Neural correlates of habituation to taste stimuli in healthy women

Angela Wagner a,b, Howard Aizenstein a, Guido K. Frank a,c, Jennifer Figurski a, J. Christopher May d,e, Karen Putnam f, Lorie Fischer a, Ursula F. Bailer a,g, Shannan E. Henry a, Claire McConaha a, Victoria Vogela a, Walter H. Kayea,⁎

⁎ Corresponding author. University of Pittsburgh, Western Psychiatric Institute and Clinic, Iroquois Building, Suite 600, 3811 O’Hara Street, Pittsburgh, PA 15213, United States. Tel.: +1 412 647 9845; fax: +1 412 647 9740.
E-mail address: kayewh@upmc.edu (W.H. Kaye).

aUniversity of Pittsburgh, School of Medicine, Department of Psychiatry, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, United States
bUniversity of Mannheim, Central Institute of Mental Health, Department of Child and Adolescent Psychiatry, Mannheim, Germany
cUniversity of California, San Diego, Department of Psychiatry, La Jolla, California, United States
dDepartment of Psychology, University of Pittsburgh, PA, United States
eCenter for the Neural Basis of Cognition, University of Pittsburgh, PA, United States
fUniversity of Cincinnati, School of Medicine, Department of Environmental Health, Division of Epidemiology and Biostatistics, Cincinnati, Ohio, United States
gMedical University of Vienna, Department of General Psychiatry, University Hospital of Psychiatry, Vienna, Austria

Received 14 January 2005; received in revised form 30 August 2005; accepted 8 November 2005

Abstract

Recent studies show that specific regions of the cortex contribute to modulation of appetitive behaviors. The purpose of this study was to determine whether neural response in these regions changes over time when a taste stimulus is administered repeatedly. Such a paradigm may be useful for determining whether altered habituation contributes to disturbed eating behavior. This study used a programmable syringe pump to compare administration of a 10% sucrose solution to distilled water in 11 healthy female subjects using functional magnetic resonance imaging. The stimuli were presented in either a sequential or pseudorandom order. An a priori ‘Region of Interest’ (ROI) based analysis method was used, with ROIs defined in the prefrontal cortex, insula, amygdala, and hippocampus. To test habituation, activation during the first half of each block was compared with activation during the second half. For the pseudorandom blocks, subjects showed habituation in almost all ROIs to water, but in none to sucrose. By contrast, for sequential blocks, both stimuli produced habituation in taste-related brain regions. These data suggest that habituation patterns in healthy subjects may depend on frequency and regularity of stimulus administration.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Taste; Habituation; fMRI; Amygdala; Insula; Eating disorder

1. Introduction

Individuals with anorexia nervosa (AN) and bulimia nervosa (BN) show behavioral disturbances which are categorized as “eating disorders”. However, it is not known whether there is a primary disturbance of appetite regulation, taste perception or habituation, or disturbed feeding behavior secondary to anxiety. The
current study was designed to identify habituation aspects of taste in healthy women, as part of a long term goal of identifying primary disturbances of eating disorders.

Recent studies have shown that specific regions of the cortex contribute to the modulation of appetitive behaviors. Studies in non-human primates suggest that the anterior insula and adjacent frontal operculum are the primary taste cortex while the caudolateral orbitofrontal cortex (OFC) is part of secondary taste cortical areas (Scott et al., 1986; Ogawa et al., 1989; Rolls et al., 1990; Yaxley et al., 1990). Studies in healthy humans using either positron emission tomography (PET) (Kinomura et al., 1994; Small et al., 1997; Zald et al., 1998; Frey and Petrides, 1999) or functional magnetic resonance imaging (fMRI) (Faurion et al., 1999; Francis et al., 1999; O’Doherty et al., 2001; De Araujo et al., 2003; Kringelbach et al., 2003) support these findings. In addition, other brain regions involved in gustatory processing have been described. The amygdala might play a role in the discrimination of a novel and aversive stimulus (Small et al., 1997; Zald et al., 1998, 2002; O’Doherty et al., 2002; Small et al., 2003). Other studies have found an activation in the cingulate cortex (Kinomura et al., 1994; Zald et al., 1998; De Araujo et al., 2003; De Araujo and Rolls, 2004). Neural activity was also described in the dorsolateral prefrontal cortex (PFC) (Gautier et al., 1999; Tataranni et al., 1999; Kringelbach et al., 2004).

