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Good taste or gut feeling? A new method in rats shows oro-sensory stimulation and gastric distention generate distinct and overlapping brain activation patterns

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Abstract

Satiation is influenced by a variety of signals including gastric distention and oro-sensory stimulation. Here we developed a high-field (9.4 T) functional magnetic resonance imaging (fMRI) protocol to test how oro-sensory stimulation and gastric distention, as induced with a block-design paradigm, affect brain activation under different states of energy balance in rats. Repeated tasting of sucrose induced positive and negative fMRI responses in the ventral tegmental area and septum, respectively, and gradual neural activation in the anterior insula and the brain stem nucleus of the solitary tract (NTS), as revealed using a two-level generalized linear model-based analysis. These unique findings align with comparable human experiments, and are now for the first time identified in rats, thereby allowing for comparison between species. Gastric distention induced more extensive brain activation, involving the insular cortex and NTS. Our findings are largely in line with human studies that have shown that the NTS is involved in processing both visceral information and taste, and anterior insula in processing sweet taste oro-sensory signals. Gastric distention and sucrose tasting induced responses in mesolimbic areas, to our knowledge not previously detected in humans, which may reflect the rewarding effects of a full stomach and sweet taste, thereby giving more insight into the processing of sensory signals leading to satiation. The similarities of these data to human neuroimaging data demonstrate the translational value of the approach and offer a new avenue to deepen our understanding of the process of satiation in healthy people and those with eating disorders.

KEYWORDS

functional magnetic resonance imaging, functional neuroimaging, rats, satiation, stomach, taste

1 | INTRODUCTION

Imbalance between the intake and burning of calories, as in over-consumption, can result in obesity, which is a major health risk in western society. Feeding behavior is tightly regulated and influenced by a variety of internal and external factors, which are processed in the brain (Smeets, Charbonnier, van Meer, van der Laan, & Spetter, 2012). Satiation, leading to ending an initiated meal, is one of

the key processes determining feeding behavior. Satiation is influenced by, among other factors, gastric distention and oro-sensory stimulation (which is the exposure of the oral cavity to sensory cues, including taste) (Blundell & Halford, 1994; Spetter, de Graaf, Mars, Viergever, & Smeets, 2014; Wijlens et al., 2012). Identification of neural correlates of satiation could aid in understanding and eventually handling of disturbances in satiation that lead to overconsumption, obesity, or different types of eating disorders, such as binge eating or bulimia nervosa. However, until now, it has been difficult to assess the specific effects of the different gustatory processes on brain activity and how these processes influence behavior. In a conscious individual, it remains difficult to dissociate responses that are directly affected by the sensory stimuli from those that are an individual's response to that stimulus. A method that permits greater specificity in differentiating gustatory processes would allow further identification of underlying mechanisms. As human studies have technical and ethical limitations in disentangling mechanisms underlying brain responses to orosensory and gastric stimulation, animal studies could provide this information.

Several brain regions, such as the brain stem nucleus of the solitary tract (NTS) and parabrachial nucleus, and the orbitofrontal cortical regions, such as the insula and anterior cingulate cortex, process visceral information that leads to satiation (Beckstead, Morse, & Norgren, 1980; Beckstead & Norgren, 1979; Flynn, 1999; Fussey, Kidd, & Whitwam, 1973; Hurleygius & Neafsey, 1986; Ongür & Price, 2000; Stephan et al., 2003; Terreberry & Neafsey, 1983; Vogt, Finch, & Olson, 1992). Processing of oro-sensory stimulation, including taste, involves partly the same brain regions, and activates the insula, the postcentral gyrus, and the hypothalamus (Gagnon, Kupers, & Ptito, 2015). Besides being involved in processing interoceptive signals from the gut, the anterior insula contains the primary taste cortex and is thus involved in processing gustatory stimulation, such as sweet and bitter taste (de Araujo & Rolls, 2004; Gagnon et al., 2015; Small, 2010; Small et al., 2003). Studies in rodents have demonstrated that the NTS is a major hub that integrates satiation signals (Chambers, Sandoval, & Seeley, 2013). Levels of the anorectic hormone cholecystokinin (CCK), released from the intestines, are strongly associated with satiation, and CCK activates the vagal nerve that projects onto NTS neurons. Higher brain centers project onto and modulate NTS neurons, such that CCK becomes less effective. By this means meal sizes become larger in negative energy balance (Grill, 2010).

During eating, gastric stretch and taste are processed simultaneously. It is not completely understood where these signals converge, whether repeated stimulation gradually builds up a neural signal, and how this contributes to satiation and meal termination (Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001). Gastric distention by itself is sufficient to increase brain activity in reward- and eating behavior-related areas such as the midbrain, hypothalamus, amygdala, and hippocampus (Geeraerts et al., 2011; Spetter et al., 2014; Van Oudenhove et al., 2009; Wang et al., 2008). Simultaneous oro-sensory stimulation and gastric distention activates more areas involved in gustation and reward than gastric distention alone, including the thalamus, amygdala, putamen, and left precuneus (Spetter et al., 2014). However, the separate effects of oro-sensory stimulation and gastric distention and their specific contribution to the process of satiation remain to large extent unknown.

