Monell Chemical Senses Center

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

The Monell Chemical Senses Center received $234,200 in formula funds for the grant award period January 1, 2013 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Early Stage Recordings of Cognitive Odor Processing – The purpose of this project is to establish a novel measure of olfactory responses occurring at the first input to the human brain, the olfactory bulb (OB). This innovation would not only enable further explorations of the role fulfilled by the olfactory bulb in the cognitive and perceptual processing of odors but would also be easily implemented as an everyday clinical tool. A technique allowing measures of human OB signals will greatly aid future olfactory-related translational work and establish a new paradigm for studies of human olfactory processing.

Duration of Project

1/1/2013 – 12/31/2013

Project Overview

The goal of the proposed research is to establish in humans the first non-invasive measure of olfactory bulb (OB) responses to odor stimuli. We hypothesize 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 are to determine whether the recorded putative EBG signal originates from the olfactory receptors by using paradigms demonstrated to clearly alter OB responses, but not olfactory receptor responses, in other animals. Moreover, we will determine whether the putative EBG signal originates from the OB or from cortical structures by using paradigms demonstrated to alter signal in piriform cortex but not the OB. The specific aims of this project are:

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.

Principal Investigator

Johan N. Lundstrom, PhD
Assistant Member
Monell Chemical Senses Center
3500 Market Street
Philadelphia, PA 19104

Other Participating Researchers

Janina Seubert, PhD; Kristen Gregory, BSc – employed by Monell Chemical Senses Center

Expected Research Outcomes and Benefits

The proposed work will establish a novel, non-invasive measure of human olfactory bulb (OB) responses to odor stimuli, and allow assessment of neural responses from one of the early stages of human olfactory processing using an inexpensive, non-invasive, and temporally-precise recording method. For the first time, we would be able to acquire data from the complete human olfactory system. If established, these measures would enable detailed investigations into the role of the OB in a wide variety of clinical disorders known to affect olfactory processing, such as neurodegenerative (Alzheimer's disease, Parkinson's disease, and multiple sclerosis) and eating disorders as well as schizophrenia, to mention only a few.

Summary of Research Completed

In this progress report, we are reporting data from three studies; two main experiments and one pilot study. In the previous annual progress report, we reported data from an incomplete

experiment (14 out of 20 subjects analyzed) and have here opted to report the full and final analyses.

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

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). Subjects participated in two separate sessions while exposed to two separate food-related 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 electroencephalography (EEG) preparations began (attachment of electrodes etc.). The order of sessions was counterbalanced. Olfactory bulb responses and EEG signals were simultaneously and continuously recorded with an Active-Two system (BioSemi, Amsterdam, 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 were segmented into epochs from -500 to 1000 milliseconds (ms), relative to the onset of the odor stimulus, which was determined using previously obtained photon ionizing detector signals (previously reported Pilot Study 2). 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 event-related potentials (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. 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 1. 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 2), [$F(1,16) = 5.12$, $p =.038$]. 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 3 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 for Experiment 1. 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 4. 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 5.

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.

Figure 1: 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.

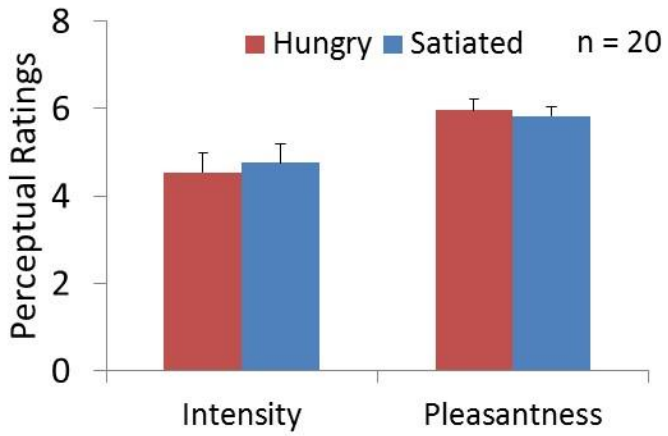


Figure 2: Putative olfactory bulb responses, pooled over the four 'bulb' electrodes, in *Experiment 1*. Mean olfactory bulb response amplitude displayed for each nutritional condition separately. Error bars in graph represent standard-error of the means. Blue bar indicates mean response during testing 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.

