Neural Correlates of Gastric Distension

Regional Cerebral Blood Flow Measured with
Positron Emission Tomography: Exploratory Investigations
of Visceral Stimulation in Healthy Young Women

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Contents

Abstract

During the last decade, anatomic and physiological neuroscience research has yielded extensive information on the physiological regulators of short-term satiety, visceral and interoceptive sensation. Distinct neural circuits regulate the elements of food ingestion physiologically. The general aim of the current studies is to elucidate the peripheral neural pathways to the brain in healthy subjects to establish the groundwork for the study of the pathophysiology of bulimia nervosa (BN). We aimed to define the central activation pattern during non-nutritive gastric distension in humans, and aimed to define the cognitive responses to this mechanical gastric distension.

We estimated regional cerebral blood flow with ^{15}O-water positron emission tomography during intragastric balloon inflation and deflation in 18 healthy young women of normal weight. The contrast between inflated minus deflated in the exploratory analysis revealed activation in more than 20 brain regions. The analysis confirmed several well known areas in the central nervous system that contribute to visceral processing: the inferior frontal cortex, representing a zone of convergence for food related stimuli; the insula and operculum referred to as "visceral cortex"; the anterior cingulate gyrus (and insula), processing affective information; and the brainstem, a site of vagal relay for visceral afferent stimuli. Brain activation in the left ventrolateral prefrontal cortex was reproducible. This area is well known for higher cognitive processing, especially reward-related stimuli. The ventrolateral prefrontal cortex with the insular regions may provide a link between the affective and rewarding components of eating and disordered eating as observed in BN and binge-eating obesity.

Gastric distension caused a significant rapid, reversible, and reproducible increase in the feelings of fullness, sleepiness, and gastric discomfort as well as a significant rapid, reversible, and reproducible decrease in the feeling of hunger. We showed that mechanical activation of the neurocircuitry involved in meal termination led to the described phenomena.

The current brain activation studies of non-painful, proximal gastric distension could provide groundwork in the field of abnormal eating behavior by suggesting a link between visceral sensation and abnormal eating patterns. A potential treatment for disordered eating and obesity could alter the conscious and unconscious perception and interoceptive awareness of gastric distension contributing to meal termination.

1   Introduction

Eating disorders constitute contemporary and frequent disorders. More than 3% of the U.S. population suffers eating disorders (Ghaderi & Scott, 2001).   Such disorders include bulimia nervosa (BN), characterized by cycles of binge eating and various compensatory purging behaviors, and anorexia nervosa, where patients restrain their eating and as a consequence fail to maintain a minimal bodyweight (American Psychiatric Association, 1994). Even though abnormal eating behavior already emerged during medieval times, the occurrence of eating disorders dramatically increased in the second half of the 20th century with a shifted motivation from the medieval seeking for religious and moral perfection to the contemporary aspiration of the achievement of an ideal body shape (Gordon, 1998). More women than men suffer eating disorders; the female-male ratio equals 10:1 (Striegel-Moore, Leslie, Petrill, Garvin, & Rosenheck, 2000), Gordon (1998) hypothesizes the increased prevalence in the female population may reflect women's challenges in self-identification during times of fast changing female social roles and their associated new expectations. Additionally, the current "thin ideal" (Stice, 2001) inheres a risk especially for women (Striegel-Moore, et al., 2000).

The consumption of abnormally large amounts of food among people diagnosed with BN suggests that mechanisms regulating meal termination operate dysfuncitonally. In fact, research on BN discovered two major findings: First, bulimia patients need to ingest more food to induce the same level of satiety than a healthy control group (Geracioti & Liddle, 1988). Second, a fixed test-meal elicits significantly lower feelings of fullness in bulimia patients than in a control group (Geliebter, Melton, McCray, Gallagher, Gage, & Hashim, 1992; Geliebter & Hashim, 2001; Kissileff, Wentzlaff, Guss, Walsh, Devlin, & Thornton, 1996). Many factors are involved in the regulation of body weight, short-term satiety and meal termination (Spiegelman & Flier, 2001) and the interaction of humoral, gastric, intestinal, oral, gustatory, and perceptional factors participating in short-term satiety is far from disclosed.

As the major symptom of BN is binge-eating (followed by purging), one attempt towards elucidating its pathophysiology is to examine the central circuits involved in the non-nutritive extension of the stomach as one contributor to meal termination and short-term satiety.   The consideration of the cognitive responses to such non-nutritive gastric distension can shed additional light on the cognitive processing during gastric distension. The greater scope of a series of studies is to determine if both the spontaneous and evoked

activity within the neurocircuitry involved in meal termination is altered in BN. Comprehension of clinical data requires the comparison to a reference group, here healthy individuals. These studies attempt to examine responses of the central nervous system to tonic activity of vagal afferents and peripherally evoked phasic vagal afferent activation in healthy young women of normal weight.

Previous anatomical and physiological animal studies revealed the involvement of the vagus nerve, the Xth cranial nerve, in gastric distension (Scratcherd & Grundy, 1982; Andrews, Grundy, & Scratcherd, 1980; Towbin, 1955; Paintal, 1953). These studies disclosed the neuroanatomy of afferent and efferent vagal fibers. Nevertheless, our understanding of the central localization and mechanisms of gastric visceral sensation in humans remain scars. Contemporary neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) offer new insights into brain processing of the awake and fully conscious human subject.

Here, we studied non-painful gastric distension in young women. A previous study revealed six activated brain regions (summarized as Experiment I, see chapter 7.3.1). We were aware of the limitations of our sample size as only representative large sample studies or replication studies justify generalized statements. We collected data from a second group of subjects and conducted an extended exploratory analysis with a split-half reliability test (Experiments II and III, see chapters 7.3.2 and 7.3.3).

This paper pursues the following outline: Chapter 2 gives background information on processing circuits of interoceptive and exteroceptive stimuli. Chapter 3 gives background information about BN and proposes the involvement of interoceptive circuits in the pathophysiology of this disorder. Chapter 4 reviews the current status of knowledge of mechanisms regulating meal termination, leading into Chapter 5, which addresses vagal anatomic pathways as they build the framework and background for our studies. Methods and Materials including a narrative of positron emission tomography, which was used to measure brain activity during gastric distension, is described in Chapter 6. Chapter 7 presents the results of all experiments which addressed the central circuits involved in and the cognitive responses to non-nutritive gastric distension. Chapter 8 discusses the results in the light of interoceptive processing. Chapter 9 offers a conclusion and an outlook on future research.

2   Background: Interoceptive Processing

The understanding of how we perceive and interpret stimuli from within the body is essential in gaining insight into the pathophysiology of psychological and physiological disorders. It is also necessary in understanding psychological distress accompanying many physical disorders. Sensation in general terms is the transduction, encoding, and the perception of information generated by stimuli arising from both the external and internal environment. Many areas in the central nervous system are devoted to these sensations. Stimuli are relayed to and represented in the central nervous system, where they are further processed to generate appropriate behavioral responses.

Sensory processing is generally classified into three groups according to where in reference to the body the perceived stimulus is generated: exteroception, proprioception, and interoception. Exteroception is the sensation and perception of stimuli from outside the body and mediates interactions between the organism and the external environment. Proprioception is the detection of mechanical forces generated by the musculoskeletal system in muscles, joints, and other deep structures by receptors called proprioceptors. These receptors detect and locate movements and position of the body in space. Interoception is the sensation and transmission of stimuli generated from within the body regarding the function of the viscera. Viscera are defined as internal organs in any of the great body cavities (thoracic, abdominal, and pelvic). The perception of pain and temperature is usually attributed to the exteroceptive system which assumes the same central representation in the somatosensory cortex as does tactile stimulation (Purves, Augustine, Fitzpatrick, Katz, Lamantia, & McNamara, 2001).

The concept of interoception is only scarcely defined in recent editions of medical dictionaries (Dorland, 2000; Miller, 1997; Stedman, 1995; Anderson, Anderson & Glanze, 1994; Dox, 1993). According to these definitions interoception is visceral sensation, that is the sensation originating from the internal organs located in one of the three great body cavities: thoracic, abdominal, and pelvic. A more inclusive perspective has been suggested by Craig (2002, 2003). The author suggests that a definition of interoception should include all afferent information, sympathetic and parasympathetic, from within the body.

Craig's concept of the interoceptive system explains how all sensations from within the organism are transmitted by the sympathetic and parasympathetic divisions of the peripheral nervous system to the central nervous system to represent the current

physiological state of the body. Such information transport is necessary to maintain homeostatis (Craig, 2002). Craig (2002) proposed that sensations from all tissues in the body are successively sent via small diameter fibers to brainstem areas, thalamic nuclei, and several areas in the insular cortex, to the anterior cingulate cortex and to the orbitofrontal cortex. At different levels of relay, information is either projected forward in the processing stream or is relayed to a motor area that signals to a target tissue via efferent fibers. The processing of interoceptive stimuli is necessary for the body to maintain homeostasis. Conscious awareness of the bodily sensation, which is irrelevant for the maintenance of homeostasis, only occurs once the information reaches cortical areas. Craig (2002) pointed out that the processing loop diversifies for organisms of higher evolutionary levels. In organisms of lower evolutionary levels, central structures are bypassed, less developed, or even absent. The processing step probably unique for humans is a re-representation of the interoceptive projections within the insular cortex which might be the basis for a conscious awareness of the current physiological state of the body. This chapter describes the anatomical and functional aspects of the concept of an interoceptive system presented by Craig (2002) followed by a review of the literature for evaluation.

2.1   Interoception: Defined by Craig (2002)

Craig (2002) described that the sensation of pain and temperature has traditionally been considered as external stimuli that are processed by the somatosensory system. The somatosensory system finds its cortical representation in the somatosensory cortex of the postcentral gyrus. Although painful and thermal stimuli are represented in the somatosensory cortex, he argues that these stimuli also find a representation in an interoceptive system. Thermal stimuli, for example, are generally applied externally in the experimental setting. Therefore, it involves cutaneous sensation that is processed in the somatosensory cortex. However, thermal stimuli also carry an interoceptive component that is dependent on the bodily state. For instance, contact with a stimulus of identical temperature can be perceived differently depending on the current physiological state of the organism. He described the perception of diving into a pool with a water temperature of 20º C when the body is hyperthermic versus when the body is hypothermic. In the first scenario, the water is perceived as pleasantly cool and the contact will be maintained (approaching behavior). In the second scenario, the water is perceived as unpleasantly cool and the contact will be terminated (withdrawal behavior). This example illustrates three essential features of the thermal stimulus; first, it has an emotional component (pleasant versus unpleasant), second, it has a motivational component (approach versus withdrawal

from stimulus), and third, the perception of the stimulus depends on the current bodily state. The motivational component requires a system of homeostasis that maintains and regulates an inner state of the organism. If the organism had no need to maintain and regulate its inner state, interpretation of characteristics of external stimuli would not be necessary. Yet we do have such a system of homeostasis which requires interpretation of the internal and external environment and its consequences for the organism. In order to assess prospective consequences, information about the current physiological state of the body is necessary. Continuous monitoring of the internal bodily state is required for such an assessment. Craig (2002) described the course of processing of internal stimuli from the periphery to their representation in the central nervous system: in brainstem areas, sub-cortical areas, and in the cerebral cortex.

2.2   Transmission of Visceral Information from the Periphery to the Brainstem

Stimuli from within the body are transmitted via small diameter fibers (Aδ and C-fibers) and synapse ipsilaterally in the superficial lamina I of the dorsal horn. They then cross and exit through the contralateral side and travel contralaterally in the spinothalamocortical system. In contrast, exteroceptive information is conveyed by large-diameter fibers that synapse in the deep dorsal horn and then travel ipsilaterally in the dorsal column/medial lemniscal system in the spinal cord.

Small-diameter fibers register the physiological condition of all tissues of the body and ascend along the neuraxis. At all levels of relay, afferent fibers synapse with ascending fibers and with cell groups that activate autonomic motor centers. Connections with motor neurons demonstrate autonomic reflex arches at different central levels. Sympathetic ascending fibers from the periphery terminate in lamina I. Projections from lamina I travel to four different sites: First, cells project from lamina I directly to autonomic motor regions in the spinal cord establishing a reflex arch at the spinal level. Second, lamina I cells project to the nucleus of the solitary tract (NTS) where the rostral ventrolateral and the ventromedial medulla project to autonomic motor regions. Third, lamina I cells project to the parabrachial nucleus (PBN) which sends information to the basal part of the ventromedial nucleus of the thalamus (VMb). Forth, lamina I cells directly project to the posterior part of the ventromedial nucleus of the thalamus (VMpo). Parasympathetic ascending fibers terminate in the NTS which projects either directly or via the PBN to VMb.

2.3   Thalamic Integration of Visceral Information

Although the PBN in the brainstem receives sympathetic and parasympathetic inflow, most of such inflow is directly sent from lamina I and NTS to the thalamic nuclei, the site of homeostatic integration. Thalamic projections are topographically organized: the VMpo is the only site that receives direct input from lamina I sympathetic afferents and the VMb is the only site that receives direct input from the NTS parasympathetic afferents. The two thalamic nuclei assume a rostro-caudal orientation with the VMpo lying rostrally. This orientation is orthogonal (perpendicular with an area of connection) to the thalamic integration area of somatosensory and proprioceptive input which assumes a medio-lateral organization. The two integration sites, homeostatic and somatosensory, connect at the representation of the mouth.

Thus, interoceptive thalamic integration areas lay adjacently and orthogonally to the exteroceptive / proprioceptive integration site in the thalamus.   Interoceptive and exteroceptive thalamic areas connect at the level of the representation of the mouth. The topographical organization of interoceptive and exteroceptive representation is maintained at the cortical level where interoceptive stimuli are represented in a rostro-caudal manner in the dorsal-posterior insular cortex and exteroceptive stimuli are represented in a medio-lateral manner in the somatosensory cortex.

2.4   Cortical Processing of Interoceptive Stimuli

Craig (2002) suggests that thalamic inputs are relayed to the dorsal-posterior insular cortex (bilateral) where spinal projections from the VMpo are represented caudally and visceral projections from the VMb are represented rostrally.   The insular cortex lies buried underneath the Sylvian fissure, covered by the ventral part of the frontal and parietal cortices and the dorsal part of the temporal cortex. The cortex that immediately covers the insula is called frontal, parietal, or temporal opercular cortex, respectivly.   The dorsal-posterior insular area comprises the limiting sulcus toward the adjacent frontal and parietal operculum. Craig (2002) assumes that the dorsal-posterior insular cortex constitutes the sensory cortex for primary afferent fibers that conduct homeostatic information. The insula is also known as a limbic sensory cortex which processes emotional stimuli (Taylor, Phan, Decker, & Liberzon, 2003).   The motivational component that is accompanied by the feeling of the bodily state is most likely represented by activation in the anterior cingulate cortex (ACC). The ACC plays an important role in limbic motor control (Zubieta, Ketter, Bueller, Xu, Kilbourn, Young, et al., 2003).   Both insula and ACC have reciprocal

projections to the amygdala and hypothalamus, and to orbitofrontal and brainstem homeostatic regions (Floyd, Price, Ferry, Keay, & Bandler, 2000). Craig (2002) believes that the concomitant activation of the ACC (motivational component) and the insular cortex (feeling component) form an emotion.

Subjective awareness of the feelings of the body and our emotions occurs via activation of a distinct area in the right ventral-anterior insula and in the orbitofrontal cortex. An initial re-representation of the visceral information is processed in the contralateral ventral-anterior insula, from where it is relayed ipsilaterally via interneurons and contralaterally via callosal fibers to the right ventral-anterior insula for secondary re-representation. This right ventral-anterior insular region projects directly to areas in the orbitofrontal cortex. Activation in the right ventral-anterior insula together with activation in orbitofrontal regions constitutes the neural basis for subjective awareness of the feelings from the body and of emotions.

2.5   Evaluation of Craig's Concept of Interoception

Craig (2002) defined itch, temperature, pain, muscle processes, and visceral stimuli as interoceptive stimuli. According to his model of interoception, these modalities all find their physiological central representation in the dorsal-posterior insular cortex. When studying interoceptive stimuli as defined by Craig (2002), neural activation in the following cortical areas can be predicted: dorsal-posterior insula, bilateral ventral-anterior insula with a predominant right representation, anterior cingulate cortex, and orbitofrontal regions. The following is a review of the literature addressing these four cortical projection sites.

2.5.1   Dorsal-Posterior Insular Cortex

The insular cortex lies buried underneath the Sylvian fissure, covered by the ventral part of the frontal and parietal cortex and the dorsal part of the temporal cortex. The cortices that immediately cover the insula are extensions of the frontal, parietal and temporal lobes and are called frontal, parietal, or temporal opercular cortex, respectively (Türe, Yasargil, Al Mefty, & Yasargil, 1999). The dorsal-posterior insular area comprises the limiting sulcus toward the adjacent frontal and parietal operculum. This area, according to Craig (2002), constitutes a sensory projection field for afferent fibers that transmit information about the bodily state. This area should represent the basic, unappraised stimulus. No evaluation or cognitive interpretation has occurred at this time.

The dorsal-posterior insula is activated in response to a variety of interoceptive stimuli such as thermal stimulation (Casey, Minoshima, Morrow, & Koeppe, 1996; Chen, Ha, Bushnell, Pike, & Duncan, 2002; Rainville, Duncan, Price, Carrier, & Bushnell, 1997), sensation of itch (Leknes, Bantick, Willis, Wilkinson, Wise, & Tracey, 2007; Drzezga, Darsow, Treede, Siebner, Frisch, Munz, et al., 2001; Hsieh, Hagermark, Stahle-Backdahl, Ericson, Eriksson, Stone-Elander, et al., 1994), and stimulation of the viscera (Aziz, Anderson, Valind, Sundin, Hamdy, Jones, et al., 1997; Binkofski, Schnitzler, Enck, Frieling, Posse, Seitz, et al., 1998; Ladabaum, Minoshima, Hasler, Cross, Chey, & Owyang, 2001; Bittorf, Ringler, Forster, Hohenberger, & Matzel, 2006). Also, cardiovascular changes activate the dorsal-posterior insular cortex independent of experimental tasks: biofeedback (Critchley, Melmed, Featherstone, Mathias, & Dolan, 2002b), exercise (King, Menon, Hachinski, & Cechetto, 1999), or changes due to isoproterenol, a drug which induces bronchial relaxation and which has positive chronotropic and inotropic effects on heart rate (Cameron & Minoshima, 2002). Swallowing (Hamdy, Rothwell, Brooks, Bailey, Aziz, & Thompson, 1999; Martin, Goodyear, Gati, & Menon, 2001; Martin, Barr, MacIntosh, Smith, Stevens, Taves, et al., 2007; Zald & Pardo, 1999) or olfactory stimulation (Kareken, Mosnik, Doty, Dzemidzic, & Hutchins, 2003; Savic & Berglund, 2004) activate this region as well.

Regional cerebral blood flow (rCBF) in the dorsal-posterior insular cortex is indeed independent of attentional (Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002; Peyron, Garcia-Larrea, Gregoire, Costes, Convers, Lavenne, et al., 1999) and qualitative stimulus characteristics (Zald, Mattson, & Pardo, 2002b). Cerebral blood flow correlates with the actual stimulus intensity (Frot, Magnin, Mauguière, & Garcia-Larrea, 2007; Craig, Chen, Bandy, & Reiman, 2000) and with physiological parameters (Darsow, Drzezga, Frisch, Munz, Weilke, Bertenstein, et al., 2000). For example, Brooks et al. (2002) and Peyron et al. (1999) applied thermal painful stimuli to the hand and varied the level of attention by directing subjects' attention toward the painful stimulus or by toward a visual or an auditory task, respectively (distracting from the painful stimulus). Both research groups found dorsal-posterior insular activation in all experimental conditions. Frot et al. (2007) found the posterior insular cortex to reliably detect different stimulus intensities at painful levels when a thermal lazer stimulus was applied to the dorsum of the hand. Craig et al. (2000) analyzed rCBF to thermal sensation with a thermode. The right palm was stimulated with decreasing temperature from a baseline level of 33° C to a non-painful sensation of cold at 20° C. Regression of rCBF and stimulus intensity (temperature) revealed a highly significant correlation with the contralateral dorsal posterior insula. This correlation was the strongest and largest in the analysis. Darsow et al. (2000) found skin

reactions (skin temperature and wheal response) to histamine application to the right forearm (sensation of itch) to be correlated with rCBF in the dorsal and ventral posterior insula. The same correlation pattern was found in a study that correlated histamine concentration, applied to the right forearm, with rCBF (Drzezga, et al., 2001).

Zald, Hagen and Pardo (2002a) orally administered bitter and sweet liquids and found dorsal posterior insular activation to both the pleasant (sweet) and unpleasant (bitter) taste. Neutral fluids as well activate the dorsal posterior insula in the vicinity of the limiting sulcus to the operculum (de Araujo, Kringelbach, Rolls, & McGlone, 2003). Swallowing, another process occurring inside the body, elicits dorsal-posterior insular activation. This activation occurs during both conscious (volitional; Zald & Pardo, 1999) and unconscious (naïve; Hamdy, et al., 1999; Martin, et al., 2001) swallowing. Dorsal-posterior insular activation arises independently from the cognitive awareness of the process.

Taken together, these findings confirm that the dorsal-posterior insular cortex encodes the actual stimulus properties without evaluative and cognitive components. This region seems to denote the projection of quantitative stimulus characteristics uninfluenced by qualitative stimulus characteristics.

2.5.2   Right Ventral-Anterior Insula

According to Craig (2002), an initial re-representation of the feeling of the bodily state is processed in the ventral-anterior insula, from where it is sent ipsi- and contralaterally to the right ventral-anterior insula as a second re-representation. This time process cannot be separated in most existing imaging research and the experiments were not designed to discover such time process. Thus, this literature review focuses on the right anterior region as a whole as the secondary re-representation area for interoceptive stimuli where subjective awareness of the bodily state is assumed.

Much research has been done that found right ventral-anterior insular activation when investigating rCBF to stimuli that Craig (2002) classified as interoceptive. For example, cardiovascular changes due to exercise (Critchley, Mathias, Josephs, O'Doherty, Zanini, Cipolotti, et al., 2003; Critchley, Corfield, Chandler, Mathias, & Dolan, 2000) and due to biofeedback (Critchley, et al., 2002b), interoceptive awareness of heartbeat and cardiovascular arousal to a physical stress task (Pollatos, Schandry, Auer, & Kaufmann, 2007), visceral stimulation (Aziz, et al., 1997; Binkofski, et al., 1998; Bittorf, et al., 2006), the sensation of itch (Hsieh, et al., 1994), and noxious and innocuous thermal stimulation

(Craig, Reiman, Evans, & Bushnell, 1996; Davis, Kwan, Crawley, & Mikulis, 1998; Hofbauer, Rainville, Duncan, & Bushnell, 2001; Rainville, et al., 1997) result in increases in brain activity in the right ventral-anterior insular cortex, mostly ventrally near the insular apex. This region is also active during gustatory stimulation (Zald, Lee, Fluegel, & Pardo, 1998b; Zald, et al., 2002a), during different states of satiation (de Araujo, et al., 2003), while watching food stimuli (LaBar, Gitelman, Parrish, Kim, Nobre, & Mesulam, 2001), or during olfactory stimulation (Kareken, et al., 2003; Qureshy, Kawashima, Imran, Sugiura, Goto, Okada, et al., 2000).

That the ventral-anterior insular region reflects subjective awareness of a bodily state becomes most apparent in experimental designs employing thermal stimulation. Such thermal stimulation paradigms examined subjects' perception of different intensity levels of the stimulus and correlated these subjective ratings with rCBF. Other paradigms inducing visceral sensation or cardiovascular arousal lack this type of analysis (Baciu, Bonaz, Papillon, Bost, Le Bas, Fournet, et al., 1999; Hsieh, et al., 1994). Further support is required from studies correlating subjective perception of visceral sensation or cardiovascular arousal with rCBF. In spite of the lack of support from visceral sensation and cardiovascular arousal studies, support for such right ventral-anterior insular activation comes from several sources, which are reviewed below.

Brooks et al. (2002) investigated brain activity to a painful thermal stimulus while subjects were either paying attention to the painful stimulus or were distracted by a visual task. Increased rCBF occurred in the right ventral-anterior insular cortex during attention to pain yet was absent during the condition of distraction. Evidently, subjects were less aware of the painful stimulation during the concomitant visual task. Rainville and coworkers (1997) hypnotized their subjects and, while keeping the actual pain intensity stable, selectively suggested increased or decreased unpleasantness of the noxious stimulus. Ventral-anterior insula activation reflects this increase in unpleasantness. Hofbauer et al. (2001) later used the same design of hypnosis and investigated the suggested change in pain intensity while again the actual intensity remained unaltered. Right ventral-anterior insular activation was apparent when attention was directed to the stimulus and when suggested pain intensity changed. It would be of interest to see a correlation analysis to examine the relationship of rCBF increase and the level of unpleasantness of the noxious stimulus. Bornhövd, Quante, Glauche, Bromm, Weiller and Buchel (2002) utilized four levels of stimulus intensities, ranging from neutral to warm to painfully hot. Pain intensity was closely correlated with activation in the ventral-anterior insular cortex, whereas actual stimulus intensity did not

correlate with activation in this region. The same experimental paradigm was employed by other independent research groups and resulted in the same activation pattern in the right ventral-anterior insula that was correlated with perceived pain intensity (Coghill, Sang, Maisog, & Iadarola, 1999; Peyron, et al., 1999).

Craig et al. (2000) illustrated thermosensory activation of the insular cortex. They utilized innocuous stimuli ranging from neutral to cold to stimulate subjects' right palm. All stimulus conditions activated the right ventral-anterior insular cortex. Correlation analyses of stimulus perception measured with subjective rating scales revealed a strong association between cortical activation in the right ventral-anterior insula with stimulus perception. The actual stimulus intensity did not correlate with the ventral-anterior insular cortex.

Craig et al. (1996) supported the notion that right insular activation is not correlated with the actual stimulus intensity. They found activation in the right insular cortex as a result to both noxious and innocuous thermal stimuli. Either cold or warm noxious and innocuous stimuli activated this region. They also showed that the alternate arrangement of innocuous cold and warm rods induces a painful sensation, which is called the illusion of pain. This sensation is also represented in the right ventral-anterior insula. The thermal grill illusion indicates that pain and thermal pathways seem to interact and that both pathways find a representation in the right ventral-anterior insular cortex (Craig, et al., 1996).

A similar pattern appears during volitional swallowing. Zald and Pardo (1999) found the right ventral-anterior insula to be activated only during conscious (volitional) swallowing. Unconscious (naïve) swallowing did not activate the very same region. The right ventral-anterior insula therefore seems to represent a volitional, conscious aspect of the swallowing act.

In addition to pain that is evoked by a potential harmful stimulus, other interoceptive experiences also elicit ventral-anterior insular activation. The group around Eisenberger studied brain activity to the experience of loss in a social setting (Eisenberger, Lieberman, & Williams, 2003). Explicitly excluding subjects from a virtual social activity (a ball game with three players) experimentally induced the experience of loss. Social pain activated similar brain regions as physical pain. Activation of the anterior yet not the dorsal-posterior insular cortex in this context provides further evidence that the ventral-anterior insula represents a subjective awareness of feelings. Confirming research comes from Lazar, Kerr, Wasserman, Gray, Greve, Treadway and coworkers (2005) who found

structural changes in the right anterior insula in subjects who had long-term meditation practice, a period of time during which people focus on the inner state of their body. The cortical area of the right anterior insula was thicker in subjects who were practiced in mediation compared with non-practiced subjects.

Taken together, functional imaging studies provide evidence that interoceptive stimuli, as defined by Craig (2002), elicit dorsal-posterior insular activation that identifies physical (objective) stimulus characteristics whereas activity of the right ventral-anterior insular represents perceived (subjective) stimulus characteristics.

2.5.3   Anterior Cingulate Cortex

The cingulate cortex lies on the medial wall and extends into each hemisphere. It follows the margin of the corpus callosum and consists of an anterior and posterior part at the level of the central sulcus. The anterior cingulate cortex (ACC) can further be divided into a dorsal and a ventral (subgenual) part according to its position in reference to the tip of the corpus callosum (genuum).

According to Craig (2002), activation of the ACC shows the motivational component of a stimulus. The decision about approaching a stimulus or withdrawing from it is represented in this area. All studies applying innocuous physical stimuli such as itch, thermal, or visceral stimulation activate the dorsal part of the ACC. Furthermore, especially noxious stimuli activate this dorsal region of the ACC (Pollatos, et al., 2007). Activation in this region correlates with pain detection (Tölle, Kaufmann, Siessmeier, Lautenbacher, Berthele, Munz, et al., 1999), perceived stimulus intensity (Bornhövd, et al., 2002; Coghill, et al., 1999; Derbyshire, Jones, Creed, Starz, Meltzer, Townsend, et al., 2002; Hofbauer, et al., 2001), and with perceived stimulus unpleasantness (Rainville, et al., 1997). The positive correlation of experienced distress with activation in the ACC illustrates the motivational component that is reflected in this cerebral region. The experience of social pain (rejection) induced by exclusion from a social setting is no exception (Eisenberger, et al., 2003).

Not only painful experiences are reflected in ACC activation. Experiencing innocuous stimulation such as itch (Darsow, et al., 2000; Mochizuki, Tashiro, Kano, Sakurada, Itoh, & Yanai, 2003; Leknes, et al., 2007), thermal innocuous sensation (Hofbauer, et al., 2001; Kwan, Crawley, Mikulis, & Davis, 2000), visceral non-painful stimulation (Aziz, Thompson, Ng, Hamdy, Sarkar, Brammer, et al., 2000; Ladabaum, et al., 2001), and

especially cardiovascular changes (Cameron & Minoshima, 2002; Critchley, et al., 2000; Critchley, Melmed, Featherstone, Mathias, & Dolan, 2001b; Critchley, et al., 2002b; Critchley, et al., 2003) are associated with activation in the dorsal part of the ACC. These findings show that a motivational component is not necessarily associated with painful stimuli but can be associated with innocuous sensation. It depends on the bodily state whether the stimulus is perceived as pleasant and will be approached or as unpleasant and will be avoided.

Activation of the ACC is also elicited by hunger (Tataranni, Gautier, Chen, Uecker, Bandy, Salbe, et al., 1999), pleasant, unpleasant or neutral taste (de Araujo, et al., 2003; Zald, Donndelinger, & Pardo, 1998a), olfactory stimuli (Qureshy, et al., 2000), and by swallowing (Hamdy, et al., 1999; Martin, et al., 2001; Martin, et al., 2007; Zald & Pardo, 1999). Swallowing, however, activates the most caudal part of the ACC. This activation pattern differs from the other stimuli. This difference could be due to the fact that swallowing not necessarily describes an interoceptive stimulus. Swallowing takes place in the esophagus, which is the elongation of the mouth area that is represented along the neuraxis in intermediate areas between the representation areas of exteroceptive and interoceptive stimuli (Craig, 2002).

2.5.4   Orbito-Frontal Regions

The orbito-frontal cortex is a region of the cerebral cortex that lies immediately above the orbit, the boney cavity containing the eyeball. It curves down from the frontal pole and reaches posteriorly to the level of the temporal pole. This region is known to be involved in higher cognitive functioning such as planning and decision-making. It is thought that consciousness is represented in this region. According to Craig (2002), the orbitofrontal cortex interprets the representation of interoceptive stimuli. Indeed, orbitofrontal and prefrontal regions of the cerebral cortex are activated by stimuli that he defined as interoceptive.

The ventral lateral and dorsal lateral prefrontal cortex in both hemispheres showed activation in pain experiments (Brooks, et al., 2002; Derbyshire, et al., 2002; Remy, Frankenstein, Mincic, Tomanek, & Stroman, 2003). Study paradigms using innocuous stimuli also caused prefrontal activation. Itch led predominantly to bilateral dorsal lateral prefrontal activation (Darsow, et al., 2000; Drzezga, et al., 2001; Hsieh, et al., 1994; Mochizuki, et al., 2003) whereas taste stimulation activated the ventral lateral prefrontal area (Zald, et al., 1998b). Thermal, non-painful stimulation and cardiovascular changes

due to exercise elicited brain activation in the lateral and medial portions of the prefrontal cortex (Bornhövd, et al., 2002; Cameron & Minoshima, 2002; Casey, et al., 1996; Craig, et al., 2000; Critchley, et al., 2000; Critchley, et al., 2003). The state of hunger and olfactory stimulation exclusively activates the ventral medial prefrontal cortex (de Araujo, et al., 2003; Kareken, et al., 2003; Tataranni, et al., 1999).

The different activation patterns within distinct areas of the prefrontal cortex seem to describe networks that process distinct stimuli. Carmichael and Price (1996) have proposed two networks within the orbital frontal cortex that can be described based on their distinct connections with other cortical and subcortical areas. They described a medial network that acts as a visceromotor entity and a lateral (orbital) network that acts as a viscerosensory entity (Carmichael & Price, 1996; Öngür & Price, 2000). Craig (2002) suggested a system for the detection of interoceptive stimuli, many of which are represented in the lateral prefrontal network as shown above. Only some studies show activation in the medial network. These results suggest that the lateral network predominantly processes sensory information. Only some study protocols activate the visceromotor system. For the studies activating both the medial and lateral orbital networks, examination of the time course of activation would be interesting. However, studies using contemporary neuroimaging techniques do not allow for interpretation of the time course of the activation patterns in the human brain. For example, brain activity measured with PET offers a maximal temporal resolution of 40-90 seconds, which is the shortest acquisition time for valid data interpretation. Although functional magnetic resonance imaging offers increased time resolution, researchers have not yet examined the data regarding the time course of emerging activations. Therefore it is challenging to give a clear account for the rapid sensory and motor components of brain activation induced by interoceptive stimuli.

Closer inspection of the relationship of prefrontal activation with subjective evaluations of the study situation and stimulus intensities revealed that the lateral prefrontal cortex seemed to act as a mediator for distress experienced during the experiment. Activation in the lateral prefrontal region correlated negatively with ratings of distress (Eisenberger, et al., 2003). That is, the less distress a participant experienced, the more active was the lateral prefrontal cortex. It seems that the prefrontal cortex adjusted the level of experienced distress and acted as a shield and safeguard. Prefrontal activation also occured in studies using painful stimuli. Prefrontal activity was highest at pain threshold indicating that most management of distress was necessary at initial pain detection (Coghill, et al., 1999).

2.6   Concluding Summary and Relevance for this Project

Craig (2002) essentially proposed an interoceptive system that transduces homeostatic information, namely, information about the physical state of the entire body, via sympathetic and parasympathetic fibers to brainstem and thalamic areas. The first integration sites of this afferent information are the nuclei of the thalamus: VMpo (basal part of the ventromedial nucleus of the thalamus) for sympathetic and VMb (posterior part of the ventromedial nucleus of the thalamus) for parasympathetic input. The segregated representation is maintained at the level of the insular cortex, where those fibers originating in the VMpo and VMb terminate caudally and rostrally. This representation of the physical state of the body finds its re-representation of this bodily feeling in a more anterior location of the insula and in the orbital cortex, which reveal the interpretation and subjective awareness of the internal state of the organism. Concomitant activation of the anterior cingulate cortex adds a motivational component that generates a behavior according to the perceived feeling.

Overall, evidence could be found for Craig's (2002) suggested central representation of interoceptive stimuli. Noxious and innocuous thermal stimuli, the sensation of itch, cardiovascular changes and visceral stimulation but also stimulation related to food intake such as the sensation of taste, olfaction and swallowing distinctly activated the dorsal and right ventral-anterior insular cortex, the ACC and the prefrontal cortex. No studies were found that specifically examined cortical processing of muscle activation, which Craig (2002) also classified as an interoceptive stimulus. Neuroimaging experiments utilizing exercising or muscle activity referred to the changes in cardiovascular activity. More explicit studies are required to clearly identify the similar processing of muscle activity and other interoceptive stimuli.

In some experiments, quantitative stimulus characteristics such as actual stimulus intensity correlated with dorsal but not ventral-anterior insular activation. Qualitative stimulus characteristics such as the subjectively perceived intensity, on the other hand, correlated with ventral-anterior insula but not with dorsal-posterior insula activation. Further support for the different functional properties of the insular cortex comes from studies examining the perception of a painful stimulus when paying attention to the stimulus or when distracted with a visual or acoustic task. The dorsal-posterior insula was active during both attention to and distraction from the painful stimulus whereas ventral-anterior insula activation only occurred when subjects paid attention to the stimulus. These findings

support the view that ventral-anterior insular activation represents the subjective awareness of stimulus characteristics.

The motivational aspect of interoceptive stimuli is represented in the ACC. Activation was highest at the threshold of pain detection, which suggests that ACC monitors the bodily state and warns the organism when the situation aggravates. Not only noxious stimulation included a motivational component that is represented in the ACC. Innocuous stimuli also generated cell activity in this region.   Thus, all stimuli have a general motivational component: to withdraw from the situation or to approach it (Craig, 2002).

The prefrontal cortex interprets the perceived situation and acts as a mediator for distress occurring from the situation. Ratings for perceived distress correlated inversely with prefrontal activation. Lowest distress was associated with highest prefrontal activation. Thus, the prefrontal region appears to mediate the level of distress.

An understanding of how interoceptive stimuli are processed is necessary to gain insight in the pathophysiology of psychological and physiological disorders. Systems maintaining homeostasis might operate dysfunctionally. For example, food intake and the regulation of energy balance are systems that are likely disrupted especially in eating disorders such as bulimia nervosa.   Bulimia nervosa is characterized by recurrent binge eating and compensatory purging behavior, mostly self-induced vomiting. The occurrence of binge eating suggests that mechanisms transmitting the signal of satiety might be impaired. The central representation of the sensation of fullness could help establish a link between visceral sensation and the abnormal eating patterns observed in bulimia nervosa yet also in other eating related disorders and phenomena such as obesity. This thesis evaluates central activation patterns and cognitive responses to non-nutritive gastric distension.   The following chapter addresses the eating disorder bulimia nervosa and gives a brief overview of the current state of knowledge regarding etiology and treatment approaches.

3   Bulimia Nervosa

This chapter reviews the current perspectives of the psychopathology and etiology of abnormal eating behavior. As it will become clear, contemporary treatment approaches have lead to good short-term results yet only fair long-term results. Considering vagal involvement as a physiological feature of bulimia nervosa (BN) introduces new treatment possibilities. Vagal involvement in BN also provides the rationale for the brain-imaging studies presented in this paper.

3.1   Psychopathology of Bulimia Nervosa

Bulimia Nervosa (BN) is an eating disorder characterized by the oscillation of binge eating and inappropriate weight control behaviors (American Psychiatric Association, 1994). According to the fourth edition of the Diagnostic and Statistical Manual for Psychiatric Disorders (DSM-IV), binge-eating is defined as eating more food in two hours than other people would normally eat and is accompanied by a sense of lack of control over eating, i.e., the feeling that one cannot stop eating or that one cannot influence the amount eaten (American Psychiatric Association, 1994). Inappropriate compensatory behaviors for the purging subtype of BN include self-induced vomiting, and, to a lesser extent, laxative, diuretic, or other medication misuse and enemas. Excessive exercising and fasting are viewed as inappropriate compensatory behaviors as well and are classified as the non-purging subtype of BN.

Other diagnostic features of BN include the following: First, a person's self-evaluation is mostly determined by body shape and weight. Second, binge-eating and compensatory behavior occur at least twice per week for a minimum duration of three months. Finally, binge-eating and compensatory behaviors are the predominant abnormal eating patterns and are not co-occurring with anorexia nervosa, an eating disorder characterized by excessive self-induced starvation.

Bulimia nervosa often adversely affects the general quality of life (Hay, 2003), especially interpersonal relationships (Johnson, Spitzer, & Williams, 2001). This is not surprising considering the co-occurring psychological factors such as feelings of frustration, helplessness, insecurity and failure and an intense feelings of self-loathing (Beumont, 1998). In addition, many medical and psychiatric comorbid conditions are often found in BN patients. Physiological changes (i.e., electrolyte imbalances) occur in a wide range but

not necessarily in every patient (Mitchell, Pyle, Eckert, Hatsukami, & Lentz, 1983) and increase as a function of symptom severity (Peeters & Meijboom, 2000; Wolfe, Metzger, Levine, & Jimerson, 2001). Common co-occurring psychiatric axis I disorders are mood and anxiety disorders, substance abuse or dependence (Ghadirian, Marini, Jabalpurwala, & Steiger, 1999; Johnson, et al., 2001; Lilenfeld, Kaye, Greeno, Merikangas, Plotnicov, Pollice, et al., 1998; Milos, Spindler, Ruggiero, Klaghofer, & Schnyder, 2002; Nishiguchi, Matsushita, Suzuki, Murayama, Shirakawa, & Higuchi, 2001; O'Brien & Vincent, 2003). Common psychiatric axis II co-morbid disorders are cluster B (impulsive cluster) personality disorders (Fernández-Aranda, Jiménez-Murcia, Álvarez-Moya, Granero, Vallejo & Bulik, 2006; Lilenfeld, et al., 1998; Nishiguchi, et al., 2001). Suicidal ideation and suicide attempts occur in up to 30% of BN patients (Corcos, Taieb, Benoit-Lamy, Paterniti, Jeammet, & Flament, 2002).

Noteworthy is that these psychiatric disorders, including suicide attempts, were found not to have a common cause and therefore are considered BN-unspecific (Lilenfeld, et al., 1998). Especially suicide attempts and substance abuse show a strong association with general life circumstances or with the diagnosis of a psychiatric illness in general (Corcos, et al., 2002; Dansky, Brewerton, & Kilpatrick, 2000). In contrast, self-injurious and impulsive behaviors, also often observed in BN, appear to be BN-specific, as they occur significantly more frequently in BN than in other psychiatric disorders, including other eating disorders (Nagata, Kawarada, Kiriike, & Iketani, 2000; Paul, Schroeter, Dahme, & Nutzinger, 2002).

Patients with bulimia symptoms usually present with normal body weight (Anderluh, Tchanturia, Rabe-Hesketh, & Treasure, 2003; Corcos, et al., 2002; Milos, et al., 2002; Vaz, Guisado, & Penas-Lledo, 2003), that is, their body mass index (BMI; weight in kg over height in m^2) falls within the normal range of 18.5 – 24.9 (National Heart, Lung, and Blood Institute, 1998). The normal bodily appearance might add to the fact that most cases remain unobserved and untreated (Johnson, et al., 2001; Striegel-Moore, Dohm, Kraemer, Taylor, Daniels, Crawford, et al., 2003). Conservative DSM-IV diagnostic criteria contribute to this situation and leave many cases undetected (Sullivan, Bulik, & Kendler, 1998). In addition, many women do not realize they have an eating disorder. Even if they seek treatment for one isolated symptom of BN, they often are simply misdiagnosed (Kaplan, 1990). The many concealed cases have stimulated a discussion about the usefulness of considering a partial syndrome of BN (sub-threshold BN) as clinically relevant. The partial syndrome is defined as meeting some but not all diagnostic criteria,

whereas the full syndrome meets all diagnostic criteria. Several researchers describe bulimic symptoms on a quantitative rather than a qualitative continuum (Crow, Stewart Agras, Halmi, Mitchell, & Kraemer, 2002; Sullivan, et al., 1998). Such a perspective allows comparisons to physiological disorders such as diabetes that are regarded as disorders of varying degrees (Tripathy, Carlsson, Almgren, Isomaa, Taskinen, Tuomi, et al., 2000). The question whether the distinction of a partial vs. a full syndrome of BN is justified remains controversial and its major impact on the reported prevalence rates needs to be evaluated.

3.2   *Prevalence and Etiology of Bulimia Nervosa*

Bulimia nervosa affects about 1% of young and middle aged women (Hudson, Hiripi, Pope, & Kessler, 2007; Hay, 2003; Striegel-Moore, et al., 2003; Al-Adawi, Dorvlo, Burke, Al-Bahlani, Martin, & Al-Ismaily, 2002; Johnson, et al., 2001; Westenhoefer, 2001). In adolescents, prevalence rates range from about 1% for a conservative diagnosis (Morande, Celada, & Casas, 1999) to up to 17 % when considering partial BN diagnosis or BN-related eating patterns (Ackard, Neumark-Sztainer, Hannan, French, & Story, 2001; Huon, Mingyi, Oliver, & Xiao, 2002; Jones, Bennett, Olmsted, Lawson, & Rodin, 2001; Rodriguez, Novalbos, Martinez, Ruiz, Fernandez, & Jimenez, 2001). Similar prevalence rates for both adolescents (Al-Adawi, et al., 2002; Jones, et al., 2001; Martin, Nieto, Jimenez, Ruiz, Vazquez, Fernandez, et al., 1999; Morande, et al., 1999; Nobakht & Dezhkam, 2000; Rhea, 1999; Rodriguez, et al., 2001) and adults occur across cultures (Nobakht & Dezhkam, 2000; Westenhoefer, 2001). There are few exceptions: Japanese and African American populations seem to be less affected than the European population (Nakamura, Yamamoto, Yamazaki, Kawashima, Muto, Someya, et al., 2000; Striegel-Moore, et al., 2003). However, if, for example, East African women are exposed to elements of the Western culture such as media exposure, they are more likely to show eating disorderd pathology (Eddy, Hennessey, & Thompson-Brenner, 2007).

Not much is known about specific factors leading to the development of BN. However, several risk factors have been suggested to contribute to its etiology. Personality, genetic and biological predispositions, family dynamics as well as socio-cultural factors seem to be involved (Jones, Leung, & Harris, 2006; Gordon, 1998). Socio-cultural influences affect individuals through internalized cultural ideals, such as the "thin ideal" (Stice, 2001). Individual factors, such as eating problems, eating conflicts, or struggle with food during childhood are risks for BN (Martin, et al., 1999). For example, dieting sets the tone for the

development of an eating disorder later on in life (Jones, et al., 2001). Restrained eating coupled with an intact appetite increases the drive to eat and leads to the dieter's dilemma (Palmer, 1998). Since individuals with BN are less capable of proper affect regulation, precursors such as anxiety, tension and boredom can lead them to succumb to their food craving (Beumont, 1998). They capitulate and binge-eat, usually experiencing a loss of control during the binge eating period (American Psychiatric Association, 1994). Guilt is the major feeling after a binge, often coupled with physical pain (Fairburn, Cooper, & Cooper, 1986). Various purging procedures bring relief, satisfaction, and relaxation (Beumont, 1998) and reduce the anxiety of weight gain (Fairburn, et al., 1986). Since purging behavior reduces aversive feelings, it represents a negative reinforcement, which may perpetuate the eating disorder. The person with low self-esteem does not quit dieting because of its failure; rather, the failed attempt to diet even intensifies the motivation and determination to achieve weight loss (Palmer, 1998). A vicious cycle establishes. Early psychopathology, e.g., obsessive-compulsive behaviors or personality disorders, increases the risk for developing an eating disorder in general (Anderluh, et al., 2003; Johnson, Cohen, Kasen, & Brook, 2006). It cannot be determined at this point why obsessive-compulsive behaviors and signs of personality disorders for some but not for all individuals lead to binge-eating and purging. Acquisition of etiology data begins only once symptoms become obvious. This reality leaves specific precursors to BN unknown. Some researchers conclude that genetic and environmental influences affect BN etiology (Nishiguchi, et al., 2001). Specifically, the role of different polymorphisms of the ghrelin gene has been discussed in the etiology of BN (Miyasaka, Hosoya, Sekime, Ohta, Amono, Matsushita, et al., 2007; Ando, Komaki, Naruo, Okabe, Takii, Kawai, et al., 2006; Cellini, Nacmias, Brecelj-Anderluh, Badía-Casanovas, Bellodi, Boni, et al., 2006). Environmental factors include, for example, a family history of eating disorders (Lilenfeld, et al., 1998) or a history of abuse (physical, sexual, or both; Lilenfeld, et al., 1998).

General factors may also partake in the etiology of bulimia. For example, major life events, family relations including parental arguments and criticism as well as abuse appear in the list of possible causes (Gordon, 1998; Palmer, 1998). Findings about major life events raise a methodological issue. In general, eating disorder onset precedes data acquisition. Thus, the disorder and possible concomitant psychopathology may color the memory and distort the actual impact as seen in studies concerning the association of critical life events and depression (Kessler, 1997). There is also the issue of base rates: Eating disordered patients may report many critical events but only a few people in the entire population with the same experience develop an eating disorder. One mechanism by

which major life events may impact eating disorders is their influence on the transformation of the self (Filipp & Ferring, 2002). Considering abuse or neglect as a specific major life event, one needs to consider its impact on development, when occurring during childhood. Different forms of child abuse, such as physical or sexual abuse, neglect or mixed maltreatment, lead to increased aggression and uncontrolled behavior (Trickett & McBride-Chang, 1995) as well as to disruptions of social and emotional development (Cicchetti & Olsen, 1990). Abuse can especially adversely change different aspects of the self, such as self-perception and self-awareness (Harter, 1999). These consequences may result in low self-esteem, possibly accompanied by self-denial and self-destructive behaviors (Harter, 1999).

Disclosing the perpetuating factors in BN is important for the development of effective treatment (Fairburn, Stice, Cooper, Doll, Norman, & O'Conner, 2003). Such perpetuating factors are still being studied. Discord prevails on how these mechanisms can maintain the illness. Two approaches have been utilized in attempts to expose the factors that turn BN into a persistent disorder: the study of predictors for outcome and the test of theoretical models (Fairburn, et al., 2003). The study of predictors for outcome has not been fruitful (Carter, Bulik, McIntosh, & Joyce, 2002; Grilo, Sanislow, Shea, Skodol, Stout, Pagano, et al., 2003; Herzog, Dorer, Keel, Selwyn, Ekeblad, Flores, et al., 1999) and has lead to contradicting results (Reas, Williamson, Martin, & Zucker, 2000; Reas, Schoemaker, Zipfel, & Williamson, 2001; Vaz, 1998). Various theoretical concepts and models have been proposed and tested by different researchers, including psychological, psychophysiological, and physiological concepts (see below).

