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

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Abstract (228 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 levels affect the processing of touch as a social reward in humans.

Sixty-eight 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 levels and pleasantness ratings. 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 levels were high. During social-affective touch, a larger ghrelin suppression following the meal was associated with higher mOFC activation, which in turn was associated with higher experienced pleasantness.

Overall, high ghrelin levels were associated with a reduction in reward-related brain activation. This suggests that ghrelin carries a negative valence signal that, however, does not alter subjective experience. Demonstrating that ghrelin is implicated in the processing of social-affective touch speaks for its versatile role in reward processes beyond motivating food search and consumption.

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

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 levels 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. On one occasion participants continued fasting throughout the session to increase ghrelin levels and on the other occasion, the same participants received a standardized meal with the aim to decrease ghrelin levels. This procedure allowed us to assess the effects of naturally varying intra-individual ghrelin levels, which is different from studies externally administering ghrelin and inducing supra-threshold levels. We pre-registered a bi-directional hypothesis stating that high ghrelin levels 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).

Results

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

Behavioral and hormonal results

Effectiveness of nutritional state manipulation: Consumption of the liquid meal reduced subjective feelings of hunger ($P<0.001$) while it had no effect on positive affect ratings ($P=0.379$). 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 levels varied based on 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 levels were comparable at the beginning of both test sessions (T0/Baseline: $P>0.999$). Similarly, ghrelin levels were comparable at the end of both test sessions (T2: $P>0.999$). At T1, about 30 min after the liquid meal, ghrelin levels were significantly decreased by 317 pg/ml compared to the same time point when participants did not receive food ($P<0.001$).

Change of pleasantness and intensity ratings of different touch velocities with nutritional state and ghrelin levels: 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 levels

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 levels 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

fMRI results

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 that brain activation following the liquid meal compared to no meal was enhanced in the right inferior frontal gyrus extending into the frontal operculum. No significant activations were observed for the interaction term (Table 2, Figure 1).

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

CONTRAST	REGION(S)	k	PEAK COORDINATES			*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.

