The “hunger” hormone ghrelin is associated with neural activation during touch, but not its affective experience

[1,2]Pfabigan, D.M., [1]Frogner, E.R., [3]Schéle, E., [4]Thorsby, P. M., [5]Skålhegg, B. S, [3]Dickson, S. L., and [1]Sailer, U.

[1] Dept. of Behavioural Medicine, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway

[2] Morbid Obesity Centre, Department of Medicine, Vestfold Hospital Trust, Tønsberg, Norway

[3] Institute for Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

[4] Hormone Laboratory, Department of Medical Biochemistry and Biochemical Endocrinology and Metabolism Research Group, Oslo University Hospital, Aker, Oslo, Norway

[5] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

* Corresponding author: Daniela M. Pfabigan, Sognsvannsveien 9, 0372 Oslo, phone: +47 22851023, mail: d.m.pfabigan@medisin.uio.no

ORCID identifiers:

Daniela M. Pfabigan: 0000-0002-4043-1695

Erik Rimestad Frogner: 0000-0003-2340-4158

Erik Schéle: 0000-0003-2693-7312

Per Medbøe Thorsby: 0000-0002-9615-1035

Bjørn Steen Skålhegg: 0000-0002-3481-8084

Suzanne L. Dickson: 0000-0002-3822-5294

Uta Sailer: 0000-0002-9728-8738

Abstract (225 words)

The gut hormone ghrelin drives food motivation and increases food intake, but it is also involved in the anticipation of and response to rewards other than food. This pre-registered study investigated how naturally varying ghrelin concentrations affect the processing of touch as a social reward in humans.

Sixty-seven volunteers received “social-affective” touch as a social reward and control touch on their shins during 3T functional imaging on two test days. On one occasion participants were fasted and on another they received a meal. On each occasion plasma ghrelin was measured at three time points.

All touch was rated as more pleasant after the meal, but there was no association between ghrelin concentrations and pleasantness. Social-affective touch was rated as most pleasant and activated somatosensory and reward networks (whole-brain). A region-of-interest in the right medial orbitofrontal cortex (mOFC) showed decreased activation during all touch when ghrelin concentrations were high. During social-affective touch, a larger satiety response (ghrelin decrease after the meal) was associated with higher mOFC activation, and this OFC activation was associated with higher experienced pleasantness.

Overall, high ghrelin concentrations appear to be related to decreased reward value. Ghrelin may carry a negative valence signal that, however, does not alter subjective experience. Ghrelin’s observed involvement in the processing of social-affective touch speaks to its multifaceted role in reward processes beyond motivating food search and consumption.

Keywords: ghrelin, affective touch, c-tactile, reward, mOFC, nutritional state

1. Introduction

In situations where food is unavailable or in limited supply, all foods are highly desirable and have a heightened reward/incentive value. Indeed, brain areas involved in reward processing are highly responsive to visual palatable food pictures when fasting (1). A question of particular interest to the present study is the extent to which hunger is able to alter the reward value and neural response to rewards other than food. That hunger might enhance reward from addictive drugs is suggested by studies in rats in which the ability of cocaine and amphetamine to condition a place preference was heightened in situations of food deprivation (2,3). For example, fasters during Ramadan were found to be more risk/loss averse in economic gambling tasks, suggesting that hunger can even reduce the subjective value of monetary rewards (4). It is not a foregone conclusion, however, that the reward value of all (including natural) reinforcers will be increased by fasting.

Linking hunger to the reward system is the stomach-derived hormone ghrelin (5). Ghrelin increases the reward value of reinforcers such as foods (6), other natural rewards (e.g., social interaction) (7) and artificial rewards (e.g., cocaine, alcohol) (7–9), revealed by conditioned place preference testing and operant conditioning in rodents. That ghrelin engages the mesolimbic reward system is suggested by studies in rodents in which ghrelin delivery (either peripherally or to the ventral tegmental area) increases dopamine release in the nucleus accumbens (9). Consistent with this, ghrelin delivery to this site increases food motivation, reflected by increased lever-pressing for sucrose in an operant responding paradigm (11,12). Moreover, endogenous ghrelin is implicated here since many of the reward-linked behaviours are decreased in rodent models of suppressed ghrelin signalling (12, 13).

The present pre-registered neuroimaging study investigated the relation between circulating ghrelin concentrations and the rewarding effects of social stimuli in humans. To implement social reward, touch in the form of slow stroking with a soft brush was used where velocities in-between 3-10 cm/s are typically experienced as particularly pleasant (e.g., 15–18). These velocities best stimulate the so-called C-tactile (CT) nerve afferents (19,20), which are assumed to transmit emotionally-relevant «social-affective» touch. Brain activation studies have shown that CT-targeted touch activates the posterior insula, superior temporal gyrus, and secondary somatosensory cortex (see 25), but also reward areas such as orbitofrontal cortex (22,23) and nucleus accumbens (24,25).

Neural activation during touch and its subjective experience were assessed in two different sessions in 67 healthy volunteers, in a cross-over within-subject fast-and-feed approach. On one occasion participants continued fasting throughout the session to increase ghrelin concentrations and on the other occasion, the same participants received a standardized meal with the aim to decrease ghrelin concentrations. This procedure allowed us to assess the effects of naturally varying intra-individual ghrelin concentrations, which is different from studies externally administering ghrelin and inducing artificially high ghrelin concentrations. We pre-registered a bi-directional hypothesis stating that high

ghrelin concentrations could be associated with increased touch reward similar to previously reported reward-enhancing effects of ghrelin (7,9,13,26,27) or with decreased touch reward as ghrelin can condition also a place aversion (28).

2. Methods

A detailed methods description can be found in Supplementary Materials (section 2).

2.1 Participants

A priori power analysis (29) was calculated for pleasantness ratings and a medium effect for the interaction between touch velocity and nutritional state (*d*=0.45). A sample size of 66 participants was recommended to reach 80% power, but the Covid-19 pandemic did not allow us to reach the planned sample size. Of the 68 recruited healthy volunteers (20 women, 47 men, 1 undisclosed; mean age: 29.1 years, SD=8.6, mean BMI: 23.83, SD=2.8), 60 completed both test sessions.