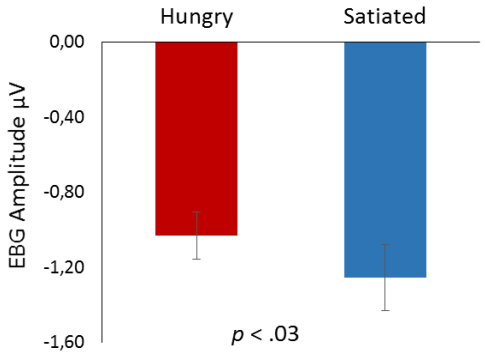


Figure 3: Mean intensity rating of each trial in *Pilot Study 4*. Due to the low number of participants, the function is smoothed with a 5-trials-wide full with at half maximum (FWHM) smoothing kernel.

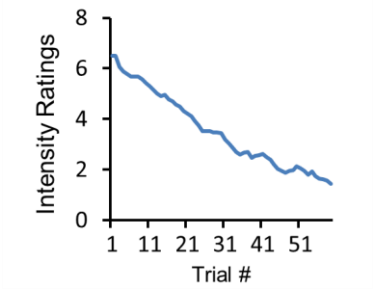


Figure 4: Mean intensity rating of each trial in *Experiment 2* (both blocks combined). Grey areas indicate trials included in the electrobulbogram and scalp event-related potentials analyses. Error bars indicate standard error of the mean. Note the clear distinction between intensity ratings in the 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.

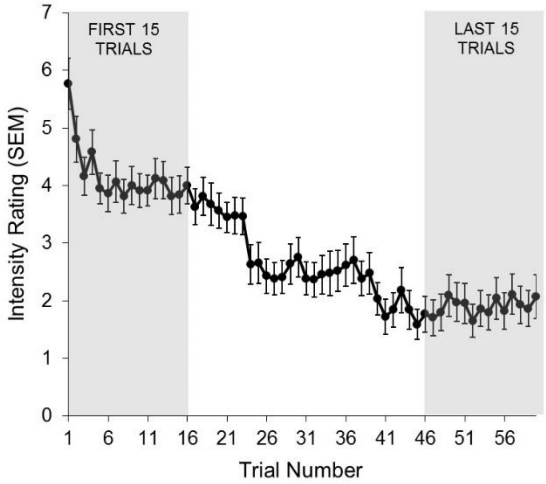
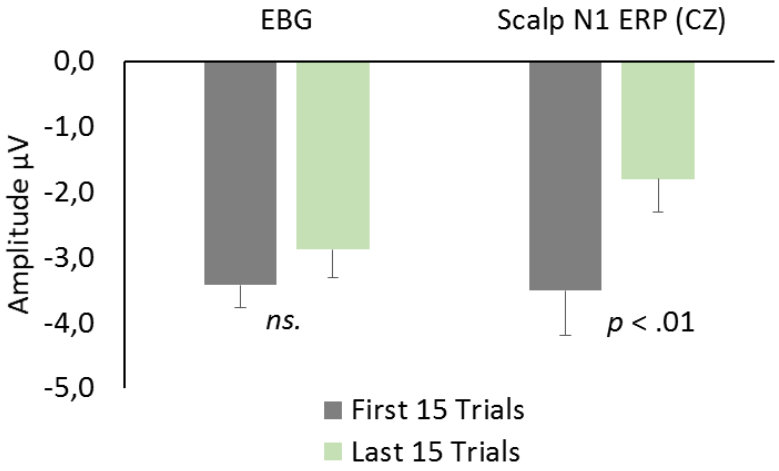


Figure 5. Putative olfactory bulb responses, pooled over the four 'bulb' electrodes, in *Experiment 2*. Mean olfactory bulb response amplitude displayed for the first 15 trials and the last 15 trials, separately. Error bars in graph represent standard-error of the means. Grey bar and line indicate mean 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.



Research Project 2: Project Title and Purpose

Bitter Taste-Induced Nausea – Bitter taste stimuli can directly elicit nausea. Usually nausea is the negative experience that accompanies toxin-induced illness. The problem lies in that some toxins are also medicines and their induced nausea is a disincentive to medical compliance, especially among children who cannot ingest encapsulated drugs. Moreover, a wide variety of gastrointestinal disorders are frequently accompanied by bitter taste reflux from gastric contents and by chronic nausea. The proposed work will determine the extent to which bitter taste evokes nausea in healthy people. This knowledge in a normative state might lead to new preventions for patients suffering from nausea due to gastric reflux or direct stimulation of bitter taste.

