Neurologic Biomarkers of Smoking Behavior

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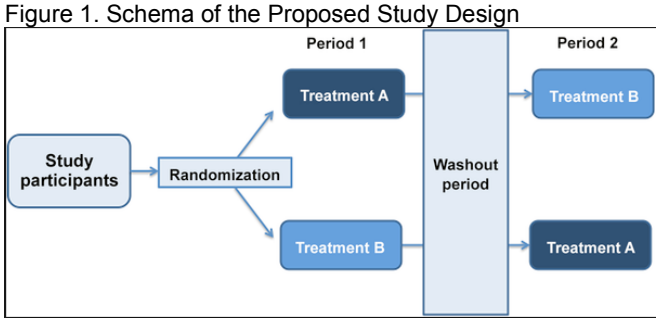
1.0 Project Summary/Abstract

This program of research focuses on identifying neurologic biomarkers of smoking behavior in order to develop individualized smoking cessation aids. The research team has carefully designed the study protocol to ensure participant safety and confidentiality. This research will include administration of intranasal insulin and a placebo comparator. The intranasal insulin administered is an investigational drug and has been granted IND status by the FDA (IND#129432). During the times of drug effects, we will evaluate a biomarker using non-invasive equipment. Researchers are trained to be vigilant in identifying any side effects associated with the study drug and associated procedures. Anticipated risks of study drugs and equipment have been recognized by the research team and will be discussed as part of the informed consent process. Access to data collection and sensitive patient information will be limited to the Principal Investigator and designated research team. Researchers have quality control metrics in place to assure collection of accurate data, proper documentation of study activities, and predeveloped contingency plans in the event unanticipated problems occur.

Schemas are welcomed and often provide a nice visual reference for the project: The proposed work is a 2-session crossover design study intended to evaluate the effects of intranasal insulin vs placebo in smokers and non-smokers. Study participants will be randomized to either receive Treatment A (intranasal insulin) or Treatment B (placebo) during each session (Figure 1). The washout period will be 1 week in duration. Study participants will consist of healthy smokers and healthy non-smokers. Period 1 represents the first testing session and Period 2 represents the second testing session. As discussed in the above-section, one specific treatment will be administered per session.

Figure 1. Schema of the Proposed Study Design



2.0 Background/Scientific Rationale

Describe the research problem and provide rationale for the research: Although there are several FDA-approved treatments for smoking cessation, only 6% of an estimated 40 million smokers successfully quit. Taking a more patient-specific approach to resolving this healthcare concern may lead to more efficacious outcomes. The purpose of finding a biomarker is to enhance future drug development of new smoking cessation pharmacotherapeutic agents. In addition to characterizing the biomarker, we will test whether intranasal insulin – an investigational drug – can improve go/no-go accuracy in smokers.

Briefly summarize prior experience and/or history relevant to the research: The rationale for using intranasal insulin is its efficacy in relieving cognitive deficits as reviewed in several papers (Shemesh et. al., 2012, de la Monte 2013, Ott et. al., 2012, Reger and Craft 2006, Strachan 2005, Fehm 2000).

It is hypothesized that intranasal insulin will relieve cognitive dysfunction in smokers as shown in previous studies (Franken et al., 2010; Luijten et al., 2011b).

Identify any investigational agents, drugs, devices, or biologics that will be administered or implanted into subjects: For this research study, smokers and non-smokers will be administered intranasal insulin (Novolin R, 60 IU) and placebo. Placebo will consist of sterile water with 14% NaCl. During administration of either intranasal insulin or placebo, participants will receive 1 spray in each nostril every 3 minutes for a total of 6 sprays. AeroPump delivers 0.1 ml of liquid per spray. Since the concentration of insulin in Novolin R is 100 IU/mL, six sprays will deliver the 60 IU dose.

Summarize relevant preclinical data: See "Briefly summarize prior experience and/or history relevant to the research" (above).

Summarize relevant clinical data to date: The clinical benefits of intranasal insulin administration have been elucidated in the context of cognitive dysfunction alleviation (Shemesh et. al., 2012). Accumulating evidence demonstrates that conditions such as diabetes and Alzheimer's disease are associated with a deficiency of insulin signaling to the brain, resulting in cognitive impairment and decline. Such cognitive effects could be reversed or slowed with medical intervention to efficiently deliver insulin to the brain. Intranasal insulin administration is a noninvasive and efficient way to bypass the blood-brain barrier; thus, it may be a useful way to reintroduce insulin to the central nervous system to alleviate cognitive dysfunction caused by a hormonal deficit.

Intranasally administered insulin has previously been shown to directly elicit changes in cognitive function with little impact on blood glucose levels (Kern et al., 1999). When insulin was administered intranasally to a cohort of healthy, euglycemic men, the dose administered was not significantly detectable from placebo by a change in blood glucose levels or cardiovascular activity. However, the treatment caused a significant reduction in the baseline-to-peak amplitude of the N1 and P3 AEP components as well as an increase in P3 latency. These results indicate that intranasal insulin administration bypasses the bloodstream to directly impact the central nervous system.

Intranasal insulin may also be a useful smoking cessation treatment, due to exploratory studies of its effects on craving in smokers (Hamidovic et al. 2017). Administration of intranasal insulin resulted in a lower self-reported urge to smoke and restored the cortisol response to experimentally induced stress. These results indicate that intranasal insulin may be an effective and viable smoking cessation product.

Provide a rationale for the dosing or use of the device, risks to subjects, and potential benefits to subjects: We conducted two clinical trials with intranasal insulin (Hamidovic et al., 2017). The 60 IU dose is identical as in those two studies (IND #116626 and IND# 120700). The first study (IND #116626), was a parallel-design clinical trial involving one-time administration of intranasal insulin (60 IU) to smokers who were abstinent from smoking overnight. The second study (IND# 120700) was a 36-hour crossover, inpatient hospital study in which smokers received two administrations of intranasal insulin (60 IU) on two separate days. None of the subjects in either of the two studies experienced hypoglycemia. Circulating glucose was not lower for the insulin group in comparison to the placebo group in either study. Olfactory function (measured with Sniffin' Sticks (US Neurologics)) was no different between the insulin and placebo groups in both studies. Sniffin' Sticks involves presentation of 12 different pens containing different smells. After the presentation of each pen,

participants complete a multiple-choice question with 4 possible choices; one of which is the answer corresponding to the correct smell. Local irritation was higher in the intranasal insulin group in comparison to the placebo group. Table 1 lists results of the Sniffin' Sticks test, which measures the ability to correctly identify various smells. Table 2 lists the all the events reported in the study by treatment group in the IND#120700 study.

Table 1. Summary of Sniffin' Sticks (US Neurologics) Scores throughout the Study by Treatment for IND#120700

Appointment	*Treatment*	*Mean*	*Std. Dev.*
Screening	NA	11	0.80
Session 1 (day 1)	Insulin	11.29	0.70
Session 1 (day 2)	Insulin	11.36	0.61
Session 2 (day 1)	Insulin	10.9	0.87
Session 2 (day 2)	Insulin	11.1	0.78
Session 1 (day 1)	Placebo	10	0.66
Session 1 (day 2)	Placebo	11.18	0.83
Session 2 (day 1)	Placebo	11.40	0.66
Session 2 (day 2)	Placebo	11.30	0.64
Follow-up	Both	11.25	0.77

Table 2. Adverse events reported by study participants.

