Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Monell Chemical Senses Center

2. **Reporting Period (start and end date of grant award period):** 1/1/13 – 12/31/13

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Holly E. Carria

4. **Grant Contact Person's Telephone Number:** 267-519-4728

5. **Grant SAP Number:** 4100062213

6. **Project Number and Title of Research Project:** 1-Early Stage Recordings of Cognitive Odor Processing

7. **Start and End Date of Research Project:** 1/1/13 – 12/31/13

8. **Name of Principal Investigator for the Research Project:** Johan N. Lundstrom, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 117,100

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Lundstrom, Johan	Associate Member	37.66%	$24,085
Seubert, Janina	Postdoctoral Fellow	37.75%	$17,330
Gergory, Kristen	Senior Research Technician	100%	$30,334

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in

Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X____ No_____

If yes, please describe your plans:

We are currently writing an application to the National Institute of Health to continue these experiments with an aim of exploring olfactory bulb processing in individuals suffering from Parkinson's disease (PD). No cure of PD currently exists or will be developed in the foreseeable future. Rather, the next breakthrough in the treatment of PD will be aimed at interference or blockade of disease progression. However, the development of this new generation of disease-modifying drugs is hampered by the lack of adequate diagnostics and biomarkers that reflects early signs of disease. The motor signs of PD are often preceded by non-motor symptoms where olfactory deficit is one of the earliest; deficits that are mediated by neural degeneration in the olfactory bulb. Today, we can acquire relatively non-invasive recordings from all main stages of human olfactory processing but one, the olfactory bulb. This lack of existing recording techniques poses a serious problem for the advancement of olfactory function as an early biomarker for PD. Further development of this method to assess OB processing in the awake human using a non-invasive technique would be of great importance for a more complete understanding of human olfactory processing in health and disease, and we are working on the aforementioned NIH application as well as applications targeted towards specialized foundations.

12. Future of Research Project. What are the future plans for this research project?

In lieu of the current funding situation, we are awaiting current and future funding decisions before continuing this line of research. Moreover, to further develop this method towards a technique more friendly to the clinical demands of easy testing and short testing times, we need collaboration from mathematicians knowledgeable in bio-signaling processing to optimize analyses. We are currently establishing working relationships with bioengineers to develop a spectral analyses software to better target these responses, thus limiting the amount of repetitions needed. If future funding is obtained, our aim is to further improve our measures within this working collaboration.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No___X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No_____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

As a direct result of obtaining these funding, we have established a collaboration with Dr. Donald Wilson, a Professor of Neuroscience at New York University. Dr. Wilson is the world leading expert on olfactory bulb processing in animals and has been an integrate part of the project and aided us in our interpretation of the results. Moreover, we have submitted two grant applications with Dr. Wilson as a co-author. In addition, we have established collaboration with Dr. Per Svenningsson, Professor of Clinical Neuroscience at the Karolinska Institutet in Sweden. Dr. Svenningsson is a world leading expert on neural processing and early biomarkers for Parkinsons' disease. Dr. Svenningsson is also a co-author on a pending grant application, as outlined above.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:
These data has yet to be either published or presented at a conference.

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate

whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Overview

The goal of the proposed research was to establish in humans the first non-invasive measure of olfactory bulb (OB) responses to odor stimuli. We hypothesized that signals obtained via recordings from extranasal electrodes at the nasal bridge represent responses from the olfactory bulb (OB), so-called Electro-Bulbograms (EBG). If our hypothesis is confirmed, this innovation would not only enable further explorations of the role fulfilled by the OB in the human olfactory system, but would also be easily implemented as an everyday clinical tool. The specific aims of this project were to determine whether the recorded putative EBG signal originates from the olfactory receptors by using a paradigm demonstrated to clearly alter OB responses, but not olfactory receptor responses, in other animals. Moreover, we wanted to determine whether the putative EBG signal originates from the OB or from cortical structures by using a paradigm demonstrated to alter signal in piriform cortex but not the OB. The specific aims of this project were:

AIM 1 - To test the alternative hypothesis that the Electro-Bulbogram (EBG) response originates from receptor processing. Nutritional state and food/non-food odor distinction have both been demonstrated to alter the signaling of the OB in non-human animals, but no effect has been demonstrated for receptor firing. We will test the hypothesis that the OB signal in response to a food odor is modulated by the individual's nutritional state. In two separate sessions, subjects will be exposed to either 'food associated odors' or 'non-food

associated odors,' or be hungry or satiated. Based on the literature demonstrating nutritional state-dependent modulation of the OB signal, we expect to find a clear difference between the two odors, as well as nutritional state, where differences would indicate an OB signal. A lack of significant differences would suggest that the signal originates from olfactory receptors.