Participants gave written informed consent prior to the experiment, which was approved by the Regional Ethics Committee (REK South-East B, project 26699).

2.2 Procedure

The same participants were invited twice to the laboratory after a 6-hours fast. Once they received a standardized meal that is expected to decrease endogenous ghrelin concentrations ("liquid-meal" session). In the other test session, participants remained fasted during the experimental procedures which ensured high endogenous ghrelin concentrations ("no-meal" session). Session order was pseudorandomized and by median four days apart. At the beginning of each session, blood glucose concentrations were assessed with a pinprick test and blood and saliva samples were collected (T0/baseline). Two further blood and saliva samples were collected, one about 30 min after the liquid meal and the respective time point in the no meal session (T1), and after the scanning (T2). The liquid meal consisted of 300 ml fermented milk (Biola®, Tine BA) and 300 ml chocolate milk (Sjokomelk, Tine BA). Participants provided ratings on subjective bodily states and their affective state after the liquid meal and a similar time point in the no meal session. The scanning took place approximately 50 min after the T1 blood sample. In the liquid meal session, participants ate a banana directly before scanning. In the no meal session, participants were fasting for more than eight hours when scanning started-see Figure 1 for the experimental time line.

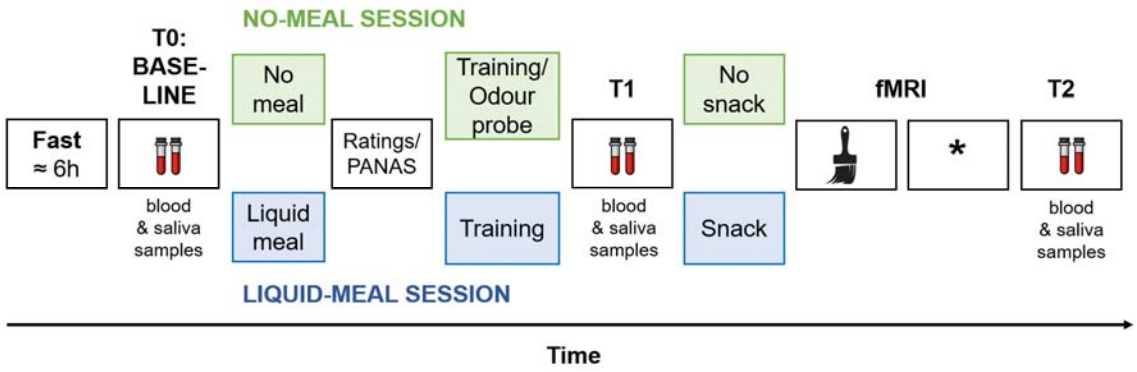

Figure 1. Sequence of experimental procedures. All participants arrived fasted at the laboratory, where a baseline assessment of blood, saliva, and blood glucose was performed first (time point T0/Baseline). Specifics for the no-meal session are illustrated in green colour, specifics for the liquid-meal session in blue colour. After the liquid-meal/no-meal, participants rated their current bodily and affective states. Subsequently, participants were instructed and trained on the upcoming experimental tasks, and were presented with an odour probe in the no-meal session only. Collection of blood and saliva samples after liquid-meal/no-meal followed (T1). Shortly before scanning, a snack was provided in the liquid-meal session only. The star represents three different experimental tasks conducted in the scanner after the touch task (brush symbol). After the scanning session, the last blood and saliva samples of the experiment were collected (T2).

2.3 Ghrelin analysis

Blood was collected in protease inhibitor-filled EDTA tubes, which were centrifuged immediately after venepuncture. Plasma samples were stabilized with HCl before storage at -80□C. Active (acylated) ghrelin concentrations were determined using the EZGRA-88K kit (Merck, Germany) in duplicates (total analytical CV at 488 pg/ml 12%). Statistical analysis of ghrelin values was conducted with a linear mixed model with nutritional state (liquid-meal/no-meal) and the three sample time points (T0/Baseline, T1, T2) as fixed factors (including a random intercept for participant and a random slope for metabolic state).

2.4 Touch task

An experimenter with a brush stood next to the participant in the scanner, whose right shin was exposed. Fifteen trials were administered, 5 trials for each of three randomly ordered stroking velocities: very slow (0.3 cm/sec), CT-targeted (3 cm/sec), and fast touch (30 cm/sec). Each touch application lasted for 15 sec, after which participants rated subjective pleasantness and intensity by answering "*How did you experience the touch?*" and "*How intense did you experience the touch?*" on visual analogue scales (VAS; anchors: unpleasant/not intense - pleasant/intense). Exact trial timing is presented in Figure S1 in Supplementary Materials.