Event	*Number of occurrences during placebo treatment*	*Number of occurrences during insulin treatment*	*Number of occurrences reported at follow-up*
Nasal occurrences			
Rhinorrhea	1	1	
Irritation	5	7	
Other			
Dizziness		1	
Sweating		2	
Headache		1	
Nonspecific pain			2
Visual disturbances			1
Confusion	1	2	
Tingling (mouth)		1	
Shaking		1	1
Anxiety	1	1	
Diarrhea			1
Restless		1	
Uncomfortable		1	
Watering eyes		2	
Menorrhagia			1

Given the growing evidence that intranasal insulin prevents neuronal damage, reduces nicotine cravings (Hamidovic et al., 2017) and improves cognition in diabetes, stroke, postoperative cognitive dysfunction, developmental delay, Parkinson's disease, bipolar disorder, Alzheimer's disease and HIV-associated neurocognitive disorders (summarized in Nedelcovych et al., 2018), a recent study compared insulin exposure in mouse brain and plasma following intranasal and subcutaneous administration of 2.4 IU (Nedelcovych et al., 2018). Whereas the subcutaneous insulin achieved therapeutically relevant concentrations in the brain (AUCbrain= 2537 h·μ IU/mL), it dramatically increased plasma insulin (AUCplasma = 520 351 h·*μ IU/mL), resulting in severe hypoglycemia and in some cases death. The intranasal administration of the same dose resulted in similar insulin levels in the brain (AUCbrain = 3442 h·μ IU/mL) but substantially lower plasma concentrations (AUCplasma = 354 h·μ IU/mL), amounting to a ~2000-fold increase in the AUCbrain:plasma ratio relative to SC. The intranasal dosing also had no significant effect on blood glucose. Intranasal insulin administration over 9 days increased brain glucose and energy metabolite concentrations (e.g., adenosine triphosphate and phosphocreatine) without causing overt toxicity, suggesting that intranasal insulin may be a safe therapeutic option.

Based on the 60-kg human, the 2.4 IU/kg dose given in the Nedelcovych et al (2018) study converts to a 144 IU dose, which is above the 60 IU dose we plan to administer as a one-time dose in the proposed study.

3.0 Objectives/Aims

Identify hypotheses being tested/primary endpoints/primary purpose of the protocol: The aim of the proposed work is to evaluate go/no-go accuracy as a neurologic biomarker of smoking behavior. Our hypothesis is that following intranasal insulin administration, smokers will have an improved go/no-go accuracy.

Study duration: This duration of the research study is 2 years starting January 2018 – January 2020.

4.0 Eligibility

Identify the subject population being evaluated by the protocol: All study participants will be healthy volunteers. Out of the sample recruited for this research, two groups will be formed based on the smoking status: Healthy non-smokers and healthy smokers. The criteria to separate the two groups is listed in Section 4.1 Inclusion Criteria.

Indicate the source of subjects: Study participants will be recruited from the general population using electronic media (ex: Craigslist), flyers, newspaper advertisements and word-of mouth referrals.

Identify who will assess and determine subject eligibility: Research staff (postdoctoral fellow(s) and research coordinator(s)) will assess eligibility criteria based on a detailed flowsheet (i.e. checklist) created specifically for this study. All research staff will be trained by the PI on assessing inclusion/exclusion criteria. The record of this training will be kept on file in the PI's laboratory. Study PI will sign off on all study participants who passed the screening session as the final approval of subject's entry into the study.

Indicate how and where eligibility will be documented: After completing each step of the flowsheet, the coordinator/postdoctoral research fellow will place time of completion and his/her initials. The PI will document approving participants for study participation on the flowsheet.

Attach an eligibility checklist to the protocol: Eligibility will be documented on the "Session Flowsheet", which is attached to this application. It is also listed in Sections 4.1 and 4.2 below.

4.1 Inclusion Criteria

- Age between 21-40 years
- Smokers only: Smoke at least 10 cigarettes daily (verified by carbon monoxide concentrations greater than 10 ppm).
- Non-smokers only: No self-reported cigarette use in the past 1-year period.
- Non-smokers only: Carbon monoxide concentration < 6 ppm.
- Normal vitals (blood pressure < 120/80 mmHg; heart rate between 60 and 100 bpm, body temperature <37 °C)
- Point-of-care (POC) blood glucose between 70 and 140 mg/dL
- Body mass index between 18.5 and 30 kg/m2

4.2 Exclusion Criteria

- Use of non-cigarette tobacco products, e-cigarettes, or smoking cessation treatment
- Positive urine drug screen test
- Current pregnancy (urine test-verified) or lactation, or a plan to become pregnant
- Breath Alcohol Concentration >0.00%
- Shipley IQ test <80
- Hyposmic or anosmic individuals (identifying less than 10 of 12 smells correctly)
- Abnormal physical exam of the nares
- Lifetime DSM-5 Axis 1 disorder (except anxiety and depression)
- Current DSM-5 Axis depression or anxiety disorder
- Over-the-counter or prescription psychotropic medications (confirmed by PI upon screening)
- Use of any medications administered intranasally
- Allergies to any ingredients in intranasal insulin or placebo

4.3 Excluded or Vulnerable Populations

Describe specifically and state the justification for any vulnerable populations (i.e., minors) or any excluded populations (i.e., non-English speaking subjects): Vulnerable populations will not be included in the study. Participants who don't speak English are not included in the study because the questionnaires and study instructions have been developed in the English language.

5.0 Subject Enrollment

Describe screening and enrollment: The telephone number and email of the CEDAR lab will be included in the advertisement. Once participants contact the lab, they will complete an initial screening survey (please see document "Initial Survey"). Study participants will have the option of completing the survey either electronically (via REDCap link) or by calling in. In the case of a phone call, study coordinator will enter participant responses into REDCap for determination of eligibility. Participants passing the "Initial Survey" will be scheduled for an in-person screening. The document "Screening Flowsheet" outlines the procedures which will be completed at the in-person screening to determine eligibility as outlined in Section 4.1 and 4.2.

Describe from where subjects will be recruited and any advertising or recruitment materials that will be used: Study participants will be recruited from the general population using electronic media (ex: Craigslist), flyers, newspaper advertisements and word-of mouth referrals. The advertising materials will include hard copy posters and Craigslist advertisement. Copies of both are attached to this application.

Describe what happens with screen failures and any data obtained from screen failures: Data gathered from participants failing the screening criteria will be kept with all study documentation, following which it will be shredded with the remaining study documentation. The reason the documentation will be kept is in the event the same subject returns with information different from what he/she reported previously. In this event, we will be able to compare responses and optimize subject protection and study integrity.

Describe the methods to minimize coercion and undue influence on the subjects: The consent procedure is detailed according to processes established in the Code of Federal Regulation and University of Illinois Institutional Review Board. Each research coordinator and postdoctoral research fellow will be trained by the PI and will complete the checklist (included in the "Screening Flowsheet" document). Study participants will be given ample time to review the form. The consent procedure will be carried out in a private and quiet setting. Prior to initiating the procedure, the person consenting will ensure that the individual is adult, able to read and understands the English language. The most recent version of the consent form will be used. The individual obtaining the consent will summarize each section of the consent. Study participants will be told the following: "Take as much time as you need to read the entire consent form. Please inform me when you are done reading the form so I may answer any questions you have." All the questions the subject had will be written on the coordinator flowsheet. The signed informed consent copy will be given to study participant.

Describe the procedures to separate clinical responsibilities and influence from research responsibilities and influence: This research study does not recruit patients; hence, there are no clinical responsibilities.

