FUNCTIONAL MAGNETIC RESONANCE STUDY OF THE CENTRAL EFFECTS OF ACUTE ACUPUNCTURE ON GLUCOSE LEVELS AND CORE BODY TEMPERATURE IN MEN

By

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To the research group in the Life Science Research Center of XiDian University and Medical Image Processing Group of the Institute of Automation in the Chinese Academy of Sciences; Beijing University of Chinese Medicine; the Yijun Liu laboratory in the Department of Psychiatry; faculty and staff at the College of Veterinary Medicine; and Jesus Christ

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
Ach	Acetylcholine
ACU	Real acupuncture
AgrP	Agouti related protein
AMY	Amygdala
ARC	Arcuate nucleus
ARN	Appetite regulating network
BA	Brodmann area
BLA	Basolateral amygdala
BMA	Basomedial amygdala
BMI	Body mass index
BMR	Basal metabolic rate
BOLD	Blood oxygen level dependent
CART	Cocaine-amphetamine regulating transcript
CBT	Core body temperature
CN	Caudate nucleus
CNS	Central nervous system
CRH	Corticotropin releasing hormone
DA	Dopamine
DCT	Discrete cosine transform analysis
DLPFC	Dorsolateral prefrontal cortex
EAS	Electroacupuncture stimulation
EPI	Echoplanar imaging
FA	Food addiction
fMRI	Functional magnetic resonance imaging

FOV	Field of view
FWHM	Full width at half maximum Gaussian kernel analysis
GABA	Gamma-aminobutyric acid
GCM	Granger causality method analysis
GH	Growth hormone
Glu	Glucose
HIPP	Hippocampus
HYP	Hypothalamus
IAPS	International Affective Picture System
ILA	Infralimbic area
LA	Lateral amygdala
LHA	Lateral hypothalamus
MC4	Melanocortin-4-receptor
MCH	Melanin concentrating hormone
MFD	Mesolimbic frontocortical dopamine system
MNI	Montreal Neurological Institute
mOFC	Medial orbitofronal cortex
mPFC	Medial prefrontal cortex
mPVN	Magnocellular paraventricular nucleus
nACC	Nucleus accumbens
NCCAM	National Center for Complementary and Alternative Medicine
NIH	National Institute of Health
NPY	Neuropeptide Y
OFC	Orbitofrontal cortex
ORX	Orexin

PET	Positron resonance imaging
PL	Paralimbic area
POMC	Pro-opiomelanocortin
pPVN	Parvocellular paraventricular nucleus
PVN	Paraventricular nuclei
PWS	Prader-Willi syndrome
ROIs	Regions of interest
SCA	Seed voxel correlation analysis
SHAM	Minimal sham acupuncture
TCM	Traditional Chinese Medicine
THC	Delta-9-tetrahydrocannabinol
VMH	Ventromedial hypothalamus
VMPFC	Ventromedial prefrontal cortex
vPFC	Ventral prefrontal cortex
WFU	Wake Forest University
α-MSH	Alpha melanocyte-stimulating hormone

Abstract of Dissertation Presented to the Graduate School
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Obesity is a major health problem worldwide and acupuncture is an effective treatment modality for this condition. This study focused on how acupoints ST 36 (Zusanli) and SP 9 (Yuan Ling Quan) and their sham acupoints acutely act on the limbic system via dopamine to affect satiety, glucose (Glu) blood levels and core body temperature (CBT) in healthy, overweight, adult, non-dieting Chinese males who have abstained from eating 12 hours prior to the functional magnetic resonance imaging (fMRI) experiment. Results for Glu and CBT indicated no significance ($p > 0.05$) in both inter- and intragroup comparisons due to variable individual responses to treatment. Hunger survey feedback was significant ($p < 0.05$) between the acupuncture (ACU) and sham control (min SHAM) groups. Soreness or Deqi, was the only significant ($p < 0.05$) intergroup sensation. Connectivity analysis utilized the amygdala (AMY) and hypothalamus (HYP) as regions of interest (ROIs). Common overlapping regions for both ACU and min SHAM using the AMY as the ROI were the putamen, caudate, parahippocampus, hippocampus (HIPP), insula, cingulate, and Brodmann areas (BA) 6, 21, 22, 24, 25, 30, 31, 34, 36, 40, and 47. The common overlapping regions for both ACU and min SHAM using the HYP as the ROI were the HIPP, putamen, thalamus, and

BA 28, 32, 38, 40, and 47. These areas are involved in dopamine, basal metabolic rate, heart rate, cognition and satiety regulation. This project will be of great importance in helping understand how acupuncture can be a safe, inexpensive, and highly effective treatment modality for weight control.

CHAPTER 1
LITERATURE REVIEW

Introduction

Acupuncture, an ancient eastern therapeutic technique, is emerging as an important modality of complementary medicine in Western countries (Li et al., 1992; Knight et al., 2001; Ng et al., 2004). The scope of my acupuncture research is new, exploratory, and is in the early stages of development that specifically addresses human health-related issues. A variety of symptoms can be treated by acupuncture clinically (Anonymous NIH, 1998). However, the mechanism of how acupuncture works has not been clearly defined. Acupoint specificity has been under much scientific debate. For example, the visual cortex was activated by laser acupuncture at points BL 67 (Zhi Yin) (Siedentopf et al., 2002), LI 4 (Hegu), ST 36 (Zusanli), BL 60 (Kunlun), and BL 67 (Litscher et al., 2004). Li et al. (2003) induced visual cortex activation at four vision-implicated acupoints (BL 60, BL 65 (Shugu), BL 66 (Zutonggu), and BL 67). Acupuncture at K 3 (Taixi) activated the auditory cortex (Parrish et al., 2005). It was reported that PC 6 (Neiguan) was used to diminish nausea (Knight et al., 2001; Yoo et al., 2004; Bai et al., 2009a). Ng et al. (2004) showed that stimulation at HN 3 (Yintang), NH 8 (Yingxiang), and ST 36 was used to treat persistent allergic rhinitis in children. ST 36 and SP 6 (Sanyinjiao) are valid for visceral disorders (Li et al., 1992). Patients with lateral epicondylitis or tennis elbow can be treated at GB 34 (Yanglingquan) and ST 36 (Tsui et al., 2002). On the other hand, Gareus et al. (2002) did not detect significant blood-oxygen level dependent (BOLD) signal changes in the visual cortex when needling GB 37 (Guangming). Kong et al. (2009) indicated that electroacupuncture stimulation (EAS) induced no significant changes in the occipital cortex at BL 60, GB 37, and an adjacent non-acupoint.

Recently, Cho et al. (2006) retracted their early research results, stating there is no acupoint specificity. Most importantly, acupuncture has been used to specifically treat obesity and weight-related issues (for recent review see Cho et al., 2009).

The development of imaging techniques, such as positron resonance imaging (PET) and functional magnetic resonance imaging (fMRI), has provided new tools for us to obtain a non-invasive appreciation of the anatomy and physiological function involved during acupuncture in humans and animals (Lewith et al., 2005; Qin et al., 2008; Bai et al., 2009b). We currently use fMRI to answer questions relating to acupoint specificity and effectiveness with respect to obesity and the physiology of metabolism. Recent research has not addressed this area in overweight individuals. We believe the physiology and response to acupuncture differs between obese versus overweight individuals. Hence, using the International Affective Picture System (IAPS), we can further evaluate the hunger signal and response to food stimuli using fMRI as well.

Obesity and factors leading to being obese or overweight are an enigmatic aspect of scientific research on a global scale. It has been shown that obesity ranks second to tobacco-related deaths at about 300,000 deaths per year (Gold, 2003). Numerous studies in the past decade have attempted to shed light on not only resolving but also preventing obesity in both animals and humans (Kumanyika and Obarzanek, 2003; Calle and Kaaks, 2004; Gill et al., 2005; Seidell et al., 2005; Rubin et al., 2007; Galani and Schneider, 2007). Two of the most common genetic and neurophysiological causes of obesity, Prader-Willi Syndrome (PWS) and food addiction (FA) respectively, will be discussed in greater detail. PWS was chosen as a well-defined genetic model to support the necessity of this project since it may help explain certain neurophysiological

mechanisms that affect appetite and FA, which eventually lead to obesity, especially in children and young adults. It is ultimately better than an animal model since it can be directly studied and applied to human obesity conditions. Both PWS and FA are driven by similar feeding behaviors, obsessive thoughts and compulsions, and hormones. Currently, the mechanisms of these two disorders are still not well understood. This dissertation presents a general overview of the characteristics, causes, endocrinology, neuroanatomy, hypotheses, treatments and preventative measures of PWS and FA. PWS provides good genetic modeling for the mechanisms of obesity and is a good candidate for acupuncture treatment.

Background

Acupuncture Overview

Acupuncture involves stimulating different anatomical points in the body that exert different desired neurophysiological effects on the appropriate organ system based on Traditional Chinese Medicine (TCM) that has been implemented for over 3000 years. There are 14 meridians along which Qi or energy flows. There are well over 360 acupoints along these meridians (Cho et al., 2002). When these acupoints are stimulated, the desirable sensation known as Deqi is felt, indicating the release of opioid peptides. There are various sensations of Deqi reported such as soreness, numbness, tingling, and so on (Park et al., 2005; MacPherson and Asghar, 2006; Hui et al., 2007).

Acupuncture was not introduced into the United States until the 1970s. It was not until 1996 when the National Institute of Health (NIH) and National Center for Complementary and Alternative Medicine (NCCAM) concluded that acupuncture was efficacious for treating conditions such as nausea, arthritis, and others (Anonymous NIH, 1998). The controversy regarding acupoint specificity continues, hence

neuroimaging studies have been crucial in addressing this issue. PET and fMRI studies started in 1997 (Cho et al., 1997). There have been no direct publications that correlated clinical outcomes in pathological conditions with induced acupuncture changes in the brain. The trial design and data interpretation have been problematic in acupuncture research (Bai et al, 2009b). We devised our control technique based on a study by Kleinhenz et al. (1999) who used the Streitberger needle method.

Acupuncture Effects on Physiology

The most important aspect of this study will be to mechanistically depict how and why acupuncture (ACU) and minimal sham (min SHAM) affect glucose (Glu) homeostasis and hypothalamic regulation of core body temperature (CBT) and basal metabolic rate (BMR). Sun et al. (2007) studied how ghrelin centrally and peripherally affects Glu homeostasis. Ghrelin causes the release of growth hormone-releasing peptides and neuropeptide Y (NPY) as well as increases appetite (Kojima et al., 1999). It has been shown ACU decreases ghrelin, hence appetite decreases as shown by our unpublished preliminary results. Other key neurocircuits that control glucose metabolism are best reviewed by Rother et al. (2008). It is expected ACU will affect liver gluconeogenesis via insulin, its mediators and gastrointestinal afferents centrally carrying information regarding energy intake.

Acupuncture Affects Satiety

One focus of this study is to delineate the neurohormonal pathways associated with the hunger response. Pissios and Maratos-Flier (2007) proposed that central serotonin affects glucose homeostasis since inhibition of serotonin reuptake decreases appetite. Apparently the arcuate nucleus pro-opiomelanocortin (ARC POMC) neurons respond to serotonin as well as leptin and Glu, which are affected by ACU treatment

(Cabioglu et al., 2006). Low leptin and other adipokine levels during fasting stimulate food intake and decrease BMR (for full review see Ahima and Lazar, 2008). Leptin controls Glu and lipid metabolism via AMP-activated protein kinase and stearoyl-coenzyme A desaturase 1 in liver and muscle (Ahima et al., 2000), which may be targeted by ACU treatment. Brain regions involved in satiety that may be involved in this study are inferior parietal lobes, dorsolateral prefrontal cortex (DLPFC), and ventromedial prefrontal cortex (VMPFC) (James et al., 2004). Our lab has shown that there is a delayed hypothalamic response to reach satiety in obese individuals, hence it would be interesting to determine if that is the case in overweight individuals.

Acupuncture Treats Obesity

Obesity research has recognized that there are many factors contributing to this devastating disorder. Much debate has surfaced amongst scientists to develop models which might be useful in understanding why obesity has become a major health problem and epidemic. Numerous studies have shown that manual ACU (Lacey et al., 2003) as well as EAS (Hsu et al., 2005a,b; Cabioglu and Ergene, 2006; Cabioglu et al., 2006; Lee et al., 2006) are effective means for weight loss and weight control. One of our initial studies may contribute to help address areas in the brain activated by ACU that may suppress appetite and prevent weight gain by decreasing food intake. Another unpublished pilot study addressed how overweight individuals respond to visual stimuli before and after ACU or min SHAM treatments. James et al. (2004) showed activation in the insula, prefrontal cortex (PFC), amygdala (AMY), thalamus, nucleus accumbens (nACC), and ventral basal ganglia in hungry subjects when viewing food versus non-food related IAPS pictures, which we hope to see as well in future studies. Data from reward pathways showed that photographs of rich, fattening food induce significantly

greater activation than non-food object photographs in the left and right striatum (ventral striatum, putamen, caudate), as well as the midbrain (including the ventral tegmental area), left AMY, and left orbitofrontal (OFC) (Schur et al., 2009). These pathways integrate aspects of motivation for feeding with hypothalamic inputs on the state of energy balance (Kelley et al., 2005).

Causes of General Obesity

Joranby et al. (2005) defines obesity as “an imbalance between energy input and energy expenditure.” Obesity has been one of the most common health disorders affecting both modern and developing countries. Although it is well-known that overeating is the leading cause of obesity, the overall etiology is not well explained. Moreover, overeating has been classified as an addiction that comes from a variety of psychological as well as physiological causes. Another factor is the effect of emotions associated with the reward system of the brain (Joranby et al., 2005).

There exist other numerous theories that are beyond the scope of this dissertation as to why this occurs, such as deviant physiological processes involved in eating and homeostasis. It is well-recognized that the hypothalamus (HYP) is the key component for maintaining homeostasis in the body, and is responsive to signals that regulate food intake. If these signals are aberrant in any way, this could lead to delayed feelings of satiety in the individual (Joranby et al., 2005). The intrinsic and extrinsic signaling pathways will be discussed in more detail. Sahu and Kalra (1993) best describe which neuropeptides regulate food intake. These will be discussed in further detail.