AIM 2 - To test the alternative hypothesis that the EBG response originates from cerebral processing. Human olfactory cortex is known to demonstrate rapid habituation to repeated odor exposure whereas the OB displays no reduced firing. Thus, a significant decline of the signal due to repetitive odor presentation would indicate that the OB signal has a major cortical source. Signals will be recorded during two sessions on two consecutive days; a 'Response session', during which odor will be delivered using long inter-stimulus-interval (ISI), and a 'Habituation session', during which odors will be delivered using short ISI. We hypothesize that: 1) there will be no significant difference in the OB response between the two sessions, 2) there will be a significant and clear difference for all scalp components between sessions in that all peak amplitudes from the Habituation session will be either reduced or non-existent.

Summary of Research Completed

In this Final Progress Report, we are reporting data from two main experiments as well as outlining results from four pilot studies; although not explicitly stipulated in the original proposal, all were conducted wholly using Pennsylvania Department of Health 2013 Formula grant funds (PA DOH, SAP grant number 4100062213), funding that created the foundation for these experiments. To directly link the outcome to the two specific aims, all studies and results will be presented under the respective specific aims.

Specific Aim 1. To test the alternative hypothesis: that the EBG response originates from receptor processing

To select and optimize odor stimuli, we initially performed three pilot studies. In Pilot Study 1, we determined iso-intense odor concentrations of two odors that previous pilot studies (not reported within this report) identified as being perceived as being food odor related.

Pilot Study 1

Aim: In Pilot Study 1, we assessed whether the two target odors we selected were perceived as iso-intense and iso-pleasant by our common testing population.

Methods*:* A total of 14 healthy individuals (8 women and 6 men; mean age 22.3 participated in Pilot Study 1. The two odors selected were both complex odor mixtures to account for odorant-dependent effects to which the olfactory bulb is known to be sensitive. Because Experiment 1 modulates nutritional state (hungry vs. satiated) as the independent variable, the two odors also varied in nutritional load. One of the two odors is commonly perceived to originate from a low energy food source (orange) whereas the other odor is commonly perceived to originate from a high energy food source (peanut). The orange odor consisted of 30% volume/volume (v/v) concentration of orange extract (Orange Oil, Sigma Aldrich) diluted by mineral oil (Sigma Aldrich) and the peanut odor consisted of 35% v/v concentration of peanut extract (roasted peanut odor, Takasago Inc.) diluted in mineral oil (Sigma Aldrich); each odor was presented using amber 150 milliliter (ml) glass bottles with a total of 10ml in each. A total of 14 participants rated the two odors for perceived intensity and pleasantness using computerized visual analogue scales ranging from 'Very Weak' to 'Very Intense' for the intensity ratings and 'Very Unpleasant' to Very Pleasant' for the pleasantness ratings. Each participant provided both ratings for each odor four times and the

geometric mean of their ratings were used for statistical analyses. Odors were presented using a fully automatic, computer operated, olfactometer with a known high temporal processing (20 millisecond [ms] odor rise-time) using a total flow of 3 liters per minutes (l/m). Each odor presentation lasted for a total of 1 second. Statistical differences were assessed using repeated-measures analyses of variance (ANOVA).

Results*:* There were no statistical differences between the two odor samples in either their perceived intensity [$F(1,13) = 1.25$, p ns.] or their perceived pleasantness [$F(1,13) = 1.9$, p ns.]. These data demonstrate that the odors we aim to use are good stimuli with respect to perceptual values.

Pilot Study 2

Contrary to presenting visual and auditory stimuli, presenting odors in a controlled manner is a non-trivial task. For each stimulus presentation, physical objects need to be delivered from a container to the odor receptors located at the roof of the nasal cavity. In addition, to this physical delivery time, one must factor in the mechanical delay, which is summed up by both the delay within each mechanical valve within the olfactometer (the odor stimulus delivery device) and the delay in TTL signal travel time and processing. The stimulus delivery time is seldom explored and more seldom reported although this has a significant impact on the reported latencies.