The psychological perspective postulates that cognitive features of the disorder perpetuate the symptoms. For example, hopeless feelings and worries about the future may make it difficult to recover. Symptom abstinence cannot be anticipated as a positive aspect in life. Binging and purging probably serve as a relief of these hopeless feelings and this relief may be one of only a few pleasurable aspects a patient experiences in her life (Godley, Tchanturia, MacLeod, & Schmidt, 2001). Negative affect and dietary constraint have also been found to be reciprocally associated with BN symptoms, suggesting a perpetuating effect (Stice, 1998).

The psycho-physiological perspective postulates that combinations of psychological with physiological factors perpetuate the illness. For example, food restriction in combination with stress increases intake of especially palatable food in rats. This suggests that food

intake is driven by a motivation for reward rather than by the necessity to meet metabolic requirements (Hagan, Wauford, Chandler, Jarrett, Rybak, & Blackburn, 2002). This relationship could apply to patients with BN, as they tend to have more stressful relationships than normal controls (Johnson, et al., 2001). Stress and dietary restrictions could predispose people to binge eating.

Fairburn and colleagues (1986) suggested another psycho-physiological mechanism for perpetuation of the illness. The researchers argue that an initial shape and weight concern leads to dietary restrictions with rigid dietary rules, which predicts lapses. Definite rules are inflexible and do not bend as opposed to general principles. Rules in general invite failure since the smallest aberration is perceived as not succeeding. When a dietary rule is broken, an all-or-nothing thinking leads to binge eating: "now that the rule is broken, it does not matter anyway how much I eat" and the meal turns into a binge-eating episode. To reduce the subsequent feelings of guilt and to compensate for the amount of food eaten, individuals engage in compensatory behavior. The experience that an eating binge seems reversible lowers the threshold to engage in binge eating again. A cycle of binge eating and purging establishes and then perpetuates itself. In accordance with this model, Byrne and McLean (2002) found a reciprocal relationship of binge eating and purging triggered by the drive of thinness. In contrast to the original model, these researchers found that the drive for thinness does not trigger dieting but rather directly induces the urge to purge. Purging can control the anxiety about weight gain since it reverses the effects of binge eating. The authors argue that purging reduces or removes the negative connotation of eating and therefore perpetuates the eating disorder.

From a physiological perspective, changes in the individual's physiology are responsible for maintaining the illness (Jones, et al., 2001; Palmer, 1998). Alterations in the serotonergic system (a blunted response of cholecystokinin to ingested food) and dysfunction in peripheral satiety mechanisms may contribute to the enlarged meal sizes observed in BN (Walsh & Devlin, 1998). When binge eating and purging behaviors occur repeatedly, satiety feelings become disturbed. The impaired satiety mechanism leads to the ingestion of enlarged meals, which again triggers purging. A cycle establishes. Geliebter and Hashim (1992) propose that an increased gastric capacity leads to enlarged meals. They suggest that due to enlarged stomach capacity, gastric stretch receptors become activated only when the stomach is filled to a certain proportion of its limit. An adapted stomach capacity (enlargement) was suggested by different research groups that investigated the Garren-Edwards Gastric Bubble in the treatment of morbid obesity

(Puglisi, Antonucci, Capuano, Zavoianni, Lobascio, Martines, et al., 2007; Totte, Hendrickx, Pauwels, & Van Hee, 2001; Vandenplas, Bollen, De Langhe, Vandemaele, & De Schepper, 1999; Rigaud, Trostler, Rozen, Vallot, & Apfelbaum, 1995).

A new perspective has been introduced by Faris, Kim, Meller, Goodale, Oakman, Hofbauer et al. (2000) who suggest that repeated bulimic behaviors stimulate the vagus nerve, the nerve connecting the gastrointestinal tract, and thus the stomach, with the central nervous system. This repeated stimulation changes the activity level of the vagus nerve, which in turn drives the urges to binge eat and vomit. The researchers hypothesized that if vagal activity could be modified, symptoms of bulimia nervosa should improve, if not cease at all. To test this hypothesis, Faris and coworkers (2000) used an anti-nausea medication that acts on the vagus nerve. Studies are ongoing and not enough knowledge has been gained to allow statements about the long-term efficacy of this approach. Nevertheless, this innovative examination seeks new avenues in the search for alternative treatments.

3.3   Contemporary Treatment for Bulimia Nervosa

The above described concepts about perpetuation of the disorder have led to contemporary treatments using psychotherapy and antidepressants, in combination or alone. Psychotherapeutic treatment includes various kinds of psychotherapy, most commonly cognitive behavior therapy (CBT; Hartmann, Herzog, & Drinkmann, 1992; Lewandowski, Gebing, Anthony, & O'Brien, 1997). Pharmacological treatment uses a variety of antidepressants (Bacaltchuk, Hay, & Mari, 2000a; Bacaltchuk, Trefiglio, de Oliveira, Hay Lima, & Mari, 2000b; Nakash-Eisikovits, Dierberger, & Westen, 2002). In general, psychological therapies find broader acceptance among patients as shown in lower dropout rates (Bacaltchuk, et al., 2000b). Experimental studies compared different treatment settings and treatments. Individual therapies are more effective than group settings (Chen, Touyz, Beumont, Fairburn, Griffiths, Butow, et al., 2003) and cognitive-behavioral approaches prove better outcome than other psychotherapies (Agras, Walsh, Fairburn, Wilson, & Kraemer, 2000). Pharmacological treatment alone can effectively reduce bulimic symptoms when compared with placebo (Goldstein, Wilson, Ascroft, & al-Banna, 1999) and can also maintain a positive outcome for about twelve months (Romano, Halmi, Sarkar, Koke, & Lee, 2002) but is less effective when compared to psychotherapeutic approaches (20% vs. 40% recovery rate post-treatment; Bacaltchuk, Trefiglio, de Oliveira, Lima, & Mari, 1999; Bacaltchuk, et al., 2000b; Cox & Merkel, 1989). Combination of antidepressants with psychotherapy proved more effective than either alone as shown in

slightly higher recovery rates of 40-50% (Bacaltchuk, et al., 2000b), a finding that is unspecific to BN-treatment (Amato, Minozzi, Davoli, Vecchi, Ferri, & Mayet, 2004; Linden, Stossel, & Maurice, 1996; Pampallona, Bollini, Tibaldi, Kupelnick, & Munizza, 2004). Recently, the administration of an antiandrogenic oral contraceptive on appetite in a group of BN patients proved successful (Naessén, Carlström, Byström, Peirre & Lindén Hirschberg, 2007). However, any treatment leaves more than half of the patients without significant improvement. Often, studies report remission rather than recovery rates indicating inefficacy of the treatment regarding recovery (Ben-Tovim, Walker, Gilchrist, Freeman, Kalucy, & Esterman, 2001).

Overall, these treatments result only in recovery rates of about 40-50%, which were reported directly after dismissal from a study. Long-term recovery is rare and no treatment effect of any kind of treatment is evident ten years post-study (Keel, Mitchell, Davis, & Crow, 2002). Fairburn already concluded in 1997 that from the state of outcome studies in the late 1990-ies, we can only draw preliminary conclusions regarding effective treatment. This notion is still valid.

Long-term outcome studies of treatment found a high rate of relapses in originally recovered patients. With drug therapy, about 30 – 50 % of recovered patients relapse (Romano, et al., 2002). With CBT, relapse rates assume more than 40 % of initially remitted patients (Halmi, Agras, Mitchell, Wilson, Crow, Bryson, et al., 2002). A 7.5 year follow-up study for anorexia nervosa and BN revealed relapse rates of one third (Herzog, et al., 1999). These findings are alarming especially in the context of the natural course of BN, which was studied over a period of five years (Fairburn, Cooper, Doll, Norman, & O'Connor, 2000). Fairburn et al. (2000) found that about one half to two-thirds of an initial group improved markedly without treatment. Most individuals did not meet diagnostic criteria at the assessments at 15-months intervals. Each year, about one third of individuals of the initial group remitted and another third relapsed. In light of these findings, Bergh's study of a computer-guided satiety training for anorexia nervosa and BN observed surprisingly high rates of recovery of about 70% and very low relapse rates of only 7% twelve months post-treatment (Bergh, Brodin, Lindberg, & Sodersten, 2002). Nevertheless, the study of perpetuating factors as well as the underlying physiology should continue and stimulate future research since contemporary treatments proved insufficient for a favorable outcome.

Studying the mechanisms maintaining the illness is crucial for the improvement of current

therapies (Fairburn, et al., 2003). The fact that short-term satiety operates inefficiently in bulimia and may therefore perpetuate the disorder shifts the focus from psychological to physiological factors, that is, the neuro-circuits underlying meal termination. The nerves mediating short-term satiety and meal termination could be one such physiological factor. The major nerve transmitting satiety signals is the vagus nerve. The following is an elaboration of potential vagal involvement in bulimia nervosa.

3.4   Vagal Involvement in Bulimia Nervosa

Two lines of research suggest vagal involvement in bulimia nervosa. First, impaired satiety feelings and increased food intake suggest a malfunctioning in the circuits regulating meal termination. As discussed in chapter 4, short-term satiety is mediated by branches of the Xth cranial nerve, the vagus nerve. Second, bulimia patients experience an elevated pressure pain threshold on the fingers, which correlates with binge eating and purging behavior (Faris, Kim, Meller, Goodale, Hofbauer, Oakman, et al., 1998). Pain perception can be modulated by stimulation of the vagus nerve (Bohotin, Scholsem, Bohotin, Franzen, & Schoenen, 2003; Kirchner, Birklein, Stefan, & Handwerker, 2000). Vagal involvement in the regulation of satiety and the regulation of pain as well as impaired satiety and elevated pressure pain thresholds in BN suggest vagal contribution to this disorder.

3.4.1   Short-Term Satiety in Bulimia Nervosa

The consumption of abnormally large amounts of food in bulimia nervosa suggests that the system(s) regulating food intake and meal termination do not respond properly to stimulation by food ingestion. Indeed, many studies have focused on the disrupted eating pattern in bulimia patients and have supported this view (Geliebter, et al., 1992; Geracioti & Liddle, 1988; Hadigan, Walsh, Devlin, LaChaussee, & Kissileff, 1992; Kissileff, et al., 1996). Researchers compared postprandial satiety feelings, the amount of food ingested, the release of cholecystokinin (CCK), a polypeptide released from the duodenum as soon as food exits the stomach into the duodenum, and gastric emptying rates of BN patients with control subjects. Although the results do not clearly match, the majority indicate that satiety feelings, CCK release and gastric emptying are disrupted in BN. There is agreement in the literature that patients with BN have a larger stomach capacity and consume larger volumes than control participants.

Geracioti and Liddle (1988) found that patients rated their satiety significantly lower than control subjects after a fixed liquid meal of 400 ml. For half of the subjects in the bulimic

group postprandial satiety levels after 45 minutes dropped below the baseline fasting levels. Several research groups reported a significantly greater volume needed for bulimia patients to induce the same level of satiety and fullness then controls subjects (Geliebter, et al., 1992; Geliebter & Hashim, 2001; Kissileff, et al., 1996). Significantly larger stomach capacities and volumes ingested for bulimia patients were reported by independent research groups in different settings (Geliebter, et al., 1992; Geliebter & Hashim, 2001; Hadigan, et al., 1992; Kissileff, et al., 1996). This finding indicates that individuals with BN seem to have larger stomach sizes than healthy subjects. Yet several research groups reported contrasting results (Devlin, Walsh, Guss, Kissileff, Liddle, & Petkova, 1997; Halmi and Sunday, 1991; Pirke, Kellner, Friess, Krieg, & Fichter, 1994).

The release of CCK, an important gut peptide involved in satiety (see chapter 4.3.1), appears to be impaired in BN as well. Yet values for baseline CCK and postprandial CCK release reported in the literature are discordant. Abnormal as well as normal baseline and postprandial levels were found. Differences occur depending on the CCK measure utilized. For example, low baseline levels of CCK seem to prevail in non-blood (non-serum) measures such as T-lymphocyte concentrations of CCK-8 (Brambilla, Brunetta, Draisci, Peirone, Perna, Sacerdote, et al., 1995) and CCK-8 levels in cerebrospinal fluid (Lydiard, Brewerton, Fossey, Laraia, Stuart, Beinfeld, et al., 1993) whereas normal levels of CCK-8 occur in blood serum (Devlin, et al., 1997; Geracioti & Liddle, 1988; Phillipp, Pirke, Kellner, & Krieg, 1991; Pirke, et al., 1994). An abnormally low level of serum CCK-8 release post-prandially has been noted by two research groups (Devlin, et al., 1997; Geracioti & Liddle, 1988) and normal CCK-8 levels are reported by Phillipp et al. (1991).

Discordant results were also found when the effects of CCK were studied in the context of gastric emptying (Devlin, et al., 1997; Geliebter, et al., 1992). Devlin and coworkers reported that the CCK response which triggers gastric emptying was overall blunted in bulimics (Devlin, et al., 1997) whereas the group around Geliebter et al. (1992) found only an early delay of gastric emptying within five to 15 minutes post-prandially. Rates 30 and 45 minutes post-prandially were similar for patients and control subjects. Despite slightly different results, these researchers agree that impaired post-prandial CCK release contributes to impaired short-term satiety in BN. However, if CCK is a satiating factor, then its administration should reduce binge size. In fact, a study administering CCK-8 orally found no decrease in the amount of food eaten in an unrestricted test meal (Mitchell, Laine, Morley, & Levine, 1986). These results question CCK contribution to the perpetuation of this eating disorder.

Overall, the short-term satiety mechanism in bulimia patients seems to be functional, yet it operates with a significant higher threshold. Although bulimia patients have a significant larger gastric capacity, both bulimia and control subjects required a certain proportion of their stomach capacity filled in order to induce maximal fullness. Both groups showed the same ratio of *volume at maximal fullness* to *volume of gastric capacity* (Geliebter, et al., 1992). Hadigan and coworkers (1992) reported that patients ate less of an unrestricted test meal after a large appetizer compared to a small appetizer. The amount eaten from the unrestricted test meal depends on the size of the appetizer. Geracioti and Liddle (1988) found a positive correlation of CCK release and satiety ratings for both control and bulimia subjects. These results assume that short-term satiety in BN is functional, but has a substantially higher threshold to be triggered.

On the other hand, these results also clearly show a dysfunctional aspect of short-term satiety mechanisms in BN. Bulimia patients experience a blunted development in the perception of satiety. More than 25 years ago, Bruch (1974) had already theorized that the basic disturbance of people suffering from eating disorders can be seen in the way the sensation of hunger is experienced.

3.4.2   *Pain Detection in Bulimia Nervosa*

It is well acknowledged that patients with eating disorders, especially with BN, have elevated pain detection thresholds (PDTs). For example, BN patients need a much stronger mechanical or thermal stimulus in order to experience pain than control subjects (de Zwaan, Biener, Schneider, & Stacher, 1996; Faris, Raymond, de Zwaan, Howard, Eckert & Mitchell, 1992; Girdler, Koo-Loeb, Pedersen, Brown, & Maixner, 1998; Lautenbacher, Pauls, Strian, Pirke, & Krieg, 1991). The site of stimulation seems to be irrelevant, as Pauls, Lautenbacher, Strian, Pirke, and Krieg (1991) did not find differences between hand and foot measures. This assumes that there is no proximal to distal change in the perception of pain. Thermal and mechanical PDTs were found to be significantly correlated, suggesting a common neural pathway for both modalities (de Zwaan, Biener, Schneider, & Stacher, 1996).

The ability to detect tactile, non-painful stimuli, however, seems to be functional. Non-noxious stimuli can be perceived with no impairment as subjects with BN and healthy control subjects had the same sensitivity measures for tactile stimuli (Faris, et al., 1992; Pauls, et al., 1991) or for temperature changes of a non-noxious thermal stimulus (Pauls, et al., 1991).

Several explanations were suggested for the elevation of pain thresholds in BN. Most of them had to be abandoned as they were falsified. The involvement of pain inhibiting opiate mechanisms was excluded since naloxone, an opiate antagonist, neither affected PDTs in BN nor in healthy control subjects (Lautenbacher, Pauls, Strian, Pirke, & Krieg, 1990). Also falsified was a deficit in neural temporal summation. Phasic and tonic thermal stimulation resulted in the same elevated PDTs (Lautenbacher, et al., 1990). Elevated PDTs could not be explained by physiological changes induced by chronic dieting. First, metabolic indicators for chronic and acute dieting did not correlate with PDTs (Lautenbacher, et al., 1990) and second, in a group of healthy subjects, dieting did not influence the PDTs (Lautenbacher, Barth, Friess, Strian, Pirke, & Krieg, 1991). Neuropathy was considered as a reason for elevated PDTs but proved to be irrelevant (Pauls, et al. 1991). Since neuropathy first involves nerve endings at distal sites, distal sites should show an increased pain tolerance over proximal sites. Such a difference could not be found (Pauls, et al., 1991). Emotional symptoms such as depression or anxiety (Raymond, Eckert, Hamalainen, Evanson, Thuras, Hartman, et al., 1999) as well as impairment in experiencing emotions (de Zwaan, Biener, Bach, Wiesnagrotzki, & Stacher, 1996) were not correlated with PDTs and were therefore disregarded as a cause for elevated pain perception.

Overall, the two lines of research examining non-painful tactile and various painful stimuli lead to the conclusion that only the pain-pathway operates dysfunctionally in BN. In fact, discriminative touch and nociception recruit different systems to relay information to the central nervous system (CNS).   Discriminative touch includes light touch, tactile information, flutter-vibration, and proprioception, which are transmitted via large diameter, fast conducting fibers.   These fast conducting fibers use two main pathways: the trigeminothalamic pathway supplying the face and the dorsal column-medial lemniscal system with the principal spinocerebellar pathways supplying the body. Nociception, in contrast, is transmitted via small diameter and slowly conducting fibers traveling within the anterolateral system.

Faris et al. (1992) showed that pain perception in BN does, in some respect, function normally. The difference in PDTs between fingers in BN was similar to values of a healthy control group, although the overall pain threshold was elevated for BN. The dysfunctional pain perception is therefore a malfunction of the basic level of operation and not an impairment of the perception per se. In other words, pain perception operates well yet needs a significantly more input to stimulate appropriate fibers to activate the pain system.

Elevated PDTs could be due to two different phenomena. First, the detecting, ascending pathways are dysfunctional, and second, the pain inhibiting, descending pathways are hyperactive. Fiber degeneration (neuropathy) of the ascending fibers was ruled out as a correlate for increased pain endurance (Pauls, et al., 1991). Impairment in the ascending fiber system would increase pain thresholds, as the pain signal could not be transmitted correctly to the CNS. Some evidence points toward the hyperactivity of the pain inhibition system which can be influenced by a modulation in vagal activity (Aicher & Randich, 1990). Faris et al. (1998) found that PDTs in BN were positively correlated with the time that had elapsed after a binge vomit cycle. That is, the more time had passed after a binge-vomit episode, the greater the elevation in PDTs. This was particularly true when the subjects had exceeded their usual inter-binge interval, which is dependent on the binge/vomit frequency. Since ondansetron, an antagonist acting on 5-HT3 receptors of the vagus nerve, abolished this correlation, there is strong evidence that the vagus nerve plays a role in elevated PDTs in BN. Raymond and coworkers (1999) could replicate the finding that engaging in bulimic behavior modulates acute pain detection. Girdler et al. (1998) found that blood pressure was positively related to pain induced by a pressure cuff only in BN patients but not in otherwise healthy subjects. Since blood pressure and cardiac function is mediated by the vagus nerve, these findings further support the hypothesis of vagal involvement in BN.

In fact, several researchers established a link between vagal afferent activity and the pain inhibitory pathway. Vagal nerve stimulation (VNS) used in refractory epilepsy has revealed a concomitant analgesic effect in humans (Ness, Randich, Fillingim, Faught, & Backensto, 2001). Studying rats, Bohotin, Scholsem, Bohotin, Franzen, and Schoenen (2003) found that stimulation at a higher intensity than used for epilepsy treatment results in a rapid decrease in pain sensitivity (increased PDTs). When stimulation parameters common for epilepsy treatment were used, pain thresholds decreased shortly after VNS onset but decreased and reached a plateau after chronic treatment of more than 48 hours. The authors argue that the analgesic effect is due to a supraspinal mechanism since VNS is effective across different spinal levels. Animal research has discovered that acute electrical stimulation of vagal afferent fibers (Ren, Randich, & Gebhart, 1988) and brainstem areas such as the nucleus tractus soletarius (NTS) receiving vagal afferent fibers (Aicher & Randich, 1990; Ren, Randich, & Gebhart, 1990) produce analgesic effects. Those findings support a direct involvement of vagal afferent fibers in nociception.

Sedan, Sprecher and Yarnitsky (2005) examined whether activation of the gastric vagal afferent fibers inhibits pain perception in healthy humans. The authors administered tonic heat pain and measured pain temporal summation to a mechanical stimulus and laser pain evoked potentials pre and post consumption of 1,500 ml water. The authors found a significantly increased threshold to heat-induced pain after water consumption and concluded that stimulation of the gastric vagal afferent fibers inhibited somatic pain perception. Significant increases in pain thresholds were found for thermally but not mechanically induced pain. The measurement of mechanical pain used the von Frey filaments commonly used to determine tactile and pain thresholds (von Frey, 1922). It would be of interest to determine if the use of an analgesiometer for administration of a mechanical painful stimulus, as used for example in the studies of Faris and coworkers (1992), would yield similar results.

3.5   Summary

There is strong evidence that the pathophysiology of bulimia nervosa (BN) involves vagal activity. Two lines of research have suggested dysfunctionality in vagal afferent neural transmission in BN: first, the study of satiation in BN and second, the study of pain detection in BN. Short-term satiety and pain detection, both modified by vagal activity, are impaired in BN. Patients with BN feel less satiated than healthy control subjects after a fixed meal and BN patients need a significantly larger amount of food in order to induce the same perceptual level of fullness than control subjects. Pain detection thresholds are elevated as bulimia patients can tolerate significantly higher intensities of thermal or mechanical stimuli before they experience pain when compared to a healthy control group. The sensitivity to non-noxious stimuli, on the opposite, operates normally. Furthermore, the mechanisms of satiety and pain detection appear somewhat functional. Pre-meal appetizer size does affect the amount eaten during a subsequent test meal as a larger appetizer results in the consumption of less food than a small appetizer. However, the overall amount eaten is significantly greater for BN subjects when compared with a normal control group. Regarding PDTs, pain detection varies across the finger studied, a phenomenon not different from the measures of normal control subjects. The latter findings not only suggest vagal involvement in BN, they imply the hypothesis that vagal hyperactivity plays a role in BN.

4   Regulators of Short-Term Satiety

Satiety is defined as "sufficiency" in general, and as "full gratification of appetite or thirst, with abolition of the desire to eat or drink" (Dorland, 2000). This definition is a very general one and it lacks the important distinction between two components of satiety: a long-term and a short-term component. Long-term satiety is an energy balance system that monitors how much energy and food the organism needs in order to function and survive. It regulates the duration of the periods of non-eating between eating episodes (inter-meal intervals). Short-term satiety addresses size and duration of one distinct eating episode. It regulates one discrete episode of eating and its termination.

Different humoral factors are indicative for either long-term or short-term satiety. Long-term satiety is mostly regulated by insulin (Chapman, Goble, Wittert, Morley, & Horowitz, 1998) and leptin (Blevins, Schwartz, & Baskin, 2002). Ghrelin also contributes to long-term satiety yet acts as a meal initiator, not as a satiety factor (Erdmann, Töpsch, Lippl, Gussmann, & Schusdziarra, 2004). Short-term satiety is mainly mediated by cholecystokinin (CCK), a polypeptide occurring in the intestines (Buchan, Polak, Solcia, Capella, Hudson, & Pearse, 1978; Polak, Bloom, Rayford, Pearse, Buchan, & Thompson, 1975) and in the brain (De Fanti, Backus, Hamilton, Gietzen, & Horwitz, 1998; Schick, Reilly, Roddy, Yaksh, & Go, 1987). The gastrointestinal hormone polypeptide YY (PYY) is thought to act as a long-term and short-term mediator of satiety (Batterham, Cowley, Small, Herzog, Cohen, Dakin, et al., 2002; Tso & Liu, 2004) and pancreatic polypeptide (PP) and glucagon-like peptide 1 (GLP-1) are considered to act as short-term mediators of satiety (Schmidt, Näslund, Grybäck, Jacobsson, Holst, Hilsted, et al., 2005; Donahey, van Dijk, Woods & Seeley, 1998). Long-term and short-term satiety need to operate interactively in order to maintain homeostasis and several mediating systems have been suggested (Fan, Ellacott, Halatchev, Takahashi, Yu, & Cone, 2004; Matson, Reid, Cannon, & Ritter, 2000; Wang, Barachina, Martinez, Wei, & Tache, 2000).

There is evidence that the systems regulating long-term and short-term satiety operate through dissociated mechanisms. Disruption of the short-term satiety system leaves the mechanism of long-term energy balance intact. For instance, interruption of the short-term satiety mechanism in mice and rats increased meal size yet had no effect on global energy intake, that is, overall caloric intake and body weight remained unchanged (Fox, Phillips, Baronowsky, Byerly, Jones, & Powly, 2001; Schwartz, Salorio, Skoglund, & Moran, 1999). This phenomenon is also apparent in the bulimic population. People diagnosed

with bulimia nervosa, characterized by binge eating followed by compensatory purging behavior, experience an insufficient short-term satiety in that they have extreme difficulty terminating a discrete eating episode yet are able to maintain a normal body weight (see chapter 3.4.1).

The initiation of an actual period of eating is influenced by different factors like habits, learned associations, opportunity and time of the day. Once eating has begun, meal size is regulated by various satiety factors (Smith, 1999). Smith (1999) suggests that gastric distention depending on meal volume and nutrient content may have a direct effect on meal termination. Besides these direct factors, indirect factors such as circadian rhythm, metabolic changes, environment, and stress affect the control of food intake. Indirect factors mediate CNS-processing of direct factors (Smith, 1999).

Once eating is initiated, the ingested food affects two different regulatory processes. One process maintains eating via a positive feedback loop (Smith, 1999). Stimuli triggering this positive feedback arise from the mouth (Blundell, 1991). The second process terminates eating via a negative feedback loop (Smith, 1999). Stimuli triggering the negative feedback system arise from the stomach and the gastrointestinal tract (Blundell, 1991). If and how the two feedback loops interact with each other is not clear at this time. Are the two systems interconnected to communicate with each other or are they two distinct entities whose signals are weighed against each other, with the stronger system influencing current behavior? Furthermore, research continues to resolve the question of how meal termination is represented in the two feedback loops. Is meal termination induced by a) a decrease in positive along with an increase in negative feedback, or b) a decrease in positive feedback with no change in negative feedback, or c) no change in positive feedback along with an increase in negative feedback (Smith, 1999)?

4.1   Methods for Studying Meal Termination

Before food is ingested, different senses detect food. Sight, smell and experience help us to decide what food we will eat. Ingested food has its first contact in the alimentary tract with taste buds on the tongue. After swallowing, it enters the stomach via the esophagus. The proximal stomach (fundus) serves as a food reservoir. Gastric pressure and contraction waves move the contents distally toward the small intestine where they are processed mechanically and chemically. Food entering the small intestine triggers the release of a variety of peptides affecting food intake directly or indirectly, such as cholecystokinin

(CCK; Liddle, 1997), enterostatin (Shargill, Tsujii, Bray, & Erlanson-Albertsson, 1991), bombesin-like peptides (Ladenheim, Wirth, & Moran, 1996), somatostatin (Lotter, Krinsky, McKay, Treneer, Porter, & Woods, 1981), pancreatic peptide, and peptide YY (Asakawa, Inui, yuzuriha, Ueno, Katsuura, Fujimiya, et al., 2003), gastric inhibitory polypeptide and glucagon-like peptide 1 (Lavin, Wittert, Andrews, Yeap, Wishart, Morris, et al., 1998). These peptides either directly or indirectly stimulate additional processes involved in digestion, for example, CCK modified gastric acid secretion via different mechanisms (Burckhardt, Delco, Ensinck, Meier, Bauerfeind, Aufderhaar, et al., 1994), affected pancreatic enzyme secretion (Louie, May, Miller, & Owyang, 1986), and modulated gastric emptying (Moran & McHugh, 1982; Reidelberger, Arnelo, Granqvist, & Permert, 2001). In the small intestine, mechanical and chemical processes stimulated by the released peptides prepare the food for nutrient and water absorption. A vast amount of research targeted two main questions about satiety: First, where along the alimentary canal does satiety develop? Second, to what extent do volume and nutrients contribute to the development of satiety? Different study designs and methodologies were used in animals to find answers to these questions. The following is a description of such methodologies.

4.1.1   Gastric Fistula

The gastric fistula is "an abnormal passage communicating with the stomach; often applied to an artificially created opening through the abdominal wall into the stomach" (Dorland, 2000). A cannula with an external opening is sutured into the stomach and abdominal walls. A lid or screw opens and closes the external opening. A closed fistula allows food to be ingested and processed naturally. An open fistula does not allow food to accumulate in the stomach as it is withdrawn immediately. Food enters yet does not accumulate in the stomach. This process is called sham feeding. Sham feeding permits observations about whether satiation occurs as a result of pre-gastric processes and whether nutritive or volumetric factors control peptide release. For example, if meal size is unaffected by an open versus a closed fistula, then satiety must occur before food enters the stomach. A gastric fistula also allows for infusion of different nutrient contents or volumes into the stomach. The effect of such infusions on a subsequent test meal can give further information about short-term satiety and hormonal mechanisms.

4.1.2   Pyloric Cuffs

Occluding the pyloric sphincter with an inflatable cuff prevents stomach contents from entering into the duodenum. All ingested food accumulates in the stomach. The pyloric sphincter is the "thickening of the circular muscle of the stomach around its opening into

the duodenum" (Dorland, 2000). It regulates stomach emptying. By blocking the pyloric sphincter, it is possible to study the satiating effects of nutrients and the satiating effects of volume of the ingested food. It is also possible to evaluate whether short-term satiety develops in the stomach. The pyloric cuff was often applied in combination with implanted gastric fistulas (Deutsch, Young, & Kalogeris, 1978; Deutsch & Gonzalez, 1980) to withdraw stomach contents or to infuse either saline or nutrients, which were previously withdrawn from the stomach. Combining the pyloric cuff with implanted gastric fistulas allows for monitoring if animals compensate for either withdrawn or infused contents with respect to nutrients and volume ingested in a subsequent test meal.

4.1.3   *Pharmacological Interventions*

Cholecystokinin (CCK) was discovered as a satiating gastric polypeptide in the early 1970s (Gibbs, Young, & Smith, 1973b). It is released from cells in the upper small intestine (duodenum and proximal jejunum) in response to bypassing food (Liddle, Green, Conrad, & Williams, 1986; Liddle, 1997). If CCK and other pancreatic and cerebral peptides are satiating factors, then administration of these agents or their agonists should increase satiation and decrease meal size. On the other hand, administration of the corresponding antagonists should decrease satiation and increase meal size. An important requirement for the satiating effects of peptides and their agonists is that they do not induce malaise or distress in the animal studied. Such aversive circumstances would question meal termination due to satiation and would suggest a decrease in food intake due to pain or illness.

4.1.4   *Vagotomy*

The vagus nerve, the Xth cranial nerve connecting the viscera with the CNS, has been suggested as a contributor to short-term satiety when activation of gastric stretch receptors was found to induce impulses in vagal nerve fibers (Paintal, 1953, 1954). Vagotomy is the vagal denervation of distinct branches of the vagus nerve. Cutting or crushing the nerve branches usually accomplishes denervation. Vagotomy has shown that peripherally administered CCK acted at abdominal sites through organs innervated by the gastric vagus nerve (Smith, Jerome, Cushin, Eterno, & Simansky, 1981). Since then, the effects of vagotomy on food intake and on CCK-satiating effects have been studied extensively in animals (Furness, Koopmans, Robbins, Clerc, Tobin, & Morris, 2001; Gonzalez & Deutsch, 1981; Moran, Baldessarini, Salorio, Lowery, & Schwartz, 1997; Phillips & Powley, 1998; Towbin, 1955) and in humans (Andrews & Taylor, 1982; Loeweneck, von Ludinghausen, & Mempel, 1967).

4.2   *Polypetides in the Regulation of Food Intake*

The involvement of the family of polypeptides in feeding was recognized in the 1970s and 1980s and Kazuhiko Tatemoto (1982) established a high degree of sequence homology between neuropeptide Y (NPY) and the peripheral peptides polypeptide YY (PYY) and pancreatic polypeptide (PP). Orexins (also called hypocretins) and glucagon-like peptide 1 (GLP-1) are other polypeptides involved in the regulation of satiey. Whereas high levels of central NPY and orexin are indicative of food deprivation and injections of NPY and orexins stimulate food intake (Stanley & Leibowitz, 1985; Taylor, Lester, Hudson, & Ritter, 2007; Asakawa, Inui, Inui, Katsuura, Fujino, & Kasuga, 2002), elevated levels of PP, PYY, GLP-1 and CCK are indicative of the postprandial state and injections of either peptide inhibit subsequent food intake (Jesudason, Monteiro, McGowan, Neary, Park, Philippou, et al., 2007; Batterham, et al., 2002; Gutzwiller, Göke, Drewe, Hildebrand, Ketterer, Handschin, et al., 1999; Gibbs et al., 1973b). The effects of CCK will be discussed in detail in chapter 4.3.1.

Pancreatic Polypeptide (PP)
PP is released in the periphery of the pancreatic islets (Adrian, Besterman, Cooke, Bloom, Barnes, & Russell, 1977; Adrian, Ferri, Bacarese-Hamilton, Fuessl, Polak, & Bloom, 1985) in response to ingestion of a meal. Plasma levels of PP in humans increased within 15 minutes after meal ingestion and peak at 25 minutes at a level almost 200-fold off baseline before they fell and remained at a 100-fold level for 6 hours (Adrian, et al., 1977). Other researchers reported a higher baseline and a 7-fold increase of plasma PP levels and confirm the fast action of PP (Inui, Okita, Miura, Hirosue, Mizuno, Baba, et al., 1993). Such release pattern indicates that human PP likely acts as a humoral meal terminator. Meal termination due to PP showed to be dependent on the nutrient contents of the meal, as the physiological release of PP after water intake (mechanical distension of the stomach) was minimal in a study examining the role of PP in short-term metabolic control (Schmidt, et al., 2005).

Polypeptide YY (PYY)
PYY is released in the gastrointestinal tract and is suggested to act centrally via the NPY Y2 receptor in the hypothalamic arcuate nucleus (Batterham, et al., 2002). Higher peripheral PYY concentrations were found the more distally the release site in the gastrointestinal tract, with maximum concentrations observed for PPY release in the terminal ileum (Adrian, et al., 1985). Concentrations of plasma PYY are dependent on the

ingested nutrient content as protein elicited the largest PYY increase followed by fat and carbohydrates (Pedersen-Bjergaard, Høst, Kelbæk, Schifter, Rehfeld, Faber, et al., 1996; Adrian, et al., 1985). Concentrations of plasma PYY are also dependent on the caloric intake as a higher calorie meal resulted in a higher plasma level of PYY (Adrian, et al., 1985). Plasma levels of PYY reach a 2 hours postprandial maximum and remain elevated for several hours (Davis, Hickner, Tanenberg, & Barakat, 2005; Monteleone, Martiadis, Rigamonti, Fabrazzo, Giordani, Muller, et al., 2005; Batterham, et al., 2002, Adrian, et al., 1985). Elevated PYY levels affect subsequent food intake over up to twelve hours and loose their effects thereafter (Batterham, et al., 2002). Thus, PYY is unlikely to contribute to meal termination. It rather regulates in-between meal times and is a longer-term regulator of energy consumption.

Glucagon-Like Peptide 1 (GLP-1)

GLP-1 is released from enteroendocrine L-cells from the distal gut in response to food intake (Herrmann, Göke, Richter, Fehmann, Arnold, & Göke, 1995). Peripheral injection of GLP-1 resulted in lower spontanuous food intake in humans in an ad libidum test meal (Flint, Raben, Astrup & Holst, 1998). Intravenous infusions of GLP-1 in human subjects dose dependently decreased food intake 20 minutes after injection (Gutzwiller, et al., 1999); the higher the dose the less food subjects ingested. However, the significant decrease in food intake was achieved with supraphysiological levels of GLP-1. The oral administration of glucose induced GLP-1 release peaking at 30-60 minutes after ingestion. A rapid release of GLP-1 in plasma could be observed at 5 minutes after the oral ingestion of a mixed liquid meal; plasma levels declined 30 minutes after meal ingestion (Herrmann, et al., 1995). Short-term but not long-term effects of GLP-1 were later confirmed in subsequent studies (Donahey, et al., 1998). This release pattern classifies GLP-1 as a short-acting satiety signal and it potentially contributes to meal termination. However, the suppressed food intake after oral ingestion but not after intravenous administration suggests that GLP-1 release is triggered by nervous reflexes (Herrmann, et al., 1995); possibly mechanical distension of the stomach and the stimulation of gastric mechano receptors. It is more likely that GLP-1 acts in synergy with gastric distension.

4.3   Meal termination

Given that the bulimic population experiences dysfunctional short-term satiety along with an intact long-term energy balance, this chapter focuses on what is known to date about short-term satiety, which is interchangeably called meal termination or satiation in the

literature. Short-term satiety is concerned with how much food is eaten at one occasion whereas long-term control of food intake is concerned with when eating is initiated. Thus, the organism requires mechanisms that monitor the amount and calories absorbed. Sight, taste, or smell which detect food to be ingested likely are involved as monitoring mechanisms (Yeomans, Weinberg, & James, 2005; Woods, 2004). French and Cecil (2001) indeed concluded that satiety is dependent on taste. They summarized that various nutrients given orally have different satiating effects than when given through an intragastric infusion. Hunger ratings were higher after intragastric infusion, which blinded subjects regarding the ingested contents; the nutrient content had less satiating effects. The authors concluded that oral sensation contributes significantly to the satiating properties of food. However, when sight, taste and smell are the only senses ingested food has contact with such as in rats with a chronic gastric fistula, rats continue eating for a prolonged period of time (Davis & Smith, 1990; Gibbs, et al., 1973b). Satiation does not occur. Thus, although orosensory aspects of the ingested food modulate satiety (French & Cecil, 2001), satiety as a whole must evolve beyond the proximal portions of the alimentary canal (Davis & Smith, 1990). In addition, gastric fistula experiments excluded the possibility that satiety develops based on a learned experience solely dependent on motor activity that accompanies ingestive behavior (Gibbs, et al., 1973b). Subsequent research has focused on the satiating effects of food beyond pre-gastric events. Gastric and post-gastric events include the satiating effects of ingested food in respect to its volume and nutrient content and the satiating effects of CCK released in the duodenum.

4.3.1   *Effects of Cholecystokinin on Food Intake*

The release of the major and most potent peptide cholecystokinin (CCK) is triggered by nutrients passing and accumulating in the small intestine. Cholecystokinin is released into the blood stream from the enteroendocrine I-cells in the duodenum (Berthoud & Patterson, 1996; Liddle, et al., 1986; Liddle, 1997; Polak, et al., 1975). I-cells become stimulated by nutrients passing through the duodenum and CCK-containing granulas release their content into the blood stream and the surrounding tissue (Buchan, et al., 1978). The amount of postprandial CCK released into the circulation varies depending on the nutrient contents of a meal (Liddle, et al., 1986). Cholecystokinin has also been detected in the CNS in cerebrospinal fluid as well as in brain tissue (Rehfeld & Kruse-Larsen, 1978). Receptors with a high affinity for CCK have been classified into two subtypes, CCK-A and CCK-B receptors, based on their location (Moran, Robinson, Goldrich, & McHugh, 1986). CCK-A receptors are most abundant in the alimentary tract in the periphery yet can be found to a lesser degree in the CNS, CCK-B receptors predominate in the CNS (Moran, et al., 1986).

The satiating effects of CCK were discovered when Gibbs, Young and Smith (1973a) illustrated that synthetic CCK octapeptide (CCK-8) injected intraperitoneally decreased food intake in normally fed rats. This finding was confirmed by several other researchers (Linden, 1989; Linden, Uvnas-Moberg, Forsberg, Bednar, & Sodersten, 1989) as well as in sham fed rats (Gibbs, et al., 1973b). Numerous subsequent studies illustrated that both exogenous CCK and endogenous CCK have satiating effects. Administration of CCK antagonists indicated that endogenously released CCK as well has satiating effects (Linden, et al., 1989; Linden, 1989; Shillabeer & Davison, 1984). Such CCK blockade abolished the satiating effects of CCK and increased food intake. Further research found CCK receptors within peripheral afferent fibers of the vagus nerve (Moran, Norgren, Crosby, & McHugh, 1990; Zarbin, Wamsley, Innis, & Kuhar, 1981) and vagal afferent sensitivity to CCK (Schwartz, Moran, White, & Ladenheim, 1997). Vagotomy abolished the satiating effect of peripheral CCK (Reidelberger, Hernandez, Fritzsch, & Hulce, 2004; Smith, et al., 1981), providing further support for vagal contribution to CCK-induced satiety.

Though peripheral action of CCK seems well supported, CCK may also act via a central mechanism in reducing food intake. It was suggested that central CCK is involved as a neurotransmitter in overall regulation of food intake (Straus & Yalow, 1979). The involvement of central CCK in feeding was confirmed by findings that injections of CCK-8 into the cerebral ventricles reduced food intake (Della-Fera & Baile, 1979) and that food intake released CCK in the hypothalamus (De Fanti, et al., 1998; Schick, et al., 1987). When injected specifically in the medial-basal hypothalamus and nucleus of the solitary tract in the brainstem, CCK-8 suppressed feeding (Blevins, Stanley, & Reidelberger, 2000). Recently, it has been suggested that CCK-8 also regulates pancreatic-exocrine secretion via efferents from the DMV projecting to the pancreas (Wan, Coleman, & Travagli, 2007). Other brain areas (medial amygdala, nucleus accumbens, posterior hypothalamus, dorsal raphe, ventral tegmental area) did not respond to CCK-8 injections. Thus, CCK-8 has distinct effects depending on its site of action in the CNS (Blevins, et al., 2000). The satiating effect of central exogenous CCK was blocked by CCK-receptor antagonists in the brain (Corp, Curcio, Gibbs, & Smith, 1997) and CCK antagonists given alone resulted in increased food intake (Corp, et al., 1997; Dorre & Smith, 1998; Ebenezer, 2002; Reidelberger, et al., 2004). However, it is not clear whether peripheral and central CCK act in synergy. Reidelberger and associates (2004) administered CCK antagonists with different blood-brain barrier permeability with an intragastric peptone infusion to release endogenous CCK. They combined drug and peptone treatment with vagotomy or sham vagotomy. Both antagonists increased food intake of a subsequent test meal in control rats,

yet only the blood-brain barrier penetrating antagonist increased food intake in the vagotomized rats. Thus, endogenous CCK released by nutrients acts peripherally via the vagus nerve and through mechanisms beyond the blood-brain barrier (Reidelberger, et al., 2004).

Peripheral CCK was found to act in synergy with gastric distension. It was first shown that slow-adapting vagal afferent fibers respond to gastric loads as well as to CCK (Schwartz, McHugh, & Moran, 1991). Vagal stimulation with CCK doubled vagal responses to a gastric load even when the initial CCK-response had ceased and vagal firing rate had declined to baseline values. This result suggests peripheral integration of responses to CCK and to a gastric load at the level of the vagus nerve (Schwartz, McHugh, & Morna, 1991). It was then shown that when a low dose of a CCK analogue, which had no effect when given alone, did significantly decrease food intake when combined with a gastric load (Schwartz, Netterville, McHugh, & Moran, 1991). It was further shown that CCK and gastric loads act in synergy (Schwartz, McHugh, & Moran, 1993). Both stimuli administered alone at a sub-threshold dose did not change vagal afferent activity patterns whereas the two sub-threshold doses together significantly changed vagal afferent firing. Moreover, supra-threshold levels of both stimuli significantly increased vagal activity over the activity elicited by each stimulus alone (Schwartz, et al., 1993). To further examine the relationship between vagal responses evoked by CCK and by gastric distension, Schwartz, McHugh and Moran (1994) administered CCK-A and CCK-B antagonists separately. The CCK-B receptor antagonist failed to affect both vagal responses evoked by CCK and by gastric distension, whereas the CCK-B antagonist only abolished vagal responses evoked by CCK and had no effect on vagal responses evoked by gastric distension. The authors concluded that vagal responses evoked by CCK and by gastric distension act via dissociable mechanisms and that the vagal afferent response to CCK is dependent on the CCK-A receptor (Schwartz et al., 1994).

4.3.2 *Effects of Nutrients and Gastric Volume on Food Intake*

The question of whether ingested nutrients or the gastric load initiate satiety dates back to the late 1970s and the early 1980s. The discussion about nutrient-induced vs. distension-induced satiety was initiated by the research group around Deutsch (Deutsch & Wang, 1977; Deutsch, et al., 1978; Deutsch & Gonzalez, 1980; Gonzalez & Deutsch, 1981). Addressing this very same issue, Gerard Smith (1998) divides satiating signals into pre-absorptive mechanical and post-absorptive chemical signals. He further classifies satiety as pre-gastric, gastric and intestinal. Some pre-gastric signals must be present since sham-fed

rats stop eating although neither volume could accumulate in the stomach nor could nutrients be absorbed (Kraly, Carty, & Smith, 1978).

Gastric satiety appears to be of mechanical nature whereas intestinal satiety appears to be of chemical (nutritional) nature (Powley & Phillips, 2004). The first indicators for gastric distension-induced satiety emerged when Paintal (1953; 1954) discovered in the mid 1950s that activation of gastric stretch receptors resulted in signals in vagal afferent fibers. Vagal involvement in short-term satiety has recently been confirmed with different study designs (van de Wall, Pomp, Strubbe, Scheurink, & Koolhaas, 2005). Deafferentiation of capsaicin-sensitive vagal C-fibers resulted in overconsumption of sucrose solution yet an adjustment in intake of additional chow. Although Deutsch and colleagues (1977, 1980) originally suggested a nutritive gastric satiation component based on experiments using occlusion of the pyloric sphincter, Phillips and Powley (1996) objected that the cuff placement used by Deutsch and colleagues was placed insufficiently. They claim that the cuff was placed to far distally so that duodenal sites were included in the sites of examination, determined as "stomach". These proximal duodenal sites are heavily innervated by chemoreceptors (Mei, 1985) and therefore detected nutrient composition of food ingested (Phillips & Powley, 1996).

Further support for volumetric, and non-nutritive, gastric satiety came from studies using inflated pyloric cuffs combined with withdrawal of stomach contents (Davis & Campbell, 1973; Deutsch, et al., 1978). These experiments showed that rats compensated for the volume that was extracted from the stomach. Pre-load infusions show a dose dependent reduction in subsequent food intake in rats independent of the nutrient content of the pre-load (Phillips & Powley, 1996). Milk drinks of different volumes yet containing the same energy content resulted in volume adjustment of a subsequent test meal in men (Rolls, Castellanos, Halford, Kilara, Panyam, Pelkman, et al., 1998), indicating that volume rather than energy content affected food intake. The overall energy intake over a 24-hour period, however, was the same for all groups indicating that systems monitoring energy balance incorporated the same energy content of the different volumes (Rolls, et al., 1998).

Intestinal satiety, however, is sensitive to the nutrients ingested. Rats consume more of low caloric solutions than of high caloric solutions when the load is allowed to pass along into the small intestine. The very same solutions differing in nutrient content had no effect on gastric satiety, which depends on volumetric signals (Phillips & Powley, 1996)

Gastric and intestinal signals of satiety operate in synergy. It was shown earlier that CCK, an intestinal satiety signal, activates vagal afferent fibers in synergy with gastric distension signals (Schwartz, et al., 1993), a finding that was later confirmed in humans by Kissileff, Carretta, Geliebter, and Pi-Sunyer (2003). The authors administered CCK octapeptide or saline intravenously with or without gastric distension. Gastric distension in combination with CCK resulted in a larger decrease of food intake than CCK or distension alone. Such integration of signals at the level of the vagus nerve indicates an interaction of gastric and intestinal satiety signals in the periphery. Sensory responses to gastric distension were indeed modified by duodenal nutrients as studied in a group of healthy volunteers (Feinle, Grundy, & Read, 1997). Gastric distension with an air-filled balloon was combined with either saline or nutrient containing duodenal infusions. Subjects reported sensations of gastric sensation. Balloon inflation in combination with nutrient infusions resulted in a meal-like satiety sensation. In contrast, subjects described balloon inflation in combination with saline infusion as pressure located in the abdomen (Feinle, et al., 1997). Experimental research with free-feeding humans has found that it is not the caloric content of a meal but its actual weight in grams in the stomach which induces the feeling of satiety (de Castro, 2005).

4.4   Summary

When food enters the alimentary tract it accumulates in the stomach and is rapidly emptied into the duodenum. With increasing volume in the stomach, vagal afferent fibers increase firing and signal stomach distension to the CNS via distinct pathways (see chapter 5). In addition to gastric distension, food emptied into the duodenum stimulates the release of CCK and other polypeptides. Although CCK mainly affects a variety of peripheral processes involved in digestion via the vagus nerve, it also acts on central structures, probably not protected by the blood-brain barrier. However, short-term satiety seems to develop peripherally triggered by the synergistic action of CCK with gastric distension. Both signals are integrated peripherally at the level of the vagus nerve. Thus, the vagus nerve represents the main mediator of short-term satiety. Infact, vagus nerve stimulation has been shown to affect cravings-ratings for sweet food in a study with human subjects (Bodenlos, Kose, Borckardt, Nahas, Shaw, O'Neil, et al., 2007), supporting the contribution of the vagus nerve in meal regulation. For better understanding of short-term satiety signaling to the CNS, the following chapter expands on vagal anatomy and vagal signal relay to the CNS.