Aside from the physiological explanations of obesity, the socioeconomic environment plays a crucial role in eating behavior. For instance, social and gender

roles, mass westernization and technological advances, and the media have all contributed to the growing population of overweight individuals. Becker et al. (2004) showed that there were three main factors that are responsible for obesity. First, many underdeveloped countries are going through westernization and are mimicking western eating habits. Second, the cultural portrayal of obesity as being undesirable and unsuccessful is changing eating habits, but not necessarily for the better. Finally, the roles of men and women have caused a shift in eating patterns as well. With more women being in the work force and often times seen as breadwinners in the family, this may lead to the entire family eating irregular, non-nutritious, fast-food meals. Although there are various genetic disorders leading to obesity, Prader-Willi syndrome (PWS) is one of its key genetic causes that will be discussed below.

Prader-Willi Syndrome Background: Causes and Symptoms

Individuals with the rare disorder, PWS, are identified as being genetically overweight since childhood. About 70% of cases are caused by a paternal genetic deletion on chromosome 15 (15q11-13), while 25% are from a maternal uniparental disomy of chromosome 15. The remaining 1-5% of PWS cases result from certain imprinting defects, which have a 50% risk potential to recur in future offspring (Glenn et al., 1997; Nichols and Knepper, 2001, Benarroch et al., 2007). There is a loss of specific brain genes such as MKRN3, MAGEL2, NDN, SNURF-SNRPN, and *sno*-RNA that are misrouted or lost resulting in abnormal cortical development in PWS (Pagliardini et al., 2005). These genetic anomalies can be detected by DNA methylation analysis and *in situ* hybridization of the alleles (Benarroch et al., 2007).

Human subjects with PWS are characterized as having dolichocephaly, almond-shaped eyes, small mouth, hands, and feet, decreased muscle mass and tone

(Cassidy, 1997), infantile hypotonia, early onset of obesity due to central dysfunction (around 18 to 36 months of age), hypogonadia, short stature (Goldstone, 2004), and show major disturbances in appetite, sleep, breathing and metabolism regulation, such as delayed satiety, premature return of hunger after eating a meal, seeking and hoarding food and food-related objects, and ingesting inanimate items (Miller et al., 2007a) as well as excessive daytime drowsiness, poor ventilation, hypercapnia, and dental caries (Nixon and Brouillette, 2002). Overall, many systems are affected by PWS such as the central nervous system (CNS), gastrointestinal, urogenital, cardiovascular, respiratory, and dermatologic resulting in numerous medical conditions and disorders (Benarroch et al., 2007).

Anatomically, PWS individuals have speech and language impairments as a result of perisylvian abnormalities including ventriculomegaly, sylvian fissure polymicrogyria, and incomplete sylvian fissure/insula closure (Miller et al., 2007a,b,c). One particular defect, the failure of growth over the insula and underdevelopment of the frontal, temporal, and parietal opercula may be caused by the lack of paternally expressed genes relating to cortical development. Insular malfunction is noted in PWS individuals since they have poorly functioning pain perception and autonomic control (Goldstone, 2004). This may be explained by incomplete insular closure leading to differences in white matter connectivity between the cortex and the insula (Miller et al., 2007a). Miller et al. (2007a) hypothesized that the aberrant appetite in this condition resulted in irregular reward processing of food stimuli in brain pathways involving the HYP, frontal cortex, insula, and limbic/paralimbic areas. Post-mortem results have shown a decreased number of cells in the paraventricular nucleus (PVN) (Swabb et al.,

1995). This is crucial evidence for explaining why satiety is difficult to attain, since the PVN is the hunger center of the brain that controls appetite (Kalra and Kalra, 2004b). What is most interesting about this condition is that PWS hyperphagia is not responsive to pharmaceutical treatment (Holland et al., 1993).

Food Addiction Background: Causes and Symptoms

Addiction is classically defined as a chronic relapsing problem caused by various fundamental factors that encourage craving for certain substances, such as food, in order to obtain a state of heightened pleasure, energy, or excitement (Tartar et al., 1998). An example of this would be carbohydrate cravers that have learned to consume high carbohydrate foods to improve their mood caused by a drop in serotonin levels (Wurtman and Wurtman, 1995). A study by Spring et al. (2008) showed convincing evidence of this phenomenon.

Most food addiction is the result of loss of control, impulsive and/or compulsive behavior that results from emotional and environmental conditions, and a psychological dependence on food. Eating behaviors are similar to those of other addictions since both affect the levels of dopamine (DA) in the mesolimbic dopaminergic system (Mogenson, 1982). DA D2 receptors have a high prevalence of Taq I A allele (Noble et al., 1994), meaning that this allele has been linked with low levels of these receptors in obese individuals (Noble et al., 1991). These patients use food to raise their DA levels, even through positive reinforcement (Noble et al., 1997). It has been shown that the activity of DA in the brain can be related to abnormal eating behavior (Jimerson et al., 1992).

One study showed that the predisposition to food addiction in offspring was caused by feeding rat mothers junk food consisting of fatty, sugary, and salty snacks

during pregnancy and lactation. Rat offspring showed increased weight gain BMI compared to controls, while their mothers displayed bingeing and overeating of junk food (Bayol et al., 2007). Thus, these findings may be applied to pregnant women's diets in order for them to have healthy children with normal appetites and weight.

Avena and colleagues (2004; 2005; 2008) found evidence for sugar addiction in rats, since it was a good animal model to describe why certain people crave sweets or other delicious foods, and why it is difficult for them to wean themselves from such an eating behavior. Sugar has been found to be an addictive substance since it releases opioids and DA, which are characteristic of addiction neurochemicals. The group classified sugar as an addictive substance because it follows the typical addiction pathway that consists of bingeing, withdrawal, craving, and cross-sensitization. The definitions of the components of this system are as follows: bingeing consists of "unusually large bouts of intake" (Colantuoni et al., 2001); withdrawal is "indicated by signs of anxiety and behavioral depression" (Colantuoni et al., 2002); craving is "measured during sugar abstinence as enhanced by responding to sugar" (Avena et al., 2005); and cross-sensitization results "from sugar to drugs of abuse" (Avena et al., 2004). Bingeing is also defined as "escalation of intake with a high proportion of intake at one time, usually after a period of voluntary abstinence or forced deprivation" (Avena et al., 2008). It consists of sensitization and tolerance, which are necessary for the initiation of any form of addiction (Koob and Le Moal, 2005). Withdrawal has been known to be caused by alterations in the opioid system (Colantuoni et al., 2002). It consists of two parts, in which DA decreases and acetylcholine (Ach) is released from the nACC. When sugar was analyzed with regards to withdrawal symptoms, it was

capable of producing DA, Ach, and opioids similar to most narcotic substances (Avena et al., 2008). It is marked by anxiety (File et al., 2004) and depression (Avena et al., 2008). Craving usually happens after a prolonged period of abstinence and is better defined by “increased efforts to obtain a substance of abuse or its associated cues as a result of dependence and abstinence” (Avena et al., 2008). Cross-sensitization is predominantly defined as “an increased locomotor response to a different drug or substance” (Avena et al., 2008). All of these definitions play a major role in helping define and classify food as an addictive substance in comparison to the criteria for drug dependence (Haddock and Dill, 2000).

On the contrary, Haddock and Dill (2000) explained that the addictions model of obesity and eating disorders was flawed and could not be compared to that of drugs. They quoted various studies (Wilson, 1991; Parham, 1995; Wilson, 1999) to state that food was not a psychoactive substance. However, the studies used to support their arguments were outdated and the studies by Avena et al. (2004; 2005; 2008) showed that sugar itself can be classified as an addictive substance.

Shared Anatomical Areas in the Brain

The brain areas involved in satiety include the HYP, orbitofrontal cortex (OFC), insula, inferotemporal cortex, nACC, ventromedial prefrontal cortex (vmPFC), limbic and paralimbic regions. They are supposed to be involved in reward, arousal, motivation, memorization, and emotional responses to food and eating (Tataranni and Delparigi, 2003). The OFC has been found to link food and rewarding experiences (O’Doherty, 2004), thus if lesions are found in this region, this may result in hyperphagia in PWS patients (Miller et al., 2006). In most obese subjects, food is more palatable and enjoyable through the postcentral gyrus of the left and right parietal cortex (Wang et al.,

2002). The nACC and ventral tegmental area (VTA) are known for reinforcing behavior such as feeding (Gold, 2003). It is a key area in the reward system, which will be described later, since it encourages food-seeking behavior, learning incentive, motivation, satiety, and stimuli processing (Bassareo and Di Chiara, 1999).

In animal models, damage to the ventromedial hypothalamus (VMH) leads to hyperphagia and increased appetite, while damage to the lateral hypothalamus (LHA) causes hypophagia and decreased appetite (Liu and Gold, 2003). However, the dorsal striatum is most important in the motivation of consuming food. In a study by Volkow et al. (2002), when DA deficient mice were treated with DA in the dorsal striatum, feeding behavior was restored. These animals also chose more palatable food over that which was not. Mice that were not given DA in the nACC were capable of initiating feeding behavior; those that were treated with DA in the nACC chose more palatable foods over non-palatable ones, even though they had no motivation to eat enough food to maintain normal function and to stay alive. In another study, DA agonists were used to increase the portion size of meals and length of feeding, while long-term administration of DA increased body mass and feeding behavior (Schwartz et al., 2000). When methylphenidate was administered to human subjects as a DA agonist into the striatum, those that got the placebo did not show any increase in appetite. Thus, the ventral striatum was localized as the site for appetite control (Wang et al., 2004). These studies are critical to localize the areas of the brain that can be identified and used to treat PWS and FA.

Hypothalamic Regulation of Appetite/Afferent Hormonal Signaling

The HYP is a key component to feeding behavior, thus it is important to look into its regulation in more detail. There have been five individual areas identified in the HYP

that regulate feeding behavior and metabolism (Kalra et al., 1999). Medial areas of the HYP control food intake and energy homeostasis. These regions obtain important information from referring organs and systems that are involved in nutrient and metabolite consumption and distribution, as well as involvement in hyperphagia and obesity (Berthoud, 2002). Ghrelin and leptin have been known to target the HYP in regulating feeding behavior. Leptin activates its receptors so that NPY, orexin (ORX), β-endorphin, and alpha melanocyte-stimulating hormone (α-MSH) can decrease appetite stimulation (Kalra and Kalra, 2004b). Leptin has been shown to have an important role in appetite control. Leptin can suppress ghrelin expression at the level of the NPY neurons (Bagnasco et al., 2002a,b). The roles of leptin and ghrelin feedback on the appetite regulating network (ARN) are crucial for energy homeostasis and appetite (Kalra et al., 2003). If there is a drop in leptin levels in the blood, the ARN is stimulated to release orexigenic NPY, agouti related protein (AgrP), and gamma-aminobutyric acid (GABA) along with an inhibition of anorexigenic α-MSH (Kalra and Kalra, 2004b). This can be best summarized by Erlanson-Albertsson (2005).

There has been interesting evidence explaining how leptin regulation is affected by factors predisposing an individual to obesity. If an animal consumes too many energy-laden calories and is inactive, this promotes hyperleptinemia and fat accumulation in the body (Kalra et al., 1999). Leptin transport and production in the HYP is limited, and excess leptin in blood circulation is unable to control appetite (Bagnasco et al., 2002a,b; Kalra and Kalra, 2002). This may be a significant factor to look into regarding overweight individuals.

Endocrinology of Obesity

Defining the endocrinology behind obesity is crucial to understanding FA and overeating behavior. Most childhood obesity is the result of genetic defects in leptin and its receptor, POMC, pro-hormone convertase-1, melanocortin-4 receptor (MC4), and ghrelin genes (Farooqi and O'Rahilly, 2000). This is why PWS is the first and foremost genetic model for obesity that demonstrates leptin resistance as one of the primary causes of obesity. In PWS cases, leptin levels are increased causing an inability to produce an anorexigenic effect (Proto et al., 2007), while numerous studies have indicated other hormonal and metabolic disorders that may be associated with hyperphagia including impaired growth hormone (GH) secretion and low insulin production (Zipf et al., 1983; Goldstone et al., 2001a,b; Cummings et al., 2002; Goldstone et al., 2002; Goldstone et al., 2004). People with FA who are obese may possibly have leptin resistance as well that leads to overeating (Liu and Gold, 2003). Hyperphagia is primarily due to continuous stimulation of NPY receptors (Kalra and Kalra, 1996). An imbalance of NPY signaling at a local level (ARC and PVN) results in unregulated eating (Kalra and Kalra, 2004b). The neurotransmitter GABA has also been known to enhance feeding behavior via its receptors or directly in the ARC, causing decreased melanocortin signaling to the PVN, which in turn results in hyperphagia (Cowley et al., 2001). It is possible that mutations or disturbances of α-MSH and other peptides involved in satiety can lead to hyperphagia and obesity (Kalra et al.,1999).

The key neurotransmitter of addiction, DA, has site-specific action regulating the intake of food; it reinforces the effects of food (Salamone et al., 1997). DA is necessary to begin the meal process (Meguid et al., 2000). It acts upon the prefornical area, VMH, and ARC to reduce the consumption of food and prevent hyperphagia, which in turn is

affected by leptin, insulin, and other hormones (Baskin et al., 1999). It may be inferred that disruptions in DA production and/or structure may predispose certain individuals to addictive behaviors and obesity.

There has been some interesting work done by Solinas and Goldberg (2005) regarding how cannabinoid and opioid interactions affect motivational effects of food reinforcement, and increase appetite and food consumption. Delta-9-tetrahydrocannabinol (THC) and morphine increased the reinforcement effects of food. *Mu* receptors are involved in the effects of THC and cannabinoid-1-receptors, and those are involved in the actions of morphine. Morphine and THC have orexigenic effects that promote appetite and food consumption. The reason for this is that high amounts of endocannabinoids (Gonzalez et al., 1999; Howlett, 2002) and high levels of opioid peptides are located in the HYP (Mansour et al., 1987). The main concept is that THC and morphine not only responded to food stimuli, but also enhanced palatability of the food (Cooper, 2004).

Abnormal hypothalamic function accounts for a variety of eating disorders, leading to hyperglycemia, which in turn causes other endocrinological problems (Liu and Gold, 2003). This may be explained by one dietary example where fructose was consumed. Fructose promotes insulin production but blocks its release (Sato et al., 1996). Insulin is known to inhibit feeding by increasing leptin which in turn leads to weight gain (Saad et al., 1998). Hence, this would be a good model to explain why FA individuals are overweight and are addicted to high carbohydrate foods containing high fructose corn syrup. A great comparison of peptides involved in appetite control and

how high fat and carbohydrate diets affect them is portrayed by Erlanson-Albertsson (2005).