Aim: In Pilot Study 2, we aimed to determine the onset time delay within our setup. In other words, we determined the time it took from stimulus trigger (simultaneous to olfactometer and EEG system) until the odor is delivery to the nasal cavity and the odor receptors.

Methods: We measured time between trigger onset to the time the odorant exited the nose piece, normally located within the nasal cavity, using a high performance Photon Ionizing Detector, PID (MiniPID 2.0, Aurora Scientific, Montreal, QC). After allowing the system to stabilize over 20 stimulus deliveries, we presented another 20 stimuli of each odorant of the two odors with a 20s inter stimulus interval in a randomized order. Odors were presented using a fully automatic, computer operated, olfactometer with a known high temporal processing (20ms odor rise-time) using a total flow of 3 liters per minutes (l/m). Each odor presentation lasted for a total of 1 second. We thereafter measured, for each stimulus, the time in ms from TTL trigger onset to the time a 50% peak-based concentration of the odorant was identified.

Results: Average stimulus onset delay for the two odors was, on average, 210ms (SD 21). There were no significant differences between the two odors with respect to stimulus odor onset time ($p > .49$). Based on this, we adjusted all event-related potentials (ERP) latencies with -210ms to adjust for the known stimulus onset time.

Pilot Study 3

In Pilot Study 3, we determined that our paradigm was able to produce clear scalp event-related potentials (ERPs) as well as clear responses from the putative olfactory bulb electrodes. This step was taken to assure that we had a working paradigm that was sensitive enough to detect the signal of interest.

Aim: In Pilot Study 3, we determined that our paradigm was able to produce clear scalp event-related potentials (ERPs) as well as clear responses from the putative olfactory bulb electrodes. The former was assessed to validate our stimulus section and temporal

presentation because scalp ERP recordings are known to be very sensitive to presentation methods with poor temporal accuracy and the latter was assessed to assure that our dependent measures performed well.

Methods*:* A total of 5 healthy individuals (4 women and 1 man; mean age 24.3) participated in Pilot Study 3. Olfactory bulb responses and EEG signals were simultaneously and continuously recorded with an Active-Two system (BioSemi, Amsterday, NL) using 32 active electrodes. The olfactory bulb response was recorded from 4 additional active electrodes placed slightly above and at the extension of the eyebrows. Signals were recorded with a sampling rate of 512 hertz (Hz) and analog filtered (0.06 and 100 Hz). All data were pre-processed using EEGLAB, a MatLab signal analyses toolbox, and data was segmented into epochs from -500 to 1000 milliseconds (ms), relative to the onset of the odor stimulus, determined using a photon ionizing detector. Extended infomax independent component analyses were applied to the concatenated single trials, and independent components representing common electroencephalography (EEG) artifacts, such as eye blinks, were identified and removed. Data was then re-referenced to the average mastoid signal; the baseline (-200 to 0 ms) was subtracted, and a 30 Hz low-pass filter was applied. Subsequently, evoked responses were computed by averaging all valid responses using a standardized algorithm within the EEGLAB software. Valid deflections in the signal were identified using multiple-comparisons-adjusted t-tests, per sampling-point, against the known baseline. Differences between conditions were assessed with adjusted t-tests of peak amplitudes. For the olfactory bulb responses, the four electrodes were merged into one response of interest to account for variations in individual electrodes. For the scalp ERP analyses, ERPs for single electrodes were merged into three regions of interest (ROIs), namely, Anterior, Central, and Posterior ROIs, to avoid a loss of statistical power and reduce variance.

Identical odors as presented above for the Pilot Studies were used. Similarly to what was described above, odors were presented using the same olfactometer with the same settings.

Results: The analyses of the olfactory bulb component demonstrated that our paradigm could indeed invoke a clear response that is not co-located in time with the scalp ERP response (Figure 1). Moreover, the analyses of the scalp ERP component over the electrode cluster centered over the Cz electrode, electrode positions known in past studies to display the most robust odor related ERP response, indicated that our stimulus paradigm could readily produce clear ERP responses (Figure 2). Taken together, this pilot study confirms that our methods and stimulus presentation paradigms are sound and are able to reliably detect our responses of interest with the parietal electrode cluster being the cluster with the best signal to noise ratio.

Experiment 1

Aim: In Experiment 1, we tested our alternative hypothesis that the putative olfactory bulb response originates from receptor processing. This alternative hypothesis is based on the basic assumption that nutritional state is not able to modulate odor receptor responses.