2.5 fMRI data acquisition and analysis

Functional and anatomical MR images were acquired with a 3T Philips Ingenia MRI scanner. After standard preprocessing steps in SPM12 (FIL Group, UC London, UK), data were analysed using (1) a whole brain, (2) a region-of-interest (ROI), and (3) a correlation approach. Single-subject analysis (first-level) was performed based on the GLM framework in SPM12 by modelling the three touch velocities and the VAS scales as regressors against the implicit baseline. Group analysis of the whole brain analysis (second-level) implemented a full-factorial model with touch velocity (very slow, CT-targeted, fast) and nutritional state (liquid-meal, no-meal) as repeated factors; 45 artefact-free datasets were available for this analysis. Results are presented at a cluster-level family-wise error (FWE) corrected threshold of $p<0.05$ (starting threshold $p<0.001$ uncorrected). For the ROI analysis, 60 liquid-meal and 51 no-meal test sessions were available for analysis. Pre-registered ROIs included left posterior insula, left and right secondary somatosensory cortex (SII), right superior temporal gyrus (STG) and left and right medial orbitofrontal cortex (mOFC). Exploratory ROIs included left and right anterior insula and left and right ventral striatum. Parameter estimates were extracted with the REX toolbox (http://web.mit.edu/swg/software.htm). Using linear mixed models, we applied a two-stage approach to disentangle (i) effects of nutritional state, and (ii) associations of ghrelin concentrations with brain activation during touch. Mean ROI activation was first modelled as a function of touch velocity (very slow/CT-targeted/fast), nutritional state (liquid-meal/no-meal), and their interaction as fixed effects (including a random intercept for participant, a random slope for nutritional state, and a random slope for touch velocity if model convergence allowed it). Secondly, mean ROI activation was modelled as a function of touch velocity (very slow/CT-targeted/fast), measurement session (1,2), and mean-centred ghrelin concentrations (sample time point T1), and the interaction of touch velocity x ghrelin as fixed effects (including a random intercept for participant and random slopes for measurement session and touch velocity, if model convergence allowed the latter). Lastly, as described in the pre-registration, potential associations between differences in CT-targeted touch ROI brain activation for liquid meal vs. no meal and differences in metabolic state measures, in particular ghrelin, were investigated. We calculated Spearman correlations between difference values (liquid-meal minus no-meal) of ghrelin (labelled as Δghrelin) and respective difference values of ROI brain activation in right mOFC (as this was the only brain region significantly associated with ghrelin variation). We also explored whether respective differences in pleasantness ratings for CT-targeted touch (Δpleasantness) were associated with differences in right ΔmOFC activation.

2.6 Behavioural data analyses

As with the ROI analysis, linear mixed models and a two-stage approach were applied to analyse subjective ratings. First, single-trial pleasantness and intensity ratings were modelled as a function of touch velocity (very slow/CT-targeted/fast), nutritional state (liquid-meal/no-meal), the mean-centred trial number, and the interaction of touch velocity x nutritional state as fixed effects (including a random intercept for participant and random slopes for touch velocity and nutritional state). Second,

single-trial pleasantness and intensity ratings were modelled as a function of touch velocity (very slow/CT-targeted/fast), measurement session (1,2), mean-centred trial number, and mean-centred ghrelin concentrations (sample time point T1), and the interaction of touch velocity x ghrelin as fixed effects (including a random intercept for participant and random slopes for touch condition and measurement session).

3. Results

A detailed results description can be found in Supplementary Materials (section 3).

3.1 Behavioral and hormonal results

3.1.1 Effectiveness of the nutritional state manipulation

Blood glucose concentrations assessed at the beginning of both measurement sessions did not differ significantly (p=.067). Consumption of the liquid meal reduced subjective feelings of hunger (p<0.001) while it had no effect on PANAS positive affect ratings (p=0.379). PANAS negative affect ratings were slightly higher when participants did not receive a meal (p=0.007). See Supplement (section 3.1) for ratings reflecting further bodily states additional to hunger.

Ghrelin concentrations varied with nutritional state (fasted and then given a liquid-meal or fasted and no-meal) and the three sampling time points (interaction with p's<0.001). Fasting ghrelin concentrations were comparable at the beginning of both test sessions (T0/Baseline: p>0.999). Similarly, ghrelin concentrations were comparable at the end of both test sessions (T2: p>0.999). At T1, about 30 min after the liquid meal, ghrelin concentrations were significantly decreased by more than 300 pg/ml compared to the same time point when participants did not receive food (p<0.001).

3.1.2 Change of pleasantness and intensity ratings of different touch velocities with nutritional state and ghrelin concentrations

To test if nutritional state had an effect on subjective touch experience, pleasantness and intensity ratings were first modelled as a function of touch velocity, nutritional state and trial number. Pleasantness ratings were higher for CT-targeted than very slow touch (p<0.001) and fast touch (p=0.002). These ratings decreased with the number of trials (p<0.001) and were higher following the liquid meal than no meal (p=0.036). Intensity ratings were higher for fast compared to CT-targeted (p<0.001), for CT-targeted than very slow touch (p<0.001) and increased with the number of trials (p<0.001).

Second, to test if ghrelin concentrations were associated with subjective touch experience, pleasantness and intensity ratings were modelled as a function of touch velocity, trial number, measurement session and ghrelin variation. The main effects for touch velocity and trial number were

similar to the first model. Ghrelin variation was not associated with pleasantness (p=0.225) or intensity ratings (p=0.882; Table 1).

Table 1. Ghrelin concentrations and touch ratings

	Liquid-meal			No-meal		
	M	*SD*	*n*	*M*	*SD*	*n*
Acylated ghrelin (pg/ml)						
Baseline (T0)	906	518	62	851	483	60
T1	502	274	57	836	445	52
T2	843	467	61	779	403	56
Pleasantness ratings						
Very slow touch	57.1	12.1	65	54.3	12.1	61
CT-targeted touch	68.5	14.5	65	66.5	14.7	61
Fast touch	63.0	15.2	65	61.9	14.0	61
Intensity ratings						
Very slow touch	28.8	17.8	65	30.10	18.60	62
CT-targeted touch	40.4	17.1	65	39.00	17.20	62
Fast touch	53.7	15.8	65	53.90	18.00	62

3.2 fMRI results

3.2.1 Change of whole-brain brain activation to touch at different velocities with nutritional state (pre-registered analysis)

Enhanced brain activation for CT-targeted than for very slow and fast touch was observed in a network of brain regions including left somatosensory cortex (SII), left middle to superior temporal gyrus (STG), bilateral putamen extending into left caudate, right precentral and inferior frontal gyrus extending into the insular cortex, bilateral supplementary motor area (SMA) and precentral gyri, and left lateral occipital cortex/middle temporal gyrus. A main effect of nutritional state indicated higher brain activation following the liquid meal in the right inferior frontal gyrus extending into the frontal operculum. Brain activation in the different nutritional states did not significantly interact with any of the touch velocities (Table 2, Figure 2).

