Protocol title:
Olfactory contributions to sleep-dependent food craving and calorie intake

NCT04179838

Document date:
4/22/2019

STU#:00203395

PROTOCOL TITLE: Olfactory contributions to sleep-dependent food craving and calorie intake

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VERSION NUMBER: 2

VERSION DATE: July 27, 2016

OBJECTIVES:

Purpose
The primary purpose of the study is to determine the effect of sleep deprivation on neural responses to food odors in the early olfactory and reward-related brain regions, and how these effects are related to appetite-regulating hormones. We will further examine how altered neural responses to food translate to enhanced appetite and food intake, and which brain processes support resilience to sleep-dependent increases in food intake. We will use appetitive food odors in combination with functional magnetic resonance imaging (fMRI) and pattern-based data analysis to test the effects of sleep deprivation on neural responses to food. We will also examine effects of sleep deprivation on appetite levels, odor pleasantness, and *ad-libitum* calorie intake.

Specific aims and Hypothesis

Aim 1. Identify sleep dependent alterations in early olfactory responses to food odors.
Food odors reliably activate both early olfactory and reward-related brain networks. Orexigenic hormones are enhanced in a sleep-deprived state and have been shown to modulate olfactory sensitivity to food. Here we aim to identify effects of sleep deprivation on early sensory processing of food odors, and how these effects are linked to changes in appetite-regulating hormones. *We hypothesize that fMRI responses to high-caloric food odors will be elevated when subjects are sleep-deprived. Specifically, we predict that sleep-deprivation will alter levels of orexigenic hormones along with early olfactory responses to food odors.* Our setup allows us to deliver appetizing food odors to human subjects undergoing fMRI scanning, and thereby to measure responses to these stimuli in olfactory cortex (i.e., piriform) as well as in reward-related regions (amygdala, orbitofrontal cortex).

2) Aim 2: Determine the link between sleep-dependent olfactory processing and calorie intake.
Sleep-restriction has been shown to enhance craving and consumption of high-calorie snacks. Here we aim to determine the relationship between altered hormone levels, neural processing of food odors, and enhanced calorie intake. *We hypothesize that fMRI responses in early olfactory and reward-related brain regions will mediate enhanced food craving and calorie intake. Moreover, we predict that brain processes related to resilience will be represented in the dorsolateral prefrontal cortex, and that these processes are characterized by enhanced connectivity with reward-related brain regions.* After the fMRI scanning session, sated subjects will be given the opportunity to consume high-calorie snacks *ad libitum* in a controlled environment. In addition, subjects' food intake will be recorded for 24 after the scanning session using dietary diaries. We will compute the total calorie intake and compare it between

sleep-deprived and non-deprived states. Critically, we will use neural mediation analyses to determine how the relationship between sleep-dependent changes in hormone levels and enhanced calorie intake is mediated by neural responses to food odors. This will reveal brain regions in which neural processing of food mediates increased food intake, and thereby identify potential therapeutic targets. We also propose to compare subjects that manage to abstain from excess calorie intake in a sleep-deprived state to those that do not. This will identify brain processes that support resilience to enhanced calorie intake. We predict that such processes will be represented in the dorsolateral prefrontal cortex and will be characterized by enhanced inhibitory connectivity with reward-related regions. Identification of these protective mechanisms may inform future clinical studies in obesity, and may help to establish novel treatment and prevention strategies for weight-gain and obesity, specifically in high-risk populations with disrupted sleep-schedules.

BACKGROUND:
About one-third of adults in the US get insufficient sleep (≤ 6 h per night; 7 h recommended) (1). Habitual sleep deprivation has been associated with adiposity and obesity in both clinical and epidemiological studies (2, 3). A primary reason for weight gain with inadequate sleep is excessive consumption of calories beyond the homeostatic energy requirement (4). Specifically, experimentally induced sleep deprivation increases the desire for foods high in sugar and fat content (5-7), and is related to excessive consumption of calories, especially from energy dense snacks (8, 9). A decline in sleep, paralleled by a steep rise in obesity rates has made it critical to understand the mechanistic link between chronic sleep deprivation and weight gain.

Sleep-deprivation has been shown to alter appetite-regulating hormones in humans (10, 11). In particular, sleep deprivation is associated with an increased availability of orexigenic hormones, such as ghrelin (10) and the *endocannabinoids* (2-arachidonoylglycerol (2-AG) and 2-oleoylglycerol (2-OG)) (11). However, how these hormonal alterations affect central processing of food stimuli, and how they result in enhanced food intake with sleep debt is not well understood in humans. Interestingly, studies suggest that orexigenic hormones may regulate appetite and food intake by altering olfactory sensitivity (12-14), hence leading to increased perception and intake of food (15). This would suggest that early olfactory cortices are among the first stages in the neural processing hierarchy at which sleep deprivation alters processing of food-related stimuli.