Orexigenic and Anorexigenic Pathways

It is necessary to discuss the interactive pathways that regulate appetite and cravings. The ARN has appetite enhancing and reducing circuits that are located in the ARC-PVN axis of the HYP. It is affected by signaling from the LHA and VMN (Kalra and Kalra, 2004b). These particular pathways have their components synthesized in the ARC and are targeted at the parvocellular PVN (pPVN) and magnocellular PVN (mPVN), which may provide insight on the mechanisms of overweight subjects. The release of these neurochemicals is regulated primarily by the VMN and LHA (Kalra et al., 1999). Kalra and colleagues (1999) showed that if there was a disruption between these two sites, then the affected individual would overeat and gain weight, as seen in PWS and FA. This suggests that the VMN is responsible for inhibiting signals to the ARC. Certain areas in the LHA that express ORX or MCH increase NPY release, thereby stimulating appetite. Thus, if there is non-stop stimulation of NPY receptors, then the satiety signal to the HYP is inhibited resulting in continuous eating (Kalra et al., 1999), which is a typical symptom in overweight individuals. What is interesting is that despite this happening, there is no known receptor down-regulation for NPY (Kalra and Kalra, 1996). It was shown that during the absence or decrease of food intake, NPY levels increased in the ARC in order to stimulate appetite (Kalra et al., 1999).

The neurotransmitter GABA has also been known to stimulate appetite by its receptor activation or by administering NPY. GABA by itself can decrease melanocortin signaling to the PVN in order to stimulate appetite (Cowley et al., 2001). Another orexigenic peptide, AgrP, enhances eating by antagonizing MC4, which are responsible

for curbing appetite (Kalra et al., 1999). Ghrelin has been shown to increase appetite by increasing NPY signaling (Kalra et al., 2003). LHA neurons express ORX and MCH, and ARC neurons coexpress NPY; AgrP and GABA are the key components of the hypothalamic orexigenic pathway (Kalra and Kalra, 2004b). Manipulation of this pathway can provide novel insight for the treatment of obesity in general.

Anorexigenic pathways are responsible for controlling the inhibition of appetite. The melanocortin pathway's crucial component is the ARC-PVN axis where POMC neurons coexpress α-MSH, while cocaine- and amphetamine-regulating transcript (CART) acts upon PVN to curb appetite (Kalra and Kalra, 2004b). Inhibition of feeding is regulated by MC4 receptors that act on α-MSH. During the hunger state, POMC gene expression is decreased, thus α-MSH release decreases as well (Kalra and Kalra, 2004b). Another anorexigenic pathway consists of corticotropin releasing hormone (CRH) neurons in the PVN, which release CRH due to stress to inhibit NPY-induced food intake. Feeding regulation by anorexigenic neurochemical signals consists of the links between NPY and POMC, and between NPY and CRH (Morley, 1987).

Food Cues: Internal and External Appetite Triggers

Food cues and motivation are crucial aspects of food intake. Most environmental cues result from Pavlovian conditioning that can overrule satiety and enhance food ingestion in the network associated with feeding including the AMY, LHA, and mPFC. Other motivational cues paired with eating during the hunger state can easily override satiety and promote eating in sated rats, which results from signals via the forebrain and LHA (Petrovich and Gallagher, 2007).

In the developed and developing world, people are constantly surrounded by food cues to enhance consumption of not necessarily healthy foods. There are diverse

examples of how food cues affect a specific group of individuals. This section will focus on how food cues affect those with obesity as compared to normal humans based on the animal model.

Certain brain circuits and networks are responsible for cue-induced eating, appetite induction, as well as specific food cravings. Kalra and Kalra (2004a) provide a thorough overview of pathways regulating appetite and cravings. The ability of food-related cues and a food-associated environment to induce eating in healthy humans can shed light on why PWS individuals overeat and become overweight. In the animal model, the brain regions consisting of the basolateral amygdala (BLA), mPFC, and LHA act as a network to regulate eating by learned, motivational cues. In neuroimaging studies, the AMY has been shown to be pivotal in its role in cue-enhanced eating (Arana et al., 2003); it has been suggested that the AMY is involved in appetite activation and maintenance in humans (Tataranni et al., 2003; Gottfried et al., 2003; Killgore et al., 2003; Kringelbach et al., 2003; Hinton et al., 2004). The OFC is also involved in food-related cues (Arana et al., 2003). The mPFC is crucial for eating due to environmental cue pressure (O'Doherty, 2004). The AMY shares tight connections with the HYP (Petrovich et al., 2001). Activations of the AMY and medial orbitofrontal cortical area (mOFC) occur when food-deprived individuals are shown food items relative to non-food items, and greater activations are seen when food items are viewed (Arana et al., 2003; Hinton et al., 2004).

On the other hand, normal eating signaling results from a response to decreased energy, but it can be triggered from environmental or learned cues, which can alter the motivation for food consumption. Petrovich et al. (2001) presented findings associated

with major connections formed between the BLA and LHA. These are responsible for processing learned cues in order to forego the satiety signal and promote eating in sated rats. The BLA shares anatomical connections with the HYP in order to control feeding behavior (Elmquist et al., 1999; DeFalco et al., 2001; Petrovich et al., 2001). A portion of the BLA, which originates in the basolateral nucleus, directly innervates the LHA (Petrovich et al., 2001). It sends vital projections to the LHA (Petrovich et al., 2001), which forms part of the feeding circuit associated with the initiation of feeding (Elmquist et al., 1999). The BLA–LHA system is crucial for allowing learned cues to override satiety signals and stimulate eating during satiation. It was shown that the BLA–LHA system is specifically associated with controlling eating via learned signals, because it does not regulate baseline eating or the rate at which rats gain weight when fed *ad libitum*. This was primarily due to an associative process in which food ingestion was directed by a cue paired previously with food but not an unpaired one (Petrovich and Gallagher, 2007).

When research animals are presented with food cues, they consume more food despite being sated. Visual cues for foods that have a higher incentive value produce greater activation in the AMY than foods that were recently eaten to satisfy hunger signals (Gottfried et al., 2003). In general, craved or highly palatable foods will activate the AMY regardless of sated state (Hinton et al., 2004).

In a recent study by Petrovich et al. (2007), cellular activation markers in the vmPFC neurons were activated following exposure to a newly-conditioned cue that stimulated eating in sated rats. When neurotoxic lesions were created in the vmPFC, this caused impaired food consumption as a result of conditioned motivational cues.

Thus, the vmPFC has a significant role in appetite influenced by motivational cues. Brain lesions did not affect eating in the pretest baseline sessions or the rate at which rats gained weight when fed freely. Rats with selective bilateral neurotoxic lesions in the central nucleus showed enhanced feeding when a conditioned stimulus was applied (Petrovich et al., 2007). Lesions in the BLA, basomedial (BMA), and lateral (LA) nuclei of the AMY as well as the LHA result in decreased food consumption and non-responsiveness to appetite stimulation cues (Petrovich et al., 2007). This part of the AMY controls food consumption via extrinsic cues (Petrovich et al., 2001). Given its important role in goal-oriented behavior (O'Doherty, 2004), the omPFC could play a pivotal role in regulating the impulse to eat in response to highly appetitive cues in PWS and overweight subjects.

From a neurohormonal perspective, glutamate is believed to be the neurotransmitter responsible for transmitting information between the areas depicted above, although the exact mechanism is still not understood (Swanson and Petrovich, 1998). Glutamatergic mechanisms within the LHA have been shown to promote feeding in sated rats (Duva et al., 2001). It is then plausible that potential feeding mechanisms involve direct glutamatergic connections from the BLA to LHA, although the exact LHA neurons involved in this process remain unidentified. It may be safe to assume that BLA outputs could influence LHA subsystems required for feeding initiation. Groups of LHA neurons express two recently discovered neuropeptides, MCH and orexin, which are regulated by the hunger–satiety state and are linked to initiation of feeding (Elmquist et al., 1999). Leptin and NPY have an opposite effect on food ingestion. Leptin inhibits feeding while NPY promotes eating (Schwartz et al., 2000). Learned cues can utilize

BLA to activate NPY or inhibit leptin. The BLA-LHA junction must be intact in order to initiate food cue-related eating (Kelley, 2004). Hunger caused by food cues is an adaptive mechanism for survival, but at the same time, learned cues can serve as a harmful force to promote overindulgence in food despite satiety. These particular learned cues can overcome specific satiety signals in order to promote continued eating (De Castro, 1997). This may be the situation in PWS and overweight patients.

Metabolic factors and non-homeostatic signals control motivational eating. Despite no clear definition of food cravings, specifically in animal models (Weingarten and Elston, 1990), cravings for food in humans can be elicited by food cues and are often associated with hedonic overeating (Jansen, 1998; Fedoroff et al., 2003; Sobik et al., 2005; Tiggemann and Kemps, 2005). Regions of the PFC may also participate in brain networks involved in cue-induced drug cravings. Other regions overlapping the vmPFC are also activated by chocolate- and nicotine-associated contextual cues in rats (Schroeder et al., 2001). Additional studies are needed to determine if this rat model parallels the role of the mOFC in human appetite and cravings. The lateral OFC is not needed for food consumption, but the ventral areas within the rat vmPFC could represent a functional counterpart for the mOFC in humans (Ongür and Price, 2000). DA also plays a critical role in food consumption stimulated by unpredictable cues (Roitman et al., 2001). DA efflux within the vmPFC resulting from signal-induced satiety was correlated with decreased consumption of high caloric, sweet and fatty foods; this may be the case in overweight individuals. Human OFC activation decreased in response to an olfactory cue of food eaten to satiety but not to an odor of uneaten food

(O'Doherty et al., 2000). This may be a key point as to why food addicted overweight individuals continue to overeat despite satiety.

Neophobia, a species-specific adaptive response to novel food, suggests a role for the intact vmPFC in regulation of unlearned adaptive feeding responses, as seen in the Petrovich group's (2007) study which showed the effects of lesions in this region. Rats with neurotoxic lesions of the prelimbic area (PL) were given a choice between familiar lab chow and a novel, preferred food in an unfamiliar, open field environment. Rats with lesions had an increased tendency to consume the novel food more and tended to eat the familiar food less as compared to controls. The amount consumed of the two foods was similar for rats with lesions than those without lesions. Food neophobia in rats was increased (Petrovich et al., 2007) and decreased with mPFC lesions. Lesions were located in the PL and infralimbic area (ILA) (Petrovich et al., 2007), along with damage to the adjacent ventral PL and mOFC regions. This may suggest different roles of the sub-regions within the vmPFC in food neophobia. The vmPFC seems to be crucial in controlling eating impulses based on environmental cues. A dysfunctional vmPFC could mechanistically depict feeding behavior in PWS or overweight humans relevant to overeating, appetite, cues and cravings.

Explicit food-associated cues during the hunger state will enhance food consumption despite subsequent satiety. Other studies link the nACC and dopaminergic brain systems to motivation and food reward (for reference reviews see Cardinal et al., 2002; Berridge and Robinson, 2003; Phillips et al., 2003; Kelley, 2004; Wise, 2004). Currently, there are no valid studies showing whether DA is necessary for cue-potentiated eating. Thus, individualized sub-circuits may be integrated into a vast,

combined system depending on the underlying processes controlling motivation to consume food or to seek out highly desired foods via external cue activation. More research is needed to elucidate the precise mechanisms of food consumption through different aspects of learning (Petrovich et al., 2007), despite a lack of a definition of food craving in animal models (Weingarten and Elston, 1990). Even human cravings have been defined with uncertainty especially in overweight individuals. Craving for food can be induced by exposure to food cues (Tiggemann and Kemps, 2005), and cue-elicited craving is associated with binge eating (for reviews see Jansen, 1998; Sobik et al., 2005). Cue-induced eating could be considered binging, since Petrovich et al. (2007) showed that sated rats consume more food pellets in a short period of time. A recent human study (Fedoroff, 2003) showed that in diet-restricted eaters, food cues elicited specific cravings for the cued food, as opposed to a general desire to partake in non-craved food. As the craving for the desired food increased, the restricted dieters consumed more of the cued food (Fedoroff, 2003).

Brain systems and mechanisms that dictate food reward learning were correlated with drug addiction (Cardinal and Everitt, 2004; Volkow and Wise, 2005). Contextual cues, used to stimulate eating, are also very powerful cues in drug addiction craving and relapse (Crombag and Shaham, 2002). Koob and Le Moal (2005) argued that drug addiction is the ‘dark side’ of the reward neurocircuitry in the form of impulsive to compulsive behavior much like that displayed in PWS. Wang et al. (2004) conceptually reviewed the similarity between obesity and drug addiction using neuroimaging techniques.

The main role of the external feeding environment in food intake depicted by an animal model might be relevant to PWS and overweight human eating. In fact, the environment in which food is consumed has been changing over the past 30 years in the United States. Increasingly disproportional food portions are served and eaten in distinct environments such as restaurants and fast food places (Nielsen et al., 2002). Advertising on television further elicits food cues encouraging even normal weight children and adults to seek out food despite lack of hunger signals. On the other hand, external food cues can be depicted in a much simpler fashion than the internal cues described above. In PWS patients, obsession and preoccupation with food, lack of satiation, and incessant food seeking are typical behaviors as compared to normal obese humans (Holm et al., 1993; Dimitropoulos et al., 2000; Lindgren et al., 2000; Ogura et al., 2008). PWS adults show preference for sweet or high carbohydrate foods over any other type of food. This is sometimes the case in normal obese individuals (Ogura et al., 2008; Singh et al., 2008). PWS patients will often eat the most desirable foods first, such as sweet, high caloric foods, and the least preferred foods last. Oftentimes, this is a ritualistic procedure in which the PWS-afflicted individual will gather the food and line it up in order of preference and ingest it sequentially (Singh et al., 2008). Since PWS cases are often highly affected by visual cues, even more so than normal overweight adults, environmental cues are of greater pertinence. For instance, passing by a bakery or restaurant, or even seeing sweet or highly palatable foods on television, will cause an enormous increase in craving and appetite despite satiety in PWS individuals as compared to normal overweight ones. Oftentimes, PWS patients will have tantrums and aberrant behavior after seeing or smelling delicious, inviting food

(Singh et al., 2008), which is highly uncommon in non-PWS people. It would almost appear that in PWS, food cues (visual) have a very high emotional attachment and significance that leads to bingeing episodes (Simmons et al., 2005).

