**Title**: Steroid Nasal Irrigation for Flavor Evaluation and Detection Study

**Name and Department of Proposed Faculty Mentor:** Jay Piccirillo, MD, Department of Otolaryngology and Sam Klein, Professor, Center for Human Nutrition, Department of Medicine and Dorina Kallogjeri, MD, MPH

**NCT number:** 201608163

**Document Date:** 05/22/2019

**Abstract**: Olfaction is the ability to perceive smell. Gustation is the ability to perceive taste. Anosmia is the inability to detect smell. Ageusia is the inability to detect taste. The senses of olfaction and gustation are often confused in people with olfactory disturbance. Despite being two different sensations, they both coincide within the human central processing system to provide the perception of "flavor." Therefore, it is common for many individuals who suffer from anosmia to also complain of deficits in taste perception. In this project, our aims are threefold: 1) to assess the efficacy of a 30-day treatment of budesonide nasal irrigation to improve the sense of smell and taste 2) to determine the impact of a 12-week period of olfactory training on patients with olfactory dysfunction who fail to improve after budesonide nasal irrigation treatment 3) to understand what, if any, neural network changes occur following olfactory training as measured by resting-state functional connectivity MRI (rs-fcMRI).

We will first identify study subjects with olfactory dysfunction based on predefined inclusion and exclusion criteria. Next, we will measure baseline perception of smell and taste using a battery of tests: University of Pennsylvania Smell Identification Test (UPSIT) and Sniffin Sticks for smell; NIH Taste Intensity Test with general Labeled Magnitude Scale (gLMS) and PROP Taster Test for taste. If subjects have hyposmia/anosmia defined by an abnormal UPSIT and/or Sniffin Sticks score, they will be treated with budesonide nasal saline irrigation for 30 days and their perception of smell and taste retested following completion of nasal irrigation using the same assessments described above. Subjects who do not improve from baseline measurements of these composite tests will undergo a 12-week smell training intervention program, after which they will repeat the battery of smell and taste tests. While several studies have demonstrated the utility of smell training intervention in the improvement of anosmia, there have not been any studies showing how much improvement budesonide nasal irrigation provides to patients with olfactory dysfunction. Upper respiratory conditions, such as rhinosinusitis, often cause patients to have a diminished sense of smell and/or taste. Budesonide is an anti-inflammatory agent that is frequently used to treat rhinosinusitis and improves olfaction in patients with upper respiratory illnesses. Similarly, budesonide may increase olfaction in individuals with olfactory dysfunction. The significance of our project will be to provide a novel way to treat olfactory dysfunction. The possible success of budesonide will manifest in the improvement in quality of life in those with olfactory dysfunction, who will hopefully experience an enhanced sense of smell, taste, and "flavor".

The olfactory system is unique in its high degree of neuroplasticity, with ongoing neurogenesis of olfactory tract cells throughout life. Because of the regenerative properties of the olfactory system, olfactory training has emerged as a promising intervention to modulate neuroplasticity in patients with smell disorders. In this study, our second and third aims will be to validate the efficacy of olfactory training and to use rs-fcMRI to evaluate changes in functional connectivity in olfactory related networks in individuals with olfactory dysfunction undergoing olfactory training, respectively.

**Statement of Research Problem:** We wish to determine the effect of budesonide nasal saline irrigation on the ability to smell and/or taste in a cohort of patients who complain of anosmia/hyposmia. It is well known that people frequently misidentify olfactory disturbances as taste disturbances.[1] Although smell and taste are separate senses, they both integrate and interact in the brain (in addition to other senses) to provide us with the perception of flavor. The intimate intertwining of these two chemical senses, combined with the erroneous use of "flavor" as a synonym for "taste," may contribute to this common confusion between symptoms arising from olfactory dysfunction as if they were caused by taste dysfunction. While literature has supported the intervention of a 12-week smell-training program in improving olfactory function[12], none have investigated the role of budesonide nasal saline irrigation in treating olfactory dysfunction and the biologic mechanism of olfactory training is not well understood. We will assess taste and smell function in subjects before and after treatment with budesonide nasal saline irrigation. Those who fail to show improvement will undergo a baseline resting state functional connectivity MRI (rs-fcMRI) and undergo 12 weeks of olfactory training while continuing the budesonide nasal irrigation. Once more, we will assess post-intervention smell and taste function in patients undergoing the smell training program to see if they display any improvement and obtain a post-intervention rs-fcMRI.

**Specific Aims:** The aims of our study will be
1) To assess the ability of a 30-day treatment with budesonide nasal irrigation to impact sense of smell.
2) To assess whether a 12 week smell training intervention is effective for subjects with anosmia or hyposmia who fail to improve after budesonide nasal irrigation treatment.
3) To determine whether improved olfaction is the result of changes in functional connectivity in one or more olfactory related networks.