Food odors are highly potent rewards. In fact, olfactory stimulation is a main component of natural food intake (16, 17) and accounts for most of “flavor” perception (18). Importantly, as discussed above, orexigenic hormones that are influenced by sleep and satiety (11, 15, 19), can modulate olfactory responses (12, 14). Moreover, the primary olfactory (i.e. piriform) cortex has strong, monosynaptic connections with brain areas related to reward, valuation, and decision-making, such as the hypothalamus, amygdala, striatum, and orbitofrontal cortex (OFC) (20). Thus, the early olfactory processes are well positioned to regulate food preference and intake. Indeed, food odors can direct choices toward specific foods, and previous research has reported increased appetite (21, 22), salivation (23), and food preference (21, 22) after exposure to food odors. However, whether sleep-deprivation increases appetite and consumption of high caloric foods by modulating early olfactory processing of food is currently not known. Based on the findings summarized above, *we hypothesize that sleep-deprivation alters neural responses to food stimuli in early olfactory brain regions*, and thereby may elicit a cascade of enhanced processing of food stimuli in reward-related brain regions that ultimately results in excess calorie intake.

Previous human functional magnetic resonance imaging (fMRI) studies have reported changes in reward-related brain regions in response to food-stimuli after sleep deprivation (24-26). However, these studies used pictures of healthy and unhealthy food items, which only capture superficial features of food and, critically, miss an olfactory component. Thus, studies were unable to examine the effects of

sleep-deprivation on early chemosensory processing of food. Moreover, these studies did not examine the effects of sleep-deprivation on appetite-regulating hormones, or how hormonal changes relate to fMRI findings. In the current application, *we propose to study the effects of sleep-deprivation on neural processing of energy-dense food odors in early sensory and reward-related brain regions, as well as on appetite-regulating hormones* (Aim 1). In a subsequent step, we aim to determine how changes neural processing of food odors translate into enhanced food craving and actual calorie intake, and to identify processes supporting resilience to enhanced food intake (Aim 2).

We have the required expertise in neural reward processing (27-29), olfactory psychophysics and neuroimaging (30-36), pattern-based fMRI (3, 37-41), and sleep research (42-44) to successfully conduct all aspects of the proposed experiments. Our preliminary data demonstrate the feasibility of our imaging protocol to measure odor-evoked activity in early olfactory cortices. Specifically, human subjects (N=23) rated the pleasantness of four sweet and savory food odors, and one sweet and one savory odor was selected based on these ratings for each participant (**Fig. 1A**). In the fMRI scanner, a visual cue indicated the type of odor that would be delivered, and subjects were then instructed to sniff the odor (**Fig. 1B**). Every 9-12 trials the association between the visual cue and the delivered odor was reversed. We observed significant food odor-evoked fMRI responses (unpredicted vs. predicted) in piriform cortex, amygdala, and OFC (**Fig. 1C**).

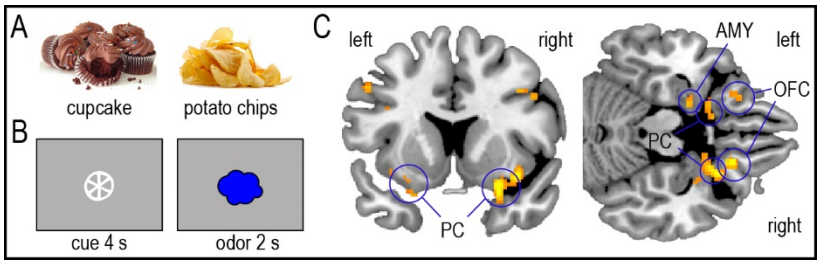

Figure 1. Food odor-evoked activity in the human brain. A. Two food odors were selected for each subject. **B.** In each trial, the odor type (sweet vs. savory) was predicted by a visual cue. At times, the association reversed and the unpredicted odor was delivered. **C.** Significant fMRI group responses to the delivery of the food odor (unpredicted vs. predicted odor type) in the piriform cortex (PC), amygdala (AMY), and OFC (N=23, P<0.05).

INCLUSION AND EXCLUSION CRITERIA:
Interested subjects will contact the PI or a research assistant at a phone number or by email indicated on the flyer. During this initial conversation, the PI or research assistant will email an online questionnaire through Google Forms that will be completed by the interested subjects. Responses will be used for screening subjects for inclusion and exclusion criteria. If they meet these initial study criteria, subjects will be invited for an in-person screening process where they will review and sign (either electronically or manually) an Informed Consent form for participation. The participants will fill sleep behavior questionnaires, an MRI-specific safety questionnaire, and undergo an odor-rating task in the lab to determine if they qualify to participate in the study.