The imaging studies described above used a variety of food administration techniques to assess taste perception and reward. For example, sweet, salty or bitter unimodal taste stimuli or multimodal taste stimuli such as tomato juice or chocolate milk were contrasted with a neutral stimulus (25 mM KCl and 2.5 mM NaHCO₃) (De Araujo et al., 2003; Kringelbach et al., 2003; De Araujo and Rolls, 2004) or water (Faurion et al., 1999). In comparison, imaging studies in AN and BN have only used pictures of food (Ellison et al., 1998; Gordon et al., 2001; Uher et al., 2004). Whether pictures of food are a relevant challenge for understanding the pathophysiology of eating disorders is unknown. Thus, our laboratory developed a standardized paradigm (Frank et al., 2003) to assess brain activation using fMRI and blind administration of pure macronutrient solutions. In the present study two taste stimuli, sucrose and water, were chosen to investigate induced neuronal activation in healthy women with an a priori region-of-interest (ROI) approach, with ROIs defined in the prefrontal cortex, medial OFC, insula, amygdala, and hippocampus.

Pathologic eating behaviors vary from severe food restriction in AN to overeating in BN. Theoretically, such alterations could be related to altered modulation of satiety or habituation. In other words, we speculate that feeding may elicit exaggerated modulation of satiety or habituation. In other words, we speculate that feeding may elicit exaggerated satiety or habituation. It is possible that sensory-specific satiety (Rolls et al., 1981), has been shown to have neural correlates. Kringelbach et al. (2003) reported a cluster of voxels in the OFC that showed a decrease in activation specific to the particular food consumed. It is also important to note that individuals with AN and BN tend to have stereotypic and monolithic eating patterns in which the same foods are consumed every day, particularly when restricting or binge eating. It is possible that sensory-specific satiety reflects habituation to specific taste stimuli. Thus the purpose of the study was to devise a paradigm that assessed habituation to the same taste stimulus. To test this, healthy subjects were administered the same stimulus 20 times in a row to determine whether they habituated to the same stimulus. This is compared to a more common design in which the stimuli alternate in a pseudorandom order.

2. Methods

2.1. Sample

Eleven healthy female volunteers were recruited through local advertisements. They were 28.6±6.75 years old and had a current Body Mass Index of 22.06±2.67 kg/m². They had no history of any psychiatric, medical or neurological illness. Participants had no first-degree relative with an eating disorder. They had normal menstrual cycles, had been within normal weight range since menarche, and were not on medication, including herbal supplements.

This study was conducted according to the institutional review board regulations of the University of Pittsburgh, and all subjects gave written informed consent. The MR imaging was performed during the first 10 days of the follicular phase for all subjects. The follicular phase was determined by history. The MR study was done at 9:00 am. All subjects had the same standardized breakfast on the morning of the study. This design was chosen to standardize the subjects’ state of satiety.

2.2. Experimental paradigm

The paradigm was based on a task developed in our group and described previously (Frank et al., 2003). In
the present task two different stimuli were used: 10% sucrose (Fisher) and distilled water. These solutions are described in the literature as pleasant and neutral, respectively (Drewnowski et al., 1987). The paradigm consisted of six blocks of 20 trials each. Within blocks, stimuli were presented either sequentially or pseudorandomly. In four sequential blocks (2 of each solution), either sucrose or water was given repeatedly for the 20 trials. Therefore the total scan number for either sucrose or water was 40. In the two pseudorandom blocks, sucrose and water were presented randomly, but in the same “random” order for all subjects (hence pseudorandom). Within these pseudorandom blocks, the number of sampling scans of sucrose and water was the same, resulting in a total of 20 for both. The six blocks were presented randomly to all subjects such that sequential blocks of the same solution were never presented consecutively. Each taste stimulus consisted of 1-ml volume of liquid and was delivered every 20 s through one of two tubes in the buccal region. Subjects were trained to perform one tongue motion (swishing the solutions across the tongue) after each application of taste stimulant, in order to wash the taste stimulus around the mouth and stimulate taste buds.

2.3. Apparatus

The macronutrient solutions were contained in two 25-ml syringes, which were attached to a semiautomatic and programmable customized syringe pump (J-Kem Scientific, St. Louis, MO), positioned in the scanner control room. Tastes were delivered to the subjects via two separate approximately 10-m long FDA approved food grade teflon tubes (Cole-Parmer Vernon Hills, IL). The syringes were also attached to a computer-controlled valve system, which enabled the two solutions to be delivered independently along the tubing. Taste delivery was controlled by E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA) software operating on a personal computer positioned in the control room. The stimuli were also synchronized with MR scanning.

