UCOPE Protocol

1. Project Title

Mechanisms of Oxytocin's Analgesia in Older Adults (Short title: UCOPE Study: **U**nderstanding **C**ognition, **O**xytocin, and **P**ain in **E**lders)

2. Investigator(s)

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3. Abstract

Osteoarthritis (OA) represents a significant cause of disability worldwide in individuals aged 65 and older, a rapidly growing segment of our population. The knee is the most commonly affected joint with pain being the primary symptom, negatively impacting physical, cognitive, and emotional functioning. Symptomatic knee OA has been traditionally attributed to peripheral mechanisms, but measures of joint damage only modestly account for the presence or severity of OA-related pain. The neuropeptide oxytocin (OT) has been recognized as a mediator of endogenous analgesia in animal and human studies. However, little is known about the neurobiological mechanisms underlying OT's pain-relieving properties. This proposal is based on a mechanistic model of OT's analgesic effects leveraging pilot data supporting efficacy and safety of self-administered intranasal OT over 4-weeks in older individuals. Relative to placebo (P), daily administration of intranasal OT diminished self-reported pain intensity, reduced experimental pain sensitivity, and increased self-reported physical and emotional functioning. Further, participants treated with OT, compared to P, showed decreases in brain metabolite concentrations associated with inflammation. Thus, our overarching goal is to evaluate the effects of intranasal OT on pain and function in aging and to determine the extent to which central and peripheral inflammatory mechanisms contribute to these analgesic responses. We aim to 1) determine the effect of intranasal OT administration on clinical and experimental pain sensitivity in older adults with symptomatic knee OA and 2) characterize inflammatory mechanisms contributing to the inter-individual variability in analgesic responses to OT. Older adults with symptomatic knee OA will self-administer intranasal OT or P over 4 weeks using a double-blinded, cross-over study design. With strong support from the University of Florida and the McKnight Brain Institute, our interdisciplinary project, using a comprehensive multi-methods approach, will be the first to determine the potential benefit of OT as a novel analgesic therapy for knee OA pain in aging. OT is currently used in obstetrics and may be an inexpensive, effective method for pain management in older adults with little potential for addiction. Embedded in a biopsychosocial framework, our proposal will help pave the way for future investigations using a mechanism-based treatment optimization strategy for individuals suffering from chronic pain.

4. Background

The prevalence and negative impact of chronic osteoarthritis pain among older adults is high: The burden of chronic pain among older adults is substantial with up to 60% of adults over 65 years reporting chronic pain in large community samples.[28] Furthermore, older compared to younger adults are at a greater risk of developing chronic pain and experience greater pain intensity and pain at more

bodily sites.[29,30] In particular, osteoarthritis (**OA**) is the leading cause of disability among older adults,[1,2] with the knee being the most commonly affected joint.[3] Lifetime risk of developing symptomatic knee OA exceeds 40%, and OA prevalence in the U.S. population is rising due to increased age and obesity.[31] *With the number of adults aged over 65 years expected to double from 40 to 88 million by the year 2050, health concerns related to OA for older individuals and society at large will increase in the coming decades. This demographic encompasses a large and growing group in need of better therapeutic modalities that are based on mechanistic translational research. In particular, there is an urgent need to develop better analgesics for individuals with OA-related pain, since currently used analgesics frequently fail to provide adequate relief or have to be discontinued owing to adverse effects.*[32]

Pain associated with OA is heterogeneous: Historically, OA has been conceptualized as a regional pain condition with symptoms driven by peripheral pathophysiology. However, a growing body of evidence supports the notion that both local and low-grade systemic inflammation contribute not only to articular damage in OA, but also to pain and reduced physical function.[33-36] The poor correspondence between measures of disease severity and clinical symptoms suggests that factors above and beyond peripheral tissue damage must contribute to OA-related pain.[9] Indeed, recent studies demonstrate widespread abnormalities in brain structure and function in persons with symptomatic OA,[37-40] including greater levels of metabolites associated with brain inflammation.[41] Quantitative sensory testing (**QST**) findings also demonstrate a maladaptive pain-modulatory profile in symptomatic knee OA,[11,42,43] characterized by heightened pain facilitation and impaired pain inhibition, consistent with significant changes in pain processing at multiple levels of the neural axis (i.e., brain and spinal cord). Furthermore, individuals with severe knee OA pain experience significant maladaptive psychological characteristics consistent with changes in brain regions associated with mood and emotional function.[5,39,44]