Methods*:* A total of 20 healthy individuals (10 women and 10 men; mean age 29.94) participated in Experiment 1. We initially determined that all participants had working olfactory functions by screening for anosmic and hyposmic individuals using a 16 item cued odor ID test where scores below 12 might indicate a deficit. All participants scored above 12 on the test with a mean score of 13.55 (range 13-15). Olfactory bulb responses and EEG signals were simultaneously and continuously recorded as described above with the exception

that measures were obtained in two separate sessions while participants were exposed to the two separate odors in each session; each odor was repeated a total of 40 times. In the nutrition-deprived session, participants commenced testing at 8am and were not allowed to ingest anything but water from 8pm until the start of testing 12h later. In the satiated session, a standardized breakfast, based on caloric intake per measured body weight was served shortly before EEG preparations began (attachment of electrodes etc.). The order of sessions was counterbalanced. All other measures, settings, and analyses were conducted as described above for Pilot Study 3. Due to bad recordings in one or both sessions, three individuals were excluded from further analyses rendering a final sample of 17 individuals.

Results: We initially analyzed whether there were any nutrition-based differences in perceived intensity or pleasantness of the odor stimuli. There were no nutrition-based differences in either perceived pleasantness or perceived intensity, as assessed by repeated measures of ANOVA [$F(1,16) = 0.92$, $p=0.35$ and $F(1,16)=1.037$, $p=0.32$, respectively], see Figure 3. These results mean that differences in our electrophysiological measures cannot be directly explained by perceptual differences in our stimuli.

We then assessed whether there was a difference in the olfactory bulb response between the two nutritional states. These analyses demonstrated that there was a significant difference in the response between nutritional states (Figure 4), [$F(1,16) = 5.12$, $p =.038$]. The significant difference between the EBG responses in the two sessions indicate that signal origin is significantly modulated by nutritional state of the individual. This result conclusively supports our a priori assumption that nutritional state modulates the putative olfactory bulb responses, thus being in line with the assumption that these signals are indeed not a measure of olfactory receptor responses. In other words, the recorded responses do not originate from peripheral sources. The results from this experiment fulfill the goal set out in Specific Aim 1.

Specific Aim 2. To test the alternative hypothesis: that the EBG response originates from cerebral processing.

Pilot Study 4

Aim: In Pilot Study 4, we aimed to select an odor concentration level and stimulus presentation time that would maintain a clear odor percept in the initial 15 trials with the perceived intensity subsiding to a minimum in the last 15 trials. This is a non-trivial and important task. Odor concentration that renders the participants to demonstrate rapid habituation and no responses will appear within the initial 15 repetitions.

Methods*:* A total of 10 healthy individuals (5 women and 5 men; mean age 31.1) participated in Pilot Study 4. Three different odors were presented 60 times each with a 4s long odor exposure and a 12s inter stimulus interval to maximize the balance between odor habituation and a clear percept in enough trials to form valid odor ERPs. Each odor was presented within separate blocks. We selected mono-molecular odors to maximize the habituation of the piriform cortex, namely isopropanol (1%), amyl acetate (80%), and geraniol (80%). After each trial, participants rated the odor for perceived intensity using computerized visual analogue scales ranging from ‘Very Weak’ to ‘Very Intense’. Odors were presented using a fully automatic, computer operated, olfactometer with a known high temporal processing (20ms odor rise-time) using a total flow of 3 liters per minute (l/m).

Results*:* Of the three odors, isopropanol was the odor that created the best psychometric function with an initial clear intensity that lasted beyond the first 15 trials before plateauing or demonstrating a complete lack of percept. See Figure 5 for perceptual values.

Experiment 2

Aim: In Experiment 2, we tested our alternative hypothesis that the putative olfactory bulb response originates from the olfactory cortex. This alternative hypothesis is based on the knowledge that the olfactory cortex and the piriform cortex demonstrate rapid habituation and adaptation; whereas the olfactory bulb is, to a large extent, insensitive to these mechanisms.