The key to these disorders is that anything that alters the satiety and hunger signaling may involve binging on food. In FA, the act of eating itself brings pleasure but in PWS, eating is pleasureless (Liu and Gold, 2003). Overall, much progress has been done in order to fully understand the anatomical, functional, and neurohormonal mechanisms of PWS and FA which would help explain the ultimate causes of obesity in the general population. Food–induced hyperphagia uses the hypothalamic neural network and afferent signaling to control appetite, but it still does not define if this mechanism is due to low energy stores. New therapeutic options, especially ACU, can then be utilized to curb food cravings and help control weight in human individuals.

Reward System Hypothesis

In drug-related addictions, it has been shown that the ventral striatum and midbrain were associated with immediate rewards and the HIPP responded to reward consequences. The globus pallidus, thalamus, and subgenual cingulate were associated with immediate rewards, while the caudate, insula, and vPFC responded to reward consequences (Elliott et al., 2000). The mesolimbic reward system is a common pathway that substances of abuse, such as food and drugs, follow in order to reinforce craving behavior (Tartar et al., 1998). Reward processing is linked to addiction and is processed only if it can promote the addicted individual in pursuit of the addiction. This then explains why addicts are more prone to seeking rewards, such as food, rather than facing the consequences of the reward behavior (Joranby et al., 2005). The fundamental idea of the reward system lies in the fact that there must be an emotional

state connected with the addiction. The stronger the emotional link, the stronger the addiction. There exist a couple of primary circuits for the reward system. The first one involves a reciprocal connection between the prefrontal areas of the brain and the AMY. The second is the limbic system that links the AMY with the HYP and septal nuclei. The Papez limbic system also joins the HYP with the HIPP and thalamus (Joranby et al., 2005). The reward system hypothesis states that appetizing food and addictive behaviors compete for reward regions (such as the nACC) in the brain. The act of overeating and obesity can lead to decreasing food reward and addiction (Kleiner et al., 2004). Anticipating and ingesting appetizing food causes an increase in DA levels in the nACC (Hernandez and Hoebel, 1988). Obesity is a “reward deficiency syndrome” (Blum et al., 1996a,b) since DA D2 receptors are mediators of reinforcement and compulsiveness, and obese subjects were found to have lower levels of these receptors in the striatum (Wang et al., 2001). The common pathway for addiction involves the mesolimbic frontocortical dopamine (MFD) system, which is a reward pathway that controls eating behavior. Addictive behaviors release DA in the reward pathway causing almost immediate positive reinforcement (Hodgkins et al., 2003). Increased activation in the somatic parietal areas in FA individuals suggests that enhanced activity in these regions involves sensory processing of food, which may make food even more rewarding (Wang et al., 2003). Morris (2001) showed that the state of hunger can be influential on the memory of food-related stimuli in fasting individuals. In the study by Joranby et al. (2005), they found that the activity of the brain was regulated depending on the stimulus it received. The right anterior OFC had a variable response to all stimuli despite hunger, while the right posterior OFC had different responses only with food-

related stimuli during hunger. The posterior area was associated with general rewards, while the anterior part was associated with abstract and goal-oriented rewards.

As mentioned earlier, studies done in sugar-dependent rats would help define the reward hypothesis (Avena et al., 2005; Rada et al., 2005; Avena et al., 2008; Bayol et al., 2007). It is well-known that certain drugs of abuse release more DA in the nACC; Rada et al. (2005) found that it was the same in rats eating highly palatable food, especially sugar. They found that sugar-dependent rats had a delayed Ach response for satiety, imbibed more sugar, and released more DA than controls. It was questionable if sugar had a similar mechanism to opioids on satiation and the lowering of Ach in the nACC. It was found that sugar indeed promoted satiety causing the release of Ach (Rada et al., 2005). Just one event of showing palatable food is enough to increase DA, which has been shown to be correlated to taste recognition (Di Chiara, 2002). Rats that were known to consume a large quantity of sugar had a delayed release of Ach, resulting in overeating of sugar (Rada et al., 2005). This may explain why FA individuals may be addicted to certain palatable foods that cause a delayed, prolonged increase in Ach levels.

Neuroimaging Studies

Neuroimaging studies, such as done by our lab, have shown that aberrant eating behaviors and obesity have altered the brain chemistry (Liu and Gold, 2003) as well as the anatomy (Miller et al., 2007a,b,c) of affected PWS and FA individuals. In an fMRI study by Shapira et al. (2005), PWS patients had delayed blood oxygen level dependent (BOLD) responses in the HYP during rest and after Glu ingestion in the frontal cortex after viewing food pictures. This was probably due to defects in the HYP resulting in abnormal reward processing that led to calorie overloading. It is well-known in fMRI that

the frontal cortex is involved in linking food and other rewarding objects with hedonism (O'Doherty, 2004). It was found that obese individuals ate more food than controls when food cues were present during a food-directed reinforcement task (Johnson, 1974). Wang et al. (2004) found that when subjects viewed delicious food, the anterior insula and right OFC, brain regions that are involved in the DA system, were activated.

Another interesting study looked at dissociated responses in the AMY and OFC using bottom-up and top-down approaches (Wright et al., 2008). The bottom-up phenomena is stimulus-driven and the top-down one is task-driven, meaning that emotional evaluation pertaining to obesity is controlled by different neural systems as depicted by fMRI. Wright and colleagues (2008) showed that the AMY is associated with bottom-up processing, and the OFC and vmPFC is linked with the top-down approach. Regarding obesity, this can explain how emotions about food are associated with the OFC, and how satiety and the reward hypothesis are correlated with the vmPFC and ACC. This information is useful in what areas of the brain need to be targeted for future treatment and intervention.

Hyperphagia is thought to be a contributing factor to increased caloric intake and hence obesity. Interestingly, there have been many unverified animal and human models that have sought to mechanistically identify the sole causes of aberrant appetite control leading to weight gain. PWS is a biological model for hyperphagia and the reward system utilized to explain human obesity using fMRI. Neuroimaging would be the most logical tool in precisely locating the brain regions responsible for controlling appetite and for being the reward centers of food addiction (Tataranni and DelParigi, 2003). In past studies, using food-related pictures or other visual means to elicit brain

responses has been a standard method of determining valid mechanisms that delineate the path to obesity (Jansen, 1998; Ernst and Epstein, 2002; Killgore et al., 2003; Hawk et al., 2004; Kemps et al., 2004; Simmons et al., 2005; Sobik et al., 2005; Beaver et al., 2006; Cornier et al., 2007; Rolls and McCabe, 2007; Stoeckel et al., 2008). Hence, the fMRI-supported hypothesis that PWS is a naturally occurring human model for food addiction or loss of control of eating or absence of satiety would be crucial for further ACU studies. In the end, what remains is how logical and effective past, present, and future research can aid and treat abnormal eating behavior and brain responses to internal and external food cues in individuals afflicted with obesity.

Treatments for PWS and FA

With respect to the obesity issue, there are two common types of non-medicinal methods to decreasing body weight and/or improving the health condition of the individual. The first one is the undieting approach which discourages the use of food restriction or dieting due to its ineffectiveness and possible health risks (Foster, 2001). The second type is isolated dieting in which one consumes less of a particular type of food or food group such as seen in the Adkins diet where carbohydrates are almost completely eliminated from the diet. A restriction diet can also be combined with supplements or specific weight-reducing herbs or even acupuncture (Foster, 2001).

Besides altering the endocrinological makeup of overweight individuals via drug therapies, alternative and complementary approaches could play a major role in the intervention and possible prevention of obesity. FA is easier to prevent than PWS. Initially, a comprehensive medical and psychological evaluation should be done on each patient to determine the root of the problem. With FA, decreasing access to highly palatable and addicting foods is necessary. On the other hand, restriction to all foods

and small inanimate objects for PWS patients is a necessity. Management includes 24 hour or constant supervision, planned physical activity, a strict diet (≤ 1200 cal/day) divided into structured, portioned meals at set times, and a static, predictable way of life (Benarroch et al., 2007). Encouraging both afflicted groups to exercise or do other enjoyable activities will discourage them from their usual eating behaviors, as well as maintaining a highly controlled eating environment and food regimen with strict, consistent and reinforced rules. Treatment of PWS and FA is a group effort that requires major lifestyle changes and dedication.

One novel treatment for obesity, FA, and PWS is acupuncture. The TCM explanation given for obesity or increased appetite for the affected individuals was described as having excess heat in the gastrointestinal system, a deficiency of the energy known as Qi in the spleen and stomach, or simply a generalized deficiency in Qi. That was one reason why we chose our acupoints.One example of a TCM formula for treating obesity consists of the acupoints Neiguan (SP 6), Fenglong (ST40), Liangmen (ST 21), Guanyuan (R 4), Zusanli (ST 36), Tianshu (ST 25), and Quchi (LI 11) (Li, 1999). We used ST36 and SP 9, which were easily accessible in the scanner. Another form of acupuncture, auricular acupuncture, has had some good results in promoting weight loss (Stux and Pomeranz, 1987). Some common auricular points for us to consider using in future studies are the hunger, stomach, and shenmen points that are indicated to promote satiety and cause sedation/analgesia respectively (Huang et al., 1996). These points are often stimulated using press needles, staples, or beads (Dung, 1986). Acupuncture may be utilized particularly in FA subjects to improve their mood, alleviate stress, and lessen depression in order to control appetite (Akil et al., 1984).

This can be achieved by the release of various neurochemicals during acupuncture treatment (Hans and Terenius, 1982) that could possibly affect our physiological results.

In one example, Sun and Xu (1993) utilized traditional ear and body acupuncture methods over a 3 month period. Interestingly, the controls only received the herb *Oenothera erythrosepala* (evening primrose oil), while the treatment group showed greater weight loss ($P<0.01$). Auricular EAS has also been a fascinating method for weight loss, such as in the study by Shafshak (1995). He found that when auricular electroacupuncture was given to overweight women 5 times a week for 3 weeks along with a low-calorie diet, the weight loss was significant ($P<0.05$). In another randomized study by Steiner et al. (1983), there were 4 experimental groups consisting of real acupuncture, sham acupuncture, eating behavior alteration only, and controls. The results concluded that weight loss was best using real auricular and body acupuncture and/or behavior modification as compared to sham acupuncture and controls ($P<0.05$). Other recent studies have all shown great success in using alternative modalities for weight loss (Hsu et al., 2005a,b; Cabioglu et al., 2006; Lee et al., 2006). We may implement such methods in future studies.

Experimental treatments in animals may have some practical application in treatment and prevention of obesity. It has been shown in mice that targeting the DA system with DA agonists promote decreased appetite and food consumption (Scislowski et at., 1999). There is a possibility such drugs can be marketed for use in human medicine and our studies.

Another suggested experimental treatment is the aid of central leptin gene therapy (Bagnasco et al., 2002a). In a study by Kalra and Kalra (2002), an injection of

recombinant adeno-associated virus vector encoding leptin into the HYP of prepubertal and adult rats resulted in weight gain and suppressed diet-induced obesity. The explanation was that it promoted loss of fatty deposits caused by a decrease in NPY and increase in MCH and thermogenesis. This is a novel approach that may not be suitable for humans at this point. Disrupting NPYergic signaling at multiple loci without affecting normal hypothalamic function would be ideal, but more research needs to be done in this area (Kalra and Kalra, 2004a).

Another experimental method is based on the theory that Ach inhibits feeding via M1 receptors if a muscarinic agonist, arecholine, is injected into the nACC. This can be reversed by using an M1 antagonist pirenzapine (Rada and Hoebel., 2000). It would be interesting to determine if arecholine would be a safe and effective method to prevent hyperphagia in overweight patients. However, there is no safe and effective pharmaceutical agent to specifically treat hyperphagia except possibly fenfluramine, which has been known to have cardiopulmonary side effects (Selikowitz et al., 1990). Some studies showed that taste aversion was a very useful therapy in which Ach levels were increased while decreasing DA levels (Mark et al., 1995), using D-fenfluramine with phentermine to control appetite using a similar mechanism (Rada and Hoebel, 2000). Others have found that baclofen, a GABA-B agonist, is useful in overeating fatty foods (Buda-Levin et al., 2005).

Current methods of treatment for PWS include supplementation with GH before 18 months of age in order to decrease body fat and increase lean muscle mass (Carrel et al., 2004). Other treatments utilize naloxone (an opioid antagonist) to block the opioid system and rimonabant (a CB1 receptor antagonist) to block the cannabinoid system;

these systems have been shown to reinforce feeding behavior, and when used together, they act synergistically to treat obesity (Berry and Mechoulam, 2002). There are other possible cannabinoid agonists and antagonists or acupoints with those properties that may potentially be used to treat FA and other eating disorders (Solinas and Goldberg, 2005).

Both PWS and FA have similar morbidities associated with them such as developing obesity, type 2 diabetes, orthopedic abnormalities, sleep apnea, gallbladder stones, hepatic lipidosis, insulin resistance, dysmetabolic syndrome, renal disease, cardiovascular disease, certain cancers, depression, anxiety disorders, sleep disturbances, and a slew of others (Miller et al., 2006). Therefore, PWS is a good genetic model to study these various obesity-related diseases and help us with our ACU studies in the overweight population.

CHAPTER 2
AIMS, MATERIALS AND METHODS

Aims

To compare the acute effects on Glu levels and CBT via fMRI in overweight Chinese males using ACU versus min SHAM protocols. To determine areas of functional connectivity in the brain following ACU and min SHAM acupuncture. To determine how ACU and min SHAM acupuncture affect the hunger state in food-deprived human subjects.

Rationale

We hypothesize that Glu levels will decrease and CBT will increase during ACU treatment and decrease post-treatment due to increased insulin production as well as other hormonal interplay via vagal stimulation due to central stimulation of the thermoregulatory center in the HYP affecting BMR. We expect to see activation due to acupuncture effects in the HYP (VMH/LHA), insula, mPFC and ventral striatal regions, nACC, caudate nucleus (CN), putamen, and globus pallidus), brain stem, PVN, ARC, cerebral cortex, subcortical structures (AMY, HIPP, cerebellum, and thalamus), inferior parietal lobes, DLPFC/VMPFC, insula, and ventral basal ganglia. The VMH, LHA and ventral striatal regions are known to regulate glycometabolism (Morton et al. 2006), while the HYP and brainstem are CNS centers that affect gastric function (Wu et al., 1999). The cerebral cortex, PVN, ARC, and subcortical brain structures are involved in cognitive function (Fuster, 2002). The inferior parietal lobes, DLPFC, VMPFC, insula and ventral basal ganglia are involved in satiety (see review by Ahima and Antwi, 2008). We expect to see hunger decrease after ACU acupuncture only (rather than min SHAM)

due to neurophysiological effects of acupuncture which are used to suppress hypothalamic activity.