6.0 Study Design and Procedures

List any collaborating sites where human subjects research will be performed, and describe the role of those sites and collaborating investigators in performing the proposed research (if applicable): This study will be only conducted at University of Illinois. Study space will be rented at Center for Clinical and Translational Science. In addition, UIC nurse will be responsible for performing nasal examination and collecting blood samples. Study personnel – working out of the PI's laboratory - will ensure regulatory compliance, recruit participants, as well as collect and analyze the data. The PI's lab is located on the first floor of UIC College of Pharmacy. Study materials, equipment and all paper copies will be locked in the office of research staff affiliated with the study.

Describe the study design. The description should be capable of meeting the study objectives: This is a two group, two session study. The only eligibility criteria separating the two groups is the smoking status, otherwise the study population will consist of healthy volunteers. Following successful completion of the "Initial Survey", study participants will be scheduled for a 3-hr In-Person Screening visit. At the in-person screening, participants will 1. be consented, 2. provide urine and breath samples, 3. complete surveys, and 4. receive study instructions. After the completion of the "In-Person Screening", study participants will be scheduled for two study sessions, separated by a minimum of 1 week. During the study session, in random order, study participants will receive either intranasal insulin (60 IU) or placebo. This is a cross-over study –participants receiving intranasal insulin will receive placebo next, and vice-versa. Please refer to Table 1 for a summary of the study

session. The sessions will be identical, other than participants receiving intranasal insulin or placebo in random order.

6.1 Procedures and Assessments

Provide a thorough description of all study procedures, assessments and subject activities in a logical and sequential format. Indicate what study activities happen when and where, including, when applicable, a study schedule that notes number and length of study visits for subjects.

Table 3. Outline of Study Session	
TIME	PROCEDURE
8:00-8:30	Determination of session eligibility (urine and breath tests)
8:30-8:50	Nicotine gum break (smokers), generic chewing gum break (non-smokers)
8:50-9:00	Nicotine blood sample
9:00-9:05	POC glucose check
9:05-9:15	Nares exam
9:15-10:00	Relaxation phase (both smokers and non-smokers)
10:00-10:06	Spray administration
10:10-10:30	Equipment setup
10:30-11:40	Go-No/Go task
11:40-11:45	Olfactory function test
11:45-12:00	Discharge procedure (POC measurement)

Study session will begin at 8:00 AM. First, the research coordinator will ensure that the lab has a signed consent form on file from the participant. Smokers will be instructed to smoke the normal amount of cigarettes they usually smoke upon waking prior to each session, then refrain from smoking until the end of the session. For example, if they usually smoke 2 cigarettes upon waking, they will be instructed to smoke 2 cigarettes prior to the first session (30 minutes upon waking) and 2 cigarettes prior to the second session (30 minutes upon waking). Participants will be asked if they have had breakfast. If they did not eat breakfast that morning, research coordinator will give them a granola bar and re-emphasize the importance of eating breakfast. Next, from 8:00 AM to 8:30 AM, urine and breath samples will be obtained by the study coordinator, for verification of absence of alcohol intake. A urine sample will be obtained to rule out illicit drug intake and pregnancy. See Section 6.2 Specimen Considerations for more information on urine sample collection and analysis protocol.

At 8:30, smokers will take a 20-minute nicotine chewing gum (Nicorette gum, 4mg) break, and non-smokers will take a 20-minute generic, non-nicotine chewing gum break. As per the instructions given by Nicorette, smokers will be instructed chew the gum slowly until they experience a tingling sensation, then to place the gum between their cheek and gum until the tingle subsides and repeat until most of the tingle is undetectable. Non-smokers will be instructed to chew the generic gum at their own pace.

At 8:50 AM, research coordinator will administer VANES Questionnaire on REDCap. Following completion of the VANES Questionnaire, a nicotine blood sample will be taken by the study nurse to ensure comparable nicotine levels in participants between the two sessions. In order to synchronize the procedure in both smokers and non-smokers, a blood sample will be taken from non-smokers as well.

Point of care measurement will occur at 9:00, followed by a physical exam of the nares at 9:05. POC glucose will be checked to ensure normal glucose levels. Study nurse will be responsible for conducting nares physical examination. Subjects will relax from 9:15 to 10:00. The blood draw at 8:50 (for the purpose of nicotine level analysis) can cause a rise in cortisol, which remains high for approximately 60 min. The purpose of the relaxation phase is to wait for the stress response to normalize, thereby minimizing its impact at the time of treatment administration and event related potential testing.

Spray administration will occur at 10:00 AM as one spray in each nostril every 3 minutes for the total of 6 sprays (two sprays at 10:00, two sprays at 10:03, two sprays at 10:06). The same spray protocol will be followed for administration of both intranasal insulin and placebo. AeroPump delivers 0.1 ml of liquid per spray. Since the concentration of insulin in Novolin R is 100 IU/mL, six sprays will deliver the 60 IU intranasal insulin dose. See Section 6.3 Drug Product Considerations for more information on insulin and placebo intranasal administration preparation.

Computerized task testing will occur at 10:30. During the Go/No-Go task, study participants will be instructed to respond as “quickly and accurately as possible” with their right index finger every time the target (“Go”) stimulus (a white “X”) appears, and to withhold a response when the distracter “No/Go” stimuli (a white “K”) appears. Targets will appear with higher frequency (84%, 412 trials with 206 for each run) than distractors (16%, 78 trials with 39 for each run) to establish a strong stimulus-response mapping on “Go” trials. In the second task, visual stimuli will be presented every 1000 ms, each for 100 ms duration, for a total of 400 trials (divided into two 200 trial blocks, separated by a 30-s break). The target stimulus will be a 3.5-cm diameter blue circle (presented 15% of trials), the standard stimulus wi ll be a 3.0-cm diameter blue circle (presented 70% of trials), and the distracter stimulus will be an 18-cm^2 black and white checkerboard (presented 15% of trials). Participants will be instructed to respond via button press as quickly as possible to target stimuli using the index finger of their dominant hand.

At 11:40 AM, the olfactory function test will be administered by a study coordinator. Olfactory function test will use the Sniffin’ Sticks protocol as outlined in Section 7.0 Expected Risks/Benefits.

Discharge procedure will occur at 11:45 AM. Study participants will be given instructions for the next session (or will be provided assent document in the event this is the second session). Their POC glucose will be checked to ensure normal glucose levels. Section 11.0 “Data and Safety Monitoring” provides further details regarding implementation of safety measures in the study, and how potential cases of hypoglycemia will be handled.

Indicate which procedures are standard of care and which procedures are being done for research purposes: None of the procedures in this research study are standard of care. They are all being done for research purposes.

6.2 Specimen Considerations

Describe the specimens to be collected: Urine drug specimens and blood samples will be collected from participants prior to screening and testing procedures. Urine specimens will be used to perform urine drug screens and pregnancy tests. Blood samples will be used to determine nicotine blood levels. Blood samples will also be used to monitor blood glucose levels during the study.

Describe aliquoting and any plans for retention specimens: Samples will be collected at the UIC Center for Clinical and Translational Science. Blood specimens used for nicotine test will be transported to UIC Research Resources Center for analysis. Urine specimens and blood samples collected for POC glucose check will not undergo aliquoting or be retained for future use.

Figure 4. Specimen Label

Subject ID: _____
Specimen: Urine/Blood

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PURPOSES ONLY

Describe tracking and labeling system: The label (Figure 4) includes the information that will be on the urine collection cup during urine drug screen and urine pregnancy testing or on the blood sample collection vial during nicotine blood test. Researchers will document test results from urine screening on the study flowsheet.

Describe where the specimens be stored and who will be responsible for care of specimens during storage: Urine specimens and blood samples will not be stored for future use.