**Background :** We will be using a battery of tests to measure subjects' baseline sensation of smell and taste before and after a 30-day trial of budesonide nasal saline irrigation and, if deemed necessary, a 12-week smell training program. The tests for each of these components are outlined below. For each test, subjects will receive a set of instructions to follow. The tests will be administered by a trained expert so that every individual experiences the tests in a uniform manner. Olfactory dysfunction affects up to fifteen percent of the general population and can cause significant psychosocial distress including decreased appetite, reduced self-hygiene, and mood disturbances. [3,4] Most causes of olfactory dysfunction are acquired and include 1) nasal inflammatory conditions, such as chronic sinusitis and viral upper respiratory tract infections; 2) traumatic brain injury and; 3) neurodegenerative disorders, such as Alzheimer's or Parkinson's disease. The pathophysiology behind olfactory dysfunction is complex and poorly understood. As a result, treatment options are limited.

The olfactory system is unique in its neuroplasticity with ongoing neurogenesis of olfactory bulb cells throughout life. Because of the neuro-regenerative properties of the olfactory system, olfactory training has emerged as a promising treatment for olfactory loss. With olfactory training, patients with olfactory dysfunction are exposed to various stimulating odors, such as resinous, fruity, spicy, or flowery scents over several weeks to months. In a large randomized, controlled multicenter trial, Damm et al. demonstrated that patients with post-infectious olfactory loss had improved olfactory function following 18 weeks of olfactory training as measured by threshold, discrimination, and identification scores. [20] While several other clinical studies have also examined the effectiveness of olfactory training with promising findings, the biologic mechanism of olfactory training is not well understood.

We hypothesize that olfactory training is associated with dynamic modulation in neural activity in the olfactory and somatosensory networks. A recent neuroimaging study by Kollndorfer et al. suggests that olfactory training enhances functional connectivity in chemosensory processing networks in anosmic patients. In their case study, seven patients with anosmia secondary to upper respiratory tract infections underwent twelve weeks of olfactory training followed by post-training fMRI. Although anosmic patients were unable to consciously detect odors, specific seed regions with increased functional connections were identified following olfactory training, which included the anterior entorhinal cortex, inferior prefrontal gyrus, and the primary somatosensory cortex. These findings suggest that olfactory related networks are capable of reorganization with training and that olfactory training may be a promising therapeutic treatment for olfactory dysfunction.[27]

Olfactory function - The olfactory portion of our testing is comprised of two exams: the UPSIT and Sniffin Sticks. The UPSIT is a test of olfactory identification and consists of four 10-page booklets, with a total of 40 items. On each page, there is a different “scratch and sniff” strip and four choice options. Subjects are asked to scratch each strip with a pencil to release the scents, detect the smell, and identify the smell from the four choice options. The UPSIT comes with a scoring rubric that identifies the normalcy benchmark based on age and gender. The UPSIT is commercially available, takes a few minutes to complete, and is the gold standard test to assess smell identification in people. [1,2] Sniffin Sticks are commercially-available felt-tip pens filled with liquid odorants or odorants dissolved in propylene glycol. Subjects are presented with odors by uncapping the pens and placing them approximately 2 cm away from each nostril for about 3 seconds. By presenting odors in this fashion, the subject’s baseline identification and discrimination score can be determined. Odor threshold intensity will be assessed by using a staircase method: 3 sticks will be presented in a random order - two with a solvent (controls) and one with the test odorant at a particular dilution (variable). Subjects will be asked to indicate which of the three sticks is the odorant. Presentation of each pen in this triplet set of Sniffin Sticks will occur every 20 seconds. The end result will be to determine the threshold at which the subject can detect odors. Starting at a lower concentration, we will present the test pen (with the two controls) at increasing concentrations until the subject is able to first detect the scent. Then,

we will present pens at continually lower concentrations until they cannot detect a scent. This will continue back and forth until we have 7 "reversals" in detection. A reversal is when the subject goes from being able to detect a scent, to being unable to detect a scent, and vice versa. The quantity we are observing is the dilution factor of the pen. At the end, we will average the dilution factors of the last 4 reversals to calculate the subject's baseline threshold for smell intensity. Following completion of the three tests, a composite threshold, discrimination, and identification (TDI) score can be determined. Use of the Sniffin Sticks have been shown to be a safe and reliable way to assess olfactory performance.[3]