A complicating factor is that the neural response to gustatory stimuli does not only depend on the type of stimulus, but also on hunger and feeding status (Del Parigi et al., 2002; Haase, Cerf-Ducastel, & Murphy, 2009; Haase, Green, & Murphy, 2011; Smeets et al., 2006; Thomas et al., 2015; van Rijn, de Graaf, & Smeets, 2015). Hungry participants have been shown to display a relatively strong neural response to tasting in the insula, thalamus and substantia nigra, while sated participants exhibited lower responses in the parahippocampus, hippocampus, amygdala, and anterior cingulate cortex (Haase et al., 2009). Another study found that the left putamen and left amygdala were more responsive to taste stimuli in a hungry compared to a fed state (Ely et al., 2017). Besides, it has been shown that the median cingulate, ventrolateral prefrontal cortex, anterior insula and thalamus play a key role in tasting calories. This process was found to be dependent on hunger status, as these regions integrate hunger status with stimulus relevance (van Rijn et al., 2015). On the other hand, studies in macaque monkeys did not find an effect of hunger on the neural response to tasting (Rolls, Scott, Sienkiewicz, & Yaxley, 1988; Yaxley, Rolls, Sienkiewicz, & Scott, 1985). This reflects the complications of comparing neural responses to taste and gastric signals under different states of energy balance in conscious animals or study participants.

Because of the complex nature of feeding behavior and eating disorders, intense manipulations are needed in order to, for example, separately assess the effects of different gustatory stimuli or the status of homeostatic energy balance on brain activity, and to assess how these brain activity patterns influence feeding behavior. As outlined above, the neural responses to gustatory stimuli depend on many factors and are confounded by conscious processing and the conscious response of humans to certain stimuli. Therefore, cross-species studies in laboratory settings are of major importance to perform these more intense manipulations. These studies allow to separate effects of gustatory and gastric stimuli without interference of conscious processing, and to relate to neural processing of feeding behavior-related stimuli in humans. Neuroimaging studies in patients with eating disorder continue to show altered responses to food-related stimuli (Frank, 2019; Simon, Stopyra, & Friederich, 2019). A better understanding of how each of these stimuli normally results in a brain response will help to provide a mechanistic understanding of what underlies brain responses to food in patients with eating disorder and healthy controls.

The aim of our study was to develop and apply a novel standardized functional imaging approach in rats, which allows separating the effects of oro-sensory stimulation and gastric distention on activation of satiation-related brain areas. Since the behavioral response to and stress induced by gastric distention and taste would compromise the processing of gastric and oro-sensory stimulation, the experiments were performed in mildly anesthetized animals. By separately assessing the effects of taste stimulation and gastric distention, we aimed to find the neural correlates of the two stimuli, to identify common activated areas, and to determine the influence of status of homeostatic energy balance on the detected neural responses. Based

on the findings in human studies, we hypothesized that taste and gastric distention would result in activation of specific brain regions, including the insula and hypothalamus in response to taste, and the NTS and the midbrain in response to gastric distention.

2 | METHODS

2.1 | Animals

Experiments were approved by the Animal Ethics Committee of the University Medical Center Utrecht, the Netherlands, and were conducted in agreement with Dutch laws ("Wet op de Dierproeven," 1996) and European regulations (Guideline 86/609/EEC).

We used adult male Wistar rats (n = 16, Crl:WU, Charles River, Sulzfeld, Germany), which were housed individually under controlled temperature and humidity conditions, and under a 12-h light/dark cycle (lights on at 7:00 am). Animals had *ad libitum* access to water, and a perspex tube was provided as cage enrichment. Half of the animals (n = 8) had *ad libitum* access to chow, while the other half (n = 8) were food-restricted starting 1 week prior to scanning. Food restriction involved providing 10 g of chow per day until the animal reached 90% of its initial body weight. Body weight was maintained at 90% of the initial weight until magnetic resonance imaging (MRI) was performed. Mean (±*SD*) bodyweight upon arrival of all animals was 240 (±6) g; the food-restricted group (n = 8) had a mean body weight upon arrival of 242 (±7) g, the *ad libitum* group (n = 8) of 239 (±6) g. Mean body weight at time of scanning for the food-restricted group was 296 (±17) g, and for the *ad libitum* fed group 354 (±34) g.

2.2 | Animal preparations and MRI

We conducted *in vivo* MRI measurements on a 9.4-T horizontal bore MR system equipped with a 400-mT/m gradient coil (Agilent). In this setup, we used a homebuilt 90 mm diameter Helmholtz volume coil for signal excitation, and an inductively coupled 25 mm diameter surface coil for signal reception.

Anesthesia was induced with 3–5% isoflurane in O_2/air (1:4) and animals were endotracheally intubated for mechanical ventilation. A homebuilt intragastric (IG) balloon device, constructed from a Portex vinyl rubber tubing (inner diameter 1 mm, outer diameter 2 mm; Smiths Industries, Hythe, Kent, England) and a thin-walled silicone 'extruding balloon' tubing (Dentsleeve BE 2.5) fixed together with silicon paste, was inserted via the esophagus. The balloon was filled with water (at ca. 37°C). For sucrose tasting, two Portex polyethylene tubes (PE90, inner diameter 0.86 mm, outer diameter 1.27 mm; Smiths Industries, Hythe, Kent, England) were placed above the tongue; one was used to flush a 30% sucrose solution over the tongue, which is palatable to rats (La Fleur et al., 2007; La Fleur, Luijendijk, Van Rozen, Kalsbeek, & Adan, 2011; La Fleur, Van Rozen, Luijendijk, Groeneweg, & Adan, 2010), the second tube was used to rinse the oral cavity with water, in order to remove sucrose from the mouth quickly.

After animals were positioned inside the scanner, anesthesia was reduced to 1.5% isoflurane in O_2/air (1:4). We maintained anesthesia at 1.5% during the entire MRI protocol. End-tidal CO_2 was monitored with a capnograph (Microcap, Oridion Medical 1987 Ltd, Jerusalem, Israel). Body temperature was maintained at 37.0 ± 1.0°C.