Duration of Project

1/1/2013 – 12/31/2013

Project Overview

Bitter taste is the evolutionary marker of poisoning, which in turn is a prime elicitor of nausea. Thus we are asking whether bitter taste is a privileged inducer of nausea. The proposed work will

determine (1) the extent to which bitter stimuli evoke nausea in healthy people, and (2) the role of perceived bitterness in nausea elicitation. In other words we will determine whether we can separate bitter perception from nausea elicitation. Bitter stimuli may indeed be able to stimulate toxin receptors that contribute to nausea in the absence of bitterness perception. In Aim 1, we will test whether different bitter tasting compounds are equally nauseating when intensity matched. Subjects will be presented with strong bitter taste stimuli in an oral stimulation protocol without swallowing. Nausea will be measured by self-assessment on a modified Muth Nausea Profile (MNP) questionnaire and by the physiological measures of electrogastrography (EGG). Four bitter solutions (SOA, denatonium benzoate, quinine sulfate (QSO4) and Phenylthiocarbamide (PTC)) will be tested for their nauseogenic effects, and compared to an equi-intense control solution (NaCl).

In Aim 2, we will test whether highly concentrated but weakly perceived bitter compounds elicit nausea to determine whether we can separate conscious bitter perception from the nausea eliciting effects of stimulation. We will manipulate perceived bitterness independently of concentration either by peripheral inhibition (using a bitter taste inhibitor: sodium glutamate) or by cognitive suppression (using mixtures of sweeteners and bitter tasting compounds). The same protocol and measurements as in Aim 1 will be used.

Principal Investigator

Catherine A. Peyrot des Gachons, PhD
Research Associate
Monell Chemical Senses Center
3500 Market Street
Philadelphia, PA 19104

Other Participating Researchers

Paul A.S. Breslin – employed by Monell Chemical Senses Center

Expected Research Outcomes and Benefits

This work has important clinical relevance. These data will help determine the basic protective physiological function of bitter taste and its elicitation of nausea as a warning against subsequent ingestion of noxious stimuli and spoiled foods. Since nausea can be a debilitating side-effect of many cancer therapies and drug treatments, and because patients with gastroparesis suffer nausea chronically as a consequence of their disorder, an improved understanding of normative nausea reflexes will provide insight into the cause of these nausea side effects and pathologies.
Many diabetic patients experience gastroparesis caused by excessive blood sugar, which inhibits gastric motility via normal feedback reflexes. The bitter gastric reflux resulting from gastroparesis is believed to be a cause of their chronic and sometimes debilitating nausea. The data obtained from this work may benefit these patients directly as we may be able to single out people at higher risk for nausea side-effects, to determine whether certain food experiences are likely to exacerbate low level ongoing nausea, and to advise vulnerable patient populations to avoid certain types of foods that are strongly bitter.

Summary of Research Completed

Aim 1 study:

Experimental design:
Healthy subjects were tested with four bitter stimuli (SOA, denatonium benzoate, quinine hydrochloride and PTC) and three control stimuli (two negative controls: an intensity matched salty stimulus (NaCl) and water, and one positive control: an opto-kinetic drum). The opto-kinetic drum (a drum with vertical white and black stripes rotating around the participant head) is an established nauseogenic stimulus, commonly used in the study of motion sickness and nausea. It was included in the study to obtain EGG recording references of nausea onset. All taste stimuli were matched for intensity. Subjects received EGG throughout testing and for 30 minutes after rinsing. They also completed modified Muth Nausea Profile (MNP) questionnaires at the beginning and at the end of the sessions and rated the intensity of the taste stimulus. To prevent adaptation to the repeated nauseogenic stimuli, subjects were tested with at least 3 days interval between test sessions. The total number of sessions per subject was eight. The first session consisted in the construction of psychophysical curves of total intensity of the compounds of interest. This first session was used to confirm that the taste stimuli given during the consecutive sessions were in the same perceived intensity range for all subjects, as well as to exclude subjects if their taste functions were not roughly ordinal over the range of concentrations tested. If the participant qualified, then, seven "EGG sessions" followed. Only one taste stimulus was tested per session: the 4 bitter stimuli were tested in random order, as well as the 3 control stimuli. The taste stimuli were exposed to the oral cavity for a total of 10 min (20 cups x 30 seconds).