5   Anatomy of the Gastric Vagus Nerve

Visceral function is regulated by the parasympathetic and the sympathetic nervous system, which together build the autonomous nervous system. The two sub-systems mostly innervate the same organs and operate antagonistically. Whereas activation of one system increases the activity of certain organs, the other exerts the opposite effect. For example, sympathetic activation leads to increased heart rate and decreased activity of the organs of the digestive system whereas parasympathetic activation results in decreased heart rate and increased activity of the organs of the digestive system (Birbaumer & Schmidt, 1991). The two systems harmonize the increase and decrease of corresponding activity and thus act in functional synergy.

Activation of the digestive system is regulated by the parasympathetic system (Birbaumer & Schmidt, 1991). Information about the body's state is transported to the brain for interpretation via afferent fibers. These afferents of the parasympathetic cranial nerves synapse in the brainstem on the nucleus of the solitary tract. Cranial nerves with visceral afferents are the facial (VII), the glossopharyngeal (IX), and the vagus (X) nerves. The vagus nerve, whose anatomy will be reviewed in this chapter, is the Xth cranial nerve and the main contributor to the parasympathetic nervous system.

5.1   Vagus Nerve: Composition and Distribution

The vagus nerve consists of afferent and efferent fibers carrying visceral and somatic information. Afferent fibers account for about 90% of the fibers in the nerve (Agostoni, Chinnock, De Burgh, & Murray, 1957). More than 95% of vagal fibers are unmyelinated, small diameter (mostly 2-4 μm) C-fibers (Agostoni, et al., 1957; Paton, Li, Deuchars, & Kasparov, 2000). Characteristic for these fibers is an average conduction velocity of about 0.9 m per second, which is considered slow conduction (Andrews, et al., 1980).

The vagus nerve is connected bilaterally by multiple rootlets to the posterolateral sulci of the medulla oblongata (Brodal, 1992; Leblanc, 2001). Composition of the vagus nerve before it leaves the cranium at the level of the medulla oblongata varies greatly among subjects. About ten to fifteen rootlets of the vagus nerve emerge in a flat bundle from the dorsal border of the olivary body (Brown, Hidden, Ledroux, & Poitevan, 2000). Rootlets may branch and join adjacent vagal fibers or may join and travel with strands of the glossopharyngeal, the spinal accessory, or the hypoglossal nerves (Brown, et al., 2000).

The rootlets merge to form a single trunk and exit the skull bilaterally through the jugular foramina (Rohen, Yokochi, & Lütjen-Dercoll, 1998). Superior and inferior to the jugular foramen, the two nerves form on each side of the neck the smaller jugular and the larger nodose ganglia respectively, which hold the cell bodies of the sensory fibers. Between the two ganglia, a first branch of the vagus nerve leaves the main trunk to innervate the skin at and near the ear (Henry, 2002). The two nerve trunks exit the cranium via the jugular foramen and enter the thorax between the brachiocephalic veins and the subclavian artery. The two trunks branch and build the esophageal plexus. Above the esophageal hiatus fibers of the esophageal plexus reunite to form two vagal trunks. Before embryonic rotation, the two trunks lie right and left of the esophagus, respectively. Rotation moves the left trunk anteriorly and the right trunk posteriorly (Harkins & Nyhus, 1986). The left anterior and right posterior trunk traverse the esophageal hiatus of the diaphragm (Aquino, Duncan, & Hayman, 2001). The two vagal trunks follow different courses when branching down into the bronchial and abdominal cavities (Aquino, et al., 2001).

In the upper neck, the left vagus nerve travels posteriorly along the internal carotid artery before it moves to a more lateral position to travel along the esophagus (Rohen, et al., 1998). The right vagus nerve travels along the jugular vein and the brachiocephalic trunk, then follows the trachea and finally moves along the esophagus (Rohen, et al., 1998). Below the neck, both trunks branch out to innervate the regions of the thorax and the abdominal cavities. They divide into the anterior and posterior gastric branch: the hepatic branch originating from the anterior branch and the celiac branch originating from the posterior branch. The branches form a dense network to connect with the target organs such as the heart, lungs, and the organs contributing to the gastrointestinal system (Tortora, 1992). Because of its branching distribution, the vagus nerve obtained its name from the Latin word for "wanderer".

5.2   *Origin of Vagal Efferent Fibers*

Vagal efferent fibers innervate striated muscles of the pharynx and larynx and most of the viscera of the thoracic and abdominal cavities such as the heart, lungs, stomach, intestines, liver, pancreas, and kidney (Henry, 2002). Right and left vagi affect organs distinctively. For example, the musculature of cervical trachea (Coon, Mueller, & Clifford, 2000) and of the cardiac arteries (Saper, Kibbe, Hurley, Spencer, Holmes, Leahy, et al., 1990) are predominantly innervated by the right vagus nerves.

Cell bodies of vagal efferent fibers reside in the motor nuclei in the medulla oblongata, mainly the dorsal motor nucleus of the vagus nerve (DMV) and the nucleus ambiguus (Hudson, 1989). The assembly of cell bodies in the medulla oblongata extends in dorsal columns in a rostro-caudal manner (Ewart, Jones, & King, 1988). Efferent cell bodies are located in the DMV without a noticeable viscerotopic organization (Hudson, 1989). Their dendrites innervate different gastric regions around the mucus-secreting cardiac glands in the upper third of the stomach, the acid-secreting gastric glands in the medial third, and the mucus- and gastrin-secreting pyloric glands, resembling mucous cells, in the lower third (Harkins & Nyhus, 1986). Only the area around the cardiac gland also receives information from cells located in the nucleus ambiguus. Although there is no obvious viscerotopic organization within the DMV, evidence exists that the anterior fundus (proximal stomach) receives more innervation from fibers originating in the left DMV whereas the posterior fundus receives more innervation from fibers originating in the right DMV (Ewart, et al., 1988).

Dendrites of cell bodies in the DMV penetrate discrete regions of the overlying nucleus tractus solitarius (NTS; Shapiro & Miselis, 1985), the main area of vagal afferent termination (Beckstead & Norgren, 1979). Such a network of afferent and efferent fibers could serve as the anatomic basis for monosynaptic vago-vagal reflexes (Shapiro & Miselis, 1985), such as stomach relaxation (Desai, Sessa, & Vane, 1991).

5.3   *Origin and First Synapses of Gastric Vagal Afferent Fibers*

At and below the jugular foramen immediately outside the cranium lie superiorly the smaller jugular ganglion and inferiorly the larger nodose ganglion, both containing the cell bodies of vagal afferent fibers (Brodal, 1992), also referred to as first order neurons. Special and general visceral sensory fibers carry gustatory, visceral sensory, and other peripheral information such as satiety signals (Henry, 2002) to the brain for processing. Since elaborations on hunger and appetite in the late 1800s and the early 1900s, it has been recognized that the vagus nerve participates in the regulation of food intake (Cannon & Washburn, 1912). In the 1950s, visceral vagal afferents signaling distension have been characterized as non- or slowly adapting mechanoreceptors that are activated *in series* (Iggo, 1955; Paintal, 1954). Characteristic for in series tension receptors is that increased distension results in more activated fibers rather than in an elevated firing rate of the same number of fibers.

Vagal mechanoreceptive afferent fibers were also found to be sensitive to chemical stimulation as they increased their firing rate depending on the dose when stimulated with cholecystokinin (CCK) or with gastrin-releasing peptide (Schwartz, et al., 1993; Schwartz & Moran, 1994; Schwartz, et al., 1997). Moreover, vagal mechanoreceptors change their sensitivity to distension when an exogenous celiac arterial CCK-infusion is given in combination with a gastric load (Schwartz, et al., 1993). Berthoud and Powley (1992) showed that such dual responsiveness can be explained by the morphological characteristics of the gastric tension receptor. These researchers anterogradely stained rat vagal afferent fibers innervating the fundus in vivo. Dissection of the tissue layers revealed that vagal afferent fibers terminated in the longitudinal and smooth muscle layers (intramuscular receptors) as well as in the myenteric plexus (intramyenteric receptors). Single vagal afferent fibers were identified with several collaterals with either intramuscular or intramyenteric receptor endings. The authors conclude that intramuscular vagal afferent receptors most likely represent gastric tension receptors. The function of the intramyenteric vagal afferent receptors, which were highly arborizing, remains unclear. Yet the authors suggest that such intramyenteric receptors might be sensitive to chemical stimulation. Such interpretation could explain the synergistic effects of mechanical distension and CCK, as found by the group around Schwartz (Schwartz, et al., 1993; Schwartz & Moran, 1994).

After entering the medulla, vagal afferent fibers travel a short distance rostrally and caudally in one or two fiber bundles and terminate in the rostral, caudal, and intermedial medulla (Beckstead & Norgren, 1979). This rostro-caudal assembly of nerve endings constitutes the NTS. The rostral distribution of fibers terminates in the lateral segment whereas the caudal and intermediate distribution terminates in the medial segment (Beckstead & Norgren, 1979). This anatomical pattern reflects functional properties: Caudal and intermediate fibers relay visceral information via general visceral afferents, rostral fibers relay gustatory information via special visceral afferents (Nieuwenhuys, Voogd, & Hiujzen, 1988). Some fibers extend to the medial segment and cross via the commissural nucleus to the contralateral medial segment of the NTS at the level of the area postrema, with a few fibers reaching into the area postrema (Beckstead & Norgren, 1979). No other contralateral areas of the NTS contain vagal afferent terminals. Few vagal afferent fibers are found in the regions adjacent to the NTS such as the DMV, nucleus intercalates, parvicellular reticular formation, and somewhat more pronounced in the area postrema. However, most vagal afferent fibers terminate ipsilaterally in the NTS (Beckstead & Norgren, 1979). Monosynaptic vago-vagal interactions, for example

between the NTS and the DMV (Shapiro & Miselis, 1985), build the basis for reflex arches as seen in stomach relaxation after distension (Desai, et al., 1991).

The NTS is viscerotopically organized (Nieuwenhuys, et al., 1988). Cardiorespiratory and gastric general visceral afferent fibers project to and intermingle in the caudal (visceral) portion of the NTS whereas special visceral afferent fibers carrying taste and odor information project to the rostral (gustatory) portion of the NTS. The oval nucleus, a rostral extension of the NTS, receives projections from the facial nerve. This rostro-caudal organization of the NTS is reflected in further projections to distinct structures within the CNS.

The anatomical concurrence of cardiorespiratory and gastric visceral afferent fibers in the caudal NTS suggests a convergence of cardiac and gastric information at the level of the NTS. Yet studies of the morphological properties of NTS neurons illustrated that subdiaphragmatic vagal afferents specifically respond to electrical stimulation but neither to aortic chemical nor to baroreceptor stimulation (Paton, et al., 2000). This suggests that vagal afferent fibers from the gastrointestinal tract represent a distinct pool of NTS neurons when compared to aortic afferents and baroreceotors (Paton, et al., 2000). However, severe vagal stimulation may affect baroreceptors as intense stomach dilatation can cause bradycardia (Ruttmann & Mandelstam, 1994) or atrioventricular block (Hmouda, Jemni, Jeridi, Ernez-Hajri, & Ammar, 1994).

5.4   *Polysynaptic Vagal Projections to the Pons, Midbrain, and Cerebellum*

Second order neurons transmitting vagal afferent information originate in the NTS. The caudal (visceral) NTS projects most densely ispsilaterally to the ventrolateral portion of the parabrachial nucleus (PBN), which is located in the dorsal pons. Only few fibers cross to the corresponding contralateral PBN region (Beckstead, Morse, & Norgren, 1980). Other ascending fibers project less densely to several forebrain structures such as the bed nucleus of the stria terminalis, the paraventricular, dorsomedial and arcuate nuclei of the hypothalamus and the medial preoptic area, as well as to the central nucleus of the amygdaloid complex, which transmit cardiopulmonary information (Ricardo & Koh, 1978). Neurons in the caudal part of the NTS also densely connect via fiber projections to the adjoining dorsal motor nucleus of the vagus nerve (DMV; Beckstead, et al., 1980; Ricardo & Koh, 1978). Projections from the caudal NTS to the nucleus ambiguous (Norgren, 1978) are thought to partake in respiratory control (Beckstead, et al., 1980).

Intermediate NTS projections most densely terminate ipsilaterally in the dorsal PBN and less densely in the lateral and medial PBN (Beckstead, et al., 1980). Fibers originating in the medial NTS also connect to the hypoglosseal, facial, and trigeminal motor nuclei (Norgren, 1978) and the nucleus ambiguus, which contributes to respiratory control (Beckstead, et al., 1980). Other fibers travel to cerebellar regions such as the cerebellar peduncles, the vermis, and inferior parts of the cerebellum (Henry, 2002). A few fibers directly project to the parvicellular subdivision of the ventral posteromedial nucleus of the thalamus (Beckstead, et al., 1980).

Fibers originating in the rostral (gustatory) NTS travel ipsilaterally with components of the central tegmental tract. Such afferents terminate in the parvicellular region of the ventral posteromedial nucleus of the thalamus (Beckstead, et al., 1980).

The PBN as the second relay for vagal afferent fibers is, as the NTS, organized topographically although its organizational pattern differs from the NTS. Gustatory information is processed in the caudal PBN, whereas visceral information finds mostly rostral PBN representation with some fibers intermingling with gustatory fibers in the caudal portion (Baird, Travers, & Travers, 2001a; Baird, Travers, & Travers, 2001b). The PBN accommodates distension-sensitive and gustatory neurons with some of these neurons responding to both classes of stimulation (Baird, et al., 2001a, b).

5.5   *Polysynaptic Vagal Projections to Cerebral Structures*

Third order neurons transmitting vagal afferent information originate in the PBN and the thalamus. The lateral PBN projects to the parvicellular region of the ventral posteromedial nucleus of the thalamus, to the amygdaloid complex, and to the paraventricular nucleus of the hypothalamus (Baird, et al., 2001a). The PBN additionally projects to the medial preoptic area in the hypothalamus and directly to the insular cortex (Baird, et al., 2001a). Direct connections exist with the septo-olfactory area, infralimbic and prefrontal cortices (Saper & Loewy, 1980). Amygdaloid and insular connections accentuate the affective component of visceral processing (Reiman, Lane, Ahern, Schwartz, Davidson, Friston, et al., 1997; Ricardo & Koh, 1978)

Cortical activation as a response to vagal activation was found in studies of human subjects measuring regional cerebral blood flow (rCBF) during cervical vagus nerve stimulation (VNS), as used in the treatment of epilepsy. For VNS, a programmable pacemaker is

implanted in the chest and electrodes are connected to the left cervical vagus nerve to deliver intervals of electrical stimulation according to programmed intervals. Comparing rCBF measured with $H_2^{15}O$ positron emission tomography during activated VNS with rCBF during deactivated VNS finds cortical effects of VNS. Such stimulation resulted in increased rCBF in many brain nuclei including the medullary nuclei, hypothalamic and thalamic nuclei, and the amygdaloid complex (Garnett, Nahmias, Scheffel, Firnau, & Upton, 1992; Henry, Votaw, Pennell, Epstein, Bakay, Faber, et al., 1999). Activation also occurred in the hippocampal formation, the entorhinal cortex, the cingulate cortex (anterior and posterior), the temporal pole, postcentral and inferior parietal cortices, bilateral insular, orbitofrontal and inferior frontal cotices (Garnett, et al., 1992; Henry, Bakay, Votaw, Pennell, Epstein, Faber, et al., 1998; Henry, et al., 1999). Yet caution needs to be taken when interpreting neuroimaging results from epilepsy patients. The occurrence of acute seizures during the actual scanning period (Garnett, et al., 1992) may skew the results as rCBF increases during seizures by about 100% (Brodersen, Paulson, Bolwig, Rogon, Rafaelsen, & Lassen, 1973).

Although knowledge about cortical activation due to VNS is growing, not much is known about the cortical representation of vagal afferent fibers originating from the stomach. Critical for the understanding of the pathophysiology of eating disorders and other disorders accompanied by dysfunctional satiety is the central processing of visceral sensation as induced by gastric distension.

6   Methods and Materials

6.1   Objectives

The objective of the described experiments was two-fold. First, the experiments attempted to evaluate the cortical representation of gastric vagal afferent stimulation as it ascends from the stomach through different levels of the central nervous system (CNS) to the cerebral cortex. Animal studies have revealed four well-known central regions associated with gastric sensation, which guided the interpretation of the results. Second, the experiments investigated the cognitive responses associated with such vagal activation. Physical, non-nutritive gastric distension by a gastric balloon was used to directly stimulate vagal afferent fibers to induce the subjective feeling of fullness in each subject. Regional cerebral blood flow was recorded with ^{15}O-water positron emission tomography (H$_2$^{15}O PET) during alternate gastric balloon inflation and deflation. A total of six experiments were conducted. Experiments I – V examined brain activation; experiment VI examined cognitive responses during gastric distension.

Experiment I aimed to generate a specific hypothesis about brain regions activated by non-nutritive gastric distension. To increase the power of the exploratory analysis (data set 1), additional data were collected for an extended exploratory analysis in experiment II (data set 2). Experiment III investigated the split-half reliability of the data. Experiment IV examined the effects of intubation alone and along with inflation; experiment V compared resting brain activation in this study with resting activity recorded in other H$_2$^{15}O PET studies. Cognitive responses to vagal activation due to gastric distension were studied in experiment VI.

6.2   Background

6.2.1   Cortical Representation of Gastric Distension

Extensive research has been conducted in animals to study the central processing of visceral sensation. Peripheral visceral sensation is mainly relayed to the central nervous system via the branches of the vagus nerve (see chapter 5). Anatomic and physiological studies in various species have identified central processing areas of visceral sensation using direct electrical stimulation of vagal afferent nerve endings (Paintal, 1973) or using visceral stimulation with gastric balloon distension, which activates vagal stretch receptors (Andrews, et al., 1980; Scratcherd & Grundy, 1982).

At the present time, the regions associated with gastric distension in humans remain unclear. The following analyses focused on four well-known central regions associated with gastric sensation in animals: brain stem nuclei such as the nucleus of the solitary tract (Beckstead & Norgren, 1979; Fussey, Kidd, & Whitwam, 1973) and the parabrachial nucleus (Beckstead, et al., 1980), the insula (Cechetto, 1987; Flynn, Benson, & Ardila, 1999), the anterior cingulate cortex (Öngür & Price, 2000; Vogt, Finch, & Olson, 1992) and orbitofrontal areas (Bailey & Sweet, 1940; Hurley-Gius & Neafsey, 1986). Although visceral sensation in general undoubtedly involves more structures than just the ones mentioned above, a targeted analysis can serve as reasonable basis for forthcoming research.

Neuroimaging techniques such as PET or magnetic resonance imaging are contemporary techniques to study visceral processing in humans. These techniques have been applied during esophageal (Aziz, et al., 2000; Schnitzler, Volkmann, Enck, Frieling, Witte, & Freund, 1999) and rectal stimulation (Hobday, Aziz, Thacker, Hollander, Jackson, & Thompson, 2001; Bittorf, et al., 2006) in healthy volunteers and in patients diagnosed with gastrointestinal diseases, such as irritable bowel syndrome (Naliboff, Derbyshire, Munakata, Berman, Mandelkern, Chang, et al., 2001). However, the field is in need of neuroimaging studies addressing non-nutritive gastric distension.

6.2.2   Cognitive Responses to Gastric Distension

Not much is known about cognitive processing during non-nutritive gastric sensation. Some previous work examined changes in cognitive responses to a gastric load yet focused exclusively on post-prandial perceptions. In those cases, changes in perception were attributed to the nutrients absorbed from the ingested food (Alfenas & Mattes, 2003) or to the nutrients absorbed in combination with gastric distension (Nolan, Guss, Liddle, Pi-Sunyer, & Kissileff, 2003; Smith, 1996). The current literature lacks information on cognitive processing of non-nutritive, gastric stimulation in humans. Such information could help establish a link between visceral sensation and abnormal eating patterns especially prominent in eating disorders such as bulimia nervosa (Geliebter, et al., 1992; Kissileff, et al., 1996).

Experiment VI aimed to assess cognitive responses during mechanical vagal stimulation. Subjective feelings of satiety (target sensation), nausea, anxiety, wakefulness, and gastric discomfort were repeatedly measured on visual analog scales (VAS) during data acquisition of brain activation. The ratings reflect the cognitive states during inflation and deflation.

6.3   Study Design

6.3.1   General Considerations

Cerebral activation was evaluated by interpretation of changes in regional cerebral blood flow using the bolus ^{15}O-water ($H_2^{15}O$) infusion technique and PET. Each study session consisted typically of six experimental scans and a resting control scan was added for data set 2. Each scan lasted 90 seconds. The scans were separated by a ten-minute inter-scan interval to allow for radioactive decay. The total administered dose of $H_2^{15}O$ was less than 155 mCi per subject (in accordance with the Radioactive Drug Research Committee guidelines). Individual intra-subject scans were normalized to adjust for changes in global cerebral blood flow (CBF) and then stereotactically averaged across subjects.

Regional cerebral blood flow (rCBF) was repeatedly measured in the same subject during the experimental state of gastric inflation and the control state of gastric deflation. We aimed at three cycles of inflation and deflation. Repeated measures in one study session had three advantages. First, averaging brain activation data of one condition resulted in more reliable rCBF measures (increased signal-to-noise ratio). Second, we avoided head alignment difficulties associated with multiple study sessions. Third, we obviated day-to-day mood fluctuations that could affect the VAS rating scores (Semmler & Brewer, 2002; Shiomura & Atsumi, 2001).

For gastric distension, we inflated the balloon with water until subjects signaled the non-painful sensation of feeling full like they would after a heavy meal (holiday meal). Subjects were instructed to close their eyes and to concentrate on their stomach during each brain scan. If subjects felt they could no longer tolerate the balloon, they could terminate the study prematurely.

We focused on the individual subjective sensation of fullness instead of a fixed volume administered to each subject. Fixed volumes could have induced rather different sensations in different subjects. Here, we intented to induce the same subjective feeling of fullness in every subject. A high VAS rating for fullness should reflect this target sensation.

The study described involved potential risks for subjects which were addressed with precautions described as follows:

- Subjects were asked to lie still in the PET scanner for about two to three hours. Some subjects may have become tired, develop transient discomfort while remaining in a fixed position, or feel claustrophobic. Subjects were excluded if they had a history of claustrophobia. Subjects could terminate the study at any time if they felt the discomfort was intolerable.

- Subjects had an intravenous (I.V.) line placed in either the left or right antecubital fossa. Subjects may have experienced some discomfort from the venipuncture. During venipuncture, subjects were in a recumbent position so as to minimize the chances of lightheadedness and fainting (none of which occurred during the study procedure). There was a low risk of infection or local bruising of the skin at the site of venous puncture. This risk was addressed by disinfecting the site of venous puncture and other standard methods for placing I.V.s. Subjects were advised of these risks. No infection occurred at the site of venous puncture for any subject.

- Subjects were exposed to an FDA[1]-approved level of radiation. Subjects were advised of radiation risks in the context of other common risks of daily living. As radiation is teratogenic to the fetus, all women were tested to exclude imminent or current pregnancy.

- Psychiatric interviews and clinical assessments were completed before entering the PET study. Subjects were advised that some questions during the interview procedures could cause emotional discomfort (none was reported).

- As a benefit, volunteers received financial remuneration and introduction to a contemporary brain imaging technology. Any questions were answered before the PET scanning procedure. After the study, subjects were debriefed and any further questions were addressed.

6.3.2   Data Collection

Subjects who responded to our advertisement initially underwent a telephone screen described in chapter 6.4.3. They then presented to the Veterans Affairs Medical Center (VAMC) in Minneapolis, MN, USA to give written informed consent of study participation and to complete further evaluation (psychiatric screening and physiological testing; see chapter 6.6.1). A study number was assigned to ensure a subject's anonymity. Study numbers all started with p0 followed by four other digits (p0xxxx). Once eligibility was

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[1] Food and Drug Administration; U.S. Department of Health and Human Services

determined, subjects arrived at the VAMC on the morning of the study day after an overnight fast (twelve hours). Subjects filled out the Positive Affect and Negative Affect Scales (PANAS; see chapter 6.4.5) and were prepared for the scanning sessions including I.V.-placement, electrocardiogram (ECG) set-up, and oral intubation (see chapter 6.6.2). Subjects' heads were adjusted and stabilized in the PET scanner and an initial transmission scan was recorded (see chapter 6.7). The PET scans were recorded alternately during inflation and deflation of the balloon for a total of three cycles (six scans). However, the number of scans was determined by the subjects' tolerance. Scans were separated by a resting interval of approximately 10 minutes to allow for tracer decay. Cognitive responses to gastric balloon distension were recorded on visual analog scales (VAS) as described in chapter 6.6.5. We aimed at VAS recordings for each inflation and deflation.

The oral tube was removed after the final experimental PET scan and a scan during a resting condition (ECR) was recorded for data set 2. After subjects had exited the scanner, the I.V. was removed. They were instructed to remain in an upright seating position for about five minutes to allow for physiological accommodation. Subjects exited the PET suite and filled out the PANAS with the time instruction "right now". Next, subjects were escorted to the VAMC cashier to receive their monetary reimbursement.

A first data acquisition (data set 1) was conducted over an eight months period. For this first data acquisition, the scanning cycles started with the deflated condition to be followed by the inflated condition. According to subjects' feedback regarding comfort and tolerance after the scan, we decided to change the order of scans to inflation followed by deflation during a second data acquisition. This second data acquisition (data set 2) was conducted over a seven months period.

Subjective ratings on VAS were collected to reflect cognitive responses during inflation and deflation. For data set 1, VAS representing inflation were administered for all subjects post-scan inflation and VAS representing deflation were administered pre-scan and post-scan deflation. For data set 2, VAS representing inflation were administered for all subjects post-scan inflation and VAS representing deflation were administered for only three subjects pre-scan and post-scan deflation. The remaining six subjects rated VAS representing deflation pre-scan deflation only. Figure 1 displays the timelines for both data acquisitions. Data from the twelve subjects who rated VAS pre- and post-deflation were investigated for differences. A Wilcoxon signed-rank test (non-parametric testing is discussed in chapter 6.8.4) was calculated for each subscale with an α-error threshold of

$\alpha = .05$ (or 5%), which was Bonferroni-corrected for six comparisons (six VAS subscales) to $\alpha = .008$ (or 0.8%). No differences were found between the pre- and post-measures for deflation (Table 1). Therefore, all inflation-deflation contrasts were calculated with post-inflation and pre-deflation measures.

Data Set 1

balloon	VAS		VAS			VAS		VAS		
intubation	pre-scan D	scan D	post-scan D	In	scan In	post-scan In	D	pre scan D	extubation	

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____ ... ____|

Data Set 2

balloon			VAS		VAS					
intubation	In	scan In	post-scan In	D	pre-scan D	scan D		In	ECR	extubation

|_____|_____|_____|_____|_____|_____|_____|_____|____ ... __|____|
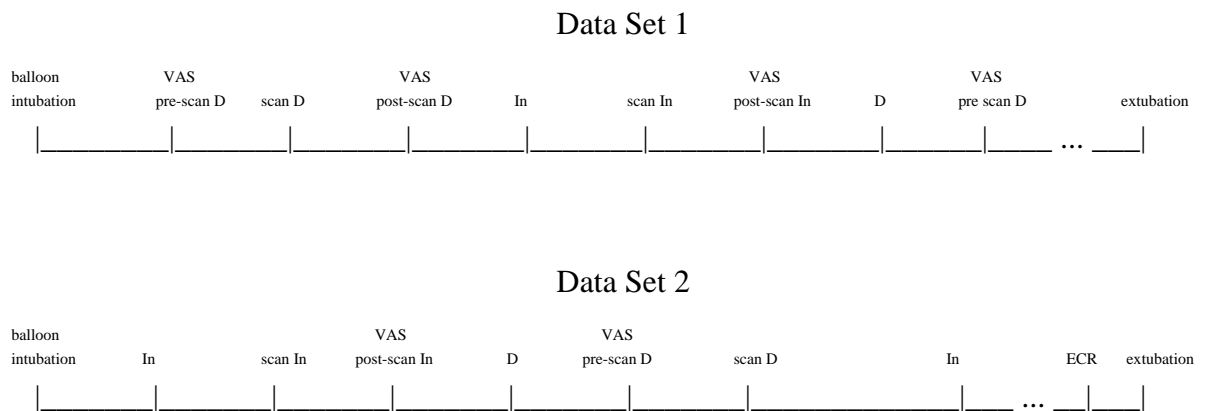
Figure 1. Data acquisition time line

Time line protocol for data acquisition for data set 1 and 2. Note for data set 1: no scan during resting condition at the end of the protocol. Note for data set 2: reversed order of inflation and deflation. Cycles of inflation and deflation were conducted up to three times depending on a subject's tolerance. ECR: resting with eyes closed (eyes closed rest); D: deflation; In: inflation; scan D: scan during deflation; scan In: scan during inflation; VAS: visual analog scales; VAS post-scan: post-scan measurement of VAS; VAS pre-scan: pre-scan measurement of VAS.

Table 1. Pre-scan versus post-scan deflation VAS ratings

	N	Wilcoxon Z	$p \leq$
Fullness	12	-1.96	.050
Hunger	12	-1.94	.052
Sleepiness	12	-1.80	.072
Gastric Discomfort	12	-0.41	.683
Nausea	12	-1.11	.271
Tension	12	-0.17	.866

N: sample size; VAS: Visual Analog Scale; acceptable α-error probability:
$\alpha = .05$ (Bonferroni-corrected for six comparisons to $\alpha = .008$).

6.4   Evaluative Screenings, Physiological and Subjective Measures

6.4.1   Inclusion and Exclusion Criteria

Only women were recruited for the experiments because of gender-based prevalence of the eating disorder bulimia nervosa, which is more prominent in the female population (Rodriguez, et al., 2001; Taraldsen, Eriksen, & Gotestam, 1996). Subjects were age-matched to the bulimic population and had to meet the following inclusion and exclusion criteria:

1) female between ages 18 and 40,
2) non-obese as identified by a body mass index (BMI; Quetelet, 1842) of lower than 30 (National Heart, Lung, and Blood Institute, 1998),
3) medical and psychiatric stability (identified by a non-eventful/normal ECG, normal electrolyte levels, and absence of a psychiatric disorders defined by the Diagnostic Interview Schedule Screening Interviews; Marcus, 1988)
4) plasma sodium and potassium within the reference range, and
5) absence of pregnancy (as verified by a negative serum beta human chorionic gonadotropin quantitative), which is a requirement for participation in PET studies in general.

The following criteria led to exclusion from the study:

1) diagnosis of current or past psychiatric disorder or a family history of a psychiatric disorder,
2) current or past eating disorder,
3) consumption of psychoactive medication during the six weeks prior to the study,
4) current abuse or dependence of drugs or alcohol, and
5) suicidal ideation.

6.4.2   Subject Recruitment

Subjects were recruited with flyers (see appendix A) advertising this study as "Brain Scan Study of Stomach Sensation" offering a reimbursement of $300. Flyers were posted at the University of Minnesota, Minneapolis campus. Potential subjects contacted the study coordinator via phone for further information. Standardized information was given with the opportunity of individual questions raised by the subject. After an initial interest was communicated, a phone screen was conducted (see chapter 6.4.3). If the subject qualified for the study and continued to communicate an interest in participating, an appointment for the pre-study evaluation was scheduled.

6.4.3   Phone Screen

The telephone screen was used to collect demographic information. It also provided a first impression of a subject's general medical and psychiatric health based on self-report. First, contact information, gender, age, handedness and general availability for the study day were assessed. Subsequent questions were developed in close relation to the inclusion and exclusion criteria (see chapter 6.4.1). The phone screen also assessed general suitability for the PET procedure (previous head trauma, seizures or strokes, difficulties lying on the back for about two hours, claustrophobic tendencies, obesity). Finally, if the subject appeared to be in good health, she was scheduled for an evaluation appointment at the VAMC. Further information (address, social security number, date of birth) was gathered in order to generate an account in the VAMC medical computer system to allow for blood sample examination and ECG processing. For a copy of the telephone screen see appendix B.

6.4.4   Diagnostic Interview Schedule Screening Interviews (DISSI)

The Diagnostic Interview Schedule Screening Interviews (DISSI) is an abbreviated computerized version of the Diagnostic Interview Schedule (DIS). The DIS is a screening instrument developed by the National Institutes of Mental Health (Robins, Helzer, Croughan, & Ratcliff, 1981). Its original version is a combination of psychiatric diagnoses quantified in the Diagnostic and Statistical Manual, third edition (American Psychiatric Association, 1980), the Feighner Criteria (Feighner, Robins, Guze, Woodruff, Winokur, & Munoz, 1972) and the Research Diagnostic Criteria (Spitzer, Endicott, & Robins, 1978). The DIS assesses psychiatric disorders from a descriptive rather than an etiological perspective. The descriptive approach is common across all diagnostic tools in psychiatry. A diagnosis is made based on symptom scores that consider the number of symptoms and their distribution in a representative sample. This procedure allows for a reduction of subjective clinical judgment necessary for diagnosis (Robins, et al., 1981). The DIS covers the following diagnoses: senile and pre-senile dementias and organic brain syndrome, schizophrenic disorders, major depression, bipolar and manic disorders, dysthymia, somatization disorder, panic and phobic disorders, obsessive compulsive disorder, antisocial personality disorder, psychosexual dysfunction, drug and alcohol abuse and dependence, eating disorders, and pathological gambling.

The DIS decision-tree structure allows for "skip-outs" after sufficient information is gathered for a diagnosis. This is the case when the answers of subsequent questions no longer affect a given diagnosis. Such interview composition permits its efficient application.

The experiments presented here utilized the Computerized-Administered Diagnostic Interview Schedule Screening Interviews Version 1.0 (Marcus, 1988), an abbreviated computerized version of the DIS (Robins, et al., 1981). Kappa values measuring the concordance between the traditionally administered full version of the DIS (paper & pencil) and the self-administered abbreviated computer version DISSI averaged .53 with a range of .34 – .87 for the individual scales (Bucholz, Robins, Shayka, Przybeck, Helzer, Goldring, et al., 1991) and are considered to be adequate. The DISSI allows for selection of specific diagnosis of interest. For our purposes, senile and pre-senile dementias and organic brain syndrome, antisocial personality disorder, and psychosexual dysfunction were not used in the screening procedure. See appendix D for an example of the DISSI result.

6.4.5 *Positive Affect and Negative Affect Schedule (PANAS)*

The Positive Affect and Negative Affect Schedule (PANAS) is a psychometric questionnaire to measure states of mood over different time periods. It consists of two ten-item self-administered mood scales to provide a brief measure of positive and negative affect (Watson, Clark, & Tellegen, 1988). The development of the PANAS was based on a factor analysis published by Zevon and Tellegen in 1982. Random descriptors addressing positive and negative affect underwent a factor analysis. Items were chosen if they had a primary minimum loading of .40 on a relevant factor representing a given descriptor across two analyses. In addition, the same item should only have a maximum secondary loading of .25 on other (irrelevant) factors not representing the given descriptor (Watson, et al., 1988). The final item list addressing positive affect consists of the following descriptors: attentiveness, interested, alert, excited, enthusiastic, inspired, proud, determined, strong, and active. The final item list addressing negative affect consists of the following descriptors: distressed, upset, hostile, irritable, scared, afraid, ashamed, guilty, nervous, and jittery. Items from both scales are randomly merged and presented in a 20-item list. Subjects are instructed to rate on a 5-point rating scale the extent to which they have experienced each feeling and emotion within a specific time frame. Rating scale anchors are introduced in the instructions and subjects give a rating in the space next to the word describing the feeling or emotion according to the following key: 1 very slightly or not at all; 2 a little; 3 moderately; 4 quite a bit; 5 very much.

Reliabilities of normative samples for the PANAS were estimated for the time instructions for "the current moment", "today", "past few days", "past few weeks", "the year" and "general" (Watson, et al., 1988). For all time instructions, negative and positive affect were only weakly negatively intercorrelated, indicating independence of the two concepts.

Internal consistency reliability as calculated with Cronbach's α revealed values between .86 and .90 for positive affect and values between .84 and .87 for negative affect (Watson, et al., 1988).

Factorial validities for each of the two factors (negative and positive affect) are high for the relevant and low for the irrelevant factor (Watson, et al., 1988). For negative affect, relevant factor loadings varied between .91 and .93 whereas irrelevant factor loadings varied between -.09 and -.18. For positive affect, relevant factor loadings varied between .89 and .95 whereas irrelevant factor loadings varied between -.02 and -.17. Correlating different instruments with the PANAS assessed external validity. Validity data were reported between .51 and .74 for negative affect and between -.19 and -.36 for positive affect (Watson, et al., 1988) with the following ratings scales: the Hopkins Symptom Checklist (Derogatis, Lipman, Rickels, Uhlenhuth, & Covi, 1974), the Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), and the State-Trait Anxiety Inventory State Anxiety Scale (Spielberger, Gorsuch, & Lushene, 1970). Overall, the PANAS can be viewed as a reliable, valid and efficient instrument to assess the described positive and negative mood states (Watson, et al., 1988).

We collected ratings for affective states with the PANAS before the study procedure with the time instruction "past month" to evaluate subjects' mood state over the past month as a baseline measure. After subjects had left the PET scanner, they rated the PANAS with the time instruction "right now". Contrast of these ratings with a normative data sample evaluated if the study group differed in their mood states from the normative sample for the time instruction "past month" and "right now". Copies of the PANAS with different time instruction can be viewed in appendix F.

Scoring:
Each descriptor can be rated on a scale from one to five, with five being the most pronounced experience of the descriptor. The scores for positive affect and negative affect are summed individually. The minimal score for each of the scales is ten; the maximal score for each of the scales is 50. Subtracting the score for negative affect from positive affect leads to a total score (Watson, et al., 1988). Here, we interpreted positive affect and negative affect separately. Scores for both dimensions were statistically compared with the normative samples for the corresponding time instruction.

6.4.6   Gastric Volumes and Pressure

The balloon set-up was connected to a water reservoir. A hand-held syringe mounted between the tube and water reservoir allowed manual inflation and deflation of the gastric balloon. We assessed gastric pressures from the balloon interior with a y-connector attached to a Stathem strain gauge wired to a paper polygraph (5/6 H Recorder, Gilson Medical Electronics, Inc., Middleton, WI). For each subject, the balloon was pressure-calibrated to 200 ml water outside the subjects' stomachs.

Gastric volumes were measured from the displacement of a large volumetric cylinder connected to the balloon. Gastric volumes were registered manually. We expected the balloon volumes within a similar range as other studies of healthy women have shown (Geliebter & Hashim, 2001). Responses of gastric relaxation served as an additional verification for proper balloon placement. Observation of gastric relaxation would indicate gastric distension, which implies proper placement in the stomach. On the other hand, the absence of gastric relaxation would indicate that the gastric balloon was placed incorrectly proximal or distal to the intended position.

Physiological recordings were acquired on a polygraph using ruled graph paper (Gilson Medical Electronics, Inc., Middleton, WI) with divisions of one mm squares. The surgeon who inserted the gastric balloon (RLG) inspected gastric pressure recordings visually to detect irregularities. Physiological recordings served monitoring purposes only.

6.4.7   Electrocardiogram (ECG)

A standard three-lead Electrocardiogram (ECG) was attached to the right and left ankle and to the left arm. We monitored ECGs with a sampling rate of 100 Hz during the entire intubation period throughout inflation and deflation. This additional procedure was chosen since stomach distension affects excitatory and inhibitory circuits that are involved in the control of cardiovascular regulation (Sabbatini, Molinari, Grossini, Mary, Vacca, & Cannas, 2004). Indeed, intense gastric expansion can result in bradycardia (Ruttmann & Mandelstam, 1994) or in atrioventricular block (Hmouda, et al., 1994). Tracings were recorded during the brain scans to later evaluate if heart rate significantly differed between gastric inflation and deflation. ECG patterns were recorded for data set 1 with a MAC 12 (Marquette Electronics, Inc., Milwaukee, WI; for an example of the ECG recordings see appendix I) and for data set 2 with the computer-aided program Biopac Student Lab Pro, version 3.6.2 (Biopac Systems, Inc., Goleta, CA, USA).

Heart rate (number of heartbeats per minute, BPM) for data set 1 was extracted manually from the printouts on ruled graph paper (Medi-Trace®, Graphic Controls Corporation, Buffalo, New York, USA and Recorder no. 40457C/40457D, Hewlet Packard, Palo Alto, CA) with divisions of one mm squares. On the graph paper, each millimeter represents 0.04 seconds (Dubin, 1990, page 28). The graph paper carried marks in one-second increments, comprising 25 mm on the paper. For each scan condition, the number of QRS-complexes in an increment of 250 mm (corresponding to 10 seconds) were counted and multiplied by six to achieve the number of heartbeats per minute. Biopac Student Lab Pro, version 3.6.2 (Biopac Systems, Inc., Goleta, CA, USA) aided the extraction heart rate for data set 2 by estimating the mean BPM during the scanning period. The two conditions inflation and deflation were contrasted for differences.

6.4.8   Visual Analog Scales (VAS)

Visual analog scales are easy to understand and easy to read graphic rating scales especially feasible for complex study situations. The rating scales are described visually as a horizontal line on a letter-size sheet of paper (Aitken, 1969), although computer-aided administration is also available. The line describes a continuum between two endpoints. One endpoint represents "not at all"; the other endpoint represents "extremely". We indicated a midpoint on the bar to provide a visial division of the bar. Subjects are asked to rate the present feeling by marking the line according to how they felt at the moment. Not providing a numbered scale as an anchor prevented the subject from assigning a number to quantify a specific feeling. This technique offers maximal convenience for the subject along with the opportunity of repeated measurement of particular feelings (Zealley & Aitken, 1969). Reliability and validity studies in clinical populations revealed adequate within-subject and within-group test re-test reliabilities and good concurrent validity (Folstein & Luria, 1973; Luria, 1975).

Subjects were presented with VAS (see appendix G) for each condition of inflation and deflation during both data acquisitions. They rated their feelings of fullness (target sensation), hunger, nausea, tension, sleepiness, and gastric discomfort. Rating scales were described visually on letter-size paper on an 11 mm horizontal line with an indicated midpoint. Each line was anchored on the left by the words "not at all" and on the right by the word "extremely". Subjective evaluations of levels of satiety, nausea, anxiety, wakefulness and gastric discomfort were assessed using six different visual rating scales at the time intervals described below (see chapter 6.6.5). Specific descriptors were used in the sentence "How _(descriptor)_ do you feel?" The corresponding specific descriptors for

satiety were "full" and "hungry", "nauseated" described the level of experienced nausea, "tense" described anxiety, "sleepy" described wakefulness, and "much gastric discomfort" described the level of gastric discomfort. The descriptor sentence was presented above the horizontal line describing the feeling (see appendix G for copies of VAS forms).

The descriptors were chosen in order to collect control values from healthy participants and are meant to be contrasted in future research with ratings from a population diagnosed with bulimia nervosa. Satiety and hunger ratings were chosen because bulimia patients have shown to experience altered post-prandial satiety and fullness responses (Geliebter, et al., 1992; Geliebter & Hashim, 2001). The rating of satiety was described in relation to the feeling of fullness after a holiday meal and served as validation of the target feeling.

The other four rating scales served as general distractors, yet each had been chosen with the intention of a later comparison with data from subjects diagnosed with bulimia nervosa. Ratings of nausea will indicate if the threshold of emesis requires less vagal activation (less gastric distension) in normal controls than in a bulimic population. Measures of anxiety (descriptor: tense) can evaluate if the study situation is perceived differently in control subjects or subjects diagnosed with BN. Wakefulness (descriptor: sleepy) was chosen to indirectly assess the level of satiety. Postprandial Sleepiness has been demonstrated in animals as resting behavior in the behavioral sequence of satiety (Antin, Gibbs, Holt, Young, & Smith, 1975). Possible changes in sleepiness would be an indicator for the successful induction of the feeling of fullness like after a heavy meal. Ratings of gastric discomfort will serve as an indirect index of distension for future experiments utilizing the rapid consumption of a liquid meal.

Scoring:
Each VAS sheet was scored manually as the distance in cm including one decimal place from the left anchor "not at all" to a subject's mark. The cm-value for each condition (inflation and deflation) and for each VAS sub-scale (a total of six) were analyzed statistically with SPSS© for Windows 10.1 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL).

6.5   Subjects

The study was conducted at the VAMC, Minneapolis, MN, USA. The Human Subject Committee and Radioactive Drug Research Committee of the Minneapolis VAMC

approved the study protocol. Subjects gave written informed consent on the approved study consent form before enrollment (see appendix C for a copy of the consent form). For evaluation criteria, see chapter 6.4.1.

We recruited ten subjects for data set 1 and eleven subjects for data set 2. After written informed consent, subjects were evaluated at the VAMC according to the procedures described in chapter 6.4. All subjects met the inclusion and exclusion criteria. One subject from data set 1 and two subjects from data set 2 terminated the study procedure prematurely. Intolerance of the balloon prevented these subjects from full participation in the study protocol. Questionnaire data from these three subjects were excluded from all data analyses. The remaining nine subjects in data set 1 and data set 2 (values for data set 2 given in parentheses), were, on average, 23.3 (24.9) years old, weighed 56.1 (63.4) kg, were 166 (168) cm tall and had a BMI of 20 (22). Table 2 describes demographic details of the two study samples separately.

Two-tailed T-tests for independent samples revealed no differences between the two groups regarding age, weight, height, and BMI. Levene's test for the equality of variances revealed no differences in the variances between the two groups in the same variables (Table 3).

Table 2: Subject demographics for data set 1 and 2

		N	Minimum	Maximum	Mean	Std. Deviation
Data Set 1	Age	9	18	39	23.3	6.2
	Weight (kg)	9	45.5	75	56.1	8.5
	Height (cm)	9	155	178	166	7
	BMI	9	17	24	20	2
	Valid N (listwise)	9				
Data Set 2	Age	9	20	34	24.9	4.5
	Weight (kg)	9	47.5	76.5	63.4	10.9
	Height (cm)	9	160	180	168	7
	BMI	9	18	27	22	3
	Valid N (listwise)	9				

Table 3: Group differences between data set 1 and 2

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	Lower	Upper
Age	Equal variances assumed	.006	.938	-.60	16	.55	-1.56	2.57	-7.01	3.90
Weight (kg)	Equal variances assumed	.850	.370	-1.60	16	.13	-7.38	4.60	-17.12	2.37
Height (cm)	Equal variances assumed	.020	.890	-.57	16	.57	-.02	.03	-.09	.05
BMI	Equal variances assumed	.184	.674	-1.78	16	.09	-2.06	1.15	-4.50	.39

6.6   Specific Study Procedures

Both data acquisitions (data set 1 and 2) were conducted with three diviations from the original protocol: First, the order of inflation and deflation was reversed for data set 2 to better accommodate to the level of subjects' comfort. Second, no VAS post-scan measures were recorded for six out of nine subjects for data set 2. Third, a post inflation protocol resting scan was recorded.

Subjects were individually introduced to the consent process and evaluated separately. No more than one subject was studied on a given day. Experiments were conducted in the PET suite of the VAMC. After study completion, subjects were accompanied to the VAMC cashier to receive immediate reimbursement. Compensation was staggered as follows yet paid in total at completion of the study: blood-draw for initial blood evaluation: $25.00; I.V. placement for radioactive tracer injection: $50; oral intubation: $250. When subjects had made a reasonable effort to tolerate the balloon, they qualified for the total reimbursement of $325. Subjects were blinded toward the composite reimbursement structure to avoid premature termination of the study procedure.

6.6.1   Pre-Study Evaluation

Subjects arrived at the VAMC on the day prior to the appointed study day for introduction to the informed consent process and for psychiatric and physiological screening. After written informed consent, subjects were screened for psychiatric disorders using the computerized and abbreviated DISSI, version 1.0 (Marcus, 1988). For a description of the interview schedule, see chapter 6.4.4. Printed results of the DISSI were readily available as soon as the subject had completed the interview. Only one case required further probing to clarify the results. All subjects met the criteria for study participation. To rule out cardiac irregularities such as previous infarct or cardiac arrhythmia, a VAMC physician independent from the study personnel recorded subjects' pre-study ECG at the VAMC. The study physician validated the ECG recordings. Additionally, blood was drawn by either an advanced medical student or the study physician and submitted to the VAMC laboratory for a test of serum sodium and potassium levels, which can be abnormal in the eating disordered population, and serum beta human chorionic gonadotropin quantitative to verify absence of pregnancy. The following morning, subjects arrived at the study site after a twelve-hour fast. See appendix E for a copy of the pre-study ECG evaluation and blood tests.

6.6.2   Subject Preparation

After arriving at the study site, subjects filled out the PANAS for trait measures of mood aspects with the time instruction "past month" as a general baseline measure of their mood state. Results of blood plasma screen were obtained through the VAMC computer system and were reported to the subjects by the study coordinator. Had irregularities occurred that needed attention, the study physician would have communicated this to the subject and would have referred her to a family physician for further evaluation. For radioactive tracer administration, an advanced medical student, the study physician or the I.V.-nurse of the VAMC placed an I.V. line in the left or right antecubital vein. Before the intubation procedure, subjects were asked to void their bladder and unbutton their trousers for increased comfort in the PET scanner. Subjects laid down in a supine position on the PET gantry and a three-lead ECG was attached.