First, we acquired anatomical images using a balanced steady-state free precession sequence, with four-phase cycling angles (0°, 90°, 180°, 270°), repetition time (TR)/echo time (TE) = 5/2.5 ms, flip angle = 20°, field-of-view (FOV) = $40 \times 32 \times 24$ mm^3, and matrix size = $160 \times 128 \times 96$ voxels (scan time: 10 min). The resulting spatial resolution was 250 μm in all directions.

Subsequently, we executed a block-design functional MRI (fMRI) protocol during which rats underwent gastric distention or oral sucrose flushing sequentially. fMRI data were acquired with a 3D gradient echo EPI sequence. The read-out and first phase-encode dimensions were covered in a single-shot EPI, the second phase-encode dimension used linear phase encoding. We acquired 680 images with an acquisition time of 974.4 ms per volume (total scan time: 11 min and 3 s), TR/TE = 34.8/20 ms, flip angle = 13°, FOV = $36 \times 36 \times 16.8$ mm^3 and matrix size = $60 \times 60 \times 28$ (isotropic spatial resolution = 600 μm). During fMRI acquisition the IG balloon was temporarily inflated with 5 ml of water for five consecutive times during 50 s each time to induce gastric distention (IG balloon inflation was done in the first 5 s, deflation in the last 5 s of this period). The volume of balloon inflation was based on a postmortem assessment of gastric volume and on another study in which gastric distention was performed in rats (Min, Tuor, & Chelikani, 2011). Each inflation period was followed by a rest period of 60 s during which the IG balloon was in a deflated state. fMRI during oral sucrose flushing was executed with a comparable block-design paradigm. An amount of 1.8 ml sucrose was flushed through the mouth over a period of 40 s, followed by a rinse with water (1 ml in 10 seconds) and a rest period of 60 s. This was repeated five times. The two fMRI paradigms were executed 10 min after each other as outlined in Figure 1a.

After MRI acquisition, we checked whether the tubes, through which sucrose and water were flushed, were still correctly positioned in the rat's mouth. In all rats, the mouth tubes were correctly placed. The rats were euthanized by an overdose of isoflurane, followed by intracardial perfusion-fixation with cold 0.1 M phosphate-buffered saline (PBS) first and 4% paraformaldehyde dissolved in PBS thereafter. Postmortem inspection of IG balloon placement demonstrated that all balloons were correctly positioned inside the stomach.

2.3 | MRI data processing and analysis

2.3.1 | Anatomical MRI

Nonuniformity correction was performed on anatomical images using *n3*, and brain masks were obtained by applying the *Brain Extraction Tool* to the anatomical images, both as provided by *FSL* (FMRIB's Software Library, http://fmrib.ox.ac.uk/fsl, Version 5.0.9). The individual anatomical images were masked and registered to an anatomical MRI