Participant demographics of Aim 1 study
A total of 43 healthy participants were enrolled in the study: 12 subjects dropped the study, and 14 were removed after the first session because the results showed that they did not perceive bitter compounds well, those numbers were higher than anticipated. 18 participants completed the study. Among those 18 subjects, 33 % were men and the median age was 25.8 ± 5.8 years.

Taste stimulus intensity results
The taste intensity rating given by the participants during the first session and after the 10 min oral exposures confirmed that all the taste stimuli tested in the study were perceived by the participants as being similarly strong to very strong.

MNP questionnaire results
On average, subjects reported feeling more strongly nauseated after oral exposure to SOA than after exposure to the other bitter compounds (Figure 1). Quinine, PTC, and denatonium were similarly nauseating in intensity. As expected the MNP scores for the negative controls (water and NaCl) were low. In this study, we defined subjects as being nauseated by a stimulus if the MNP score given by the subject after exposure to this compound was higher than 15. Following this rule, the distribution of participants nauseated for each bitter compound was as follow: SOA (56%) > denatonium (44 %) = PTC (44 %) > quinine (33%). Taken together it seems that SOA is the most nauseogenic compound among the four bitter compounds tested.

EGG results

- *Positive control: opto-kinetic drum*

This is a potent and standardized tool to study nausea elicitation. In this study, we used the opto-kinetic drum as a reference to interpret the EGG results obtained with the taste stimuli. Figure 2 displays the changes in gastric myoelectrical activity (GMA) obtained with the EGG recordings. They reflect the change in activity between the pre-stimulus period (drum not rotating) and the stimulus period (drum rotating) for the dysrhythmia and normal frequency ranges. Values were natural log transformed (zero represents no change). Our results are in accordance with published results showing a strong dysrhythmia associated with nausea induction. The increase in dysrhythmia appears higher than the increase in normal rhythm during nausea induction, showing a shift toward dysrhythmia when nausea is triggered. To examine this potential shift after taste stimulation, in the report we determined the delta between GMA in dysrhythmia and GMA in normal rhythm.

- *Taste stimuli*

Figure 3 presents the effects of oral exposure to bitter compounds and control solutions on the GMA in dysrhythmia and normal rhythm for both categories of subjects (nauseated and non nauseated, based on MNP scores). For all the bitter compounds we observed an increase in dysrhythmia for the subjects who reported being nauseated by the compound and a decrease in dysrhythmia for the subjects who reported not being nauseated by the compound. A decrease in dysrhythmia was also observed after exposure to the control solutions (water and NaCl). The difference in dysrhythmia activity between the two groups reached significance for PTC and denatonium (respectively $p=0.03$ and $p=0.004$). Moreover, the overall GMA of the non nauseated subjects decreases after exposure to the bitter stimuli, which is similar to the responses to the control solutions (water and NaCl). It means that when subjects are not nauseated by the bitter compound the changes in GMA after bitter taste exposure are similar to exposure to control stimuli (water and NaCl).

The GMA delta (dysrhythmia - normal rhythm) was calculated (Figure 4). The graph shows that for the non nauseated subjects there is no shift of GMA toward dysrhythmia; the deltas were close to zero, if anything there is a slightly stronger activity increase in the normal frequency ranges. By contrast, nauseated subjects show a positive delta which indicates that after exposure to the bitter stimuli the GMA shifts toward more dysrhythmia than normal rhythm (significance was reached for denatonium, $p=0.02$). As mentioned before, such shift toward dysrhythmia has been shown to correlate with nausea induction.

<u>Aim 2 study:</u>

Experimental design:

The general protocol was the same as used with Aim 1 except for the taste stimuli. In study 1 (peripheral inhibition) subjects were tested with one bitter compound (1 μM denatonium) presented alone or in mixture with bitter blockers (15 mM zinc sulfate + 75 mM L-Glutamic acid potassium (MPG)) and with the blockers alone as negative control. And in study 2 (central inhibition) subjects were tested with denatonium presented alone or in mixture with 2M sucrose,

and with the sucrose solution alone as negative control. Because in this aim we examined the effect of decreased perceived bitter taste on perceived nausea elicited by denatonium, we had first to select participants who were nauseated by denatonium oral exposure. This was a limiting factor in the total number of participants who could complete the study. Those subjects tested all the stimuli in duplicate to increase the data reliability.

Participant of Aim 2 study

A total of 33 healthy participants were enrolled in the study: 5 subjects dropped the study, and 19 were removed after the first session because they were not nauseated by denatonium. 5 participants completed the study and 4 are still currently enrolled.