Prior to balloon insertion, the surgeon (RLG) gave a light oral anesthetic (20% Benzocaine spray, Beutlich L.P. Pharmaceuticals, Waukegan, IL) followed by 3 cc of 2% Lidocaine Viscous gel (Roxane Laboratories, Inc., Columbus, OH) to facilitate intubation. The surgeon placed a small plastic mouthpiece between the teeth and orally inserted the tube with the deflated balloon while verbally comforting and soothing the subject. Markings in distances of 10 cm on the tube guided correct placement. The tube went downward until a 45 cm mark opposite incisors indicated the approximate location of the upper end of the balloon in the stomach. A test inflation of the balloon to 200 ml water and gentle retraction for a variable length until cardia (proximal third of stomach) resistance gave evidence of proper placement. Umbilical tape tied to the tube and positioned around the neck stabilized the tube position and minimized pharyngeal stimulation and oral movement. We decided to exclude fluoroscopic control of balloon placement from the protocol for two reasons: First, C-arm equipment was incompatible with the PET gantry. Second, transportation to and from an x-ray facility would have prolonged the study time significantly.

The tube featured separate inflation and deflation channels with distal openings. A third channel with a proximal opening allowed for aspiration of saliva from the esophagus. The fourth distal channel was left unused and therefore sealed. The latex balloon (non-lubricated condom, Trojan, Carter-Wallace Inc., New York, NY) was extended over the three distal openings to the 20 cm indicator to a length of 22 cm. The balloon was secured with multiple wrappings of 3-0 surgical silk below the opening for esophageal saliva extraction. For the gastric balloon set-up see Figure 2.
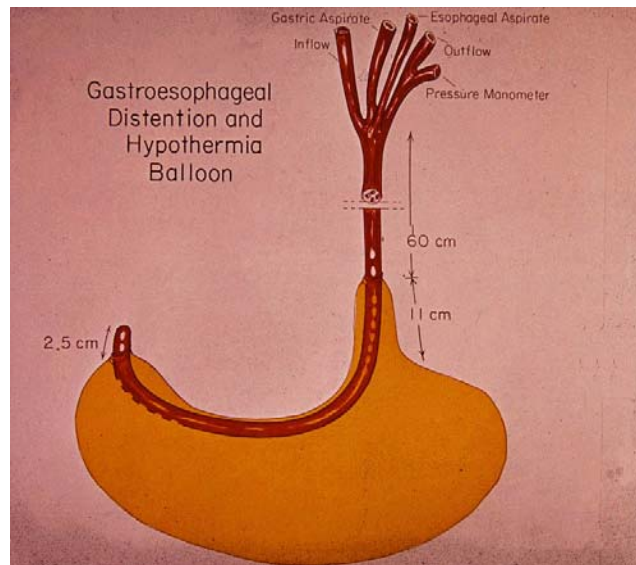
Figure 2: Balloon set-up

Note that most distal opening was sealed for the study and covered with the balloon.

Once the balloon was positioned in the stomach, the subject's head was immobilized in a cushioned head holder to maintain initial head position. Tension of a medical tape (Johnson & Johnson, New Brunswick, NJ, USA) connecting the subject's forehead with the head holder reminded the subject of any head twists and further reduced head movements. With subjects in a supine position, their heads were then positioned in the PET scanner.

6.6.3   *Inflation and Deflation Procedures*

The balloon was inflated with room-temperature water over a two-minute period until the participant reported feeling full like after a holiday meal. The displacement from the water reservoir connected to the balloon indicated the volume of inflation. After two minutes during which brain activity was recoreded, the visual analog scales were administered and subjects rated their feelings of fullness, hunger, nausea, tension, sleepiness, and gastric discomfort. The balloon was deflated after subjects had reported their ratings. We aimed for three consecutive cycles of inflation and deflation. The conditions of inflation and deflation were separated by a 10-minute resting period. Intragastric balloon pressure was monitored and recorded (5/6 H Recorder; Gilson Medical Electronics, Inc., Middleton, WI) during inflation and deflation. Heart rate was monitored for data set 1 with a MAC 12 (Marquette Electronics, Inc., Milwaukee, WI) and for data set 2 sith Biopac Student Lab Pro, version 3.6.2 (Biopac Systems, Inc., Goleta, CA, USA).

6.6.4   PET scanning

The study aimed at three consecutive cycles of inflation and deflation (i.e., a total of six scans). Prior to the inflated brain scan, we filled the balloon with water over a two-minute period until subjects signaled a non-painful sensation of feeling full like they would after a heavy meal. We darkened the room; subjects were instructed to close their eyes and to concentrate on their stomach during the brain scan. Similarly, after stomach deflation and the resting interval, we recorded the next scan using the same procedure and instructions. The scans were separated from each other by a resting interval of approximately 10 minutes to allow for radioactive tracer decay. See appendix H for an example of a subject protocol sheet during data acquisition, PET enrollment form and PET protocol record.

6.6.5   Visual Analog Scales

To aid the scoring process for subjects, a research assistant positioned a fresh set of VAS attached to a clipboard in front of the subjects. Immobilization of subjects in the scanner prevented them from managing VAS scales themselves. The research assistant placed a thick marker in the subject's preferred hand. Subjects were told not to move their head for reading but to direct the research assistant to position the scales so they could read them. Subjects then placed a vertical pencil mark on the horizontal rating bar to indicate the magnitude of the probed feeling. After rating a particular feeling, the research assistant changed to the VAS rating scale with the next descriptor.

Cognitive responses representing the state of gastric distension and the state of an empty stomach were obtained during inflation and deflation respectively. During the first data collection, VAS describing inflation were administered for all subjects after the scanning process (post-scan inflation) and VAS describing deflation were administered before and after the scanning process (pre-scan and post-scan deflation). During the second data collection, VAS describing inflation were administered for all subjects post-scan inflation. Ratings describing deflation were administered for three subjects pre-scan and post-scan deflation.   The remaining six subjects rated VAS describing deflation only pre-scan deflation. No differences were found between the pre- and post-scan measures for deflation (see corresponding section in chapter 6.3.2).

6.7   PET Imaging and Image Reconstruction

We measured regional cerebral blood flow (rCBF) with positron emission tomography (PET) using $H_2^{15}O$ to estimate brain activity during gastric distension. Since rCBF is

tightly coupled with neuronal activity (Raichle, 1987), increased rCBF indicates dynamic activation of the brain. Such functional activation of brain areas is based on changes in the electrical and chemical activity of large populations of neurons which requires energy (Matthews, 2001). Energy metabolism in the brain is mainly oxidative; glucose oxidizes to CO_2; adenosine triphosphate (ATP) and water are produced (Gjedde, 2001). Even during resting states a high metabolic demand exists, probably due to ongoing synaptic processes (Schwartz, et al., 1979). Such ongoing metabolic events at baseline must be distinguished from increased metabolic demands during activation. Neuronal activity correlates positively with rCBF, glucose utilization, and to some degree with oxygen delivery (Raichle & Gusnard, 2002). However, the relationship between blood flow and oxygen utilization is not linear. Changes in rCBF are much larger than accompanying changes in oxygen consumption (Fox, Raichle, Mintun, & Dence, 1988). In addition, the amount of oxygen delivered exceeds the actual increase in demand. The oxygen extraction fraction, which is the portion of oxygen used by the brain out of the total oxygen available, quantifies this difference between oxygen delivery and utilization. During activations in the brain, blood flow increases more than the utilization of oxygen delivered. Positron emission tomography (PET) can examine the oxygen extraction fraction of the brain (Gusnard, Raichle, & Raichle, 2001).

Brain mapping using PET defines cortical and subcortical areas of activation during a particular task by subtracting brain activation in a control (comparison) task from an experimental task (e.g., inflation minus deflation). The selection of the control (comparison) task becomes essential as interpretations of functional activation data depend on them. For example, if a simple comparison task like resting with eyes closed (ECR) is selected, tonic activity during the resting state may mask changes in neuronal activity associated with the experimental task. We countered this difficulty by selecting a control task that differs from the experimental task by only one feature of interest: gastric distension.

For actual blood flow tracings, the radioactive tracer $H_2^{15}O$ was prepared by deuteron bombardment of a gas mixture of 1% oxygen in nitrogen in the Minneapolis VAMC Scanditronix 40 MeV cyclotron. The gas mixture was directed through charcoal and soda lime traps to remove byproducts, mixed with hydrogen gas, and passed through a palladium catalyst at 150 degrees Celsius to produce $H_2^{15}O$. The radioactive water vapor was bubbled through a sterile saline solution to provide for an injectable tracer (West & Dollery, 1961). The $H_2^{15}O$ was analyzed for radiochemical purity by gas chromatography (Porapak N

column) with a radiochemical detector. Sterility and pyrogenicity testing was performed at regular intervals. Immediately prior to injection, the radioactivity of $H_2^{15}O$ was assayed. Standards for quality control were adapted from the United States Nuclear Regulatory Commission Guide 10.8, "Guide for the Preparation of the Applications for Medical Programs" (Revision 1, October 1980).

We acquired brain activation data using an ECAT 953B camera (Siemens, Knoxville, TN) with septae retracted (3d-mode). The scanner produced 31 slices spaced 3.3 mm apart. A transmission scan was performed to correct the subsequent experimental emission images for radiation attenuation. A slow bolus of $H_2^{15}O$ (0.25 mCi/kg body weight) was injected intravenously at a constant rate over a 30-second interval. After proportional distribution of radioactivity across the brain, the camera started recording for 90 seconds. Automated PET software (Minoshima, Koeppe, Frey, & Kuhl, 1993) estimated rCBF from normalized tissue radioactivity (normalized to 1,000 counts). Image processing for each subject included normalization for global activity, co-registration, and nonlinear warping (Minoshima, Koeppe, Frey, & Kuhl, 1994) to a defined stereotactic space (Talairach & Tournoux, 1988). We smoothed images with a four-pixel (9 mm), three-dimensional, Gaussian filter, resulting in images with a final resolution of 12.4 and 12.0 mm full-width at half-maximum (FWHM) for data set 1 and 2 respectively.

To increase the signal-to-noise ratio, we averaged the subtraction images across subjects through stereotactic normalization (Talairach & Tournoux, 1988). Areas of increased rCBF were color-coded in the average image. The brighter the color, the greater the difference in regional cerebral blood flow, which is also referred to as greater "activation" (e.g., relative to a control condition, here deflation).

6.8   *Statistical Analyses*

Subjective feelings measured with VAS are of ordinal character (ordinal scale data). Equal distances in the rating values cannot be assumed to represent equal distances in the psychological construct of fullness. For example, a qualitative similar experience may find a quantitative different expression on the horizontal line bounded by opposite poles and vice versa. Such data display continuous (ordered) categories and represent ratio-level measurements. Ordinal (categorical) data do not allow for parametric analysis. Therefore, all VAS data analysis used non-parametric procedures.

6.8.1   Target Sensation: Fullness

Subjective feelings of fullness were recorded using VAS. Group differences between the two data sets were calculated for the target sensation of fullness to verify similarity of samples. Four different analyses were computed to answer four questions regarding the similarity of groups. All analyses of VAS scores used non-parametric procedures (see above and chapter 6.8.4) and were computed on a personal computer using SPSS© for Windows 10.1 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). Tables and graphs describe VAS scores of fullness for each data set separately. Cases with missing values and outliers were excluded from the specific data analysis. We aimed to induce the same subjective feeling of fullness in all subjects and sought to answer the following questions:

1. Were there any outliers in the data set?
    This question was examined for each data set separately with exploratory statistics using stem-and-leaf plots and boxplots.

    Stem-and-leaf plots. For stem-and-leaf plots, the numeric data values for each inflation and each sample were collected into intervals and displayed in horizontal bars. The digits of each numerical value with one decimal point were separated into a stem (integral number) and a leaf (decimal; SPSS, 1997). Each integral number has an assigned frequency. Each part is listed under its respective headline (frequency, stem, leaf). Extreme values (outliers) are identified and labeled as such (Figure 3, Figure 4). The bottom part of each diagram informs about the stem width and the number of cases each leaf represents. The shape of the stem-and-leaf display indicates the distribution of the data values (symmetric or skewed).

    Boxplots. For each group of data (here: three inflations), the horizontal line in the middle of the box marks the median of the sample. The edges of each box, called hinges, mark the 25th and 75th percentiles. The median is the midpoint in the series of numbers and splits the values of the sample in half. The hinges split the remaining halves in half again. Thus, the central 50% of the data values fall within the range of the box. The length of the box is defined as an hspread and corresponds to the inter-quartile range. The vertical lines (whiskers) extend up and down from each box and show the range of values that fall within 1.5 hspreads of the hinges. The tips of the whiskers describe the largest value at the upper whisker and the smallest observed value at the lower whisker which are not described as outliers. Boxplots allow the visual

comparison of groups by identifying their variations between medians and spreads. Outliers (outside values) are defined as values between 1.5 and 3 hpsreads outside the lower or upper hinges (marked by an open circle: o). Extreme values are defined as values greater than 3 hspreads beyond the lower or upper hinges (marked by an asterisk *). In addition to stem-and-leaf and boxplot display, fullness ratings are presented visually in bar graphs (Figure 7 through Figure 9).

2. Did the two groups (data set 1 and 2) differ from each other regarding their ratings of fullness? In other words, were the two groups comparable with regards to the target sensation?

The Mann-Whitney test for independent samples was applied to the ratings of fullness for each of the three consecutive inflations to investigate group differences in the feeling of fullness. The Mann-Whitney test is a non-parametric alternative to the independent-samples t-test. Like the t-test, Mann-Whitney tests the null hypothesis that two independent samples originate from the same population. Rather than being based on parameters of a normal distribution like mean and variance, the Mann-Whitney test statistic, U, is based on ranks. It is computed by counting the number of times an observation from the group with the smaller sample size precedes an observation from the larger group. The equation for the Mann-Whitney U is

Equation 1 $$U = N_1 N_2 + \frac{N_1(N_1 + 1)}{2} - T_1$$

where N_1 and N_2 are the sample sizes of the two groups, and T_1 is the sum of ranks of sample 1. The α-error threshold was set to α = .05 and Bonferroni-corrected for three comparisons to α = .016.

3. Did subjects feel full during each of the three consecutive inflations?

This question was examined with three Wilcoxon signed-rank tests for related samples comparing the values of the three successive inflations. The Wilcoxon signed-rank test considers information about the sign of the differences and the magnitude of the differences between pairs. The testing procedure involves the calculation of differences, here: for each inflation-deflation cycle, the numeric valueof deflation was subtracted from the numeric value of inflation. The differences are then ranked by their absolute values (from low to high). The sign of each difference is then affixed to the corresponding rank (Zar, 1984). Ranks are summed according to their sign (positive and negative sum of ranks: T_+ and T_-). For a two-tailed test, the null hypothesis H_0 is

rejected if either T_+ or T_- is less than or equal a critical value listed in the table for critical values of the Wilcoxon T distribution (McCornack, 1965). Calculating either T_+ or T_- determines the corresponding value according to

Equation 2
$$T_- = \frac{n(n+1)}{2} - T_+$$

or respectively,

Equation 3
$$T_+ = \frac{n(n+1)}{2} - T_-$$

where n is the sample size and T_+ and T_- are the positive and negative sum of ranks.

The ratings for fullness during each inflation were compared with those during the corresponding deflation. The threshold of $\alpha = .05$ for α-error probability was Bonferroni-corrected for three comparisons (three inflation/deflation cycles) to $\alpha = .016$.

4. Did subjects as a group feel similarly full during each of the three inflations?

This question was examined with the Friedman test statistic. The Friedman test is the non-parametric alternative to a one-way repeated measures analysis of variance. Like the Mann-Whitney test statistic, the calculation of the Friedman test statistic is based on ranks within each case. The scores for each variable are ranked and the mean ranks for the variables are compared. Here, each subject's VAS responses for fullness were ranked for three consecutive inflations. The mean ranks were then calculated and compared for the three inflations. The Friedman test is used to test the H_0: it assumes there is no difference in the feeling of fullness for the three consecutive inflations. The Friedman test statistic is approximately distributed as a chi-square distribution. If there is no difference between the three measures, each subject's rank would be random, and there would be no difference in the mean ranks across the variables. The equation for the Friedman test statistic is

Equation 4

$$X^2 = \frac{12}{Kj(j+1)} [\Sigma\, T_j^2] - 3K(j+1)$$

where K is the number of sets of matched observations (here: $K = 18$ for 18 subjects), j is the number of groups (here: $j = 3$ for three consecutive inflations), and T_j is the sum of ranks for each group.

6.8.2   *Physiological Measures and Mood Questionnaire*

All statistical analyses for physiological measures and the mood questionnaire used the pooled data set of all 18 subjects. Measures of volume and pressure as well as heart rate are continuous measurements and examples of ratio scale data. If the assumptions of normal distribution and equal sphericity of sample populations for the distributions of the samples were met, parametric procedures were the appropriate methods for data analysis. Cases with missing values, outliers and/or extreme values were excluded from all data analysis. Tables and graphs describe data for the pooled data set. Analyses used parametric procedures (see below) and were computed on a personal computer using SPSS© for Windows 10.1 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL).

6.8.2.1   Gastric Volume and Pressure

Exploratory (stem-and-leaf and boxplots) and descriptive statistics were determined for maximal tolerated volume and gastric pressure measured in mmHg for the three inflations to determine outliers and extreme values. The effects of repeated inflation on maximal tolerated volume and on gastric pressure were examined separately with a general linear model (GLM) for repeated measures with one within-subject factor with three levels (three repeated inflations). The GLM repeated measures procedure provides analysis of variance when the same measurement is recorded several times on each subject, here, three measures of volume and pressure for three inflations. For our calculations, the threshold for α-error probability was set to $\alpha = .05$.

6.8.2.2   Electrocardiogram (ECG)

Heart rate was measured in beats per minute (BPM) by ECG recordings. Exploratory (stem-and-leaf and boxplots) and descriptive statistics were determined for heart rate for the three inflations. The effects of repeated inflation and deflation on heart rate were examined with a GLM for repeated measures with two within-subject factors in a 2x3 design: *Condition* with two levels (inflation and deflation) and *Time* with three levels (three repeated inflations). The threshold for α-error probability was set to $\alpha = .05$.

6.8.2.3   Positive Affect and Negative Affect Schedule (PANAS)

Descriptive statistics were determined for the group scores for positive affect (PA) and negative affect (NA) and for the two time instructions separately. Group scores were compared with the normative samples reported by Watson and cowrokers (1988) with four independent samples t-tests using the group means with corresponding standard deviations. The chosen threshold for α-error probability of α = .05 was Bonferroni-corrected for four comparisons to α = .0125. Group means and standard deviations of the study sample and the normative sample were used to calculate the t-value according to Zar, 1984.

6.8.3   Experiment I-V: Brain Activation Data

6.8.3.1   Experiment I: Exploratory Analysis

The general methods were presented in the Masters Thesis "Neural Correlates of Gastric Distension", submitted to the University of Trier, Trier, Germany on April 9, 2002 by the author of this dissertation thesis. The main points will be summarized here. Regional CBF was quantified as a pixel-wise count of radioactive decay. Individual activation minus control scans were calculated pixel-wise, then normalized to 1000 counts and transformed into stereotactic space (Talairach & Tournoux, 1988). Responses were identified using a roving cube.   After image reconstruction and processing (see chapter 6.7), statistical analysis used the global variance of all intra-cerebral pixels (Minoshima, et al., 1993) and calculated pixel-wise t-statistics (Friston, Frith, Liddle, & Frackowiak, 1991). The t-image was transformed into a z-map image with a chosen significance threshold of a z-score of 3.3 (Zald, et al., 1998a). When at least six subjects contributed to an activation peak, it was considered a reliable activation. Identification of areas of increased rCBF was focused on four key areas involved in the central processing of gastric distension previously identified in anatomical and physiological studies: brain stem nuclei, the insular cortex, the anterior cingulate cortex, and orbitofrontal areas (see chapter 6.2.1).

6.8.3.2   Experiment II: Extended Exploratory Analysis

To increase the power of the results (Andreasen, Arndt, Cizadlo, O'Leary, Watkins, Ponto, et al., 1996) which were obtained with data set 1 and analyzed in experiment, additional nine subjects participated in the study (data set 2). For an extended exploratory analysis, data from both data sets were combined. The pooled data set contained reconstructed and preprocessed data from all 18 subjects. Statistics used the global variance of all intra-cerebral pixels (Minoshima, et al., 1993) and calculated pixel-wise t-statistics (Friston, et al., 1991). The t-image was transformed into a z-map image with a chosen threshold of $z \geq 3.3$ (Zald, et al., 1998b). We increased the minimal number of subjects contributing to

an activation peak to ten in order to consider a particular peak as reliable activation (Andreasen, et al., 1996; Friston, Holmes, & Worsley, 1999). As for experiment I, identification of areas of increased rCBF was focused on four key areas involved in the central processing of gastric distension: brain stem nuclei, the insular cortex, the anterior cingulate cortex, and orbitofrontal areas (chapter 6.2.1).

6.8.3.3   Experiment III: Split-Half Reliability

For this reliability experiment, we hypothesized a significant increase in rCBF in the described regions of increased blood flow identified in experiment II for two halves of the pooled data set. Data were split into two groups as follows: Data were arranged chronologically in the order they were recorded. Each case was assigned a running number ranging from 1 through 18. Cases with odd numbers defined group 1, cases with even numbers defined group 2. Each brain region involved in human processing of gastric distension as identified in experiment II is referred to as a region of interest (ROI). Regional CBF in the identified ROIs was quantified as the mean counts of radioactive decay in a 6.75 mm spherical region (three voxels of 2.25 mm) around the peak coordinate. For each group, rCBF during inflation was contrasted with rCBF during deflation with one-tailed paired t-tests for dependent samples ($\alpha = .05$).

6.8.3.4   Experiment IV: Effects of Intubation with or without Inflation

These analyses interpreted both deflation and inflation as *activation conditions* and contrasted each with resting brain activity during the resting condition eyes closed rest (ECR). Resting brain activity was recorded when subjects rested in the PET-scanner with their eyes closed after removal of the gastric balloon (ECR without intubation). The contrast *Deflation minus ECR* reveals the effect of intubation alone on brain activation. This analysis illustrates esophageal stimulation. The subtraction *Inflation minus ECR* reveals the combined effects of intubation and gastric distension which is vagal afferent stimulation through gastric balloon distension. This analysis illustrates esophageal stimulation along with vagal afferent stimulation. Resting activity was recorded for data set 2 after the experimental procedures and after balloon removal. No resting activity was recorded for data set 1. Subjects were instructed to close their eyes and relax. Image processing and subtraction procedures followed the same calculations as previous analyses.

6.8.3.5   Experiment V: Resting State of the Brain

Ongoing metabolic events at baseline are reflected in increases and decreases of rCBF when compared to an experimental task (Raichle, MacLeod, Snyder, Powers, Gusnard, & Shulman, 2001). Such a default mode of brain function, when measured after experimental tasks, might reflect brain activation carried over from those preceding experimental

conditions. Therefore, we investigated if brain activation elicited by the stress of intubation carried over into brain activation measured during ECR. Brain images representing ECR in our study (acquired for data set 2) were compared with ECR scans of other study protocols without the component of the physical stress inherent in this study of gastric distension. Such experiments used cognitive, motor, and sensory tasks. A pool of 58 ECR scans from healthy subjects in our database met the matching requirements for age, sex and weight. All ECR scans were warped into Talairach space (Talairach & Tournoux, 1988) and normalized. Images were filtered to remove brain activity below 30% of peak activation. From both sets of images (database-ECR and study-ECR), an average image and a standard deviation image were used to calculate a pixel-wise t-test resulting in a t-image. A search algorithm detected extrema (t-values with an associated probability of $\alpha \leq 0.1\%$) in the t-image and described their distribution. The gamma-z procedure split the distribution of peaks in the t-image at the mean and tested the distribution for leptokurtosis. Kurtosis in general pertains to the shape of a distribution and is described as the fourth power (momentum) about the mean (κ_4) as

Equation 5 $$\kappa_4 = \frac{\Sigma(x_i - \mu)^4}{N} \ .$$

Since κ_4 describes units to the fourth power and results in large numbers, the measure of kurtosis is often referred to as κ_4 / σ^4, which equals 3 for a normal (mesokurtic) distribution (Zar, 1984). Therefore, the measure of kurtosis can be expressed as

Equation 6 $$\gamma_2 = \frac{\kappa_4}{\sigma^4} - 3$$

where γ_2 serves as a population parameter for kurtosis. If $\gamma_2 = 0$, the distribution is normal (mesokurtic). If $\gamma_2 > 0$, the distribution is leptokurtic. If $\gamma_2 < 0$, the distribution is platykurtic. Statistical significance of a γ_2-value is met when it is greater (leptokurtosis) or smaller (platykurtosis) than a critical value. A significant leptokurtosis detected with the gamma-z procedure would indicate that the average images from the two ECR populations (database-ECR and study-ECR) differ regarding their distribution of peaks. A non-significant test of leptokurtosis confirms that no outliers occur at the tails of the distribution of peaks. The distribution of peaks in the two images would be considered to be similar.

6.8.4   Experiment VI: Visual Analog Scales (VAS)

Experiment VI aimed to assess cognitive responses during mechanical vagal stimulation. Subjective feelings were repeatedly measured on VAS during data acquisition of brain activation. The ratings intended to reflect the cognitive states during inflation and deflation of the stomach. We intended to investigate if and how cognitive responses change due to gastric inflation and deflation. Ratings of VAS subscales to assess the feelings of hunger, sleepiness, nausea, gastric discomfort and tension during inflation were contrasted with their corresponding ratings during deflation.

As explained above, subjective feelings measured with VAS are of ordinal character (ordinal scale data). Equal distances in the rating values cannot be assumed to represent equal distances in the psychological construct of fullness. For example, a qualitative similar experience may find a quantitative different expression on the horizontal line bounded by opposite poles and vice versa. Such data display continuous (ordered) categories and represent ratio-level measurements. Categorical data do not allow for parametric analysis. Therefore, all VAS data analysis used non-parametric procedures.

Exploratory (stem-and-leaf and boxplots) and descriptive statistics were determined for each VAS subscale for the three inflations and deflations to determine outliers and extreme values. All inflation-deflation contrasts were calculated with post-scan inflation and pre-scan deflation measures (see chapter 6.3.2) using Wilcoxon signed-rank tests for the subscales hunger, sleepiness, nausea, gastric discomfort and tension (SPSS© for Windows 10.1, Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). Cases with missing values and outliers / extreme values were excluded from data analysis. The chosen threshold for acceptable α-error probability of $\alpha = .05$ was Bonferroni-corrected for each subscale for three comparisons (three cycles of inflation and deflation) to $\alpha = .016$.

7   Results

7.1   Target Sensation: Fullness

A summary of the VAS ratings for fullness for data sets 1 and 2 is presented in Table 4. Visual inspection of the data revealed that all ratings of fullness during inflation were, with one exception higher than ratings during deflation (subject p05705 for the first cycle of inflation [score: 1.6] and deflation [score: 1.9]). The questions propounded in chapter 6.8.1 were addressed as followed:

Table 4. Descriptive statistics for VAS scores of Fullness

		Inflation 1	Inflation 2	Inflation 3
Data Set 1	N	9	8	7
	Median	9.2	9.8	9.4
	Minimum	1.2	6.4	7.7
	Maximum	11	11	11
Data Set 2	N	9	9	7
	Median	8.1	9.2	8.4
	Minimum	1.6	5.4	6.4
	Maximum	11.2	11.2	11.2

N: sample size; VAS: Visual Analog Scale.

1.   Were there any outliers in the data set?

Outliers and extreme values for the feeling of fullness were identified using stem-and-leaf (Figure 3 and Figure 4) and boxplot (Figure 5 and Figure 6) displays. These exploratory statistics detected one extreme value for data set 1 (p02895) and one outlier for data set 2 (p05705). Extreme values and outliers were defined in chapter 6.8.1Both subjects rated their feeling of fullness during inflation 1 considerably lower than the other subjects. Such a low rating, however, was only evident during the first inflation. For the following ratings, the scores of both subjects were within the range of other subjects' scores.

```
a) VAS score: Full
   Stem-and-Leaf Plot for Inflation 1

   Frequency     Stem &  Leaf

   1.00 Extremes    (=<1.2)
   1.00          6 .  6
   1.00          7 .  9
   1.00          8 .  6
   2.00          9 .  28
   2.00         10 .  07
   1.00         11 .  0

   Stem width:      1.00
   Each leaf:       1 case(s)



b) VAS score: Full
   Stem-and-Leaf Plot for Inflation 2

    Frequency     Stem &  Leaf

       1.00          6 .  4
       1.00          7 .  4
       1.00          8 .  4
       2.00          9 .  69
       2.00         10 .  78
       1.00         11 .  0

    Stem width:      1.00
    Each leaf:       1 case(s)




c) VAS score: Full
   Stem-and-Leaf Plot for Inflation 3

    Frequency     Stem &  Leaf

       1.00          7 .  7
       1.00          8 .  2
       2.00          9 .  04
       1.00         10 .  7
       2.00         11 .  00

    Stem width:      1.00
    Each leaf:       1 case(s)
```

Figure 3. Stem-and-leaf plots: Fullness (data set 1)

Ratings of fullness for data set 1 for three consecutive inflations presented in a) through c). VAS: Visual Analog Scale.

```
a) VAS score: Full
   Stem-and-Leaf Plot for Inflation 1

    Frequency     Stem &  Leaf

        1.00 Extremes     (=<1.6)
        1.00          6 .  6
        1.00          7 .  0
        2.00          8 .  11
        2.00          9 .  08
         .00         10 .
        2.00         11 .  22

    Stem width:      1.00
    Each leaf:        1 case(s)



b) VAS score: Full
   Stem-and-Leaf Plot for Inflation 2

    Frequency     Stem &  Leaf

        1.00          5 .  4
        1.00          6 .  3
        1.00          7 .  9
        1.00          8 .  9
        3.00          9 .  267
         .00         10 .
        2.00         11 .  12

    Stem width:      1.00
    Each leaf:        1 case(s)



c) VAS score: Full
   Stem-and-Leaf Plot for Inflation 3

    Frequency     Stem &  Leaf

        1.00          6 .  4
        2.00          7 .  88
        1.00          8 .  4
         .00          9 .
        1.00         10 .  7
        2.00         11 .  22

    Stem width:      1.00
    Each leaf:        1 case(s)
```

Figure 4. Stem-and-leaf plots: Fullness (data set 2)

Ratings of fullness for data set 2 for three consecutive inflations presented in a) through c). VAS: Visual Analog Scale.
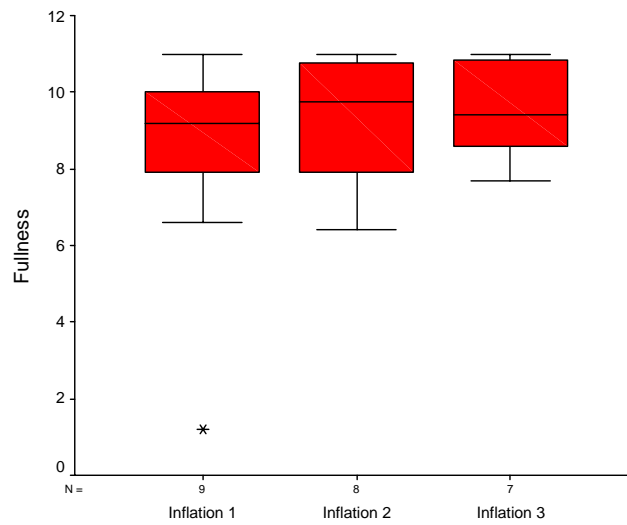
Figure 5. Boxplot: Fullness (data set 1)

Ratings of fullness for data set 1 for three consecutive inflations presented in boxplots. * extreme value, more than 3 hspreads below the lower hinge; N: sample size.
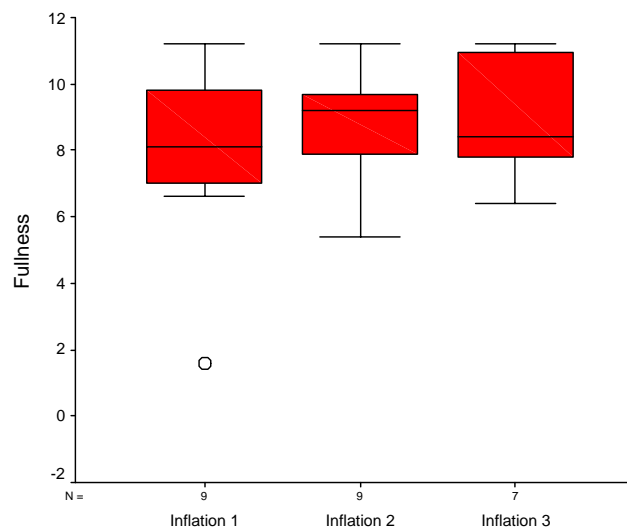
Figure 6. Boxplot: Fullness (data set 2)

Ratings of fullness for data set 2 for three consecutive inflations presented in boxplots. O outlier, value between 1.5 and 3 hspreads below the lower hinge; N: sample size.

2. Did the two groups (data set 1 and 2) differ from each other regarding their ratings of fullness? In other words, were the two groups comparable with regards to the target sensation?

No group differences in the feeling of fullness between data set 1 and data set 2 were found with Mann-Whitney U-tests (Table 5). The chosen significance threshold of $\alpha = .05$ was Bonferroni-corrected for three comparisons to $\alpha = .016$ ($\alpha = .01$ corrected to $\alpha = .003$).

3. Did subjects feel full during each of the three consecutive inflations?

Subjects felt full during each of the three inflations. Wilcoxon's signed ranks tests for related samples comparing inflation with deflation for each inflation/deflation cycle excluded outliers and extreme values. Results were statistically significant at the threshold of $\alpha = .016$. For Wilcoxon's Z-scores and associated p-values, see Table 6.

4. Did subjects as a group feel similarly full during each of the three inflations?

Subjects felt similarly full during each of the three inflations. Although ratings of fullness across subjects varied considerably (range: 5.4 – 11, 0 = not full at all, 11 = extremely full), within-subject values remained consistent for three or two consecutive inflations (Figure 7 through Figure 9). The Friedman test for 3 related samples revealed that fullness ratings did not change over the three consecutive inflations at the chosen significance threshold of $\alpha = .05$. For mean ranks and the Friedman test statistics, see Table 7.

Table 5. Group differences in the feeling of Fullness

	N	Mann-Whitney U	p ≤
Inflation 1	16	29	.752
Inflation 2	17	31.5	.665
Inflation 3	14	22.5	.797

N: sample size; VAS: Visual Analog Scale; α = .05 (Bonferroni-corrected for three comparisons to α = .016).

Table 6. Feeling of Fullness for three inflations

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	16	-3.464	.0005**
Inflation 2 vs. Deflation 2	17	-3.622	.0003**
Inflation 3 vs. Deflation 3	14	-3.061	.0022**

N: sample size; VAS: Visual Analog Scale; **significant at α = .01 (Bonferroni-corrected to α = .003).

Table 7. Changes in the feeling of Fullness

a)	Mean Rank	b)	N	12
Inflation 1	1.63		Chi-Square*	2.905
Inflation 2	2.21		df	2
Inflation 3	2.17		Asymp. Sig.*	.234

Changes in the feeling of fullness for three consecutive inflations. a) Mean rank of values, b) Friedman test for k related samples (repeated measures). N: sample size; * α = .05.
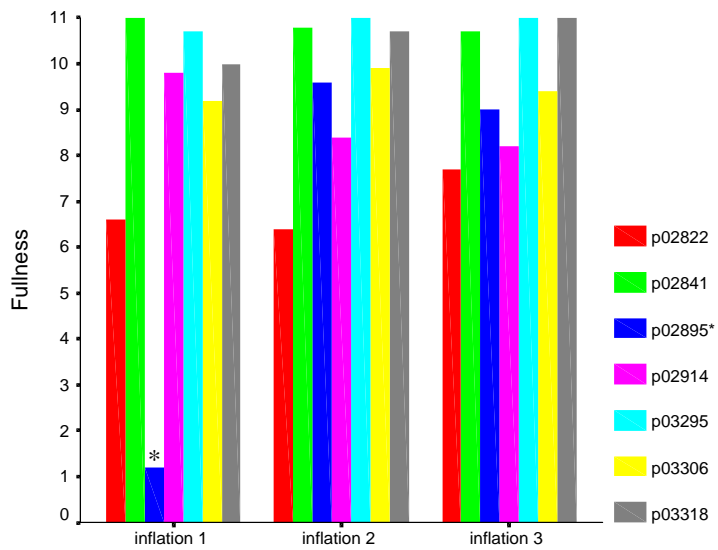
Figure 7. Fullness ratings for three inflations (data set 1)

Ratings of fullness for seven subjects for three consecutive inflations. One subject terminated the study prematurely after two cycles, one subject after one cycle of inflation/deflation. *outlier.
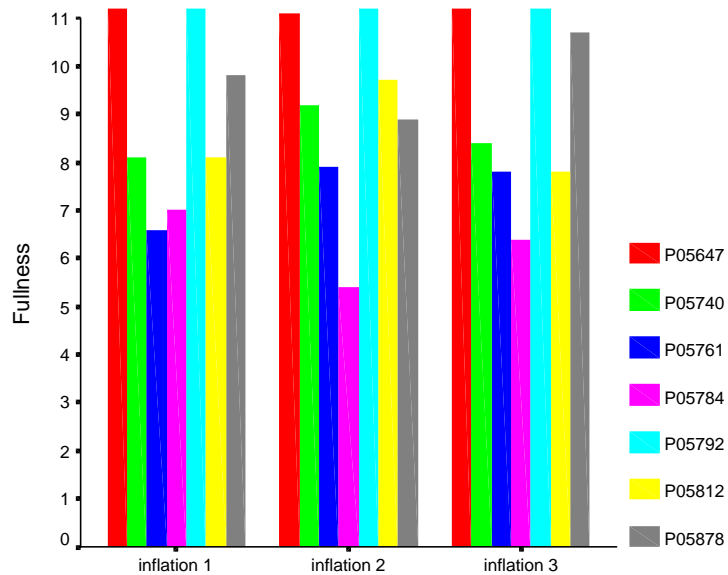
Figure 8. Fullness ratings for three inflations (data set 2)

Ratings of fullness for six subjects for three consecutive inflations. Three subjects terminated the study prematurely after two cycles of inflation/deflation.
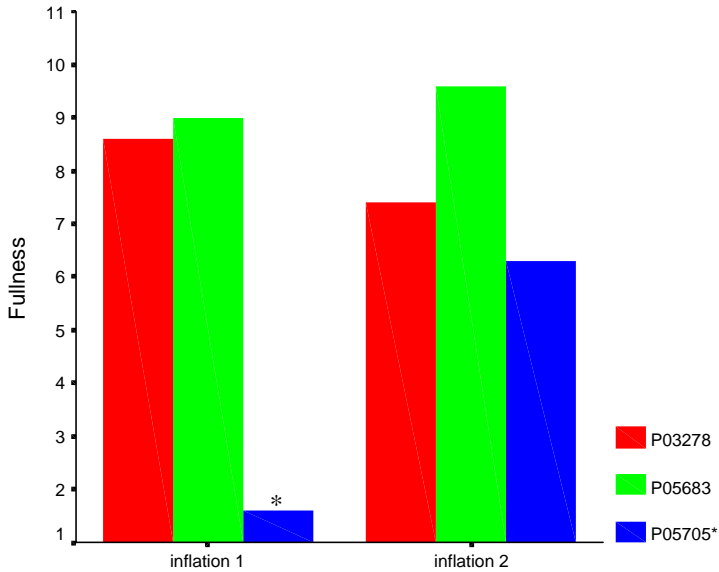
Figure 9. Fullness ratings for two inflations (data set 1 and 2)

Ratings of fullness for three subjects for two consecutive inflations (premature termination after two cycles of inflation/deflation). *outlier.

7.2   Physiological Measures and Mood Questionnaire

7.2.1   Gastric Volume and Pressure

Maximal tolerated volume (mean ± standard deviation) during inflation across subjects and across three inflations was 612 ± 214 ml (group mean). Table 8 displays descriptive statistics for each inflation. Exploratory statistics for maximal tolerated volume are given in Figure 10 (stem-and-leaf plots) and in Figure 11 (boxplots). Exploratory investigation of the data revealed three outliers (p05792 during inflation 2 and inflation 3; p03306 during inflation 3). A repeated-measures GLM with one within-subject factor with three levels (three repeated measures of inflation) excluded outliers. The threshold for acceptable α-error probability was set to $\alpha = .05$. Mauchly's test of sphericity assumed sphericity for the data (Mauchly's $W_{df=2} = 0.856$, $p \leq .732$). The GLM revealed no effect of the three consecutive inflations on maximal tolerated volume (main effect *Inflation* $F_{df=2} = 2.108$, $p \leq .148$, sphericity assumed).

Gastric pressure (mean ± standard deviation) during inflation across subjects and across three inflations was 6.7 ± 3.9 mmHg. Table 9 displays descriptive statistics for each inflation. Exploratory statistics for gastric pressure are given in Figure 12 (stem-and-leaf plots) and in Figure 13 (boxplots). Exploratory investigation of the data revealed one outlier (p05783) during inflation 2. A repeated-measures GLM with one within-subject factor with three levels (three repeated measures of inflation) excluded outliers. The threshold for acceptable α-error probability was set to $\alpha = .05$. Mauchly's test of sphericity assumed sphericity for the data (Mauchly's $W_{df=2} = 0.806$, $p \leq .524$). The GLM revealed no effect of the three consecutive inflations on maximal tolerated volume (main effect *Inflation* $F_{df=2} = 2.219$, $p \leq .146$, sphericity assumed).

Table 8. Maximal tolerated volume (ml) during inflation

	N	Minimum	Maximum	Mean	Std. Deviation
Inflation 1	18	350	1075	653	216
Inflation 2	17	200	1175	584	231
Inflation 3	13	150	1175	600	246

N: sample size; Std. Deviation: Standard Deviation.

Table 9. Average gastric pressure (mmHg) during inflation

	N	Minimum	Maximum	Mean	Std. Deviation
Inflation 1	15	2.7	15.6	6.8	3.8
Inflation 2	14	1.9	17.8	6.4	4.3
Inflation 3	10	1.8	12.7	6.0	3.8

N: sample size; Std. Deviation: Standard Deviation.

```
a)  Volume in ml:
    Stem-and-Leaf Plot for Inflation 1

      Frequency     Stem &  Leaf

         1.00           3 .  5
         3.00           4 .  007
         4.00           5 .  0000
         2.00           6 .  05
         1.00           7 .  0
         5.00           8 .  00009
          .00           9 .
         2.00          10 .  07

     Stem width:     100.00
     Each leaf:        1 case(s)



b)  Volume in ml:
    Stem-and-Leaf Plot for Inflation 2

      Frequency     Stem &  Leaf

         1.00           2 .  0
         1.00           3 .  0
         4.00           4 .  0005
         4.00           5 .  2558
         1.00           6 .  5
         3.00           7 .  005
         2.00           8 .  00
         1.00 Extremes     (>=1175)

     Stem width:     100.00
     Each leaf:        1 case(s)



c)  Volume in ml:
    Stem-and-Leaf Plot for Inflation 3

      Frequency     Stem &  Leaf

         1.00 Extremes     (=<150)
         1.00           3 .  0
         2.00           4 .  57
         1.00           5 .  5
         6.00           6 .  055588
          .00           7 .
         1.00           8 .  0
         1.00 Extremes     (>=1175)

     Stem width:     100.00
     Each leaf:        1 case(s)
```

Figure 10. Stem-and-leaf plots: Maximal tolerated volume (ml)

Volume in ml for three inflations presented in a) through c).

Figure 11. Boxplot: Maximal tolerated volume (ml)

Volume in ml for three consecutive inflations presented in boxplots. O outlier, value between 1.5 and 3 hspreads below the lower hinge; N: sample size.

a) Gastric pressure in mmHg
Stem-and-Leaf Plot for Inflation 1

```
 Frequency      Stem &   Leaf

      5.00          0 .   23333
      7.00          0 .   5567779
      2.00          1 .   02
      1.00          1 .   5

 Stem width:       10.00
 Each leaf:         1 case(s)
```

b) Gastric pressure in mmHg
Stem-and-Leaf Plot for Inflation 2

```
 Frequency      Stem &   Leaf

      7.00          0 .   1223344
      5.00          0 .   57889
      1.00          1 .   1
      1.00 Extremes    (>=18)

 Stem width:       10.00
 Each leaf:         1 case(s)
```

c) Gastric pressure in mmHg
Stem-and-Leaf Plot for Inflation 3

```
 Frequency      Stem &   Leaf

      5.00          0 .   12234
      3.00          0 .   578
      2.00          1 .   12

 Stem width:       10.00
 Each leaf:         1 case(s)
```

Figure 12. Stem-and-leaf plots: Gastric pressure (mmHg)

Gastric pressure in mmHg during maximal tolerated volume for three
consecutive inflations presented in a) through c).
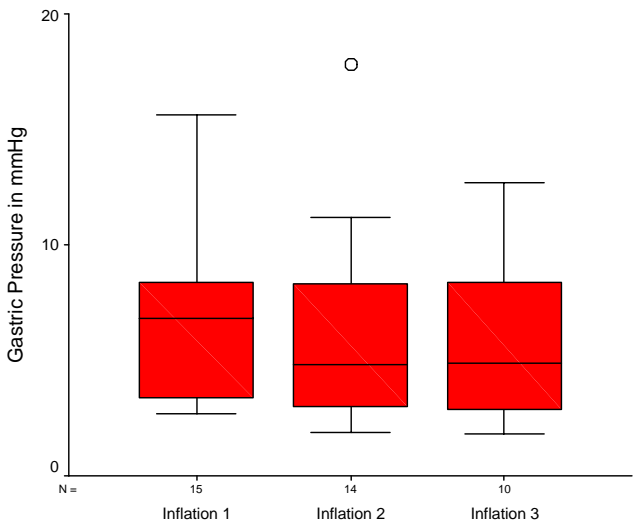
Figure 13. Boxplot: Gastric pressure (mmHg)

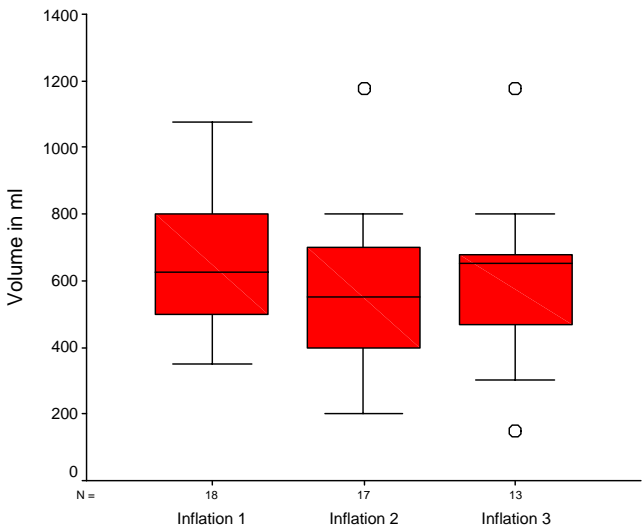Gastric pressure in mmHg during maximal tolerated volume for three consecutive inflations presented in boxplots. o outlier, value between 1.5 and 3 hspreads below the lower hinge; N: sample size.

7.2.2   Electrocardiogram (ECG)

Heart rate in beats per minute (BPM) was recorded with ECGs from twelve subjects (eight for data set 1, four for data set 2). Exploratory statistics for heart rate in BPM during inflation are given in Figure 14 (stem-and-leaf plots), for BPM during deflation in Figure 15 (stem-and-leaf plots) and for BPM during inflation and deflation in Figure 16 (boxplots). Exploratory investigation of the data revealed no outliers or extreme values for inflation. However, three outliers were found for deflation (p02914, p03295, p05784; all occurring during the third deflation).

Mean BPM (± standard deviation, calculated for all subjects with ECG data) during inflation across subjects and across three inflations was 86.38 ± 13.48. Descriptive statistics for each inflation are given in Table 10. For the investigation of the effects of condition (inflation vs. deflation) and time (repeated measures), data from a total of seven subjects were excluded (four subjects with missing values, three subjects with outlier values). A repeated-measures GLM (N=5) with two within-subject factors (*Condition*: inflation, deflation; *Time*: three repeated cycles of inflation/deflation) with an acceptable α-error probability of $\alpha = .05$ was conducted. Sphericity was assumed for the main effect of *Time* (Mauchley's $W_{df=2} = 0.275$, $p \leq .275$) and the interaction of *Condition * Time* (Mauchley's $W_{df=2} = 0.235$, $p \leq .235$). The GLM revealed neither main effects nor an interaction effect (main effect *Condition* $F_{df=1} = 1.263$, $p \leq .343$, main effect *Time* $F_{df=2}$ 0.590, $p \leq .584$, interaction *Condition * Time* $F_{df=2} = 0.268$, $p \leq .774$).