Gustatory function - Subject's gustatory function will be evaluated using the NIH Taste Intensity Test and the PROP Taster Test. NIH Taste Intensity Test examines taste identification by determining subjects' perception of salty, sweet and bitter solutions on the tip of the tongue and in the whole mouth. Salty taste will be identified as the predominant taste quality from 150 mM sodium chloride, sweetness as the predominant quality from 300 mM sucrose and bitterness as the predominant quality from 0.5 mM quinine hydrochloride. One concentration of each of the 3 taste compounds will be presented in two test series (one for tip of the tongue and one for whole mouth taste sensation). For assessment of taste function at the tip of the tongue, solutions will be presented on a cotton swab.[5] Between each exposure to the cotton swab, subjects will rinse with distilled water to prevent adaptation or interference between samples. After a 3-minute break, the test will be repeated with samples presented in medicine cups (whole mouth procedure). Subjects will sip and expectorate each sample and rinse with distilled water between samples. The rationale behind using both a regional (tip of the tongue) and whole mouth taste test is intensity perception evaluated using a whole mouth procedure may fail to detect damage to the taste component perceived by the anterior portion of the tongue due to the central release of inhibition from other oral sensory cranial nerves.[5] However, the regional application of taste at the tip of the tongue isolates taste perception that is carried solely by the chorda tympani nerve, and therefore allows detection of specific nerve damage, which can occur due to early history of frequent ear infections, some dental procedures, and viral illness. The NIH Taste Intensity Test has been shown to adequately assess the patient's ability to identify tastants.[5] The ability to taste PROP and phenylthiocarbamide (PTC), both bitter compounds, is an inherited trait present in 70% of the US adult Caucasian population.[11] We will use the PROP Taster Test to evaluate subjects' taster classification. After rinsing with distilled water, subjects will be asked to place 10 mL of 0.32 mM PROP in their mouth, expectorate it, and rate the intensity of the sensation. They will then rinse three times with distilled water and wait 45 seconds. After this break, subjects will be asked to place 10 mL of 0.1 M NaCl in their mouth, expectorate it, and rate the intensity of the sensation. Non-tasters are classified as those who rate the sensation of NaCl as higher than that of PROP, medium tasters are those who rate the two sensations similarly, and supertasters are those who rate the intensity of the PROP sensation higher than that of NaCl. The PROP test has been proven to be a valid and reliable means to test PROP taster status.[14] The general Labeled Magnitude Scale (gLMS) will be used to rate sensations for both the NIH Taste Intensity Test and the PROP Taster Test. This is a psychophysical tool that requires subjects to

rate the perceived intensity along a vertical axis lined with adjectives that are spaced semi-logarithmically based upon experimentally determined intervals to yield ratio-quality data.[6] Seven anchor labels are provided: no sensation, barely detectable, weak, moderate, strong, very strong, and strongest sensation of any kind. Each of the labels will correspond to a predominant taste sensation - bitter, salty, sweet. Before using the gLMS scale to assess taste intensity and quality, subjects will be trained on the use of the gLMS.[7] That is, at their baseline visit, subjects will be first familiarized with the use of the gLMS by rating the intensity of 10 oral and non-oral sensations (e.g. the tingling of a carbonated beverage, the warmth of lukewarm water) and then being told how intense the average individual perceives these stimulants. That way, subjects will have a standardized comparison model for quantifying their detection of taste intensity.

Clinical Global Impression, Questionnaire for Olfactory Dysfunction, and ODOR questionnaire: At each phase of the study, subjects will be asked to fill out an intervention-specific form rating how they feel they've responded to treatment as well as a Questionnaire for Olfactory Dysfunction, which is a validated quality of life questionnaire for patients with olfactory disorders. Together, these two forms will provide a subjective measurement to go along with our objective measures of smell and taste test performance. With the results from the UPSIT, Sniffin Sticks ,Clinical Global Impression forms and Questionnaire for Olfactory Dysfunction, we hope to have a well-rounded sense of the utility of budesonide nasal irrigation in affecting olfactory and gustatory sense for phase one of the study. In phase two, the smell training program will be initiated in the group of subjects who have abnormal results on either the Sniffin Sticks or UPSIT tests after 30 days of budesonide nasal saline irrigation. Subjects will smell 4 odors (phenyl ethyl alcohol, eucalyptus, lemon, eugenol) twice a day for 12 weeks. The odors will be placed in separate jars at the same concentration in each jar. Each jar will be labeled with the specific odor. Subjects will be advised to sniff each of the jars for 10 seconds (~30-second interval in between) twice a day. This will continue for 12 weeks. During this period, the subjects will continue the budesonide nasal irrigation. After the intervention, they will be asked to return and be retested with the aforementioned battery of tests along with completing an additional quality of life questionnaire (ODOR). The ODOR questionnaire will be completed during a telephone call or via a link sent by email. The smell training program has been performed and shown to be safe and successful in a previous study of patients with olfactory dysfunction without sinonasal disease.[12]