Describe how long the specimens will be kept: Urine specimens will be disposed immediately after drug and pregnancy screen. Blood samples used for POC blood glucose testing will be disposed immediately after blood glucose check. Following study completion, blood specimens for nicotine testing will be frozen at the UIC Center for Clinical and Translational Science for up to 6 months before transport on dry ice to UIC Research Resources Center. Urine drugs screens, pregnancy tests, and POC glucose checks are expected to take approximately 5 minutes each.

Describe how specimens will be destroyed at study completion: Urine and blood specimens will not need to be retained by researchers until study completion. Urine specimen will only need to be collected for drug and pregnancy screen during the in-person screening and prior to both testing session. Following drug and pregnancy screen, all urine specimens will be disposed immediately as noted above. Following POC blood glucose check, all blood specimens will also be disposed immediately. Following study completion, blood specimens for nicotine testing will be stored and shipped to UIC Research Resources Center as noted above.

Describe how specimens will be analyzed (type and state of development of assay, controls): The research team will conduct and analyze urine drug screens at the beginning of each testing session. The research team is equipped with supplies and has undergone training to perform urine drug

screens and pregnancy screens. For the drug screening, participants will first be asked to provide a urine specimen. A member of the research team will verify the temperature strip on the collection cup is between 90 – 100°F. Researchers will immerse a 12-panel drug screen dipcard into the urine specimen for 10 seconds and analyze the results in 5 minutes. For pregnancy testing, researchers will immerse an HCG test strip into the urine specimen for 3 seconds and analyze results in 5 minutes. Testing supplies used for drug and pregnancy screens are FDA approved with approximately 99% accuracy of test results.

The research team will perform pregnancy testing using AccuMed HCG test strips by immersing the HCG test strip into the urine specimen for approximately 3 seconds and reading the results after 5 minutes. AccuMed HCG test strips are easy-to-use and provide quick and accurate test results. The test strips provide early pregnancy detection with HCG sensitivity levels as low as 25 mIU/mL. This product is FDA approved and provides over 99% accuracy of results.

Each POC blood glucose check will be conducted by a study nurse using the Accu-Check Performa glucometer. A glucose test strip will be inserted into the glucometer. The study nurse will then prick the participant's finger with the lancing device and place the end of finger with the blood drop to the test strip. Blood glucose will be read by the meter and displayed within 5 seconds.

6.3 Drug Product Considerations

Name and description of product: The drug substance used for preparation of the final drug product will be bulk Novolin R. Novolin R is a sterile, clear, aqueous, and colorless solution that contains human insulin (rDNA origin) 100 units/mL, glycerol 16 mg/mL, metacresol 3 mg/mL, zinc chloride approximately 7 mcg/mL and water for injection. The pH is adjusted to 7.4. Hydrochloric acid 2N or sodium hydroxide 2N may be added to adjust pH. Novolin R vials are latex-free. The drug substance is being purchased from McKesson. The placebo utilized in this study is 14% sodium chloride in sterile water.

Route of administration, dosing, dosage regimen and duration: Intranasal insulin will be prepared by University of Illinois Hospital and Health Sciences System Investigational Drug Service. The service is located in the Hospital Pharmacy and contains a segregated compounding area with a biological safety cabinet (BSC). The drug product will be compounded in the BSC using aseptic technique. The pharmacist or a pharmacy technician designee will prepare the requisite drug product following personnel hand hygiene and garbing procedures according to USP<797>. Aero Pump nasal spray system is manufactured in ISO Class 7 environment, hence sterilization of the system prior to compounding procedure will not be necessary. Aero Pump and Novolin R vial will be placed into the BSC. A volume of 3.3 mL Novolin R will be withdrawn using a set of sterile needle and syringe followed by emptying the contents of the syringe into the AeroPump glass bottle. The bottle will then be capped with AeroPump nasal spray. Finally, sterile gauze will be sprayed with sterile IPA following which 20 sprays will be pumped into the gauze for the purpose of priming the final drug product.

Placebo will be prepared using the aseptic technique as described for intranasal insulin. The pharmacist or a pharmacy technician designee will transfer 2.7 mL of sterile water and 4 mL of sterile 23.4% NaCl solution into a sterile container with 10 mL capacity. A volume of 3.3 mL of 14% NaCl

solution will be aseptically placed in the Aeropump spray as describeda. The final step will be the same priming procedure as for insulin spray.

The directions for spray administration will be one spray in each nostril every 3 minutes for the total of 6 sprays delivered over 6 minutes. AeroPump delivers 0.1 ml of liquid per spray. Since the concentration of insulin in Novolin R is 100 IU/mL, six sprays will deliver the 60 IU dose. Aero Pump and Novolin R vial will be placed into the BSC. A volume of 3.3 mL Novolin R will be withdrawn using a set of sterile needle and syringe followed by emptying the contents of the syringe into the AeroPump glass bottle. The bottle will then be capped with AeroPump nasal spray. Finally, sterile gauze will be sprayed with sterile IPA following which 20 sprays will be pumped into the gauze for the purpose of priming the final drug product.

Placebo will be prepared using the aseptic technique as described in the above paragraph. The pharmacist or a pharmacy technician designee will transfer 2.7 mL of sterile water and 4 mL of sterile 23.4% NaCl solution into a sterile container with 10 mL capacity. A volume of 3.3 mL of 14% NaCl solution will be aseptically placed in the Aeropump spray as described in the "Drug Product" paragraph. The final step will be the same priming procedure as for insulin spray.

Describe how any adverse events or serious adverse events will be handled: Section 11.0 describes in detail study procedures which will be implemented to 1. Ensure thorough and accurate collection of AEs and UPs, 2. Timely reporting to the Institutional Review Board, and 3. Follow-up and resolution of all potential AEs and UPs.

List/describe the expected adverse events based on the product label: Expected adverse events from an investigational study conducted with intranasally administered Novolin R (IND#120700) are listed below:

- Local (nasal): anosmia, nasal irritation, local tissue damage
- Cognitive: confusion, visual disturbances
- Others: sweating, shaking, hunger, tingling sensation around the mouth

Expected adverse events from the product label for Nicorette gum are listed below (Nicorette Informational Pamphlet, 2017):

- Central nervous system: Depression, dizziness, headache, insomnia, lack of concentration, nervousness, pain, paresthesia
- Dermatologic: Diaphoresis
- Gastrointestinal: Aphthous stomatitis, constipation, diarrhea, dysgeusia, dyspepsia, flatulence, gingival hemorrhage, glossitis, hiccups, nausea, sialorrhea, stomatitis, tooth enamel damage (abrasions), xerostomia
- Dermatologic: Skin rash
- Hypersensitivity: Hypersensitivity reaction
- *Local: Application site reaction, localized edema, localized erythema*
- *Neuromuscular & skeletal: Arthralgia, jaw pain, myalgia*
- Respiratory: Cough, sinusitis

7.0 Expected Risks/Benefits

Include expected risks and benefits to subjects and/or society: There are no expected benefits to the participants of this research study, however, it is expected that a better understanding of biomarkers will lead to more sophisticated smoking cessation treatments.

There may be side effects associated with placebo and intranasal insulin administration. One common side effect of placebo is nasal burning and irritation due to a high salt content. Based on the results of our earlier INDs with the same 60 IU dose of intranasal insulin, we don't anticipate any risks of particular severity or seriousness. Nonetheless, as in our previous IND studies, we will complete a detailed evaluation of circulating glucose and olfactory function. Following each of the three rounds of administration, on a scale of 1-10, the following will be assessed: 1. Pain, 2. Burning, 3. Taste, 4. Cough and 5. Anything else participants wish to rate with regards to local irritation.