Table 10. Mean heart rate (BPM) during inflation

	N	Minimum	Maximum	Mean	Std. Deviation
Inflation 1	12	66	108	83.9	13.0
Inflation 2	11	60	117	83.8	16.3
Inflation 3	8	66	102	81.6	12.2

BPM: beats per minute; N: sample size; Std. Deviation: Standard Deviation.

```
a)  Heart rate in BPM
    Stem-and-Leaf Plot for Inflation 1

     Frequency      Stem &   Leaf

         2.00          6 .   69
         3.00          7 .   269
         3.00          8 .   144
         2.00          9 .   06
         2.00         10 .   28

     Stem width:      10.00
     Each leaf:        1 case(s)




b)  Heart rate in BPM
    Stem-and-Leaf Plot for Inflation 2

     Frequency      Stem &   Leaf

         2.00          6 .   08
         4.00          7 .   2888
         2.00          8 .   49
         1.00          9 .   6
         1.00         10 .   2
         1.00         11 .   7

     Stem width:      10.00
     Each leaf:        1 case(s)



c)  Heart rate in BPM
    Stem-and-Leaf Plot for Inflation 3

     Frequency      Stem &   Leaf

         2.00          6 .   68
         2.00          7 .   58
         1.00          8 .   4
         2.00          9 .   00
         1.00         10 .   2

     Stem width:      10.00
     Each leaf:        1 case(s)
```

_____

Figure 14. Stem-and-leaf plots: BPM during inflation

Heart rate in BPM during three inflations presented in a) through c).
BPM: beats per minute.

```
a) Heart rate in BPM
   Stem-and-Leaf Plot for Deflation 1

    Frequency     Stem &  Leaf

      10.00          0 .  5667788999
       2.00          1 .  13

    Stem width:    100.00
    Each leaf:       1 case(s)
```

```
b) Heart rate in BPM
   Stem-and-Leaf Plot for Deflation 2

    Frequency     Stem &  Leaf

       3.00          6 .  666
       4.00          7 .  2888
       4.00          8 .  0444
       1.00          9 .  6

    Stem width:     10.00
    Each leaf:       1 case(s)
```

```
c) Heart rate in BPM
   Stem-and-Leaf Plot for Deflation 3

    Frequency     Stem &  Leaf

       2.00 Extremes    (=<66)
       3.00          7 .  888
       1.00          8 .  4
       1.00          8 .  5
       1.00          9 .  0
       1.00 Extremes    (>=102)

    Stem width:     10.00
    Each leaf:       1 case(s)
```

_____

Figure 15. Stem-and-leaf plots: BPM during deflation

Heart rate in BPM during three deflations presented in a) through c).
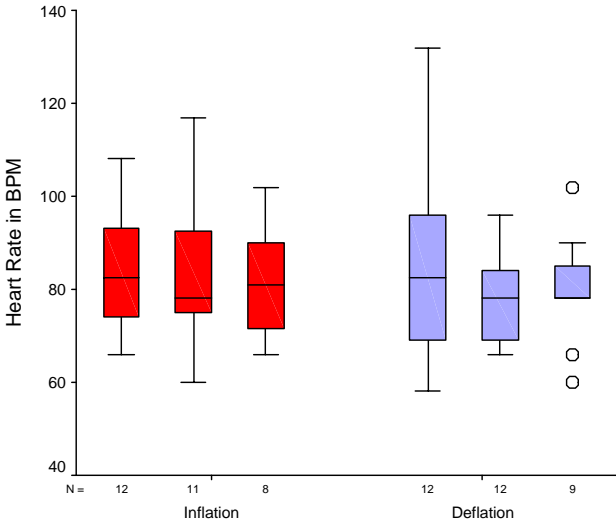BPM: beats per minute.

Figure 16. Boxplot: BPM during inflation and deflation

Heart rate in BPM during three cycles of inflation and deflation presented in boxplots. O outlier, value between 1.5 and 3 hspreads below the lower hinge; BPM: beats per minute; N: sample size.

7.2.3   Positive Affect and Negative Affect Schedule (PANAS)

A summary of PANAS scores from these studies is presented in Table 11. T-tests comparing PANAS scores for both time instructions (right now and past month) with a normative sample (Watson, et al., 1988) revealed that study scores did not differ from the normative sample. Test results are shown in Table 12. Normative data are presented in Table 13.

Table 11. Descriptive statistics for PANAS scores

	N	Minimum	Maximum	Mean	Std. Deviation
Positive Affect (past month)	15	31	42	36.5	3.3
Negative Affect (past month)	15	11	25	16.5	3.5
Positive Affect (right now)	15	13	45	29.4	8.4
Negative Affect (right now)	15	10	19	12.4	2.8

N: sample size; PANAS: Positive Affect and Negative Affect Schedule; Std. Deviation: Standard Deviation.

Table 12. Normative sample of PANAS scores

	N	Mean	Std. Deviation
Positive Affect (past month)	586	32.0	7.0
Negative Affect (past month)	586	19.5	7.0
Positive Affect (right now)	660	29.7	7.9
Negative Affect (right now)	660	14.8	5.4

N: sample size; PANAS: Positive Affect and Negative Affect Schedule; Std. Deviation: Standard Deviation.

Table 13. Comparison of PANAS scores with normative data (t-test for independent samples)

	t	df	$p \leq$
Positive Affect (past month)	2.481	599	.013
Negative Affect (past month)	-1.654	599	.099
Positive Affect (right now)	-0.145	673	.885
Negative Affect (right now)	-1.715	673	.087

PANAS: Positive Affect and Negative Affect Schedule. $\alpha = .05$ (Bonferroni-corrected for four comparisons to $\alpha = .0125$).

7.3   Experiment I-V: Brain Activation Data

7.3.1   Experiment I: Hypothesis Generation

Regional cerebral blood flow (rCBF) increased in several brain regions during gastric distension when compared to non-distention. Eight areas of brain activation met or exceeded the significance threshold of $z \geq 3.3$.

Table 14 presents areas of increased rCBF during gastric distension expressed as z-scores in stereotactic space (Talairach & Tournoux, 1988) in accordance with Damasio (1995) for more detailed description. On the lateral surface, increased rCBF was detected in two distinct sites of the right precentral gyrus, the middle frontal gyrus and in the intraparietal sulcus. On the medial surface, increased rCBF was detected in two distinct sites of the right superior frontal gyrus, the right paracentral lobe in the ascending branch off the cingulate sulcus, and in the left cingulate gyrus bordering the beak of the corpus callosum. Figure 17 represents a transverse display of peaks across the brain. Since the results acquired from nine subjects lack power (Andreasen, et al., 1996; Friston, et al., 1999), we collected data from nine additional subjects to achieve a sample size of 18 subjects for increased power (see chapter 7.3.2).

Table 14. Brain activation during gastric distension (Experiment I)

Cerebral Structure*	BA	Coordinates* x	y	z	z-score	p-value	N
Right intraparietal sulcus	40	42	42	47	4.60	.000002156216	9
Left middle frontal gyrus	6	-44	14	47	4.39	.000005539830	9
Left superior frontal gyrus, medial surface	6	-1	-1	52	4.18	.000014468897	8
Left paracentral lobe (within ascending branch of cingulate sulcus)	6	-10	-24	54	3.73	.000096877826	7
Right precentral gyrus, within central sulcus	4	30	-22	56	3.53	.000210453290	6
right precentral gyrus, motor fibers near central sulcus	4	46	-13	56	3.35	.000407455838	9
Superior frontal gyrus, medial surface	6/8	6	21	54	3.31	.000469183113	8
Left cingulate gyrus, bordering beak of corpus callosum	24	-12	28	14	3.30	.000482359959	9

* Coordinates and cerebral structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area; N: number of subjects contributing to an activation peak.

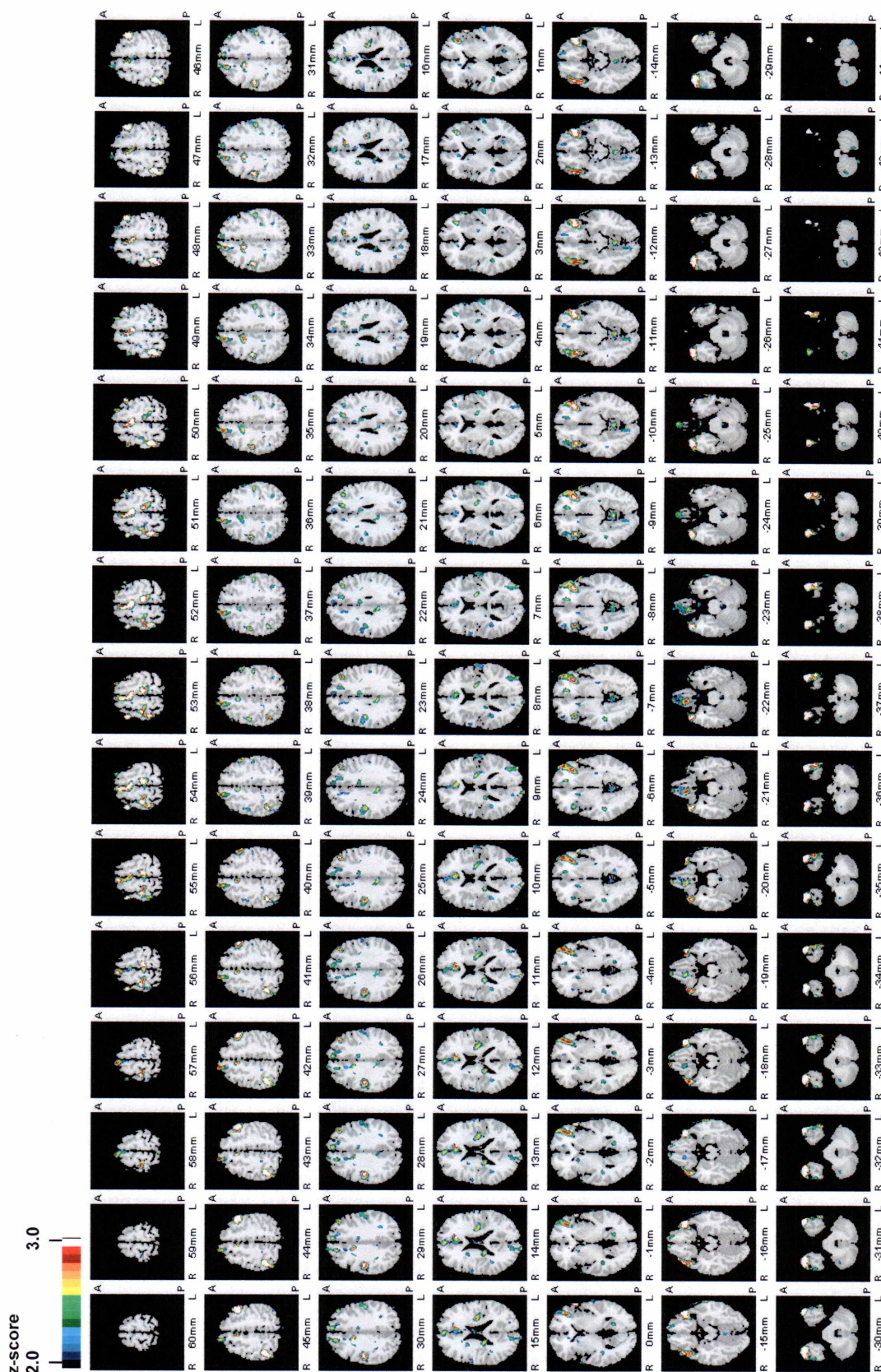**Figure 17.** Increased rCBF during gastric distension across the brain (N = 9)

Transverse (horizontal) display of the brain. Letters and numbers below the slices indicate their orientations and stereotactic z-plane coordinate (Talairach & Tournoux, 1988). Colored areas are coded to represent the magnitude of change in rCBF as z-sores $(2.0 \leq z \leq 3.0)$. A: anterior; L: left; N: sample size; P: posterior; R: right.

7.3.2   Experiment II: Extended Exploratory Analysis

Regional CBF increased in more than 20 different areas when comparing inflated to deflated scans (threshold: $z \geq 3.3$; Zald, et al., 1998b). Table 15 gives an overview of all brain areas activated during gastric inflation when compared to gastric deflation and Figure 18 presents a transverse display of these peaks across the brain. Furthermore, Table 16 lists brain activation during gastric inflation in the four well-known regions involved in visceral sensation as described above.

Figure 19 displays activation centers in two activation peaks in the in the dorsal brainstem (section A); left inferior frontal cortex (LIFC, section B); several activation areas in the left insular cortex / claustrum, and bilateral operculum (section B and C); activation in the right subgenual anterior cingulate cortex (ACC, section D). Table 16 lists the coordinates of these areas according to Talairach and Tournoux (1988) with corresponding z-scores, uncorrected p-values, and number of subjects contributing to each activation focus (see chapter 6.8.3.2). Figure 20 gives a detailed picture of activated regions in the claustrum, insula, and the operculum.

Table 15. Gastric distension reflected in the entire brain (inflation minus deflation)

Cerebral Structure*	BA	x	y	z	z-score	p-value	N
			Coordinates*				
Left inferior frontal gyrus	47	-55	19	-4	5.35	.00000004	11
Left medial superior frontal gyrus	6	-8	-26	61	5.11	.00000016	8
Left inferior frontal gyrus	47	-44	30	-14	4.92	.00000043	10
Right precentral gyrus	4	35	-24	52	4.75	.00000103	17
Left superior temporal gyrus	22/42	-62	-6	11	4.31	.00000807	18
Right inferior parietal lobule, intraparietal sulcus	7/40	42	-40	50	4.26	.00001035	17
Left medial superior frontal gyrus	6	-1	-1	52	4.18	.00001475	16
Left lateral middle frontal gyrus	6	-46	12	47	4.16	.00001599	17
Right medial superior frontal gyrus, cingulate sulcus	6	6	10	52	4.03	.00002762	16
Right postcentral gyrus	3	21	-28	58	4.02	.00002940	11
Left lateral middle frontal gyrus	6	-53	8	43	3.97	.00003574	14
Right inferior parietal lobule, intraparietal sulcus	40	44	-49	47	3.91	.00004621	17
Left anterior insula, near insular apex		-28	14	-11	3.60	.00015877	18
Right lateral middle frontal gyrus	6	44	5	54	3.59	.00016690	14
Right insula / parietal operculum		39	-13	22	3.58	.00017385	18
Right anterior subgenual cingulate cortex	24/32	15	19	-9	3.45	.00027806	18
Left insula, superior periinsular sulcus		-30	-4	22	3.43	.00030242	18
Left insula		-39	1	0	3.38	.00035605	18
Left claustrum		-33	1	-4	3.35	.00039844	18
Right brainstem		1	-33	-18	3.34	.00041737	18

* Coordinates and cerebral structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area;
N: number of subjects contributing to an activation peak.

Figure 18. Increased rCBF during gastric distension across the brain (N = 18)

Transverse (horizontal) display of the brain. Letters and numbers below the slices indicate their orientations and stereotactic z-plane coordinate (Talairach & Tournoux, 1988). Colored areas are coded to represent the magnitude of change in rCBF as z-sores ($2.0 \leq z \leq 3.0$). A: anterior; L: left; N: sample size; P: posterior; R: right.

Table 16. Gastric distension reflected in four key brain structures involved in visceral sensation: brainstem nuclei, insular and anterior cingulate cortex, and ventrolateral prefrontal cortex (Experiment II)

Cerebral Structure*	BA	Coordinates*			z-score	p-value	N
		x	y	z			
Left inferior frontal gyrus	47	-55	18	-4	5.35	.00000004	11
Left inferior frontal gyrus	47	-44	30	-14	4.92	.00000043	10
Left insular pole		-28	14	-11	3.60	.00015877	18
Left insula (intermediate)		-39	1	0	3.38	.00035605	18
Left claustrum		-33	1	-4	3.35	.00039844	18
Left insula / parietal operculum		-30	-4	22	3.43	.00030242	18
Right insula / parietal operculum		39	-13	22	3.58	.00017385	18
Right anterior cingulate cortex		15	19	-9	3.45	.00027806	18
Brain stem (parabrachial nucleus)		1	-33	-18	3.34	.00041737	18

* Coordinates and cerebral structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman Area; N: number of subjects contributing to the activation peak.
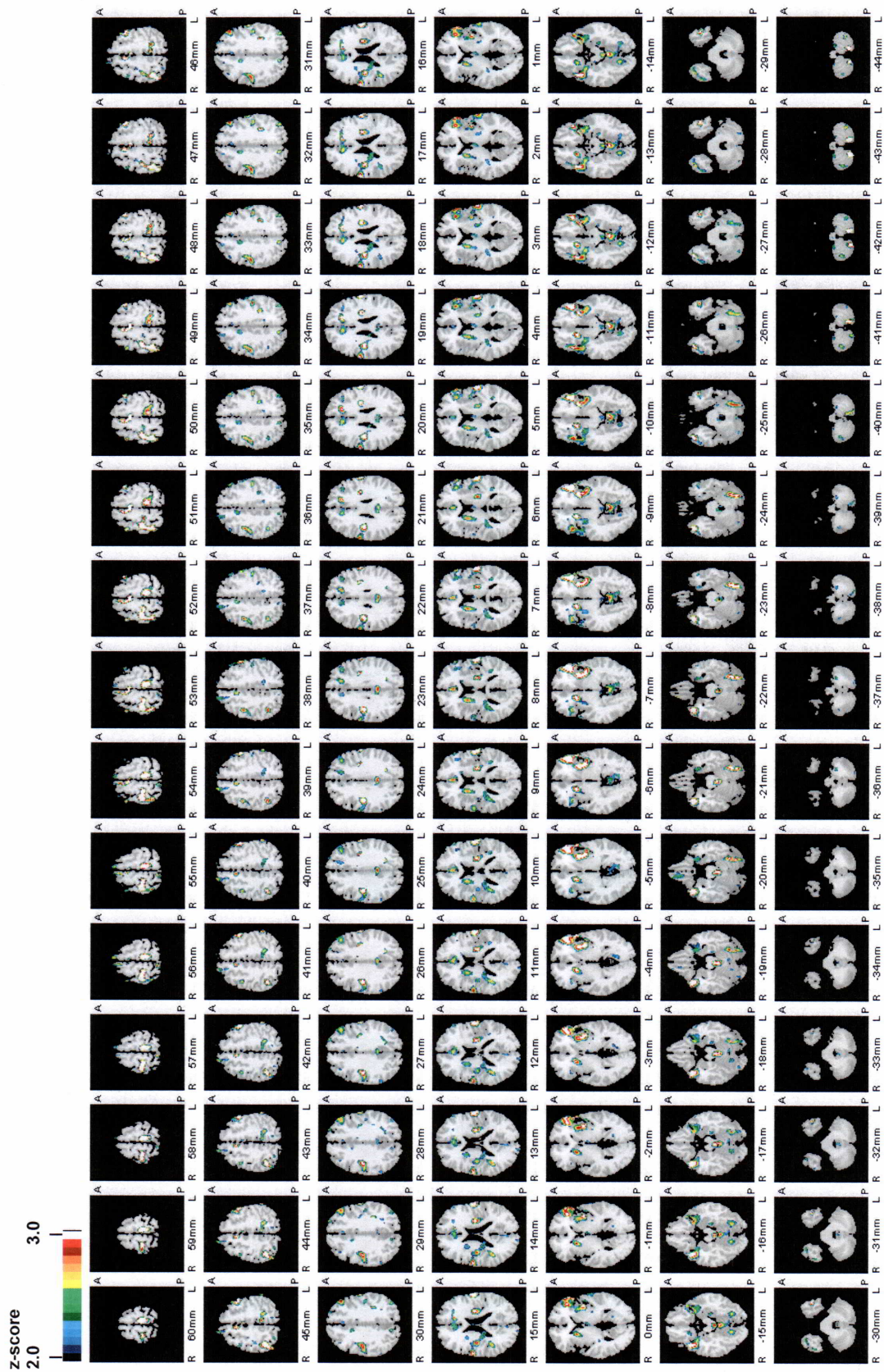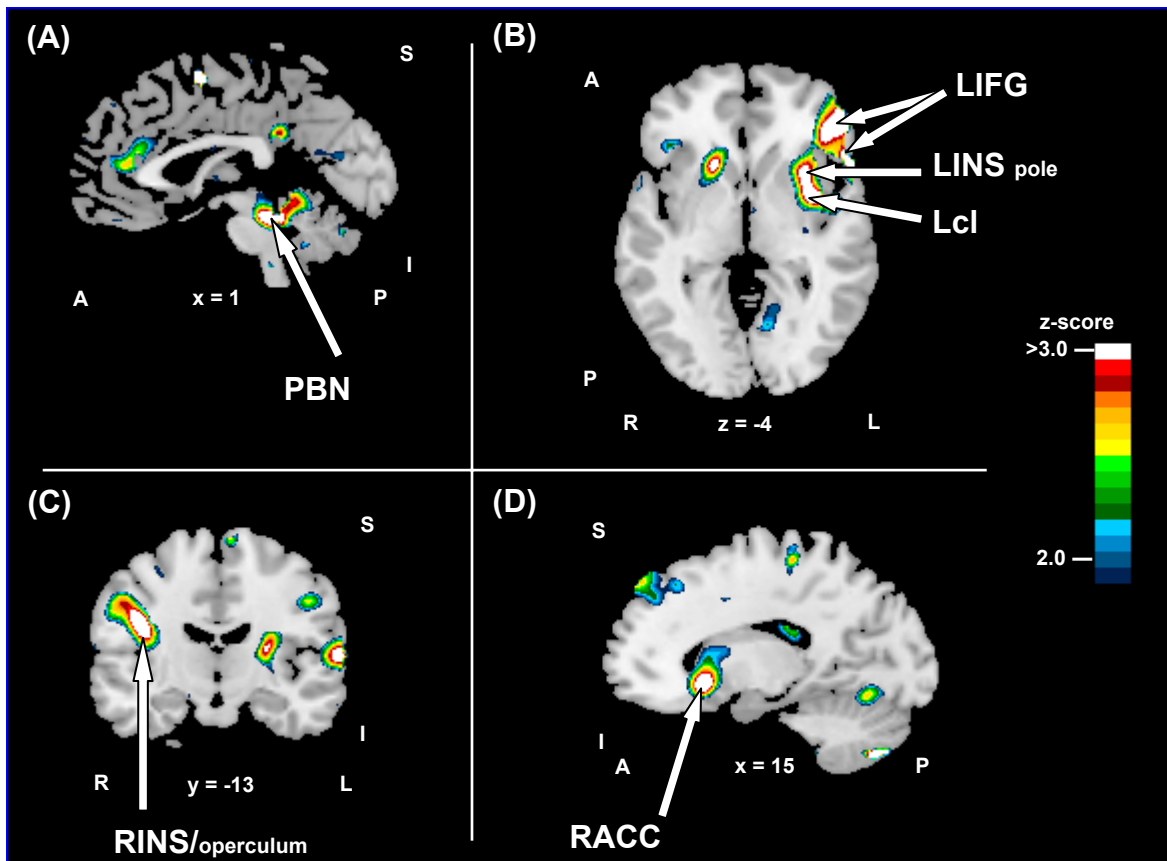
Figure 19. Increased rCBF during gastric distension in four key brain regions

Arrows point to areas with significant changes in rCBF in four a priori defined regions of intereste involved in visceral sensation: (A) dorsal brain stem nuclei; (B) inferior frontal gyrus; (B) and (C) insular cortex and claustrum; (D) subgenual anterior cingulate cortex. Letters and numbers below the segments indicate their orientations and stereotactic coordinates (Talairach & Tournoux, 1988). A: anterior; I: inferior; L: left; Lcl: left claustrum; LIFG: left inferior frontal gyrus; LINS: left insula; LINS pole: left insular pole; P: posterior; PBN: parabrachial nucleus; R: right; RACC: right anterior cingulate cortex; RINS/oper: right insula/operculum; S: superior; x: sagittal plane; y: coronal (verticofrontal) plane; z: transverse (horizontal) plane.
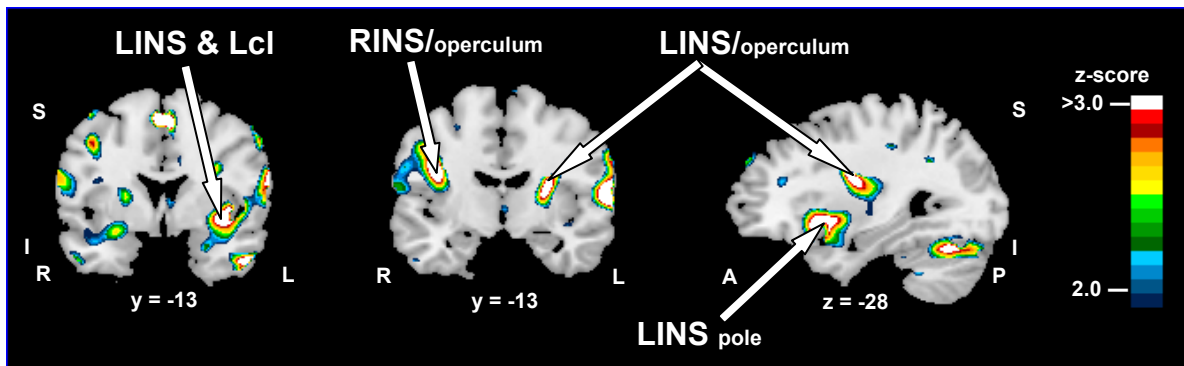

Figure 20. Increased rCBF during gastric distension in the insular cortex

Arrows point to areas with significant changes in rCBF in insular regions. LINS/oper: left insula/operculum; all other abbreviations as in Figure 19.

7.3.3 Experiment III: Split-Half Reliability

The contrast of brain activation expressed as counts of radioactive decay between the two conditions inflation and deflation revealed that only one region in group 1 (left inferior frontal gyrus, x/y/z = -55/19/4) and two regions in group 2 (both activation peaks in the left inferior frontal gyrus, x/y/z = -55/19/4 and x/y/z = -44/30/-14) reached the threshold of significance at α = .05 (Bonferroni-corrected for nine comparisons to α = .006). For the specific results of the one-tailed paired t-tests for dependent samples for group 1 and group 2, see Table 17 and Table 18.

Table 17. Split-half reliability: Group 1 in nine ROIs

Cerebral Structure[†]	BA	Coordinates[†]			$t_{df=8}$	p ≤
		x	y	z		
Left inferior frontal gyrus	47	-55	19	-4	-3.71	.006*
Left inferior frontal gyrus	47	-44	30	-14	-1.44	.188
Left insula, apex		-28	14	-11	-2.67	.028
Left insula		-39	1	0	-2.80	.023
Left claustrum		-33	1	-4	-1.33	.220
Left insula		-30	-4	22	-1.33	.220
Right insula		39	-13	22	-2.02	.078
Right anterior cingulate cortex	24/32	15	19	-9	-2.16	.062
Parabrachial nucleus		1	-33	-18	-2.42	.042

[†] Coordinates and structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area; *significant at α = .05 (Bonferroni-corrected for nine comparisons: α = .006).

Table 18. Split-half reliability: Group 2 in nine ROIs

Cerebral Structure[†]	BA	Coordinates[†]			$t_{df=8}$	p ≤
		x	y	z		
Left inferior frontal gyrus	47	-55	19	-4	-4.67	.002*
Left inferior frontal gyrus	47	-44	30	-14	-4.03	.004*
Left insula, apex		-28	14	-11	-2.71	.027
Left insula		-39	1	0	-3.34	.010
Left claustrum		-33	1	-4	-2.86	.021
Left insula		-30	-4	22	-2.86	.021
Right insula		39	-13	22	-2.12	.067
Right anterior cingulate cortex	24/32	15	19	-9	-1.39	.202
Parabrachial nucleus		1	-33	-18	-2.42	.042

[†] Coordinates and structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area; *significant at α = .05 (Bonferroni-corrected for nine comparisons: α = .006).

7.3.4   Experiment IV: Effects of Intubation with or without Inflation

When investigating the effects of intubation, nine distinct regions in the brain could be identified as active. Activity in these regions reached a z-score ≥ 3.3 and a minimum of six subjects contributed to the activation peak. These results represent esophageal stimulation without gastric distension. Comparing brain activation during deflation to the resting condition ECR activated brain structures listed in Table 19. This table presents the coordinates according to Talairach and Tournoux (1988) in accordance with Damasio (1995), z-scores ≥ 3.3 with associated p-values and the number of subjects contributing to the activation peak. Deactivation below the threshold of $z \leq -3.3$ occurred in the left precuneus in the vicinity of the tip of the intraparietal sulcus (x/y/z = -21/-46/45, z-score: -4.03, $p \leq .00003$). Figure 21 represents selective activation peaks. Figure 22 represents a transverse display of peaks across the brain.

Table 19. Effects of intubation without inflation (deflation minus ECR)

Cerebral Structure*	BA	Coordinates* x	y	z	z-score	p-value	N
Left postcentral gyrus: SI (lips/mouth)	4	-55	-13	27	6.47	.000000000049	8
Right postcentral gyrus: SI (tongue/pharynx)	43	55	-10	14	5.08	.000000191336	8
Left medial lingual gyrus, visual cortex	17/18	-1	-89	-7	4.68	.000001432899	8
Right precentral gyrus: MI (lips/mouth)	4	55	-8	27	4.60	.000002093965	8
Left gyrus rectus	11	-6	12	-20	4.33	.000007334274	8
Right posterior cingulate sulcus	7	12	-37	50	4.03	.000027573118	6
Left extrastriate cortex / cerebellum		-17	-53	-11	3.64	.000134840375	8
Left intraparietal sulcus (valley)	7/40	-39	-46	40	3.63	.000140873191	8
Left posterior cingulate sulcus	6	-3	-22	50	3.51	.000224089483	7

* Coordinates and structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area; ECR: eyer closed rest; N: number of subjects contributing to an activation peak.
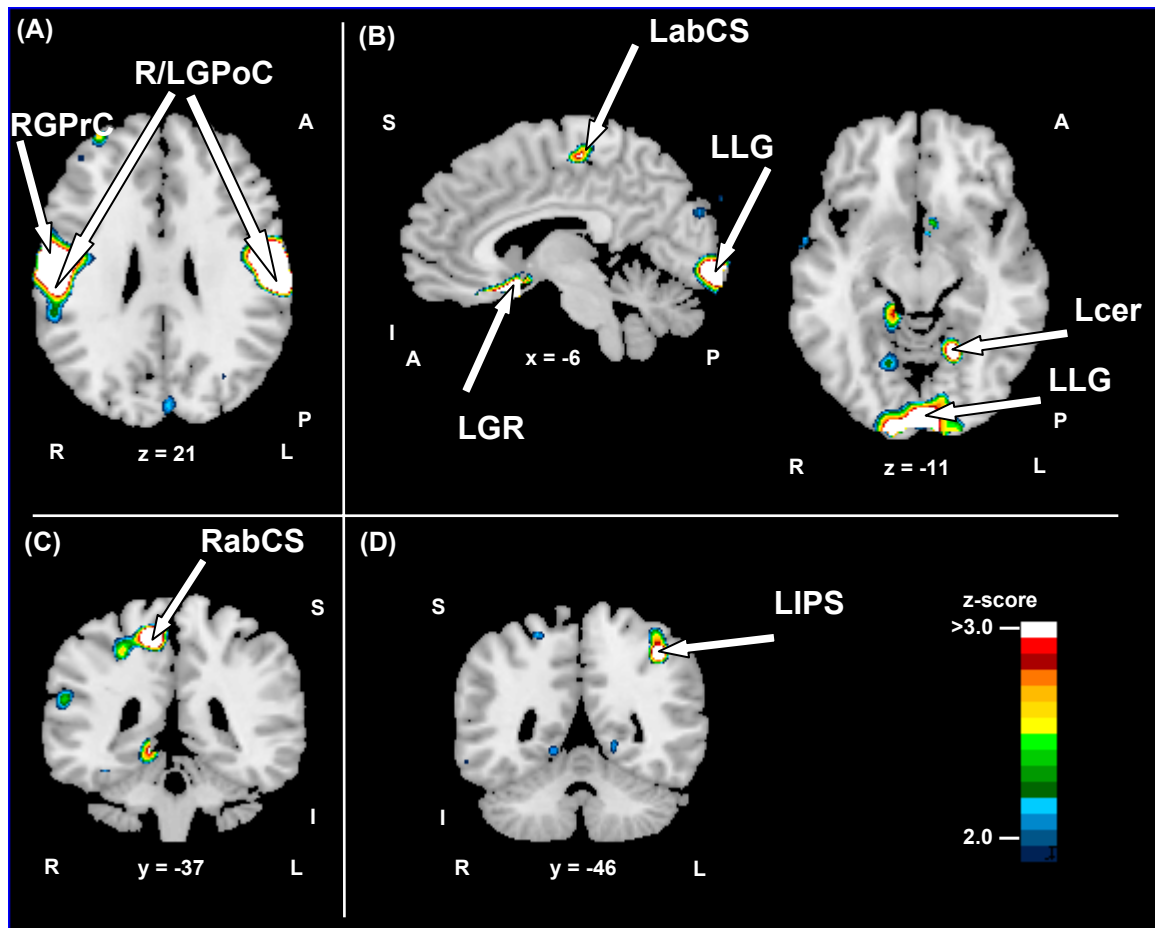
Figure 21. Increased rCBF during intubation without gastric distension

(A) Right/left postcentral gyrus and right precentral gyrus; (B) left gyrus rectus, left ascending branch of cingulate sulcus, left lingual gyrus and left cerebellum; C) right ascending branch of cingulate sulcus; (D) left intraparietal sulcus (valley). Letters and numbers below the segments indicate their orientations and stereotactic coordinates (Talairach & Tournoux, 1988). A: anterior; I: inferior; L: left; LabCS: left ascending branch of cingulate gyrus; Lcr: left cerebellum; LGR: left gyrus rectus; LIPS: left intraparietal sulcus; LLG: left lingual gyrus; P: posterior; R: right; RabCS: right ascending branch of cingulate gyrus; RGPrC: right precentral gyrus; R/LGPoC: right and left postcentral gyrus; S: superior; x: sagittal plane; y: coronal (verticofrontal) plane; z: transverse (horizontal) plane.
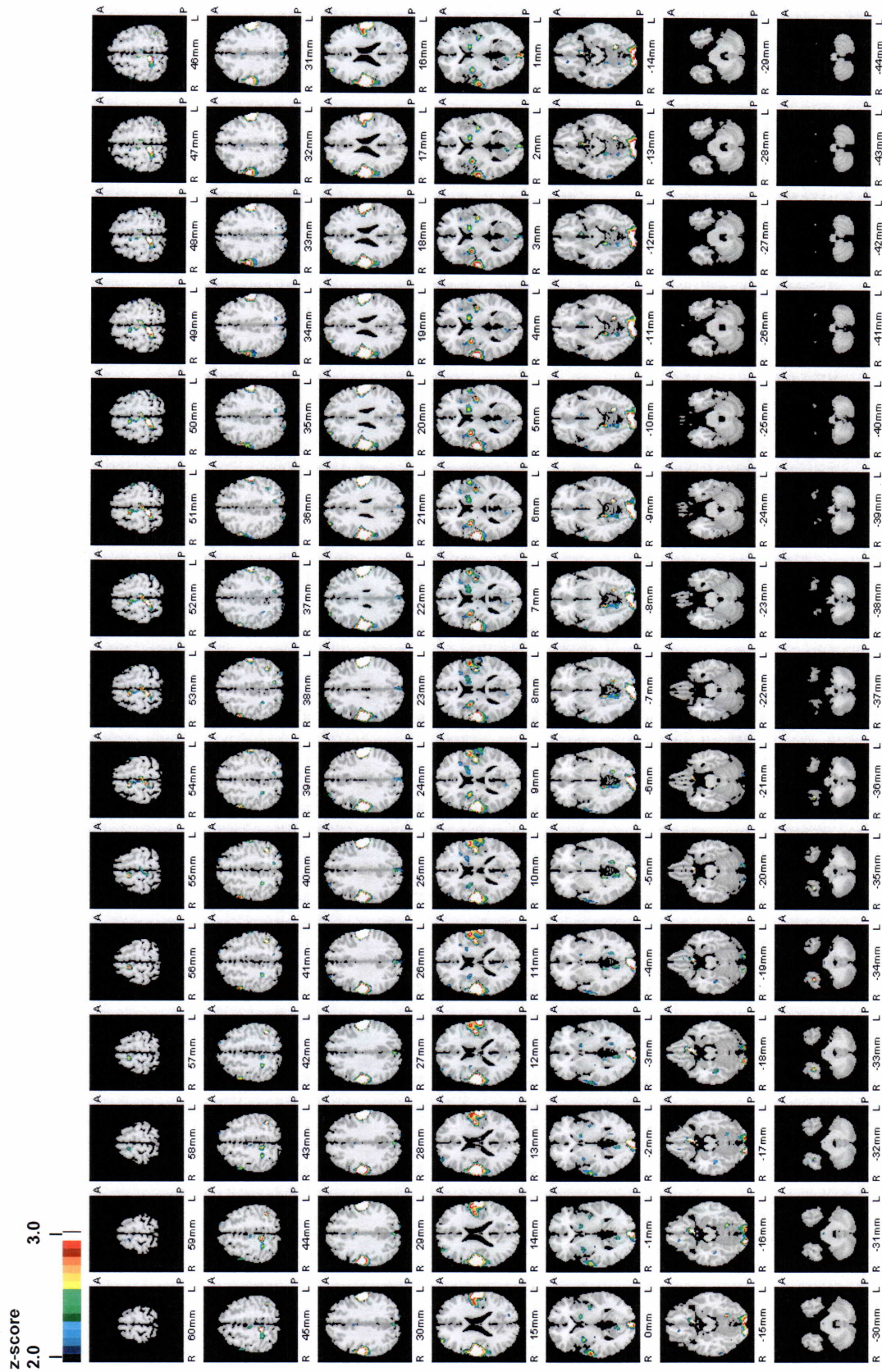
Figure 22. Increased rCBF during intubation without gastric distension across the brain (N = 8)

Transverse (horizontal) display of the brain. Letters and numbers below the slices indicate their orientations and stereotactic z-plane coordinate (Talairach & Tournoux, 1988). Colored areas are coded to represent the magnitude of change in rCBF as z-sores ($2.0 \leq z \leq 3.0$). A: anterior; L: left; N: sample size; P: posterior; R: right.

When investigating the effects of intubation along with gastric inflation, 29 distinct regions in the brain could be identified as active. Activity in these regions reached a z-score $\geq$ 3.3 and a minimum of six subjects contributed to the activation peak. These results represent esophageal stimulation along with stimulation of gastric vagal afferent fibers. Comparing brain activation during inflation toECR activated brain structures listed in Table 20 (sorted by z-score). This table presents the coordinates according to Talairach and Tournoux (1988) in accordance with Damasio (1995), z-scores $\geq$ 3.3. Thalamic activation was included in this table (z = 3.06, p $\leq$ .0011) although it did not reach the significance threshold. Deactivated brain areas did not occur below the z-threshold of -3.3. Figure 23 represents selective slices in various orientations to display the brain areas with peak activation. Figure 24 represents a transverse display of peaks across the brain.

7.3.5   *Experiment V: Resting State of the Brain*

Comparison of the ECR images from this study (acquired for data set 2; N = 9) with ECR images from 58 subjects matched according to age, sex, and weight recruited from our database revealed no significant difference between the two sets of images. A total of 1,893 data points (843 positive data points above the mean and 1,050 negative data points below the mean) were included in the analysis. The gamma 2 (γ_2) statistic was calculated for three sets of data points: 1) all data points, 2) positive data points and 3) negative data points. The gamma 2 (γ_2) statistic for 1) of all points resulted in γ_2 = -1.260 (critical value for leptokurtosis = 0.191 for α = .05); for 2) the top of the curve (positive data points) γ_2 = -1.413 (critical value for leptokurtosis = 0.281 for α = .05); and 3) the bottom of the curve (negative data points) γ_2 = -1.182 (critical value for leptokurtosis = 0.255 for α = .05) respectively. The gamma-z test did not find significant leptokurtosis in the distribution of peaks in the database ECR and study ECR images (see appendix J).

Table 20. Effects of intubation with inflation (inflation minus ECR)

Cerebral Structure	BA	x	y	z	z-score	p-value	N
		Coordinates*					
Left postcentral gyrus: SI (lip/mouth)	3	-60	-10	27	7.74	.000000000000	8
Right postcentral gyrus: SI (tongue/pharynx)	43	55	-8	14	7.13	.000000000001	8
Left cerebellum, hemisphere, lobule V¶		-17	-55	-14	4.74	.000001050484	8
Right postcentral sulcus	7	37	-37	52	4.70	.000001272770	6
Right cerebellum, hemisphere, lobule V¶		15	-64	-16	4.67	.000001513634	7
Left lingual gyrus (medial)	18	-3	-85	-9	4.67	.000001472963	8
Right putamen (intermediate, anterior)		26	-1	7	4.66	.000001543808	8
Left insula / claustrum		-33	1	0	4.35	.000006830721	8
Right postcentral gyrus: SI (arm/hand)	3	35	-26	52	4.23	.000011552721	7
Right putamen (ventral, anterior)		26	5	-4	3.87	.000053320862	8
Right ventral insula/claustrum, near apex		24	8	-9	3.85	.000060184684	8
Left posterior cingulate sulcus,	24	-1	-6	47	3.82	.000066145811	8
Left putamen (dorsal, anterior)		-19	5	11	3.74	.000092205293	8
Right precentral gyrus: MI (arm/hand)	4/6	30	-15	52	3.69	.000112951842	8
Left inferior frontal gyrus, merging with insula	45	-37	23	4	3.65	.000129120337	8
Right anterior insula, apex		35	17	4	3.61	.000154214358	8
Right superior temporal gyrus, extending to operculum	42	57	-31	20	3.60	.000161076590	8
Right middle frontal gyrus, sulcus	9/10	39	48	20	3.58	.000169687963	8
Right inferior parietal lobe, extending to operculum	40	62	-28	27	3.54	.000202220981	8
Left putamen/claustrum (dorsal, intermediate)		-24	-6	14	3.42	.000309137278	8
Left posterior cingulate sulcus		-15	-37	52	3.42	.000310433563	6
Right intraparietal sulcus	7/40	37	-55	50	3.39	.000345010543	6
Right posterior cingulate sulcus	5	10	-33	50	3.38	.000360453036	7
Left intraparietal sulcus	7	-37	-46	52	3.37	.000371838163	6
Right intraparietal sulcus	7	26	-46	52	3.36	.000389448891	6
Right cerebellum, hemisphere, lobule VI¶		28	-51	-20	3.31	.000465254358	7
Right superior temporal gyrus		51	-46	18	3.30	.000476808375	8
Left thalamus (medial-dorsal nucleus)		-3	-15	9	3.06	.001094138599	8

* Coordinates and structures according to Talairach and Tournoux (1988) and Damasio (1995). BA: Brodman area; ECR: eyes closed rest; N: number of subjects contributing to an activation peak.
¶ Structures according to Schmahmann, Doyon, Toga, Petrides and Evans (2000).
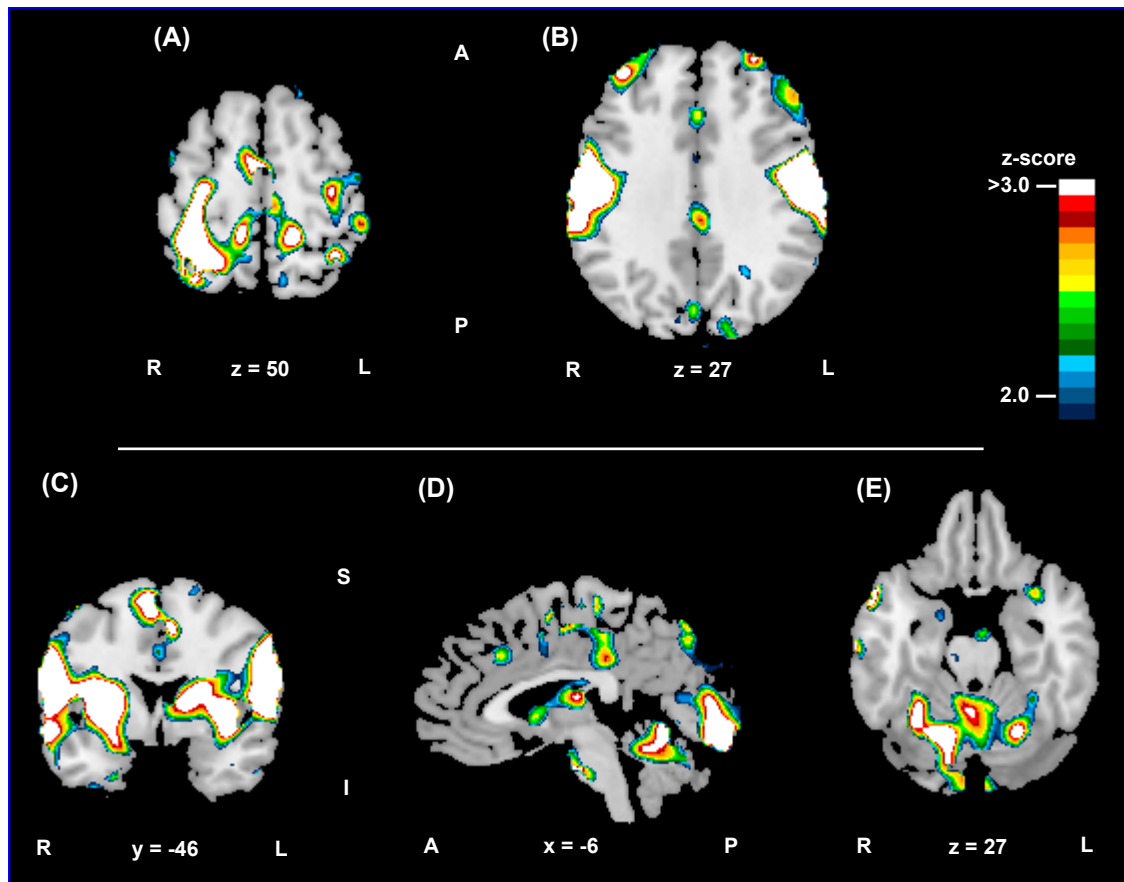
Figure 23. Increased rCBF during intubation with gastric distension

(A) left (medial) cingulate gyrus, right pre-/postcentral gyrus (central sulcus), right/left intraparietal sulcus, right/left ascending branch of cingulate sulcus; (B) right inferior parietal lobe, right superior temporal gyrus, left postcentral gyrus (central sulcus); C) right postcentral gyrus, right/left putamen, claustrum, and insula; (D) left thalamus, left cerebellum, left lingual gyrus; (E) right/left cerebellum. Letters and numbers below the segments indicate their orientations and stereotactic coordinates (Talairach & Tournoux, 1988). A: anterior; I: inferior; L: left; P: posterior; R: right; S: superior; x: sagittal plane; y: coronal (verticofrontal) plane; z: transverse (horizontal) plane.

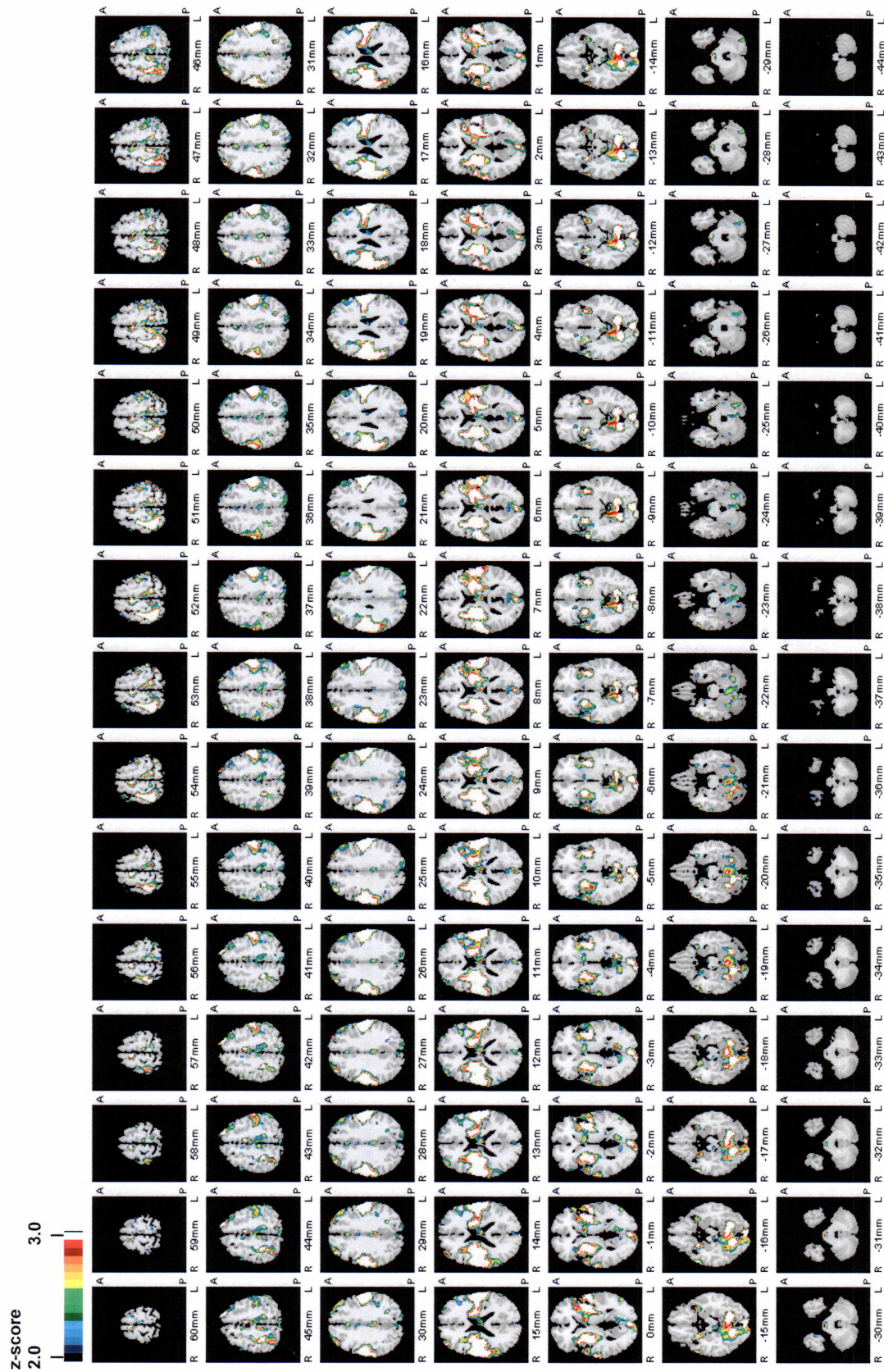**Figure 24.** Increased rCBF during intubation with gastric distension across the brain (N = 8)

Transverse (horizontal) display of the brain. Letters and numbers below the slices indicate their orientations and stereotactic z-plane coordinate (Talairach & Tournoux, 1988). Colored areas are coded to represent the magnitude of change in rCBF as z-sores (2.0 ≤ z ≤ 3.0). A: anterior; L: left; N: sample size; P: posterior; R: right.