Table 2. Whole brain results for the main effects of nutritional state and touch velocity

CONTRAST	REGION(S)	k	PEAK COORDINATES X	Y	Z	*t* VALUE	*p* VALUE (FWE-corr.)
liquid-meal >no-meal	R inferior frontal gyrus, pars opercularis	199	58	22	10	4.3	0.032
			56	16	2	3.99	
			58	14	20	3.94	
CT-targeted>very slow touch	R putamen	2229	26	4	0	5.86	< 0.001
	L putamen		-22	-4	-8	5.68	
			-24	4	4	5.53	
	L precentral gyrus	289	-38	-4	56	5.85	0.007
			-32	-6	64	4.39	
	L secondary somatosensory cortex	2568	-36	-44	60	5.80	< 0.001
			-48	-36	24	5.68	
			-52	-20	30	5.42	
	L lateral occipital cortex/area MT	570	-42	-66	8	5.42	< 0.001
	L middle temporal gyrus		-50	-58	6	4.00	
			-40	-54	10	3.24	
	R supramarginal gyrus	748	56	-38	18	5.02	< 0.001
			46	-40	10	4.82	
			64	-44	14	4.22	
	R lateral occipital cortex/area MT	226	54	-66	4	4.91	0.020
			42	-58	8	3.53	
	L thalamus	256	-10	-18	6	4.85	0.012
			-4	-30	0	4.17	
	R precentral gyrus	219	42	-4	56	4.63	0.022
			52	6	44	3.76	
			40	-4	44	3.72	
CT-targeted>fast touch	R central opercular cortex/insular cortex	2813	42	-2	14	6.28	< 0.001

R precentral gyrus		60	10	24	6.11	
R postcentral gyrus		58	-14	32	6.02	
R supplementary motor area	669	6	0	56	4.76	< 0.001
		2	-8	54	4.22	
		4	-12	66	4.21	
L postcentral gyrus	476	-56	-22	36	4.61	< 0.001
		-62	-14	28	4.29	
		-60	-2	22	3.78	

Note: k = cluster size.

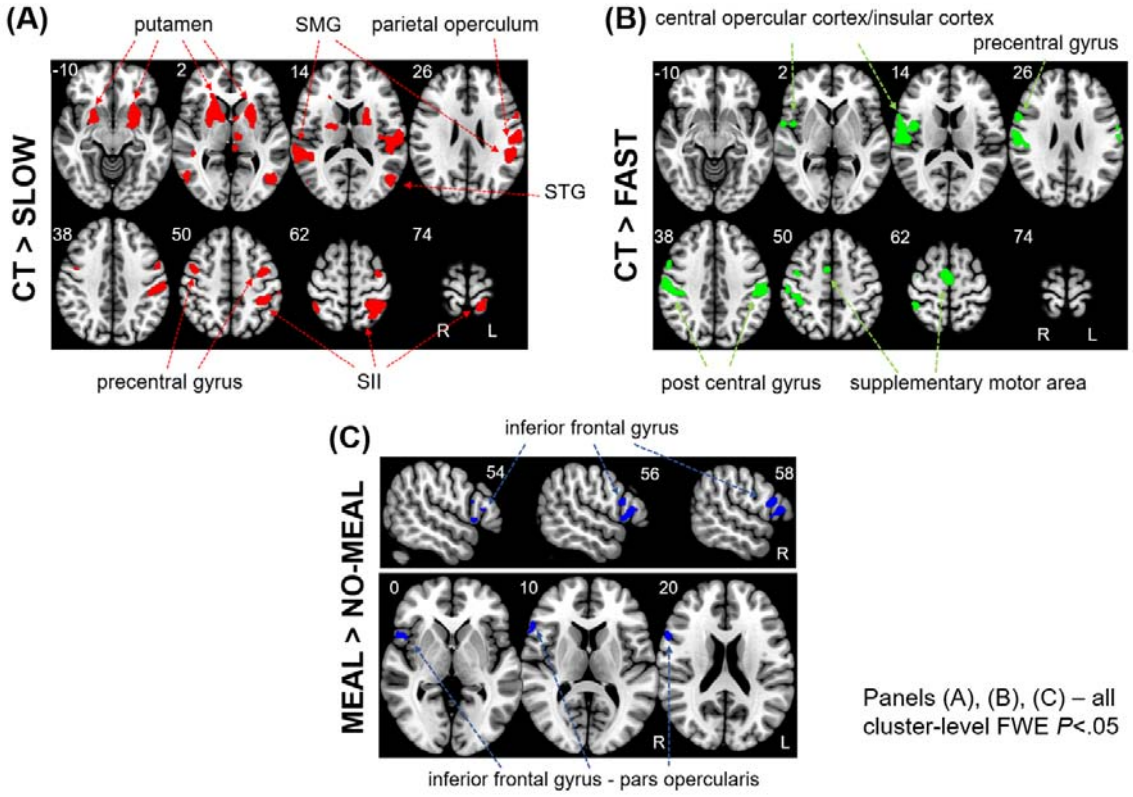

Figure 2: **Whole brain results for touch velocity and nutritional state.** Panel (A) depicts significant activation clusters for the contrast CT-targeted > very slow touch (in red) in 8 axial slices (Z coordinates reported). Panel (B) depicts significant activation clusters for the contrast CT-targeted > fast touch (in green) in 8 axial slices (Z coordinates reported). Panel (C) depicts the significant activation cluster for the contrast liquid-meal > no-meal (in blue) in 3 sagittal slices in the upper part (right hemisphere, X coordinates reported) and in 3 axial slices in the lower part (Z coordinates reported). All results are presented at a cluster-level FWE corrected level of $p<0.05$.

3.2.2 Change of brain activation in different regions-of-interest (ROIs) with touch velocity, nutritional state and ghrelin concentrations (exploratory analysis)

To test if nutritional state changes the brain activation in pre-defined ROIs, we first modelled ROI activation as a function of touch velocity and nutritional state. This showed enhanced activation for CT-targeted than for very slow touch in right STG, bilateral SII and right ventral striatum (VS) (*p's*<0.004). Enhanced activation for CT-targeted than for fast touch was found in right SII ($p<0.001$). None of the ROIs showed significant differences between liquid meal and no meal (*p's*>0.055).

