Monell Chemical Senses Center

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2011 – December 31, 2011

Formula Grant Overview

Monell Chemical Senses Center received $216,916 in formula funds for the grant award period January 1, 2011 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

*Effects of Environmental Tobacco Smoke Exposure on Cough in Adolescents and Adults* - Cough is a reflex that protects the lungs against noxious airborne molecules and smokers have impaired cough sensitivity, which contributes to their higher rates of respiratory illness. Parents who smoke expose their children to environmental tobacco smoke (ETS), but it is not known how ETS affects the cough sensitivity of these children. This question is especially important in Pennsylvania because the smoking rate here is in the top quintile. In our research study, we will determine whether adolescents who are exposed to ETS (because one or both parents smoke) have impaired cough sensitivity relative to children of non-smokers. The information gleaned will set the stage for investigating whether reduced sensitivity contributes to illness and early initiation of smoking during adolescence.

Duration of Project

1/1/2011 - 12/31/2011

Project Overview

Cigarette smoke is a common source of chemical and particulate irritants, and cough is an obvious and healthy response to this airway threat. The cough response becomes desensitized in smokers, which helps them tolerate exposure and may contribute to higher rates of respiratory illness. Further, almost half of the children in the United States are exposed to environmental tobacco smoke (ETS) in the home because one or both parents smoke. ETS-exposed children have higher rates of pneumonia, bronchitis, wheezing, and ear infections. In addition, children of smokers are more likely to experiment with smoking during early adolescence, increasing their risk of becoming habitual smokers. Since cough sensitivity could play a role in these negative outcomes, the objective is to understand how ETS exposure affects cough sensitivity in adolescents.
The specific aim is to test the hypothesis that ETS-exposed children are more likely than non-exposed children to suffer from impaired cough sensitivity. Subjects will include 40 racially and ethnically diverse, healthy adolescents aged 10 to 17 years (a critical time for experimenting with tobacco) and their mothers (40 child-mother pairs). The sample will comprise two groups: Non-ETS Exposed (neither the child nor parents has ever smoked or been exposed to ETS in the home) and ETS-Exposed (the mother has smoked at least three cigarettes per day for at least five years in the home, with the child living in the home continuously). Cough sensitivity will be measured using a standard single-inhalation challenge, a test of the minimum concentration of capsaisin (the spicy chemical in hot peppers) needed to elicit cough. Measures of breath carbon monoxide will validate the smoking status of mothers and their adolescent children. The key comparison will be between Non-ETS Exposed and ETS-Exposed children, with the difference between smoking and non-smoking mothers as a positive control. Because smoking and non-smoking families may differ in ways besides tobacco exposure, we will obtain health histories (with a focus on respiratory illness), smoking histories, measures of body weight, diet, and responses to personality tests (including susceptibility to addiction). We will also obtain genomic DNA from saliva samples. Genes for chemosensory receptors that are part of the cough reflex pathway and genotype may account for aspects of cough sensitivity. Some of these variables may be co-variates to control for possible confounds that could affect the conclusions, but we specifically plan to determine whether cough sensitivity correlates with history of respiratory illnesses.

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Expected Research Outcomes and Benefits

Smoking not only affects the health of the smoker, increasing their risks for chronic diseases including those of the heart and respiratory system, but it adversely affects the health of their children who are passively exposed to ETS. We know from previous studies that the smokers have a reduced cough sensitivity, which can have an impact on their health because cough serves the physiologic function of clearing excessive secretions and debris from the throat – a sentinel portal to the airways. It is not known whether ETS exposure affects cough sensitivity in the children of smokers, but reduced sensitivity could contribute to the increased rates of various respiratory illnesses that are seen in this population. Increased knowledge of the risks associated with ETS exposure would provide physicians, policymakers, and others responsible for health education with clearer information for smoking parents. Of course, better information will fail to
deter many parents from smoking in the home. Thus, increased knowledge of the underlying causes of negative health outcomes is the first step toward effective treatment. If the proposed work finds reduced cough sensitivity in ETS-exposed children and increased frequency and severity of respiratory illness, then future research efforts can consider measures to counteract reduced sensitivity as a possible therapeutic strategy.