Bitterness intensity results

Immediately after exposing their mouth to the taste stimulus for 10 min, the subjects were asked to rate the overall perceived taste and the perceived bitterness. Figure 5A shows that the presence of the bitter taste blockers or high concentration sucrose decrease the perceived bitterness elicited by denatonium, in a similar range. In both cases, the denatonium elicited bitterness was perceived as lower. Nevertheless, the ratings were still placed just under the descriptor strong on the Labeled Magnitude Scale (LMS).

MNP questionnaire results

On average, subjects reported feeling less nauseated after oral exposure to denatonium when the bitter compound was in mixture compared to alone. Both the blockers and sucrose diminished the self-reported nausea sensation (Figure 5B) As an indicator, in Aim 1 we placed participants providing MNP scores lower than 15 to a stimulus in the non-nauseated category. MNP scores after denatonium + inhibitors and denatonium + sucrose were lower than 15 on average. Thus it appears that bitter taste blockers and sweeteners are inhibitors of bitter taste elicited-nausea induction. When perceived bitterness is decreased either at the peripheral level by inhibitors or the central cognitive level, the induced nausea is also diminished. As expected, the three negative controls (blockers alone, sucrose alone and water) were given low MNP scores by the participants.

EGG results

In Figure 6 , the EGG results are presented as GMA delta (dysrhythmia - normal rhythm). First they confirm the results obtained in Aim 1 study; that is, they show the shift of GMA toward dysrhythmia after oral exposure to a strong bitter taste (denatonium). Such a shift was not observed when the compound was presented in mixture with the bitter taste inhibitors, in mixture with sucrose (2M), or when the negative controls were tested. Thus the EGG results and the MNP results are in agreement. And the results so far support the idea that decreasing perceived bitterness decreases nausea induction.

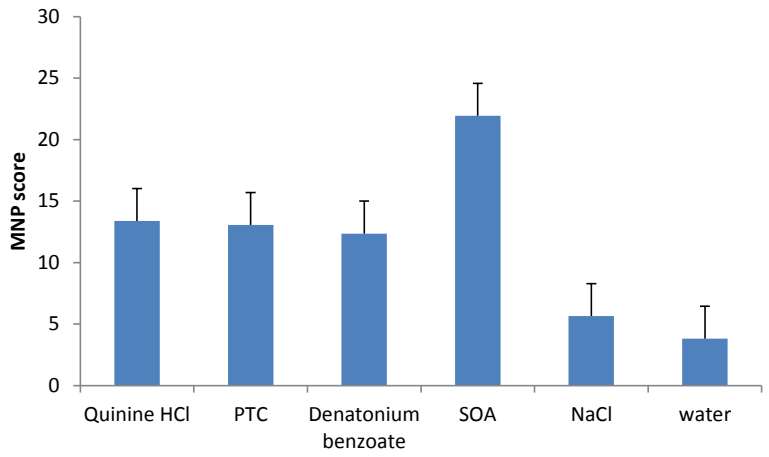


Figure1: Muth Nausea Profile (MNP) scores collected after oral exposure to taste stimuli (n=18; means ± SEM) (SOA = sucrose octoacetate and PTC = phenylthiocarbamide).

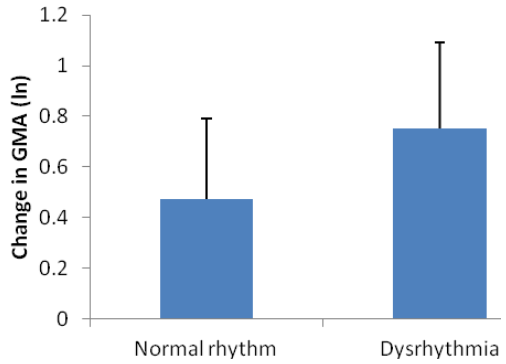


Figure 2: Changes in gastric myoelectrical activity (GMA) between pre-stimulation and stimulation with the rotating opto-kinetic drum, in dysrhythmia and normal frequency ranges (means ± SEM) (n=18). Values were natural log transformed (ln).