To test if ghrelin concentrations were associated with brain activation in those pre-defined ROIs, we modelled ROI activation as a function of touch velocity, measurement session and ghrelin concentrations. This showed enhanced activation for CT-targeted than for very slow touch in right

STG, bilateral SII and right VS (*p's*<0.007). Enhanced activation for CT-targeted than for fast touch was found in right SII (*p*<0.001). Higher ghrelin concentrations were associated with decreased brain activation in the right medial orbitofrontal cortex (mOFC) (*p*=0.016) irrespective of touch velocity.

3.2.3 Relationship between nutritional state differences in brain activation during social-affective (CT-targeted) touch and ghrelin concentrations (pre-registered analysis)

Since CT-targeted touch is particularly tuned to social interactions (19,20), we were especially interested in ghrelin's association with this touch velocity. A significant negative association between Δghrelin and right ΔmOFC activation was observed (*p*=0.013), suggesting that the greater the suppression of ghrelin by the liquid meal compared to no meal (i.e., the satiety response induced by the meal), the higher the brain activation following the liquid meal during CT-targeted touch (compared to no meal; Figure 3B). Furthermore, a significant positive association between right ΔmOFC activation and Δpleasantness was found (*p*=0.012, Figure 3A), while Δghrelin and Δpleasantness were not associated (*p*=0.914). This means that the higher the brain activation during CT-targeted touch following the liquid meal (compared to no meal), the higher was touch pleasantness.

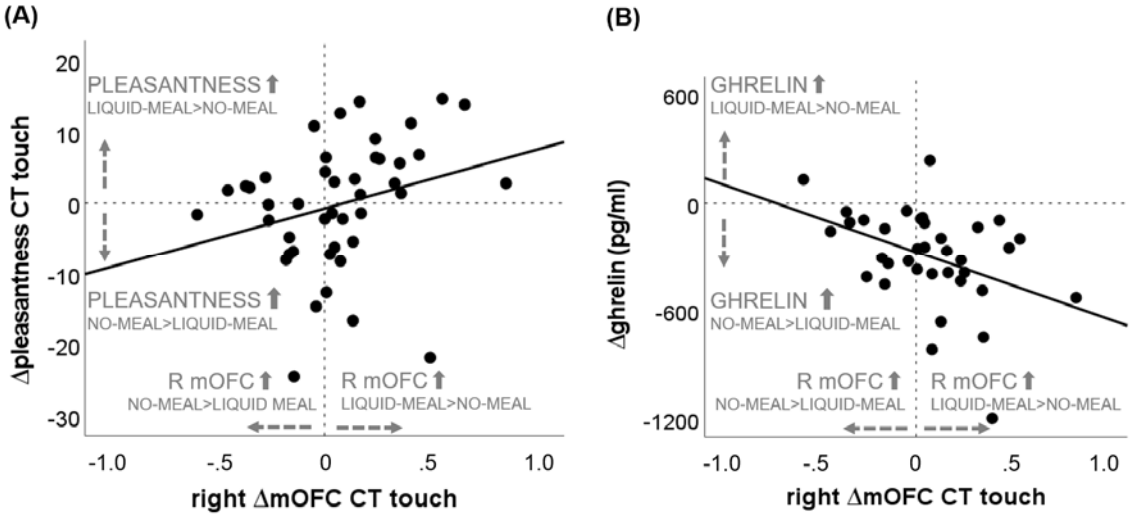

Figure 3: **Nutritional state-dependent differences in brain activation and pleasantness ratings during CT-targeted touch and ghrelin variation.** (A) "liquid-meal minus no-meal" session differences of right mOFC activation during CT-targeted touch on the x-axis (right ΔmOFC CT touch; positive values indicate higher right mOFC activation in the liquid-meal than the no-meal session; negative values indicate higher right mOFC activation in the no-meal than the liquid-meal session). On the y-axis, "liquid-meal minus no-meal" session differences in mean pleasantness ratings are depicted (Δpleasantness CT touch; positive values indicate higher reported pleasantness of CT-targeted touch in the liquid-meal than the no-meal session; negative values indicate higher reported pleasantness in the no-meal than the liquid-meal session). (B) "liquid-meal minus no-meal" session differences of right mOFC activation during CT-targeted touch on the x-axis (right ΔmOFC CT touch). On the y-axis, "liquid-meal minus no-meal" session differences in ghrelin concentrations at T1

are depicted (Δghrelin (pg/ml); positive values indicate higher ghrelin concentrations in the liquid-meal than the no-meal session; negative values indicate higher ghrelin concentrations in the no-meal than the liquid-meal session), this represents the satiety response induced by the meal resulting in ghrelin suppression in the liquid-meal session. The solid black lines depict the regression lines per correlation.

4. Discussion

This study investigated reward responses to social-affective touch when fasting participants either received a liquid meal aiming to decrease endogenous ghrelin concentrations or received no meal aiming to maintain high ghrelin concentrations. All touch was experienced as more pleasant after participants had eaten, and this was accompanied by enhanced frontal brain activation. In contrast to our pre-registered hypothesis, subjective pleasantness did not vary with ghrelin concentrations, although higher ghrelin concentrations were associated with reduced brain activation during all touch in the right medial orbitofrontal cortex (mOFC), a brain region known for encoding reward value (30). Indeed, mOFC activation was also related to pleasantness in the present study.

4.1 Ghrelin's impact on brain activation during touch

We pre-registered the mOFC as a ROI because it has been associated with ascribing hedonic value to different types of experimental stimuli (30) including touch (31). Higher ghrelin concentrations were associated with reduced right mOFC activation in response to all touch velocities. In addition to reduced mOFC activation with higher ghrelin concentrations during all types of touch, there was also an activation change specific to social-affective touch. The larger the individual ghrelin suppression following the liquid meal (i.e., the satiety response induced by the meal), the higher the mOFC activation during social-affective touch (pre-registered correlation). Thus, both pre-registered and exploratory analyses suggest that ghrelin is related to mOFC activation in a way that high ghrelin concentrations reduce mOFC activation during touch. In accordance with the literature on mOFC function, mOFC activation appears to represent reward valuation, as the difference in mOFC activation between liquid meal and no meal for social-affective touch was positively associated with the respective difference in pleasantness ratings for social-affective touch. Since ghrelin was related to reduced mOFC activation and mOFC activation in turn was related to pleasantness, ghrelin rather seems to reduce than enhance touch reward. This is in line with previous findings suggesting that ghrelin acts as a negative valence signal (28). By this, concurrent experiences such as touch become negative (or less positive) simply because they are paired with this negative valence signal, i.e. via classic conditioning.