Resting-state functional connectivity MRI: We propose to study changes in functional connectivity in the olfactory and somatosensory networks in patients with olfactory dysfunction following olfactory training. We hypothesize that olfactory training is associated with dynamic modulation in neural activity in the olfactory and somatosensory networks. A recent neuroimaging study by Kollndorfer et al. suggests that olfactory training enhances functional connectivity in chemosensory processing networks in anosmic patients. In their case study, seven patients with anosmia secondary to upper respiratory tract infections underwent twelve weeks of olfactory training followed by post-training fMRI. Although anosmic patients were unable to

consciously detect odors, specific seed regions with increased functional connections were identified following olfactory training, which included the anterior entorhinal cortex, inferior prefrontal gyrus, and the primary somatosensory cortex (Figure 1). [27] These findings suggest that olfactory related networks are capable of reorganization with training and that olfactory training may be a promising therapeutic treatment for olfactory dysfunction.

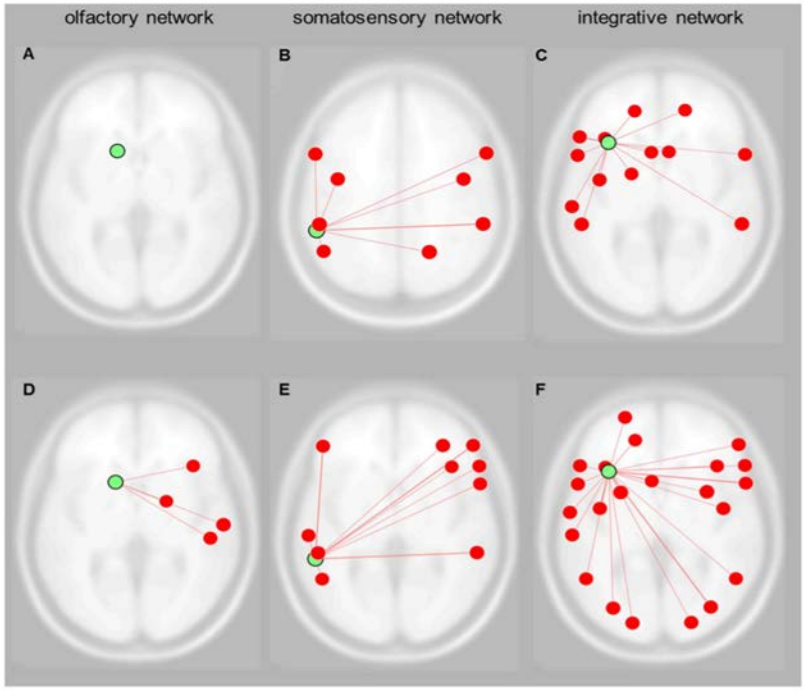


Fig. 1. Functional connectivity during chemosensory stimulation in anosmic patients before (A/B/C) and after the olfactory training (D/E/F), overlaid on an axial template in MNI space (p = 0.005, uncorrected). The green dot represents the selected ROI (A/D (olfactory network): −14,14,2 (caudate nucleus); B/E (integrative network): −34,22,10 (insular cortex); C/F somatosensory network: −58,−42,36 (supramarginal gyrus)); the red dots capture the statistically significant functionally connected brain areas. [27]

A Siemens 3 Tesla PRISMA MRI scanner will be used to capture resting-state blood oxygenation level-dependent (BOLD) fc-MRI and structural scans. The rs-fcMRI acquisition procedure will take advantage of state-of-the-art sequences (developed originally for the Human Connectome Project(HCP) that provide unprecedented spatial and temporal resolution, which should increase the sensitivity and power of network-based methods: use of a 32-channel head coil with multiband EPI, 8-fold acceleration (repetition time [TR] = 800 ms, echo time [TE] = 33 ms, flip angle = 90º, 2 × 2 × 2 mm voxels). Scans take place while participants are awake in the scanner performing no task (i.e., eyes open, no music, headphones and earplugs in place). The rs-fcMRI activity of predefined cortical networks of interest will be measured before the intervention and after the completion of the intervention. Preprocessing steps include best practices for rs-fcMRI (again primarily developed at Washington University), including motion

correction, atlas registration, and temporal filtering. Critically, we will identify time periods of high motion and model these out of the analysis to avoid spurious connectivity results.