Cough sensitivity is likely to have several determinants and in addition to exposure to ETS in the home, inborn differences in response to ETS may be important in determining how much exposure to chemicals is required to elicit cough. Reduced cough sensitivity may also contribute to the tendency for the children of smokers to become smokers themselves. New information about the role of chemosensory receptors in cough suggests that ETS exposure may interact with genotype to determine cough thresholds. Better information on the interaction between genes and environment will increase our understanding of who is at risk, and why. Better knowledge of underlying mechanisms and the factors which contribute to early onset of smoking can guide the search for more effective interventions.

**Summary of Research Completed**

**Methods and procedures**

The methods and procedures were presented in detail in the previous progress report. A brief outline of key procedures is presented here for convenience. Two groups of healthy children between the ages of 10 and 17 years and their mothers were studied. One group included children currently exposed to ETS in the home by their mothers, who smoked (on average, 9.5±3.5 cigarettes per day). All but one child had been exposed to ETS from birth and 35.29% had another smoker besides their mother living in the home. The comparison group included age- and sex-matched children who were never exposed to ETS because neither parent (nor any other members of the household) smoked in their lifetimes.

Each parent and child was tested on two days, separated by at least two days, following not having consumed food, beverages (except for water), or smoked for at least 1 hour (for those parents who are smokers). Subjects completed questionnaires to assess general respiratory health, smoking behavior (for adults), and ETS exposure. Saliva samples were collected to extract Genomic DNA (for later exploratory analyses on associations between genetic polymorphisms and individual differences in cough sensitivity). Cough sensitivity was measured using a modified single-inhalation capsaicin challenge. Using identical procedures for children and adults, aerosolized capsaicin solution was delivered using a nebulizer that controlled the duration, timing, and flow rate of inhalations. After practice trials with saline blanks, subjects inhaled increasing concentrations of capsaicin (0.98 to 1,000 µM in 11 serial doubling steps) with blanks randomly interspersed to increase the blindness of the test. After each inhalation, the subject indicated whether they felt throat irritation (e.g., tickle, sting, or burn), and the experimenter recorded the number of coughs elicited, if any (expulsive; throat clearing was not counted). The lowest concentration for which a subject reported throat irritation was defined as irritation threshold. The lowest concentration that triggered at least 2 coughs was defined as cough threshold.
Progress in recruitment and testing

During the second half of the project period, we continued to test subjects. Between 7/1/2011 and 12/31/2011, we tested 15 parents (all mothers) and 22 children. Mothers were recruited from newspaper ads and extant databases of past subjects who had agreed in writing to be contacted regarding future studies. Initial interviews were conducted over the telephone. Those who had a history of chronic respiratory problems, current respiratory infections, were pregnant, lactating, or on any medication (except for birth control pills), were excluded from the study. The study has been reported to the IRB as closed to further enrollment.

Together with the subjects tested during the first project period, we have now met the original goal of enrolling 80 subjects. The total sample now includes 46 children and 34 mothers. The number of children tested was larger than the number of mothers because some mothers had more than one eligible child who participated in the experiment. Specifically, there were 8 sibling pairs and 2 sibling triads. The results on children were the primary focus of the study, so a larger sample of children was deemed acceptable. Some subjects will be excluded from final analyses due to non-compliance with instructions or other issues. Work on this issue and analyses are ongoing.

Progress in genotyping

Saliva samples from all subjects have been collected and stored in a -80°C freezer. From these samples we have extracted, purified and quantified the DNA. This procedure involves the lysis of cells and the removal of protein to obtain DNA. The DNA is further purified by removing residual proteins, salts and small molecules, and quantified through spectrophotometry or flurometric methods. Each sample purified for this project meets the standards needed for genotyping (optical density ratio of A260/A280>1.8, concentration >5 ng/ul). Genotyping has been completed for 21 alleles of common chemosensory receptors known to be expressed in the airways. Targets included different forms of bitter receptors (which are found in the airways and are known to play a role in smooth muscle response and breathing dynamics in animal models), TPV1 (capsaicin/heat receptor), TRPA1 (known to detect various reactive volatile irritants), and TRPM8 (menthol/cooling receptor). Power is very limited due to the relatively small sample size. However, preliminary analyses suggest that polymorphisms in a particular bitter receptor (TAS2R20) is associated with individual differences in cough reflex sensitivity. Analyses are ongoing.

