

Monell Chemical Senses Center

Annual Progress Report: 2009 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2010

Formula Grant Overview

The Monell Chemical Senses Center received \$240,428 in formula funds for the grant award period January 1, 2010 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Relationship between Parental Smoking, Dietary Habits, Salt and Sweet Preferences and Blood Pressure among Children - Children whose parents smoke eat a poorer quality diet (e.g., high in sodium) than children of nonsmokers, thus further increasing their future risk for chronic disease. Because primary hypertension, an important risk factor for cardiovascular disease, is rooted early in life and because taste and flavor preferences are important determinants of food choice, this project will evaluate for the first time the relationships among preferences and sensitivity for salty and sweet foods, dietary intake of these nutrients, and blood pressure among children, half of whom have mothers who smoke. This project will also consider recent advances in genetics to genotype all participants for selected genes related to taste perception, blood pressure and addiction. The information gleaned will set the stage for developing strategies to reduce salt and sugar intake in children.

Anticipated Duration of Project

1/1/2010 – 12/31/2010

Project Overview

In some cases, smoking appears to change food preferences directly whereas in other cases the relationships appear to arise from a common mechanism that affects both smoking habits and food choice, such as genetics. This project builds upon our expertise in taste psychophysics, development and genetics and focuses on children, some of whom have a parent who smokes tobacco. We will focus on salty and sweet foods because there is growing agreement that children whose parents smoke eat a poor quality diet, high in salt and sugars, thus further increasing their future risk for chronic disease. A group of 50 racially and ethnically diverse, healthy children aged 5 to 10 years and their mothers will be tested on two days separated by one week. Half of the mothers will be women who smoke five or more cigarettes daily for at least the past year, and the other half will have both parents who never smoked in their lifetimes. Each mother-child dyad will be phenotyped for preferences and sensitivity for salt (NaCl) and

sugar, blood pressure and body weight characteristics, and dietary intake will be assessed by using a 24-hour dietary recall instrument developed by the National Cancer Institute. Twenty-four hour urine samples will be obtained to assess urinary sodium excretion, the gold standard indicator of dietary sodium, and cheek swabs will be obtained from which DNA will be isolated, purified and quantitated for alleles of the human taste receptors and other candidate genes previously identified as important in taste perception, blood pressure and addiction. Smoking history, family history of addictions, and food habits/cravings will be assessed by standardized questionnaires.

We hypothesize that children of smokers and mothers who smoke will prefer a higher level of salt which, in turn, will be related to dietary salt intake and blood pressure. We also expect that these children will prefer more intense sweetness and eat more sweets, as assessed by dietary records, than those of nonsmokers, and that familial history of addiction will be an important determinant of sweet preferences *per se*. Women who smoke may have different alleles for genes relevant to taste preferences and addictions, or allele frequencies between smokers and nonsmokers might not differ, but within smoking and non-smoking groups, genotype might modify taste perception and hedonics.

Principal Investigator

Julie A. Mennella, PhD
Member
Monell Chemical Senses Center
3500 Market St
Philadelphia, PA 19104

Other Participating Researchers

Danielle R Reed, PhD, Susana Finkbeiner, BS, Aleida Silva Garcia, BA, Fujiko Duke, BS – employed by Monell Chemical Senses Center

Expected Research Outcomes and Benefits

People's behavior directly affects their health. Nowhere is this clearer than in the realm of smoking, which is responsible for the largest number of deaths in the United States, and dietary factors such as high dietary salt intake. Both contribute to the risks for chronic diseases such as heart disease in both adults who smoke and in their children who are passively exposed to tobacco smoke and exposed to a lower dietary quality. For both smoking and diet, there is a strong behavioral component, e.g., people choose to eat certain foods or choose to smoke. While many people endanger their lives by smoking and by eating an unhealthy diet (e.g., too much sodium), not everyone does so. An important question is why people differ in these behaviors. In this project we focus on two aspects, the mother-child environment and genetic variation.