Inclusion criteria: a) ages 18 – 40; b) right-handed as defined by a modified Edinburgh score > 60 (131); c) Body mass index (BMI) between 18.5 and 24.9; and d) native English speakers.

Exclusion criteria: All subjects will be screened for significant neurological or psychiatric disease, chronic medical illness, pulmonary disease, or cerebrovascular risk factors (cardiovascular disease, cerebrovascular disease, hypertension, diabetes, elevated cholesterol, or smoking). Examples in each of these categories would include a history of seizures, multiple sclerosis, meningitis, major affective disorder, obsessive-compulsive disorder, schizophrenia, cancer, chronic obstructive pulmonary disease, severe asthma requiring hospitalization for treatment, renal insufficiency requiring dialysis, etc. Additional exclusion criteria are as follows: a) past or current history of alcoholism or consistent drug use; b) use of medications that affect alertness (e.g., barbiturates, benzodiazepines, chloral hydrate, haloperidol, lithium, carbamazepine, phenytoin, etc.); c) history of major head trauma with sustained

loss of consciousness; d) ongoing or possible pregnancy; e) history of metal working, or injury with shrapnel or metal slivers; f) history of neurosurgery or cardiac surgery; g) history of pacemaker or neurostimulator implantation; i) known smell or taste dysfunction; j) history of significant food or non-food allergy or asthma requiring hospitalization; k) depressed mood (Center for Epidemiologic Studies-Depression Scale score of >15); l) Pittsburgh Sleep Quality Index score >10); m) night work, variable sleep habits, or habitual daytime naps; n) habitual caffeine intake >300 mg/d

Children will not be included in our subject group. Brain organization changes with age. Therefore, to reduce neural variability across the subject group, we plan to limit our studies to adults between the ages of 18 and 40.

STUDY-WIDE NUMBER OF PARTICIPANTS:
A total of 40 normal-weight male and female subjects will participate in all aspects of the proposed study.

STUDY-WIDE RECRUITMENT METHODS:
Subjects will be recruited from flyers posted around Northwestern, DePaul, and Loyola University, or by email. Interested subjects will contact the PI at a phone number or by email indicated on the flyer. During this initial conversation, the PI or a research assistant will email an online questionnaire through Google Forms that is completed by interested subjects; this is used for screening subjects for inclusion and exclusion criteria. If subjects meet these initial study criteria, they will review and sign an Informed Consent form for participation in the study. Subjects will also review and sign an MRI-specific safety form. A copy of all consent forms will be provided to the subject. Subjects will be paid a total of $240 for participation in the complete study. If the subject is only able to complete a portion of the study, monetary compensation will be prorated on the basis of procedures completed. The 90-minute fMRI scanning procedure will also be prorated independently in both the sleep deprived and non-sleep deprived stages. Subjects will receive all payment in the form of a check (or in the case of a Northwestern University employee, direct deposit) at the completion of participation in the study, and payment may be subject to tax withholding.

The recruitment process will encourage the participation of women through widespread flyers aimed at the students and staff of Northwestern University. Based on subject recruitment patterns through the Department of Neurology over the last 3 years, we expect equal numbers of men and women to participate in the studies.

Similarly, the recruitment process will encourage the participation of minorities through flyers aimed at the students and staff of Northwestern University that have a wide readership. Recent recruitment patterns achieved at the Department of Neurology reflect a diverse ethnic assortment of subjects.

MULTI-SITE RESEARCH: N/A

STUDY TIMELINES:
Each participant will follow a ~ 6 week within-subject, crossover partial sleep-deprivation protocol to test the effect of sleep deprivation (SD) and non-sleep deprivation (NSD) on neural processing of food odors in sensory and reward related regions (**Fig. 2**). Qualified subjects will be randomly assigned to either participate in the SD or the NSD condition first. Subjects will then undergo a week of sleep stabilization phase to stabilize circadian rhythms for a week. After the sleep stabilization week, the night before the fMRI session, subjects will either sleep normally (NSD, 8 h of sleep between 11 pm and 7 am), or sleep only for 4 h between 1 am and 5 am (SD) at home. All subjects will participate in both sleep conditions (SD and NSD) in randomized order, separated by ~2.5 week washout period. The

evening of the sleep manipulation, subjects will visit the lab to consume dinner and pick up their sleep monitors. We will also provide a take-out breakfast and lunch to be consumed at 8:00 am and 12 noon, respectively. All meals will be provided by the CRU. Subjects will perform an odor-rating task inside the MRI scanner on the same evening. Serial blood samples will be collected for measurement of appetite-regulating hormones. At the completion of the fMRI scan, participants will be provided with snacks in a buffet-style setting where they will have *ad libitum* access to food items corresponding to the odors used during fMRI scanning (sweet and savory high-calorie snacks). They will be instructed to consume as much food as they like. We anticipate to enroll all participants within a 12-month period and plan to complete the study data collection and analyses within a period of 3 years from the date of IRB approval.