Methods*:* A total of 22 healthy individuals (11 women and 11 men; mean age 29.55) participated in Experiment 2. We initially determined that all participants had working olfactory functions by screening for anosmic and hyposmic individuals using a 16 item cued odor ID test where scores below 12 might indicate a deficit. All participants scored above 12 on the test with a mean score of 13.5 (range 13-15). Olfactory bulb responses and EEG signals were simultaneously and continuously recorded with four experiment-specific exceptions. First, only one odor was used throughout the experiment to maximize the likelihood of perceptual habituation. Second, recordings were obtained within only one session divided into two identical blocks with a 20 minute break in-between to allow the sensory system to recopy from the sensory habituation. Third, analyses were done by merging the 15 first stimulus presentations in the two blocks together (First 15 Trials) and merging the last 15 stimulus presentations in the two blocks together (Last 15 Trials). The ISI was a mere 16s with a 4s long odor to render a habituation curve that would allow a clear odor percept for the first 15 presentations but with a sharply reduced odor percept for the last 15 trials. Within each block, the odor was repeated a total of 60 times. All other measures, settings, and analyses were conducted according to what is described above for Pilot Study 3. Due to bad recordings in one or both sessions, three individuals were excluded from further analyses rendering a final sample of 19 individuals. We extracted maximum peak amplitudes within the a priori identified time window for the EBG response (150-250ms) as well as the a priori identified time window for the N1 component of the ERP (380-450), the component most likely to be derived directly from intensity perception.

Results: Although a slightly sharper reduction in intensity perception over time was evident in this study compared to what was found in Pilot Study 4, the intensity perception of the participants demonstrated a similar psychometric function to what we predicted and based our analyses on, see Figure 6. We initially assessed the statistical difference between the N1 amplitude at the beginning of the experiment (First 15 Trials) and at the end of the experiment (Last 15 Trials). As fully expected, there was a significant and large reduction in N1 signal amplitude in response to the presented odor [$F(1,18) = 8.66$, $p > .01$]. We thereafter assessed the statistical difference between EBG at the beginning of the experiment (First 15 Trials) and at the end of the experiment (Last 15 Trials). As predicted, even though there was a nominal reduction in EBG amplitudes due to adaptation, this reduction did not reach statistical significance, [$F(1,18) = 2.57$, $p = .13$], see Figure 7.

This result conclusively supports our a priori assumption that an odor habituation paradigm would significantly reduce the scalp ERP response, based on the fact that the piriform cortex is known to be very receptive to odor habitation, whereas there would only be a slight reduction in olfactory bulb signaling, based on the fact that the olfactory bulb is mostly receptive to adaptation processes originating in the olfactory receptor neurons. These results

are therefore in line with the assumption that the so-called EBG signals are indeed not a measure of responses within the olfactory cortex. In other words, the recorded responses do not originate from central sources, but rather from the olfactory bulb. The results from this experiment fulfill the goal set out in Specific Aim 2.

Figures and Figure Legends

Figure 1: Putative olfactory bulb responses, sampled from the four 'bulb' electrodes, in *Pilot Study* 3. Data from the two odors combined for maximum power. Note the large negative deflection around 200ms after odor onset, a time window where odor ERPs are not commonly developed.

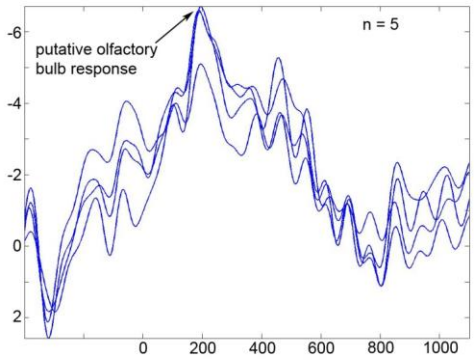


Figure 2: Scalp ERP response, sampled from the Cz electrode cluster, from the two odors combined in *Pilot Study 3*. Note the large positive deflection around 620ms after odor onset typical for odor-evoked ERPs.

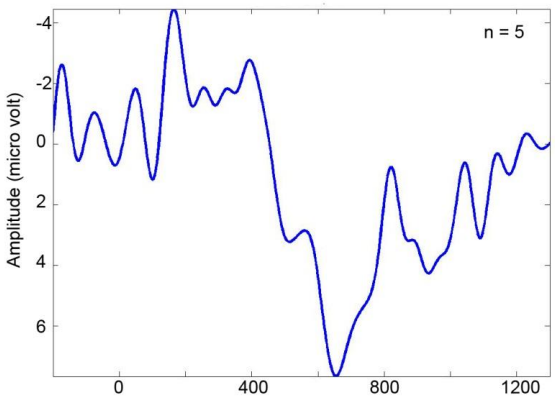


Figure 3: Average perceptual ratings (n=20) of perceived odor intensity and pleasantness within *Experiment 1*, divided per nutritional state. Error bars in graph represent standard-error of the means. There were no statistical differences between the two nutritional states for either perceived intensity or pleasantness. Note that the scale is ranging from 0 to 10.