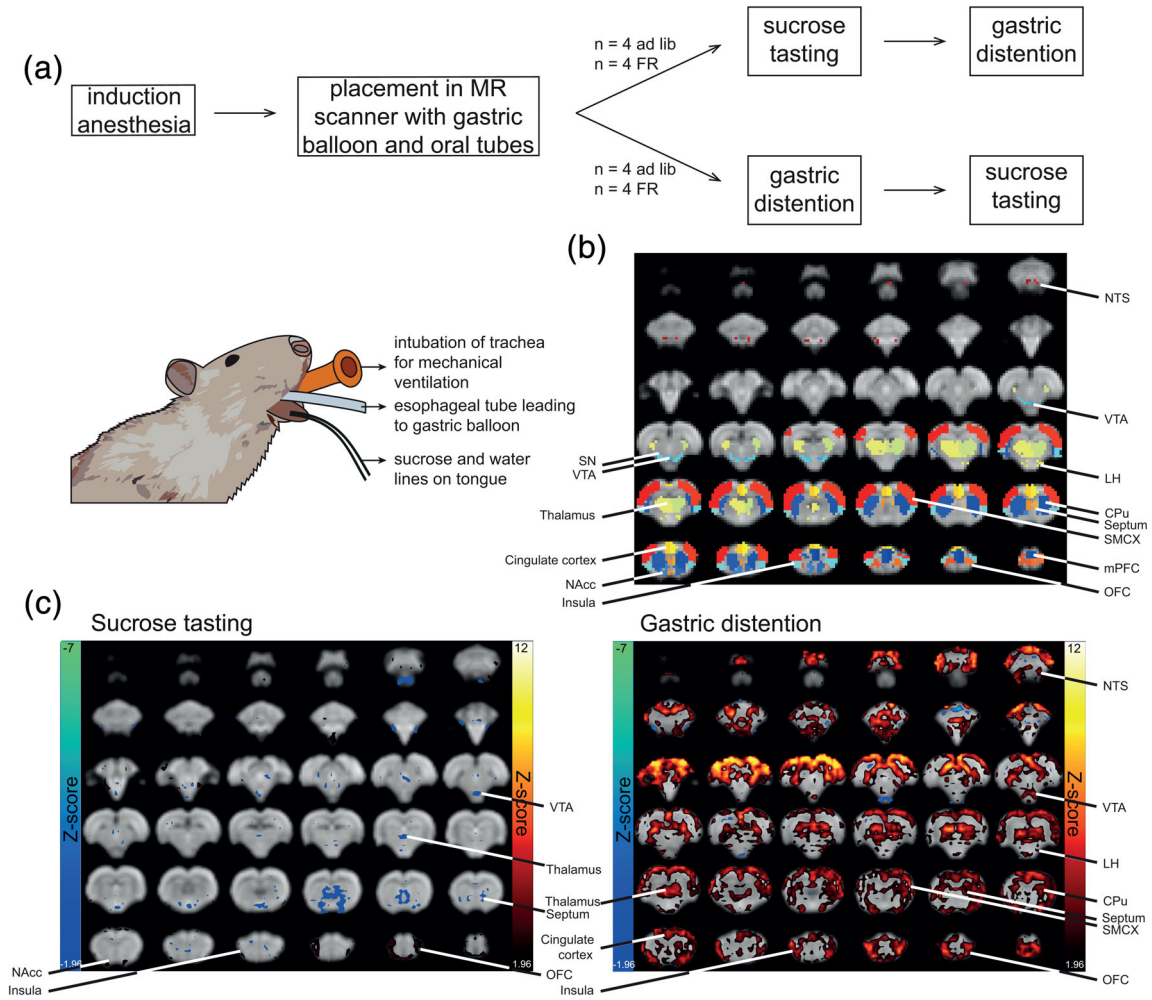

FIGURE 1 Experimental overview and whole-brain pattern of activation to sucrose tasting and gastric distention. (a) Overview of experimental protocol and setup. (b) Regions of interest overlaid on fMRI template. (c) Whole-brain group average activation patterns in response to sucrose tasting (left) and gastric distention (right). *Z*-scores represent FDR-corrected values. Significantly activated regions are shown in hot colors; significantly deactivated regions are shown in cold colors. CPu, caudate putamen; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens; NTS, nucleus of the solitary tract; OFC, orbitofrontal cortex; SMCX, sensorimotor cortex; SN, substantia nigra; VTA, ventral tegmental area [Color figure can be viewed at wileyonlinelibrary.com]

template that was matched to a 3D model of a rat brain atlas (Paxinos & Watson, 2007) using the affine intermodal image registration tool *FLIRT* (FMRIBs Linear Image Registration Tool, v6.0). Coregistered anatomical images were averaged to acquire an anatomical template in atlas space. The original individual anatomical images were registered to this anatomical template using *FLIRT* followed by *FNIRT* (FMRIBs Nonlinear Image Registration Tool, build 508). Inverse coefficients were calculated to register regions of interest (ROIs) from atlas space to individual anatomical space.

2.3.2 | Functional MRI

All block-design fMR images were corrected for subject motion using *MCFLIRT* and image intensity nonuniformity correction was performed using *n3* (solely for registration purposes), both as provided by *FSL*. Using *FLIRT*, fMRI images were registered to the same fMRI image of a single representative rat. Coregistered images were averaged to create a template specific for each fMRI paradigm (i.e., gastric distention and sucrose tasting). These templates were made to obtain proper brain masks that excluded any tissue outside the brain from analyses. Therefore, we applied the *Brain Extraction Tool* from *FSL* to the templates, registered the individual data to the templates using *FLIRT* and calculated the inverse coefficients, to be able to register the brain masks from template space to individual fMRI space.

fMRI data were normalized to the baseline (corresponding to the first 90 MR volumes) using *FSLMATHS* from *FSL*. In order to create a general fMRI template, that is, a template for all block-design fMRI data of this experiment, on which a two-level generalized linear model (GLM)-based analysis could be performed, individual fMRI data were registered to a representative fMRI dataset (from a single animal's sucrose flushing experiment) using *FLIRT* followed by *FNIRT*. All

coregistered fMRI images were averaged to create this general fMRI template. Individual normalized fMRI data were again registered to this template using *FLIRT*, to ensure the same amount of registration deformations per data set. On first level of the GLM-based analysis we analyzed the individual normalized fMRI data comparable to the approach as described in (Mandeville, Liu, Vanduffel, Marota, & Jenkins, 2014). For this analysis a block-design function with 40 (for sucrose tasting) or 50 (for gastric distention) seconds on-periods and 70 (for sucrose tasting) or 60 (for gastric distention) seconds off-periods, convolved with a hemodynamic response function (HRF), was used as a regressor. This resulted in whole-brain activation maps (*Z*-maps) for each animal for both fMRI paradigms. Subsequently, we assessed the differences in brain responses to sucrose tasting and gastric distention on the group level by comparing the specific *Z*-maps resulting from the subject level analysis in a GLM-based analysis. We also compared the *Z*-maps of the hungry and satiated groups to assess possible effects of status of homeostatic energy balance on brain activation responses to the treatments. False discovery rate (FDR)

TABLE 1 Brain regions exhibiting significant positive or negative BOLD responses during sucrose tasting or gastric distention

Treatment	Activated regions	*Z*-score	Activated part of ROI (%)	Deactivated regions	*Z*-score	Deactivated part of ROI (%)
Sucrose tasting	Insula left	2.5	17	Septum left	−2.4	30
	Insula right	2.6	30	Septum right	−2.3	17
	OFC left	2.5	38	Thalamus left	−2.1	4
	OFC right	2.6	30	Thalamus right	−2.2	4
	NAcc left[a]	2.8	6	Voxels caudal to the VTA		
	NAcc right[a]	3.1	6			
	VP left[a]	2.1	9			
	VP right[a]	–	0			
	NTS left	2.1	22			
	NTS right	–	0			
Gastric distention	Insula left	3.8	86	Voxels in/around pontine nucleus		
	Insula right	4.2	81			
	OFC left	4.2	85			
	OFC right	4.2	85			
	Cingulate cortex left	3.9	74			
	Cingulate cortex right	4.0	88			
	CPu left	3.4	83			
	CPu right	3.2	64			
	LH left	3.4	46			
	LH right	3.3	50			
	Septum left	2.7	50			
	Septum right	2.9	55			
	Thalamus left	4.1	84			
	Thalamus right	4.1	75			
	Sensorimotor cortex left	3.9	78			
	Sensorimotor cortex right	3.7	59			
	VTA left	3.7	60			
	VTA right	2.7	43			
	NTS left	4.0	78			
	NTS right	4.2	69			
	Caudal cortical areas, like the RSC					

Note: *Z*-scores are calculated as mean FDR-corrected *Z*-score across significantly (de)activated voxels within regions of interest. A *Z*-score of (−)1.96 corresponds to a *p* value of .05.