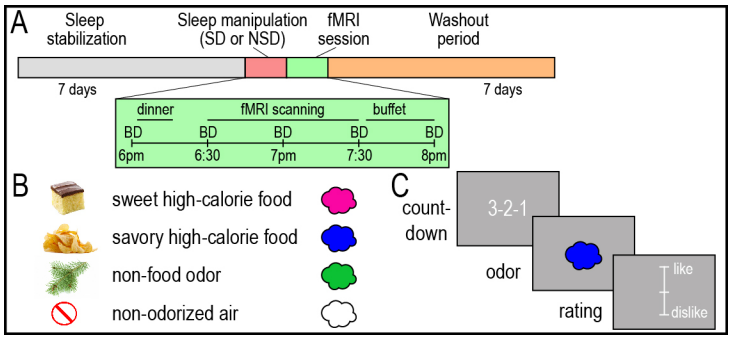

Figure 2. Study design and scanning protocol. A. Timeline of the sleep-deprivation protocol. All subjects will participate in both sleep conditions (SD and ND), in randomized order. On the scanning day, blood (BD) will be drawn every 30 min. **B.** For each subject, two sweet and two savory high-calorie food odors will be selected and presented during the scanning session, in addition to two non-food odors and trials in which non-odorized air is presented. **C.** Example trial of the fMRI task. After a countdown, an odor is presented, after which subjects have to rate the pleasantness or the intensity of the delivered odor.

STUDY ENDPOINTS:

Primary endpoints

The primary endpoints of this proposal are the analysis of neural representations of high-caloric food odors in early olfactory cortex and food reward regions, and levels of orexigenic hormones using the serial blood samples.

Secondary endpoints

Secondary endpoints of this proposal are the total calories of food consumed during the ad-libitum food intake phase, subjective ratings of hunger, fullness, prospective food consumption, and satiety using the visual analogue scale, food craving questionnaire, and total calorie intake using the self-reported food intake for 24 h following the fMRI scan.

PROCEDURES INVOLVED:

We propose to implement a randomized within-subject, crossover partial sleep-deprivation protocol to examine the effects of sleep deprivation (SD) on neural processing of food odors in early sensory and reward-related brain regions. In brief, after a one-week sleep stabilization period, subjects will spend a night either sleep deprived (SD, 4 h of sleep) or with normal sleep (non-SD, NDS, 8 h of sleep) at home. On the next day, fMRI scanning will be conducted at 7 pm after dinner, when sleep-deprivation induced changes in hormonal levels are most prominent (11). Hormone levels will be monitored using serial blood sampling in response to a meal (dinner).

Sleep-deprivation protocol. Subjects (N=40) will be randomly assigned to either participate in the SD or the NSD condition first (**Fig. 2A**). Subjects will then undergo a sleep stabilization phase to stabilize circadian rhythms. During this week, subjects will be instructed to keep a regular sleep schedule of 7-9 hours in bed between 10:30 pm and 7:30 am. Participants will abstain from alcohol, caffeine, and drugs in this phase. After the sleep stabilization week, the night before the fMRI session, subjects will either sleep normally (NSD, 8 h of sleep between 11 pm and 7 am), or sleep only for 4 h between 1 am and 5 am (SD) at home. We opted for a partial sleep-deprivation protocol because this situation (late bedtime and early wakeup) is ecologically valid as it reflects the most realistic form of sleep-deprivation in

everyday life. All subjects will participate in both sleep conditions (SD and NSD) in randomized order, separated by a 2.5-week washout period.

Sleep-monitoring. To ensure that subjects follow the sleep schedule during the sleep stabilization phase and sleep manipulation days, we will record sleep duration, as well as sleep and wake-up time using a daily sleep diary and wrist actigraphy (ActiGraph, LLC, Pensacola, Florida (45)). Additionally, subjects will rate their subjective sleep quality, alertness, and restfulness every morning using an online questionnaire. Subjects will also rate their subjective sleepiness using the Stanford Sleepiness Scale (46) before the fMRI scan during both the SD and NSD phase. Participants will spend the night of sleep manipulation (both SD and NSD) at home and food will be provided for these days.