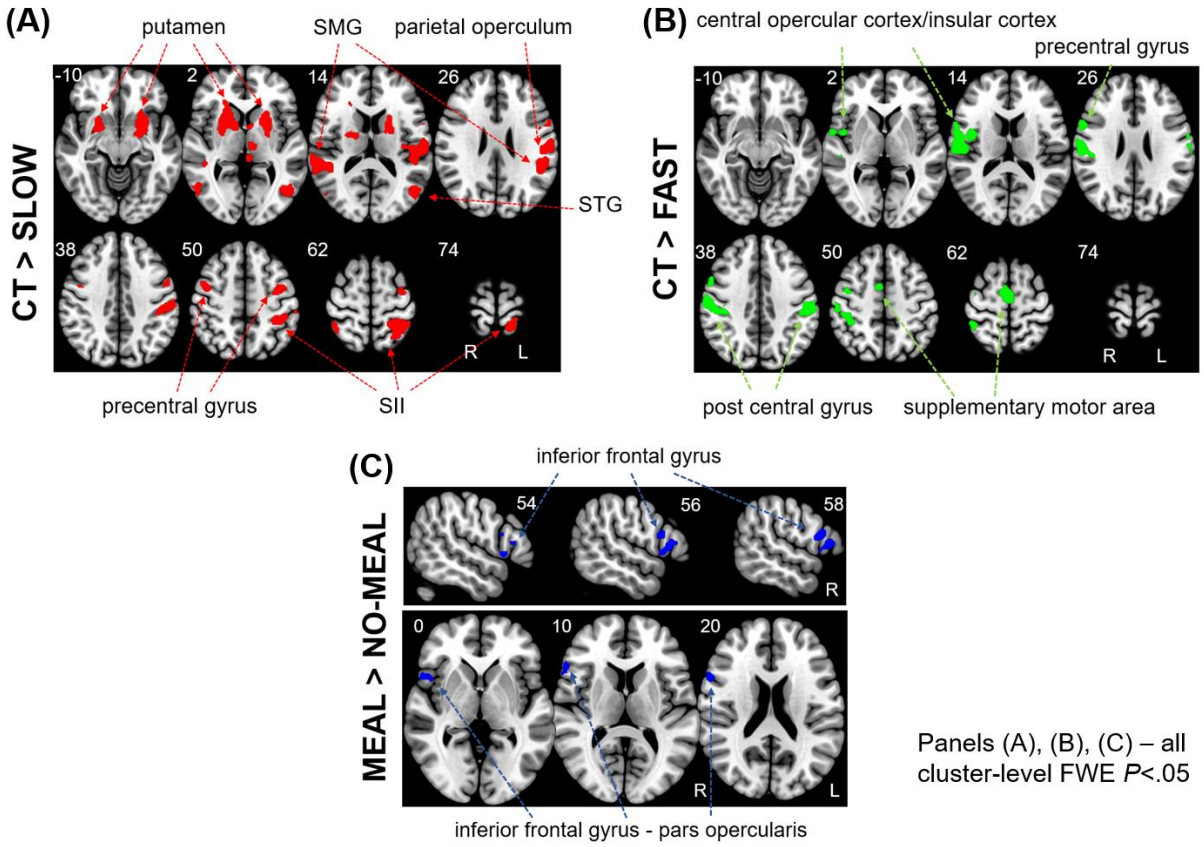

Figure 1: **Whole brain results for touch velocity and nutritional state.** Panel (A) depicts significant activation clusters for the contrast CT-targeted > very slow touch (in red) in 8 axial slices (Z coordinates reported). Panel (B) depicts significant activation clusters for the contrast CT-targeted > 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 $P<0.05$.

<u>Change of brain activation in different regions-of-interest (ROIs) with stroking velocity, nutritional state and ghrelin levels (exploratory analysis)</u>: To test if nutritional state had an effect on 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 levels were associated with brain activation in those pre-defined ROIs, we modelled ROI activation as a function of touch velocity, measurement session and ghrelin variation. 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 values were associated with decreased brain activation in the right medial orbitofrontal cortex (mOFC) (*P*=0.016) irrespective of touch velocity.

Relationship between nutritional state differences in brain activation during social-affective (CT-targeted) touch and ghrelin levels (pre-registered analysis): Since CT-targeted touch is assumed to reflect social reward (19, 20), we were particularly 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), the higher the brain activation following the liquid meal during CT-targeted touch (compared to no meal; Figure 2B). Furthermore, a significant positive association between right ΔmOFC activation and Δpleasantness was found (*P*=0.012, Figure 2A), while Δghrelin and Δpleasantness were not associated (*P* =0.914). This means that the higher the brain activation following the liquid meal (compared to no meal) during CT-targeted touch, the higher was touch pleasantness.