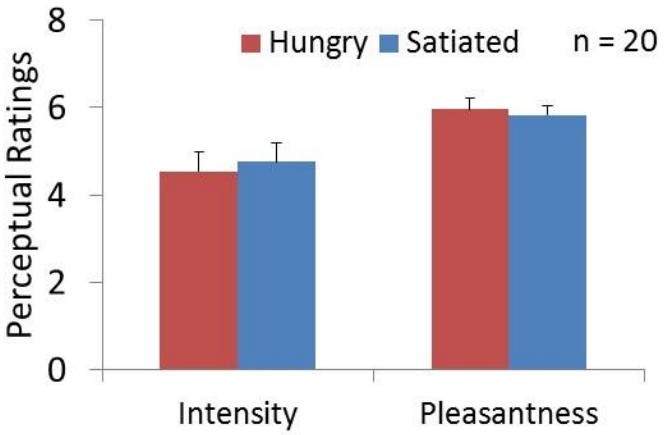


Figure 4: Putative olfactory bulb responses, pooled over the four 'bulb' electrodes, in *Experiment 1*. Mean olfactory bulb response amplitude displayed for each nutritional condition separately. Error bars in graph represent standard-error of the means. Blue bar indicates mean response during testing session where participants felt satiated and red bar indicates mean response where participants felt hungry. Data from the two odors were combined for maximum power. Max peak lasting longer than 10ms were extracted in the time window 150-250 post odor onset for each participant and condition. Time window selection was identified by both the grand average and based on previous pilot data.

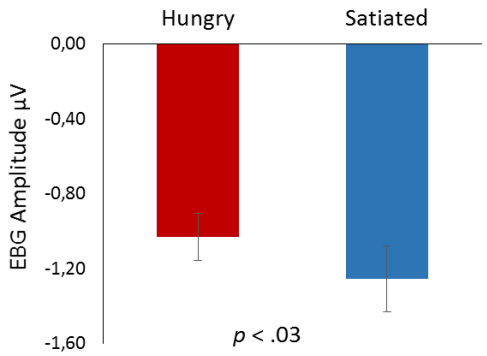


Figure 5: Mean intensity rating of each trial in *Pilot Study 4*. Due to the low number of participants, the function is smoothed with a 5-trials-wide full with at half maximum (FWHM) smoothing kernel.

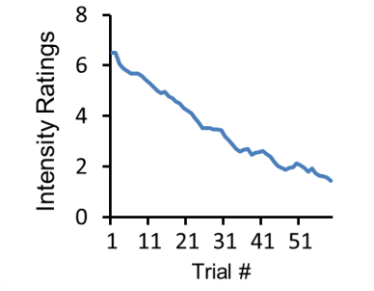


Figure 6: Mean intensity rating of each trial in *Experiment 2* (both blocks combined). Grey areas indicate trials included in the electrobulbogram and scalp event-related potentials analyses. Error bars indicate standard error of the mean. Note the clear distinction between intensity ratings in the first 15 trials and intensity ratings in the last 15 trials, thus indicating a strong habituation effect. Note that the scale is ranging from 0 to 10.