**Methods**

**Overview of design:** This is a single pre- and post-intervention study. Potential participants will be identified from recruiting methods described below. After an initial screening for inclusion and exclusion criteria, potential participants will be scheduled for an in-person assessment. Consent will be obtained. The UPSIT and Sniffin Sticks test will then be conducted to determine if the participant has a composite score of less than 33/34 (male/female) and/or 30.5, respectively, and thus qualifies for the study. A limited nasal cavity exam will be conducted by a licensed physician (the Nasal Exam Form will be completed by the physician) and the rest of the battery of smell and taste function tests will be performed. Phase 1 of the study will consist of 30-days of budesonide saline nasal irrigation treatment. All subjects will be provided with the 8-ounce (240 ml) NeilMed Sinus Rinse Regular Bottle Kit and a one-month supply of USP Grade Sodium Chloride & Sodium Bicarbonate Mixture (pH balanced, Isotonic & Preservative & Iodine Free) commercially prepared packettes. Subjects may substitute the NeilMed Sinus Rinse Regular Bottle Kit for a nasal irrigation system, which in the opinion of the Principal Investigator, is similar to the NeilMed system and embodies the low-pressure, high-volume concept of nasal irrigation. Examples of such systems include, but are not limited to, ceramic or plastic neti pot or nasal douch (Nasendusche). Subjects will need to purchase distilled water or boil tap water for five minutes for use with the saline irrigation. Subjects will be required to dissolve the contents of two capsules into the 8-ounce (240 ml) NeilMed Sinus Rinse Regular Bottle along with the saline rinse. All subjects will be instructed to irrigate both right and left nasal cavity with one-half of the contents of the nasal rinse once daily. After this treatment has been completed, participants will return for a follow-up appointment, at which time the same battery of smell and taste tests will be performed. We will compare the results of these tests before and after 30 days of budesonide.

The primary purpose of budesonide nasal irrigation in our study is to rule out inflammation as a reversible cause of olfactory dysfunction. Evaluating otolaryngologists at our institution and in the community sometimes prescribe budesonide nasal irrigation to patients with post-viral olfactory dysfunction as part of their initial work-up. As a result, if patients are referred to our study having already completed budesonide nasal irrigation and have no improvement on smell with objective smell testing, we will enroll participants directly into olfactory training.

If the participant scores abnormally on the Sniffin Sticks or UPSIT test, they will be eligible to continue the second phase of the study, the smell training program. Participants who obtain normal scores on the UPSIT and Sniffin Sticks at follow-up will be excluded from Phase 2. Prior to starting the 12 week smell training program subjects will have a baseline rs-fcMRI. At the conclusion of the 12 week smell training subjects will return for the third and final sitting of the

smell and taste function tests and final MRI. During this period, the subjects will continue the budesonide nasal irrigation. We will examine: a) whether olfactory training improves conscious smell through intervention-based assessments; b) differences in functional networks in patients with olfactory dysfunction before and after 12 weeks of olfactory training.

In addition, 20 age- and gender-matched healthy controls with normal smell will also undergo baseline rs-fcMRI. Control participants will have their smell assessed using the UPSIT to confirm normosmia and will be given a baseline questionnaire regarding overall health and smell. Control participants will be compensated $50.00 for the MRI scan.

**Study subjects:** Subjects include adults age 18-70 with post-infectious smell loss only. Age (+/- 5 yrs) - and gender-matched healthy controls with normal smell will also be recruited.

**Sampling criteria:** Inclusion criteria are adults age 18-70 with anosmia or hyposmia, or normosmia. Exclusion criteria are inability to speak/understand English, current smoking or history of smoking within the past 6 months, nasal polyps, phantosmia, exposure to head and/or neck radiation, exposure to chemotherapy, neurologic disorder, magnetic implantation devices (e.g. pacemaker, cochlear implant) that would preclude the use of MRI, pregnancy, prior smell training, and current cold or rhinitis.

**Plans for recruitment:** We will recruit subjects using a variety of means. Patients scheduled in the Barnes-Jewish ENT clinic with complaints of trouble smelling will be introduced to the study during their clinic appointment and, if they meet the criteria, consented to participate. Advertisements will be posted in Barnes-Jewish ENT clinic for patients with complaints of anosmia/hyposmia to enroll in the study as well, if they meet the criteria above. The ads will briefly describe the concept of the study. Lastly, we will recruit subjects from the Washington University Volunteers for Health Research Participant Registry and the Otolaryngology Research Participant Registry via telephone by using a uniform script delivered by a trained researcher.

**Measurements**

**Main predictor variables** include baseline olfactory and gustatory identification and intensity perception. Baseline olfactory ability will be determined by the subjects' performance on the UPSIT and Sniffin Sticks; Baseline gustatory identification will be determined by subjects' performance of detecting the correct flavor during the NIH Taste Intensity Test; gustatory intensity will be determined by their ratings on the gLMS; PROP taster status will be determined by their rating on the gLMS.