Abbreviations: BOLD, blood oxygenation level-dependent; CPu, caudate putamen; LH, lateral hypothalamus; NAcc, nucleus accumbens; NTS, nucleus of the solitary tract; OFC, orbitofrontal cortex; ROIs, regions of interest; RSC, retrosplenial cortex; VP, ventral pallidum; VTA, ventral tegmental area.

[a]Activation was detected at the border of the NAcc and VP; when these areas are considered as one ROI the left NAcc-and-VP had a mean *Z*-score of 2.6 and 7% of the total voxels in this region was significantly activated; the right NAcc-and-VP had a mean *Z*-score of 3.1 in 4% of the voxels.

correction for multiple testing was performed on first-level (when shown separately) and second-level GLM results, and an FDR-corrected *Z*-value of (–)1.96, corresponding to a *p* value of .05, was taken as cutoff value for activation maps.

To calculate mean blood oxygenation level-dependent (BOLD) signal intensity over time per ROI, the inverse coefficients for the above described registration from individual anatomical space to anatomical template space were applied to register the ROIs to individual anatomical space. Individual fMRI data were registered to individual anatomical images using *FLIRT*, the inverse coefficients were calculated, and individual anatomical images were then registered to individual fMRI images using *FNIRT*. These coefficients were applied to the ROIs for registration to individual fMRI space. Mean signal intensity was calculated per ROI from normalized fMRI data. ROIs

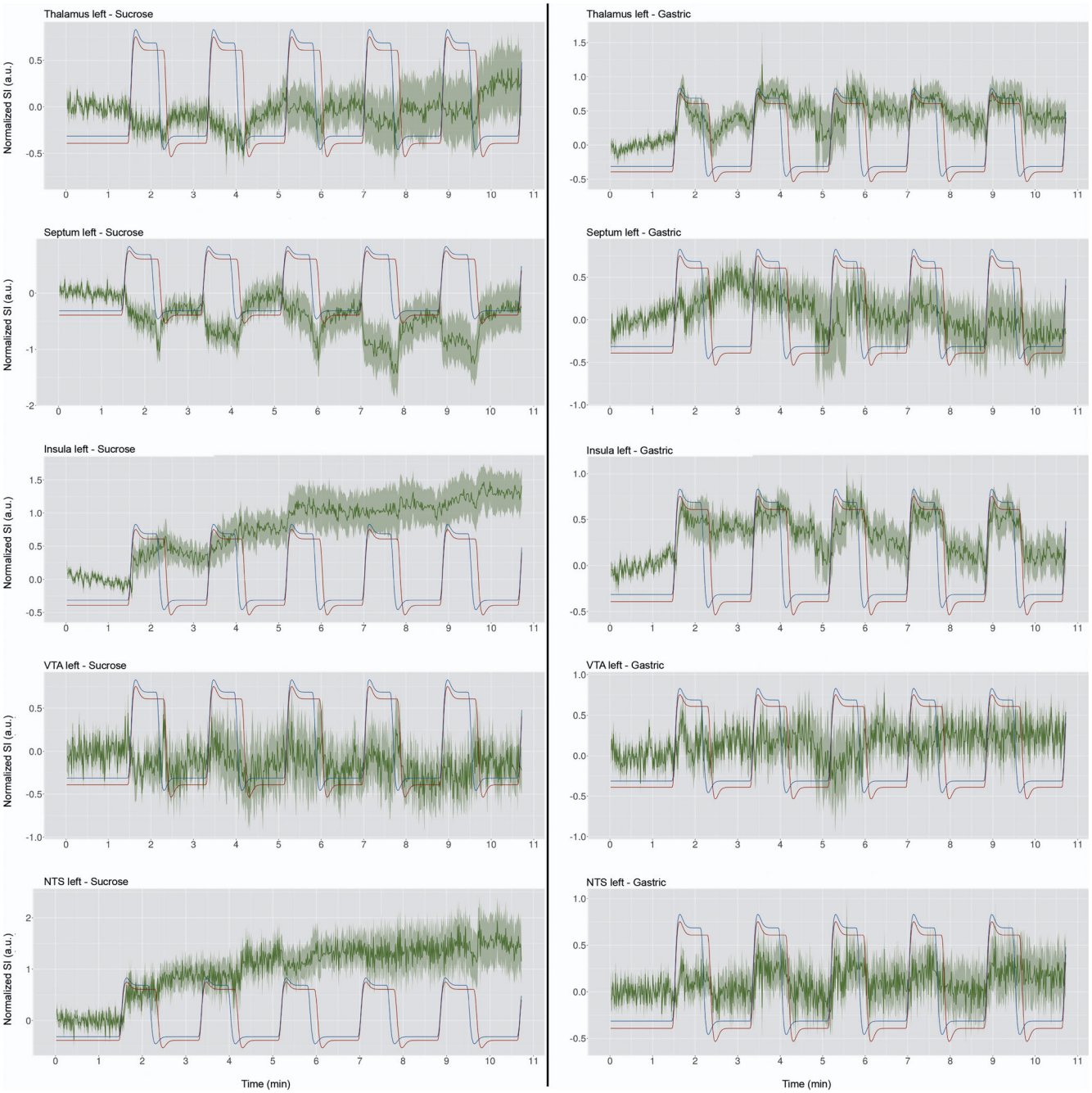

FIGURE 2 Mean BOLD responses to sucrose tasting (left) and gastric distention (right). Normalized BOLD signal time-courses are shown for different ROIs, displayed over the entire scan period. The blue line represents the HRF for the sucrose tasting paradigm; the red line represents the HRF for the gastric distention paradigm. Difference in the downward phases of both regressors is time needed to flush the mouth with water after a sucrose tasting. a.u., arbitrary units; BOLD, blood oxygenation level-dependent; HRF, hemodynamic response function; NTS, nucleus of the solitary tract; ROIs, regions of interest; SI, signal intensity; VTA, ventral tegmental area [Color figure can be viewed at wileyonlinelibrary.com]

extracted from two rat brain atlases (Paxinos & Watson, 2007; WaxholmSpace, 2014) included the left and right medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), cingulate cortex, insula, nucleus accumbens (NAcc), caudate putamen (CPu), septum, sensorimotor cortex, thalamus, lateral hypothalamus (LH), ventral tegmental area (VTA), substantia nigra (SN), and nucleus of the solitary tract (NTS) (Figure 1b).