Subjects

The study was performed on 19 right-handed volunteer Chinese males aged 21-45 years (10 for ACU treatment and 9 for min SHAM) who had no history of major neurologic and psychiatric disease. All subjects were acupuncture naïve and gave written informed consent as approved by West China University of Medical Science. All subjects were in accordance with the Declaration of Helsinki. All patients were free to withdraw at any time from the study without obligation. Table 2-1 shows inclusion and exclusion criteria used to recruit subjects.

Table 2-1. Inclusion and exclusion criteria used to select subjects for this experiment.

Inclusion Criteria	Exclusion Criteria
Right-handed adult (age 21-45 years) Chinese males	Left-handed non-Chinese males or females (age <21 and >45 years)
BMI >18 and <30	Normal weight/BMI
Non-smoker	Smokers
Non-dieter (regular diet and exercise program in past 3 months)	On a weight loss program in past 3 months
Not on prescription or non-prescription medication especially anti-depressants and appetite suppressants	Taking antidepressants or appetite suppressing drugs (i.e. loperamide)
Never experienced acupuncture (acupuncture naïve)	Had major acupuncture treatment in the past, especially recently
Healthy (no neurological and endocrine problems)	Neurological or endocrine disorders
Not claustrophobic	Claustrophobic
12 hour fast prior to experiment	Ate within 12 hours of the experiment

Experimental Design

Subjects were recruited and pre-screened based on a standard questionnaire. Subjects were randomly assigned by a computer program to groups A and B (the acupuncturist is the only non-blinded individual in the research group). Group A received standard ACU treatment. Group B was treated with min SHAM. Session I Experiment I (which included group A) and II (which included group B) consisted of the following protocols.

Physiological Measurements

Height (cm) and weight (kg) were measured for each subject in order to calculate the body mass index (BMI). A brief chest and heart auscultation was performed for each patient. Prior to scan, initial CBT was measured sublingually with an Omron® electronic thermometer (MC-142L). Initial Glu was taken from the left index finger and was measured via OneTouch® Ultra™ Blood Glucose Monitoring System (Lifescan; Johnson & Johnson Company). Instrument used glucose oxide (>0.8 IU) and buffer (0.05 mg). Range was 20-600 mg/dL or 1.1-33.3 mmol/L. Accuracy was a slope of 0.986, y-intercept = -5.5 mg/dL, and CC = 0.984. Precision was 1.6-3.2% for blood and 2.4-4.4% for the control. Blood pressure was measured via Omron® electronic blood pressure monitor (HEM-645). Sensitivity was ±4 mmHg (±5% accuracy) with a range of 0-299 mmHg. The hunger survey was then conducted asking the patient to evaluate his hunger on a standard scale from 0 (no hunger) to 10 (starvation).

After the 21 min scan, CBT, Glu (from right index finger), and a hunger survey were conducted. The patient was asked to evaluate the Deqi sensations he felt during the treatment. A standard scale (0 being no sensation and 10 being most intense sensation felt) was used to evaluate Deqi sensations listed. When the anatomic scan

and post-scan were done, the final CBT, Glu (left middle finger), and a hunger survey were conducted. Subjects were asked if they think they received real or sham acupuncture.

Treatment Methods

After a 5 min prescan, the certified acupuncturist set up for either ACU or min SHAM procedure, depending on random patient assignment. Scan began when needles were in at time 0 min. For ACU, 4 acupoints were used bilaterally, ST 36 and SP 9. ST 36 is 3 cun below ST 35 (Dubi), which is in the depression lateral to the patellar ligament on the lower border of the patella when the knee is flexed, and 1 cun lateral to the anterior crest of the tibia. When the knee is flexed, SP 9 is located along the posterior border of the upper tibia. For min SHAM, the SHAM acupoints were located 2 cun lateral and dorsal to ST 36 and 2 cun medial to SP 9 on the same plane bilaterally. Acupuncturist used paramagnetic (0.18 mm x 40mm) needles for both ACU and min SHAM.

For ACU, after a 1 min pause, the acupuncturist inserted needles vertically to a depth of 2-3 cm and rotated needles in a “tonifying and reducing” technique clockwise and counterclockwise at a rate of 60 times per minute or 2 Hz in an alternating bilateral diagonal manner at 30 sec intervals for a total of 2 min. The subject was allowed to raise his right index finger if the Deqi sensations were painful. The lower legs were covered to mask the treatment choice. Scan continued for 21 min.

For min SHAM, after a 1 min pause, the acupuncturist inserted needles to a depth of 2-3 cm and immediately removed them, but pretended to rotate the needles as described for the ACU procedure. The lower legs were covered to mask the treatment choice. After treatments, a 7 min anatomic scan and a 9 min post-scan were conducted.

fMRI Parameters

The functional MRI experiment was performed using a 3.0 Tesla Signa (GE) MR with a standard head coil. The images covered the entire brain and were parallel to the AC-PC line. Functional images were acquired with a single-shot gradient–recalled echo planar imaging (EPI) sequence (TR/TE: 2000ms/30ms, field of view (FOV): 240mm×240mm, matrix size: 64×64, flip angle: 90°, i n-plane resolution: 3.75mm×3.75mm, slice thickness: 5mm thick with no gaps, 43 sagittal slices). A set of T1-weighted high-resolution structural images was collected (TR/TE: 5.7ms/2.2 ms, FOV: 256mm×256 mm, matrix size: 256×256, flip angle: 7°, in-plane resolution: 1mm x 1mm, slice thickness: 1mm with no gaps).

Preprocessing of Data and Analysis

The first 5 time points were discarded to avoid the instability of the initial MRI signal. The fMRI runs were intensity-scaled to yield a whole brain mode value of 1,000. Data sets were preprocessed using SPM5 (www.fil.ion.ucl.ac.uk/spm). Images were realigned to the first image. If translation and rotation was > 1 mm in any direction or > 1 degree, the subject was excluded. The images were then normalized to a Montreal Neurological Institute (MNI) template and re-sampled to 3mm x 3mm x 3mm. Resting data used a band-pass filter of 0.01-0.1 Hz. Finally, the images were smoothed using 6mm x 6mm x 6mm.

For regions of interest (ROIs), the HYP and AMY were chosen initially. The first 0.5 min of data were omitted and 8.5 min of the ACU data were extracted. The HYP (7 grey voxels) and AMY masks (2 grey voxels) were selected using WFU (Wake Forest University) Atlas software. The data was smoothed with a 12-mm full width at half maximum (FWHM) Gaussian kernel for the discrete cosine transform (DCT) analysis.

The DCT analysis was followed by steps depicted in Liu et al. (2009). The discrete cosine bias set contained 60 regressors spanning the frequency of 0–0.1Hz. Statistical parametrical maps were constructed by computing F-contrasts, which compared the effect of signal fluctuations in the range of 0.01–0.1Hz. Statistical parametrical maps were created under the threshold $P<0.005$ (corrected for multiple comparisons) at the first level. The final overlapping mask was created by multiplying the binary values of the individual mask in each group. Finally, the conjunction analysis of the two group masks was applied to detect inter-group similarities of spatial patterns, which was adopted as the ROI for the functional connectivity analysis. The ROI was applied for further functional connectivity analysis. First, the data were processed with a bandpass filter of 0.01–0.1 Hz. The data sets were then spatially smoothed with 6 mm FWHM Gaussian kernel. Second, linear regressions were used to remove several spurious variances along with their temporal derivatives: head motion parameters, signals from a region centered in the white matter, and a region centered in the cerebrospinal fluid. Third, correlation maps were created by computing the correlation coefficients between the BOLD time course from the seed region, and the BOLD time course from all of the other brain voxels. Finally, correlation coefficients were converted to an approximately normal distribution using Fisher's z-transformation. At the second-level analysis, a two sample *t*-test was applied to evaluate the baseline scan of the two acupoint groups before ACU or min SHAM. Finally, the test for differences of brain networks between the two groups was evaluated using a two sample *t*-test. All contrasts had a threshold at $P<0.005$ (uncorrected) and a cluster size >3 voxels. Seed voxel correlation analysis (SCA) was then applied for connectivity results. SCA is based on extracting the BOLD

time course from a predefined seed region and calculating cross-correlation coefficients with all other voxels in the brain (Liu et al., 2009).

CHAPTER 3
RESULTS

Parametric Test Statistics

There was an outlier present for subject 067 (CBT 1 = 35.34) which was substituted with the average of the remaining values (36.31), and that value was used in the following calculations. Using Repeated Measures ANOVA, there was no significant within group difference ($p>0.05$) found in ACU and min SHAM acupuncture groups for CBT and GLU. There were no significant group differences ($p>0.05$) seen in the intergroup analysis using the same test. These results point out that despite visual trends seen in CBT and Glu in the two different groups, no significance was found (see Figures 3-1 and 3-2).

There were individual differences that are of interest. In CBT, all ACU patients had a higher CBT during acupuncture which was lower at the completion of the treatment except for patients 028 (CBT values: 36.25, 36.21, 36.43) who had a BMI of 19.76, and 045 (CBT values: 36.59, 36.54, 36.46) whose BMI was 26.89. On the other hand, individual min SHAM patients differed in CBT outcomes. In general, there was slight increase or no change from before, during or after treatment. Patient 020 showed a major decrease in CBT (CBT values: 37.12, 36.67, 36.71). Patients 029 (CBT values: 36.61, 36.62, 36.56), 046 (CBT values: 36.87, 36.89, 36.91), and 055 (CBT values: 36.93, 36.94, 36.77) had a slight increase followed by a decrease post-treatment. Patients 066 (CBT values: 36.42, 37.34, 37.23) and 087 (CBT values: 36.82, 36.96, 36.98) had major continuous increases in CBT after treatment (Figure 3-1).

In most ACU individuals, Glu decreased during treatment and post-treatment except in patient 018 (Glu values: 4.3, 4.3, 4.2) who remained relatively the same. In

patients 045 (Glu values: 5.5, 5.8, 6.7) and 089 (Glu values: 4.2, 4.3, 4.9) there was a major increase during and after treatment. Patient 054 (Glu values: 5.3, 4.7, 5.6) had a decrease during treatment followed by an increase in Glu. Patient 067 (Glu values: 4.8, 5.2, 4.8) showed an increase only during treatment. On the other hand, min SHAM patients showed a decrease in Glu during treatment except patient 020 (Glu values: 4.6, 4.7, 4.7) who had a slight increase during treatment. Patients 055 (Glu values: 4.9, 4.6, 6) and 068 (Glu values: 5.5, 4.8, 6.2) showed major Glu increases at the end of treatment (Figure 3-2).

Nonparametric Test Statistics

The original categorical Deqi data was best analyzed using a Mann-Whitney rank sum test based on the analysis done by Park et al. (2005). The results indicated that out of the 12 different sensations, only soreness, numbness, and fullness were significant ($p<0.05$, 1 tailed). The remaining sensations were insignificant ($p>0.05$, 1 tailed) when compared between ACU and min SHAM acupuncture Deqi. A two sample Kolmogorov-Smirnov rank sum test was conducted to determine if there were intergroup differences between the ACU and min SHAM group Deqi sensations. Only soreness was found to be significantly different ($p<0.05$, 2 tailed) between the two groups (see Figure 3-3). For individual Deqi data in the ACU group, there were no reportings of warmth, tingling, itching, aching, pressure or heaviness sensations. For soreness, the highest score was 8.5 and the lowest was 0. For numbness, the highest score was 7 and the lowest was 0. For fullness, the highest was 8 and the lowest was 0. Only one patient felt coolness (score of 2). For sharp pain, the highest score was 7 and the lowest was 0. Only one patient felt dull pain (score 10). For other sensations reported, one patient felt pain for 1 min after needle insertion. On the other hand, in the SHAM group, there were no

reported sensations of warmth, dull pain, tingling, itching, aching, and pressure. For soreness, the highest score was 3 and the lowest was 0. For numbness, the highest score was 3 and the lowest was 0. For fullness, the highest score was 4 and the lowest was 0. Only one patient reported coolness (score of 4). For sharp pain, the highest score was 4 and the lowest was 0. For heaviness, the highest score was 2 and the lowest was 0. For other sensations felt, patients 026, 047, 053, and 055 were sore for 1 sec after needle insertion and felt that the needle insertion was painful (Figure 3-3).

For the ordinal repeated measures hunger data, we used the Kruskal-Wallis rank sum test to determine that there was no significant within-group difference between hunger 1, 2, and 3, and no interaction between time and group ($p>0.05$). However, the intergroup comparison showed a significant difference between ACU and min SHAM hungers ($p<0.05$) (see Figure 3-4). For individual hunger data in the ACU group, most patients had a slight increase or no change during treatment except for patients 017 (HUNGER values: 4, 6, 7), 027 (HUNGER values: 3.5, 9, 9), 028 (HUNGER values: 5.5, 7, 7.5), and 067 (HUNGER values: 3, 6, 7) who showed dramatic increases in hunger values during and after treatment. Patient 018 (HUNGER values: 7, 5, 6) had a steady decrease during and after treatment. On the other hand, in the SHAM hunger group, most patients showed a steady increase or no change in hunger values except patient 020 (HUNGER values: 5, 3, 1) who had a steady decrease in hunger values (Figure 3-4).