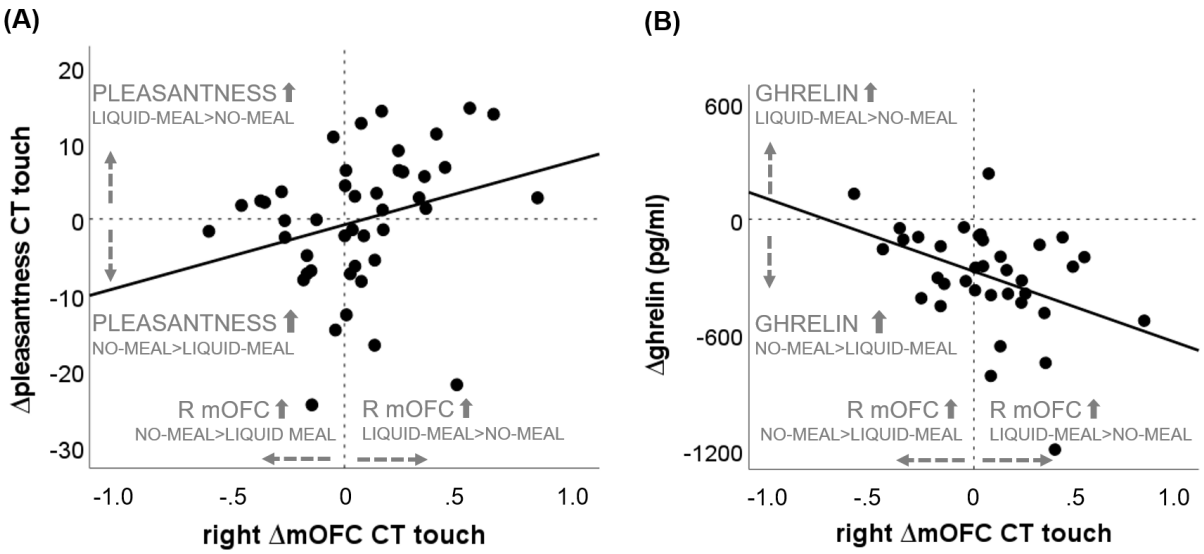

Figure 2: **Nutritional state-dependent differences in brain activation and pleasantness experience during CT-targeted touch and ghrelin variation.** (A) "liquid-meal minus no-meal" session differences of right mOFC activation during CT-targeted touch on the x-axis (right ΔmOFC CT touch; positive values 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 levels at T1 are depicted (Δghrelin (pg/ml); positive values indicate higher ghrelin levels in the liquid-meal than the no-meal session; negative values indicate higher ghrelin levels in the no-meal than the liquid-meal session). The solid black lines depict the regression lines per correlation.

Discussion

This study investigated reward responses to social-affective touch when fasting participants either received a liquid meal aiming to decrease endogenous ghrelin levels or received no meal aiming to maintain high ghrelin levels. 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, variations in ghrelin levels were not associated with subjective experience of touch, despite that higher ghrelin levels were associated with reduced brain activation during touch in the right medial orbitofrontal cortex (mOFC), an area involved in reward valuation (29).

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 (29) including touch (30). Higher ghrelin levels were associated with reduced right mOFC activation in response to all touch velocities in the exploratory analysis. In addition to reduced mOFC activation with higher ghrelin levels 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, the higher the mOFC activation during social-affective touch (pre-registered correlation). Thus, both pre-registered and exploratory analyses point to a negative association between ghrelin levels and mOFC activation. This negative association between ghrelin levels and mOFC activation may suggest that high ghrelin levels reduce right mOFC activation during touch. 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 levels 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 (31). 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, Cassidy and Tong suggested that the hypothalamic hunger system, which is the primary target region for ghrelin (32), influences the reward system in a two-fold way (33). It may increase the salience and value of food rewards, and concurrently decrease the salience and value of non-food rewards, unless non-food rewards are drugs that lead to a strong dopamine release overriding the reduction in salience. 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. Supporting this suggestion, 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 (33, 34). For example, dopamine-deficient mice reduce food intake until they are provided with a dopamine precursor (35). Moreover, food-restricted hungry mice showed enhanced responses and reinforcement to drugs such as amphetamine and cocaine that substantially increase dopamine release, while external administration of satiety hormones reduced the drive to self-administer these drugs (36, 37).

<u>No association between ghrelin levels on subjective experience during touch</u>

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 levels 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.

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. Contrary to the pre-registered hypothesis, the whole-brain analysis showed higher brain activation in the right inferior frontal gyrus and frontal operculum following eating than fasting.

Also pleasantness ratings of all touch velocities were higher 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 therefore have been distracted by hunger-related interoceptive feelings caused by an empty stomach. Another explanation may 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 differed only slightly with nutritional state. Participants reported slightly 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 negative affect scores on brain activation or ratings (see Supplementary Materials, section 3.6).

Advantages of "endogenous" ghrelin variation

One of the strengths of the present study was to induce and utilize intra-individual changes in ghrelin levels caused by fasting and eating. Most previous imaging studies compared intravenous ghrelin administration vs. placebo, and thereby induced short-lasting supra-threshold levels of ghrelin that are often physiologically impossible (46). Ghrelin levels after

external administration were previously associated with enhanced activation in OFC in response to food pictures (1, 31), which was interpreted as enhanced hedonic response to food cues. In contrast, with naturally varying ghrelin levels, the relationship between ghrelin levels 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 variation than external administration in humans.

Conclusion

Naturally circulating ghrelin levels 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 levels seemed to reduce mOFC activation without exercising a direct effect on subjective experience. 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.

Methods

A detailed methods description can be found in Supplementary Materials (section 2). Of the 68 recruited healthy volunteers (20 women, 47 men, 1 undisclosed; mean age: 31.9 years), 60 completed both test sessions. A priori power analysis recommended a sample size of 66 participants to reach 80% power for a middle-sized behavioural effect (d=0.45), but the Covid-19 pandemic did not allow us to reach the planned sample size. Participants gave written informed consent prior to the experiment, which was approved by the Regional Ethics Committee (REK South-East B, project 26699).