To ensure that participants' blood glucose levels are not adversely affected by intranasal insulin administration, we will measure blood glucose levels for an immediate glucose level reading to ensure that participants are not hypoglycemic. If a study subject tests below euglycemic level (<70 mg/dL) at any point during the study, the session will be stopped and the subject will be treated. A hypoglycemic subject will be given 15 grams of glucose (4 oz of orange juice) and their glucose level will be rechecked in 15 minutes. The process will be repeated if they are still hypoglycemic. Once treated, they will be given a snack (a granola bar) to eat in order to prevent recurrence. Subjects will then engage in a light activity such as reading for an hour. Following this hour, subjects will drink 4 ounces of orange juice and eat a granola bar. At this time, we will retest their blood glucose levels. Upon confirmation that the subject is still euglycemic, he/she will be discharged.

To assess olfactory function, all subjects will undergo a baseline smell test, and only individuals with normal smell function (identifying 11 or 12 out of 12 total smells) will be eligible to participate in the study. Sniffin' Sticks involves presentation of 12 different pens containing different smells. After the presentation of each pen, participants complete a multiple-choice question with 4 possible choices; one of which is the answer corresponding to the correct smell.

This research study involves risks to pregnant women and/ or an unborn baby which are currently unforeseeable. To protect against possible side effects of nicotine, pregnant and nursing women will not be eligible to participate in the study. Pregnancy will be ruled out prior to initiating any of the two study sessions.

Additionally, there is a potential risk of loss of confidentiality. Information that identifies subjects will only be shared between research staff involved in this particular study. The research team will make every effort to protect subject's private health information and guard against any loss of privacy. Participant information will be securely stored and only accessible to authorized personnel as described in section 8.0 "Data Collection and Management Procedures".

Describe the expected frequency, degree of severity, and potential reversibility of the risks:

As investigation of intranasal insulin effects on brain has gained considerable interest both in the United States and internationally, a group of authors (Schmid et al., 2018) recently conducted a systematic review on the safety of intranasal insulin. They identified thirty-eight studies, with the total of 1092 study participants with acute (i.e. one time) intranasal insulin application and 18 studies with chronic treatment lasting between 21 days and 9.7 years (total of 832 study participants). The authors listed all adverse events reported by study authors and noted that there were no cases of hypoglycemia or severe adverse events (Figure 3).

Figure 3. Adverse events of intranasal insulin identified by Schmid et al., 2018.

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Kern et al., 1999 (50)	120 U over 90 minutes versus Placebo (cross-over)	18 volunteers	H-Insulin 100 Sanofi; Sanofi	Changes in auditory evoked potentials, no effect on plasma insulin, glucose or plasma norepinephrine levels	No information	none
Born et al., 2002 (51)	40 U once versus placebo (randomized parallel)	8 healthy subjects (insulin arm), 5 healthy subjects (placebo arm)	No information	Increase in CSF insulin, no effect on serum insulin	No information	No information
Reger et al., 2006 (19)	20 or 40 U or placebo once (cross-over)	26 memory-impaired subject and 35 normal controls	Novolin R, Novo Nordisk	Improved verbal memory, no effect on plasma insulin or blood glucose	Nose bleed (1 subject at 40 U), nose soreness for about 24h (treatment not specified)	none
Benedict et al., 2008 (44)	160 U versus placebo once (cross-over)	32 normal-weight subjects (18 females)	Actrapid, Novo Nordisk	Decreased food intake in men, but not in women, improved memory in women, but not in men	Decrease in blood glucose (by about 0.24 mmol/l)	none
Bohringer et al., 2008 (52)	40 U versus placebo once (randomized parallel)	26 healthy young males	Actrapid, Novo Nordisk	Diminished cortisol response to the Trier social stress test	none	none
Mark A. Reger et al., 2008 (53)	10, 20, 40, 60 U versus placebo once (cross-over)	59 controls and 33 memory-impaired patients	Novolin R, Novo Nordisk	Improved verbal memory, peak effect with 20 U no effect on plasma insulin or blood glucose	Personal communication: Some cases of rhinitis, no other side effects.	none
Guthoff et al., 2010 (54)	160 U versus placebo once (cross-over)	9 healthy subjects (5 males)	Actrapid, Novo Nordisk	Altered processing of food pictures (assessed by fMRI)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Krug et al., 2010 (55)	160 U versus placebo once (cross-over)	14 healthy postmenopausal women	Actrapid, Novo Nordisk	No effect on food intake, enhanced memory	Personal communication: tingling or mild burning sensation in the nose in some participants; no other AEs	none
Stingl et al., 2010 (56)	160 U once versus. placebo (cross-over)	10 lean/10 overweight healthy volunteers	Actrapid, Novo Nordisk	Changes in resting state dynamics (assessed by MEG)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Benedict et al., 2011 (57)	160 U versus Placebo once (cross-over)	19 healthy men	Actrapid, Novo Nordisk	Enhanced postprandial thermogenesis, lower postprandial circulating insulin and C-peptide levels	Personal communication: tingling or mild burning sensation in the nose in some participants; no other AEs	none
Fan et al., 2011 (28)	40 U versus placebo once (randomized parallel)	30 patients with schizophrenia	Humulin, Eli Lilly	No effect on verbal memory or sustained attention	No effect on plasma glucose	none
Guthoff et al., 2011 (58)	160 U versus placebo once (cross-over)	10 lean and 10 obese subject	Actrapid, Novo Nordisk	Altered cerebral processing of food pictures in lean, but not obese (assessed by MEG)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Stockhorst et al., 2011 (48)	20 U once (randomized parallel)	32 healthy young subjects	Insuman Rapid, Sanofi	Conditioned increase in peripheral insulin	Slight decline in blood glucose, no adverse side effects	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Stein et al., 2011 (21)	60 U (n=16) versus placebo (n=16) 4 times daily for 2 days (total 480 U insulin) (parallel-design)	32 patients with mild to moderate Alzheimer's disease (16 females treated with nasal insulin)	Humulin, Eli Lilly	No effect of nasal insulin on memory	Personal communication: no side effects	Personal communication: No hypoglycemia was measured
Grichisch et al., 2012 (59)	160 U versus oral caffeine (200mg) once (cross-over)	8 healthy subjects (3 males)	Actrapid, Novo Nordisk	No effect on global cerebral blood flow, i.e. no direct vasodilatatory effect of nasal insulin	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	Personal communication: none
Hallschmid et al., 2012 (45)	160 U versus placebo once (randomized parallel)	30 healthy women	Actrapid, Novo Nordisk	Decreased postprandial appetite, decreased postprandial intake of chocolate cookies	Slight decrease in plasma glucose	none
M Heni et al., 2012; Ketterer et al., 2014 (43,60)	160 U versus Placebo once (cross-over)	103 volunteers	Actrapid, Novo Nordisk	Change in HOMA-IR and change in regular brain activity, no effect on cortisol levels	Decline in blood glucose by about 0.2 mmol/l, burning sensation in the nose in some participants (no more than 5 minutes)	none
Jauch-Chara et al., 2012 (61)	40 U versus placebo once (cross-over)	15 healthy men	Actrapid, Novo Nordisk	Increased brain ATP and phosphocreatine levels	No effect on blood glucose (measured every 5 minutes), no other side effects reported	none
Brünner et al., 2013 (47)	40 U once versus placebo (cross-over)	14 healthy subjects (7 females)	Actrapid, Novo Nordisk	Decrease in olfactory threshold	Slight decline in blood glucose (about 0.2 mmol/l)	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Kullmann et al., 2013 (62)	160 U once versus placebo (cross-over)	17 female volunteers	Actrapid, Novo Nordisk	Modification of reward processes and prefrontal brain activity, assessed by fMRI	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Heni et al., 2014 (40)	160 U once versus placebo during systemic hyperinsulinemia (cross-over)	10 lean and 5 obese healthy men	Actrapid, Novo Nordisk	Improved peripheral insulin sensitivity, modulation of hypothalamic activity (assessed by fMRI), change in heart rate variability	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Iwen et al., 2014) (63)	160 U once versus placebo (cross-over)	14 healthy men	Actrapid, Novo Nordisk	Decrease in circulating free fatty acids and lipolysis	Personal communication: tingling or mildly burning sensation in the nose in some participants; no other AEs	none
Novak et al., 2014; Zhang et al., 2015 (64,65)	40 U once versus saline (randomized parallel)	15 patients with type 2 diabetes, 14 controls	Novolin R, Novo Nordisk	Improvement in cognitive function, change in cerebral blood flow (assessed by MRI)	No serious adverse events, no nasal irritation, no allergic reactions	none
Ferreira de Sá et al., 2014; Schilling et al., 2014 (66,67)	40 U insulin (n=13), 30mg Cortisol (n=12), 30mg Cortisol + 40 U insulin (n=15), placebo (n=14) (parallel-design)	54 healthy volunteers	Actrapid, Novo Nordisk	No effect of insulin on processing of food cues	Personal communication: tingling or mildly burning sensation in the nose in some participants; no other AEs	Personal communication: none
Brünner et al., 2015 (68)	40U once versus placebo (cross-over)	18 male subjects	Actrapid, Novo Nordisk	Improved, delayed but not immediate odor-cued recall of spatial memory	None	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Gancheva et al., 2015 (38)	160 U once versus placebo (cross-over)	10 patients with type 2 diabetes (1 female), 10 healthy volunteers (3 females)	Actrapid, Novo Nordisk	Improvement in hepatic energy metabolism and decline in liver fat content in lean subjects	Slight decline in blood glucose	none
Kullmann et al., 2015, 2017b, 2017a (69–71)	160 U once versus placebo (cross-over)	25 lean (10 female) and 23 (11 female) overweight healthy volunteers	Actrapid, Novo Nordisk	Change in regional brain activity (assessed by fMRI)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Schöpf et al., 2015 (27)	40 U insulin (n=10), NaCl at later time point (n=7) once	10 patients with smell loss	Actrapid, Novo Nordisk	Improved olfactory sensitivity and intensity	Personal communication: No adverse events	Personal communication: none
Feld et al., 2016 (41)	160 U versus placebo (cross-over)	16 healthy men and 16 healthy women	Actrapid, Novo Nordisk	Increased growth hormone concentrations in the night-half following nasal insulin, impaired memory encoding on subsequent day	Temporary, slight decline in blood glucose	none
Brünner et al., 2016 (72)	40 U versus placebo once (cross-over)	16 healthy men	Actrapid, Novo Nordisk	No effect of nasal insulin on declarative memory or hippocampal activity	none	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Hamidovic et al., 2017 (26)	60 U versus placebo once	19 healthy smokers (cross-over) and 37 healthy smokers (parallel) abstained from smoking for 36h	Novolin R, Novo Nordisk	Reduction in nicotine craving, Increase in circulating cortisol during psychosocial stress	- Slight decrease in blood glucose - Rinorrhea (Placebo: N=1; Insulin: N=1) - Nasal irritation (Placebo: N=5, Insulin N=7) - Dizziness (Placebo: N=0, Insulin N=1) - Sweating (Placebo: N=0, Insulin N=2) - Headache (Placebo: N=0, Insulin N=1) - Confusion (Placebo: N=1, Insulin N=2) - Tingling in mouth (Placebo: N=0, Insulin N=1) - Shaking (Placebo: N=0, Insulin N=1) - Anxiety (Placebo: N=1, Insulin N=1) - Restlessness (Placebo: N=0, Insulin N=1) - Discomfort (Placebo: N=0, Insulin N=1) - Watering eyes (Placebo: N=0, Insulin N=2)	none