**Main outcome variables** include the objective pre- and post-intervention differences within and between subject performance on the UPSIT and Sniffin Sticks tests before and after budesonide (Phase 1) and within and between changes in network connectivity between the olfactory and

somatosensory network post smell intervention training (Phase 2). We will also evaluate a subjective response by having subjects complete an intervention-specific Clinical Global Impression form after each intervention. To determine subjects eligible for phase 2 of the study, we will only select subjects with scores that fall below normal on either the UPSIT or Sniffin Sticks tests. For the UPSIT, subject scores will be compared to a rubric containing normal age- and gender-based scores. For the Sniffin Sticks, patients whose composite score remains below the normal cutoff of 30.5 will be eligible for Phase 2. Upon completion of the smell training program, subjects will once again complete the UPSIT, Sniffin Sticks, NIH Taste Intensity and PROP Taster tests. We will use results on the UPSIT, Sniffin Sticks, Clinical Global impression form and Questionnaire for Olfactory Dysfunctionto gather information on the objective and subjective differences in smell before and after smell training.

**Materials**

Olfaction: UPSIT: 4 different 10-page booklets, with a total of 40 items. On each page, there is a different "scratch and sniff" strip and four choice options. Sniffin Sticks: A triplicate set of 16 felt-tip pens filled with liquid odorants or odorants dissolved in propylene glycol at different concentrations. We will use a blindfold and cloth gloves to protect against the incidental detection of external olfactory stimuli.

Gustation: NIH Taste Intensity Test solutions: 1.0 M sucrose (sweet), 1.0 M NaCl (salty),[10] and 0.001M Quinine hydrochloride (bitter)[5]. PROP Taster Test: 0.320 mM Prop and 0.1 M NaCl. Stimuli will be prepared in the metabolic kitchen of the Clinical Research Unit at Washington University School of Medicine.

**Approach to statistical analyses:** We will use standard descriptive statistics to describe the study population and distribution of pre- and post-intervention test scores on the UPSIT and Sniffin Sticks, for both Phase 1 (Specific Aim 1) and Phase 2 (Specific Aim 2). Paired-sample t-tests or its non-parametric equivalent, the Wilcoxon signed rank test, will be used to compare pre- and post-intervention changes. Effect size and 95% confidence interval will be calculated and reported. The impact of any potential confounders will be explored through mixed model analysis.

Our primary analyses of the resting state will be network-based approaches that quantify properties of network organization based on nodes (brain regions) and the connections between them (identified by correlations in their time series). To define the nodes, we will use a recently-developed multimodal atlas of human cerebral cortex produced for the HCP20, the most current parcellations of brain areas based on a large number of participants' resting state data. Having extracted time series data from each brain region, we will then capitalize on recent applications of graph theoretic approaches to identifying and characterizing network connectivity.[21] To analyze the functional networks in terms of their integration and segregation, four graph

measures will be computed: global efficiency, network modularity, characteristic path length, and clustering coefficient. [22,23]

Our primary focus will be on the integration of the olfactory and somatosensory networks, which is most strongly reflected in the global efficiency measure (intuitively, an integrated network will have a higher number of short paths between nodes; the other measures will be used to provide convergent support). [24] We will also conduct exploratory analyses using these measures on whole-brain functional connectivity maps, to detect more general network effects we may have missed.

To supplement these graph theoretic analyses, we will also conduct more conventional seed-based analyses as we have done in our prior work, placing seeds in included the anterior entorhinal cortex, inferior prefrontal gyrus, and the primary somatosensory cortex regions to produce whole-brain voxelwise functional connectivity maps. Doing so helps foster continuity in our research program and comparison of our new results to prior published work. [20-24]

A strength of our network analysis of the rs-fcMRI data is that we are able to numerically quantify network properties (e.g., global efficiency) in a way that can be easily combined with our behavioral and clinical data. We will include our measures of neural network processing in the statistical models described above in order to see whether degree of clinical improvement is related to changes in neural network processing.

We predict that both olfactory training will be associated with increased integration between the olfactory and somatosensory networks, and that the degree of improved integration will correlate with clinical outcomes. We will also investigate changes in cortical connectivity by comparing integration changes within subjects, and whether certain signatures of network organization before intervention predicts whether olfactory training will be effective.

In secondary analyses, we plan to explore the relationship between our two main networks of interest and the anterior entorhinal cortex, inferior prefrontal gyrus, and the primary somatosensory cortex region networks. Each of these additional networks has been hypothesized to contribute to olfaction, and by broadening our network analyses we hope to increase our understanding of the large-scale brain changes associated with anosmia/hyposmia.