2.4. Scanning procedures

Imaging data were collected with a 3T Signa scanner (GE Medical Systems). High-resolution anatomical images (1.5 mm³) were acquired for each subject. Additionally, T1 structural images were acquired with a 3.2-mm thickness (in-plane with the functional images). These had 36 oblique axial slices oriented parallel to a line from the anterior to posterior commissures (the AC–PC line), with an in-plane resolution of 0.78 mm² and with a field of view of 200 mm². Functional images were acquired using a one-shot reverse spiral pulse sequence (Noll et al., 1995; Bomert et al., 2000) with TE=26 ms and TR=2000 ms; 30 oblique axial slices were acquired with an in-plane resolution of $64 \times 64$ with 3.125 mm² pixels and a slice thickness of 3.2 mm, with a field of view of 200 mm². Functional images were acquired in six blocks of 20 trials each over the course of learning. The presentation of stimuli was synchronized with the scanning such that ten 2-second images were acquired per trial.

2.5. Image analysis

2.5.1. Data preprocessing

Motion correction was performed using a six-parameter linear algorithm (Woods et al., 1998a). A linear detrending algorithm was also done, using only data within 3 standard deviations of the mean to estimate the linear trend. Global normalization was performed multiplicatively to give each subject a mean intensity of 3000. All analyses were conducted on a single subject basis on non-cross-registered data.

2.5.2. Automated labeling pathway

We used an a priori ROI approach, with the regions defined anatomically in each individual’s anatomic space. An automated approach for defining the ROIs was used, as described in Rosano (Rosano et al., 2005) and Aizenstein (Aizenstein et al., 2004). The anatomical ROIs were chosen based on a literature review of previous studies. Bilateral amygdala, BA10, BA11, BA46, hippocampus, and insula were defined in the Automatic Anatomical Labeling (aal) map obtained from the MRIcro software package (Tzourio-Mazoyer et al., 2002). The caudal anterior cingulate cortex (ACC) was chosen from a previous functional imaging study (Carter et al., 2000). To define each ROI for each single subject in her own space, the following steps were performed. First, each subject’s low-resolution anatomical image was cross-registered to her high-resolution anatomical image using a second order warping algorithm with 30 parameters (Woods et al., 1998b). Each ROI from the aal map was then put onto each subject’s SPGR. Then a gray matter mask was applied to each label, using the...
FAST algorithm from the FSL package (Zhang et al., 2001). The labeling of each subject’s ROIs was then visually inspected to assure accurate mappings.

2.5.3. Time series analysis

For each subject and for each ROI, a mean time series was extracted. The time series for each voxel in each region was first calculated as the percent signal change across the 10 different scans of a trial, that is, the percent change of signal intensity in that voxel from the first scan of the trial. The mean signal for each trial type and for each region was then calculated. The mean time series across all blocks was used to test the effect of condition type, and the difference in peak signal across the blocks (i.e., first half of trials of one condition versus second half of trials for the same condition) was used to test for habituation.

A significance level was computed for each test result presented in Table 1 using t-tests of percentage signal change at expected HRF peak versus 0 \((df=10)\) as well as in Table 2 using paired t-tests \((df=10)\). These levels were then adjusted to an overall family-wise error rate of 0.05 for each condition using the Hochberg procedure, a “step-up” modified Bonferroni method for multiple comparisons (Hochberg, 1988). Using this approach, significance levels are ordered from largest to smallest and compared to the Hochberg adjusted alpha. The Hochberg adjusted alpha is calculated by multiplying the P value by the rank number it has in the list of comparisons.

3. Results

3.1. Pseudorandom blocks

For the distilled water stimuli, there was significant neuronal activation for all of the ROIs, except for the medial OFC and dorsolateral prefrontal cortex after correcting for multiple statistical testing (Table 1a). In contrast, the neuronal activation following sucrose stimuli did not stay significant in all ROIs after correction for multiple comparison (Table 1b).

A contrast analysis of sucrose versus distilled water revealed significant differences, at a level of \(P<0.05\), for bilateral amygdala, hippocampus, insula, caudal ACC, dorsolateral prefrontal cortex and medial OFC (Brodmann area, BA 11). However, only the contrast for the bilateral amygdala, hippocampus, medial OFC and right insula remained significant after correction for multiple statistical testing (Table 2a).