We know from previous studies that the experience children receive from their mothers, for instance, the smell of cigarette smoking if she smokes, or the foods she chooses to feed, can have an impact on the health and behavior of the child. We also know that a person's genetic

constitution can markedly affect their behavior. Therefore we will examine the effect of genetic variation on the liking and perception of salt and sweet in mothers and children as well as how the variation interacts with whether or not the mother is a smoker. Using this family structure to collect data gives us several advantages: we can assess the heritability of traits (like salt preference), as well as the effect of development, e.g., similarities and differences between the mother and the child. This study design also allows us to understand how the maternal likings for salty foods and smoking habits of the mother, which may be genetically mediated, impacts the child. For instance, if a mother is genetically predisposed to prefer a high-salt, high-sugar diet, this is the diet she is more likely to feed to the child, who may or may not have the same genetic propensity. Another benefit to this model is that it may reveal that children may be more sensitive to genetic effects. The information gleaned from this project will enable us to design effective interventions to reduce the sodium intake in children's diets.

Summary of Research Completed

The goal of this research project is to characterize the preferences of children and their mothers for sweet and salt tastes and the hypothesis is that women who smoke and their children will prefer higher concentrations of salt and sugar and will consume more foods of low quality, e.g., high sugar/ high salt junk food, which will be related to blood pressure. We also examine whether high salt preferences are related to high sweet preferences (e.g., a liking for extremes of tastes often found in highly processed food), or whether there is the opposite relationship, e.g., that some children prefer high salt foods whereas others have a sweet tooth. These individual differences in taste preferences are being evaluated for their genetic roots through association studies. Sophisticated psychophysical methods, as reviewed below, are being used to measure important aspects of sensory perception, such as sensitivity and preference, in tandem with recent techniques to measure genotypes, such as single nucleotide polymorphisms. The following describes the research methods used. This is followed by a progress report on recruitment and the number of subjects enrolled in the study to date (*Milestone #1*), genotyping (*Milestone #2*), and data entry and analysis (*Milestone #3*).

Overall Method. Each mother-child dyad is being phenotyped for preferences and sensitivity for salts and sugar using age-appropriate psychophysical testings (e.g., two-alternative, forced-choice tracking procedure), dietary intake using diet records, blood pressure, and obesity (height, weight, percent body fat). Each is weighed and measured for height and their body mass index (kg/m^2) computed. For children, their BMI are then classified in one of four categories (underweight, healthy weight, overweight, or obese) using the CDC pediatric growth charts. Twenty-four hour urine samples are obtained to assess urinary sodium excretion, an indicator of dietary sodium, and cheek swabs or saliva samples are obtained from which DNA is isolated, purified and quantitated for alleles of the human sweet receptor (*TAS1R3*) and other alleles previously identified as important in sweet taste perception. For salt, alleles of the putative receptor (sodium channel delta, *SCNN1D*) are being genotyped, as well as alleles that contribute to blood pressure based on association studies. Smoking history, family history of addictions, and food habits/cravings are being assessed by standardized questionnaires, as described herein.

Experimental Design/Overview As shown in Table 1, each mother and child is tested on two separate days, approximately 1 hour before their next scheduled meal and 1 week apart. Testing

lasts 1.5-2 hours each day and occurs in a newly renovated testing facility designed for psychophysical testing of children and their parents. At the beginning of each test day, the mother and child breath into a Vitalograph (Lenexa, KS) to obtain a measure of carbon monoxide levels. Subjects are tested in counterbalanced order, such that half of the mother-child dyads undergo Day A on the first day of testing whereas the other half undergo Day B on the first day of testing. On day A, we perform psychophysical tests to determine salt and sucrose preferences for both mother and child, using salt and sugar solutions and on Day B we measure their taste thresholds (sensitivity) and preferences for salt and sweet in real foods. On each testing day, blood pressure readings are obtained to determine whether salt liking is related to blood pressure in children and adults. Dietary recall data is obtained to determine whether higher intake of salt relates to higher salt liking. At the end of each test day, mothers complete questionnaires related to their and their children's food habits and mood states. Table 2 describes the psychophysical testing and biometric procedures and Table 3 describes the questionnaires used to assess mood states, family history and personality characteristics in the mother-child dyads.

Genotyping for Salt and Sweet Taste, Addiction, and Hypertension. Genomic DNA are being extracted from saliva and cheek cells following the directions of the manufacturer (Epicenter, Madison, WI). As stated, the DNA will come from either cheek swabs or saliva samples because we are in the process of transitioning from an older method (cheek swabs) to a newer method (saliva collection). Cheek swab samples are useful for most applications but as we transition from low-to-high throughput genotyping methods, we need more DNA, which is readily obtained from saliva samples.