7.4   Experiment VI: Visual Analog Scales (VAS)

A summary of the Visual Analog Scale ratings for hunger, sleepiness, nausea, gastric discomfort, and tension is presented in Table 21 and Table 22. Outliers and extreme values for VAS subscales were identified using stem-and-leaf (not displayed) and boxplot displays. For a complete presentation, Figure 25 displays boxplots of VAS subscales including the feeling of fullness (target sensation). Exploratory investigation of the feeling of hunger revealed three outliers (p02822 and p05761 during inflation 2 and p03306 during inflation 3) and two extreme values (p02822 and p05761 during inflation 3). Exploratory investigation of the feeling of tension revealed four outliers (p02895, p03306, and p05647 during inflation 1, p03306 during deflation 3). No other outliers were found.

Wilcoxon signed-rank tests illustrated that inflation caused an immediate significant decrease in the feeling of hunger along with an immediate significant increase in the feelings of fullness (target sensation) and sleepiness for all inflation-deflation cycles. Gastric discomfort increased significantly during inflation 2 and inflation 3.   Nausea increased significantly during inflation 1 only and tension increased significantly during inflation 2 only. For results of Wilcoxon signed-rank tests for all VAS subscales see Table 24 through Table 28. (For a complete overview of all VAS data, Table 23 duplicates data for fullness, which were presented in Table 6 [see chapter 7.1])

Table 21. Descriptive statistics for VAS scores (inflation)

VAS	Statistic	Inflation 1	Inflation 2	Inflation 3
Hunger	N	18	17	14
	Median	0.8	0.2	0.1
	Minimum	0.0	0.0	0.0
	Maximum	8.4	6.7	7.2
Sleepiness	N	18	17	14
	Median	4.7	4.2	5.3
	Minimum	0.1	0.0	0.0
	Maximum	8.8	8.8	8.4
Nausea	N	18	17	14
	Median	4.6	5.8	6.7
	Minimum	0.0	0.0	0.0
	Maximum	9.2	10.8	11.0
Gastric Discomfort	N	18	17	14
	Median	6.1	7.8	8.0
	Minimum	0.0	0.0	0.0
	Maximum	10.8	10.8	11.0
Tension	N	18	17	14
	Median	5.7	5.9	5.5
	Minimum	0.0	1.8	0.0
	Maximum	10.8	11.0	10.7

Table 22. Descriptive statistics for VAS scores (deflation)

VAS	Statistic	Deflation 1	Deflation 2	Deflation 3
Hunger	N	18	18	15
	Median	6.4	7.4	6.5
	Minimum	0.0	2.7	1.6
	Maximum	10.6	11.0	10.6
Sleepiness	N	18	18	15
	Median	2.7	2.1	2.1
	Minimum	0.0	0.0	0.0
	Maximum	8.4	7.0	5.8
Nausea	N	18	18	15
	Median	1.5	3.5	3.0
	Minimum	0.0	0.0	0.0
	Maximum	8.0	11.2	9.0
Gastric Discomfort	N	18	18	15
	Median	3.9	3.2	2.8
	Minimum	0.0	0.0	0.0
	Maximum	7.9	8.7	7.8
Tension	N	18	18	15
	Median	5.9	5.9	4.4
	Minimum	1.7	0.1	1.1
	Maximum	11.0	9.1	10.4
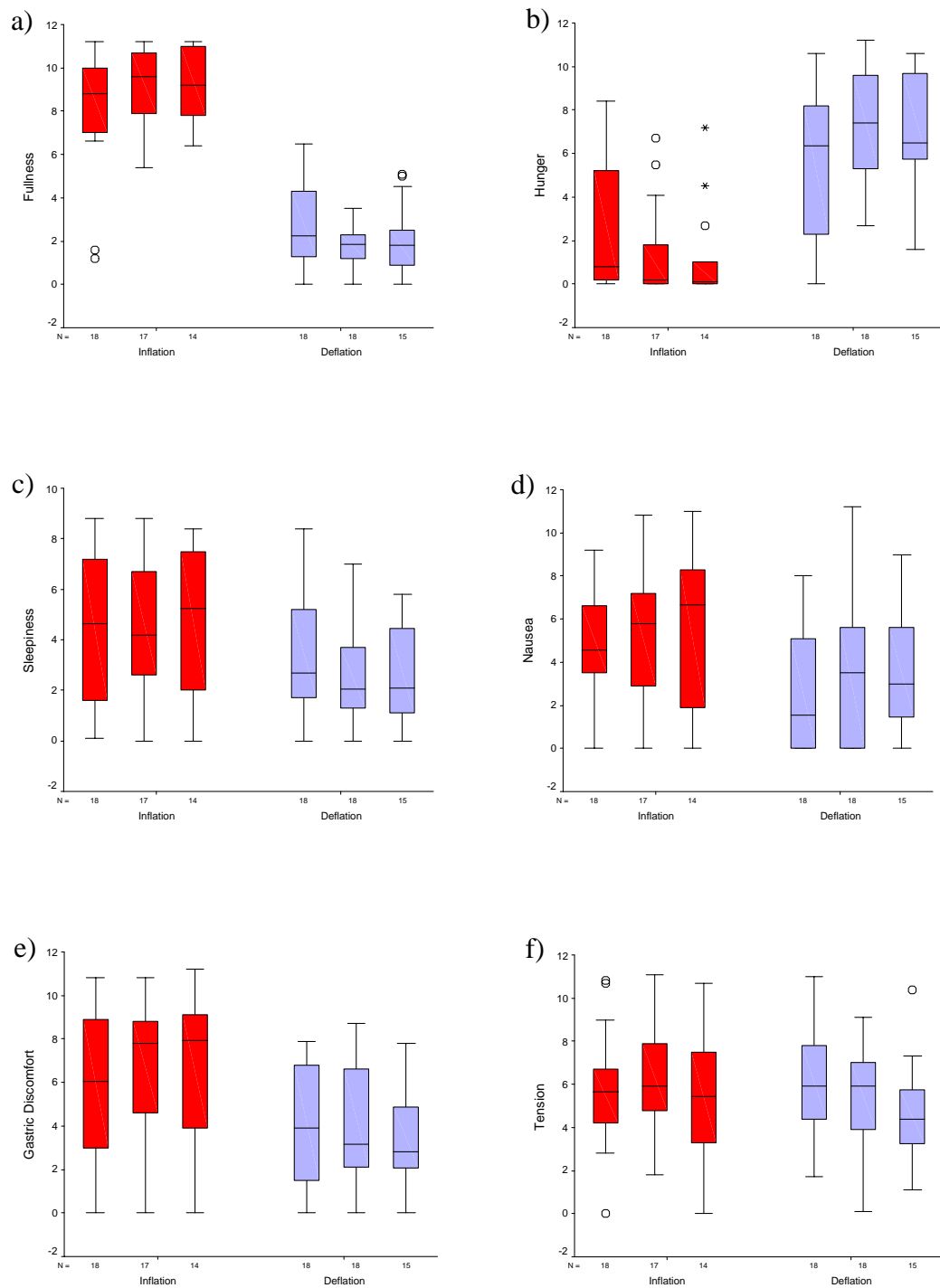
Figure 25. Boxplot: VAS scores

Ratings for three inflations and three deflations of a) fullness, b) hunger, c) sleepiness, d) nausea, e) gastric discomfort, and f) tension for three cycles of inflation and deflation presented in boxplots. * extreme values, more than 3 hspreads above the upper hinge; O outlier, value between 1.5 and 3 hspreads above the upper hinge or between 1.5 and 3 hspreads below the lower hinge; N: sample size; VAS: visual analog scale.

Table 23. Wilcoxon signed-rank test: VAS Fullness

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-3.516	.0004**
Inflation 2 vs. Deflation 2	17	-3.622	.0003**
Inflation 3 vs. Deflation 3	14	-3.061	.0022**

N: sample size; VAS: Visual Analog Scale; **significant at α = .01 (Bonferroni-corrected for three comparisons to α = .003).

Table 24. Wilcoxon signed-rank test: VAS Hunger

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-3.527	.0004**
Inflation 2 vs. Deflation 2	17	-3.409	.0007**
Inflation 3 vs. Deflation 3	14	-2.934	.0033*

N: sample size; VAS: Visual Analog Scale; *significant at α = .05 (Bonferroni-corrected for three comparisons to α = .016); **significant at α = .01 (Bonferroni-corrected to α = .003).

Table 25. Wilcoxon signed-rank test: VAS Sleepiness

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-1.546	.122
Inflation 2 vs. Deflation 2	17	-2.586	.010*
Inflation 3 vs. Deflation 3	14	-2.412	.016*

N: sample size; VAS: Visual Analog Scale; *significant at α = .05 (Bonferroni-corrected for three comparisons to α = .016).

Table 26. Wilcoxon signed-rank test: VAS Nausea

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-2.939	.003**
Inflation 2 vs. Deflation 2	17	-1.474	.140
Inflation 3 vs. Deflation 3	14	-2.180	.029

N: sample size; VAS: Visual Analog Scale; no significant results at α = .05 (Bonferroni-corrected for three comparisons to α = .016); **significant at α = .01 (Bonferroni-corrected to α = .003).

Table 27. Wilcoxon signed-rank test: VAS Gastric discomfort

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-2.320	.020
Inflation 2 vs. Deflation 2	17	-2.508	.012*
Inflation 3 vs. Deflation 3	14	-3.061	.002*

N: sample size; VAS: Visual Analog Scale; *significant at α = .05 (Bonferroni-corrected for three comparisons to α = .016); no significant results at α = .01 (Bonferroni-corrected to α = .003).

Table 28. Wilcoxon signed-rank test: VAS Tension

	N	Wilcoxon's Z	p ≤
Inflation 1 vs. Deflation 1	18	-0.625	.532
Inflation 2 vs. Deflation 2	17	-2.431	.015*
Inflation 3 vs. Deflation 3	14	-1.855	.064

N: sample size; VAS: Visual Analog Scale; *significant at α = .05 (Bonferroni-corrected for three comparisons to α = .016).

8   Discussion

8.1   Target Sensation: Fullness

The present study used proximal gastric distension with a water-filled balloon to activate vagal afferent fibers and to induce the feeling of fullness (satiety) in normal subjects. All but two subjects rated their feeling of fullness significantly higher during inflation than deflation. The two outliers were detected during inflation 1 only where two subjects gave a lower rating of fullness during inflation than during deflation. This direction was in contrast to the ratings of all other subjects and inflation-deflation cycles. For the following inflation-deflation cycles, fullness scores for the two outlier-subjects were significantly higher during inflation than during deflation and were within the range of values of other subjects. We attributed the low scores of fullness during inflation 1 for these two subjects to the novelty of the stimulus and excluded those outliers as invalid values.

Subjects felt full after each inflation. The level of fullness was equal for all three inflations. Despite int*er*individual variance in the perceived level of fullness, int*ra*individual variance was marginal. In fact, during each inflation subjects gave a similar rating for the feeling of fullness on the visual analog scales. The similar fullness ratings indicate that we were able to induce the same feeling of fullness with each of the three consecutive inflations. Subjects rated fullness in the upper third of the VAS rating scale. Values clustered around 8 to 9 cm on the VAS ranging from 0 to 11 cm. Fullness ratings after the consumption of a yoghurt meal were found in the upper fourth of the rating scale in healthy subjects (Kissileff, et al., 1996). In the present study, non-nutrient gastric balloon distension induced a similar level of fullness as the ingestion of a meal. Thus, the study reached its goal of mimicking a meal was reached.

8.2   Physiological Measures and PANAS

Gastric Volume and Pressure. We reached our study goal of mimicking a meal simply with mechanical gastric balloon distension. Observed volumes causing fullness and associated pressures at these volumes resembled the values of those reported in normal subjects after a meal (Geliebter & Hashim, 2001; Jones, Hoffman, Shah, Patel, & Ebert, 2003). We also found gastric relaxation after stomach distension as others have found after a meal (Desai, et al., 1991; Takahashi & Owyang, 1997; Zerbib, Bruley, Scarpignato, Leray, D'Amato, Roze, et al., 1998). A previous study revealed similar physiologic stomach relaxation for post-balloon and post-prandial measurements (Vu, Straathof,

v. d. Schaar, Arndt, Ringers, Lamers, et al., 1999). For each subject, gastric distension volumes and pressures remained consistent for up to three subsequent inflations, showing no trend or shift of threshold to the distending stimulus. No gastric habituation occurred over this time interval. Interindividual differences in maximal tolerated volumes demonstrate the variable subjective thresholds of fullness. Geliebter and Hashim (2001) also described the variability of maximal tolerated volumes and reported mean interindividual differences of this measure as high as 475 ml among normal eating subjects. Such interindividual variability of tolerated volumes in combination with the intraindividual stability of tolerated volumes mirrors the findings of the target sensation of fullness. Taken together, the VAS ratings of fullness and the maximally tolerated volumes indicate that non-nutrient gastric distension imitated the ingestion of a meal (Geliebter & Hashim, 2001; M. P. Jones, et al., 2003; Kissileff, et al., 1996).

Electrocardiogram (ECG). All subjects demonstrated consistent and normal ECG patterns and heart rate throughout the study. Heart rate did not change from deflation to inflation, indicating no effect of inflation on heart rate. Anatomical studies have shown that stomach distension affected excitatory and inhibitory circuits that are involved in the control of cardiovascular regulation (Sabbatini, et al., 2004). Additionally, a case study found that extreme stomach inflation caused bradycardia (Ruttmann & Mandelstam, 1994) with harmful effects on the subject. Therefore, we monitored cardiac function as a preventive measure and for safety purposes. However, we did not find an indication in our study for such an effect. Heart rate did neither significantly change from deflation to inflation nor during the course of the study. This finding converges with results from Rossi, Andriesse, Oey, Wieneke, Roelofs and Akkermans (1998) who measured cardiovascular function in response to stomach distension in healthy humans. They inflated a gastric balloon with air and obtained various measures of cardiovascular function including heart rate expressed as beats per minute. Whereas other measures such as blood pressure did vary with gastric distension, heart rate changed only with extreme distension pressures and in unpredictable patterns (Rossi, et al., (1998).

PANAS. Baseline ratings for the Positive Affect and Negative Affect Schedule (PANAS) did not differ from the normative sample. Subjects experienced a similar mood during the previous month when compared to the normative data set. Post-study scores describing the current mood state also corresponded to the normative sample. Thus, we observed mood ratings within a normal range. Caution is necessary when interpreting these results. The unequal sample sizes along with unequal variances present limitations. The normative data

samples are considerably larger. Additionally, the variances of the study sample and the normative sample differ. The variance of Positive Affect (PA) *right now* is larger for the smaller sample size, which indicates that the real probability is greater than α (Kohr & Games, 1974; Ramsey, 1980). The variances for Negative Affect (NA) *right now* and *past month* and the variances for PA *past month* are larger for the larger normative sample, which indicates that the real probability is smaller than α (Kohr & Games, 1974; Ramsey, 1980). The latter case of larger variances for the larger sample sizes in our data requires caution since actual α-values could be smaller then the critical α for NA and PA *past month* and NA *right now* indicating a significant difference between the study and the normative samples.

8.3   *Experiment I and II: Exploratory Analysis*

Gastric distension altered regional cerebral blood flow (rCBF) in many areas of the central nervous system. To increase power, results from experiment I were expanded by additional data collection and analysis for an extended exploratory analysis. Therefore, we decline to interpret results of data set 1 alone. Extended exploratory analysis with 18 subjects (the subtraction of measure of brian activation during inflation minus measures of brain activation during deflation) revealed that rCBF increased in more than 20 different areas when inflated and deflated scans were compared. Increases in rCBF in the targeted visceral projection areas occurred in the dorsal brainstem, the bilateral insular region, the right subgenual anterior cingulate cortex, and in the left inferior frontal gyrus. These visceral projection areas found in animal research are addressed in light of Craig's concept of interoception (Craig, 2002) followed by the discussion of additional areas of increased rCBF during inflation (activation in fronto-parietal regions, somatosensory cortex and temporal poles).

<u>Brain Stem</u>
The activation evident in the brainstem during inflation most likely corresponds to the parabrachial nucleus (PBN). The PBN receives projections from the nucleus of the solitary tract (Herbert & Saper, 1990), which is the primary site of subdiaphragmatic vagal afferent termination (Appleyard, Bailey, Doyle, Jin, Smart, Low, et al., 2005; Norgren & Smith, 1988). The vagus nerve as the main contributor to the parasympathetic system consists mostly of unmyelinated, small diameter fibers (Agostoni, et al., 1957; Paton, et al., 2000; see chapter 5.1). These small-diameter fibers register the physiological condition of all tissues of the body (Craig, 2002). In turn, the PBN projects to many brainstem and

forebrain regions, including the ventromedial hypothalamus, amygdaloid nuclei, and thalamic areas (Jhamandas, Petrov, Harris, Vu, & Krukoff, 1996; Jia, Rao, & Shi, 1994; Krout & Loewy, 2000; Saleh & Cechetto, 1995; Takaki, Nagai, Takaki, Yanaihara, & Nakagawa, 1990). It is also reciprocally connected with the motor cortex at the level of the larynx (Simonyan & Jürgens, 2003; Simonyan & Jürgens, 2005). These interconnections provide the neural substrates for the transmission of proprioceptive and tactile information from the larynx and oral cavity (Simonyan & Jürgens, 2005) and could serve the purpose of swallowing food (Hartnick, Rudolph, Willging, & Holland, 2001). Functionally, the PBN is a well-recognized relay and integration site for peripheral vagal afferent information of food intake (Bray, 2000; Ritter, 1994; Scharrer, 1999). Activation of this brainstem area likely represents the neural processing of the visceral sensation resulting from balloon inflation.

Insula / Opperculum

We observed activation in the insular cortex during gastric balloon inflation. The insular cortex represents a visceral projection field for a variety of autonomic and visceral functions (Flynn, et al., 1999; Saper, 1982). It constitutes the base of the Sylvian fissure and lies buried under the frontal, parietal and temporal lobes (Türe, et al., 1999). The portions of the overlapping lobes define the frontal, parietal or temporal operculum, respectively. We found two activation peaks in the left insula, bilateral activation in the insula/parietal operculum and one activation peak in the left claustrum.

Peaks in the bilateral insula/parietal operculum lie dorsally and extend caudally, probably across the periinsular sulcus into the parietal operculum. They also extend posteriorly. The dorsal insula/operculum reflects general visceral modalities and responds to visceral stimuli such as gastric distension (Cechetto, 1987) and thus constitutes a sensory projection field for visceral afferent fibers. According to Craig (2002), information about interoceptive stimuli find their initial cortical representation in this dorsal insular area. At this point, no cognitive appraisal has occurred.

Visceral stimulation in humans previously activated the dorsal insular cortex in a study conducted by Aziz and coworkers (1997). They distended the distal esophagus in three conditions: baseline, definite sensation, and pain. Dorsal insular activation occurred for *definite sensation* and increased during *pain*. Other groups showed in humans that the rCBF response in the dorsal insula was dependent on stimulus intensity across a variety of interoceptive stimuli (Chen, et al., 2002; Hsieh, et al., 1994; King, et al., 1999). Our

findings parallel these observations as prevalent in the contrast inflation minus deflation. Bilateral dorsal insular activation in the present study indicates the objective representation of the change in the bodily state, here induced by visceral stimulation.

We also found activation in the left insula. These activation areas lie ventrally and extend rostrally from the intermediate insula to the anterior insular pole. This ventral anterior portion of the insula was previously found to reflect taste and smell processing (Cechetto, 1987), and also responded especially to internally generated emotional states and potentially distressing stimuli (Lane, Reiman, Ahern, Schwartz, & Davidson, 1997a; Reiman, et al., 1997). The present finding of ventral-anterior insular activation affirms these previous results. Visceral sensation represents an internal stimulus, which, according to Craig (2002), finds a first and second re-representation in the ventral anterior insula. Craig (2002) describes the first re-representation to occur bilaterally and the second re-representation to occur in the right anterior insula only. We found left anterior insular and bilateral posterior insular activation. Right anterior insular activation did not reach significance.

In the present experiment, ventral anterior insular activation occurs only in the left hemisphere. We inspected the images visually for right ventral anterior insular activation, yet could not verify such increased rCBF in the subtraction analysis inflation minus deflation, as it would be predicted by Craig (2002). Left and bilateral anterior insular activation has been reported in the literature when thermal (interoceptive) stimuli were applied (Bornhövd, et al., 2002; Casey, et al., 1996; Davis, et al., 1998). Davis and coworkers (1998) found bilateral anterior insular activation to thermal stimulation of the right hand. Casey's group (1996) found left anterior insular activation when subjects' left hands experienced heat applied with a thermode or cold through cold-water emersion. Bornhövd and his colleagues (2002) compared warm and painfully hot stimulation of the left hand and found bilateral ventral insular activation associated with pain intensity. The critical voice could argue that ventral anterior insular activation is a response to pain and therefore represents a structure that is part of the pain processing network. Craig (2002) however argues that painful stimuli find a representation both in the somatosensory and the interoceptive system as painful stimuli are only evaluated as painful based on the current environment the body experiences (see chapter 2).

Other interoceptive stimuli such as cardiovascular changes and physical exercise activated the left and bilateral anterior insular cortex (Critchley, et al., 2003; Williamson, McColl,

Mathews, Ginsburg, & Mitchell, 1999). Critchley and coworkers (2003) found that activation in this area correlated with heart rate variability. Williamson et al. (1999) correlated insular activation with measures of cardiovascular function (blood pressure and heart rate) and found peaks in the bilateral insular cortex to be positively correlated with both measures although the correlation with the right insular activation was much stronger (Williamson, et al., 1999). Ratings of perceived effort, however, were positively correlated with the right anterior insula only. The bilateral insula has also been suggested to be involved in the addiction of cigarette smoking (Naqvi, Rudrauf, Damasio, & Bechara, 2007). Although the left insula showed a similar effect, the authors conclude that the effects in the right insula were somewhat larger than in the left insula. Subjects with insular damage quit smoking immediately (within one day) after the lesion, did not start smoking once quit, did not feel any urges to smoke again and rated the difficulty of quitting marginal. This fininding is supported by imaging studies which showed that exposure to drug-associated cues activated the ACC, orbitofrontal cortex and the insula (Brody, Madelkern, London, Childress, Lee, Bota, et al., 2002; Bonson, Grant, Contoreggi, Links, Metcalfe, Weyl, et al., 2002; Olbrich, Balerius, Paris, Hagenbuch, Ebert, & Juengling, 2006). The bilateral anterior insula has also been reported to respond to internally generated emotional states and potentially distressing stimuli (Lane, et al., 1997a; Reiman, et al., 1997). Increased ratings of gastric discomfort reflect the distress experienced by subjects in the present study of non-painful proximal gastric distension. Such distress could result from the evaluation of the visceral sensation as a potential danger to the body.

Taken together, insular activation in the present study could indicate a neural correlate of the process of evaluation of the interoceptive stimulus. Imaging the distinct stages of re-representation of an interoceptive stimulus and its evaluative appraisal is currently unfeasible given the temporal resolution of contemporary neuroimaging techniques. Also, the current study did not assess different levels of stimulus intensity with step-wise balloon inflation. Such separate intensity levels would have allowed for correlation analysis of stimulus intensity with rCBF in specific brain regions.

Considering the current knowledge of the functional anatomy of visceral processing, activation of the dorsal posterior and ventral anterior insular regions in the present study could reflect two different features of stimulus processing: first, the detection of gastric distension as the objective stimulus and second, the subjective evaluation of this sensation. Although we cannot distinguish between a first and second re-representation in the ventral anterior insular cortex, this area is a likely candidate for evaluative processing of

interoceptive stimuli. While the dorsal posterior insular cortex probably partakes in objective stimulus sensation, the anterior insula seems to evaluate the stimulus depending on the environment and could alert the organism to potential internal threats. Such alert signals support the organism to maintain homeostasis and permit survival.

Claustrum

We found increased rCBF in the dorsal claustrum during gastric distension. The claustrum is a subcortical band of gray matter extending in a dorsal-ventral manner located in the basolateral telencephalon bordering the insula medially and the putamen laterally (Edelstein & Denaro, 2004; Kowianski, Dziewiatkowski, Kowianska, & Morys, 1999). The claustrum is separated from the insula by the extreme capsule and from the putamen by the external capsule (Edelstein & Denaro, 2004). Because insula and claustrum have similar reciprocal connections with the motor cortex, Simonyan and Jürgens (2005) suggest that the clausturm should rather be considered a derivative of the insula than of the striatum. The claustrum participates in auditory (Beneyto & Prieto, 2001) and visual (Baizer, 2001) stimulation and lesion studies suggest its involvement in Alzheimer's (Morys, Bobinski, Wegiel, Wisniewski, & Narkiewicz, 1996) and Parkinson's (Sener, 1998) disease. The claustrum, which activates during stomach distension, reciprocally connects to all regions of the cortex except the posterior temporal cortex (Guldin, Markowitsch, Lampe, & Irle, 1986). It probably transfers information among various cortical regions (Kowianski, et al., 1999). Reciprocal connections of the claustrum and PBN have been found with the motor cortex at the level of the larynx (Simonyan & Jürgens, 2003; Simonyan & Jürgens, 2005). Via this interconnection, it is likely that information about tactile and proprioceptive stimulation of the larynx and the oral cavity is exchanged with the motor cortex (Simonyan & Jürgens, 2005). Claustral activation in combination with increased rCBF in the primary motor cortex in the present study (see below) could indicate communication of these two areas. Intubation of subjects in our study provided the tactile and proprioceptive stimulation of the larynx. This activation could be communicated to the primary motorcortex to initiate a motor response, e.g. swollowing.

Anterior Cingulate Cortex

The activation peak in the right anterior cingulate cortex (ACC) during inflation lies subgenually. This region represents a key area involved in central autonomic and visceral processing (Elliott, Frith, & Dolan, 1997; Vogt, et al., 1992) and connects to limbic regions (Vogt, Pandya, & Rosene, 1987). The ACC is part of the medial, visceral, motor network

within the orbitofrontal cortex (Öngür & Price, 2000; Price, 1999). Via its connections to the hypothalamus and brainstem, it provides frontal cortical influence over autonomic functions. The subgenual ACC projects through a ventral pathway to the thalamus, hypothalamus, the amygdala, the bed nucleus of the stria terminalis and also sends descending projections to autonomic cell groups in the brainstem such as the periaqueductal gray (PAG) and the PBN (Freedman, Insel, & Smith, 2000). There is discordance regarding the existence of projections to the NTS, dorsal motor nucleus of the vagus, and the spinal cord (Freedman, et al., 2000). The widespread projections to central autonomic nuclei may participate in the generation of visceral responses to emotional stimuli, such as stress (Vogt & Pandya, 1987). Critchley, Rotshtein, Nagai, O'Doherty, Mathias and Dolan, (2005) investigated cardiovascular function and brain activation to pictures showing faces expressing different emotions. ACC activation correlated positively with heart rate depending on the emotional stimulus material. Moreover, the authors found that heart rate predicted the type of emotion in the visual stimuli. Sadness and anger elicited a greater increase of heart rate than disgust or happiness. Heart rate did not respond differentially to the valence of the emotion. Happiness and disgust, for example, showed a similar increase in heart rate. Heart rate to misinterpreted stimuli (e.g., anger interpreted as disgust) resembled the pattern of the misinterpreted emotion (disgust). Thus, visceral function reflected how the subject evaluated and interpreted an emotion, not what was actually shown in the pictures. Activation in several brain regions besides the ACC correlated with increases in heart rate: the insular cortex, the amygdala, and brainstem nuclei. These results are in agreement with the complex connection pattern of the ACC with autonomic structures (Vogt & Pandya, 1987).

Some projections from the subgenual ACC terminate in the PAG. There, they predominantly end in the dorsolateral columns (Price, 1999). The PAG in the brainstem coordinates visceral and behavioral responses especially to inescapable stress or threatening stimuli (An, Bandler, Öngür, & Price, 1998). The ACC also processes sadness in healthy subjects (Liotti, Mayberg, Brannan, McGinnis, Jerabeck, & Fox, 2000; Mayberg, Liotti, Brannan, McGinnis, Mahurin, Jerabek, et al., 1999), and contributes to the activation pattern observed in pathologic depression (Drevets, Öngür, & Price, 1998; Mayberg, et al., 1999). Activation in the ACC was also found when drug users were confronted with drug related cues or memories (Olbrich, et al., 2006; Brody, et al., 2002) and a significant deactivation was detected when drug users had to perform a cognitive GO – NOGO task (Kaufman, Ross, Stein, & Garavan, 2003). Here, we interpret subgenual ACC activation as visceral processing with a negative affective component. This area is a likely candidate for

associating affect with behavior, e.g. eating. Non-painful yet definite stomach distension, as induced in this study and as seen in overeating episodes, induced gastrointestinal discomfort, which is here accompanied by ACC activation. The ACC seems to process the feeling of discomfort.

Orbitofrontal Cortex

The subgenual ACC is also interconnected with various medial and lateral areas in the orbitofrontal cortex (Vogt & Pandya, 1987). Lateral inferior frontal and orbitofrontal areas, which in the present study also showed increases in rCBF during inflation, are part of a visceral network including brainstem nuclei, the insula and the ACC. Besides its contribution to visceral function, the ventrolateral portion of the prefrontal cortex (VLPFC) has been involved in a variety of tasks, for example, vocalization (Romanski, Averbeck, & Diltz, 2005), numerous cognitive tasks (Bunge, Burrows, & Wagner, 2004; Menon, Mackenzie, Rivera, & Reiss, 2002) as well as tasks involving food (Thorpe, Rolls, & Maddison, 1983). Thorpe and coworkers (1983) for example, recorded single cell activity from the VLPFC. These neurons indicated selective responses to specific foods and to visual and gustatory stimuli (Thorpe, et al., 1983). These findings together underline the multi-modal properties of the VLPFC. Tasks utilizing emotional stimuli have repeatedly activated the VLPFC in humans (Habel, Klein, Kellermann, Shah, & Schneider, 2005; Levesque, Eugene, Joanette, Paquette, Mensour, Beaudoin, et al., 2003; Royet, Zald, Versace, Costes, Lavenne, Koenig, et al., 2000) and was involved in fear processing in the rat (Morgan & LeDoux, 1999). Different types of social exclusion actually activated the VLPFC differentially (Eisenberger, et al., 2003). Activation in this area correlated negatively with perceived distress during exclusion and negatively with activation in the ACC. Eisenberger, et al. (2003) suggested that VLPFC mediated the perceived distress by suspending ACC activity. According to Craig (2002), the orbitofrontal cortex decodes the representation of interoceptive stimuli, thus adding humans' conscious interpretation to the interoceptive experience. Conscious interpretation includes a subjective evaluation of the stimuli, including their affective features. Affective components related to different objects, for example, have been reported to be associated with VLPFC activation in single cell recordings in the rhesus monkey (Thorpe, et al., 1983). Two tubes dispensing either fruit juice (rewarding) or hypertonic saline (aversive) were coupled with specific objects. Thus, monkeys learned an association of a specific object with a particular consequence, rewarding or aversive. Reversing the learned associations between the objects and their consequences revealed that cells responded independently of the objects yet their response was dependent on the rewarding aspects of the consequences (Thorpe, et al., 1983). Cells

that initially responded to the association "object A – fruit juice" now responded to "object B – fruit juice" after reversal of the consequences (Thorpe, et al., 1983). Thus, cell activity is dependent on the emotial association with objects, not on its physical aspects.

The VLPFC extending to its junction with the anterior insular cortex has been described as projection area for visceral stimulation (Cechetto & Saper, 1987; Cechetto, 1987) based on its input from the PBN (Saper & Loewy, 1980), a major relay nucleus for vagal afferent activation (Beckstead, et al., 1980). In early studies, selective electrical stimulation of the ventrolateral orbitofrontal cortex in cats and monkeys (macaca mulatta) has induced changes in respiration, blood pressure and gastric motility (Bailey & Sweet, 1940) which suggested orbitofrontal involvement in visceral function. This brain region also responded strongly to stimuli associated with food. Various stimulus modalities such as visual, olfactory and gustatory stimuli activate cells in the VLPFC in the monkey (Rolls & Baylis, 1994). Activation of this area to the rewarding or aversive features of liquid food indicates the affective features of food (Thorpe, et al., 1983). Children with Prader-Willi syndrome showed indreased BOLD response in the ventromedial proforontal cortex when viewing food related pictures compared with healthy controls (Miller, James, Goldstone, Couch, He, Driscoll, et al., 2007). Miller and colleagues (2007) conclude that this elevated activity could be an indicator for a higher reward value for food in Prader-Will children which could explain the occurrence of their excessive hunger. The present study revealed that gastric distension by itself activated this VLPFC region, thus adding the finding that neurons in this area also respond to a mechanical signal associated with food, namely stomach distension as a signal of satiety. The lateral frontal cortex thus seems to represent an area of convergence for many food-related stimuli.

Activation in the lateral orbitofrontal cortex has been demonstrated in craving cocain users and smokers (Bonson, et al., 2002; Brody, et al., 2002). Subjects exposed to a drug-related video while watching or handling drug related objects exhibit higher activity in the orbitofrontal cortex (BA 46 and 47) when compared to a neutral video coupled with neutral objects. The orbitofrontal cortex has been hypothesized to mediate drug craving through its many connections with brain areas known to be involved with reinforcing effects of addictive drugs (Volkow & Fowler, 2000). As seen above, it is also involved in establishing the motivational value of a stimulus based on its potential reward (see above; Thorpe, et al., 1983).

Lateral and Medial Frontal and Parietal Lobes

Non-nutritive gastric distension also activated a fronto-parietal network including structures on the lateral and on the medial surface (medial wall). Activation peaks in the medial wall, the lateral middle frontal gyrus, the precentral gyrus and the inferior parietal sulcus are probably motor-related activation peaks. Fronto-parietal activation was consistently found in functional imaging studies that included movement inhibition as a common component (Ehrsson, Geyer, & Naito, 2003; Kuhtz-Buschbeck, Mahnkopf, Holzknecht, Siebner, Ulmer, & Jansen, 2003; Solodkin, Hlustik, Chen, & Small, 2004). Major and minor interconnections between the frontal and parietal cortex build a network of fronto-parietal circuits (Rizzolatti, Luppino, & Matelli, 1998) which are thought to work in parallel to translate different features of sensory information into motor commands (Geyer, Matelli, Luppino, & Zilles, 2000). The circuits can be divided into lateral fronto-parietal circuits and into medial fronto-parietal circuits. The lateral circuits involve Brodman area (BA) 6 on the superior and inferior lateral surface of the frontal lobe. The medial circuits involve BA 6 on the medial surface of the superior frontal gyrus. Medial connections of area 6 extend to the prefrontal cortex as well as to the parietal lobe. Each fronto-parietal circuit is suggested to serve a distinct functional purpose. Circuits involving the lateral frontal areas of BA 6 are thought to be active during the analysis of proprioceptive information, the encoding of immediate personal environment including all objects and the direction of action toward them (Kakei, Hoffman, & Strick, 2001). The lateral circuits are further divided into ventrolateral and a dorsolateral circuits. The ventrolateral circuits are thought to transform intrinsic properties of an object into appropriate hand movements and to observe an action. The dorsolateral circuits are thought to control eye movements, to plan and control movements of the limbs on the basis of somatosensory information, and to conditionally select movement selection dorsolateral circuits (Rizzolatti, et al., 1998).

Circuits involving the medial areas of BA 6 possibly control motor activity in general, e.g., the posture and postural adjustments preceding voluntary movements. A cuircuit connecting the prefrontal lobe and the pre-supplementary motor area (pre-SMA) is thought to control the potential actions encoded in the lateral parieto-frontal circuits (Rizzolatti, et al., 1998). In the present study, activation on the lateral convexity occurs on the dorsal frontal cortex. This activation could reflect the planning and control of movements and their conditionality. The condition under which the movement is planned is that the subjects are in the PET scanner. Such environment requires the subjects to remain as still as possible. In this study, subjects were required not to move. The planning of movement consists of movement inhibition. Although this requirement is unchanged between

inflation and deflation, the increased discomfort during inflation could intensify the desire to move. Enhanced effort likely is necessary to inhibit movement.

Medial wall activation occurred in the left hemisphere in the pre-SMA that lies rostral to the vertical anterior commissure line (Picard & Strick, 1996; Picard & Strick, 2001). In previous studies, the pre-SMA was found to be active during higher-order movement preparation in complex tasks (Picard & Strick, 1996) and when sensory-motor associations are formed independent of task modality (Sakai, Hikosaka, Takino, Miyauchi, Nielsen, & Tamada, 2000). This region seems to process and preserve relevant sensory information and is active during the preparatory period preceding the movements when cognitive, sensory or motivational information related to motor behavior is processed on an abstract level (Picard & Strick, 1996; Picard & Strick, 2001). Such abstract processing is supported by the poor somatotopic organization of the pre-SMA (Picard & Strick, 1996) and its direct interconnections with prefrontal but not motor regions (Bates & Goldman-Rakic, 1993; Lu, Preston, & Strick, 1994; Luppino, Matelli, Camarda, & Rizzolatti, 1993). In this study, subjects were asked to lie still for the duration of the study, which lasted between one to one and one-half hours including the initial transmission scan and depending on the number of inflation-deflation cycles a subject could tolerate. Medial wall activation confirms that considerable movement inhibition was necessary to comply with the study instructions.

Significant parietal activation in the present study occurred only in the right hemisphere. Increases in rCBF in the posterior parietal lobe have been previously reported as pain-related activation (Derbyshire, Jones, Gyulai, Clark, Townsend, & Firestone, 1997; Remy, et al., 2003). Derbyshire and coworkers (1997) reported that parietal activity correlated positively with the level of pain experienced. Other researchers have interpreted parietal activation during the experience of pain with attentional networks (Peyron, et al., 1999; Pardo, Fox, & Raichle, 1991). Peyron and coworkers (1999) administered pain to the back of hands during ongoing auditory stimulation. Subjects' attention was either directed toward the auditory stimulation (distraction from pain) or toward the thermal stimulus (focus on pain). Only during selective attention toward the painful stimulus, rCBF increased significantly in the parietal cortex. The researchers explained parietal activation in this context as attentional-cognitive activity prompted by the noxious stimulus. In the past, the inferior parietal cortex has been described as part of a posterior attentional network thought to be responsible for preconscious orientation (Posner & Rothbart, 1992). Here, parietal activation could be explained with an increased demand of attention. The requirement to inhibit movement interferes with the subjects' desire to stretch and move

after a period of immobilization in the PET scanner. Such interference demands effort to remain still. In fact, other researchers have found inferior parietal lobe activation in a task with interfering features, which increased subjects' requirements for behavioral inhibition (Bunge, Ochsner, Desmond, Glover, & Gabrieli, 2001).

Overall, we interpret activation in the medial pre-SMA and SMA and activation in the lateral pre-motor and parietal areas in this study as repression of movements. Although active movement inhibition was present during both conditions, inflated and deflated, increased discomfort during inflation (see chapter 7.4) likely has amplified the necessary demands and efforts to remain still in the scanner. Subjects were asked not to move during the actual scanning period of 90 seconds as well as during the break periods. Scan comparability requires an unchanged head position for each scan. This requirement is challenging and the desire to move in the scanner after a period of movement restriction likely increased.

Primary Somatosensory Cortex

We also observed activation in the primary somatosensory cortex (SI) at the level of the hand/finger representation. Activation in SI was previously found in experiments of visceral sensation. Cell activation in SI was associated with volitional control of autonomic arousal, heart rate variability, parasympathetic and sympathetic influence on cardiac rhythm, and itch processing (Critchley, et al., 2003; Darsow, et al., 2000; Drzezga, et al., 2001) as well as with esophageal and lower gastrointestinal tract stimulation (Aziz, et al., 1997; Aziz, et al., 2000; Baciu, et al., 1999; Binkofski, et al., 1998; Hobday, et al., 2001; Kern, Jaradeh, Arndorfer, Jesmanowicz, Hyde, & Shaker, et al., 2001; Naliboff, et al., 2001). Lotze, Wietek, Birbaumer, Ehrhardt, Grodd and Enck (2001) reported SI activation during anal but not during rectal distension, arguing that the anal canal has a rich supply of nerve endings which allows for localized sensation. The rectum, on the other hand, senses mechanical distention through mechanoreceptors located deep in-between the muscle layers of the gut wall. Thus, rectal stimulation leads to diffuse perception (Lotze, et al., 2001). Activation of SI reported in this study for the inflation minus deflation subtraction cannot be explained with esophageal stimulation as it occurs throughout the study. Any stimulation occurring during both inflation and deflation should be cancelled out by subtraction. Activation in SI in this study could contribute to the additional visceral sensory processing during gastric distension. Such activation was reported in studies of itch processing and other visceral stimulation studies (Critchley, et al., 2003; Critchley, et al., 2002b; Binkofski, et al., 1998).

Activation of SI has been reported during pain processing (Bornhövd, et al., 2002; Casey, et al., 1996; Hofbauer, et al., 2001; Rainville, et al., 1997). The application of painful stimuli activated SI, and rCBF was associated with stimulus intensity rather than perceived pain intensity. Especially experiments applying pain under hypnosis revealed a relationship between the level of SI activation and the physical characteristics of the painful stimulus (Bornhövd, et al., 2002). The authors excluded tactile stimulation as the cause for SI activation; a laser heat stimulus was utilized preventing activation of the fibrotactile system. In regards to the perceived qualities of painful stimuli, opposing results have been found for SI activation (Hofbauer, et al., 2001; Rainville, et al., 1997). Hofbauer et al. (2001) found a positive correlation between SI activation and the perceived pain intensity whereas Rainville et al. (1997) found lower SI activation during suggested increased unpleasantness and stronger SI activation during suggested decreased unpleasantness of pain perception. While a number of studies have reported significant pain-related rCBF increases in SI (Becerra, Iadarola, & Borsook, 2004; Bornhövd, et al., 2002; Casey, et al., 1996; Chen, et al., 2002; Coghill, et al., 1999; Craig, et al., 1996; Hofbauer, et al., 2001; Kwan, et al., 2000; Schulz-Stubner, Krings, Meister, Rex, Thron, & Rossaint, 2004), many other studies have failed to show such activation (Brooks, et al., 2002; Davis, et al., 1998; Derbyshire, et al., 2002; Rainville, et al., 1997; Remy, et al., 2003; Tölle, et al., 1999; Wise, Williams, & Tracey, 2004). Thus, SI contribution to pain processing remains controversial. In this Study, S1 activation is unlikely to reflect pain as subjects were instructed to indicate a non-painfullevel of fullness. Yet it coud indicate the uncomfortable aspect of visceral perception.

We see here bilateral temporal pole activation. Interconnections of the temporal pole with the medial and orbital frontal cortex have been found (Kondo, Saleem, & Price, 2003). The superior temporal pole areas connect with the orbital network, which is suggested to operate as a sensory related network (An, et al., 1998). The ventral temporal pole areas, on the other hand, connect with the medial prefrontal network (Kondo, et al., 2003), which is suggested to operate as a visceral motor network (Carmichael & Price, 1995; Öngür & Price, 2000). However, in this study all temporal pole activation was located beyond brain tissue according to Talairach and Tournoux (1988) or was excluded due to a low number of subjects contributing to the activation peaks. Temporal pole activation was previously interpreted as changes in rCBF due to contraction of extracranial muscles of the jaw during anxiety (Benkelfat, Bradwejn, Meyer, Ellenbogen, Milot, Gjedde, et al., 1995; Drevets, Videen, MacLeod, Haller, & Raichle, 1992). In this study, increased muscle contraction of the jaw could reflect the greater tension subjects were experiencing during inflation.

8.4 Experiment III: Split-Half Reliability

This quasi-replication analysis computed the split-half reliability and hypothesized a significant increase in rCBF in the key regions of visceral processing revealed in experiment II. Two groups were defined (see chapter 6.8.3.3) and the increase in rCBF was quantified for each group separately for nine regions within the visceral network containing the inferior frontal gyrus, the insula, anterior cingulate cortex and the parabrachial nucleus. Only two out of nine regions showed significantly increased rCBF in group 2 and only one region in group 1. The three regions of significant rCBF increase fall into the area of the inferior frontal gyrus. This area has been shown to respond to a diversity of food-related stimuli (Rolls & Baylis, 1994) including tasks in which food served as a rewarding stimulus (Thorpe, et al., 1983). Non-significant rCBF increase in all other regions of interest are possibly due to a lack of power. This split-half reliability analysis only included nine subjects for each group. Similar results were found by another replication study which used substantially larger samples of 51 and 38 subjects and could replicate activation in only two brain areas (Zald, et al., 2002b). Thus, replicating changes in rCBF in regions of interest remains challenging.

Nonetheless, a new finding should not be accepted unless it was replicated. This statement holds true for any science. For example, physicists first replicate a new finding before they elaborate its possible impacts (Hendrick, 1990). The social and behavioral sciences, however, lack explicit replication studies (Hendrick, 1990). According to Hendrick (1990), major journals often reject articles with the argument that new components were missing. At the same time, replication research in any field provides confirmation of a finding and builds scientific knowledge (Allen & Preiss, 1993). Successful replication indicates reliability as well as internal, external and contextual validity (Allen & Preiss, 1993; Lamal, 1990) and should constitute the basis of scientific research (Allen & Preiss, 1993).

8.5 Experiment IV: Effects of Intubation with or without Inflation

8.5.1 Intubatin whithout Inflation

Intubation without inflation, represented by the subtraction deflation minus ECR, activated several brain areas in SI and MI at the level of the lips, mouth, pharynx and larynx. Increased rCBF was also observed in the inferior parietal sulcus, the striate cortex (visual field), the extrastriate cortex/cerebellum, the medial prefrontal cortex (gyrus rectus) and the posterior cingulate sulcus.

Primary Somatosensory Cortex

We found increased rCBF in the primary somatosensory cortex. The role of the primary somatosensory cortex (SI) in encoding intensity discrimination of somatic heat sensation has been confirmed by PET studies (Bornhövd, et al., 2002; Casey, et al., 1996; Hofbauer, et al., 2001; Rainville, et al., 1997). It probably plays a similar role in the processing of visceral sensation. Activation of similar regions in SI were seen bilaterally after distal esophageal stimulation (Binkofski, et al., 1998). Binkofski and coworkers (1998) administered painful and non-painful esophageal distension using a pneumatic pump. Both types of stimulation resulted in increased rCBF in SI, a finding supported by bilateral SI activation to proximal and distal non-painful esophageal stimulation (Aziz, et al., 2000). However, the researchers found differences in proximal and distal stimulation in that proximal esophageal stimulation activated SI unilaterally on the left, whereas distal esophageal stimulation activated SI bilaterally. This differential pattern could result from different sensory sensation in the two parts of the esophagus as the proximal esophagus consists of striated muscle tissue facilitating somatic sensation whereas the distal esophagus consists of smooth muscle tissue facilitating visceral sensation (Kahrilas, 1992). In our study, stimulation of the esophagus included both proximal and distal parts. Thus, a distinction between those two types of stimulation is impossible.

Primary Motor Cortex

Activation of the primary motor cortex (MI) along with primary sensory activation has been repeatedly reported during visceral stimulation (Aziz, et al., 2000; Binkofski, et al., 1998) as well as during cutaneous heat stimulation (Casey, et al., 1996). However, interpretation of MI activation in a task that required motor inhibition is challenging. Considering MI activation with rCBF increases in the inferior parietal lobule, these areas could represent parts of an attentional network (Posner & Rothbart, 1992) which activates with the requirement of motor inhibition (Ehrsson, et al., 2003; Kuhtz-Buschbeck, et al., 2003; Solodkin, et al., 2004).

Ventromedial Occipital Cortex

Activation of the ventromedial occipital cortex (primary visual and the visual association cortex, BA 17/18) was previously described during the central processing of rectal pain (Baciu, et al., 1999). In our study, all conditions required subjects to keep their eyes closed. We speculate that intubation lead to an increased bodily awareness combined with increased visualization of the subjects' situation. Activation of the visual cortex (BA 17/18/19) without actual visual stimulation was reported in visual imagination tasks

Kosslyn, Pascual-Leone, Felician, Camposano, Keenan, Thompson, et al., 1999; Kosslyn, Shin, Thompson, McNally, Rauch, Pitman, et al., 1996) and in studies comparing different resting conditions (Marx, Stephan, Nolte, Deutschländer, Seelos, Dieterich, et al., 2003; Marx, Deutschländer, Stephan, Dieterich, Wiesmann, & Brandt, 2004). Kosslyn and coworkers (1999) found that activation of BA 17 was necessary for actual mental visualization and not only its epiphenomenon. In our study, activation of BA 17/18 could result from the imagination of the subject's situation in the PET scanner. Marx and her colleagues (2003, 2004) explain increased neuronal activation in the visual and other sensory systems during ECR with increased imagination and describe the condition ECR as an interoceptive mental state. We believe that activation of the ventromedial occipital cortex indicates visual imagery. Although we know that BA 17 is necessary for visual imagery (Kosslyn, et al., 1999), the reverse of such knowledge into "activation in BA 17 indicates visual imagery" needs further elaboration.