3 | RESULTS

3.1 | No effect of status of homeostatic energy balance on brain responses to sucrose tasting or gastric distention

To measure brain responses to tasting (sucrose flushing over the tongue) and gastric distention (water-filled IG balloon inflation), we developed and performed a block-design fMRI protocol in 16 rats that received both treatments sequentially and that were either fed *ad libitum* or food restricted (Figure 1a). Due to artefacts in the fMRI data (probably Nyquist N/2 ghosts) resulting from technical issues, three animals had to be excluded from all analyses. Final group sizes were: food-restricted group, $n = 6$; *ad libitum* fed group, $n = 7$. We performed a two-level GLM-based analysis. On the first level, we calculated individual whole-brain activation maps (Z-maps) per treatment, and on second level we assessed the differences in brain responses to the two treatments as well as the differences in responses between food-restricted and *ad libitum* fed rats. The latter part of the second level GLM-based analyses revealed no significant differences in activation responses between food-restricted and *ad libitum* fed rats (Figure S1) Therefore, we pooled the data from both groups for further analyses.

3.2 | Neural responses to oro-sensory stimulation and gastric distention

Group average brain activation patterns in response to sucrose tasting and gastric distention are shown in Figure 1c. Significantly activated or deactivated brain regions are listed in Table 1. Sucrose tasting induced brain activation in the anterior region of the insular cortex, the OFC, the NTS, and in voxels at the border of the NAcc and ventral pallidum (VP) (Figure 1c, left panel; Table 1). Negative BOLD responses were detected in the septum, the central region of the thalamus, and in voxels right below the VTA. Gastric distention led to brain activation in the insular cortex, OFC, cingulate cortex, CPu, LH, septum, thalamus, sensorimotor cortex, VTA, caudal cortical areas, for example, the retrosplenial cortex (RSC), and NTS (Figure 1(c), right panel; Table 1). Some voxels in and around the pontine nucleus (caudal to the VTA) showed a negative BOLD response upon gastric distention.

Comparison of the responses to the two treatments with a second-level GLM-based analysis showed that the insular cortex, cingulate cortex, CPu, LH, thalamus, some parts of the sensorimotor cortex, VTA, RSC, and some NTS voxels were more significantly activated in response to gastric distention than to sucrose tasting (Figure S2). There were no brain areas exhibiting a significantly stronger response to sucrose tasting as compared to gastric distention.

Figure 2 displays the normalized BOLD signal time course in ROIs in which we detected significant activation or deactivation in response to sucrose tasting or gastric distention. In response to gastric distention, BOLD signal intensity in the left thalamus increased upon inflation of the balloon and (partially) recovered upon deflation. Comparable response patterns to gastric distention were detected in the insula and NTS. On the contrary, in response to sucrose tasting, we detected a decrease in signal intensity in the thalamus, followed by recovery after water flushing. In the septum we found a similar but stronger negative correlation of the BOLD response to sucrose tasting, which was more prominent during water flushing (which was done in the time frame between the downward phases of the two regressors in Figure 2). Although we did not find a significant correlation to the regressor in the GLM-based analysis in the insula nor the NTS in response to sucrose tasting (Figure 1c), we did detect a considerable gradual BOLD signal elevation in these ROIs; signal intensity started to increase with the first sucrose tasting challenge, further increased upon the second and third sucrose tasting challenges, and remained elevated during the rest of the scan period. Although the signal in the VTA was more affected by noise than the other ROIs, and the response to the first sucrose tasting challenge was, if anything, negative, we detected an increase in mean signal intensity in response to the second, third, and fourth sucrose tasting challenge in the VTA. However, the GLM-based analysis in voxels right below the VTA revealed a negative response (Figure 1c). We found no significant VTA response to gastric distention (Figure 2). Signal intensity time courses for all left and right ROIs are shown in Figure S3.

4 | DISCUSSION

In this study, we developed and applied a new method that enables functional neuroimaging during oral and gastric sensory stimulation in rats in a single experimental setting. fMRI during manipulation of the gastric environment in rats has already been proven feasible (Min, Tuor, & Chelikani, 2011; Min, Tuor, Koopmans, & Chelikani, 2011), but to the best of our knowledge this had not yet been accomplished together with a taste stimulation paradigm in a single experimental setting in rats. This novel method enabled us to differentiate the effects of different gustatory processes, and to assess the effects of these different stimuli on brain activity. Thereby, this method could also contribute to the elucidation of mechanisms underlying different aspects of consumption. Direct comparison of the brain activation patterns in response to sucrose tasting and to gastric distention enabled us to distinguish specific contributions of both factors to satiation-related neural signaling, as outlined below. Our findings align with what we hypothesized (activation in specific brain regions: the insula and hypothalamus in response to taste, and the NTS and midbrain in response to gastric distention), but are more encompassing and complex in nature, as described below. Furthermore, our findings

are in agreement with results from human studies, but are now also reported for rats, thereby allowing cross-species comparison of neural mechanisms underlying feeding behavior. The findings also provide novel insights in the involvement of brain regions in processing oro-sensory and gastric information, like the involvement of mesolimbic regions in the processing of both sweet taste and gastric distention. Also, the gradual increase in signal intensity as detected in the NTS and insula provides mechanistic information on the processing of sensory signals leading to satiation.