**Hypotheses, sample size and power:** Our hypothesis is if individuals with persistent complaints of anosmia or hyposmia, who fit our inclusion and exclusion criteria, are given a 30-day treatment of budesonide nasal saline irrigation, then they will demonstrate an improvement in their sense of smell and thus, perception of taste and flavor. We also predict that those who do not experience improvement with the budesonide treatment will experience improvement after the smell training intervention program.

Sample size: As this is a pilot study and there is no previous research to guide our sample size calculation, we derived our planned sample size of 125 subjects as follows:

For the imaging portion of the study, we would like to have 20 participants with post-viral olfactory dysfunction and 20 age- and gender-matched healthy controls with normal smell. In this way, we should have a minimum number of subjects for neuroimaging and behavioral analyses to obtain more robust sample size estimates for future definitive studies. Studies using 12 weeks of OT in patient populations similar to ours report improvement in 30% to 85% of the subjects undergoing training. [12, 21, 26] Therefore, in order to obtain 20 subjects who do not experience improvement, we will need to have 96 subjects complete budesonide therapy. Assuming a 20% drop-out rate, we will need to enroll 120 subjects. As this is a pilot study, we will use the data herein to power our next study. Stopping rule: If after treating with budesonide and assessing the first 25 subjects, we observe 100% improvement of smelling and identify no need for smell training, we will stop the study and report the results.

**Limitations and issues:** Possible experimental issues include subject compliance in returning for retesting; subject ability to successfully use the gLMS scale after training; proper use of budesonide nasal irrigation; possible interference between samples during smell and taste battery testing; and compliance in performing the daily smell training intervention. Inability to use nasal irrigation seems unlikely, as Dr. Piccirillo has treated hundreds of patients with budesonide nasal saline irrigation and has experienced few patients who have difficulty.

**Ethical considerations:** Each of the tests (UPSIT, Sniffin Sticks, NIH Taste Intensity Test, PROP Taster Test) and the smell training intervention have been safely administered in previous studies. They are all non-invasive and pose no harm to the subjects. The budesonide nasal irrigation has been previously used in another study without harm to human subjects. Each test is commercially available and there are no financial conflicts of interest. Limited identifying information will be collected to protect patient confidentiality.

**References**

1 Deems, D. A. *et al.* Smell and taste disorders, a study of 750 patients from the University of Pennsylvania Smell and Taste Center. *Arch Otolaryngol Head Neck Surg* **117**, 519-528 (1991).

2 Doty, R. L., Frye, R. E. & Agrawal, U. Internal consistency reliability of the fractionated and whole University of Pennsylvania Smell Identification Test. *Percept Psychophys* **45**, 381-384 (1989).

3 Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E. & Kobal, G. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem Senses* **22**, 39-52 (1997).

4 Kobal, G. *et al.* "Sniffin' sticks": screening of olfactory performance. *Rhinology* **34**, 222-226 (1996).

5 Coldwell, S. E. *et al.* Gustation assessment using the NIH Toolbox. *Neurology* **80**, S20-24, doi:10.1212/WNL.0b013e3182872e38 (2013).
6 Bartoshuk, L. M., Duffy, V. B., Hayes, J. E., Moskowitz, H. R. & Snyder, D. J. Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. *Philos Trans R Soc Lond B Biol Sci* **361**, 1137-1148, doi:10.1098/rstb.2006.1853 (2006).
7 Pepino, M. Y., Finkbeiner, S., Beauchamp, G. K. & Mennella, J. A. Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women. *Obesity (Silver Spring)* **18**, 959-965, doi:10.1038/oby.2009.493 (2010).
8 Cicerale, S., Breslin, P. A., Beauchamp, G. K. & Keast, R. S. Sensory characterization of the irritant properties of oleocanthal, a natural anti-inflammatory agent in extra virgin olive oils. *Chem Senses* **34**, 333-339, doi:10.1093/chemse/bjp006 (2009).
9 Keast, R. S. & Roper, J. A complex relationship among chemical concentration, detection threshold, and suprathreshold intensity of bitter compounds. *Chem Senses* **32**, 245-253, doi:10.1093/chemse/bjl052 (2007).
10 Cowart, B. J. Relationships between taste and smell across the adult life span. *Ann N Y Acad Sci* **561**, 39-55 (1989).
11 Blakeslee, A. F. & Fox, A. L. Our different taste worlds: P.T.C. as a demonstration of genetic differences in taste. *Journal of Heredity*. **23**, 97-107 (1932).
12 Hummel, T., Rissom, K., Reden, J., Hähner, A., Weidenbecher, M. and Hüttenbrink, K.-B., Effects of olfactory training in patients with olfactory loss. *The Laryngoscope*, **119**: 496–499, doi: 10.1002/lary.20101(2009).
13 Stamps JJ, Bartoshuk LM, Heilman KM, A Brief Olfactory Test for Alzheimer's Disease. *Journal of the neurological sciences*. **333**, 19-24, doi:10.1016/j.jns.2013.06.033 (2013).
14 Tepper, B. J., Christensen, C. M., Cao, J. Development of brief methods to classify individuals by PROP taster status. *Physiology & Behavior*. **73**, 571-577 (2001).
15 Pekala, K., R.K. Chandra, and J.H. Turner. 2016. Efficacy of olfactory training in patients with olfactory loss: a systematic review and meta-analysis. International Forum of Allergy & Rhinology 6: 299-307.
16. Liu G, Zong G, Doty RL, Sun Q. Prevalence and risk factors of taste and smell impairment in a nationwide representative sample of the US population: a cross-sectional study. BMJ Open 2016;6:e013246.
17.. Wilson DA, Best AR, Sullivan RM. Plasticity in the Olfactory System: Lessons for the Neurobiology of Memory. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 2004;10:513-24.
18. Hummel T, Nordin S. Olfactory disorders and their consequences for quality of life. Acta oto-laryngologica 2005;125:116-21.
19. Murphy C, Schubert CR, Cruickshanks KJ, Klein BE, Klein R, Nondahl DM. Prevalence of olfactory impairment in older adults. Jama 2002;288:2307-12.
20. Damm M, Pikart LK, Reimann H, et al. Olfactory training is helpful in postinfectious olfactory loss: a randomized, controlled, multicenter study. The Laryngoscope 2014;124:826-31.