Posterior Cingulate Sulcus

Not much is known about neuronal activation in the posterior cingulate sulcus which we found to be active in the subtraction analysis deflation minus ECR. The posterior cingulate cortex lining the posterior cingulate sulcus, though, reflected increased rCBF in a variety of tasks. A muscle stimulation task in humans (Niddam, Chen, Wu, & Hsieh, 2005) as well as an action-reward association task in rats (Tabuchi, Furusawa, Hori, Umeno, Ono, & Nishijo, 2005) detected neural activation in the posterior cingulate cortex. Common to both tasks was their interoceptive nature. Niddam et al. (2005) stimulated sensory fibers within the muscles with intramuscular electro-stimulation and Tabuchi et al. (2005) recorded neuronal activity while rats were performing a licking task to acquire distinct rewards. Both tasks required the observation of the internal state of the organism, thus interoception. While Niddam and coworkers (2005) do not further discuss neuronal activation in the posterior cingulate cortex, Tabuchi and coworkers (2005) suggest that this region observes and generates associations between actions and their specific rewarding outcome. The posterior cingulate cortex, together with the ventromedial prefrontal cortex (VMPFC), which in our study shows more activation during intubation than during the basic resting condition ECR, are part of a network that shows relatively more activation during rest than during active task performance (Raichle, et al., 2001). Raichle and coworkers (2001) suggest that this network, including the VMPFC, posterior cingulate cortex and the precuneus in the posterior dorsomedial cortex may be tonically active to constantly observe and monitor information from the interior and exterior environment. The VMPFC, for example, is involved in internal processes such as emotional and autonomic regulation

(Craig, 2002; Öngür, An, & Price, 1998; Zald, et al., 2002b). When specific goal-directed activities are introduced to the organism, attention is focused away from monitoring the internal milieu to the task performance and a relative decrease of activation in these brain regions occurs (Raichle, et al., 2001). In this study, we observed increased activation in the posterior cingulate sulcus as well as in the gyrus rectus (located in the VMPFC) after the gastric balloon was orally inserted and subjects were verbally instructed to focus on their stomach. Such activation could reflect a heightened state of internal monitoring. The two conditions compared in this analysis were almost the same as subjects were asked to rest with their eyes closed. The two conditions differed in that, during deflation, subjects were intubated and were instructed to pay attention to their stomach. The deflated condition with the unpleasant sensation of intubation along with the requirement to remain still in the PET scanner may have increased the demands of internal monitoring combined with movement inhibition. In this case, the deflated condition may have rather increased the level of interoception than shifting attention to an active task performance.

8.5.2   Intubatin with Inflation

Intubation with inflation, represented by the subtraction inflation minus ECR, activated visceral projection fields that were identified in the subtraction analysis inflation minus deflation. (For a discussion see chapter 8.3.) Activation in the parietal lobule and in primary somatosensory and primary motor regions identified in this subtraction was also identified in the subtraction analysis deflation minus and interpreted as reflecting tactile sensation of the tube in the oro-pharyngeal areas and as attentional processes involved in movement inhibition. Activation in the visual cortex, which was found when subtracting ECR from inflation as well as from deflation, is challenging to interpret. Although such activation might reflect a visual imagination of the subject's situation in the PET scanner, we cannot conclude that activation of visual fields represents visual imagery.

Posterior Cingulate Sulcus

We found activation in the posterior cingulate sulcus in the region of the posterior cingulate cortex but no increased activation in the VMPFC as during deflation minus ECR. We suggested earlier that activation in these areas reflected increased interoceptive monitoring due to intubation. Intubation with inflation did not result in increased VMPFC activation. We assume that the additional stimulus of inflation below pain threshold in combination with the verbal instruction to focus on the stomach redirected subjects' attention from the observation of the general internal environment to the inflated stomach. Subjects were no longer just resting with their eyes closed and focusing on an empty stomach as they did

during deflation. Rather, they now were directing their attention to the gastric inflation. It seems like the inflated condition was no longer a resting task and rather turned into a condition requiring active task performance. As detailed above, engagement in active task performance reduced neuronal activation in the VMPFC (Raichle, et al., 2001).

Putamen

The subtraction inflation minus ECR also activated the putamen, a nucleus in the basal ganglia. The putamen is located beneath the claustrum and is divided from the claustrum by the external capsule (Nieuwenhuys, et al., 1988). Traditionally, the basal ganglia in general and the putamen in particular have been associated with the generation and control of voluntary movement (Hoover & Strick, 1993; Nakano, Kayahara, Tsutsumi, & Ushiro, 2000). This view was supported by the reciprocal connections of the putamen with primary, supplementary and pre-motor areas via the thalamus (Hoover & Strick, 1993; Kelly & Strick, 2004; Middleton & Strick, 2002). Recently, anatomical studies revealed interconnections of the putamen with prefrontal areas (Middleton & Strick, 2002) and thus suggested its involvement in cognitive functions. Moreover, amygdaloid afferents to the putamen were found and limbic influences to motor control were proposed (Kelly & Strick, 2004). These neuroanatomical results support present and previous neuroimaging findings in humans in which the putamen was involved in the processing of emotional stimuli (Lane, et al., 1997a; Phillips, Young, Senior, Brammer, Andrew, Clader, et al., 1997; Phillips, Young, Scott, Calder, Andrew, Giampietro, et al., 1998; Sinha, Lacadie, Skudlarski, & Wexler, 2004; Surguladze, Brammer, Keedwell, Giampietro, Young, Travis, et al., 2005; Surguladze, Brammer, Young, Andrew, Travis, Williams, et al., 2003; Sambataro, Dimalta, Di Giorgio, Taurisano, Blasi, Scarabino, et al., 2006). Increased rCBF in the putamen was associated with positive as well as negative emotions (Lane, et al., 1997a; Phan, Wager, Taylor, & Liberzon, 2002; Surguladze, et al., 2005; Surguladze, et al., 2003). Emotional processing by the putamen was further supported by clinical studies in which depressed subjects and healthy controls showed reversed effects of changes in rCBF in the putamen (Surguladze, et al., 2005). Furthermore, a case study showed that lesions in the putamen prevented the experience and accurate identification of disgust (Calder, Keane, Manes, Antoun, & Young, 2000). Here, we explain activation in the putamen as associated with the unpleasant feelings and sensations related to intubation in combination with gastric distension.

Cerebellum

Bilateral cerebellar activation in the present study was identified in the subtraction inflation minus ECR. These activation peaks lie in the anterior cerebellum symmetrically in the right and left hemisphere in lobule V/VI (Schmahmann, Doyon, Toga, Petrides, & Evans, 2000). The cerebellum has been associated with the processing of pleasant and unpleasant emotional stimuli (Lane, et al., 1997a; Lane, Reiman, Bradley, Lang, Ahern, Davidson, et al., 1997b; Reiman, et al., 1997). Reiman and colleagues (1997) as well as Lane and colleagues (1997a, b) provoked emotional states in their subjects to either visual cues (film clips) or to autobiographical scripts of recent incidents. These types of presentation were described as external and internal generation of emotions respectively. Both conditions resulted in increased rCBF in the bilateral cerebellum. Cerebellar activation peaks in those studies lie more lateral and inferior to the peak locations identified in the present study. Thus, it is unlikely that cerebellar activation in this study is associated with unpleasant feelings experienced during inflation.

Cerebellar involvement was also suggested in pain perception and sensory functions (Helmchen, Mohr, Erdmann, Petersen, & Nitschke, 2003; Strigo, Duncan, Boivin, & Bushnell, 2003) and a model of cerebellar contribution to nociceptive processing was suggested (Saab & Willis, 2003). Helmchen et al. (2003) applied noxious and non-noxious stimuli to the right hand with a thermode and measured brain activation with fMRI. The contrast noxious versus non-noxious stimuli revealed cerebellar activation in lobules III - VI, VIII, IX, Crus I, and the deep cerebellar nuclei (Schmahmann, et al., 2000). Helmchen and corworkers (2003) could further show that cerebellar activation in the ipsilateral anterior hemisphere (lobule III-VI), the anterior vermis, the deep cerebellar nuclei and activation in the contralateral lobule VIII correlated with perceived pain intensity. Strigo et al. (2003) studied visceral and cutaneous pain and found bilateral cerebellar activation during esophageal stimulation. However, activation sites in lobule V and VI are only somewhat similar to our results: cerebellar activation foci in the present study lie more dorsally in the anterior cerebellum.

The cerebellum has been considered to partake in a diversity of tasks regarding movement control and movement generation such as motor imagery, oculomotor processes, and spatial event processing (Bobee, Mariette, Tremblay-Leveau, & Caston, 2000; Gonzalez, Rodriguez, Ramirez, & Sabate, 2005; Mandolesi, Leggio, Graziano, Neri, & Petrosini, 2001; Straube, Deubel, Ditterich, & Eggert, 2001) but also in speech perception (Ackermann, Graber, Hertrich, & Daum, 1997). The involvement in motor processes is

supported by clinical studies that showed impairment in non-executive motor functions after cerebellar stroke (Gonzalez, et al., 2005). Anatomical studies identified dense projections from lobules V and VI to the primary motor cortex (Kelly & Strick, 2003), suggesting cerebellar motor functions. Studies using fMRI have shown that activation in the lateral hemispheric cerebellum is associated with hand and finger movements (Grodd, Hulsmann, Lotze, Wildgruber, & Erb, 2001; Takanashi, Yanagihara, Sakoda, Tanaka, Hirabuki, et al., 2003). The cerebellar areas indicated by Grodd et al. (2001) representing finger and hand movement in lobule V and VI (Schmahmann, et al., 2000) correspond to the areas identified in the present study. Cerebellar activation at very similar locations resulted from swallowing (Hamdy, et al., 1999; Zald & Pardo, 1999) and clinical studies suggest that cerebellar lesions result in dysphagia (Bhatoe, 1997). Activation peaks in the present study lie on similar coordinates as found in hand movement and voluntary swallowing studies. Although the cerebellum may have an abundance of functions that could be interesting in light of the present study (e.g., visceral sensation, emotional processing, pain processing), the location of the peak rCBF increase in the dorsal anterior hemispheric cerebellum indicates movement-related function. Zald and Pardo (1999) described such activation pertaining to the coordination, sequencing and timing of swallowing. Since cerebellar activation in lobule V and VI was associated with different motor-related tasks such as finger movement and swallowing (Grodd, et al., 2001; Hamdy, et al., 1999; Takanashi, et al., 2003; Zald & Pardo, 1999), it might be involved in the coordination, sequencing and timing of motor behavior in general. In the present study, cerebellar activation could reflect the requirement of movement inhibition, which involves the coordination, sequencing and timing of motor behavior.

8.6   Experiment V: Resting State of the Brain

In this analysis, we explored if the stress of intubation experienced during our experiment was reflected in the baseline condition ECR, which was recorded after the study procedure of recurrent inflation and deflation and after extubation. We investigated the question if activation during preceding experimental conditions might have had a lasting effect on the resting state and might have carried over into the ongoing metabolic events at baseline ECR. We compared brain activation images obtained in our study with a pool of other ECR images from age, weight, and sex-matched subjects from our database. The gamma-z test statistic which was used to compare the two sets of images did not find any differences between ECR images obtained during this study and ECR images obtained during other studies. Thus, our data do not support such carry-over effect of brain activation from the

experimental task into the resting condition and accentuates the transient changes of neuronal activity during task performance.

8.7   *Experiment VI: Cognitive Responses to Gastric Distension*

Experiment VI observed cognitive responses to a non-nutritive gastric load utilizing a gastric balloon. It has been shown that gastric distension activated gastric vagal afferent fibers (Mathis, Moran, & Schwartz, 1998; Scratcherd & Grundy, 1982). Vagal afferent stimulation critically contributes to meal termination (Smith, 1998). Here, intragastric balloon distension provoked vagal afferent activation and caused a significant rapid, reversible, and reproducible increase in the feelings of fullness (target sensation), sleepiness, and gastric discomfort as well as a significant rapid, reversible, and reproducible decrease in the feeling of hunger.

Fullness

Increased fullness in response to non-nutritive gastric distension supports previous research that showed that a gastric load itself, not its nutrient contents, activate vagal afferent fibers singalling satiety (Mathis, et al., 1998). A gastric load stimulated vagal mechanoreceptors that relay the satiety response to central areas and finally terminated a meal. Mathis and colleagues (1998) addressed the nutrient sensitivity of gastric vagal afferent fibers and found that they re indeed insensitive to the chemical composition of the ingested food. Gastric loads containing saline, glucose, or peptone elicited the same activity level in vagal afferent fibers as shown with neurophysiological recordings. However, nutrients do have an effect on CCK release and CCK acting on mechanoreceptors does amplify the satiety response to a gastric load (Nolan, et al., 2003; Schwartz, et al., 1993). These findings suggest that the satiety response to a meal is produced by an interaction of several variables. The actual impact of each one of those variables remains to be determined.

Nausea and Gastric Discomfort

Gastric distension also caused significant changes in the feelings of nausea in the first inflation and significant changes in gastric discomfort during the second and third inflation. These findings support previous research showing increased nausea to increasing gastric inflation with an air-filled balloon (Rossi, et al., 1998; Verhagen, Rayner, Andrews, Hebbard, Doran, Samsom, et al., 1999). Together, these data converge with a report on NTS involvement in the processing of nausea via vagal pathways (Vrang, Phifer, Corkern, & Berthoud, 2003). Here, the increased feeling of nausea is attributed to vagal stimulation

which is relayed through the NTS. Data on changes in nausea and gastric discomfort will serve as comparison for future experiments with a population diagnosed with bulimia nervosa.

Hunger and Sleepiness

Particularly interesting is the finding that ratings of hunger and sleepiness changed reflexively with vagal afferent activation induced with non-nutritive, mechanical stimulation of the stomach. Previously, a change in hunger ratings was hypothesized to be dependent on the nutrients in a meal and its energy content (Kirkmeyer & Mattes, 2000). The energy content of a meal was considered to be the principal factor in reducing hunger. Kirkmeyer and Mattes (2000) found that food with higher energy content is more potent in decreasing hunger values than food with low energy content. Different types of fat, on the other hand, did not have any effect on ratings of hunger (Alfenas & Mattes, 2003). The authors recorded hunger and fullness ratings after the intake of muffins containing various levels of saturated fats. The ingestion of different fat-containing muffins resulted in similar appetitive ratings. However, the comparison of ratings collected after the intake of fat-containing vs. fat-free muffins revealed a significant difference in hunger and fullness ratings. Fat-free muffins were less satiating and less capable of reducing hunger, a result that could be caused by the lower energy content of fat-free food. A further explanation offered here is that fat-free and low-energy food tends to be less dense and possibly of lighter weight (Alfenas & Mattes, 2003). Less weight in the stomach could either activate fewer vagal afferent fibers or could activate the same amount of fibers, yet to a lesser degree. Such a reduced activation of vagal fibers could result in the smaller satiating effect, as reported, and could explain the smaller decrease in hunger ratings.

A decline in hunger has also been attributed to post-prandial humoral release. For example, CCK is released when food enters the small intestines (Liddle, et al., 1986) and acts on vagal afferent fibers (Schwartz, et al., 1991). If CCK is responsible for a reduction of hunger, then the CCK response and hunger ratings should exhibit similar patterns of change after meal ingestion. If a change in hunger could solely be explained by CCK release, then the relationship between hunger and CCK should be linear. Yet Nolan et al. (2003) collected CCK measures and recorded hunger ratings after two consecutive meals and found that the two measures did not follow similar patterns. This finding suggests that CCK release only partially explains the variance in the changes of hunger ratings. Thus, a decline in hunger can be attributed to an interaction between the distending properties of ingested food accumulating in the stomach and to post-prandial CCK release. A similar

interaction effect was also demonstrated by Smeets, Vidarsdottir, de Graaf, Stafleu, van Osch, Viergever, and colleagues (2007). They administered golucose either intravenously or orally and matched each condition with a control condition (intravenous saline or water ingestion). They measured plasma glucose levels and hypothalamic blood oxygen level-dependend response with fMRI. Whereas intravenous glucose resulted in threefold higher glucose plasma levels than ingestion of glucose, it did not show a change in hunger ratings and only a moderate decline in hypothalamic activity. Oral water ingestion, a gastric distending stimulus alone, did also not result in hypothalamic changes or decrease hunger ratings. However, oral glucose administration, on the contrary, resulted in significantly lower glucose plasma levels and in significant hypothalamic deactivation in combination with significantly lower hunger ratings.

Rolls et al. (1998) showed previously that the volume of stomach contents has a rapid and transient effect on hunger ratings. The authors found that volume overrides information about energy content when a test meal was ingested 30 minutes after different volume appetizers with the same energy. Study participants ate significantly less after a large volume appetizer. Such an adjustment in the amount eaten did not occur with a test meal that subjects ingested four or more hours after the appetizer. With time elapsing, the body analyzed the energy content of the appetizer and the early effect of volume ceased. Although these findings are flawed by the fact that food volume is not independent from its energy density, they provide a good approximation of responses to a volumetric gastric stimulus.

Because the stimulus used in our study was a non-nutritive stimulus, namely inflation of a gastric balloon, the rapid decline of hunger in our study cannot be explained by the nutrient composition of the gastric load. We activated vagal afferent fibers with gastric balloon distension. The rapid reduction of hunger in this study is better explained with coordinated cell activity along the neuraxis initiated by the distending stomach. Gastric distension activates vagal afferent fibers and increases neuronal activity in the ventromedial nucleus of the hypothalamus whereas it inhibits neuronal activity in the lateral hypothalamus. This pattern immediately reverses with deflation of a gastric balloon in cats (Anand & Pillai, 1967). Such activation pattern could explain the reflexive and rapid nature of hunger changes detected in our study. Verhagen, et al. (1999) conducted a similar study using non-nutrient gastric distension with an air-filled balloon and could not find any effects of volume on hunger ratings. However, they used air to distend the stomach which lacks the weight component of the gastric load.

We also detected rapid, reversible, and reproducible changes in sleepiness after non-nutritive gastric distension. Resting behavior has been observed in animals as part of the post-prandial behavioral sequence (Antin, et al., 1975). In humans, a change in sleepiness after a meal was typically attributed to the general circadian rhythm, to the intake of especially fatty food, and to humoral release after a meal (Wells, Read, Fried, Borovicka, & D'Amato, 1997; Wells, Read, Uvnasmoberg, & Alster, 1997; Wells, Read, Idzikowski, & Jones, 1998). However, circadian rhythm and food composition alone could not fully explain the changes in sleepiness in these studies, indicating that other factors contributed to the change in sleepiness after a meal.

Until recently, sleep research chiefly focused on central and autonomic systems regulating arousal, autonomic, limbic and circadian functions. Several regions along the neuraxis coordinate the level of arousal and sleep patterns (Chou, Bjorkum, Gaus, Lu, Scammell, Saper, et al., 2002). Studies in rats showed that lesions in the ventrolateral preoptic nucleus in the hypothalamic region caused insomnia (Lu, Greco, Shiromani, & Saper, 2000). The ventrolateral preoptic nucleus in the hypothalamic region receives considerable input from the parabrachial nucleus (Chou, et al., 2002), which is the major site of relay for vagal afferent fibers. Activation of vagal afferent fibers with gastric balloon distension was relayed through the different nuclei along the neuraxis as described above. We conclude that non-nutritive gastric balloon distension resulted in the changes of sleepiness observed in this study via these pathways.

With the discovery of orexins (Sakurai, Amemiya, Ishii, Matsuzaki, Chemelli, Tanak, et al., 1998), new connections between feeding and sleep mechanisms are surfacing. Sakurai et al. (1998) isolated two neuropeptiedes, orexin A and orexin B, by screening rat and bovine brains for possible ligands of orphan G protein coupled receptors. Both neuropeptides are derived from the same precursor (prepro-orexin) that bind and activate closely related orphan G protein-coupled receptors named OX_1R and OX_2R for orexin receptor 1 and 2. Orexin A and B are localized predominantly in the lateral and posterior hypothalamus and central administration in the rat stimulates food intake, hence their name from the Greek word *orexis*, meaning appetite (Sakurai et al., 1998; Asakawa et al., 2002). At the same time orexins were discovered, an independent research group identified two hypothalamic neuropeptides deriving from the same precursor (preprohypocretin) and sharing substantial amino acid identities with the gut hormone secretin, hence the name hypocretin (de Lecea, Kilduff, Peyron, Gao, Foye, Danielson, et al., 1998). For the remainder of this paper, these two neuropeptides will be referred to as orexins (orexin A and orexin B).

The involvement of orexins in the regulation of sleep became apparent when the effects of orexin deficiency were studied in orexin knockout mice, mice completely lacking orexin production due to the missing precursor prepro-orexin (Chemelli, Willie, Sinton, Elmquist, Scammell, Lee, et al., 1999). Orexin knockout mice behave normally during daytime when mice are usually quiet. Yet orexin knowckout mice exhibit disturbances during nighttime, when they are usually active. Chemelli and coworkers (1999) demonstrated that orexin knockout mice show clear behavioral arrest with or without consciousness during the dark phase when compared to wild-type littermates. Periods of behavioral arrest were characterized by abrupt cessation of purposeful motor activity and associated with a sudden, lasting change in posture that continued throughout the episode. The episode ended abruptly with complete resumption of purposeful motor activity. The arrest occurred in 100% of the orexin knockout mice and serum electrolyte imbalance or hypoglycemia was excluded as a cause for the behavioral arrrest. As these symptoms resemble the pathophysiology of patients suffering narcolepsy, a sleep disorder associated with excessive daytime sleepiness, involuntary daytime sleep episodes, disturbed nocturnal sleep and cataplexy (Aldrich, 1996), Chemelli et al. (1999) proposed that orexin knockout mice are a model of human narcolepsy. Indeed, a disruption in the orexin system was confirmed in human narcolepsy by Nishino, Ripley, Overeem, Nevsimalova, Lammers, Vankova, et al. (2001). They found that orexin was significantly decreased in about 85% of narcoleptic patients yet it was easily identified and abundant in healthy control subjects. Post mortem studies found that orexin mRNA and immunoreactivity was markedly reduced (Thannickal, Moore, Nienhuis, Ramanathan, Gulyani, Aldrich, et al., 2000; Peyron, Faraco, Rogers, Ripley, Overeem, Charnay, et al., 2000) confirming a deficiency in the orexin system in human narcolepsy. Orexin involvement in sleep/wakefulness in rats was further supported by the finding of positive correlations of orexin neuronal firing with wakefulness and negative correlations of orexin neuronal firing with sleep (Estabrooke, McCarthy, Ko, Chou, Chemelli, Yanagisawa, et al. 2001). Additionally, modafinil, an anti-narcoleptic drug with unidentified mechanism of action, stimulates orexin-containing neurons (Chemelli, et al., 1999).

Mieda, Williams, Richardson, Tanaka and Yanagisawa (2006) recently found another interesting connection between feeding behavior and the wake/sleep cycle in mice. They revealed a food entrainable oscillator that controls the wake/sleep cycle in the suprachiasmatic nucleus and the dorsomedial hypothalamus which seems to act independently of the main circadian light entrainable oscillator in the suprachiasmatic nucleus (sensitive to the light/dark cycle). Mice re-learned behavioral rhythms including

feeding anticipatory behavior according to feeding cycles which deviated from behavioral rhythms associated with the light/dark cycle. This re-learned behavior persisted even when no food was given at the learned and anticipated feeding time. The search for the location of this food entrainable oscillator revealed time dependent cell activity in the compact part of the dorsomedial hypothalamus with projections to the medial part of the NTS and the area postrema. We hypothesize that our study of gastric distension has probably inhibited the orexin system via NTS projections through the PBN to the medial hypothalamus.

Vagal contribution to sleepiness (measured by vigilance) was found by vagus nerve stimulation (VNS) in epileptic patients, in whom chronic VNS impacted vigilance during daily performance (Galli, Bonanni, Pizzanelli, Maestri, Lutemberger, Giorgi, et al., 2003). Patients receiving VNS were sleepier by the measures of prolonged visual reaction times and shortened sleep latencies. Chronic VNS suggests a direct vagal impact on general sleep patterns. Through the same pathways, and likely involving the orexin system (Takahashi, Okumura, Yamada, & Kohgo, 1999), vagal stimulation can explain the rapid, reversible, and reproducible changes in sleepiness that were found in this study.

The present experiment of non-nutritive, gastric distension showed rapid, reversible, and reproducible effects of cognitive responses to the distending stimulus. Cognitive processing of visceral sensation transmitted by the vagus nerves in healthy participants will increase our understanding of how food intake, for example, is linked to our subjective perceptions. Such knowledge about the central and cognitive processing of visceral sensation could lead to new perspectives on the perpetuating factors of eating disorders and obesity.

8.8   General Discussion

The present results indicate that, in humans, non-nutrient proximal gastric balloon distension activated an expansive cortical and subcortical network consistent with previous anatomical findings. Non-nutrient gastric distension mimicked the ingestion of a meal by inducing the feeling of fullness with balloon volumes similar to volumes ingested during test meals in other studies. The purpose of the current study was to identify brain regions involved in and cognitive responses to the processing of gastric-visceral sensation induced by stimulation of gastric vagal afferent fibers. Our results demonstrate that the stomach projects via vagal afferent pathways to visceral cortical areas that also participate in emotional and affective processing such as the parabrachial nucleus in the brainstem, the

insula, anterior cingulate and ventrolateral prefrontal cortices. Those visceral projection areas show increased rCBF to vagal afferent stimulation induced by gastric distension. The visceral cortex connects with limbic areas and actually represents paralimbic structures. The limbic system generally aims to contribute to the survival of an organism, which includes initiation of feeding and drinking with the appropriate preceding behaviors including agonistic activities (defense and attack); the latter initiated via emotions (Nieuwenhuys, et al., 1988). In this respect, limbic and paralimbic areas involved in the processing of signals of satiety serve the survival of the organism.

Stimulation from the esophageal tube is visible in sensory and motor cortices along the central sulcus at the level of the larynx and pharynx when comparing the inflated and deflated conditions with ECR respectively. Such sensory stimulation is eliminated in the comparison of inflation minus deflation. Sensory and motor activation in this subtraction occurred at the level of the hand and arm, indicating limb movements. We hypothesize that these movements served as a balance for the otherwise confined body and head posture in the PET scanner.

Attentional networks in the parietal and posterior cingulate regions also showed increased rCBF during gastric distension. Such activation, as well as activation in pre-motor and supplementary motor regions, is most likely due to active movement inhibition. Subject were instructed to not to move in the PET scanner. Cerebellar activation was dominant in the subtraction analysis inflation minus ECR and seems to participate in the coordination, sequencing and timing of motor behavior such as swallowing and movement inhibition.

Activation of the ventrolateral prefrontal cortex (VLPFC) in combination with visceral cortical areas in the insular and anterior cingulate cortices emphasizes the emotional aspect of gastric distension and in particular, the evaluative aspect of this signal of satiety. Increased activity in the VLPFC has been associated with processing reward-related information in humans (McClure, Laibson, Loewenstein, & Cohen, 2004; Ursu & Carter, 2005), primates (Ohgushi, Ifuku, Ito, & Ogawa, 2005; Thorpe, et al., 1983) and non-primates (Peters, O'Donnell, & Carelli, 2005). Activation in the VLPFC occurred in response to the increasing unpleasantness of taste (O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001b; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001), to sadness (Habel, et al., 2005; Levesque, et al., 2003), and to increasing punishments (O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001a). In humans specifically, lateral prefrontal areas seem to assist in the quantitative analysis of economic alternatives and the assessment

of future opportunities for reward (Kringelbach, O'Doherty, Rolls, & Andrews, 2003). These findings suggest that the VLPFC partakes in the processing of negative consequences of presented stimuli or choices. Such activation could indicate the modulation of distress associated with the negative experience. In the present study, subjects' negative experiences are reflected by increased gastric discomfort during inflation. In fact, the study of subjective negative experiences by varying the levels of social exclusion in a group setting revealed that VLPFC activation was negatively correlated with the level of distress subjects experienced during the study. Thus, these authors conclude, the VLPFC modulated the level of distress when experiencing social exclusion in that the more active VLPFC was, the less distress subjects experienced (Eisenberger, et al., 2003).

Rewarding and punishing consequences of task performance resulted in dissociated rCBF changes in medial and lateral regions of the prefrontal cortex (O'Doherty, et al., 2001a). O'Doherty and coworkers (2001a) found that rCBF in the ventromedial prefrontal cortex (VMPFC) increased after reward and that rCBF in the VLPFC increased after punishment. When increased rCBF was observed in the VMPFC, rCBF decreased at the same time in the VLPFC, and vice versa. The authors explain this dissociation with the distinct connectivity of the medial and lateral areas of the prefrontal cortex (Carmichael & Price, 1996). Carmichael and Price (1996) described two networks in the prefrontal cortex; an orbital (lateral) network that receives sensory inputs from other cortical and subcortical areas and a medial network that receives almost all of the sensory inputs via few yet dense connections with the orbital network (Carmichael & Price, 1996). Visual, somatosensory, gustatory/visceral, and olfactory peripheral projections terminate in the caudal and lateral orbital cortex within the orbital network (Carmichael & Price, 1995). Thus, the orbital network can be considered as a viscerosensory system within the prefrontal cortex. In contrast, the medial network receives fewer direct sensory inputs, yet provides the major cortical output to both the hypothalamus and midbrain, including the PAG (An, et al., 1998; Öngür, et al., 1998). The medial prefrontal network has therefore been characterized as a visceromotor system within the prefrontal cortex. Some areas within the orbital and the medial prefrontal networks interconnect substantially (Carmichael & Price, 1996). These interconnected areas may serve as points of interaction between them (Carmichael & Price, 1996). As discussed by Öngür, An and Price (1998), this may suggest that most sensory information is received in the orbital network, then transferred to the medial network via the links between the networks, and finally sent from the medial network to subcortical autonomic, endocrine, and somatic integrating areas such as hypothalamus and PAG.

Activation in the VLPFC was the only brain activation that could be replicated in the split-half reliability analysis. This finding emphasizes the importance of VLPFC contribution to the processing of gastric distension as a satiety signal, a viscerosensory signal from within the body. Activation in the VLPFC also occurred across many other study paradigms with one common denominator: the subjects' aversive/negative experiences due to aversive/negative consequences of the study task. Strong correlations of activation in the left VLPFC with the amygdala were found during aversive olfactory stimulation, which emphasizes the contribution of VLPFC in emotional processing related to food stimuli (Zald & Pardo, 1997). The VLPFC also contributes to the central processing of satiety, as rewarding features and aspects of pleasantness partake in the experience of food intake (O'Doherty, et al., 2001b; Small, et al., 2001; Zald, et al., 2002a). Via connections between the lateral and medial prefrontal areas (Carmichael & Price, 1996), viscerosensory information manipulates subcortical autonomic, endocrine, and somatic integrating areas such as the hypothalamus and PAG.

We conclude that activation in the ventrolateral prefrontal cortex in the present study indicates the modulation of the evaluative processing of gastric distension as one signal of satiety. As discussed above, this ventrolateral prefrontal area represents a convergence zone for numerous food-related stimuli including visual, gustatory (Thorpe, et al., 1983), and rewarding (O'Doherty, et al., 2001a,b; O'Doherty, Deichmann, Critchley, & Dolan, 2002) characteristics of food as well as gastric motility (Bailey & Sweet, 1940) which occurs as a consequence of food ingestion.

The likely involvement of orexin neurons in gastric distension could explain features of eating disorders, especially bulimia nervosa, resembling addictive behaviors. Orexin neurons in the ventral tegmental area appear to mediate morphine's action on the mesolimbic system inducing hyperlocomotor activity and reward effects (Harris, Wimmer, Randall-Thompson, & Aston-Jones, 2007). Zhou, Bendor, Hofmann, Randesi, Ho and Kreek (2006) found that orexin levels in the lateral hypothalamus are enhanced by morphine withdrawal. This relationship of the orexin neurons with reward systems could be an indicator for the rewarding features of food and could possibly elucidate the addictive features of bulimia nervosa.

The present study illustrates one of the signaling pathways related to food intake. We think that brain activity during proximal gastric distension in healthy subjects leads to further understanding of mechanisms of satiety and may shed light on the behavior of overeating.

The group around Faris has previously suggested that frequent binge-vomit cycles, as observed in bulimia nervosa, could cause structural or functional changes in vagal afferent nerves (Faris, et al., 1992; Faris, et al., 1998; Faris, et al., 2000; Hartman, Faris, Kim, Raymond, Goodale, Meller, et al., 1997). Such nerve density remodeling has been observed in the heart (Lowes, Gilber, Abraham, Minobe, Larrabee, Ferguson, et al., 2002) and it is plausible that the biomechanical stress incurred on the vagus nerve could have similar effects. Those possible changes in the vagus nerve functionality may complement habitual and recurrent bouts of over-eating, as present in binge-eating disorder and bulimia nervosa. With the vagus nerve as the major transmitter of visceral information to visceral projection areas and limbic structures, it defines a link between gastric vagal afferent activation induced by meal ingestion and affective characteristics of the ingested food.

We used a temporarily inserted gastric balloon set-up to study brain activity during acute gastric distension. This method should not be mistaken as a suggested treatment for disordered eating or obesity. Such treatment would include the implantation of a permanent gastric balloon, which, used alone or as an adjunct to behavioral programs had only transient effects to induce weight loss (Barkin, Reiner, Goldberg, & Phillips, 1988; Hogan, Johnston, Long, Sones, Hinton, Bunge, et al., 1989; Mathus-Vliegen, Tytgat, & Veldhuyzen-Offermans, 1990; Martinez-Brocca, Belda, Parejo, Jimenez, del Valle, Pereira, et al., 2007). Gastric balloon implants could only temporarily change the feelings of hunger (Rigaud, et al., 1995), a possible indicator for stomach adaptation (Rigaud, et al., 1995; Totte, et al., 2001; Vandenplas, et al., 1999). Moreover, gastric balloon implants produced side effects such as gastric ulcers, gastric erosion, abdominal cramps, balloon displacement and deflation (Hogan, et al., 1989; Kirby, Wade, Mills, Sugerman, Kellum, Zfass, et al., 1990; Kramer, Stunkard, Spiegel, Deren, Velchik, Wadden, et al., 1989; Mathus-Vliegen, et al., 1990; Meshkinpour, Hsu, & Farivar, 1988). Hence, contemporary indication of gastric implants is restricted to preoperative weight loss to reduce the operative risk for morbidly obese patients undergoing surgery (de Waele, Reynaert, Urbain, & Willems, 2000; Doldi, Micheletto, Prisco, Zappa, Lattuada, & Reitano, 2000; Evans & Scott, 2001).

8.9   Study Limitations

The current study and the choice of gastric distension as a measure of satiety could be challenged on several grounds. The following eight issues will be addressed below: First, secondary effects such as humoral release could have produced the brain activation and

cognitive appraisal patterns. Second, the experiences of non-nutrient gastric balloon inflation could also differ substantially from the normal eating experience. Third, the subjective feelings could have been generated by a conditioned response learned during previous meal ingestions. Fourth, the length of the study could have affected our results and fifth, the variation in VAS acquisition for inflation and deflation did not meet the standard of a constant study protocol. Sixth, we did not use stepwise inflation to identify the progressive neural and cognitive processing of the distending stimulus. Seventh, the neural activation might be colored by the tactile stimulation of the esophagus. And last but not least, within-subject image averaging is preferred over image averaging across subjects.

Humoral Release

It is possible that sensory oral stimulation from the tube could cause cephalic phase insulin responses (Ahren & Holst, 2001). Also, gastric balloon distension could have triggered the release of gastric hormones, for instance, ghrelin (Cummings, Weigle, Frayo, Breen, Ma, Dellinger, et al., 2002; Flier & Maratos-Flier, 2002), incretin (Rask, Olsson, Soderberg, Johnson, Seckl, Holst, et al., 2001), CCK, or gastrin (Koop, Ruppert-Seipp, Koop, Schafmayer, & Arnold, 1990). Such humoral releases could have led to the observed changes in neural activity and subjective feelings. Humoral release detected in plasma levels, however, has a longer than short-term effect and would have affected all data recorded after hormone release into the blood stream. Gastrin or Peptide YY, for example, are released with a time-lag (Koop, et al., 1990; Batterham, et al., 2002; Monteleone, et al., 2005; Davis, et al., 2005) and plasma levels remain elevated for several hours (Pedersen-Bjergaard, et al., 1996; Batterham, et al., 2002). If humoral release had affected the appetitive ratings in our study, the subsequent measures on the VAS should reflect continuously decreased hunger even during balloon deflation. Yet the rapid, reversible and reproducible pattern of rCBF changes and VAS rating changes argue against such humoral effects. Also, in a pilot study we did not find elevated CCK plasma levels following balloon distension and only a 30-minute delayed gastric acid release (unpublished data). This outcome is also supported by the finding that CCK or PYY levels remained unaffected by gastric balloon inflation (Oesch, Rüegg, Fischer, Degen, & Beglinger, 2006) and by the finding that water intake only minimally released PP (Schmidt, et al., 2005). We did not study neuropeptides, such as melanin concentrating hormone or orexins/hypocretins, which project to numerous central and peripheral areas (Date, Ueta, Yamashita, Hamaguchi, Matsukura, Kangawa, et al., 1999). Nor did we study hormones released at sites other than gastrointestinal sites, the adipocyte hormones resistin (Shuldiner, Yang, & Gong, 2001) and leptin (Elmquist, Elias, & Saper, 1999), which represent important regulators in eating.

Normal Eating Experience

Neural and cognitive responses during non-nutritive gastric inflation could differ from the normal eating experience. Different sensations and atypical stomach relaxation could be expected during gastric distension when compared with postprandial values. Yet subjective ratings after gastric distension in our study show very similar values as reported by others after meals (Ladabaum, et al., 2001; Tataranni, et al., 1999). A comparison of post-balloon and post-prandial physiologic stomach relaxation in the same subjects found similar responses for both conditions (Vu, et al., 1999). We conclude that the non-nutritive, mechanical stimulus used in the present study evoked the same physiological gastric relaxation response and the same subjective feelings as a meal.

Conditional Responses to VAS

The VAS ratings might represent a conditioned response previously acquired during meal ingestions. Conditioned responses to food intake during normal meal ingestion have been studied in rats. A conditioned response obtained in free-feeding rats extinguished rapidly when only one of several satiation stimuli was absent (Davis, 1998; Davis, Smith, Singh, & McCann, 2001). The acquired conditioned response (quantified as the rate of licking) during free-feeding trials vanished within the initial trials and had completely disappeared with a fifth trial of sham-feeding. If, in our study, the subjective rating values had reflected a conditioned response originally acquired during normal meal ingestion and then present during gastric distension, we would have anticipated a change in values over the three cycles to reflect the organism's ability to detect the sham feeding induced by non-nutritive gastric distension. Instead, no such change occurred. Thus, we conclude that non-nutritive gastric distension has evoked both a real physiological and cognitive response.

Length of Study

VAS rating scores could be affected by the confining, lengthy nature of the study, which could have blunted subjects' sensations. On the contrary, we reproduced all subjective feelings, maximal tolerated volumes and gastric relaxation to the same degree with the three consecutive inflations; no effects of habituation occurred. Our results do not support the view that the length of the study affected the results.

Variation of VAS Acquisition Protocol

Cognitive responses were collected pre-scan in the deflated condition but were collected post-scan for the inflated condition. However, VAS ratings from twelve subjects were collected pre-scan and post-scan for deflation. The statistical comparison of the ratings

acquired at divergent time points revealed no significant difference between the values. Therefore, we conclude that there was no difference in cognitive processing of deflation at divergent time points.

Incremental Balloon Inflation

Step-wise balloon inflation with intermittent VAS ratings would have given us the opportunity to more closely follow the changes in the cognitive appraisal of the distending stimulus. This extension of the protocol would have allowed for the distinction between the objective and subjective evaluation of the physical stimulus. Correlation of physical stimulus characteristics (various volumes) with rCBF as well as correlations of rCBF with the subjective appraisal of different levels of gastric distension could have shed light on the differential neural processing of subjective and objective stimulus features as suggested by Craig (2002).

Esophageal Stimulation

Increases in rCBF in the current study could result from esophageal but not gastric sensation (Aziz, et al., 1997; Aziz, et al., 2000; Binkofski, et al., 1998). In the present study, rCBF increases in SI/MI occurred at the level of the lips/mouth, larynx/pharynx in the subtraction analyses inflation minus ECR and deflation minus ECR. Such activation is due to tactile esophageal stimulation of the tube. Since the tube stimulated the esophagus during both conditions, subtraction of deflated from inflated activation should have eliminated esophageal activation. In fact, no SI/MI activation at the level of the lips/mouth, larynx/pharynx could be found in the inflation minus deflation analysis. Instead, SI/MI activation was obvious at the level of the arm/trunk indicating limb movements (see chapter 8.3 for a discussion).

Image Averaging across Subjects

Last but not least, an important issue to address is image averaging across subjects. Between-subject averaging is the standard analysis paradigm for PET studies. We felt it was unreasonable to prolong the study session to allow for a sufficient number of cycles of inflation and deflation for within-subject averaging. Each session lasted for about one to one and one-half hours once subjects were intubated and secured to the PET scanner. A few subjects aborted the study prematurely due to discomfort. Although the average is the best group estimate, we included in our results the number of subjects contributing to each activation focus to highlight the prevalence of responses across the group. Therefore, averaging across the group of subjects was appropriate.

9   Conclusion and Outlook

The present study illustrates one of the signaling pathways in food intake. Greater knowledge of active brain areas during proximal gastric distension in healthy subjects could lead to further understanding of the mechanisms of satiety. Understanding the neural correlates of stomach distension may shed light on the behavior of overeating ocurring in eating disorders and obesity. Faris and coworkers (1992, 1998, 2000) as well as Hartman and coworkers (1997) have previously suggested that frequent binge-vomit cycles, as evident in bulimia nervosa, could cause structural or functional changes in vagal afferent nerves. Such nerve density remodeling has been noticed in the heart (Lowes, et al., 2002) and it is plausible that the biomechanical stress incurred on the vagus nerve could have similar effects. Those possible changes in the vagus nerve may complement habitual and recurrent bouts of over-eating, as it occurs in bulimia nervosa and in obese binge-eating disorder. With the vagus nerve as the major transmitter of visceral information to visceral projection areas, limbic structures, and especially the prefrontal cortex involved in processing reward-relevant information, it defines a link between gastric vagal afferent activation induced by meal ingestion and affective characteristics of the ingested food.

Craig (2002) classifies the processing of visceral sensation and stimulation as interoceptive processing. As vagal afferent transmission is suggested to operate dysfunctionally in the population experiencing severe bulimia nervosa (Faris, et al., 2000), it is conceivable that interoceptive processing operates dysfunctionally in a population with abnormal eating behaviors. Cortical processing of interoceptive stimuli involves the prefrontal areas concerned with processing of reward-related stimuli in healthy subjects. Activation in those areas might differ in subjects diagnosed with bulimia nervosa. Overeating and extensive stretching of the stomach might be interpreted as positive and rewarding and might induce a subjective feeling of pleasantness. Since the organism strives for a positive emotional state, brain activation in the prefrontal cortex might contribute to the perpetuation of the abnormal eating pattern. How non-nutritive gastric distension is processed centrally in a population characterized by disordered eating remains to be determined in future research. Comparison of those data would allow establishing a hypothesis about the underlying pathophysiology of abnormal eating habits.

The current project did not permit the close examination of changes in the cognitive processing of the distending stimulus. Additional valuable information could be derived from step-wise balloon inflation alternating with the acquisition of VAS ratings and

measures of rCBF. Implementing a study protocol which includes the step-wise gastric distention could yield information about the objective and subjective evaluation of the physical stimulus. Separate rCBF and VAS data from distinct intensity levels would allow for meaningful interpretation of correlational analyses. Correlating the various volumes (physical stimulus characteristics) with rCBF as well as correlating the VAS rating scores at different levels of gastric distension (subjective appraisal) with rCBF could illuminate the differential neural processing of subjective and objective stimulus features as suggested by Craig (2002). Such data would clarify the association between the physical and perceived distending stimulus intensity and its relationship with subjective perception. This suggested study design, however, requires a neuroimaging technique other than PET to allow for frequent and repetitive recording of brain activity.

Another variation of the study protocol for further valuable information on this topic derives from the group around Patricia Faris at the University of Minnesota, MN. Faris has suggested that afferent vagal hyperactivity is a possible factor in the pathophysiology of BN (Faris, et al., 2000). She studied the effects of blockage of vagal activity with the 5-HT$_3$ antagonist ondansetron in subjects diagnosed with severe bulimia nervosa (Faris, et al., 1998, 2000). According to Faris and coworkers (2000), the rationale to examine 5-HT$_3$ receptor blockage with ondansetron is based on several pieces of evidence. The 5-HT$_3$ receptor is found abundantly in gastrointestinal sites (Champaneria, Costall, Naylor, & Robertson, 1992; Kidd, Levy, Nielsen, Hamon, & Gozlan, 1993) and 5-HT$_3$ receptor antagonists block the chemosensitive vagal afferent fibers in the gastric mucosa (Blackshaw & Grundy, 1993). Although 5-HT$_3$ receptors also occur in central structures (Kidd, et al., 1993; Ohuoha, Knable, Wolf, Kleinman, & Hyde, 1994), ondansetron acts mostly peripherally as it only minimally penetrates the blood brain barrier (Simpson, Murphy, Colthup, & Whelan, 1992). Therefore, ondansetron blocks peripheral vagal afferent activity. Studying the effects of non-nutritive gastric distension in combination with the administration of ondansetron will give further insight into the peripheral and central processing of gastric distension.

Afferent vagal hyperactivity as a possible factor in the pathophysiology of BN has been suggested by Faris et al. (2000) as ondansetron reduced the frequency of the behavioral cycles of binge-eating and vomiting and at the same time reestablished normal eating habits. The authors attributed these positive effects to the pharmacological regulation of abnormal vagal neurotransmission by ondansetron. The positive effects of different 5-HT$_3$ antagonists on gastric tone, feelings of fullness and nausea (Feinle & Read, 1996), and on

symptoms of irritable bowl syndrome and rCBF during rectal distension (Berman, Chang, Suyenoby, Derbyshire, Stains, Fitzgerald, et al., 2002) have also been examined previously. Since 5-HT$_3$ antagonists did change symptoms of the eating disorder bulimia nervosa and of irritable bowl syndrome, since satiety and meal termination are mainly vagally mediated processes, and since vagal afferent stimulation with non-nutritive gastric distension resulted in significant activation of central structures, the effects of 5-HT$_3$ antagonists on rCBF during non-nutritive gastric distension could yield further helpful information in the study of visceral (interoceptive) processing.

Strongest brain activation found in the present study was the ventrolateral prefrontal cortex (VLPFC). Increased rCBF in this area was found in two subtraction analyses: inflation minus deflation and inflation minus ECR. Since no such activation was observed in the subtraction deflation minus ECR and since gastric distension activates vagal afferent fibers, we suggest that rCBF changes prevalent in the analyses inflation minus deflation and inflation minus ECR are due to vagal afferent stimulation. The VLPFC responds to food stimuli and satiety signals, modulates emotional distress, is involved in craving states of addictive drugs and processes reward-related information. We hypothesize that the administration of a peripheral 5-HT3 receptor antagonist will decrease vagal neurotransmission and thus would result in reduced rCBF in the VLPFC as compared to the current study. Other central effects of the blockage of peripheral vagal afferent neurotransmission are likely. Incorporating vagal blockade in a study design using stepwise balloon inflation as suggested above could elucidate vagal contribution to the objective and subjective processing of visceral (interoceptive) stimuli. Experiments addressing the cognitive appraisal of non-nutritive, gastric distension after the administration of 5-HT3 receptor antagonists could further expand our knowledge of vagal effects on cognitive processing during visceral sensation. Hence, results could clarify vagal involvement in emotional and behavioral aspects of eating.

The current interest in the central processing of interoceptive stimuli is based on William James' (1894) proposal of the physical basis of emotion. According to James (1894), physiological arousal unconsciously evoked by a situation triggers a behavior that is interpreted by the brain as a particular emotion. In fact, it was shown that inducing different emotional states in humans is associated with different autonomic response patterns (Collet, Vernet-Maury, Delhomme, & Dittmar, 1997; Levenson, Ekman, & Friesen, 1990). More recently, heart rate patterns were elicited as a function of the emotion that was presented in a facial expression (Critchley, et al., 2005). There is clinical evidence

that the way we perceive our bodily state reflects on the emotional experience we have. Patients with peripheral autonomic denervation or with pure autonomic failure experienced blunting of their emotional experience (Critchley, Mathias, & Dolan, 2001a; Critchley, Mathias, & Dolan, 2002a). It has been suggested that subjective feeling states as the individual expression of emotions involve the representation of bodily responses that were elicited by specific situations (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Different levels of sensitivity to internal bodily responses then are the basis for the individual differences in the intensity of emotional experiences (Critchley, et al., 2004). The attempt to associate autonomic responses as markers of emotional experience, such as galvanic skin response and measures of cardiovascular outcome, with measures of bodily awareness and brain activation patterns could describe the physiological and neurological underpinnings of individual differences. Extending the study of individual differences based on the level of sensitivity and awareness to bodily states to populations with abnormal expressed behavior could provide an inroad into the pathology of personality disorders.