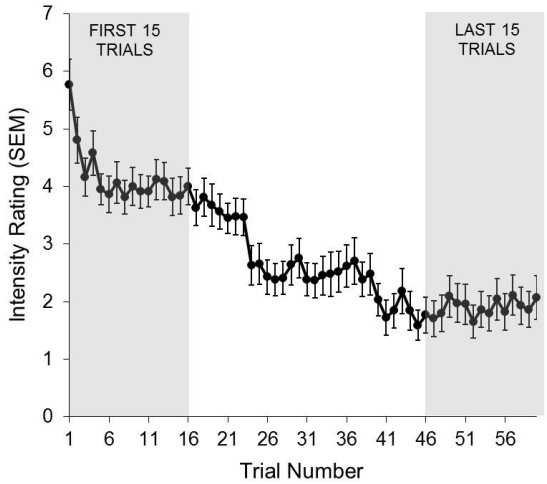
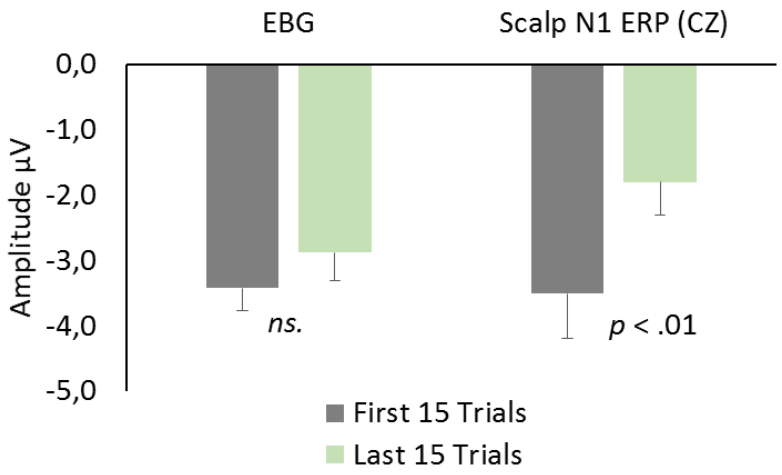


Figure 7. Putative olfactory bulb responses, pooled over the four 'bulb' electrodes, in *Experiment 2*. Mean olfactory bulb response amplitude displayed for the first 15 trials and the last 15 trials, separately. Error bars in graph represent standard-error of the means. Grey bar and line indicate mean response during the initial 15 trials in both sessions whereas the red bar indicates mean response during the last 15 trials in both sessions. Max peaks for the electrobulbar response were extracted as described above for Experiment 1 and max N1 peak for the scalp electrode cluster centering on the central midline electrode were extracted based on time window selection identified by both the grand average and based on previous pilot data. Statistical differences were assessed by repeated measures ANOVAs where ns. indicates no significant difference.



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes

___X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____X____ No_____

If yes, please describe your plans:

Our aim is to publish the two studies described above within one publication.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This project lends support to the notion that the EBG measure is a valid measure of olfactory bulb signaling. Further testing must, however, be done to further characterize the signal before a definitive answer is at hand. Nonetheless, the variation in signal strength of the EBG measure between the two experiments implies that although this signal may eventually be demonstrated, beyond critique, to originate from the olfactory bulb, its clinical usefulness might be limited. Future studies should explore whether this variation is an experimental artifact or a defining character.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

A. Personal Statement

The goal of my present research is three-fold; (1) to define the behavioral effects of social chemosignals and their neural substrates, (2) to map the neural network underlying multimodal sensory integration, and (3) develop novel odor stimulus presentation and measurement techniques. In doing this, we use a multifaceted approach to explore the neural networks responsible for processing established behavioral effects using various neuroimaging techniques, such as electroencephalography, functional magnetic

resonance imaging, positron emission tomography, and transcranial magnetic stimulation. These methods are used in complement with each other to triangulate the precise location and function of the neural connections. I have a broad background in biological psychology with extensive training in psychophysical and psychophysiological measures during my graduate school and extensive training in various neuroimaging methods during my postdoctoral training at the Montreal Neurological Institute, McGill University. Moreover, I have through successfully finishing multiyear projects, acquired skills to administer advanced research projects as well as coordinate multi-site collaborative studies.

B. Positions and Honors.

Positions and Employments

2005-2007, Postdoctoral fellow, Dept. of Psychology, McGill University
2007 – Present, Assistant Member, Monell Chemical Senses Center
2008 – Present, Adjunct Assistant Professor, Dpt. of Psychology, University of Pennsylvania
2009 – Present, Adjunct Assistant Professor, Dpt. of Clinical Neuroscience, Karolinska Institute

Honors

2006 The Royal Swedish National Academy of Sciences Price to Promising Young Researcher in Psychology
2009 Moskowitz Jacobs Inc. Award for Research Excellence in the Psychophysics of Taste and Smell, Association for Chemoreception Sciences
2012 Wallenberg Academy Fellow. A competitive fellowship program open for young researchers across all sciences and countries. The Academy of Sciences select individuals after interviews. The fellowship includes financial and mentoring resources. [~US$ 2 million]

Peer-reviewed Publications Last 3 Years (selected from a total of 48 peer-reviewed publications)

Olsson, M.J., Lundström, J.N., Gordon, A,R., Karshikoff, B., Hosseini, N., Sorjonen, K., Kimball, B.A., Hoglund, C.O., Soop, A., Axelsson, J., and Lekander, M. (2014). The smell of disease: Human body odor contains an early chemosensory cue of sickness. Psychological Sciences.