In a previous study on intranasal insulin (IND #120700), smokers received two administrations of intranasal insulin (60 IU) on two separate days. Table 2 (see Section 2.0 Background/Scientific Rationale) lists all the adverse events reported in the study by the treatment group. Common reported events included nasal occurrences such as rhinorrhea and irritation, which are to be expected given the exploratory nature of intranasal administration of Novolin R, which is designed for subcutaneous delivery.

Single uses of nicotine chewing gum use can have nicotine-mediated cardiovascular effects, including an acute heart rate increase of up to 10-15 beats/min and a blood pressure increase of up to 5-10 mmHg (Benowitz et al., 1997). Both of these effects are similar to those of cigarette smoking as well as to other nicotine replacement therapies, such as intravenous nicotine and nicotine nasal spray.

Describe any dose modifications as a result of adverse events, as applicable: Based on the results of our earlier INDs with the same 60 IU dose of intranasal insulin, we don't anticipate any risks of

particular severity or seriousness. Nonetheless, as in our previous IND studies, we will complete a detailed evaluation of circulating glucose and olfactory function (see first subheading) and make the appropriate dose modifications if adverse events occur.

8.0 Data Collection and Management Procedures

Outline the process for data procurement: Table 3 below lists data collection tools. The list of data collection materials attached to this application is after the reference section (14.0).

Table 3. Data procurement layout for "Neurologic Biomarkers of Smoking Behavior"

Data Collection Tool	REDCap	Hard copy	Hard Drive	Used for Determination of Study Eligibility
1. Initial Survey	✓			✓
2. Urine Drug Test Results		✓ (Flowsheet)		✓
3. Urine Pregnancy Tests		✓ (Flowsheet)		✓
4. Cigarette Smoking Quantity	✓			✓
5. Carbon Monoxide Level		✓ (Flowsheet)		✓
6. Breath Alcohol Level		✓ (Flowsheet)		✓
7. Medication and Health Review		✓ (Flowsheet)		✓
8. Mental Health Interview		✓		✓
9. Hearing Test		✓ (Flowsheet)		✓
10. Shipley IQ Test		✓ (Flowsheet)		✓
11. Demographics Survey	✓			
12. Substance Use Survey	✓			
13. Visual Analog Nicotine Effects Scale (VANES)	✓			
14. POC Glucose Level		✓ (Flowsheet)		
15. Adverse Event Questionnaire		✓		
16. Adverse Event Tracking Form		✓		
17. Spray Effect Rating Questionnaire	✓			

The mental health document includes a question about suicide risk. This question is a not part of an evaluation of suicide risk *per se*. Rather, it is one of the questions under the category "Recent Major Depressive Episode". Study coordinator will call Dr. Hamidovic regarding any person reporting that he/she is suicidal (as part of Depression assessment). Dr Hamidovic will conduct an assessment by asking further questions related to suicidality using the attached MINI-International Neuropsychiatric Interview. Individuals scoring "low suicidality" and without imminent threat will be given referral to UIC Adult Psychiatry Service (312-996-2200) and contacted for follow-up after the screening. Dr. Hamidovic will contact Dr. Holden for an in-person assessment for individuals with moderate/high suicidality. For those exhibiting an immediate danger, authorities may be called if Dr Holden or Dr. Hamidovic view it necessary.

Describe source documents and how data will be collected from source documents and incorporated into the database: During the screening session for both smokers and non-smokers, the research

team will have a hard copy of the screening flowsheet. The flowsheet will be used as a guidance document for researcher staff to conduct the series of screening procedures including obtaining informed consent, breath tests, questionnaires, specimen analysis, and health screens. Researchers will document on the flowsheet whether or not participants passed or failed each screening procedure and add any additional significant notes throughout the process. Similarly, the research team will also follow the flowsheet when running each of the two study sessions. For testing sessions, research data will be collected using software discussed above and all other study information will be entered into the REDCap database. All flowsheets will be stored in the PI's UIC lab located on the first floor of the UIC College of Pharmacy.