F i g u r e s

T a b l e s

A b b r e v i a t i o n s

A	anterior
ACC	anterior cingulate gyrus
ATP	adenosine triphosphate
BA	Brodman area
BMI	body mass index
BN	bulimia nervosa
BPM	beats per minute
CBF	cerebral blood flow
CBT	cognitive behavior therapy
CCK	cholecystokinin
CCK-8	cholecystokinin octapeptide
CNS	central nervous system
D	deflation
DIS	Diagnostic Interview Schedule
DISSI	Diagnostic Interview Schedule Screening Interviews
DMV	dorsal motor nucleus of the vagus nerve
DSM-IV	Diagnostic and Statistical Manual for Psychiatric Disorders, 4th edition
ECG	electrocardiogram
ECR	eyes closed rest, resting condition
FDA	Food and Drug Administration
fMRI	functional magnetic resonance imaging
FWHM	full width at half maximum
GLM	general linear model
GLP-1	glucagon-like peptide 1
I	inferior
In	inflation
I.V.	intravenous
Lcl	left claustrum
Lcr	left cerebrellum
LGR	left gyrus rectus
LIFC	left inferior frontal cortex
LINS	left insula
LINS pole	left insular pole

LINS/oper.............................. left insula/operculum

LIPS .... left intraparietal sulcus

LLG...................................... left lingual gyrus

N.. sample size

NA.. negative affect

NPY...................................... neuropeptide Y

NTS .... nucleus of the solitary tract (nucleus tractus solitarius)

P .... posterior

PA.. positive affect

PAG...................................... periaqueductal gray

PANAS.................................. Positive Affect and Negative Affect Scales

PBN...................................... parabrachial nucleus

PDTs..................................... pain detection thresholds

PET....................................... positron emission tomography

PP .... pancreatic polypeptide

PYY...................................... polypeptide YY

R .... right

R/LGPoC............................... right and left postcentral gyrus

RabCS .... right ascentding branch of cingulate gyrus

RACC.................................... right anterior cingulate cortex

rCBF...................................... regional cerebral blood flow

RGPrC................................... right precentral gyrus

RINS/oper .... right insula/operculum

RLG...................................... Robert L. Goodale, study surgeon

ROI....................................... region of interest

S .... superior

SI .... primary somatosensory cortex

SMA .... supplemental motor area

SPSS..................................... Statistical Package for Social Sciences

Std. Deviation........................ Standard Deviation

U.S.. United States

VAMC................................... Veterans Affairs Medical Center

VAS...................................... visual analog scales

VLPFC .... ventrolateral prefrontal cortex

VMb .... basal part of the ventromedial nucleus of the thalamus

VMPFC .... ventromedial prefrontal cortex

VMpo .... posterior part of the ventromedial nucleus of the thalamus

VNS...................................... vagal nerve stimulation

vs. .... versus

R e f e r e n c e s

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Appendix   A

Healthy female **Volunteers** age 18-40 needed for neuroimaging research studies of human brain function related to the sensation of fullness

Subjects will be paid $300.00 for participating.

Requires less than 3 hours of your time.

Receive a color picture of your brain activity.

UNIVERSITY OF MINNESOTA
conducted at the
MINNEAPOLIS VA MEDICAL CENTER

For further information and scheduling, please call:

(612) 725-2000, extension 1758

sensorimotor area

frontal eye field

prefrontal area

Broca's area (in left hemisphere)

visual

visual association

auditory association (including Wernicke's area, in left hemisphere)

auditory

A-1

Sensation of fullness
Brain Scan
(612) 725-2000,
ext. 1758

Sensation of fullness
Brain Scan
(612) 725-2000,
ext. 1758

Sensation of fullness
Brain Scan
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ext. 1758

Sensation of fullness
Brain Scan
(612) 725-2000,
ext. 1758

Appendix B

Medical screening questions: control subjects

Date ___/____/____

Name:_____   Gender (CIRCLE): F   M

Day phone: _____   Eve. phone:_____

Age: _____yrs.   *MUST BE AT LEAST 18YRS*

Handedness (CIRCLE) : R,   L,   or   Ambidextrous

Available (CIRCLE ALL TIMES AVAIL.):
Tues. 8 a.m. - 11:30 am   Tues. 11 a.m -2:30 p.m.   Thurs.: 8 am - 11:30 a.m.

NOW, I NEED TO ASK YOU A FEW QUESTIONS SINCE WE ARE TRYING TO MATCH OUR NORMAL PET SUBJECTS WITH DIFFERENT PATIENT GROUP SUBJECTS

1. Are you currently in good health? Y   N => What conditions are you being treated for?
_____

2. Are you currently or have you recently been on a prescribed medication?
    N   Y => Name of medications?_____
              For what condition?_____
    Medications on, but not affecting study (PLEASE CIRLCE ALL THAT APPLY):
    Tylenol   aspirin   ibuprofen (ex.Advil, Motrin) Aleve   other anti-inflammatory:_____
    Vitamins   Synthroid (for thyroid)   Birth Control Pills   Estrogen
    Antibiotics (which one &what for):_____for what condition ?_____
    high blood pressure medication (which one):_____

3. Are you seeing a doctor now? N   Y   =>   If so, for what:_____

4. Have you seen a doctor for a moderate to severe medical problem in the last year?
    N   Y   =>   If so, for what:_____

5. Have you ever had one of the following: a stroke, a seizure, head injury or other neurological problem which required a doctor's care? (head injury not serious if not knocked unconscience & brief seizure during childhood during fever not serious)?.   N   Y
(If yes)   stroke   seizure   head injury   other _____
                  when did this occur:_____ & how serious:_____

7. Do you have any physical condition which may make it difficult for you to lie still and on your back for two hours: N   Y   If so, what?_____

8. How much do you weigh ?_____   (exclude if above above 270 pounds)

9. Do you ever have problems with claustrophobia?
        N   Y =>   If so, how freqently (CIRLCE ONE)? daily   weekly   monthly

10. Do you have a history of any psychiatric problems (for example, depression, alcoholism)?
        N   Y   If so what ?_____

11. Do you have normal vision or are you 20/20 corrected with glasses or contacts? Y   N

12. Do you have a hearing problem?
        N   Y: => Is it corrected (CIRCLE)?   Y (hearing aid)   No, not corrected

13. Any problems with smell or taste? (optional)   Y   N

Schedule if in good health, make sure to write info on calendar

If scheduled: **** <u>**Subject scheduled for scan on**</u>:   Day:_____   Time:_____ am   pm.***
   1.) SS#_____-_____-_____
   2) Date of Birth: _____/_____/_____
   3.) Address: _____
   4) Are you a United States veteran?   Y   N
   5 If female: Are you non-menopausal?   N   Y => You will be required to give a
   blood sample within 1-2 days before the study. When can you come in for a blood test
   between 8:00 a.m. and 5:00 p.m.?
   Day:_____   Time:_____ am   pm. When you arrive here for the blood test or the PET
   scan study, go to the information desk & call extension #2473 & someone will come to
   meet you there. *YOU CANNOT BE PREGNANT AT THE TIME OF THE STUDY.*
Each study costs over 2,000 dollars. Please let us know immediately if you need to cancel.
Call 725-2000, ext. 2473 to cancel.   {now go get voucher ready for this subject}

<u>Notes about what being a PET subject involves:</u>

Risk: The radiation in a study has the equivalent cancer risk to that of smoking 2 cartons of cigarettes.
Procedure: An IV line is placed in your arm, & radioactive water is delivered thru it to the
bloodstream. This type of radiation is a gamma type, like x-rays, and is completely gone in ten
minutes. After we inject the positron-labeled water (0^{15}), we detect where it goes in the brain using a
PET scanner, which looks like an MRI scanner. Cerebral blood flow is an excellent marker of brain
activity as the neurons require more oxygen & glucose when they are active & this leads to more
increased blood flow to brain areas that are more active. For ex., when we are looking at something,
the brain's visual areas in the occipital lobe show increased blood flow. We use this same strategy to
identify brain areas active during other tasks, such as speaking, memorizing, etc.
Also: You will also be asked to also take a health screening questionnaire. Additionally, non-
menopausal females will be required to have a blood pregnancy test prior to scanning.

Voucher procedures for PET:
  Make sure that you have the subject's full name, SS#, and address typed on the payment voucher
and take to Wanda prior to scan day. Wanda Tel =ext 2034 (Research Service), office = 3N-107.

Appendix   C

Protocol #2007
Subject Name: Date

Title of Study: Neuroimaging Studies of Appetitive Behaviors

Principal Investigator: José V. Pardo, M.D., Ph.D.   VAMC: <u>Minneapolis</u>

INTRODUCTION

It is important that you read and understand the following explanation of the proposed research study before you agree to participate. This consent form describes:
* The purpose,
* The description of the study,
* The benefits,
* The risks and/or discomforts (including any potential for pain),
* Steps taken to decrease or eliminate the risks, discomforts, or possible pain,
* Any other treatments that may be available, and
* Confidentiality and use of research results.

Whether you decide to participate or not, treatment at the VA for which you are eligible will not be affected.

This consent form may contain words you do not understand. Please ask the study doctor or the study staff to explain any words or information unclear to you.

PURPOSE OF THE STUDY

You are being asked to voluntarily participate in a research study for the purpose of investigating brain function related to eating. You have been asked to participate in this study because you are an adult, non-pregnant female having good health or having problems with eating. Your participation is expected to last about three hours during one or two sessions; and approximately 40 people will be in the study.

DESCRIPTION OF STUDY

The following information describes what will happen while you participate in the study.

A 30 minute screening session will ensure that you meet the criteria required for this study. This session involves completing a mental health questionnaire, giving a blood sample (see below), an getting an electrocardiogram (EKG). If you are a woman of child-bearing potential, the date of your last menstrual period will be recorded, and a serum pregnancy test will be performed if indicated. You may be asked to participate in a brain imaging study called positron emission tomography. This method involves having blood flow in your brain measured using the PET scanner and radioactive water. A picture of your brain activity will be taken while certain tasks or procedures are performed. Examples of these include: thinking about food after an overnight fast; seeing pictures of food or smelling foods; tasting substances such as sugar, vinegar, mint; or distending your stomach like after a large meal with a volume of about a pint to a quart (the

_____
Subject Signature

Protocol #2007
Subject Name: Date

Title of Study: Neuroimaging Studies of Appetitive Behaviors

Principal Investigator: José V. Pardo, M.D., Ph.D.   VAMC: Minneapolis

latter will require your prior participation in Dr. Goodale's study and a separate informed consent).

During the PET study you will need to lie still for about two and a half hours. You will have a small catheter (plastic tube) in a vein (intravenous line) in one of your arms, so that you can be given the radioactive water directly into your vein (intravenously). About every ten minutes you will receive an intravenous injection of radioactive water while you perform the task for about one minute. The scanner will take a picture of your brain activity while you do the tasks. Then you will lie still with eyes closed for about ten minutes until the next task is started.

RISKS, DISCOMFORTS, and PAIN

A. **Blood Drawing:** Approximately 2 tablespoons of blood will be drawn from your arm. Possible side effects from blood drawing include faintness, inflammation of the vein, pain, bruising, or bleeding at the site of the puncture. There is also a sight possibility of infection.
B. **Catheter placement:** A small plastic tube will be inserted into your arm vein to permit injection of the radioactive water. This experience is similar to getting blood drawn.
C. **Radiation:** You will be given a prescribed amount of radiation. The amount of radiation is less than what is allowed for research subjects. **However, since the effects of radiation add up, it is important for your regular physician to know about your participation in this study and any other studies involving radiation, including x-rays.** The researcher will talk with you regarding radiation. Ask if you have any special concerns.
D. **Psychological Distress:** You may feel uncomfortable answering questions about your medical or mental health. You may refuse to answer any questions which you do not feel comfortable answering. Depending on the particular task or procedure, you may feel hungry or full, or taste different flavors (sour, bitter, sweet), etc.
E. **Female Participation:** Radiation can cause damage to an embryo or fetus. Therefore, if you are pregnant or planning to become pregnant, you should not participate in this study. If you are a female of childbearing age, you will get a serum pregnancy test, if indicated, to minimize the risk of radiation exposure to an embryo or fetus.

BENEFITS
No benefit is guaranteed from your being in this study. The knowledge gained from this study may benefit others in the future.

COMPENSATION You will be paid $25 for the screening session and $50 for the PET study.

_____
Subject Signature

Protocol #2007
Subject Name: Date

Title of Study: Neuroimaging Studies of Appetitive Behaviors

Principal Investigator: José V. Pardo, M.D., Ph.D.   VAMC: <u>Minneapolis</u>

CONFIDENTIALITY AND USE OF RESEARCH RESULTS

The results of this study may be published or presented but your identity and records will not be revealed unless required by Federal Law. A Federal Law allows the U.S. Food and Drug Administration and the Institutional Review Board to review records. Because of the need for these inspections, absolute confidentiality cannot be guaranteed.

COSTS TO YOU FOR PARTICIPATING

There is no cost to you for taking part in this study. All the study costs, including any study medications and procedures related directly to the study, will be paid for by the VA Medical Center. Veterans who must make a co-payment for their usual medications or treatments will continue to be required to make such a co-payment for non-study related drugs. There should be no additional medical costs to you for taking part in this study. However, frequent clinic visits may result in transportation costs and possible wages lost due to time missed from work.

MEDICAL CARE IF YOU ARE INJURED

In case you are injured from this research study, treatment will be available, including first aid, emergency treatment and follow-up care, as needed. The VA Medical Center will provide necessary medical treatment for any injury or illness that may arise from your participation in this research study.

The VA Medical Center will provide payment for necessary emergency medical treatment. However, the VA Medical Center must be contacted at (612) 725-2003 within 72 hours in the event of any non-VA treatment or else you may lose any eligibility for VA payment of emergency bills.

COMPENSATION FOR ANY INJURIES

You have not released the VA Medical Center for liability by signing this form. This includes but is not limited to, free medical care other than as described in this consent form, payment of lost wages, or compensation for pain and suffering. Compensation for those items from the VA may be available under applicable Federal Law. You should immediately report any injuries resulting from your participation in this study to Dr. <u>Jose Pardo</u> at <u>612-725-2000, x2473</u> during the day, and during the evenings or week-ends, by calling the <u>psychiatry resident on call 612-725-2000 (please request the doctor be paged).</u>

NEW INFORMATION

You will be given any new significant information which is discovered during the course of this study which may influence your willingness to continue the study.

_____
Subject Signature

Protocol #2007

Subject Name: Date

Title of Study: Neuroimaging Studies of Appetitive Behaviors

Principal Investigator: José V. Pardo, M.D., Ph.D.   VAMC: Minneapolis

---

RESEARCH SUBJECT'S RIGHTS: I have read or have had read to me all of the above. Dr. Pardo has explained the study to me and answered all of my questions. I have been told of the risks or discomforts and possible benefits of the study.

I understand that I do not have to take part in this study, and my refusal to participate will involve no penalty or loss of rights to which I am entitled. I may withdraw from this study at any time without penalty or loss of VA or other benefits to which I am entitled.

The results of this study may be published but my identity and records will not be revealed unless required by law.

As part of my participation in this research study, a VA Medical Center permanent chart with my essential identifying information (names, permanent address, gender, date of birth, and social security number) as well as the procedure performed (e.g., PET study, gastric intubation, dose and type of isotope) will be generated. If I wish to participate in other research studies involving radiation, I can readily find from this record the dose I have had to determine eligibility for participating in other research studies.

In case there are medical problems or questions, I have been told I can call <u>Dr. Jose Pardo</u> at <u>(612) 725-2000, x2473</u> during the day and the Psychiatry Resident on Call at (612) 725-2000 (please request the resident be paged) after hours.

If I have any question about the rights of a research subject, I understand that I may contact the VA Patient Representative at (612) 725-2106. If I have any questions and concerns and would like to talk to someone other than the researchers, I can contact Fairview Hospital Patient Relations, 2-499 Fairview University Medical Center, 500 Harvard Street SE, Minneapolis, MN 55455.

If any medical problems occur in connection with this study the VA will provide emergency care.

My questions have been answered and I voluntarily consent to participate in this study. By signing this form, I have not given away any of my legal rights, which I have as a subject of this research study. I will receive a signed copy of this consent form.

Subject's Signature   Date

Subject Signature

Protocol #2007
Subject Name: ___ ___   Date

Title of Study: Neuroimaging Studies of Appetitive Behaviors

Principal Investigator: José V. Pardo, M.D., Ph.D.   VAMC: <u>Minneapolis</u>

_____   _____
Signature of Investigator   Signature of Person obtaining consent if
                                         other than the investigator

_____
Signature of Witness

Approved: _9/1/00__
Approval expires: _10/18/00_
Chair, HSS: _Billington_

Approved: _10/16/00_
Approval expires: _10/16/01_
Chair, HSS: _Billington_

_____
Subject Signature

Appendix   D

DIS

<<012670.PAT>>

F:DEMOGRAPHICS   05-24-2000   3:53:42 PM
SEX=FEMALE, MARRIED=NO, CHILDREN=NO, AGE=30

Z:SOMATIZATION   05-24-2000   3:57:14 PM
    Q1 1   : dizziness
    Q2 1   : poor physical health
    Q3 1   : nausea
    Q4 1   : excessive gas
    Q5 1   : pain when having sexual relations
    Q6 1   : back pain
    Q7 1   : trouble urinating
    Q8 1   : blurred vision
    Q9 1   : being unconscious
    Q10 1   : lack of sexual pleasure
    Q11 0   : excessive bleeding during your periods
    Q12 0   : abdominal pain
    Q13 0   : sex life being unimportant
    Q14 0   : a lump in your throat
    Q15 0   : periods of weakness
    Q16 0   : pain in the joints
    Q17 0   : burning pain
    Q18 0   : losing your voice
    Q19 0   : deafness
    Q20 0   : amnesia
    Q21 0   : palpitations
    Q22 0   : trouble walking
    Q23 0   : pain
    Q24 0   : chest pain
    Q25 0   : menstrual pain
    Q26 0   : fainting spells
    Q27 0   : shortness of breath
    Q28 0   : being paralysed
    Q29 0   : arm or leg pain
    Q30 0   : strange problems
    Q31 0   : being blind
    Q32 0   : being made ill by foods
    Q33 0   : menstrual irregularity
    Q34 0   : vomiting all through pregnancy
    Q35 0   : vomiting
    Q36 0   : seizure
    Q37 0   : diarrhea
    DIAGNOSIS IS NEGATIVE
    ** THE SCREENER EXITED ON BRANCH 2

$\mathcal{DIS}$

<<012670.PAT>>

F:DEMOGRAPHICS   05-24-2000   3:53:42 PM
SEX=FEMALE, MARRIED=NO, CHILDREN=NO, AGE=30

Z:SOMATIZATION   05-24-2000   3:57:14 PM
Q1 1   : dizziness
Q2 1   : poor physical health
Q3 1   : nausea
Q4 1   : excessive gas
Q5 1   : pain when having sexual relations
Q6 1   : back pain
Q7 1   : trouble urinating
Q8 1   : blurred vision
Q9 1   : being unconscious
Q10 1   : lack of sexual pleasure
Q11 0   : excessive bleeding during your periods
Q12 0   : abdominal pain
Q13 0   : sex life being unimportant
Q14 0   : a lump in your throat
Q15 0   : periods of weakness
Q16 0   : pain in the joints
Q17 0   : burning pain
Q18 0   : losing your voice
Q19 0   : deafness
Q20 0   : amnesia
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Q22 0   : trouble walking
Q23 0   : pain
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Q25 0   : menstrual pain
Q26 0   : fainting spells
Q27 0   : shortness of breath
Q28 0   : being paralysed
Q29 0   : arm or leg pain
Q30 0   : strange problems
Q31 0   : being blind
Q32 0   : being made ill by foods
Q33 0   : menstrual irregularity
Q34 0   : vomiting all through pregnancy
Q35 0   : vomiting
Q36 0   : seizure
Q37 0   : diarrhea
: DIAGNOSIS IS NEGATIVE
  ** THE SCREENER EXITED ON BRANCH 2

N:PANIC DISORDER   05-24-2000   3:57:28 PM
Q1 1   : attack of fear or anxiety
Q2 0   : feel like choking or smothering
Q3 0   : feel faint
Q4 0   : hear your heart pound
Q5 0   : things felt unreal
Q6 0   : hot or cold flashes
Q7 0   : tightness or pain in your chest
Q8 0   : trembling or shaking
Q9 0   : afraid that you might die or act in a crazy way
Q10 0   : fingers or feet tingling
Q11 0   : sweating
Q12 0   : short of breath
Q13 0   : dizzy or lightheaded
Q14 0   : three spells within 3 weeks
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 0

X:GENERALIZED ANXIETY   05-24-2000   3:57:41 PM
Q1 1   : 6 months or more of being worried
Q2 0   : several different worries
Q3 0   : keyed up or on edge
Q4 0   : being easily startled
Q5 0   : trouble keeping your mind on what you were doing
Q6 0   : trouble falling asleep or staying asleep
Q7 0   : irritable
Q8 0   : being short of breath or feeling as though you were smothering
Q9 0   : sweating a lot
Q10 0   : aware of your heart pounding or racing
Q11 0   : a dry mouth
Q12 0   : dizzy or lightheaded
Q13 0   : nausea or diarrhea
Q14 0   : cold and clammy hands
Q15 0   : urinate too frequently
Q16 0   : trouble swallowing
Q17 0   : restless
Q18 0   : getting tired easily
Q19 0   : trembly or shaky
Q20 0   : tense or aching muscles
Q21 0   : hot flashes or chills
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 0

P:PHOBIC DISORDER   05-24-2000   3:59:00 PM
Q1 1   : fear of spiders, bugs, mice, snakes, bats, birds or cats
Q2 1   : fear of heights

Q3 1 : fear of being in a crowd
Q4 1 : fear of being in water
Q5 1 : fear of being in a closed place
Q6 1 : fear of speaking in front of people
Q7 1 : fear of storms, thunder, or lightning
Q8 1 : fear of being alone
Q9 1 : fear of speaking to strangers
Q10 1 : fear of being on airplanes, buses or elevators
Q11 1 : fear of going out of the house alone
Q12 1 : fear of being near an animal
Q13 1 : fear of tunnels or bridges
Q14 1 : fear of eating in front of others
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 2

D:DEPRESSION OR DYSTHYMIA   05-24-2000   3:59:51 PM
Q1 1 0 : period of depression
Q2 1 0 : worthless
Q3 1 0 : loss of appetite
Q4 1 0 : trouble concentrating
Q5 1 0 : two or more weeks of talking or moving slowly
Q6 1 0 : keep moving all the time
Q7 0 0 : tired all the time
Q8 0 0 : felt sad, blue, depressed or lost interest in things
Q9 0 0 : crying spells
Q10 0 0 : decreased interest in sex
Q11 0 0 : slow or mixed up thinking
Q12 0 0 : trouble sleeping
Q13 0 0 : thought about death
Q14 0 0 : sleeping too much
Q15 0 0 : increased eating
Q16 0 0 : wanting to die
Q17 0 0 : hopeless
Q18 0 0 : losing weight
Q19 0 0 : thought of committing suicide
Q20 0 0 : attempted suicide
Q21 0 0 : month period of depression and other symptoms
Q22 0 0 : sad, blue or depressed and other problems
Q23 0 0 : several problems in same month
Q24 0 0 : low, gloomy, blue and other problems
Q25 0 0 : two weeks of feeling blue with other problems
Q26 0 0 : two years of feeling blue with other problems
Q27 0 0 : spell occurred after someone close died
Q28 0 0 : depression other than after death of someone close
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 2

M:MANIA   05-24-2000   4:00:14 PM
Q1 1   0 : period when you were happy or excited or high
Q2 1   0 : being easily distracted
Q3 0   0 : you had a special gift or special powers
Q4 0   0 : being more active than usual
Q5 0   0 : increased interest in sex
Q6 0   0 : going on spending sprees
Q7 0   0 : not sleeping
Q8 0   0 : racing thoughts
Q9 0   0 : talking fast
Q10 0   0 : manic and other symptoms together in same month
Q11 0   0 : never been a period of being manic with other symptoms
Q12 0   0 : some symptoms together in same month
Q13 0   0 : being irritable
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 1

S:SCHIZOPHRENIA OR SCHIZOPHRENIFORM   05-24-2000   4:00:44 PM
Q1 1   : hearing things other people couldn't hear
Q2 0   : hearing voices commenting on what you were doing
Q3 1   : hearing two or more voices talking to each other
Q4 0   : unusual feelings inside or on your body
Q5 1   : others were controlling how you moved or what you thought
Q6 1   : people were following you
Q7 1   : someone was trying to hurt you
Q8 1   : you were being sent special messages
Q9 0   : you could hear what another person was thinking
Q10 0   : smelling things that nobody else seemed to smell
Q11 0   : people were watching you or spying on you
Q12 0   : visions
Q13 0   : someone put thoughts into your mind or stole your thoughts
Q14 0   : someone was reading your mind
Q15 0   : nervous or upset while having problems
Q16 0   : nervous or upset for two weeks
Q17 0   : first had problems before 12
Q18 0   : first had problems before 45
Q19 0   : first had problems when 45 or 46
Q20 0   : length of period at least six months
Q21 0   : length of period at least two weeks
Q22 0   : less able to do work well
Q23 0   : less able to enjoy social relationships
Q24 0   : two years before period not being normal
Q25 0   : not seeing your friends during that period
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 2

R:ANOREXIA NERVOSA   05-24-2000   4:00:59 PM
Q1 1   : you were too fat
Q2 0   : losing 15 pounds or more
Q3 0   : weight
Q4 0   : height
Q5 0   : thought you were overweight when others said you were too thin
Q6 0   : missing menstrual periods
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 0

B:BULIMIA   05-24-2000   4:01:08 PM
Q1 1   : binge eating
Q2 0   : several periods of binge eating
Q3 0   : period of 3 months of binge eating at least twice a week
Q4 0   : being afraid of not being able to stop eating binge
Q5 0   : doing something special to stop eating
Q6 0   : stopping only because of belly pain
Q7 0   : worrying about your weight or looking fat
Q8 0   : exercising to control your weight
Q9 0   : fasting all day to control your weight
Q10 0   : dieting
Q11 0   : taking water pills or diuretics
Q12 0   : taking laxatives
Q13 0   : vomiting
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 0

L:ALCOHOL ABUSE / DEPENDENCE   05-24-2000   4:03:05 PM
Q1 1   : drinking as much as a fifth of liquor in one day
Q2 1   : two weeks when every day you were drinking 7 or more drinks
Q3 1   : blackouts
Q4 1   : wanting to stop drinking
Q5 1   : controlling your drinking by making rules
Q6 1   : shaking
Q7 1   : continuing to drink after illness
Q8 1   : binges
Q9 1   : needing a drink just after you had gotten up
Q10 0   : family objections to your drinking
Q11 0   : physical fights
Q12 0   : trouble driving
Q13 0   : friends or any professional saying you were drinking too much
Q14 0   : being arrested because of your drinking
Q15 0   : job or school troubles
Q16 0   : losing a job because of drinking
DIAGNOSIS IS NEGATIVE

** THE SCREENER EXITED ON BRANCH 2

O:OBSESSIVE COMPULSIVE   05-24-2000   4:04:31 PM
    Q1 1   : unpleasant thoughts
    Q2 0   : unpleasant thoughts for several weeks
    Q3 0   : unpleasant and persistent thoughts
    Q4 0   : unpleasant thoughts
    Q5 0   : unpleasant thoughts
    Q6 1   : obsessive thoughts
    Q7 0   : obsessive thoughts for several weeks
    Q8 0   : unreasonable thoughts
    Q9 0   : unreasonable thoughts
    Q10 0   : unreasonable thoughts
    Q11 1   : do something over and over
    Q12 0   : do things over and over
    Q13 0   : do things over and over
    Q14 1   : do something in a certain order
    Q15 0   : do something in a certain order for several weeks
    Q16 0   : do things in a certain order
    Q17 0   : do things in a certain order
    Q18 1   : count something
    Q19 0   : count things
    Q20 0   : count things
    DIAGNOSIS IS NEGATIVE
    ** THE SCREENER EXITED ON BRANCH 0

G:DRUG ABUSE / DEPENDENCE   05-24-2000   4:04:51 PM
    Q1 1   : taken drugs 5 times without prescription
    Q2 0   : emotional or psychological problems from using drugs
    Q3 0   : needing larger amounts of drugs to get an effect
    Q4 0   : problems with your family, friends, on the job
    Q5 0   : withdrawal symptoms because you cut down on drugs
    Q6 0   : taking drugs every day for two weeks or more
    Q7 0   : becoming dependent on drugs
    Q8 0   : not being able to cut down on drugs
    Q9 0   : health problems because of drugs
    DIAGNOSIS IS NEGATIVE
    ** THE SCREENER EXITED ON BRANCH 0

C:CHILDHOOD CONDUCT DISORDERS   05-24-2000   4:07:00 PM
    Q1 1   : misbehaving at school
    Q2 1   : stealing
    Q3 1   : run away from home
    Q4 1   : getting drunk before 15
    Q5 1   : playing hooky 5 times a year
    Q6 1   : damaging someone's property

Q7 1   : suspended or expelled from school
Q8 1   : arrested
Q9 1   : fighting
Q10 0   : sexual relations before 15
Q11 0   : telling lies
Q12 0   : bad grades in school
Q13 0   : taking drugs before 15
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 1

I:PATHOLOGICAL GAMBLING   05-24-2000   4:07:10 PM
Q1 1   : ever gambled or bet
Q2 0   : spent a lot of time gambling
Q3 0   : gambled longer than you intended
Q4 0   : had to increase the size of your bets to stay interested
Q5 0   : felt restless or irritable if you could not gamble
Q6 0   : would try to win back what you lost from the place you lost it
Q7 0   : wanted to cut down on your gambling
Q8 0   : gambled when you should have been doing something else
Q9 0   : gave up doing other things to gamble
Q10 0   : had legal, debt, or family problems from gambling
Q11 0   : gambled even though you knew it was causing problems
DIAGNOSIS IS NEGATIVE
** THE SCREENER EXITED ON BRANCH 0

Appendix E

30years
Female
62in
Caucasian
112lbs

Loc: 1

Technician: 79

Vent. rate   55 bpm
PR interval   158 ms
QRS duration   82 ms
QT/QTc   432/413 ms
P-R-T axes   60 61 36

23-May-2000 14:25:54   VAMC MINNEAPOLIS MN

Sinus bradycardia
Otherwise normal ECG
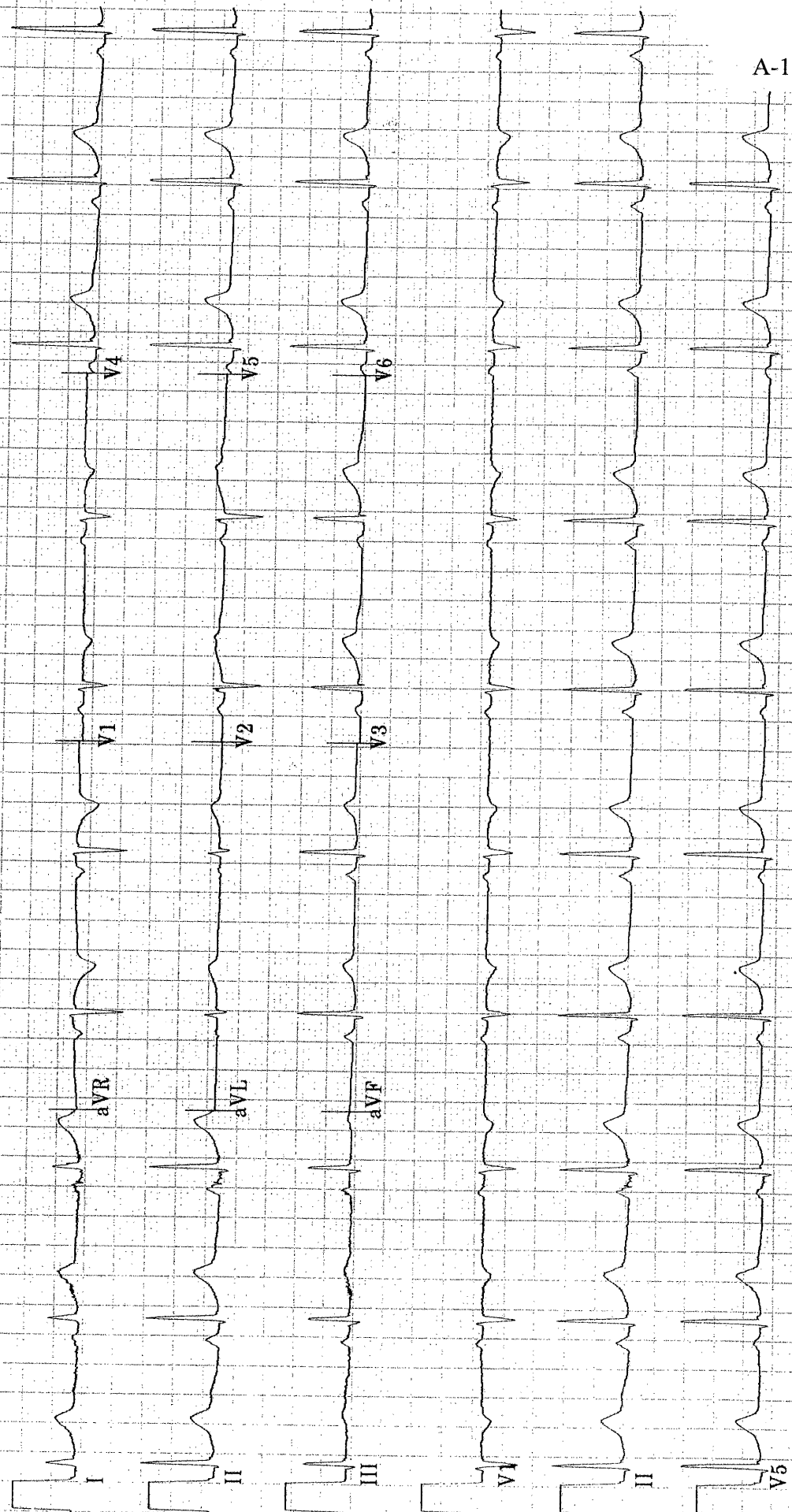
Unconfirmed

Comments:: RESEARCH

CLINIC:: CNU

A-17

100 Hz   25.0 mm/s   10.0 mm/mV   4 by 2.5s + 3 rhythm lds   12SL™ v250

MEDI-TRACE   GRAPHIC CONTROLS CORPORATION · BUFFALO, N.Y. / OH.   MACVU 003C

FORM 10-1338 FINAL COPY
                    MINNEAPOLIS VAMC CLINICAL LABORATORY REPORT

                                                06/21/2000 4:28 pm
        SSN: SEX: F   AGE: 30   LOC: 2290

        Provider: PARDO,JOSE V
        Specimen: PLASMA
Accession [UID]: CH 0621 489 [0401730489]

                              06/21/2000 15:48
        Test name   Result   units   Ref.   range
        SODIUM   144. H   mmol/L   136 -   143
        POTASSIUM   3.8   mmol/L   3.5 -   4.7
          Eval: Serum ref. range = 3.7-5.2 mmol/L
          Eval: Plasma ref. range = 3.5-4.7 mmol/L
==
          KEY: "L"=Abnormal low, "H"=Abnormal high, "*"=Critical value

FORM 10-1338 FINAL COPY
                    MINNEAPOLIS VAMC CLINICAL LABORATORY REPORT

                                               06/21/2000 4:28 pm
     SSN: SEX: F   AGE: 30   LOC: 2290

        Provider: PARDO,JOSE V
        Specimen: PLASMA
Accession [UID]: CH 0621 489 [0401730489]

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     Test name   Result   units   Ref.   range
     SODIUM   144. H   mmol/L   136 -   143
     POTASSIUM   3.8   mmol/L   3.5 -   4.7
        Eval: Serum ref. range = 3.7-5.2 mmol/L
        Eval: Plasma ref. range = 3.5-4.7 mmol/L
==
     KEY: "L"=Abnormal low, "H"=Abnormal high, "*"=Critical value

Appendix   F

Name:_____   Date:_____

The PANAS

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you have felt this way during the past month.

1	2	3	4	5
Very slightly or not at all	A little	Moderately	Quite a bit	Extremely

_____ Interested
_____ Distressed
_____ Excited
_____ Upset
_____ Strong
_____ Guilty
_____ Scared
_____ Hostile
_____ Enthusiastic
_____ Proud

_____ Irritable
_____ Alert
_____ Ashamed
_____ Inspired
_____ Nervous
_____ Determined
_____ Attentive
_____ Jittery
_____ Active
_____ Afraid

Subject Name_____ ID_____ Date_____
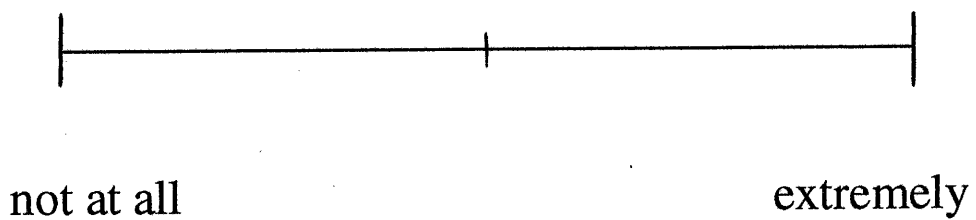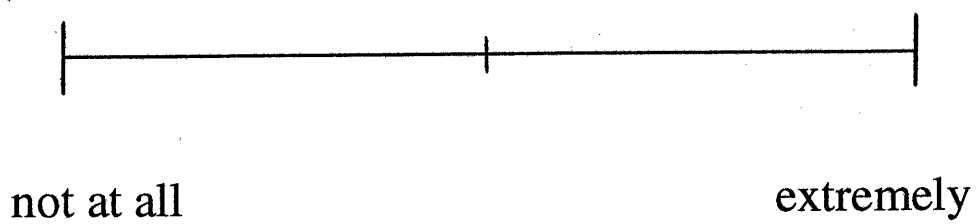
Modified PANAS

Current Ratings

Read each mood descriptor and rate the extent to which you feel this way
right now. Use the following scale to make your ratings.

1	2	3	4	5
Very slightly or not at all	A little	Moderately	Quite a bit	Extremely

Interested	___	Distressed	___
Excited	___	Upset	___
Strong	___	Guilty	___
Scared	___	Hostile	___
Enthusiastic	___	Proud	___
Irritable	___	Alert	___
Ashamed	___	Inspired	___
Nervous	___	Determined	___
Attentive	___	Jittery	___
Active	___	Afraid	___

Appendix   G

How hungry do you feel?

|—————————————————|—————————————————|

not at all   extremely

How tense do you feel?

not at all   extremely

How much gastric discomfort do you have?

not at all   extremely
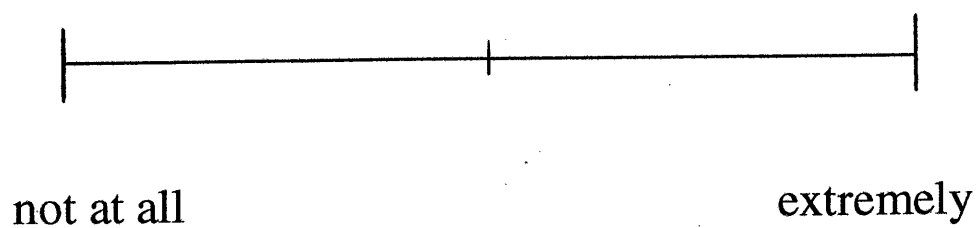
How full do you feel?

not at all   extremely
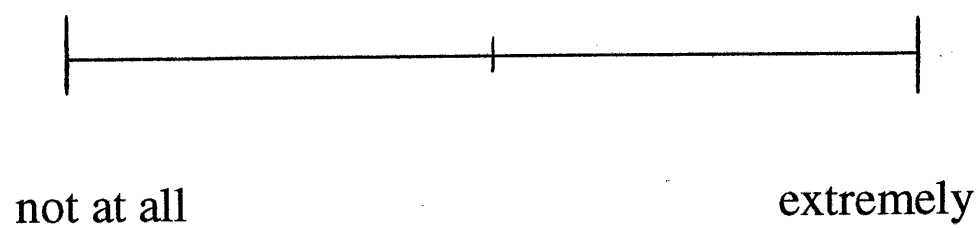
How sleepy do you feel?

|—————————————————|—————————————————|

not at all   extremely
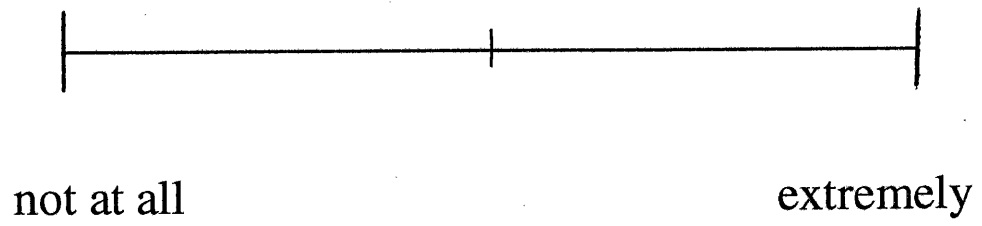
How nauseated do you feel?

not at all   extremely
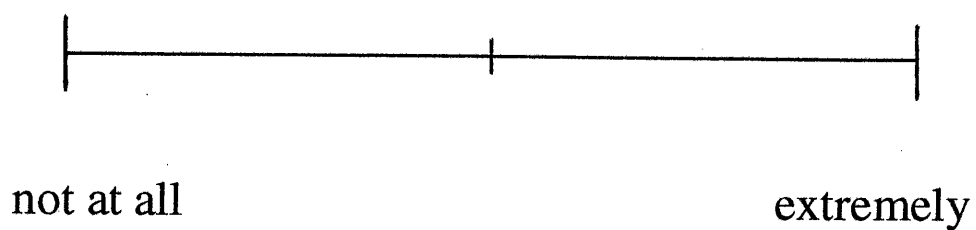
Appendix H

SUBJECT PROTOCOL SHEET

SUBJECT NAME: _____ SEX: ___ AGE _____ PO# 5705 DATE 8/10/00

RDRC Protocol # 2007 PPU# ____ HEAD ANGLE: -6.9 deg. HEAD POSITION +3 MODE: (3-D) 2-D (circle)
Stimuli Name: _____

HO₁ BALLOON INFLATED

blank p∅5703
3d norm
phantom p∅5698

Sc On 1:55 :   dose: _22_ mCi (circle)   or 19 mCi (write in amount)   Comments:

HO2 BALLOON EMPTY

Sc On 2:08 :   dose: _22_ mCi (circle)   or 19 mCi (write in amount)   Comments:

HO3 Balloon inflated

Sc On 2:15 :   dose: _22_ mCi (circle)   or 19 mCi (write in amount)   Comments:

HO4 Balloon deflated

Sc On 2:23 :   dose: _22_ mCi (circle)   or 19 mCi (write in amount)   Comments:

HO5 GCR

Sc On 2:34   dose: _22_ mCi (circle)   or 19 mCi (write in amount)   Comments:

HO6 Th 2

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO7

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO8

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO9

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO10

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO11

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO12

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

HO13

Sc On   :   dose: _22_ mCi (circle)   or ____ mCi (write in amount)   Comments:

TOTAL # OF IMAGES: _____   Flip over for the rest of the scan list: Yes___ No___
STUDY CONTINUED? No___ Yes_____, from what study number? _____

PET Volunteer Enrollment Form

Name (last, first, initial): ███████████████████

Address: ███████████████ Egjan, MN 55122

Home Telephone: ███████████ Work Telephone:_____

Telephone & name of contact should you move:_____

Birth Date (m/d/y): ████████ Social Security # ██████████████

Veteran:(circle one) Yes (No) Occupation: Student

Weight: 115 lbs. Height: 5'2" Sex: (circle one) male (female)

Race or Ethnic Group: White   Handedness: R   Native Language German

Prior Research Radiation Studies: (circle one) Yes No   Education:_____

Medications:_____

Medical Conditions:_____

_____

Head Trauma:_____ Eye Conditions: 20/20 corrected Onset Last Menstrual Period_____

Surgery:_____

Psychiatric or Neurologic Conditions:_____

Drug or Alcohol Problems:_____ Learning Disability:_____

NOTE: *Please be sure to void just before starting the study.*

_____

PET Staff Use:

PPU# 38   Scanner Study ID P05647   Study Date 6/22/00 Study Time 130PM
Scanner 953 B   Mode 3D   Gantry Angle -6.9°   Head Holder Position_____
IV Line Locations (L) forearm Art Line ___ IV Lock Locations ___
Type of Infusion slow bolus Dose 13mCi Scan Duration 90s p̄ manual

petnrol.frm

Nursing intake notes:

_✓_ Arrived in PET suite per (amb), wc, stretcher

_✓_ Consent form signed. Questions:

Do what? _✓_; 2 risks? _✓_; If distressed? _✓_; No participate _✓_

_✓_ Protocol, risk, & benefits reviewed

Nursing exit notes

_____ left PET suite per (amb), wc stretcher

_✓_ self, with _____, to _____

Post scan instruction given _✓_ verbally ___ in writing ___ to other, confirmation of understanding of instructions.

Vitals: WT _115 lb_   HR _~60_   BP _____
measured   strong

José V. Pardo M.D. Ph.D. _____

Date: _6/22/00_   Name: _____

Cognitive Neuroimaging Unit

Rationale: _✓_ Research Volunteer

_____ Clinical

Indiction:

Dose: _91 mCi_ H_2 ^{15}O 13 mCi × 7

_____ FDG

Protocol # _2007_

SS# ███████████

Psychiatry PET Service

(Identify actions that were addressed)

1. _✓_ Protocol diet followed (Yes) No, N/A, (if NO explain)
2. _✓_ Voided _____ Urine/Blood test for pregnancy test POS, NEG, N/A
3. _✓_ IV placed, Right Arm, (Left Arm)
4. _____ A-Line placed, (N/A)
5. _____ EEG leads placed, (N/A)
6. _✓_ Safety and Comfort: _✓_ Allergies
   _✓_ Elbows and wrists padded
   _✓_ Pillows under knee _____ Blanket
   _____ Safety Belt (N/A)
7. _____ Blood sample drawn and taken to lab, (N/A)
8. _____ Voided _____ Instructed to drink fluids to flush urinary bladder, (N/A)
9. _____ Toleration of protocol _✓_ well tolerated;
   _____ other (Hypoglycemia, hypotension, anxiety, back pain) (state action on back of sheet).
10. _____ Follow up of clinically significant finding, N/A

W 116 16
H 5' 3"   } measured
160 cm

ECAT Study Number: p05647
Study Description: 1H2O-Auto
Date(dd.mm.yy):   22.06.00
Radiopharmaceutical: H2O
Radioisotope: O15
Patient Name: ████████
Patient ID (ss#):   ████████
Target Flush Period: 60 s
Pressure Release Period: 45 s
Target Fill Period: 20 s
Beam Period: 110 s
Create Period: 30 s
Transfer Period: 75 s
Lead Period: 10 s
Reservoir Volume: 15.00 ml
Reservoir Dead Volume: 0.65 ml
PreSyringe Dead Volume: 0.55 ml
Syringe Dead Volume: 1.00 ml
Patient Dead Volume: 5.89 ml
Syringe Diameter: 26.60 mm

PO 5647

```
Set Volume:          10.000 ml
Set Activity:        13.000 mCi
Set Duration:        30.000 s
Flush Rate:          60.000 ml/min
Scan w Activity (0) or Infusion (1): 0

Required Spec Act:   1.603 mCi/ml
Infusion Rate:       20.000 ml/min
Flush Volume:        29.450 ml
Flush Period:           29 s
```

Appendix I
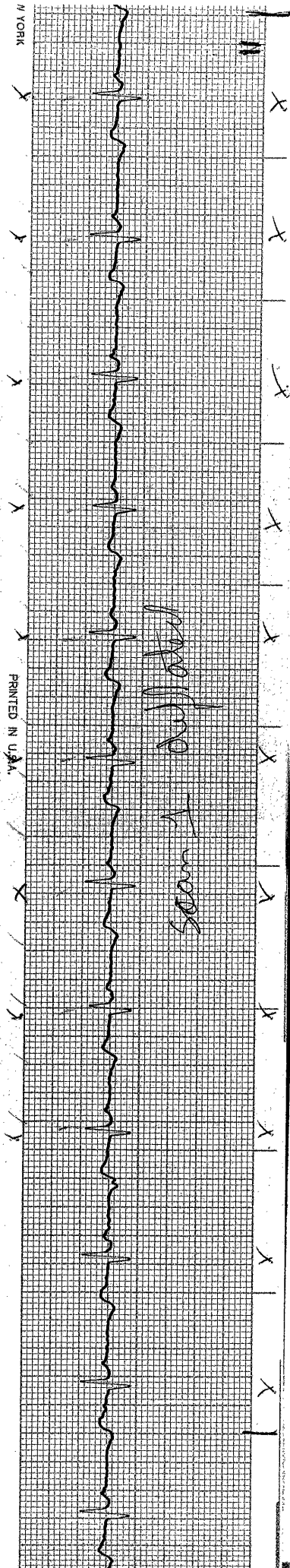
Appendix   J

GammaZ version 1.0.1

Number of data points: 1893
Number of positive data points: 782
Number of negative data points: 1050

Mean: -.3141E+00
  Number of points above the mean: 843
  Number of points below the mean: 1050

Distribution is split at the mean.

Standard deviation of all points: 0.2028E+01
Stnd. dev. of the top of the curve: 0.2249E+01
Stnd. dev. of the bot. of the curve: 0.1831E+01

Gamma 1 statistic of all points: 0.2129E+00
Gamma 1 t-statistic for all points: 0.3785E+01

  Too many points to test skew.

Gamma 2 statistic of all points: -.1260E+01
Gamma 2 of the top of the curve: -.1413E+01
Gamma 2 of the bot. of the curve: -.1182E+01

Gamma 2 t-statistic for all points: -.1121E+02
Gamma 2 t-statistic for the top points: -.8399E+01
Gamma 2 t-statistic for the bottom points: -.7839E+01
  Nota bene: Do NOT use the t-statistic for less than 2000 points

  Kurtosis:

  Bottom of curve:
  There is no significant leptokurtosis.
  For 1050 points significance levels are:
     .01 : 0.4000E+00   .05 : 0.2550E+00

  All points:
  There is no significant leptokurtosis.
  For 1893 points significance levels are:
     .01 : 0.2907E+00   .05 : 0.1907E+00

  Top of curve:
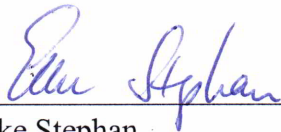 There is no significant leptokurtosis.
  For 843 points significance levels are:
     .01 : 0.4514E+00   .05 : 0.2814E+00

Cutoff at 0.1000E-02

   x,   y,   z,   max,   z-score,   significance level

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

_____

Elke Stephan   7.1.2008

                                                   Date