As a control, alleles of the bitter receptor *TAS2R38* gene (accession no. NM_176817) are being genotyped using real time PCR single nucleotide polymorphism (SNP) genotyping assays (*rs713598*, *rs1726866*, and *rs10246939*) with the Prism 7000, manufactured by Applied Biosystems (Foster City, CA). This gene is routinely typed in the Reed laboratory as a check on DNA quality and genotype accuracy. To that end, the resulting data will be checked for appropriate segregation to detect genotyping or family history errors. Genes that may play a role in salty or sweet taste perception or nutrient selection or addiction are also being genotyped, such as the putative salt receptor (commonly known as ENAC, official gene symbol *SCNN1D*), a bitter receptor associated with addiction (*TAS2R16*), a sweet receptor (*TAS1R3*), a gene associated with obesity (*FTO*), dopaminergic receptors, i.e. *DRD2*, opioidergic receptor, i.e. *OPRM* and *CNRI*, a cannabinoid receptor. Likewise new discoveries about hypertension genes will be investigated. Variant sites are being genotyped using the subject's DNA, and the correlation between nucleotide variants within these genes with the phenotypic responses of the subjects will be examined.

Milestone #1: Progress Report on Recruitment and Testing. The recruitment goal was to test at least 50 mother-child dyads. Excellent progress has been made on subject recruitment and testing. Mothers were recruited from newspaper ads and initial interviews were conducted over the telephone. Those who have a history of chronic rhinitis or sinusitis, were diabetic, pregnant, lactating or on any medication, with exception of birth control pills, were excluded from the study. There have been no problems with subject recruitment or enrollment.

As shown in Table 4, we have exceeded our recruitment goals. We have tested 54 children (36 girls, 18 boys) who were between the ages of 5 and 10 years and their mothers (N=44); among the children there were 8 siblings pairs and 1 sibling triad.

Of the mothers, 65.9% never smoked in their lifetime and 34.1% were current smokers who reported that they have been smoking 14.5 ± 5.6 (range 5.1-24.4) years and were currently smoking 10.3 ± 6.6 (range 5-30) cigarettes daily. They first experimented with a cigarette when they were 15.1 ± 2.7 (range 10-21) years of age but did not begin smoking regularly until they were 17.6 ± 3.6 (range 11-26) years of age. On average, current smokers scored 8.1 ± 2.7 (range: 5-13) on the Fagerstrom questionnaire of nicotine dependence (scores above 7 indicate high nicotine dependence whereas scores below 7 indicate low to moderate nicotine dependence). Therefore, the mothers in the current sample represent women with varying ranges of nicotine dependence.

The majority of the children were healthy weight (74%), 15% were overweight, 11% were obese. The children had their blood pressure (systolic and diastolic) measured 4 times using an automated pediatric BP cuff. From these measurements, the children were classified as being normotensive (49%), prehypertensive (25%) or hypertensive (26%) using BP tables developed by the National High-Blood Pressure Education Program Working Group on High-Blood Pressure in Children and Adolescents (*Pediatrics* 2004; 114:555–576).

The subject population is racially/ethnically diverse (54% Black, 26% White, 20% Hispanic/Mixed Race). Because some of the genotypes under study are rare and because of our intriguing preliminary findings (e.g., *SCNN1D* alleles; see Milestone #2), our goal is to test at least 10 additional mother-child dyads by November 2010. After this, final data analyses will be conducted and manuscript preparation will begin. An abstract describing these findings will also be submitted for presentation at the 2011 Annual Meeting for the Association for Chemoreception Sciences, the leading scientific meeting on taste and smell research and a grant will be submitted to the National Institutes of Health in early 2011.