To test for effects of habituation, the activation that occurred during the first half of the blocks was compared to activation that occurred during the second half of the blocks. For the water trials, there were significant reductions in activation during the second half of the trials for all ROIs (Table 2b) except medial OFC. However, there were no differences in activation for sucrose, when activation during the first half of the sucrose trials was compared with the activation during the second half of the trials (Table 2c). An interaction analysis comparing response to sucrose and water
confirmed that there were differences in response to water and sucrose over time for all ROIs (P<0.05) except the medial OFC (BA 10). Figs. 1 and 2 show representational findings for the left amygdala and left insula. In summary, for pseudorandom blocks, sucrose and water showed different neuronal responses over time for almost all ROIs, with subjects showing habituation to water but not sucrose.

3.2. Sequential blocks

For the distilled water stimuli, there was significant neuronal activation for all of the ROIs, except for the medial OFC and dorsolateral prefrontal cortex after correcting for multiple statistical testing (Table 1c). For the sucrose stimuli, there was significant neuronal activation for the amygdala, hippocampus, and both sides of the insula after correcting for multiple statistical testing (Table 1d). A contrast analysis between sucrose and distilled water stimuli showed no significant differences for all ROIs (Table 2d).

To test for effects of habituation, the activation that occurred during the first half of the blocks was compared with activation that occurred during the second half of the blocks. There were reductions, significant at a 0.05 level, in activation in the second half of the blocks for water in all ROIs except the medial OFC (BA 10). After adjustment for multiple comparisons, only neural response in amygdala, hippocampus and insula remained significant (Table 2e). Similarly, when activation during the first half of the study block was compared to activation during the second half of the study block for sucrose, significant reductions in neuronal activation were found in all ROIs except the medial OFC (BA 10) (Table 2f). These data showed that sucrose and water had similar responses over time for a block design (Figs. 1–4).

4. Discussion

The purpose of this study was to test methods that might characterize changes in neuronal activation to food stimuli over time, and thus reflect habituation. In this way we intended to test methods that might replicate, in a laboratory setting, the patterns of eating behaviors commonly seen in AN and BN.

4.1. Pseudorandom design versus sequential design

This study found that healthy control women respond differently when sucrose and water are administered...
in different patterns. Reported studies tend to use designs with a stimulus delivery in an unpredictable pseudorandom order (Berns et al., 2001; De Araujo and Rolls, 2004; Kringelbach et al., 2004) to compare sweet and neutral stimuli. Those studies, and the pseudorandom design described in this article, tend to show activation of the insula, medial OFC (BA 11), amygdala, hippocampus, caudal ACC and dorsolateral PFC. Consistent with the literature, the region of the medial OFC described as BA 11 was activated (O’Doherty et al., 2000; Berns et al., 2001; Kringelbach et al., 2003; De Araujo and Rolls, 2004). By contrast BA 10 was as expected not significantly activated indicating the specific neural response of the taste paradigm.

However, we questioned whether a design with unpredictable food stimulus delivery was the best method for studying subjects with eating disorders, because individuals with AN and BN do not have normal feeding patterns (Kaye et al., 1993). Instead, they tend to restrict or binge by consuming a narrow range of foods. Because food choice is not varied during aberrant extremes of feeding in AN and BN, we wanted to test the use of a predictable, sequential design.

Anticipation of a taste experience may result in different activation patterns for unpredictable pseudorandom blocks in contrast to more predictable sequential blocks. There was no significant difference in neural activation for all ROIs when sucrose was compared with water in the sequential blocks. Our findings are consistent with Berns who described that the medial OFC displayed a significant activity for fruit juice versus water when the stimuli were unpredictable (Berns et al., 2001). Predictability (both stimuli were alternated) was only correlated with activity in the right superior temporal gyrus. In contrast, O’Doherty found that expectation of a pleasant taste produced activation in the posterior dorsal amygdala, striatum and OFC, while apart from the OFC, these regions were not activated by reward receipt (O’Doherty et al., 2002). Our paradigm, in which the stimulus becomes predictable because it is sequentially administered, may not be comparable to one using a visual cue, which indicates, before the taste stimulus is delivered, whether a sweet taste or water is to be administered.