Controlled diet protocol. During the 24 h before the fMRI session, we will provide subjects with a controlled diet based on their estimated energy requirements. Specifically, the evening of the sleep manipulation, subjects will consume dinner at 7 pm at our lab and will also be provided with a take-out breakfast and lunch to be consumed at 8:00 am and 12:00 noon the next day. No other calorie intake will be permitted. Participants will be instructed to consume only non-caffeinated and non-caloric drinks.

fMRI scanning protocol. On the first day of the study, before being assigned to the SD or NSD phase, subjects will rate the pleasantness of six food odors, including three high caloric sweet (caramel, yellow cake, ginger bread) and three high caloric savory odors (potato chips, pot roast, garlic butter). Based on each participant's ratings, we will select two sweet and two savory odors matched in rated pleasantness. We will also include two control non-food odors (fir and celery seed). On the day of fMRI scanning, subjects will arrive at the imaging center at 6 pm and will consume a dinner before entering the scanner. This pre-feeding will satisfy metabolic hunger (i.e., energy requirements), and thus render the fMRI measures more sensitive to sleep-dependent changes in hedonic hunger, i.e., food intake exceeding energy requirements (47). During fMRI data acquisition, odors will be delivered directly to the subject's nose via a MR-compatible olfactometer. Neuroimaging will be performed at the Northwestern University Center for Translational Imaging (CTI) on a state-of-the-art Siemens PRISMA system with a 64 channel head coil, using imaging sequences optimized for signal recovery in olfactory and orbitofrontal cortices (48). Specifically, subjects will perform 4 runs of an odor detection task inside the MRI scanner while blood oxygen-level dependent (BOLD) responses will be acquired with high spatial (2 mm isotopic) and temporal resolution (2000 ms). On each trial, subjects will be cued to sniff, and an odor corresponding to one of the three experimental groups (sweet, savory, non-food) or clean air will be delivered pseudo-randomly. Subsequently, subjects will rate the pleasantness or the intensity (pseudo-randomized) of the delivered odor. Sniffing behavior will be monitored using an MR-compatible breathing belt and a spirometer. A high-resolution anatomical image will be acquired for the purpose of spatial normalization and anatomical localization of the fMRI responses.

Hormone levels. As soon as subjects arrive at the imaging center, an intravenous sterile heparin-lock catheter will be inserted in a forearm vein and blood sampling will be initiated by the CRU mobile nurse. We will draw blood every 30 min after arrival, resulting in 5 samples, including a fasted baseline (6 pm), after dinner (6:30 pm), before (7 pm), during (7:30 pm, after the second scanning run), and after the scanning session (8 pm) for leptin and ghrelin analysis. An additional sample will be collected at 7:30 pm time point for 2-AG and 2-OG analysis. Plasma and serum samples will be frozen at −80°C until assay for ghrelin, leptin, insulin using commercially available ELISA kits, and 2-AG and 2-OG using published procedures (49, 50). Phase of menstrual cycle in women has an impact on these hormonal levels. The 2.5-week washout period will ensure that the blood samples are collected in the same phase of the menstrual cycle

Ad-libitum food intake. At the completion of the fMRI scan, participants will be provided with snacks in a

buffet-style setting where they will have *ad libitum* access to food items corresponding to the odors used during fMRI scanning (sweet and savory high-calorie snacks). They will be instructed to consume as much food as they like. To measure food intake, food will be weighed pre- and post- consumption, and calories consumed will be calculated. Because subjects have been provided with dinner before the scanning session, food intake at the buffet will mainly reflect hedonic, rather than metabolic eating (47). *Appetite and hunger ratings.* Subjective food craving, ratings of hunger, fullness, prospective food consumption, and satiety will be measured using a visual analogue scale. These ratings will be acquired on the fMRI scanning day upon arrival, at all blood collection time points, and after the *ad libitum* meal. Subjects will also complete the food craving questionnaire on the day of fMRI scan.

24 h post-fMRI food intake diary. Subjects will be instructed to carefully record the food items, quantity consumed, place of consumption, and the food brands, for 24 h following the fMRI scan. We will calculate the total energy, fat and carbohydrate consumption for SD and NSD states.

DATA AND SPECIMEN BANKING:
Once the blood samples are obtained, we will store them in a freezer in the lab at -80 Celcius until assay. Blood samples will be sent to the Comprehensive Metabolic Core lab at the Northwestern University for hormone analyses. Blood samples will also be sent to the lab of Dr. Cicila Hillard at the Medical College of Wisconsin, Milwaukee for endocannabinoid analysis. These labs will be instructed to destroy the samples immediately once the desired data is obtained. All samples will be labeled with the subject study codes and date of collection. The lab technicians of the lab where the samples will be sent will be completely blinded to the purpose of the sample collection. The lab results will be returned to the research personnel with the subject codes who will then enter the raw data in the study spreadsheet. Lab technicians will not have access to the spreadsheet and will not be able to identify subjects. Only research personnel included in the IRB application will have access to the raw data.

DATA AND SPECIMEN MANAGEMENT:
Sample size is based upon the requirements for group analysis, particularly when random-effects analyses are performed. Random-effects analyses require a minimum of 14 -18 subjects in each group in order to obtain statistically meaningful fMRI effects. Our target subject number also accounts for attrition in the subject pool due to unanticipated claustrophobia, technical/mechanical issues, or other problems during the fMRI session.