Boesveldt, S. and Lundström, J.N. (2014). Smelling fat content of food from a distance: olfactory-based fat discrimination in humans. PLoS ONE. 9(1): e85977.

Freiherr, J., Lundström, J.N., Habel, U., and Reetz, K. (2013). Multisensory integration mechanisms during aging. Frontiers in Human Neuroscience. 7:836.

Ohla, K. & Lundström, J.N. (2013). Sex differences in chemosensation: sensory or emotional? Frontiers in Human Neuroscience. 7:607.

Lundström, J. N., Schaal, B., Mathe, A., Frasnelli, J., Nitzsche, K., Gerber, J., and Hummel, T. (2013). The body odors of newborns activate women's neuronal reward system. Frontiers in Psychology. 4:597.

Seubert, J., Freiherr, J., Frasnelli, J., Hummel, T., and Lundström, J.N. (2013). Orbitofrontal cortex and olfactory bulb volume predict distinct aspects of olfactory performance in healthy subjects. Cerebral Cortex. 23(10), 2448-56.

Schriever, V., Hummel, T., Lundström, J.N., and Freiherr, J. (2013). Size of nostril opening as a measure of intranasal volume. Physiology and Behavior.110-111, 3-5.

Åhs, F., Miller, S.S., Gordon, A.R., and Lundström, J.N. (2013). Aversive learning increases sensory detection sensitivity. Biological Psychology. 92(2), 135-41.
Miller, S.S., Gordon, A.R., Olsson, M.J., Lundström, J.N., and Dalton, P. (2013). Mind over age: Social priming and olfactory function. Chemical Senses.38(2), 167-74.
Seubert, J., Freiherr, J., Djordjevic, J., and Lundström, J. N. (2013). Statistical localization of human olfactory cortex. NeuroImage.66(1), 333-342.
Frasnelli, J., Lundström, J. N., Schöpf, V., Negoias, S., Hummel, T., and Lepore, F. (2012). Dual processing streams in chemosensory processing. Frontiers in Human Neuroscience. 6, 288. doi: 10.3389/fnhum.2012.00288
Wise, P., Wysocki, C.J., and Lundström, J.N. (2012). Stimulus selection for intranasal sensory isolation: eugenol is an irritant. Chemical Senses. 37(6), 509-14.
Mitro, S., Gordon, A.R., Olsson, M.J., and Lundström, J.N. (2012). The smell of age: perception and discrimination of body odors of different ages. PLoS ONE. 7(5): e38110.
Jadauji, J.B., Djordjevic, J., Lundström, J.N., and Pack, C.C. (2012). Modulation of olfactory perception by visual cortex stimulation. Journal of Neuroscience. 32(9), 3095-100.
Lundström, J. N., Gordon, A.R., Wise, P., and Frasnelli, J. (2012) Individual differences in the chemical senses: is there a common sensitivity? Chemical Senses. 37(4) 371-8.
Freiherr, J., Boesveldt, S., Gordon, A. R., Alden, E. C., Hernandez, M., and Lundström, J. N. (2011). The 40-item Monell Extended Sniffin' Sticks Identification Test (MONEX-40). Journal of Neuroscience Methods. 205(1),10-16.
Frasnelli, J., Albrecht, J., Bryant, B., and Lundström, J. N. (2011). Perception of specific trigeminal chemosensory receptor agonists. Neuroscience. 189, 377-83.
Boesveldt, S., Tessler-Linday, S., Schumm, L. P., McClintock, M.K., Hummel, T., and Lundström, J. N. (2011). Gustatory and olfactory dysfunction in older adults: a national probability study. Rhinology. 49(3), 324-30.
Veldhuizen, M. G., Albrecht, J., Zelano, C., Boesveldt, S., Breslin, P., and Lundström, J. N. (2011). Identification of human gustatory cortex by activation likelihood estimation. Human Brain Mapping. 32(12), 2256-66.
Frasnelli, J., Lundström, J. N., Boyle, J. A., Katsarkas, E. and Jones-Gotman, M. (2011). The vomeronasal organ is not involved in the perception of endogenous odors. Human Brain Mapping. 32(3), 450-460.