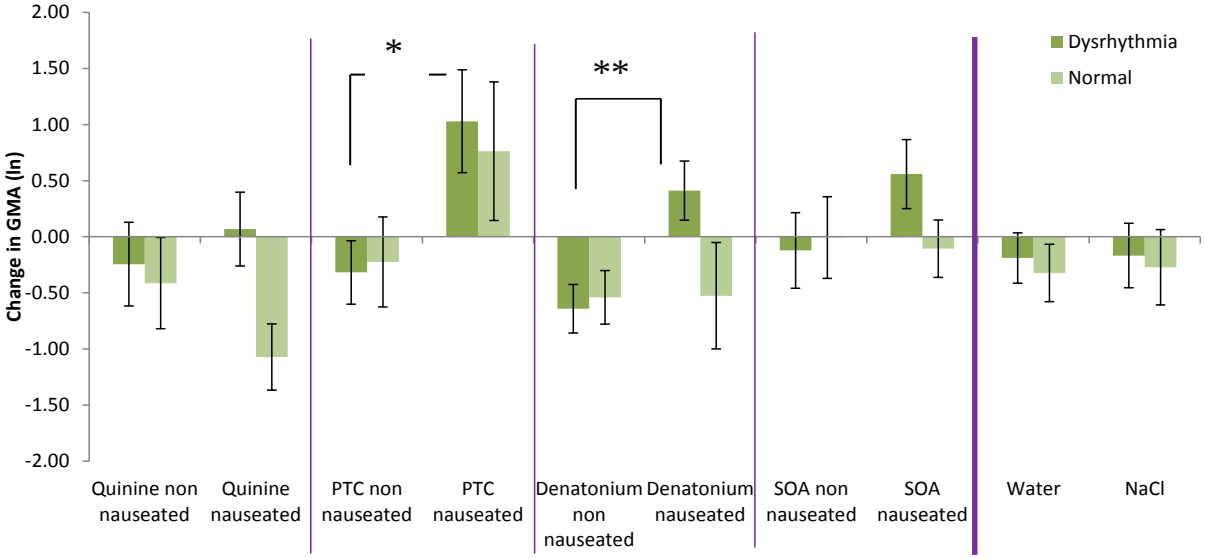


*Figure 3: Change in gastric myoelectrical activity (GMA) after oral taste stimulus exposure in dysrhythmia and normal frequency ranges (means ± SEM) for participants nauseated and participant not nauseated by the compound tested (Quinine: non nauseated n=12, nauseated n= 6 ; PTC : non nauseated n=10, nauseated n=8 ; Denatonium: non nauseated n=10, nauseated n=8 ; SOA : non nauseated n=8, nauseated n=17). Asterisks show significance: * p<0.05 and ** p<0.01 (SOA = sucrose octoacetate and PTC = phenylthiocarbamide).*

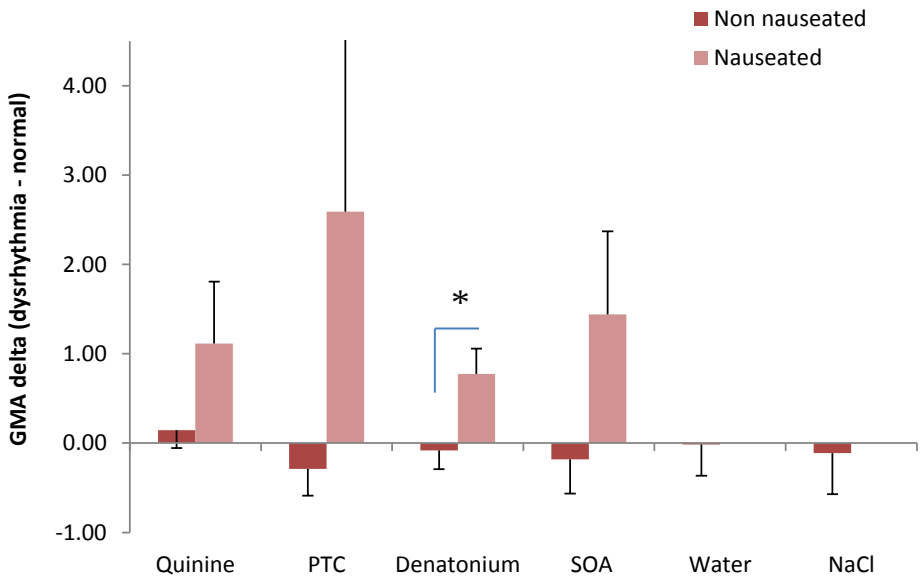


*Figure 4: Shift in gastric myoelectrical activity (GMA) between pre and post exposure to the bitter stimuli. Each bar corresponds to the GMA delta: dysrhythmia – normal frequency for both categories of participants (nauseated and non nauseated by the compound tested). Asterisks show significance: * p<0.05 (SOA = sucrose octoacetate and PTC = phenylthiocarbamide).*