Milestone #2. Project Report of Genotyping. Currently we have extracted, purified and genotyped DNA from 37 children and 30 mothers. This DNA was obtained from saliva rather than using an older method (cheek swabs) and thus far the DNA from saliva has been, on average, of higher yield, purity and concentration than that obtained from cheek swabs. Twelve variant sites from the following genes have been typed: *SCNN1D* (a subunit of the putative sodium taste receptor), *TAS2R16* (a bitter receptor gene), *TAS2R38* (a bitter receptor gene), the obesity gene *FTO*, and two genes associated with addiction - the opiate receptor *OPRM1* and the dopamine receptor *DRD2*. Drawing conclusions from analysis of the data at this point would be premature because the study is still open to recruitment and not all subjects have been tested, but preliminary examination of the results suggests that *SCNN1D* alleles are associated with blood pressure in the African American adults and children tested. This result is novel but is consistent with the role of the salt receptor in sodium balance and the relationship of other subunits of the sodium receptor with blood pressure in other subjects.

Milestone #3. Data Entry and Analysis. To date, the psychophysical data for these 54 children and 44 mothers have been de-identified and entered into the database. The final analysis will be

conducted when the study is closed to enrollment. The data analysis and the questions we intend to address fall into three categories. The first category of data exploration is a description of the relationship between maternal smoking and the effects on the behavior of the children, with a focus on food intake and taste preferences. For instance, previous findings from our laboratory indicate that depressed children like more concentrated sweet solutions compared with children who do not experience symptoms of depression. Therefore we might expect that children of smokers, a group with higher rates of addiction and depression, might have children with an exaggerated sweet preference. A second category of data exploration will be to ask whether children with a particular pattern of taste preferences also have a particular pattern of physiological outcomes important for disease. As an example, we wonder whether children who like highly concentrated salt solutions are prone to hypertension. In the last category of analytical approaches, we will investigate the extent to which inborn genetic variation contributes to taste preferences, behavioral traits and physiological outcome measures. Our earlier work indicated that children differ in bitter perception as a function of the pattern of genetic variation in bitter taste receptors. We will explore whether alleles of the sweet receptor predict liking for sugar in children. Using similar logic, we will test whether children differ in salt preference as a function of the genetic variation in their salt receptor.

<i>Table 1. Schedule of Events for mother and child</i>	
<i>Day A</i>	<i>Day B</i>
Carbon Monoxide Levels (2 min each)	Carbon Monoxide Levels (2 min each)
Cheek Swab and Saliva Collection (5 min)	Urine Collection Questionnaire (5 minutes)
Blood Pressure Readings (5 min each)	Blood Pressure Readings (5 min each)
Anthropometry (5 min each)	Anthropometry (5 min each)
Sucrose Preference Test in Solutions (10 min each)	Salt Preference Test in Food (crackers) (10 min each)
Salt Preference Test in Broth (10 min each)	Sweet Preference Test (Grape Jelly) (10 min each)
24-hour Dietary Recall (20 min)	24-hour Dietary Recall (20min)
Questionnaires (30 min)	Questionnaires (30 min)

Table 2. Description of Psychophysical Testing and Anthropometry/Biometric Procedures

<i>Name of Task/Measure</i>	<i>Description</i>
Sweet Preference	Forced-choice tracking technique that measures the most preferred level of sucrose in solution.
Sweet Preferences in a Food Matrix (Grape Jelly)	Forced-choice tracking technique to determine individual preferences for sweet taste in a food matrix (grape jelly). In counterbalanced order, subjects were presented with pairs of grape jelly samples that differ in sucrose content. They were presented with pairs of samples and asked to taste each, without swallowing, and then to point to which of the pair is most preferred.
Salt Preference (Vegetable broth)	Forced-choice tracking technique that measures the most preferred level of salt added in vegetable broth.
Salt Preference in a Food Matrix (Crackers)	Forced-choice tracking technique that measures the most preferred level of salt added in a cracker.
BMI	Each child and mother are weighed and measured so that body mass index (BMI; the weight in kilograms divided by the square of the height in meters) can be computed.
Body Composition/Waist-to-Hip Ratio	Total body water, fat free mass and fat are estimated by bioelectrical impedance analysis (BIA) using the Quantum X instrument (RJL Systems, MI). Abdominal circumference (waist) was measured by having the subject stand comfortably with his or her weight evenly distributed on both feet, and the feet about 25-30 cm apart. The measurement is taken midway between the inferior margin of the last rib and the crest of the ileum, in a horizontal plane.
Blood Pressure	Blood pressure is measured using an automated cuff (DINAMAP®, GE Medical Systems, FL) in children and adults. Appropriate child-sized cuffs, based on the participant's arm size, are used for the children. The average of four blood pressure measurements was used for preliminary analyses.
Urine Collection	At the end of the 1 st day of testing, mothers and children are given containers to collect 1-day of urine. Because most of the children will be in school, this day of collection is typically on the weekend. To date, all have complied with these procedures.

