatty acid used as intervention. Such small effect size may not be strong enough to influence participants' behaviors in other domains.

Furthermore, our interoceptive stimulus in the food domain did not influence decision making in the financial domain. However, we found a significant association between subjective hunger ratings and participants' performance in the intertemoral monetary choice task. As aforementioned, activating exteroceptive stimulation in food or sex reward domains facilitate reward sensitivity in the financial domain (2, 4, 19). The fatty acid differs from activating signals in prior works in that it was applied subliminally, and thus might not have been strong enough to trigger generalization to the financial domain. Skrynka & Vincent (19) performed intertemporal choice task using both food and monetary rewards in fasting and fed states. They

found that participants' preference switched from smaller-sooner food rewards to later-larger food rewards after they were offered a meal, with an approximately 25% spillover effect to monetary rewards. However, Skrynka & Vincent did not measure subjective appetite related sensations. Therefore, it would be interesting to investigate the role of subjective hunger on reward sensitivity in the financial domain in both fasting and fed states.

A limitation of the current study is that we only recruited healthy heterosexual males because the brain responses to visual sexual stimuli (21) or food images (18) are different between men and women. Moreover, we did not acquire test-retest reliability data for the picture rating tasks. Laus et al. (22) tested both high calorie and low calorie food pictures among healthy adults and found good reliability among men (κ = 0.75-1.00), whereas the test-retest reliability of face picture ratings is, to our knowledge, not well-documented. We evaluated correlation of averaged picture ratings between fat condition and saline condition, and found significant correlations between conditions with median to high effect sizes. Although these were not appropriate analysis for test-retest reliability, the significant correlations indicated that participants' picture ratings of food images and female face pictures were consistent between conditions. Another limitation is that we did not offer pictures with neutral items as a non-reward control. A change in mood following a meal could cause a general effect. Therefore, we would not know whether the effects were a general attenuation effect on picture ratings regardless of the content of the pictures.

Our study constitutes an initial investigation of how a subliminal intragastric nutrient signal influences the motivational value of exteroceptive stimuli in the food domain, in another primary domain (i.e. sex), and the secondary domain (i.e. money). Further, it will be interesting to replicate our findings in a population of different sexes and sexual orientation profiles.

5. Conclusion

A purely subliminal interoceptive nutrient signal induced a subtle but significant decrease in sensitivity to exteroceptive reward stimuli within the food domain, but not across domains in the sex domain or in the financial domain, among healthy heterosexual male participants. These findings are partly in line with our hypothesis that interoceptive nutrient stimulation influences reward sensitivity to exteroceptive food cues. However, such effect does not significantly generalize to other domains.

Data availability statement

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

DZ, MVG, NW, MMJ, JT, LW, and LVO designed the research; DZ and MVG conducted research; DZ, MMJ, LW, and LVO analyzed data and performed statistical analysis; DZ and LVO wrote the manuscript. MMJ and LW provided critical feedback and revised the manuscript. LVO and LW had primary responsibility for the final content. All authors read and approved the final manuscript.

Additional information

Competing financial interests

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Figure legend

Figure 1. Overview of the recruitment procedure and flow of participants.

Figure 2. Schematic overview of a study visit. *Participants came after an overnight fast. They were then intubated with a nasogastric tube. After an adaptation period, they received either 2.5 g fat or saline intragastrically within 2 min, while they were watching a neutral movie (10 min). Before and after the neutral movie, appetite-related sensations, nausea, and unpleasant feeling of the tube were measured via visual analogue scales (VAS). The nasogastric tube was then extubated, after which the reward sensitivity tasks (the picture rating tasks, the intertemporal choice tasks, and the progressive ratio task) were consecutively performed.*

Figure 3. Appetite-related sensations [hunger, satiety, fullness, 'how much you think you will be able to eat' (PFC1), and 'how much you want to eat' (PFC2)] on the visual analogue scales (VAS) were not different between fat and saline conditions. *The x-axis indicates the time points of the measurements (before and after infusion), and the y-axis shows participants' VAS ratings on a 0 – 100 scale, where 0 indicated 'none' and 100 indicated 'extreme.'*

Figure 4. Averaged image ratings in fat condition were significantly correlated to the averaged image ratings in saline condition in all categories: *(A) high calorie food images ($r = 0.44$, $p=0.0004$), (B) low caloriec food images ($r = 0.71$, $p<0.0001$), (C) highly-attractive female face pictures ($r = 0.63$, $p<0.0001$), and (D) moderately-attractive female face pictures ($r = 0.77$, $p<0.0001$).*

Figure 5. Average image ratings in fat and saline condition were presented in the (A) sex and (B) food domains, with different reward values. *The image ratings were significantly lower in the fat condition in the food domain but not in the sexual domain, regardless of their reward values. Moreover, there was no interaction between the reward value of images and conditions in either domains.*