For the statistical analyses, comparisons of subjective appetite ratings and calorie intake between the SD and NSD state will be carried out using paired t-tests. Spearman’s correlation analysis will be used to test the relation between appetite ratings, neural responses to food odors, and the total calories consumed. Moreover, we will use whole-brain mediation analysis (51) to identify the brain regions in which neural responses to food odors mediate the link between sleep-dependent changes in hormone levels and enhanced calorie intake. To identify brain mechanisms related to resilience, we will compare fMRI responses to food odors between subjects that manage to abstain from excess calorie intake in a sleep-deprived state to those that do not. We will use fMRI connectivity analyses (psychophysiological interaction, PPI (52)) to test the hypothesis that resilience to enhanced food intake in a SD state is associated with enhanced functional connectivity between the dorsolateral prefrontal cortex and reward-related brain regions (53). We will monitor sniffing using a breathing belt and spirometer, and model trial-by-trial sniff parameters will be included as covariates in all fMRI analyses following established procedures to control for respiration-induced effects.

To preserve subject privacy and confidentiality, all research materials (questionnaires, behavioral testing results, and magnetic resonance imaging data) will be coded by a different study code number unique for each subject, in order to keep the identity and personal information of each subject as confidential as possible. This number will be recorded on all testing sheets instead of the subject's

name. In addition, if the individual results of this study are used for teaching, research, publications, or presentations at scientific meetings, subject identity will be protected by using the study code number rather than subject name or other identifying information, including (but not limited to) Social Security number, date of birth, or address. In order to secure the data, all paper data will be de-identified and kept in a locked cabinet. The de-identified data will also be entered into spreadsheets and saved in the encrypted form on the password protected University's secure server, protected by the Northwestern's firewall. This dataset will also be stored within password protected folders to which only the PI and study personnel will have access. Only the PI and the key personnel will have access to the identifiable information and the data. All electronic data and records of the individuals who after screening are deemed ineligible for the study will be completely de-identified and destroyed. Finally, unless required by law, only the study investigator, members of the investigator's staff, representatives from the Cognitive Neurology and Alzheimer's Disease Center, and the Northwestern University Institutional Review Board will have the authority to review the study records. They will all be required to maintain the strictest confidentiality regarding subject identity.

As explained above, blood samples will be stored in a freezer in the lab at -80 Celcius until assay. All samples will be labeled with the subject study codes and date of collection. The lab technicians of the lab where the samples will be sent will be completely blinded to the purpose of the sample collection. The lab results will be returned to the research personnel with the subject codes who will then enter the raw data in the study spreadsheet. Lab technicians will not have access to the spreadsheet and will not be able to identify subjects. Only research personnel included in the IRB application will have access to the blood samples and raw data, and will be able to transport the samples to the Comprehensive Metabolic Core lab and the Medical College of Wisconsin lab for hormonal analysis.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS:
This study does not involve more than minimal risk to the participants and therefore this section is not applicable.

WITHDRAWAL OF PARTICIPANTS:
Subjects will be informed at the onset of the study that they are free to terminate the study for any reason whatsoever. They will be assured that such a decision will not influence their treatment or relationships with health care providers at the Northwestern Medical Faculty Foundation or Northwestern Memorial Hospital. If the subject is a student or employee of Northwestern University, they will be informed that participation will not adversely affect their class ranking or employment status.

Any participant that experience adverse event during the study, which makes it no longer safe to for the individuals to participate problems, will be withdrawn from the study without their consent. Any unanticipated problems will be reported to the IRB in accordance with posted guidance. In addition, if a participant fails to follow the procedure he/she will be excluded from the study.

RISKS TO PARTICIPANTS:
Potential risks of participating in the proposed research:

1. Sleep deprivation - The risks associated with acute sleep deprivation in the study will be similar to risks experienced with habitual sleep deprivation. Some participants may feel fatigued, sluggish, irritable, and may complain of lack of energy and concentration on the day following study induced sleep deprivation. To mitigate these risks, participants will be advised to get adequate recovery sleep during the week following the study procedure. Participants will also be advised to refrain from driving and using heavy machinery on the day following the sleep deprivation.

2. Blood draw - Participants may feel bruising or bleeding at the site of venipuncture. Venipuncture will be performed by the trained nursing staff at the Clinical Research Unit. This should reduce the chance of pain, discomfort, and bruising during the blood draws.