21. Fleiner F, Lau L, Goktas O. Active olfactory training for the treatment of smelling disorders. Ear, nose, & throat journal 2012;91:198-203, 15.
22. Hummel T, Rissom K, Reden J, Hahner A, Weidenbecher M, Huttenbrink KB. Effects of olfactory training in patients with olfactory loss. The Laryngoscope 2009;119:496-9.
23. Konstantinidis I, Tsakiropoulou E, Constantinidis J. Long term effects of olfactory training in patients with post-infectious olfactory loss. Rhinology 2016;54:170-5.
24. Knudsen K, Flensborg Damholdt M, Mouridsen K, Borghammer P. Olfactory function in Parkinson's Disease - effects of training. Acta neurologica Scandinavica 2015;132:395-400.
25. Haehner A, Tosch C, Wolz M, et al. Olfactory training in patients with Parkinson's disease. PloS one 2013;8:e61680.
26. Konstantinidis I, Tsakiropoulou E, Bekiaridou P, Kazantzidou C, Constantinidis J. Use of olfactory training in post-traumatic and postinfectious olfactory dysfunction. The Laryngoscope 2013;123:E85-90.
27. Kollndorfer K, Fischmeister FP, Kowalczyk K, et al. Olfactory training induces changes in regional functional connectivity in patients with long-term smell loss. NeuroImage Clinical 2015;9:401-10.
28. Siegel JS, Power JD, Dubis JW, et al. Statistical improvements in functional magnetic resonance imaging analyses produced by censoring high-motion data points. Hum Brain Mapp 2014;35:1981-96.
29. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. Neuroimage 2014;84:320-41.
30. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 2012;59:2142-54.
31. Glasser MF, Coalson TS, Robinson EC, et al. A multi-modal parcellation of human cerebral cortex. Nature 2016;536:171-8.
32. Bassett DS, Sporns O. Network neuroscience. Nature neuroscience 2017;20:353-64.
33. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. NeuroImage 2010;52:1059-69.
34. Gard T, Taquet M, Dixit R, et al. Fluid intelligence and brain functional organization in aging yoga and meditation practitioners. Frontiers in aging neuroscience 2014;6:76.
35. Kinnison J, Padmala S, Choi JM, Pessoa L. Network analysis reveals increased integration during emotional and motivational processing. The Journal of neuroscience : the official journal of the Society for Neuroscience 2012;32:8361-72.

1. Konstantinidis I, Tsakiropoulou E, Bekiaridou P, Kazantzidou C, Constantinidis J. Use of olfactory training in post-traumatic and postinfectious olfactory dysfunction. The Laryngoscope 2013;123:E85-90.

2. Hummel T, Rissom K, Reden J, Hahner A, Weidenbecher M, Huttenbrink KB. Effects of olfactory training in patients with olfactory loss. The Laryngoscope 2009;119:496-9.
3. Geissler K, Reimann H, Gudziol H, Bitter T, Guntinas-Lichius O. Olfactory training for patients with olfactory loss after upper respiratory tract infections. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery 2014;271:1557-62.
4. Fleiner F, Lau L, Goktas O. Active olfactory training for the treatment of smelling disorders. Ear, nose, & throat journal 2012;91:198-203, 15.