Progress in data entry and analysis

Collected psychophysical, biometric, and other data from this reporting period have been de-identified, completed and entered into a database and statistical analyses are ongoing. Preliminary analyses suggest that (averaged across the two test sessions) cough thresholds were higher (cough sensitivity was reduced) in ETS exposed children relative to non-exposed children, t(36)=2.35, p=0.03. This result suggests that ETS exposure impairs the sensitivity of a vital airway protective mechanism in children, a previously unknown consequence of exposure that may be related to increased risk of respiratory illness.
Research Project 2: Project Title and Purpose

Effects of Chemotherapeutic Agents on the Peripheral Taste Structure and Function - Cancer patients undergoing chemotherapy frequently experience taste abnormalities. The severity of taste dysfunction is associated with high rates of weight loss and poor prognosis. To date, the underlying mechanisms of chemotherapy-associated taste disorders remain unclear. The purpose of this project is to investigate how chemotherapeutic agents affect the peripheral taste structure and function. The ultimate goal of this research is to identify approaches that can prevent or minimize the side effects of chemotherapy on the taste system.

Duration of Project

1/1/2011 - 12/31/2011

Project Overview

Many chemotherapeutic agents can affect taste. Studies have shown that up to two thirds of patients receiving chemotherapy can experience taste alterations. Although it has been speculated that chemotherapy can directly affect taste buds, the experimental evidence is lacking and the underlying mechanisms remain elusive. Our long-term goal for this research is to understand the molecular and cellular bases of chemotherapy-associated taste disorders.

Anticancer drugs kill primarily fast-proliferating cancer cells. However, many of these drugs also show toxicity towards fast-dividing progenitor cells in normal tissues. We hypothesize that the progenitor cells for the taste bud, the basic functional unit of the peripheral taste system, are among the fast-dividing cells affected by anticancer drugs. In this project we will investigate the effects of three chemotherapeutic agents, 5-fluorouracil (5-FU), cisplatin, and paclitaxel (PTX), on the peripheral taste tissues. These three drugs belong to different categories of anticancer agents, are currently being used to treat a variety of malignancies, and have been shown clinically to be associated with taste alterations. We will investigate both the structural and functional effects of these drugs on the peripheral taste system. We will carry out these studies in two specific aims:

Aim 1: determine the effects of 5-FU, cisplatin, and PTX on the taste bud structure. We will use methods, such as histology and immunohistochemistry, to examine changes in the gross structure of taste buds, as well as in cell proliferation and death in the taste epithelium after drug treatments.

Aim 2: determine the effects of 5-FU, cisplatin, and PTX on taste function. We will study the effects of drug treatments on taste responses to the five basic taste qualities, sweet, bitter, umami, sour, and salty tastes, using brief-access or lickometer tests during this funding period.

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Expected Research Outcomes and Benefits

Anorexia and weight loss are among the major concerns of cancer patients. Taste abnormality-associated with chemotherapy is one of the contributing factors for food aversion and decreased caloric intake in these patients. Our project is designed to investigate the underlying causes of taste alterations led by chemotherapeutic agents. We expect that results from this research will provide important information on how some anticancer drugs affect the taste bud structure and function. Knowledge gained from this study may facilitate future development of treatment options and strategies that minimize taste alterations caused by cancer therapy. In addition, this project will further our understanding of the regulatory mechanisms that control taste bud renewal and turnover, an understudied area in the chemosensory field.

Summary of Research Completed

There are two specific aims for this project. **Aim 1** is to determine the effects of 5-FU, cisplatin, and PTX on the taste bud structure. **Aim 2** is to determine the effects of 5-FU, cisplatin, and PTX on taste function. During the previous reporting period, our study was mostly focused on Aim 1. During this reporting period, our major focus was on Aim 2. To examine taste function as proposed in Aim 2, we performed taste behavioral tests by brief-access assay or lickometer assay. Consistent with our previous results on the effects of chemotherapy drugs on the taste bud structure (Aim 1), behavioral tests show that mice injected with these drugs display abnormal taste responses to several taste compounds. Our study suggests that chemotherapy drugs have detrimental effects on taste bud structure and function.

Experimental procedures:

*Drug administration and lickometer test schedules:*

We have carried out two experiments with different drug dosages and testing schedules. These experiments are described below.