3. Functional Magnetic Resonance imaging (fMRI) - There are no known risks associated with undergoing fMRI scanning. All fast imaging sequences (those provided by Siemens Medical Systems or those developed at Northwestern) are monitored by safety devices built into the MR scanner to ensure that pulse sequences perform within FDA guidelines for human safety. Of major concern is the specific absorption rate (SAR) of radiofrequency energy used in generating an image. This value is physically measured and calculated three different ways. The largest value is used in determining if that particular scan is safe to use. This method and its implementation are currently FDA approved. There is no way to override this safety feature on our scanner.

   During fMRI scanning, some subjects report discomfort from having to lie still in the scanner for a long period of time, and some subjects feel anxious or claustrophobic in the scanner. Highly trained staff members will be working with the subjects throughout the study and will take appropriate steps to minimize anxiety and fatigue. Although smelling the odors should not present any significant health risks, it is possible that a subject could have an allergic reaction. For this reason, any subject with a history of serious food or non-food allergies, or a history of asthma requiring hospitalization, will be unable to participate in the study. Additionally, subjects may be asked to refrain from eating for 6 hours prior to participation in the experiment. Some subjects may experience dizziness due to fasting

   If an abnormal finding is seen on the anatomical MRI scan, the PI will contact the subject and recommend a clinical evaluation. Copies of the abnormal scan will be sent to the patient's physician upon request.

4. Olfactory stimulation- Although smelling the odors should not present any significant health risk, it is possible that a subject could have an allergic reaction. Therefore, subjects with a history of serious food or non-food allergies, or a history of asthma requiring hospitalization, will be excluded from the study. Selected odorants will be presented at concentrations and durations that have been safely used in prior olfactory experiments and should not present any health risks to the subjects. Stimulus parameters will be adjusted to ensure that the exposure limits, where indicated on the odorant material data safety sheets, are not exceeded.

5. Privacy and Confidentiality- To preserve subject privacy and confidentiality, all research materials (questionnaires, behavioral testing results, and magnetic resonance imaging data) will be coded by a different study code number unique for each subject, in order to keep the identity and personal information of each subject as confidential as possible. This number will be recorded on all testing sheets instead of the subject's name. In addition, if the individual results of this study are used for teaching, research, publications, or presentations at scientific meetings, subject identity will be protected by using the study code number rather than subject name or other identifying information, including (but not limited to) Social Security number, date of birth, or address. Finally, unless required by law, only the study investigator, members of the investigator’s staff, representatives from the Cognitive Neurology and Alzheimer's Disease Center, and the Northwestern University Institutional Review Board will have the authority to review the study records. They will all be required to maintain the strictest confidentiality regarding subject identity.

POTENTIAL BENEFITS TO PARTICIPANTS:
There are no direct benefits of participation in the study.

VULNERABLE POPULATIONS:

N/A

COMMUNITY-BASED PARTICIPATORY RESEARCH:
N/A

SHARING OF RESULTS WITH PARTICIPANTS:
If requested by the participant, study results may be shared with the participant. We are not performing any diagnostic procedures in the study, therefore will not be able to identify any abnormalities. However, if an abnormal finding is seen on the anatomical MRI scan, the PI will contact the subject and recommend a clinical evaluation. In this case, copies of the abnormal scan will be sent to the patient's physician upon request.

SETTING:
Prior to recruitment in the study, consent and screening process will be performed at our lab at the Department of Neurology. Once deemed eligible to participate in the study, participants will be instructed to follow a one-week sleep stabilization period at home. After a week subjects will spend a night either sleep deprived (SD, 4 h of sleep) or with normal sleep (non-SD, NDS, 8 h of sleep) at home. On the next day, fMRI scanning will be conducted and blood samples will be collected at the Center for Translational Imaging (CTI), Northwestern University.

RESOURCES AVAILABLE:
Our lab has conducted multiple studies and published manuscripts in the area of neural imaging of olfaction and reward regions in high impact peer reviewed journals. This study will combine our expertise in neural reward processing, olfactory psychophysics, and food-related choices in obesity to address the posed research question. All research personnel involved in the study have extensive experience in conducting all aspects of research study including consent, screening, recruitment, and use of olfactometer and scanner. Some members of the study team are also certified to conduct the fMRI in the CTI independent of the CTI staff. In addition, the primary researcher of this project also has previously worked with the Clinical Research Unit at a different institution and has extensive knowledge of the process involved. Overall, all research personnel involved in the study are well equipped to conduct the study.

We have access to several research related resources to successfully conduct this study. We have a custom built olfactometer in the lab that will be used to deliver odors both outside and inside the scanner. The other site~~s~~ where the study will be conducted (CTI) are also within walking distance from our lab. The screening and the olfactometer behavioral tasks will be conducted in a quiet ventilated room in our lab, accessible only by the research personnel. Brain imaging will be performed at the CTI using the state-of-the-art 3T Prisma platform by a certified and MRI safety trained study personnel. On the day of the scan, subjects will fill out the safety form prior to the scan. The form will be submitted to the MR tech to verify subject safety. In addition, two safety-trained study team members will be present for all human scans. In case of emergency CTI staff will be present to assist the study team. The primary researchers on the project will contribute 100% of her time on the project. Other staff involved in the study is expected to contribute 20-25% of their time on the project.