Fig. 1. Time course of BOLD signal (mean of all 11 subjects) for taste-related response in the left amygdala during sequential blocks and pseudorandom blocks comparing the activation during the first 10 trials versus the second 10 trials.
4.2. Habituation effects

Rolls (Rolls, 2001) suggested that the brain regions investigated in our study contribute to the modulation of the reward value of a sensory stimulus such as the taste of food. Normally, food tastes pleasant when someone is hungry. However, after a food is eaten to satiety, there is a reduction in the pleasantness of its taste. In fact, neurons in the OFC decrease their response to a food eaten to satiety, but remain responsive to other foods, contributing to a mechanism for sensory-specific satiety. In addition, O’Doherty et al. found sensory-specific satiety effects for some subjects also in the insula, amygdala and anterior cingulate cortex (O’Doherty et al., 2000). A similar effect in the OFC, middle part of the insula and rostral ACC is described for water and a subject’s thirst level (De Araujo et al., 2003b). It is possible that sensory-specific satiety and habituation are related phenomena. However, the paradigms used to address them are different. The Rolls group first gave a complex food stimulus to satiety and then tested fMRI response to further administration of this stimulus. In comparison, we tested the administration of a pure macronutrient or water given repeatedly. These differences in method may explain different findings, especially for the pseudorandom blocks. Interestingly, we did not find habituation effects for sucrose when the stimuli were given in an unpredictable alternated order. In contrast, this paradigm did reveal a habituation effect to distilled water. It is not clear why subjects did not habituate to sucrose. Perhaps the block length of around 6 min is too short for habituation effects of strong stimuli like sucrose. It should be noted that the overall percentage signal change for the pseudorandom blocks (trials 1–10) for sucrose was significantly lower ($t(10)=4.50; P=0.001$) than for the sequential blocks (trials 1–10) in the amygdala (Fig. 1). It is possible that in the sequential blocks there is a more robust early amygdala activation due to the anticipation of sucrose.

Another way to look at a different kind of habituation effect could be to compare the entire first block versus the entire last block. O’Doherty et al. revealed this type of habituation effect over the time course of their experiment only in the orbital frontal region when glucose was compared with a neutral
(25 mM KCl and 2.5 mM NaHCO₃) stimulus, but not when a salty stimulus was compared with a neutral stimulus (O’Doherty et al., 2002). We did not detect this kind of habituation in our data set comparing the first versus the last of the same block type for all ROIs (data not shown). It appears, therefore, that there is more habituation within blocks of repeating stimuli than across blocks when blocks of repeating stimuli intervene.

4.3. Limitations

In terms of limitations, the delivery interval of 20 s was not long enough to return the activation back to baseline and therefore could drive the analysis. But we expect a continuing central activation over several minutes following a taste stimulus (Liu et al., 2000), which does not fit into any event-related study design. It has to be mentioned that twice as many sucrose/water stimuli appear in sequential blocks than in pseudorandom blocks. It could be argued that stimulus numbers influence overall magnitudes of habituation between sequential and pseudorandom blocks. It is known that neurons in taste-modulating brain regions do not respond exclusively to taste but also to mechanical stimulation related to a fluid or plastic tube (Katz et al., 2002). The purpose of this study is to report a method to assess possible habituation effects in eating disorders. Whether mechanical stimulation effects might be washed out in a contrast analysis of eating-disordered subjects versus control subjects remains to be determined. Additionally, we studied female subjects only. However, since gender may have an impact on the result of the neuronal activation (Cailhol and Mormede, 2002), we made this choice as this study was designed as a comparison for women with eating disorders.

4.4. Summary

This study confirms and extends our understanding of neuronal activation patterns in taste processing. In summary, we replicated the activation of the primary and secondary taste cortex as well as other taste-specific brain regions such as caudal ACC and dorsolateral PFC. To our knowledge, this is the first study assessing a neuronal correlation of habituation effects in taste using...
fMRI. This study suggests that habituation patterns in healthy subjects may be related to methods of stimulus administration, predictability of stimuli or duration of blocks. Habituation effects can play a role in reinforcement of eating. Thus, the predictable, sequential experimental design may be useful for assessing taste processing in eating-disordered patients and may shed light on their eating behavior.

As noted above, future studies will seek to determine whether individuals with AN and BN have an abnormal physiologic response to foods, such as carbohydrates. Furthermore, we might test the hypothesis that individuals with AN and BN have disturbances of these higher order taste centers that regulate the satiating aspects of feeding or related emotional behavior.

Acknowledgments

The authors thank Eva Gerardi for manuscript preparation and C. Carter for comments on an earlier draft. The authors are indebted to the participating subjects for their contribution of time and effort in support of this study.

Financial support was provided by the following grants: NIMH MH046001, MH04298 and K05-MH01894.

References