Our study demonstrates that several brain regions are activated upon oro-sensory and gastric stimulation in anaesthetized rats, which underscores that these areas are activated in the absence of consciousness. Our data indicate that gastric distention and taste engage distinct neural circuits. Gastric distention resulted in activation of the brainstem (NTS) and midbrain, among others, and taste induced activation in the hypothalamus and insula, among others. This implicates these areas in satiation. As bulimia nervosa patients may have a deficit in satiation (Keel et al., 2018), we speculate that neuroimaging studies could aid in further understanding of disease mechanisms, through assessment of activation in the brainstem and midbrain in patients and controls in response to experimental gastric distention. Furthermore, in patients with anorexia nervosa, who may have altered taste processing (Kot, Kucharska, Monteleone, & Monteleone, 2020), neuroimaging studies could aid in elucidating the role of hypothalamic and insular activation in response to taste.

In accordance with the literature, we detected activation in the anterior part of the insular cortex upon sucrose tasting (de Araujo & Rolls, 2004; S. Frank, Kullmann, & Veit, 2013; Gagnon et al., 2015; Rolls, 2006; Small, 2010; Small et al., 2003; Small & Prescott, 2005; Turner, Byblow, Stinear, & Gant, 2014). In a human fMRI study with healthy volunteers (de Araujo & Rolls, 2004), significant activation upon sucrose tasting was detected in the anterior insular (putative primary taste) cortex. Other studies (Gagnon et al., 2015; Small et al., 2003; Spetter, Smeets, de Graaf, & Viergever, 2010; Turner et al., 2014) also detected activation in the middle and anterior parts of the insula upon sweet taste. In our rat study, we found that the insula became activated in response to the first sucrose stimulation, and subsequent mouth rinses with sucrose had an additive effect on insula activation, as seen from a regional rise in BOLD signal intensity. However, it must be noted that the gradual increase in signal intensity in the insular cortex did not clearly covary with the manipulation, which might suggest that this was induced by residual sucrose that was not washed out with the 10-s rinses of water. However, we think this is unlikely as other brain areas did not show this effect and responded according to the block-designed treatment paradigm. We speculate that the rising response in the anterior insula, which contains the primary taste cortex, reflects lasting neuronal activation that builds up with reexposure to sucrose. Perhaps, this neural correlate contributes to an increase in satiation by longer oral exposure to food or taste (Bolhuis et al., 2014; Lasschuijt et al., 2017; Zijlstra et al., 2009; Zijlstra, Mars, De Wijk, Westerterp-Plantenga, & De Graaf, 2008). We found a comparable gradual increase in BOLD signal intensity upon sucrose tasting in the NTS, which is also known to be involved in processing visceral signals and thereby contributing to the process of satiation. The NTS is known as a major hub that integrates satiation signals (Chambers et al., 2013). Therefore, even though the rise in BOLD signal intensity did not clearly correlate with the manipulation, its activation in the current study can reasonably be explained by lasting neuronal activation.

Since sucrose tasting is rewarding for rats, we also expected a response in the VTA, which is an essential part of the reward system. Although the activation maps revealed a negative correlation between stimulus presentation and BOLD signal intensity in some voxels caudal to the VTA, the mean signal time course in the whole VTA demonstrated activation responses to individual taste challenges. The difference in findings between the whole-brain voxel-based GLM analysis and the ROI analysis may be explained by the difference in spatial dimensions of the two analyses: whereas the ROI analyses encompassed the mean response for the entire VTA region, the voxel-based GLM analysis allowed for detection of sub regional (de)activation at the single voxel level.

The detected activation in some voxels at the border of the NAcc and VP in response to sucrose tasting is in line with an activated VTA. The VTA projects directly to the NAcc, as part of the mesolimbic projection. We speculate that upon sucrose-induced activation of the mesolimbic projection, dopamine is released in the NAcc, which binds to inhibitory D2 receptors on local GABAergic neurons that project to the VP. Consequent disinhibition would lead to activation of the VP (Humphries & Prescott, 2010; Kenny, Voren, & Johnson, 2013; Russo & Nestler, 2013).

We also observed deactivation of the septum upon sucrose tasting, which aligns with findings from Sweeney and Yang (Sweeney & Yang, 2016), who showed that chemogenetic or optogenetic inhibition of the septum increased food intake. Thus, perhaps food intake and consequent tasting deactivate the septum and its inhibitory action in a feedback loop.

We detected significant activation in the NTS, which is known to be involved in the processing of visceral signals, in response to gastric distention. This effect was stronger as compared to sucrose-induced activation of the NTS. Min and colleagues previously reported that gastric distention increases BOLD signal in regions such as the NTS and hypothalamus (Min, Tuor, & Chelikani, 2011). Furthermore, they detected activation in the hippocampus, amygdala, thalamus, cerebellum, and the cingulate, insular and motor and sensory cortices. We detected a comparable activation pattern, with additional activation in the OFC, cingulate cortex, dorsal and ventral striatal regions, VTA, and caudal cortical areas. Also in human studies, similar areas were found to be activated in response to gastric distention: Wang et al. detected activation in the sensorimotor cortices, right insula, left posterior amygdala, left posterior insula and left precuneus in response to gastric balloon inflation (Wang et al., 2008). Spetter et al. found that gastric distention with a liquid increased brain activity in reward- and eating behavior-related areas such as the midbrain, hypothalamus, amygdala, and hippocampus (Spetter et al., 2014). Other studies reported activation in the dorsal brain stem, the left inferior frontal gyrus, the bilateral insula, and the right subgenual anterior cingulate

cortex in response to gastric distention (Stephan et al., 2003); activation in the right insula in response to balloon-induced distention (Ly et al., 2017); and activation in the opercular part of the inferior frontal gyrus upon manipulation of gastric content volume (Camps, Veit, Mars, de Graaf, & Smeets, 2018) or upon gastric stimulation with a liquid meal after 36 h of fasting (Del Parigi et al., 2002).