Meetings will be conducted with the CRU team to discuss the protocol and procedure flow chart will be developed to ensure that the mobile nurse assisting with the research is adequately informed about the protocol and the research procedures.

PRIOR APPROVALS:
N/A

RECRUITMENT METHODS:
Subjects will be recruited from flyers posted around Northwestern, DePaul, and Loyola University, or by email. Interested subjects will contact the PI or a research assistant at a phone number or by email indicated on the flyer. During this initial conversation, the PI or research assistant will email an online questionnaire through Google Forms that is completed by the interested subjects; this is used for screening subjects for inclusion and exclusion criteria. If they meet these initial study criteria, subjects will review and sign (either electronically or manually) an Informed Consent form for participation in the study. The subject will also review and sign an MRI-specific safety form. A copy of all consent forms will be provided to the subject; if signed electronically, a printed form will be provided. Subjects will be paid a total of $240 for participation in the complete study. If the subject is only able to complete a portion of the study, monetary compensation will be prorated on the basis of procedures completed. The 90 minute fMRI scanning procedure will also be prorated independently in both the sleep deprived and non-sleep deprived stages. Subjects will receive all payment in the form of a check (or in the case of a Northwestern University employee, direct deposit) at the completion of participation in the study, and payment may be subject to tax withholding.

The recruitment process will encourage the participation of women through widespread flyers aimed at the students and staff of Northwestern University. Based on subject recruitment patterns through the Department of Neurology over the last 3 years, we expect equal numbers of men and women to participate in the studies.

Similarly, the recruitment process will encourage the participation of minorities through flyers aimed at the students and staff of Northwestern University that have a wide readership. Recent recruitment patterns achieved at the Department of Neurology reflect a diverse ethnic assortment of subjects.

NUMBER OF LOCAL PARTICIPANTS:
A total of 40 participants will be enrolled locally. This allows for the possibility of excluding subjects from analysis due to failure to comply with the sleep protocol, claustrophobia, technical issues, or other unanticipated situations which may arise during MRI scanning. We expect a drop-out rate of 15-25%.

CONFIDENTIALITY:
This is not a multicenter study therefore this section is not applicable.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS:
To ensure subject privacy, no data collection will occur in a group setting or in public. Study personnel will keep participation and results confidential by using code numbers to identify subjects on all reports and samples. Only the mobile CRU nurse will have access to the patients' medical records

Potential psychosocial risks to subjects are that some of the questions asked may be upsetting or may make the subject feel uncomfortable. Some participants may also find these questionnaires frustrating and time consuming. There may also be a potential risk of loss of confidentiality. To minimize the risks associated with loss of confidentiality, strict measures will be taken as detailed elsewhere in the application. In particular, participants will be given the option to skip certain questions if they do not feel comfortable answering them. However, if that question is part of inclusion/exclusion criteria, the prospective participant may not participate in the study to maintain the purpose of the study. Study staff will not have access to the medical records of the participants.

COMPENSATION FOR RESEARCH-RELATED INJURY:
This research does not involve more than minimal risk.

ECONOMIC BURDEN TO PARTICIPANTS:

Participants will not be responsible for any cost related to the participation in the study.

CONSENT PROCESS:
All subjects will provide their written informed consent at the screening visit. The consent process will take place in our lab at the Department of Neurology, Feinberg School of Medicine. Information on the purpose of the study and routine potential risks associated with the study procedures will be provided to the potential subjects by the research personnel. All risks, costs, and benefits will be discussed. All potential participants will be given time to ask questions and make the decision on whether they would like to participate in the study. Subjects will be assured of their right to withdraw at any time without prejudice to their care. They will also be assured of confidentiality in maintaining records and reporting of results. Written informed consent will be obtained before entry into the study form all participants. Subjects will also review and sign an MRI-specific safety form. A copy of all consent forms will be provided to all subjects. To minimize possibility of coercion, participants will be given a choice to have a digital version of the consent form with all study information sent to them prior to the screening visit. Participants will be given one week to decide if they want to participate in the study. They will also be given a copy of the consent form if they want to read it again.

For Non-English Speaking Participants
Non-English speakers will not be included in the study.

Waiver or Alteration of Consent Process
N/A

Participants who are not yet adults (infants, children, teenagers)
N/A

Cognitively Impaired Adults
N/A

Adults Unable to Consent
N/A

PROCESS TO DOCUMENT CONSENT IN WRITING:
We will document consent in writing. The consent form is attached to the eIRB application.

DRUGS OR DEVICES:
N/A

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