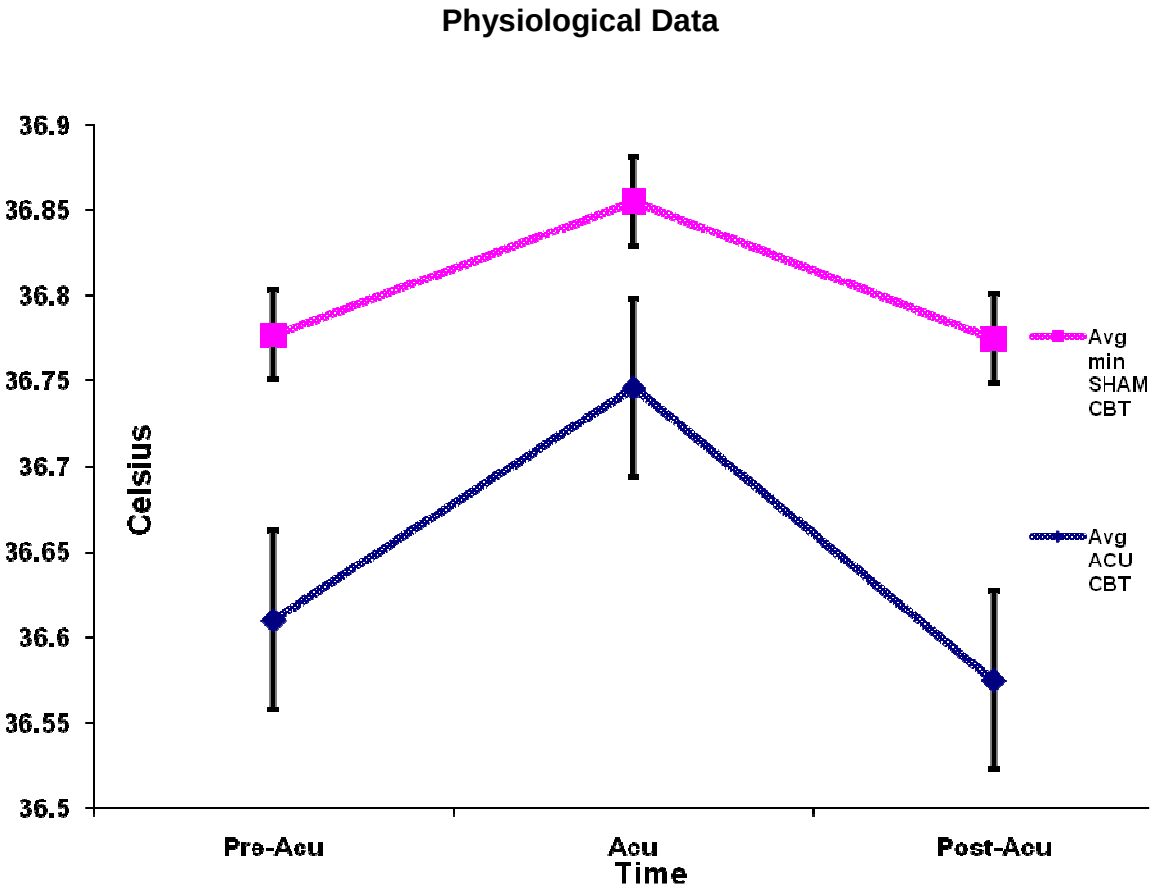

Figure 3-1. Measurement of corrected average core body temperature (CBT) in degrees Celsius shown before (Pre-Acu), during (Acu) and after (Post-Acu) acupuncture (ACU) or minimal sham (min SHAM) treatments in overweight adult Chinese males (n = 10 for ACU and n = 9 for min SHAM). There was no significant difference between groups as shown by the student *t*-test ($p>0.05$) despite a visual increase in CBT during both treatments. Standard errors bars are shown for each time point.

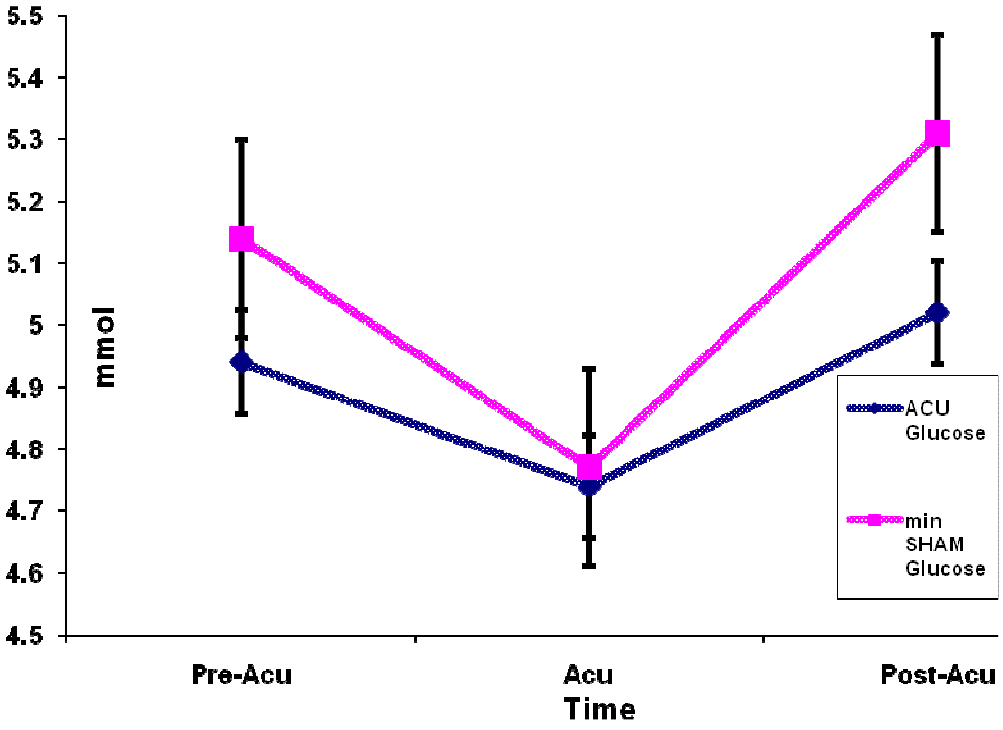

Figure 3-2. Measurement of uncorrected average blood glucose (Glu) shown before (Pre-Acu), during (Acu) and after (Post-Acu) real acupuncture (ACU) or minimal sham (min SHAM) treatment in overweight adult Chinese males (n=10 for ACU and n = 9 for min SHAM). There was no significant difference between or within the two groups as shown by a student *t*-test ($p>0.05$) despite a visual decrease in blood Glu during treatments. Standard errors bars are shown for each time point.

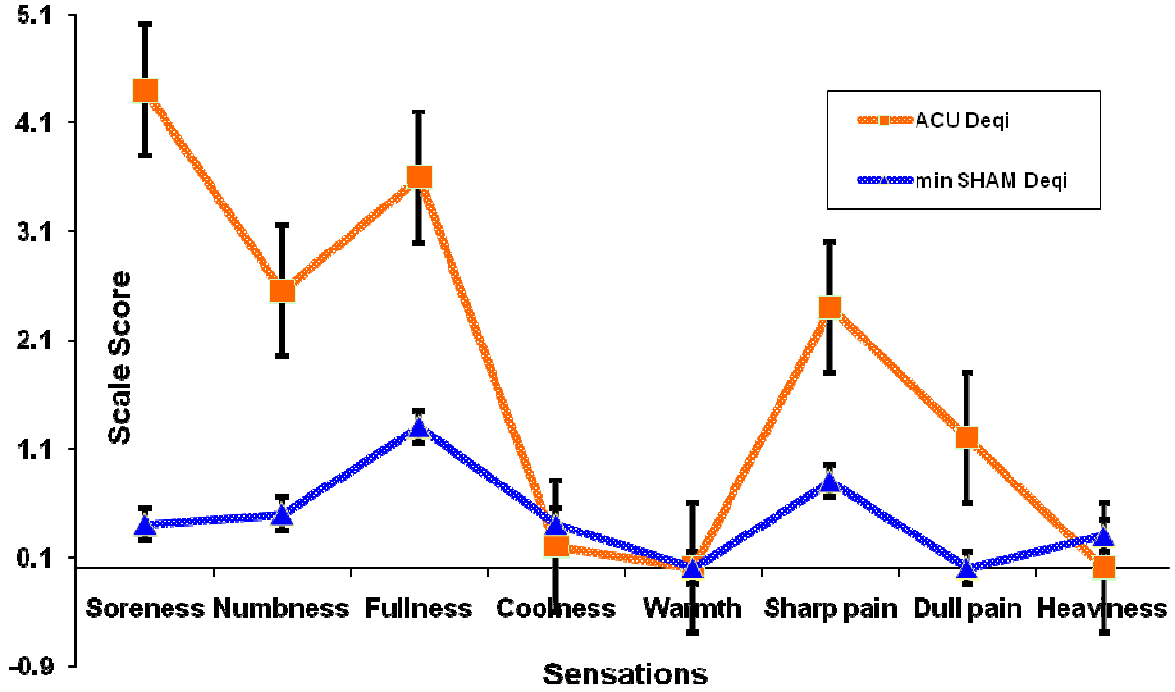

Figure 3-3. Averaged major Deqi sensations (soreness, numbness, fullness, coolness, warmth, sharp pain, dull pain, and heaviness) comparison between real acupuncture (ACU) and minimal sham (min SHAM) treatments in overweight adult Chinese males (n=10 in ACU and n = 9 min SHAM). Significant intra-group differences were only found between soreness, numbness, and fullness ($p \leq 0.05$; 1 tailed; Mann-Whitney Rank sum test). Soreness was significant in an inter-group comparison ($p \leq 0.05$; 1 tailed; Kolmogorov-Smirnov Rank Sum test). Standard errors bars are shown for each sensation.

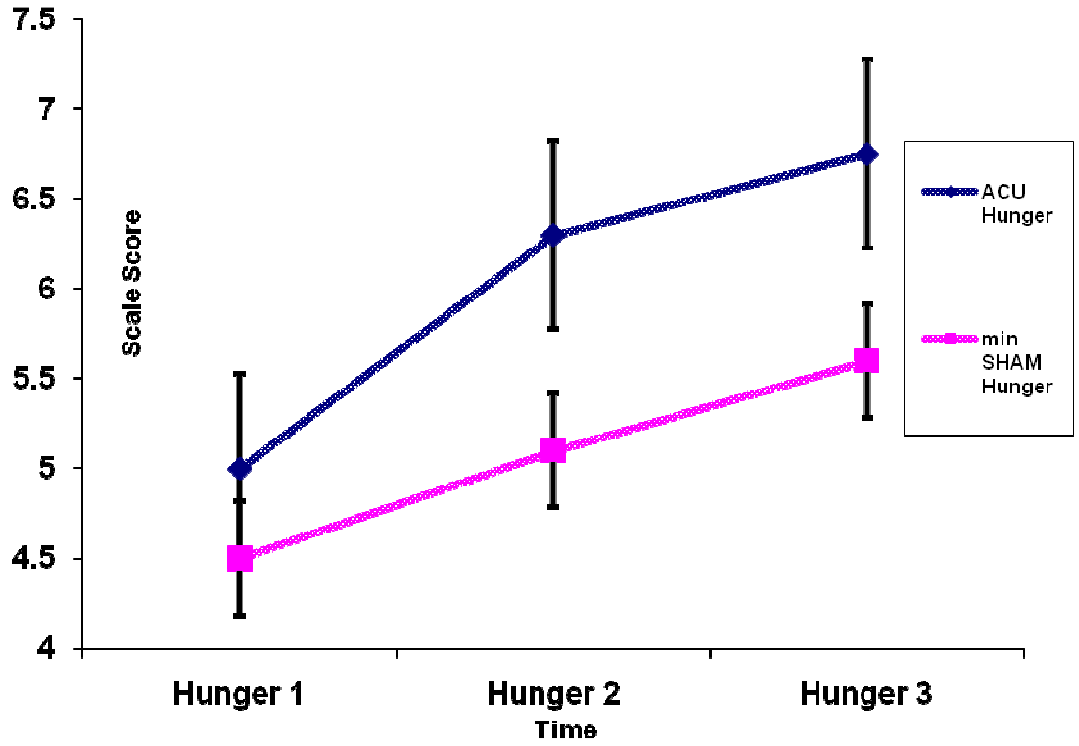

Figure 3-4. Averaged hunger sensation comparison between real acupuncture (ACU) and minimal sham (min SHAM) treatments in overweight adult Chinese males (n=10 in ACU and n = 9 in min SHAM). Significant inter-group differences were found before (Hunger 1), during (Hunger 2), and after (Hunger 3) treatment ($p \leq 0.05$; 1 tailed; Kruskal-Wallis Rank Sum test). There was no interaction between time and ACU or min SHAM groups. Standard errors bars are shown for each time point.

Neuroimaging Data

Using Talairach coordinates and SPM5, the AMY and HYP were chosen as ROIs for the functional connectivity results (see methods section and Figures 3-5 and 3-6). SCA results showed overlapping areas from functional connectivity analysis listed in Tables 3-1 to 3-4. For the AMY connectivity analysis during ACU, the major overlapping regions are listed in Table 3-1. The key regions were the uncus, putamen, caudate, para-HIPP, HIPP, lateral/medial globus pallidus, insula, cingulate, substantia nigra and BA 2, 3, 4, 5, 6, 9, 21, 22, 24, 25, 28, 29, 30, 31, 34, 35, 36, 37, 38, 40, 44, 45, and 47. For the AMY connectivity analysis during min SHAM, the major overlapping regions are listed in Table 3-2. The most important areas were the caudate, cingulate, insula, putamen, HIPP, para-HIPP, ACC, and BA 6, 7, 10, 11, 21, 22, 24, 25, 30, 31, 32, 34, 35, 36, 40, 41, 42, 43, and 47. For the HYP connectivity analysis during ACU, the major overlapping regions are listed in Table 3-3. The main areas were the HIPP, para-HIPP, AMY, putamen, thalamus, ACC, and BA 2, 6, 8, 9, 10, 24, 28, 32, 34, 36, 38, 40, 44, 45, 46, and 47. For the HYP connectivity analysis during min SHAM, the major overlapping regions are listed in Table 3-4. The key areas were the HIPP, substantia nigra, insula, caudate, putamen, thalamus, and BA 17, 21, 28, 32, 35, 38, 39, 40, and 47.

Comparisons of overlapping regions between the ROIs during ACU and min SHAM produced interesting results (see Table 3-5). Common overlapping regions for both ACU and min SHAM using the AMY as the ROI were the putamen, caudate, para-HIPP, HIPP, insula, cingulate, and BA 6, 21, 22, 24, 25, 30, 31, 34, 36, 40, and 47. The common overlapping regions for both ACU and min SHAM using the HYP as the ROI were the HIPP, putamen, thalamus, and BA 28, 32, 38, 40, and 47.