The decreased mOFC activation with high ghrelin concentrations during touch is in contrast to a previous study with visual stimuli. When participants looked at food compared to landscape pictures, left mOFC activation was increased following exogenous ghrelin administration, but not following

saline administration (32). Both of these findings can be reconciled by assuming that ghrelin may indicate metabolic stimulus salience. In other words, ghrelin may amplify the salience of stimuli that can restore homeostatic balance, and may dampen the salience of stimuli that fail to do so. Along these lines, the hypothalamic hunger system as the primary target region for ghrelin (33), has been suggested (34) to increase the salience and value of food rewards, and to concurrently decrease the salience and value of non-food rewards. The function of this may be that survival is best served by focusing on food search and this is, to some extent, accomplished by increasing the rewarding value of food stimuli. However, the postulated salience reduction of non-food rewards might be impaired when these rewards consist of drugs that lead to a strong dopamine release. Indeed, food-restricted hungry mice showed enhanced responses and reinforcement to drugs such as amphetamine and cocaine that substantially increase dopamine release, and the inhibition of the satiety hormone leptin led to upregulated dopamine levels and increased preference for cocaine-conditioned reward (35,36). Providing a physiological basis for the assumption that the hypothalamic hunger system dynamically modulates reward salience, studies in rodents have demonstrated that neural circuits of reward processing – in particular the mesolimbic dopamine system – and neural circuits of appetite in the hypothalamus are closely interconnected and interdependent (34,37). By modulating the firing rate or synaptic activity of mesolimbic dopaminergic neurons, ghrelin could be crucially involved in tuning these neurons to enhance or attenuate the salience of potential reward stimuli.

4.2 No association between ghrelin concentrations on subjective touch experience

As in many previous studies, subjective pleasantness ratings showed an inverted U-shaped curve with social-affective touch being rated as most pleasant (38–40) on the group level, with expected inter-individual variations (41). Moreover, whole brain results as well as ROI analyses demonstrated that social-affective touch was accompanied by enhanced brain activation in somatosensory and social cognition areas, as previously reported (21,22). The absent association of ghrelin with explicit ratings of touch pleasantness may have several explanations. First, circulating ghrelin concentrations might not have been high enough to lead to measurable changes in participants' subjective experience. Consistent with this, animal studies suggest that the effects of ghrelin could be dose-dependent. Systemic administration of very high doses of ghrelin induced avoidance behaviours in a place preference study (42), while a lower dose induced preference behaviours in a similar experimental set-up (10). Second, a number of additional processing steps take place in between neural activation in response to stroking and the subsequently given pleasantness ratings: participants need to bring touch experience to their conscious awareness, compare this experience to the presented rating scale, determine where on the presented scale this experience is best represented, prepare the response while remembering the decision and execute the motor response. Ghrelin's effects might be limited to earlier

stages of brain representation, whereas later stages of deciding and responding might be less sensitive to ghrelin's influence. Overall, it appears that functional imaging was able to reveal subtle associations of ghrelin that might otherwise have been missed if only explicit ratings would have served as outcome variables.

4.3 Impact of nutritional state on brain activation and subjective experiences during touch

Independent of ghrelin variation, participants' nutritional state affected brain activation during touch and the associated subjective experience. Brain activation in the right inferior frontal gyrus and frontal operculum was higher following eating than fasting. This was contrary to the pre-registered hypothesis and will therefore not be discussed any further.

Also, all touch velocities were rated as more pleasant when participants had eaten. Previous studies found that hunger states divert participants' attention from a current task (43,44). Participants in the current study may have been distracted by hunger-related interoceptive feelings caused by an empty stomach. Another explanation might be that being hungry is often accompanied by an unpleasant subjective feeling in humans (45), which could have decreased the experienced pleasantness of touch. However, explicit reports of negative emotions (assessed with the PANAS) differed only slightly with nutritional state. Participants reported marginally higher negative affect following no meal vs. the liquid meal, but affect was on average neutral (liquid-meal: M=11.8; no-meal: M=12.7; range 10-50 where a mean value of 10 corresponds to an agreement of "not at all" with various negative emotions). Moreover, exploratory analyses showed no main effects of PANAS negative affect on brain activation or pleasantness, and ghrelin concentrations and PANAS affective states were also not associated (see Supplementary Materials, section 3.6).

4.4 Advantages of "endogenous" ghrelin variation

One of the strengths of the present study was to induce and utilize intra-individual changes in ghrelin concentrations caused by fasting and eating. Most previous imaging studies compared intravenous ghrelin administration vs. placebo, and thereby induced short-lasting artificially high ghrelin concentrations that are often physiologically impossible (46). Ghrelin concentrations after external administration were previously associated with enhanced activation in OFC in response to food pictures (1,32), which was interpreted as enhanced hedonic response to food cues. In contrast, with naturally varying ghrelin concentrations, the relationship between ghrelin concentrations and such hedonic reactivity to food cues was only modest (47). We postulate therefore that it would be more informative and ecologically more valid to investigate physiologically plausible intra-individual ghrelin concentrations than external administration in humans.

4.5 Limitations

While the applied within-subject fast-and-feed approach is a strength of the current study, we acknowledge that it does not allow to infer causal relationships between ghrelin concentrations and the assessed outcome measures.