Table 3. Description of Questionnaires

<i>Name of Task/Measure</i>	<i>Description</i>
24 Hour Dietary Intake (ASA24).	An automated, web-based, self-administered 24 hour dietary recall instrument developed by the National Cancer Institute. Outcomes measures include calories, sodium content, and amount of fast foods.
Fat Preference Questionnaire	Self-administered instrument to assess preferences for dietary fat (for adults).
Sweet Taste Questionnaire (STQ):	12-item questionnaire which assesses sensitivity to the mood-altering effects of sweet foods and impaired control over eating sweet foods (for adults).
Food Craving Inventory (FCI)	28-item validated questionnaire designed to measure the frequency of overall food cravings as well as cravings for specific types of foods (high fats, sweets, carbohydrates/starches, fast foods) (for adults).
Restrained Eating Behavior	21-item questionnaire that measures conscious restriction of food intake in order to control body weight or to promote weight loss (for adults).
Profile of Mood States Questionnaire (POMS)	65-item questionnaire which measures six independent, transient mood states: tension, depression, anger, vigor, fatigue, and confusion (for adult).
Becks Depression Inventory (BDI-II)	21-item questionnaire which measures characteristic attitudes and symptoms of depression.
Nicotine Dependence and Family History of Addictions and Obesity	Mothers complete standardized questionnaires to assess nicotine dependence (e.g., Fagerstrom, Michigan Nicotine Reinforcement questionnaire) as well as weight histories of themselves and their child (e.g., Weight and Lifestyle Inventory, family history of alcoholism and family history of obesity because there are studies showing a relationship between obesity, addiction and taste preferences).
Temperament and Food Neophobia Questionnaire	25-item scale completed by mothers that measures their child's temperament dimensions of emotionality, shyness, activity, sociability, negative reactivity to food and food neophobia (children).
Children Liking Score	Children will be presented with several pairs of foods and asked to indicate which they prefer; one of each pair tastes sweet or fatty whereas the other tastes salty and/or savory. From these data, liking scores are calculated (for children).
Pictorial Depression Scale	23-item scale that measures depressive symptomology in children which has been shown to be related to sweet liking (for children)

Table 4. Characteristics of Subjects Tested to Date

4A. <u>Mothers</u>	N=44
Age in years, Mean (SD)	34.8 (8.6)
Race/Ethnicity	
%African American	59.1
%Caucasian	27.3
%Other/More than One	6.8
%Hispanic	6.8
Yrs of school, Mean (SD)	14.2 (2.0)
Family yearly income	
%<15,000	15.9
%15,000-35,000	34.1
%35,000-75,000	38.6
%>75,000	9.1
%Unknown	2.3
Smoking status	
%Never-smokers	65.9
%Current-smoker	34.1
BMI, Mean (SD)	28.3 (6.8)
Weight category by BMI	
%Underweight	2.3
%Normal-weight	34.1
%Overweight	20.4
%Obese	40.9
%Unknown	2.3

4B. <u>Children</u>	N=54
Age in years, Mean (SD)	7.4 (1.8)
Sex	
%Female	66.7
%Male	33.3
Yrs of school, Mean (SD)	2.1 (1.8)
By smoking status of the mother	
%Never-smokers	68.5
%Current-smokers	31.5
Weight category	
%Underweight	0.0
%Healthy-weight	74.1
%Overweight	14.8
%Obese	11.1

SD=standard deviation; Yrs=Years. Family yearly income is reported in US dollars (\$). Smoking status was determined through questionnaire and confirmed with empirical measures (breath carbon monoxide concentrations). School refers to formal education, high school, community or 4-year college. BMI=body mass index, a measure of obesity. BMI=kg/m² where kg is weight in kilograms and m is height measured in meters. Categories to classify BMI are from the Center for Disease Control.