In addition to activation in orbitofrontal regions and insular regions, analogous with findings in humans, our study in rats exhibited gastic distention-induced responses in mesolimbic areas like the VTA, NAcc, and CPu. These areas are known to be involved in motivation- and reward-related behaviors (Humphries & Prescott, 2010; Russo & Nestler, 2013) and may therefore be associated with the rewarding effects of a full stomach.

Because of the use of anesthesia, gastric distention should have been a neutral stimulus to the animals in our study. Inflation of a gastric balloon with 5 ml may be aversive to awake rats, however, since animals in our study were under anesthesia we can reasonably exclude consious processes of possible aversion. Moreover, balloon inflation of 5 ml is within the phsyiological range of gastric distention, since the volume was based on postmortem assessment of gastric volume in a pilot animal as well as on a previous study in which gastric balloon inflation was performed under comparable conditions (Min, Tuor, & Chelikani, 2011). In the latter study, gastric balloons were inflated with a higher volume, that is 8 ml of saline, but at a slower rate (2 ml/min during 4 min).

In contrast to human fMRI studies (Ely et al., 2017; Haase et al., 2009, 2011; Smeets et al., 2006; Thomas et al., 2015; van Rijn et al., 2015), but in line with early macaque studies (Rolls et al., 1988; Yaxley et al., 1985), we did not detect differences in the response to tasting (or gastric distention) between *ad libitum* fed and food-deprived animals. In another cohort of animals we have tried to assess differences in the responses to gustatory stimuli between *ad libitum* fed and food-deprived animals with a similar protocol as used in human literature, that is, involving assignment to a fasted of fed state immediately preceding the experiment instead of a manipulation of energy balance for 1 week. With this protocol that was more comparable to protocols used in the human literature, we did not detect any differences between fasted or fed rats. Unfortunately, data from that measurement were inconclusive. Our measurements in the current study may have lacked sensitivity to detect possible subtle differences in status of homeostatic energy balance. Moreover, in our study rats were mildly anesthetized, which would obscure potentially augmenting effects of awareness of physiological state.

The need for anesthesia may appear as a limitation, however, the alternative of scanning awake rats with a paradigm as used in the current study comes with more limitations, such as confounding effects of consciousness on the responses to either stimuli; stress induced by the scanning procedure itself or by the stimuli; and motion artefacts. Yet, it is important to note that isoflurane anesthesia may have influenced the responses to tasting and gastric distention differently, as full taste sensation may rely stronger on conscious awareness. To our knowledge there are currently no studies investigating the possible different influences of anesthesia on taste and gastric distention responses.

The sample size of the current study was relatively small, which may have limited the power to validly identify differences in brain activity between taste and gastric distention. Due to the unfortunate drop out of three animals, our sample size decreased further. However, even with this relatively low number of animals per group, the power was still high enough to detect (at least some of) the effects of taste and gastric distention, which were found to be comparable to human data.

Another limitation of this study is that we only assessed brain responses to gastric distention using a gastric balloon. Studies have shown that gastric distention is different from gut nutrient sensing, and as a consequence brain patterns in response to gastric balloon distention are different from those in response to actual feeding, thus nutrients entering the stomach. A difficulty with the latter is that a block-design paradigm is impossible when food is entering the stomach, which has to be emptied first. Using a balloon, we were able to scan animals using the same block-design for gastric distention as well as for sucrose tasting. In a follow-up study it should be investigated how brain responses differ between different paradigms of gut stimulation.

In conclusion, we developed and applied a translational fMRI approach that can be used to study feeding-related neural signals in rats under standardized and controlled settings. The detected patterns of brain activation in response to oro-sensory stimulation and gastric distention aligned well with findings from human neuroimaging studies (e.g., Small et al., 2001; Spetter et al., 2014), and uncovered additional brain regions involved in processing taste and gastric distention signals. Future studies may use the described neuroimaging approach to assess the effects of manipulations of specific satiation pathways to further unravel the role of these pathways and elucidate mechanisms underlying regulation of satiation. This may, for example, involve chemo- or optogenetics, simultaneous oro-sensory and gastric stimulation, or manipulations of feeding and hunger status. We suggest that clinical studies, using a similar neuroimaging approach, should further dive into the role of specific brain regions in altered satiation in patients with eating disorders, such as binge eating, anorexia nervosa, or bulimia nervosa. Thereby, this type of functional imaging methodology may ultimately aid in the identification of therapeutic targets and establishment of intervention strategies for eating disorders and the development of novel strategies to reduce overconsumption and prevent obesity.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Theresia J. M. Roelofs, Rick M. Dijkhuizen, and Roger A. H. Adan designed the experiments. Theresia J. M. Roelofs, Mieneke C. M. Luijendijk, and Annette van der Toorn performed the experiments. Theresia J.M. Roelofs analyzed the data. Guido Camps, Paul A. M. Smeets, Rick M. Dijkhuizen, and Roger A. H. Adan inputted on data interpretation and the manuscript. Theresia J.M. Roelofs, Rick M. Dijkhuizen, and Roger A. H. Adan wrote the manuscript. All authors reviewed the article.

DATA AVAILABILITY STATEMENT

The data sets generated during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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