Power calculations were based assuming a medium-sized effect, which resulted in a sample size that was feasible to collect with functional imaging. It is, however, possible that effects of ghrelin concentrations on subjective experience during the task are small-sized. Therefore, future studies should aim to investigate the effects of ghrelin variation on touch experience in sample sizes large enough to detect small effects. It might further be interesting to assess additional aspects of subjective experience during touch, such as comfort and arousal (e.g.; 48), for a more comprehensive description of potential ghrelin effects.

5. Conclusion

Naturally circulating ghrelin concentrations after eight hours of fasting were associated with neural activation in response to touch in the right mOFC, a brain area involved in hedonic valuation. Higher ghrelin concentrations seemed to reduce mOFC activation without exercising a direct effect on subjective experience in terms of pleasantness, intensity, or affective states. The current findings on ghrelin's involvement in the processing of social-affective touch contribute to the knowledge of the comprehensive role of ghrelin in the response to reward.

Data availability:
All data, code, and materials will be made publicly available via the Open Science Framework: ***
Group-level T maps are available via https://neurovault.org/collections/12572/
The study was pre-registered on the Open Science Forum (https://osf.io/f9rkq). This manuscript was deposited at a preprint server bioRxiv (https://doi.org/10.1101/2022.05.10.491384) under a CC-BY-NC-ND 4.0 International license.

Acknowledgements

The study was funded by the Norwegian Research Council (grant number 275316), ERA-NET-NEURON, JTC 2020/Norwegian Research Council (grant number 323047), and the South-Eastern Norway Regional Health Authority (grant number 2021046). SLD received addition support from the

Swedish Research Council for Medicine and Health (2019-01051), the Novo Nordisk Foundation (NNF19OC0056694) Hjärnfonden (FO2019-0086) and the Swedish state under the agreement between the Swedish Government and the county councils in the ALF agreement (ALFGBG- 965364).

We thank TINE BA for providing us with the products used as liquid meal for free. We thank Aiste Gvildyte Næss, Pietro Aleksander Rocco Berger Lapolla, Aleksandra Pusica, Thea Wiker Engelund, and Anbjørn Ree for their work with participant recruitment and data collection, and Federica Riva for helpful comments on a previous version of the manuscript.

References

1. Goldstone AP, Prechtl CG, Scholtz S, Miras AD, Chhina N, Durighel G, et al. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. Am J Clin Nutr. 2014 Jun 1;99(6):1319–30.

2. Bell SM, Stewart RB, Thompson SC, Meisch RA. Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology (Berl). 1997 May;131(1):1–8.

3. Stuber GD, Evans SB, Higgins MS, Pu Y, Figlewicz DP. Food restriction modulates amphetamine-conditioned place preference and nucleus accumbens dopamine release in the rat. Synap N Y N. 2002 Nov;46(2):83–90.

4. Rad MS, Ginges J. Loss of control is not necessary to induce behavioral consequences of deprivation: The case of religious fasting during Ramadan. Behav Brain Sci. 2017/11/29 ed. 2017;40:e338.

5. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999 Dec;402(6762):656–60.

6. Egecioglu E, Jerlhag E, Salomé N, Skibicka KP, Haage D, Bohlooly-Y M, et al. PRECLINICAL STUDY: FULL ARTICLE: Ghrelin increases intake of rewarding food in rodents. Addict Biol. 2010;15(3):304–11.

7. Schéle E, Pfabigan DM, Simrén J, Sailer U, Dickson SL. Ghrelin Induces Place Preference for Social Interaction in the Larger Peer of a Male Rat Pair. Neuroscience. 2020 Nov 1;447:148–54.

8. Wellman PJ, Clifford PS, Rodriguez JA. Ghrelin and ghrelin receptor modulation of psychostimulant action. Front Neurosci. 2013 Sep 25;7:171.

9. Jerlhag E, Egecioglu E, Landgren S, Salomé N, Heilig M, Moechars D, et al. Requirement of central ghrelin signaling for alcohol reward. Proc Natl Acad Sci U S A. 2009 Jul 7;106(27):11318–23.

10. Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict Biol. 2008 Sep;13(3–4):358–63.

11. Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly targets the ventral tegmental area to increase food motivation. Neuroscience. 2011 Apr 28;180:129–37.

12. Skibicka KP, Hansson C, Egecioglu E, Dickson SL. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. Addict Biol. 2012 Jan;17(1):95–107.

13. Jerlhag E, Engel JA. Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. Drug Alcohol Depend. 2011 Sep 1;117(2–3):126–31.

14. Park SB. Contribution of growth hormone secretagogue receptor (GHSR) signaling in the ventral tegmental area (VTA) to the regulation of social motivation in male mice. 2021;14.

15. Kass-Iliyya L, Leung M, Marshall A, Trotter P, Kobylecki C, Walker S, et al. The perception of affective touch in Parkinson's disease and its relation to small fibre neuropathy. Eur J Neurosci. 2016/11/20 ed. 2017 Jan;45(2):232–7.

16. Essick GK, McGlone F, Dancer C, Fabricant D, Ragin Y, Phillips N, et al. Quantitative assessment of pleasant touch. Neurosci Biobehav Rev. 2010 Feb 1;34(2):192–203.

17. Triscoli C, Olausson H, Sailer U, Ignell H, Croy I. CT-optimized skin stroking delivered by hand or robot is comparable. Front Behav Neurosci. 2014/01/07 ed. 2013;7:208.

18. Morrison I, Loken LS, Minde J, Wessberg J, Perini I, Nennesmo I, et al. Reduced C-afferent fibre density affects perceived pleasantness and empathy for touch. Brain. 2011/03/08 ed. 2011 Apr;134(Pt 4):1116–26.

19. Löken LS, Wessberg J, Morrison I, McGlone F, Olausson H. Coding of pleasant touch by unmyelinated afferents in humans. Nat Neurosci. 2009/04/14 ed. 2009 May;12(5):547–8.

20. Ackerley R, Backlund Wasling H, Liljencrantz J, Olausson H, Johnson RD, Wessberg J. Human C-tactile afferents are tuned to the temperature of a skin-stroking caress. J Neurosci. 2014/02/21 ed. 2014 Feb 19;34(8):2879–83.