Experiment 1: Twenty-seven male C57BL/6 mice (about 2-months-old in the beginning of the experiment) were separated into three groups with 9 mice in each group. The three groups were: PBS, 5-FU, and PTX. Due to limited number of lickometers available, we could not include a cisplatin group in these experiments. Mice were individually caged and trained for four days to
familiarize with the lickometer (see details in the next section). Mice were then tested on two consecutive days for responses to NaCl (0.1, 0.3, and 0.6 M), quinine (QHCl, 0.1, 0.3, and 3 mM), and citric acid (3, 10, and 100 mM) on the first day and to sucrose (0.1, 0.2, and 0.6 M), saccharin (4, 16, and 64 mM), and inosine monophosphate (IMP, 1, 10, and 30 mM) on the following day. The taste tests were repeated once before drug injection.

Drugs and the control buffer were administered to mice by intraperitoneal (i.p.) injection. On day 1, mice in the PBS group received 200 µl of phosphate-buffered saline (PBS); mice in the 5-FU group received 150 mg/kg (body weight) of 5-FU; and mice in the PTX group received 115 mg/kg (body weight) of PTX. On day 3, all mice received another i.p. injection of PBS, 5-FU (150 mg/kg body weight), or PTX (115 mg/kg body weight) according to their group assignments. Taste tests were performed on day 5 for NaCl (0.1, 0.3, and 1 M), citric acid (3, 10, and 100 mM), and QHCl (0.1, 0.3, and 3 mM) and on day 6 for sucrose (0.1, 0.2, and 0.6 M), saccharin (4, 16, and 64 mM), and IMP (1, 10, and 30 mM). These tests were repeated on days 7 and 8, and again on days 11 and 12. Because several 5-FU and PTX treated mice became ill and some of them died on days 11 and 12, the experiment was stopped on day 12.

Experiment 2: Twenty-eight male C57BL/6 mice (about 3-months-old in the beginning of the experiment) were separated into three groups with 9 mice each for the PBS and 5-FU groups and 10 mice for the PTX group. Before drug administration, mice were trained and tested for taste responses as described above. For this experiment, only one dose of drugs was given to mice by i.p. injection. On day 1, mice were injected with PBS (200 µl each), 5-FU (150 mg/kg body weight), or PTX (115 mg/kg body weight). Taste tests were performed on days 5, 8, 11, 15, 18, 22, 25, 29, and 32 for NaCl (0.1, 0.6, and 1 M), citric acid (3, 10, and 100 mM), and QHCl (0.1, 0.3, and 3 mM) and on days 6, 9, 12, 16, 19, 23, 26, 30, and 33 for sucrose (0.1, 0.2, and 0.6 M), saccharin (4, 16, and 64 mM), and IMP (1, 10, and 30 mM).

**Lickometer tests:**

Lickometer tests were conducted using the Davis MS-160 mouse gustometer (Dilog Instruments, Tallahassee, FL). We followed the standard procedure developed by several laboratories for testing mice. Before testing of responses to taste solutions, mice were subjected to a few days of water training to familiarize with the lickometer and to learn to lick from the sipper tube to obtain fluid. To motivate mice to lick from the sipper tube, they were water-deprived for 22.5 hr prior to the training sessions. Each training session lasted 30 min. For training session 1, mice were allowed to drink water freely from a single stationary spout throughout the training session. Immediately after this training session, mice were given 1 hr of ad libitum access to water. Mice were water-deprived again for another 22.5 hr for training session 2. During training session 2, mice received water during 5 s trials (30 trials total). Mice initiated these trials by licking of the sipper tube. After the training session, mice were given free access to water and food. These training sessions were repeated until all mice learned how to perform.

Taste tests were performed on the days described above. We tested three concentrations each of NaCl, QHCl, and citric acid on the same day, which were followed by tests of three concentrations each of sucrose, saccharin, and IMP on the next day. For testing bitter, salty, and sour taste compounds, mice were water-deprived for 22.5 h prior to the tests. After the tests,
mice were given free access to water and food for 1 h. Then for the next 22.5 h, they were given 1.5 ml of water and 1 g of food. Mice were then tested for responses to sweet and umami compounds. For all tests, thirty-six 5 s trials were included, and taste solutions and water were randomly presented to mice according to computer generated random sequences. When the tests for sweet and umami compounds were completed, mice were given free access to water and food until the next set of tests. We measured mouse body weight before water and food restriction and before each taste tests. Mice adapted to water and food restriction quickly and their body weights were stabilized at about 85-90% of pre-restriction level.

Data analysis:

Lickometer test results were presented as tastant over water lick ratios which were calculated by dividing the number of licks for each taste solution by the number of licks for water. T-tests were performed in Excel to compare taste responses between PBS control group and 5-FU- or PTX-treated groups. A p value less than 0.05 was considered statistically significant.