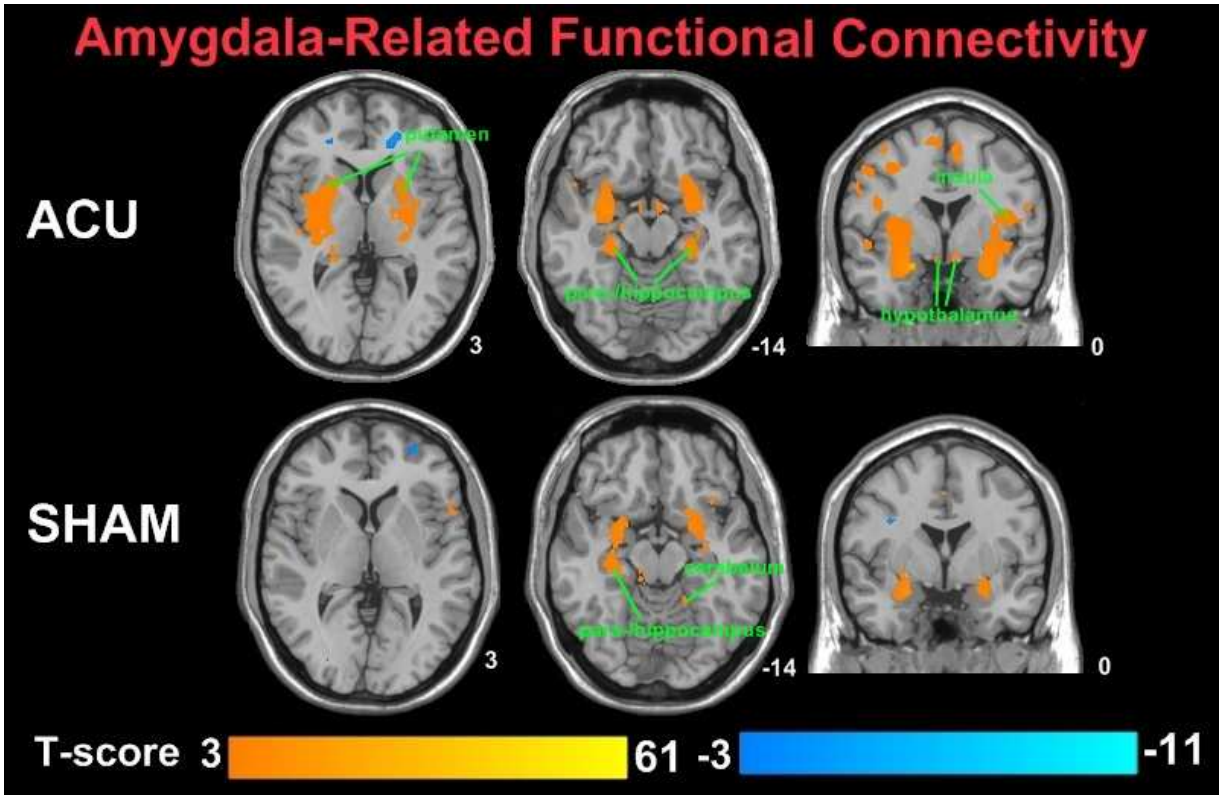

Figure 3-5. Blood oxygen level dependent (BOLD) significant brain regions from an amygdala-related functional connectivity analysis comparing real acupuncture (ACU) versus minimal sham (SHAM)-treated individuals. Epoch of treatment lasted for 9 min. Results from the conjunction analysis were based on the discrete cosine transform (DCT) group results of the two acupoints. The overlapping areas are the putamen, insula, parahippocampus, hippocampus, hypothalamus, and cerebellum. T-value scales located on bottom of picture (p<0.05).

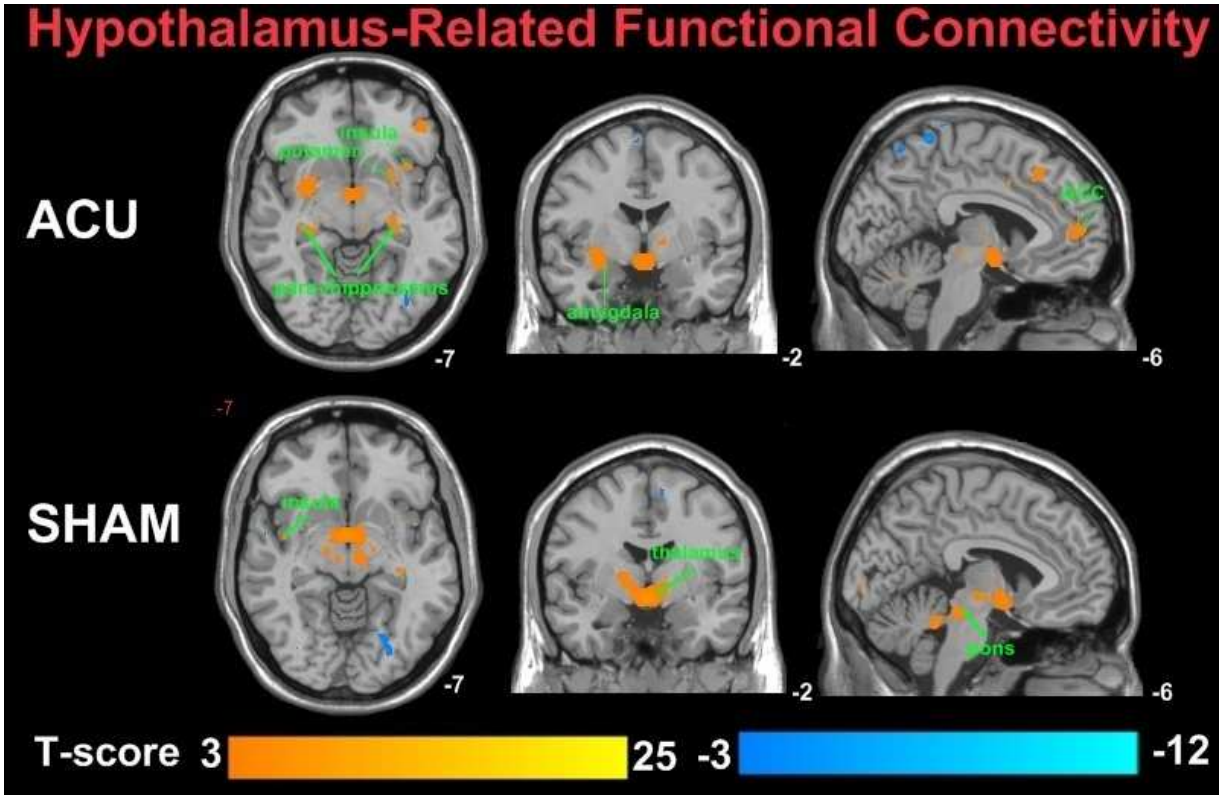

Figure 3-6. Blood oxygen level dependent (BOLD) significant brain regions from a hypothalamus-related functional connectivity analysis comparing real acupuncture (ACU) versus minimal sham (SHAM)-treated individuals. Epoch of treatment lasted 9 min. Results from the conjunction analysis were based on the discrete cosine transform (DCT) group results of the two acupoints. The overlapping areas are the putamen, insula, parahippocampus, hippocampus, amygdala, anterior cingulate cortex (ACC), thalamus, and pons. T-value scales located on bottom of picture (p<0.05).

Table 3-1. Overlapping areas during acupuncture treatment from functional connectivity analysis using the amygdala as the region of interest.

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	*t*-Value
-27	-10	-20	Left Cerebrum	Parahippocampal Gyrus	Hippocampus	6.35
27	-4	-20	Right Cerebrum	Uncus	Amygdala	8.13
-24	-4	-20	Left Cerebrum	Uncus	Amygdala	6.84
-48	-4	-20	Left Cerebrum	Middle Temporal Gyrus	Brodmann area 21	4.51
-24	-27	-16	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 35	5.25
33	-15	-17	Right Cerebrum	Parahippocampal Gyrus	Hippocampus	7.39
-30	2	-18	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 34	5.74
27	8	-18	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 34	5.026
30	-33	-14	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 47	4.12
27	-33	-14	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 36	3.50
-21	-30	-14	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 36	6.90
-24	-15	-14	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 28	3.51
-30	2	-15	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 34	5.90
30	5	-15	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 38	5.24
27	8	-16	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	4.53
-30	8	-16	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	5.55
27	-36	-11	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 37	4.43
-9	-18	-9	Left Brainstem	*	Substania Nigra	3.88
36	11	-11	Right Cerebrum	Inferior Frontal Gyrus	Substania Nigra	4.23
-21	-32	-6	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 30	4.04
-9	-21	-7	Left Brainstem	*	Brodmann area 28	3.57
12	-18	-7	Right Brainstem	*	Substania Nigra	4.31

Table 3-1. continued

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	*t*-Value
18	11	-8	Right Cerebrum	Lentiform Nucleus	Putamen	6.20
-3	11	-8	Left Cerebrum	Anterior Cingulate	Putamen	4.33
-18	11	-8	Left Cerebrum	Lentiform Nucleus	Brodmann area 25	3.73
21	-12	-4	Right Cerebrum	Lentiform Nucleus	Lateral Globus Pallidus	4.79
-24	-12	-4	Left Cerebrum	Lentiform Nucleus	Medial Globus Pallidus	4.43
-27	-9	-5	Left Cerebrum	Lentiform Nucleus	Lateral Globus Pallidus	4.80
-24	-3	-5	Left Cerebrum	Lentiform Nucleus	Brodmann area 22	5.02
-18	12	2	Left Cerebrum	Lentiform Nucleus	Caudate Head	4.20
-9	-43	8	Left Cerebrum	Posterior Cingulate	Brodmann area 29	3.80
42	-2	8	Right Cerebrum	Insula	Brodmann area 44	4.53
59	4	14	Right Cerebrum	Precentral Gyrus	Brodmann area 6	4.63
-59	7	16	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 6	4.59
-42	-34	21	Left Cerebrum	Insula	Brodmann area 44	5.65
-45	-2	22	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 45	4.92
-45	4	27	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 9	5.66
-45	-7	34	Left Cerebrum	Precentral Gyrus	Brodmann area 40	3.96
9	-16	39	Right Cerebrum	Cingulate Gyrus	Brodmann area 3	3.56
-12	-7	39	Left Cerebrum	Cingulate Gyrus	Brodmann area 24	6.08
-9	-9	45	Left Cerebrum	Cingulate Gyrus	Brodmann area 31	7.47
9	-4	44	Right Cerebrum	Cingulate Gyrus	Brodmann area 31	5.58
-3	-21	48	Left Cerebrum	Medial Frontal Gyrus	Brodmann area 5	3.69
-24	-26	57	Left Cerebrum	Precentral Gyrus	Brodmann area 3	3.79
-27	-32	60	Left Cerebrum	Postcentral Gyrus	Brodmann area 2	7.78
-21	-26	59	Left Cerebrum	Postcentral Gyrus	Brodmann area 3	6.02
-33	-23	59	Left Cerebrum	Precentral Gyrus	Brodmann area 4	4.33
9	-12	59	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 4	3.79

Table 3-2. Overlapping areas during minimal sham acupuncture treatment from functional connectivity analysis using the amygdala as the region of interest.

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	*t*-Value
-24	-27	-16	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 35	3.71
-30	-15	-14	Left Cerebrum	Parahippocampal Gyrus	Hippocampus	6.31
30	-12	-15	Right Cerebrum	Parahippocampal Gyrus	Hippocampus	3.78
21	2	-15	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 34	6.23
-24	-30	-11	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 36	4.52
-24	2	-13	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 34	7.82
36	17	-13	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	4.54
18	-32	-8	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 35	4.00
-3	49	-13	Left Cerebrum	Medial Frontal Gyrus	Brodmann area 11	3.65
6	52	-13	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 11	4.24
15	-35	-6	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 30	4.93
-18	-32	-6	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 30	4.41
6	11	-8	Right Cerebrum	Anterior Cingulate	Brodmann area 25	4.27
-24	8	-5	Left Cerebrum	Lentiform Nucleus	Putamen	5.99
21	11	-6	Right Cerebrum	Lentiform Nucleus	Putamen	4.92
-9	11	-6	Left Cerebrum	Caudate	Caudate Head	4.04
12	14	-6	Right Cerebrum	Caudate	Caudate Head	4.73
6	46	-7	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 10	3.50
-59	-6	-2	Left Cerebrum	Superior Temporal Gyrus	Brodmann area 21	3.75
56	11	-3	Right Cerebrum	Superior Temporal Gyrus	Brodmann area 22	4.64
12	46	-5	Right Cerebrum	Anterior Cingulate	Brodmann area 32	3.70
-59	0	8	Left Cerebrum	Precentral Gyrus	Brodmann area 6	3.69
-56	-17	12	Left Cerebrum	Transverse Temporal Gyrus	Brodmann area 41	3.86
-62	-17	12	Left Cerebrum	Transverse Temporal Gyrus	Brodmann area 42	3.91

Table 3-2. continued

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	*t*-Value
3	-12	45	Right Cerebrum	Paracentral Lobule	Brodmann area 31	5.53
-3	-12	45	Left Cerebrum	Paracentral Lobule	Brodmann area 31	3.75
-3	8	44	Left Cerebrum	Medial Frontal Gyrus	Brodmann area 32	4.05
3	-9	56	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 6	4.81
-62	-52	16	Left Cerebrum	Superior Temporal Gyrus	Brodmann area 22	4.25
-48	-17	17	Left Cerebrum	Postcentral Gyrus	Brodmann area 43	3.60
-48	-22	20	Left Cerebrum	Insula		4.39
-6	22	24	Left Cerebrum	Anterior Cingulate	Brodmann area 24	3.58
-6	19	29	Left Cerebrum	Cingulate Gyrus	Brodmann area 32	4.16
-62	-30	37	Left Cerebrum	Inferior Parietal Lobule	Brodmann area 40	3.89
3	-12	42	Right Cerebrum	Cingulate Gyrus	Brodmann area 24	4.41
-3	-12	42	Left Cerebrum	Cingulate Gyrus	Brodmann area 24	3.75
3	11	41	Right Cerebrum	Cingulate Gyrus	Brodmann area 32	6.35
15	-49	66	Right Cerebrum	Postcentral Gyrus	Brodmann area 7	3.62

Table 3-3. Overlapping areas during acupuncture treatment from functional connectivity analysis using the hypothalamus as the region of interest.