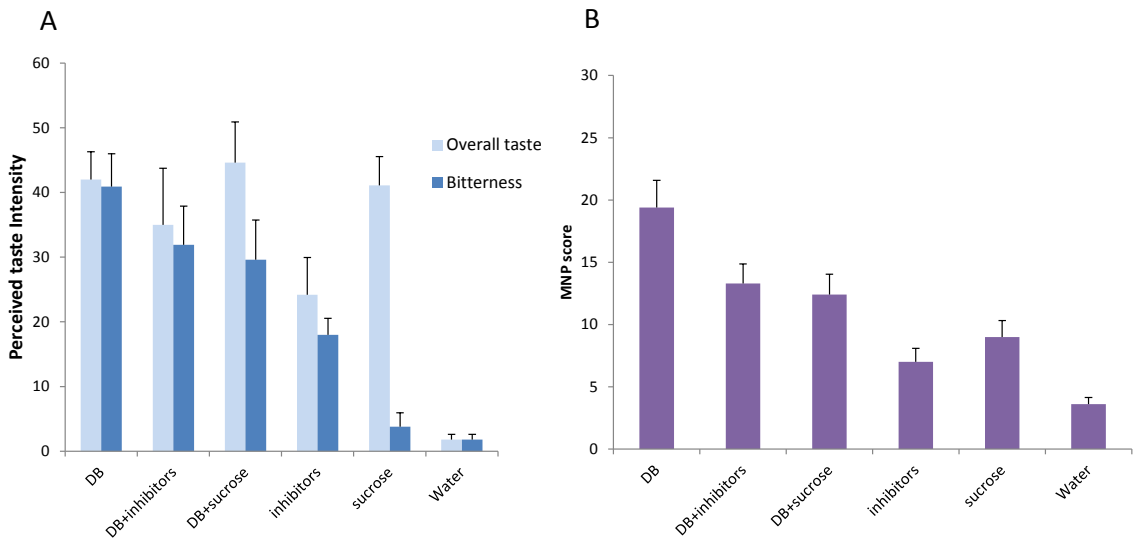


Figure 5: (A) *Perceived overall taste and bitterness intensities rated after oral exposure to taste stimuli.* (B) *Muth Nausea Profile (MNP) scores collected after oral exposure to taste stimuli (means ± SEM). (DB = denatonium benzoate).*

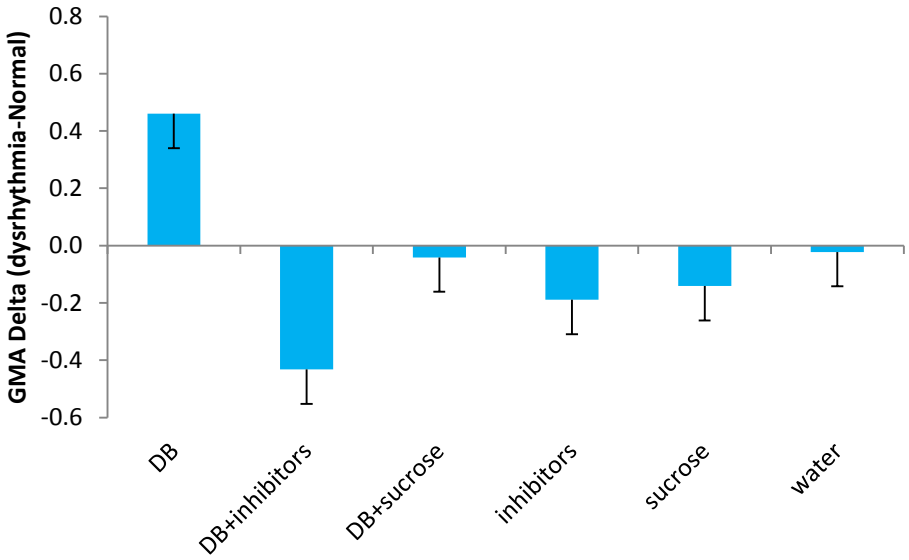


Figure 6: Shift in gastric myoelectrical activity (GMA) between pre and post exposure to the taste stimuli. Each bar corresponds to the GMA delta: dysrhythmia – normal frequency. (DB = denatonium benzoate).