Results:

Because in Experiment 1, 5-FU- and PTX-treated mice showed stronger effects on taste responses, only data from this experiment are discussed here.

Before drug administration, mice in all groups were tested for their responses to NaCl, QHCl, and citric acid on one day and to sucrose, saccharin, and IMP on the following day. The tests were repeated once and the results from the two sets of tests were averaged and shown in Figure 1. For all the taste solutions tested, there were no significant differences in tastant to water lick ratios among the mice assigned to PBS, 5-FU, and PTX groups, suggesting that no significant variations existed in taste responses before drug administration. All mice showed typical avoidance responses (tastant/water lick ratio < 1) to QHCl, citric acid and high concentrations of NaCl and preference responses (tastant/water lick ratio > 1) to sucrose, saccharin and IMP.

After drug administration, PTX-treated mice showed significantly altered taste responses on day 7 to three concentrations of NaCl, two concentrations of QHCl and one concentration of citric acid when compared with PBS-treated mice (Figure 2D, 2E, and 2F). On day 7, PTX-treated mice showed preference to 0.1 and 0.3 M NaCl (tastant/water lick ratios > 1) and indifference to 1 M NaCl (tastant/water lick ratio close to 1), whereas control mice showed indifference to 0.1 and 0.3 M NaCl and strong avoidance to 1 M NaCl. In behavioral tests, normal mice may show preference responses to low concentrations (< 0.1 M) of NaCl which were not included in our tests. Before drug administration, all mice showed close to indifference to 0.1 and 0.3 M NaCl but avoidance to 0.6 M NaCl (Figure 1A). Indifference to 1 M NaCl in PTX-treated mice suggests that these mice had become a lot less sensitive to the salty compound NaCl. On day 7, these mice also showed reduced avoidance behavior to 0.3 and 3 mM QHCl and 100 mM citric acid, suggesting decreased sensitivities to bitter and sour taste compounds. However, PTX-treated mice did not display significant differences in responses to sweet and umami taste compounds (sucrose, saccharin and IMP) compared with control mice (Figure 3), showing specificity in the altered taste responses. On the contrary, 5-FU-treated mice only showed significantly altered responses to sweet compounds (Figure 2 and 3). Interestingly, these mice
showed heightened preference responses to sucrose on day 6, but much reduced preference to sucrose and saccharin on day 8, indicating that the changes in the taste buds may temporarily enhance sweet responses before the loss of sweet receptor cells overriding the enhancement.

Conclusions:

Our results show that the chemotherapy drugs, 5-FU and PTX, can alter behavioral responses to taste compounds. In general, mice treated with these drugs showed reduced sensitivities to some taste solutions tested. The results also suggest that 5-FU and PTX may affect different taste qualities differently with 5-FU primarily affecting sweet taste and PTX affecting salty, bitter and sour tastes.
Figure 1. Lickometer test results in the three groups of mice before drug administration. Results are presented as tastant/water lick ratio. No significant differences in response to these taste compounds were detected among these mice without drug treatments. All groups showed avoidance to QHCl, citric acid and high concentrations of NaCl and preference to sucrose, saccharin and IMP.
Figure 2. Lickometer test results in the three groups of mice after drug administration. Responses to NaCl (A, D), QHCl (B, E) and citric acid (C, F) are presented as tastant/water lick ratio. Drugs or PBS were given to mice on days 1 and 3. Test results from days 5 and 7 are shown. On day 7, PTX-treated mice showed significantly altered responses to 0.1, 0.3, and 1M NaCl, to 0.3 and 3 mM QHCl, and to 100 mM citric acid when compared with PBS-treated control mice. * p < 0.05, ** p < 0.01 (vs. PBS control group).
Figure 3. Lickometer test results in the three groups of mice after drug administration. Responses to sucrose (A, D), saccharin (B, E) and IMP (C, F) are presented as tastant/water lick ratio. Drugs or PBS were given to mice on days 1 and 3. Test results from days 6 and 8 are shown. On day 6, 5-FU-treated mice showed significantly heightened preference to 0.1, 0.2, and 0.6 M sucrose, whereas on day 8, these mice showed significantly reduced preference to 0.1, 0.2, and 0.6 M sucrose and to 16 and 64 mM saccharin when compared with PBS-treated control mice. * p < 0.05, ** p < 0.01 (vs. PBS control group).