21. Morrison I. ALE meta-analysis reveals dissociable networks for affective and discriminative aspects of touch. Hum Brain Mapp. 2016 Apr;37(4):1308–20.

22. Sailer U, Triscoli C, Haggblad G, Hamilton P, Olausson H, Croy I. Temporal dynamics of brain activation during 40 minutes of pleasant touch. Neuroimage. 2016/06/25 ed. 2016 Jun 20;139:360–7.

23. Grabenhorst F, D'Souza AA, Parris BA, Rolls ET, Passingham RE. A common neural scale for the subjective pleasantness of different primary rewards. Neuroimage. 2010/03/25 ed. 2010 Jul 1;51(3):1265–74.

24. Kreuder AK, Scheele D, Wassermann L, Wollseifer M, Stoffel-Wagner B, Lee MR, et al. How the brain codes intimacy: The neurobiological substrates of romantic touch. Hum Brain Mapp. 2017 Sep;38(9):4525–34.

25. McCabe C, Rolls ET, Bilderbeck A, McGlone F. Cognitive influences on the affective representation of touch and the sight of touch in the human brain. Soc Cogn Affect Neurosci. 2008/11/19 ed. 2008 Jun;3(2):97–108.

26. Maric T, Sedki F, Ronfard B, Chafetz D, Shalev U. A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. Addict Biol. 2012 May;17(3):613–22.

27. Egecioglu E, Prieto-Garcia L, Studer E, Westberg L, Jerlhag E. The role of ghrelin signalling for sexual behaviour in male mice. Addict Biol. 2016 Mar;21(2):348–59.

28. Schéle E, Cook C, Le May M, Bake T, Luckman SM, Dickson SL. Central administration of ghrelin induces conditioned avoidance in rodents. Eur Neuropsychopharmacol. 2017 Aug 1;27(8):809–15.

29. Westfall J. PANGEA: Power ANalysis for GEneral Anova designs (working paper). URL Jakewestfall Orgpangea. 2016;

30. Rolls ET. The orbitofrontal cortex, amygdala, reward value, and emotion. In: Brain Computations [Internet]. Oxford: Oxford University Press; 2020 [cited 2021 Dec 31]. Available from: https://oxford.universitypressscholarship.com/10.1093/oso/9780198871101.001.0001/oso-9780198871101-chapter-11

31. Rolls ET, O'Doherty J, Kringelbach ML, Francis S, Bowtell R, McGlone F. Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. Cereb Cortex N Y N 1991. 2003 Mar;13(3):308–17.

32. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. Cell Metab. 2008 May;7(5):400–9.

33. Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, et al. The Distribution and Mechanism of Action of Ghrelin in the CNS Demonstrates a Novel Hypothalamic Circuit Regulating Energy Homeostasis. Neuron. 2003 Feb 20;37(4):649–61.

34. Cassidy RM, Tong Q. Hunger and Satiety Gauge Reward Sensitivity. Front Endocrinol [Internet]. 2017 [cited 2021 Jun 2];8. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2017.00104/full

35. Zheng D, Cabeza de Vaca S, Carr KD. Food restriction increases acquisition, persistence and drug prime-induced expression of a cocaine-conditioned place preference in rats. Pharmacol Biochem Behav. 2012 Jan;100(3):538–44.

36. Shen M, Jiang C, Liu P, Wang F, Ma L. Mesolimbic leptin signaling negatively regulates cocaine-conditioned reward. Transl Psychiatry. 2016 Dec 6;6(12):e972.

37. Abizaid A. Ghrelin and dopamine: new insights on the peripheral regulation of appetite. J Neuroendocr. 2009/06/16 ed. 2009 Sep;21(9):787–93.

38. Gentsch A, Panagiotopoulou E, Fotopoulou A. Active Interpersonal Touch Gives Rise to the Social Softness Illusion. Curr Biol. 2015/09/15 ed. 2015 Sep 21;25(18):2392–7.

39. Hielscher E, Mahar D. An Exploration of the Interaction Between Touch Avoidance and the Pleasant Touch (C-Tactile Afferent) System. Perception. 2017 Jan 1;46(1):18–30.

40. Croy I, Luong A, Triscoli C, Hofmann E, Olausson H, Sailer U. Interpersonal stroking touch is targeted to C tactile afferent activation. Behav Brain Res. 2016 Jan 15;297:37–40.

41. Croy I, Bierling A, Sailer U, Ackerley R. Individual Variability of Pleasantness Ratings to Stroking Touch Over Different Velocities. Neuroscience. 2021 Jun 1;464:33–43.

42. Lockie SH, Dinan T, Lawrence AJ, Spencer SJ, Andrews ZB. Diet-induced obesity causes ghrelin resistance in reward processing tasks. Psychoneuroendocrinology. 2015 Dec;62:114–20.

43. Sänger J. Can't take my eyes off you – How task irrelevant pictures of food influence attentional selection. Appetite. 2019 Feb 1;133:313–23.

44. Stamataki NS, Elliott R, McKie S, McLaughlin JT. Attentional bias to food varies as a function of metabolic state independent of weight status. Appetite. 2019 Dec 1;143:104388.

45. MacCormack JK, Lindquist KA. Feeling hangry? When hunger is conceptualized as emotion. Emot Wash DC. 2019 Mar;19(2):301–19.

46. Kunath N, Müller NCJ, Tonon M, Konrad BN, Pawlowski M, Kopczak A, et al. Ghrelin modulates encoding-related brain function without enhancing memory formation in humans. NeuroImage. 2016 Nov 15;142:465–73.

47. Wever MCM, van Meer F, Charbonnier L, Crabtree DR, Buosi W, Giannopoulou A, et al. Associations between ghrelin and leptin and neural food cue reactivity in a fasted and sated state. NeuroImage. 2021 Oct 15;240:118374.

48. Guest S, Dessirier JM, Mehrabyan A, McGlone F, Essick G, Gescheider G, et al. The development and validation of sensory and emotional scales of touch perception. Atten Percept Psychophys. 2011/01/26 ed. 2011 Feb;73(2):531–50.