Describe the methods in which the data will be collected and stored (i.e., electronic, hard copy, specimens, artifacts, etc.): Please see Table 3.

Describe where each method of data will be stored and how each method will be maintained in a secured manner (i.e., encryption, password protection, use of Qualtrics or REDCap, etc.): REDCap will only be accessed by the PI and her research team on password protected computers in the PI's lab located on the first floor of UIC College of Pharmacy. This research project will be shared amongst the research team in REDCap and each member will be able to access the information by logging into the REDCap database under their individual profiles. Participants' personal information will be stored in REDCap to ensure protection of personal identifiers. Software needed to collect study data from research devices during testing sessions will be downloaded on PI's research laptop. As discussed, the PI's laptop is password-protected and only available to members of the research team. No identifiers will be stored on the laptops. Hard copy files will be stored in locked cabinets inside the locked offices in the PI's lab located in the UIC College of Pharmacy - 833 South Wood Street – Chicago, IL 60612. The PI's lab consists of 3 rooms within UIC College of Pharmacy – room 117A, 115, 112C.

Describe who will have access to each method of data and how each method of data will be transferred to any collaborators: The Principal Investigator and her designated research team will have access to the research study information. There will be no collaborators involved in this study. Electronic data and hard copy files including research information will be stored and secured as noted above.

11.0 Data and Safety Monitoring

Addresses how problems/side effects will be identified and handled:
Subjects will be asked to report any adverse events by specific questioning. The questions will specifically refer to symptoms of hypoglycemia and local irritation, but they will include additional questions about general adverse events as specified in the Consent Form. In addition, subjects will be asked to report any adverse events not listed on the Consent Form. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. If any serious events are detected, they will be followed up to determine the final outcome. Following completion of the study, participants will be instructed to report any adverse events and if the Principal Investigator determines that it is possibly related to the study treatment or participation, will be recorded and reported.

Subjects will be asked to report any adverse events they may have experienced related to the spray that was administered, the Nicorette nicotine gum, and the general study protocol. Information on all adverse events will be recorded immediately on the "Adverse Event Questionnaire" form provided by the PI's lab. The "Adverse Event Questionnaire" lists all the expected AEs of intranasal insulin and nicotine gum as listed in the Nicorette information sheet and IND#120700 for intranasally administered Novolin R. In addition, blank space is included for study participants to list any additional AEs not listed on the form.

Per policy of UIC OPRS, depending on the seriousness of the event, an adverse event will be reported either within 5 business days (in case of a serious event) or 15 business days (in an event the serious criteria is not met) as outlined in the UIC HSPP form "Prompt Reporting to the IRB". Clinical course of each event will be followed until resolution, stabilization or until it has been determined that the study treatment or participation is not the cause. In addition to reporting adverse events related to spray administration, should they occur, the following non-drug related events be reported within 5 business days as well: 1) Breach of confidentiality, 2) Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant, 3) Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team, or 4) Major protocol violations that are unplanned and unintentional, and 5) Apparent serious or continuing noncompliance. Anticipated and non-serious adverse events will be collected and entered into the study database for the purpose of comparing their incidence following intranasal insulin in comparison to the placebo spray administration.

This form will be reviewed and signed first by the research team member, and also by the study PI. At that time, the PI will determine whether the reported events fall in the category of unanticipated AEs. Depending on the seriousness of the event, it will be reported either within 5 business days (in case of a serious event) or 15 business days (in an event the serious criteria is not met) as outlined in the UIC HSPP form "Prompt Reporting to the IRB". Clinical course of each event will be followed until resolution, stabilization or until it has been determined that the study treatment or participation is not the cause.

In addition to reporting adverse events related to spray administration or nicotine gum, should they occur, the following non-drug related events be reported within 5 business days as well: 1) Breach of confidentiality, 2) Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant, 3) Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team, or 4) Major protocol violations that are unplanned and unintentional, and 5) Apparent serious or continuing noncompliance.

Anticipated and non-serious adverse events will be collected and entered into the study database for the purpose of comparing their incidence following intranasal insulin in comparison to the placebo spray administration.

For studies that are minimal risk, describe how potential problems will be monitored and handled (e.g., breaches of confidentiality, emotional upset): This is not a minimal risk study.

For research involving more than minimal risk to subjects, describe: Who will monitor adverse events (AEs) and unanticipated problems (UPs) involving risks to subjects or others and when events will be assessed: All research staff will be responsible for collecting AEs and UPs, but the ultimate responsibility and classification determination will rest with the PI. Whereas the study

coordinator/postdoc will sign the completed Adverse Event form, that form will be given to the PI who will use the “Adverse Event Tracking Form” for final classification of the AE. It will be documented on the form whether the event listed in the blank space on the Adverse Event form falls into anticipated/unanticipated and whether the nature of the event is serious/non-serious. In the event of unanticipated and/or serious event, the resolution of the AE will be tracked by the PI and all communications will be related to the Institutional Review Board.

How AEs or UPs will be recorded and communicated amongst research team members and who is responsible for making the reports: At the end of each testing session, participants will be asked to complete “Adverse Event Questionnaire”. The research member facilitating the session and the PI will review and sign the form. The PI will record the determination of expectancy and seriousness of each event in the “Adverse Event Tracking Form”, and will communicate the result of this determination using UIC HSPP form “Prompt Reporting to the IRB”.

The composition of the Data and Safety Monitoring Board (DSMB) and how frequently the DSMB meets, if one has been formed for the study: The Data and Safety Monitoring Board will be chaired by a physician – Dr. Mark R. Burge – and two additional members – Dr. James Nawarskas and Dr. Gretchen Ray – who are also clinicians. Dr. Burge is the Deputy Director of the Clinical and Translational Science Center at the University of New Mexico and a Regents’ and Distinguished Professor of Medicine. Dr. Nawarskas is an Associate Professor at the University of New Mexico’s College of Pharmacy. Dr. Gretchen Ray practices as a pharmacist clinician in the University of New Mexico Family Medicine clinics. The DSMB will meet every six months on a teleconference call. The type of safety information that will be assessed is the number and nature of adverse events that have been reported in the time since the previous call, results of olfactory function testing (i.e. Sniffin’ Sticks), POC glucose testing as well as the outcome of events discussed in previous meetings. The summary of the meetings and recommendations will be reported to the Principal Investigator as well as Human Research Protections Office within 15 days after the meeting. The board will meet on an interim basis (within 7 days) in a case of expedited cases in which the probability of treatment causality is high or a case of a change to the risks or potential benefits of the research (such as safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected). In such cases, the Principal Investigator will supply results of interim analysis including plans/safeguards to keep any data or DSMB analyses confidential.

Identify how often AEs and UPs will be monitored and what events will be reported to the sponsor and/or the IRB: Adverse events and unanticipated problems will be monitored after each testing session as described above. Per policy of HSPP, expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at an increased risk of harm, will occur within 5 working days from the time the investigator became aware of the event. This is the case for any adverse that occurs any time during or after research study, which in the opinion of the Principal Investigator is unexpected and related to the study procedures. The reporting process will consist of sending a written report to the IRB that includes a description of the event with information regarding the fulfillment of expedited reporting, follow-up/resolution and need for revision of the consent and/or other study documentation. Copies of each report and documentation of IRB notification and receipt will be kept in the Principal Investigator’s study file.