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 levels ("liquid-meal" session). In the other test session, participants remained fasted during the experimental procedures which ensured high endogenous ghrelin levels ("no-meal" session). Session order was pseudorandomized and by median four days apart. At the beginning of each session, blood glucose levels 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 S1 for the experimental time line.

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 -80ºC. Active (acylated) ghrelin levels 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).

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 S2.

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 levels 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 condition (very slow/CT-targeted/fast), measurement session (1,2), and mean-centred ghrelin values (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.

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 values (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).

Data availability: All data, code, and materials will be made publicly available via the Open Science Framework upon acceptance. The study was pre-registered (currently under an embargo).

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References

1. A. P. Goldstone, *et al.*, Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *Am. J. Clin. Nutr.* **99**, 1319–1330 (2014).

2. S. M. Bell, R. B. Stewart, S. C. Thompson, R. A. Meisch, Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. *Psychopharmacology (Berl.)* **131**, 1–8 (1997).

3. G. D. Stuber, S. B. Evans, M. S. Higgins, Y. Pu, D. P. Figlewicz, Food restriction modulates amphetamine-conditioned place preference and nucleus accumbens dopamine release in the rat. *Synap. N. Y. N* **46**, 83–90 (2002).

4. M. S. Rad, J. Ginges, Loss of control is not necessary to induce behavioral consequences of deprivation: The case of religious fasting during Ramadan. *Behav. Brain Sci.* **40**, e338 (2017).

5. M. Kojima, *et al.*, Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656–660 (1999).

6. E. Egecioglu, *et al.*, PRECLINICAL STUDY: FULL ARTICLE: Ghrelin increases intake of rewarding food in rodents. *Addict. Biol.* **15**, 304–311 (2010).

7. E. Schéle, D. M. Pfabigan, J. Simrén, U. Sailer, S. L. Dickson, Ghrelin Induces Place Preference for Social Interaction in the Larger Peer of a Male Rat Pair. *Neuroscience* **447**, 148–154 (2020).

8. P. J. Wellman, P. S. Clifford, J. A. Rodriguez, Ghrelin and ghrelin receptor modulation of psychostimulant action. *Front. Neurosci.* **7**, 171 (2013).

9. E. Jerlhag, *et al.*, Requirement of central ghrelin signaling for alcohol reward. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 11318–11323 (2009).

10. E. Jerlhag, Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict. Biol.* **13**, 358–363 (2008).

11. K. P. Skibicka, C. Hansson, M. Alvarez-Crespo, P. A. Friberg, S. L. Dickson, Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* **180**, 129–137 (2011).

12. K. P. Skibicka, C. Hansson, E. Egecioglu, S. L. Dickson, Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict. Biol.* **17**, 95–107 (2012).

13. E. Jerlhag, J. A. Engel, Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. *Drug Alcohol Depend.* **117**, 126–131 (2011).

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

15. L. Kass-Iliyya, *et al.*, The perception of affective touch in Parkinson's disease and its relation to small fibre neuropathy. *Eur J Neurosci* **45**, 232–237 (2017).

16. G. K. Essick, *et al.*, Quantitative assessment of pleasant touch. *Neurosci. Biobehav. Rev.* **34**, 192–203 (2010).

17. C. Triscoli, H. Olausson, U. Sailer, H. Ignell, I. Croy, CT-optimized skin stroking delivered by hand or robot is comparable. *Front Behav Neurosci* **7**, 208 (2013).

18. I. Morrison, *et al.*, Reduced C-afferent fibre density affects perceived pleasantness and empathy for touch. *Brain* **134**, 1116–26 (2011).

19. L. S. Löken, J. Wessberg, I. Morrison, F. McGlone, H. Olausson, Coding of pleasant touch by unmyelinated afferents in humans. *Nat Neurosci* **12**, 547–8 (2009).

20. R. Ackerley, *et al.*, Human C-tactile afferents are tuned to the temperature of a skin-stroking caress. *J Neurosci* **34**, 2879–83 (2014).

21. I. Morrison, ALE meta-analysis reveals dissociable networks for affective and discriminative aspects of touch. *Hum. Brain Mapp.* **37**, 1308–1320 (2016).