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	t-Value
-24	-30	-14	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 36	3.68
-24	-7	-15	Left Cerebrum	Parahippocampal Gyrus	Amygdala	3.91
30	-1	-15	Right Cerebrum	Parahippocampal Gyrus	Amygdala	3.65
33	5	-15	Right Cerebrum	Superior Temporal Gyrus	Brodmann area 38	3.89
27	5	-15	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 34	3.75
-27	5	-15	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 34	3.72
30	14	-16	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	3.79
-24	-30	-11	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 36	3.56
18	-15	-12	Right Cerebrum	Parahippocampal Gyrus	Brodmann area 28	4.01
30	-27	-9	Right Cerebrum	Sub-Gyral	Hippocampus	4.84
-30	-27	-9	Left Cerebrum	Sub-Gyral	Hippocampus	3.90
-24	-21	-9	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 28	3.99
45	37	-9	Right Cerebrum	Middle Frontal Gyrus	Brodmann area 47	4.33
42	37	-9	Right Cerebrum	Middle Frontal Gyrus	Brodmann area 47	3.61
27	-3	-5	Right Cerebrum	Lentiform Nucleus	Putamen	3.63
-24	6	-5	Left Cerebrum	Lentiform Nucleus	Putamen	6.46
15	-23	1	Right Cerebrum	Thalamus	Ventral Posterior Medial Nucleus	3.51
50	17	-1	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	3.69
45	47	-2	Right Cerebrum	Inferior Frontal Gyrus	Brodmann area 10	3.76
6	47	-2	Right Cerebrum	Anterior Cingulate	Brodmann area 32	3.65
6	49	-2	Right Cerebrum	Anterior Cingulate	Brodmann area 10	3.65
12	-9	3	Right Cerebrum	Thalamus	Ventral Lateral Nucleus	5.33
-3	32	1	Left Cerebrum	Anterior Cingulate	Brodmann area 24	4.37
21	-23	7	Right Cerebrum	Thalamus	Ventral Posterior Lateral Nucleus	5.09
6	47	3	Right Cerebrum	Anterior Cingulate	Brodmann area 32	4.82
6	50	3	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 10	4.48
59	15	8	Right Cerebrum	Precentral Gyrus	Brodmann area 44	4.81
-56	18	7	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 45	4.06

Table 3-3. continued

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	t-Value
9	33	15	Right Cerebrum	Anterior Cingulate	Brodmann area 24	3.68
45	39	15	Right Cerebrum	Middle Frontal Gyrus	Brodmann area 46	3.83
-6	39	15	Left Cerebrum	Anterior Cingulate	Brodmann area 32	4.02
3	33	18	Right Cerebrum	Anterior Cingulate	Brodmann area 32	3.67
48	42	17	Right Cerebrum	Middle Frontal Gyrus	Brodmann area 46	5.70
-3	22	24	Left Cerebrum	Anterior Cingulate	Brodmann area 24	3.54
36	22	32	Right Cerebrum	Middle Frontal Gyrus	Brodmann area 9	4.91
-3	34	34	Left Cerebrum	Medial Frontal Gyrus	Brodmann area 6	3.92
-24	22	38	Left Cerebrum	Middle Frontal Gyrus	Brodmann area 8	3.53
6	28	37	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 6	4.16
56	-27	43	Right Cerebrum	Postcentral Gyrus	Brodmann area 2	4.22
3	22	40	Right Cerebrum	Cingulate Gyrus	Brodmann area 32	3.51
6	28	40	Right Cerebrum	Medial Frontal Gyrus	Brodmann area 8	5.86
-56	-36	46	Left Cerebrum	Inferior Parietal Lobule	Brodmann area 40	3.85
-3	37	42	Left Cerebrum	Medial Frontal Gyrus	Brodmann area 8	8.19

Table 3-4. Overlapping areas during minimal sham acupuncture treatment from functional connectivity analysis using the hypothalamus as the region of interest.

Talairach X	Talairach Y	Talairach Z	Direction	Region	Area	*t*-Value
-36	5	-18	Left Cerebrum	Superior Temporal Gyrus	Brodmann area 38	3.97
-24	11	-16	Left Cerebrum	Inferior Frontal Gyrus	Brodmann area 47	4.70
-18	-18	-12	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 35	7.59
-33	-18	-12	Left Cerebrum	Parahippocampal Gyrus	Hippocampus	3.81
-18	-15	-12	Left Cerebrum	Parahippocampal Gyrus	Brodmann area 28	5.58
12	-21	-9	Right Brainstem	*	Substania Nigra	6.13
-9	-18	-9	Left Brainstem	*	Substania Nigra	4.68
-56	-15	-9	Left Cerebrum	Middle Temporal Gyrus	Brodmann area 21	3.49
-42	-6	-7	Left Cerebrum	Sub-Gyral	Brodmann area 21	3.95
42	-12	-4	Right Cerebrum	Insula		3.66
6	-93	2	Right Cerebrum	Lingual Gyrus	Brodmann area 17	4.47
9	9	-3	Right Cerebrum	Caudate	Caudate Head	3.94
-18	9	-3	Left Cerebrum	Lentiform Nucleus	Putamen	4.49
-6	15	-1	Left Cerebrum	Caudate	Caudate Head	4.54
-12	-8	6	Left Cerebrum	Thalamus	Ventral Lateral Nucleus	5.92
-12	-6	6	Left Cerebrum	Thalamus	Ventral Anterior Nucleus	3.57
-45	-75	12	Left Cerebrum	Middle Temporal Gyrus	Brodmann area 39	4.56
-12	-20	12	Left Cerebrum	Thalamus	Medial Dorsal Nucleus	4.50
-15	-20	12	Left Cerebrum	Thalamus	Lateral Posterior Nucleus	3.89
-12	-17	15	Left Cerebrum	Thalamus	Lateral Dorsal Nucleus	5.27
-6	16	30	Left Cerebrum	Cingulate Gyrus	Brodmann area 32	3.85
53	-53	41	Right Cerebrum	Inferior Parietal Lobule	Brodmann area 40	4.30

Table 3-5. Common overlapping areas during acupuncture and minimal sham acupuncture treatments from functional connectivity analysis using the amygdala and hypothalamus as the regions of interest.

Amygdala Region of Interest	Hypothalamus Region of Interest
Caudate	Thalamus
Putamen	Putamen
Parahippocampus	Hippocampus
Hippocampus	Brodmann areas 28, 32, 38, 40, 47
Insula	
Cingulate	
Brodmann areas 6, 21, 22, 24, 25, 30, 31, 34, 35, 36, 40, 47	

CHAPTER 4
DISCUSSION AND CONCLUSION

Discussion

As mentioned in the literature review, acupoint specificity has been studied and questioned. We specifically chose ST 36 and SP 9 based on their functions in TCM and acupuncturist recommendation and approval. However, it was noted by another experienced acupuncturist that for our specific hypotheses and goals, other acupoints could have been chosen instead. For glucose metabolism and thermoregulation, acupoints LI 4, LIV 3, GV 3, GV 4, LI 10, and ST 36 bilaterally were recommended. For HYP activation and hunger suppression, acupoints GB 34, LIV 3, LI 4, SP 6, and SP 9 bilaterally were suggested. Most importantly, ST 36 and SP 9 are crucial points in obesity and weight loss acupuncture studies as summarized by Cho et al. (2009).

Choosing appropriate acupuncture controls is difficult in this field of study, hence the choice of our control method was unique with noteworthy results. Kleinhenz and peers (1999) used the Streitberger needle method, which mimics needle penetration in real acupuncture but does not fully penetrate the skin layers. We combined this method with the standard sham placebo acupuncture protocol. Surprisingly, all subjects believed they received real acupuncture, thus this proves we used an effective control method.

Our neurophysiological results were rather unexpected. Most of our results were insignificant although visually, it could be perceived that Glu decreased during ACU and min SHAM, and CBT increased during ACU and min SHAM. Some significance was found in certain Deqi sensations and hunger ($p < 0.05$; Kruskal-Wallis Rank Sum test and Mann-Whitney test respectively). Based on previous studies, fasting plasma levels

in obese subjects showed that levels of ghrelin, adiponectin, CCK, and NPY decreased, while leptin levels increased (Cabioglu and Ergene, 2006; Pissios and Maratos-Flier, 2007). Unlike the findings in obese individuals, we are led to believe that the specificity of the acupoints chosen caused a release in ghrelin during ACU to stimulate appetite, hence increasing hunger in our overweight population. This is different compared to other studies that show ACU suppresses appetite by increasing serotonin levels (Wenhe and Yucun, 1981) and promotes satiety in the HYP (Shiraishi et al., 1995). The VMH, LHA and ventral striatal regions are known to regulate glycometabolism (Morton et al. 2006), while the HYP and brainstem are CNS centers that affect gastric function (Wu et al., 1999). ST 36 is supposed to promote satiety, regulate intestinal motility, cause sedation (Cabioglu and Ergene, 2006), and increase excitability of the satiety center in the VMH (Zhao et al., 2000). Acupuncture at this point and SP 9 caused a significant increase in hunger in our subjects, which would be useful to treat anorexic patients or individuals receiving chemotherapy. Zusanli has also been reported to increase motility in individuals with hypoactive intestines and vice-versa (Li et al., 1992). Stimulation of ST 36 also increases the amplitude and frequency of gastric peristalsis in normal individuals, shortening the gastric emptying time and delaying the contractions (Li et al., 1992). Therefore, it would have been useful to auscultate the subjects' intestines and stomach before, during, and after ACU or min SHAM procedures to verify these findings.

Studies have shown that mere electrical stimulation of the VMH caused increased Glu uptake via the sympathetic nervous system in skeletal muscle without increasing plasma insulin concentration (Minokoshi et al., 1994; Lang et al., 1995). In

our study, ACU stimulation could be correlated to the electrical stimuli by causing Glu to decrease during treatment due to ACU having a central effect on the overall physiology. The ARC and VMH may even be activated centrally to uptake Glu (Morton et al., 2006) via the effects of ACU. If we used EAS or prolonged manual ACU treatment in our experiment, we would probably have significant physiological results. It is also necessary to look at deactivations and activations in specific brain areas during rest, stimulation, and post-stimulation in order to verify this assumption in future studies. ACU treatment in our study had no statistically significant effect on Glu, hence it may be a time effect only; as hunger increases, Glu decreases then increases. A possible interaction may be occurring, or this difference is probably contributed to hormonal interplay and acupuncture stimulation of the CNS.

The AMY and HYP were chosen as ROIs for the simple reason that the various regions of the HYP are involved in appetite control and thermoregulation. The AMY is often linked with the HYP (Joranby et al., 2005). As shown in Figures 3-5 and 3-6, there was a significant difference in the spatial patterns of the distinct brain regions between the two treatment groups. Functional connectivity describes the temporal synchrony or correlation of the BOLD fMRI signal from two or more anatomically separated brain regions (Friston et al., 1993). Therefore, the spatial and temporal patterns of brain responses would be modulated by the sustained effects of ACU versus min SHAM. We derived the functional connectivity networks from the temporal pattern during the states during and after stimulation associated with the ROIs and the overlapping regions. This is along the lines of our hypotheses that the mPFC, cerebral cortex, cerebellum, DLPFC, and VMPFC would be activated due to the acute ACU effects affecting

primarily satiety and some cognitive functions. With respect to the connectivity analyses, primary somatosensory, motor function, visual stimulation, language, limbic system (pain), and cognitive function centers were involved in both ACU and min SHAM. This was expected since the sensation of the needle and the surrounding environment stimulated all subjects' brain areas such as noted in BA 2-47. One important observation was that with activations in the AMY, ACC, and BA 9, 20, and 36, the subjects were thinking about food, Deqi sensations and/or hunger. Therefore, there was a direct correlation between behavioral data and the functional connectivity results.

Conclusion

Based on connectivity results with the AMY and HYP, it can be assumed that the mode of action for ACU and min SHAM is mediated by the limbic system specifically the neurotransmitter DA. DA is known to increase heart rate and blood pressure (Benes, 2001), hence it would affect CBT in our subjects. DA also has a role in pain processing (Flores et al., 2004), which would explain Deqi or sensations felt during ACU stimulation. This conclusion is based on ACU activation of the insula (responsible for homeostasis), ACC, HYP, putamen, globus pallidus, substantia nigra, and HIPP. The ACC is of great importance in this study since it is involved in blood pressure regulation and heart rate, but it also shares direct connection with the AMY, HYP, nACC, and insula (Bush et al., 2000). As for the physiological data, it can be inferred that the reason for the variability amongs treatment groups was due to the fact that ACU is tailored to the unique physiology of each individual despite having a homogeneous experimental population.

Study Limitations

Limitations in most acupuncture studies regard obtaining individuals that meet the inclusion criteria and adequate sample size. A large group size is needed to do a power analysis in multiple sessions to capture the activation and deactivation patterns evoked by acupuncture stimulation at particular acupoints. Confounding factors include Deqi mixed with pain, artifactual activation, appropriate controls, patient anxiety and anticipation of pain or discomfort from acupuncture treatment. Hui et al. (2007) described this in their study regarding the influence of patient sensations on fMRI BOLD signal changes. It was difficult to obtain an ideal overweight population in our study as well. In the Sichuan Province (China), the BMI of overweight individuals was much lower than in other areas or countries. It could be due to the diet (hot, spicy food) and lifestyle (genetic hypertension). As discussed in another one of our studies, individual differences in response to acupuncture should be taken into account as seen by the variable results in our study population. More subjects are needed to verify our pilot study results, as well as implementing a different experimental design.

CHAPTER 5
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BIOGRAPHICAL SKETCH

Karen Milada von Deneen was born in Ostrava, Czech Republic. She came from a large, well-rounded family who decided to emigrate to the United States in 1984. English was her 4th language at that time out of the 9 that she has studied so far. Ever since the age of 5, Karen wanted to be a veterinarian and pursue academia. Her family moved all over North America and so have her academic endeavors. She graduated from Barstow High School in California as the salutatorian and chose to attend Morehead State University in Kentucky where she obtained an associate's degree in veterinary technology, a bachelor's degree in general education, graduated from the highly competitive Honors Program, and played National Collegiate Athletic Association (NCAA) women's tennis. In 1998, Karen won the coveted NCAA Woman of the Year and Ohio Valley Conference Scholar-Athlete of the Year. She then obtained her Master of Science in animal sciences with an integrated minor in equine reproduction from Oregon State University. She was accepted to the College of Veterinary Medicine and Biological Sciences (CVMBS) at Colorado State University to become a veterinarian. It was there that she was exposed to veterinary acupuncture and alternative medicine. In 2004, Karen was recruited by the Department of Pathobiology at the University of Florida to begin her doctorate training in immunology and infectious diseases. In 2007, she transferred to the Department of Psychiatry to study neuroimaging and physiology. Her mentor enabled Karen to go to China from 2007 to 2008 to do alternative medicine research and promote the scientific method at different universities. Some of her other research orientation has been in food addiction, Prader-Willi Syndrome, and Parkinson's disease. Karen currently has a faculty position at XiDian University in Xi'an, China.