More rapid reporting requirements will be followed when deaths occur during the course of the research study. The event will be reported within 24 hours when the death is unforeseen (unexpected) and indicates participants (or others) are at an increased risk of harm. Report of the event within 72 hours will be filed for all other deaths, regardless of whether the death is related to study participation.

Less serious events (1. Local adverse events or problems that are unanticipated and, while not meeting the criteria of serious, indicate research is associated with a greater risk of harm to participants or others than previously known, 2. External adverse events that are unanticipated, indicate research associated with a greater risk of harm to participants or others than previously known and more likely than not to have been caused by the procedures associated with or subject's participation in the research 3. New information indicating an unexpected change to the risks or benefits of the research (i.e., an unanticipated problem), and 4. administrative hold by investigator, sponsor, regulatory authorities or other entities) will be reported within 15 business days.

Describe stopping rules for the study: Stopping rules for the study will apply to expedited cases in which the probability of study treatment causality is high or a case of a change to the risks or potential benefits of the research (such as safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected). In the case of an expedited event, the study will be stopped until further information is gathered and a potential plan is formulated through consultation with the IRB.

Describe what occurs if a subject withdraws prematurely: The PI and an appointed research team member will provide guidance to participants on the most appropriate method of discontinuation based on where they are in the study protocol. Recommendations will be given based on what procedures the subject has completed. For example, if the participant decides to withdraw participation immediately after receiving a spray, researchers will counsel the participant on common side effects they may experience. Researchers will provide a discharge summary of the session based on what the participant has completed. The team will provide supportive care to subjects as needed following all premature discontinuations.

12.0 Statistical Considerations

If a study incorporates qualitative rather than quantitative methods, indicate this and describe qualitative analysis and disregard the rest of this section: This study does not incorporate qualitative data analysis.

Describe how the data will be examined and statistically analyzed to answer the objectives: Go/no-go accuracy will be evaluated in a 2 x 2 repeated measure ANOVA, with group, treatment and treatment x group interaction entered into the model.

Provide a brief sample size calculation or description of sample size calculation. Include methods and assumptions such as loss to follow-up, as appropriate: Effect size is estimated from Luijten et al (2011). In that study, effect size was 0.24.

We anticipate 120 participants completing the study. An a priori power analysis was conducted using G*power 3.1.9.2 for an ANOVA test, repeated measures and within-between interaction. With 2

within and 2 between factors; a base correlation=0.6; power=0.95; alpha=0.05, the total of 120 participants (60 per group) is required to capture an interaction between group and treatment on ERN.

13.0 Regulatory Requirements

13.1 Informed Consent

Describe how informed consent will be obtained and who will obtain it: If a participant passes the initial REDCap survey, he/she will be contacted by a member of the research team to schedule an in-person screening session. In-person screenings will take place in a private clinic room located in the Center for Clinical and Translational Science. Informed consent will be obtained at the beginning of the screening session. The "Screening Flowsheet" (submitted as part of this application) was developed by the PI including all the sections that need to be checked by the member of the research team obtaining the consent.

Study participants will be given ample time to review the form. The consent procedure will be carried out in a private and quiet setting. Prior to initiating the procedure, the person consenting will ensure that the individual is adult, able to read and understands the English language. The most recent version of the consent form will be used. The individual obtaining the consent will summarize each section of the consent. Study participants will be told the following: "Take as much time as you need to read the entire consent form. Please inform me when you are done reading the form so I may answer any questions you have." All the questions the subject had will be written on the coordinator flowsheet.

The research team will retain the original consent form and store the document in a locked cabinet in a locked office within the PI's lab. Only designated research team members appointed to this research study will be granted approval by the PI to obtain informed consent.

Describe the training that will be completed by each member of the research team prior to being delegated to obtaining informed consent: Study personnel have completed the required CITI Training for Human Subject Protection. In addition to completion of the online training modules, study personnel will practice obtaining informed consent in the PI's lab. The PI will provide feedback and expert tips to the research team for the consent form process. The PI has over 10 years of research experience and has lead numerous clinical research studies involving obtaining informed consent from human subjects. The record of training of all research members will be kept on file in the laboratory.

Describe where the informed consent document will be stored and who will have access to the informed consent documents: Informed consent documents will be stored as hard copy files in the PI's lab located in the UIC College of Pharmacy. Only the PI and her designated research team for this study will have access to the informed consent. The research team is committed to being vigilant in the protection of patient information.

If research involves minors, describe assent process, as applicable: The research study does not involve minors. This population will not be allowed to participate in the study.

If research involves non-English speaking subjects, describe the consenting process, as applicable: The research study does not involve non- English speaking subjects.

Describe the use of any waivers, if applicable: Waivers will not be used for this research study.

13.2 Subject Confidentiality

Describe how the subject's confidentiality will be maintained: Research personnel will take all feasible measures to ensure subject information remains secure and protected at all times. Paper files will be stored in a locked cabinet located in the PI's UIC lab located in the College of Pharmacy located at 833 South Wood Street, Chicago, IL 60612. Electronic files will be stored on a password protected computer that will only be accessible to the research team for this study. REDCap, a widely used secure web application, will be the storage location for electronic files for this study. The details of the research project will be shared electronically in REDCap amongst the study team. Patient identifiers will be stored in REDCap, however, to ensure confidentiality subjects will be tracked by subject ID number throughout the study. Researchers will refer to subjects by their assigned ID number instead of using personal identifying information in research discussions. Unauthorized individuals who are not affiliated with the research study will not have access to sensitive subject information.

Describe who will have access to the data: The PI and her appointed research team will have access to the study data. Study generated data and sensitive participant information will only be accessible to researchers affiliated with this study. Study data will be stored on password-protected computers in the PI's lab. Sensitive patient identifiers will be stored in REDCap on password protected computers only accessible to authorized individuals.

Describe whether the data will be de-identified, coded, or retain identifiers: The REDCap database used for this research will include identifiable information in order for researchers to track participants for both research and safety purposes. Upon enrolling in the study, participants will be assigned a subject ID number. Throughout the study, participants will only be tracked by their subject ID number, not any personal identifiers. Hard copy files including screening documents for each participant, will only list the subject ID number. The exception to this are the forms which the subjects will have to sign (for example, the consent form and adverse event form). Those forms will be locked in a filing cabinet in a lab office which will also be locked.

Describe plans for the destruction of any identifiers: Researchers will destroy participant identifiers after all analyses, publications, reports, and presentations are complete – i.e., 6 years following study completion. Identifiable information will not be used in these discussions. Following completion of the study, the research team will submit a final report to the UIC IRB as directed by the Office of the Vice Chancellor for Research.

Provide justification for use of personally identifiable data or private health information (PHI): Researchers will not access any patient charts for this research study. As discussed, personal identifying information will be requested from participants at the beginning of the study for research and safety purposes. Researches will need documentation that participants meet study criteria (i.e. age between 18 – 40 years old) and will also need participant's contact information for scheduling

testing session appointments. Participants will be asked detailed questions about their health to ensure it is safe to participate in the research. Throughout the study, participants will only be tracked by their subject ID number, not any personal identifiers.

Describe whether a Certificate of Confidentiality will be required: A Certificate of Confidentiality will be required and obtained for this research study. Participants will be asked personal questions about their substance use history. Researchers are committed to protecting study participants' confidentiality. The research team will inform participants about the purpose of the Certificate of Confidentiality prior to asking questions about substance use.

13.3 Unanticipated Problems

Describe process for reporting any unanticipated problems to IRB, sponsor (if applicable), and FDA (if an FDA-regulated study): Details of this process are outlined in Section 11.0.

14.0 References

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