22. U. Sailer, *et al.*, Temporal dynamics of brain activation during 40 minutes of pleasant touch. *Neuroimage* **139**, 360–367 (2016).

23. F. Grabenhorst, A. A. D'Souza, B. A. Parris, E. T. Rolls, R. E. Passingham, A common neural scale for the subjective pleasantness of different primary rewards. *Neuroimage* **51**, 1265–74 (2010).

24. A.-K. Kreuder, *et al.*, How the brain codes intimacy: The neurobiological substrates of romantic touch. *Hum. Brain Mapp.* **38**, 4525–4534 (2017).

25. C. McCabe, E. T. Rolls, A. Bilderbeck, F. McGlone, Cognitive influences on the affective representation of touch and the sight of touch in the human brain. *Soc Cogn Affect Neurosci* **3**, 97–108 (2008).

26. T. Maric, F. Sedki, B. Ronfard, D. Chafetz, U. Shalev, A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. *Addict. Biol.* **17**, 613–622 (2012).

27. E. Egecioglu, L. Prieto-Garcia, E. Studer, L. Westberg, E. Jerlhag, The role of ghrelin signalling for sexual behaviour in male mice. *Addict. Biol.* **21**, 348–359 (2016).

28. E. Schéle, *et al.*, Central administration of ghrelin induces conditioned avoidance in rodents. *Eur. Neuropsychopharmacol.* **27**, 809–815 (2017).

29. E. T. Rolls, "The orbitofrontal cortex, amygdala, reward value, and emotion" in *Brain Computations*, (Oxford University Press, 2020) https:/doi.org/10.1093/oso/9780198871101.003.0011 (December 31, 2021).

30. E. T. Rolls, *et al.*, Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. *Cereb. Cortex N. Y. N 1991* **13**, 308–317 (2003).

31. S. Malik, F. McGlone, D. Bedrossian, A. Dagher, Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. *Cell Metab.* **7**, 400–409 (2008).

32. M. A. Cowley, *et al.*, The Distribution and Mechanism of Action of Ghrelin in the CNS Demonstrates a Novel Hypothalamic Circuit Regulating Energy Homeostasis. *Neuron* **37**, 649–661 (2003).

33. R. M. Cassidy, Q. Tong, Hunger and Satiety Gauge Reward Sensitivity. *Front. Endocrinol.* **8** (2017).

34. A. Abizaid, Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J Neuroendocr.* **21**, 787–93 (2009).

35. Q. Y. Zhou, R. D. Palmiter, Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell* **83**, 1197–1209 (1995).

36. D. Zheng, S. Cabeza de Vaca, K. D. Carr, Food restriction increases acquisition, persistence and drug prime-induced expression of a cocaine-conditioned place preference in rats. *Pharmacol. Biochem. Behav.* **100**, 538–544 (2012).

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

38. A. Gentsch, E. Panagiotopoulou, A. Fotopoulou, Active Interpersonal Touch Gives Rise to the Social Softness Illusion. *Curr Biol* **25**, 2392–7 (2015).

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

40. I. Croy, *et al.*, Interpersonal stroking touch is targeted to C tactile afferent activation. *Behav. Brain Res.* **297**, 37–40 (2016).

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

42. S. H. Lockie, T. Dinan, A. J. Lawrence, S. J. Spencer, Z. B. Andrews, Diet-induced obesity causes ghrelin resistance in reward processing tasks. *Psychoneuroendocrinology* **62**, 114–120 (2015).

43. J. Sänger, Can't take my eyes off you – How task irrelevant pictures of food influence attentional selection. *Appetite* **133**, 313–323 (2019).

44. N. S. Stamataki, R. Elliott, S. McKie, J. T. McLaughlin, Attentional bias to food varies as a function of metabolic state independent of weight status. *Appetite* **143**, 104388 (2019).

45. J. K. MacCormack, K. A. Lindquist, Feeling hangry? When hunger is conceptualized as emotion. *Emot. Wash. DC* **19**, 301–319 (2019).

46. N. Kunath, *et al.*, Ghrelin modulates encoding-related brain function without enhancing memory formation in humans. *NeuroImage* **142**, 465–473 (2016).

47. M. C. M. Wever, *et al.*, Associations between ghrelin and leptin and neural food cue reactivity in a fasted and sated state. *NeuroImage* **240**, 118374 (2021).