Oxytocin as a multifunctional analgesic targeting altered central and peripheral pain mechanisms in older adults with OA: A promising treatment candidate for pain that is increasingly discussed in the literature, is the neuropeptide oxytocin (**OT**).[13,14] While best known for its roles in parturition and lactation,[45] OT has been shown to play a role in endogenous analgesia with animal studies demonstrating anti-nociceptive effects.[46-48] In humans, low plasma OT levels are associated with increased prevalence of chronic pain,[15-17] and acute (i.e., one-time) intranasal OT administration decreases experimental pain sensitivity, increases pain inhibition, and improves mood and positive affect in younger inidividuals.[18,19] However, the analgesic effects of chronic OT administration remain understudied in persons with chronic pain, and in particular, in older adults affected by symptomatic knee OA. The proposed work will address this research gap.

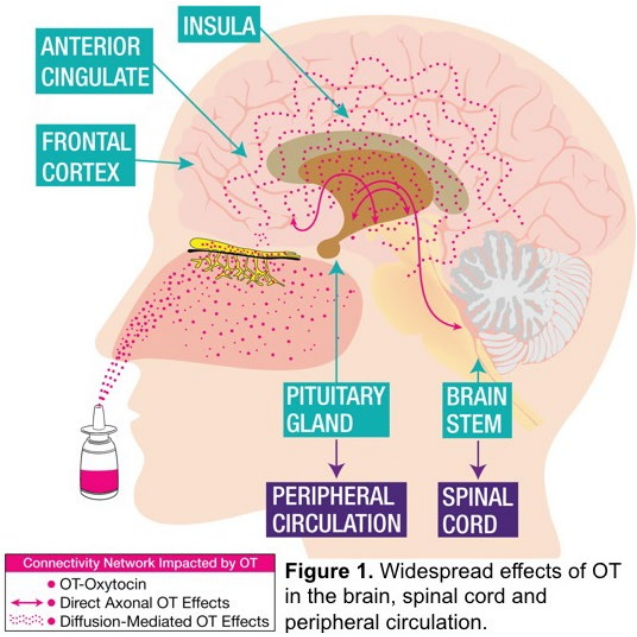


Figure 1. Widespread effects of OT in the brain, spinal cord and peripheral circulation.

A notable characteristic of OT is that it is multifunctional in ways that may be leveraged therapeutically.[14] OT's potential analgesic mechanisms may be explained by its roles as both a neurotransmitter and a paracrine hormone (**Figure 1**). As a neurotransmitter, OT may provide analgesia via widespread effects on the brain[22,26] and spinal cord.[22] As a hormone, OT is released into the peripheral circulation and acts directly on multiple organ systems. Possibly connecting these two routes, the OT-secreting system

within the hypothalamus integrates neural, endocrine, and immune function.[26] Indeed, animal studies suggest that OT can inhibit inflammation both in the brain and spinal cord,[22] with recent evidence supporting that OT's effect on inflammation may contribute to its analgesia. Similarly, in humans, OT decreases pro-inflammatory cytokines in the peripheral circulation,[23] and our preliminary data implicates both peripheral and central inflammatory processes in OT's analgesia.
Further, OT's effects in mood and affect regulation are likely explained by its interaction with the hypothalamic-pituitary-adrenal (**HPA**) axis and effects on stress regulation.[26,49,50] Based on this combined evidence, our working hypothesis is that older adults with chronic knee OA may benefit therapeutically from OT's multiple pain-modulatory mechanisms.

5. Specific Aims

Specific Aim 1: To determine the effect of intranasal OT administration on clinical and experimental pain in older adults with symptomatic knee OA. We will assess self-reported pain along with a standardized, multimodal experimental pain battery, and plasma levels of OT as modifier of a significant treatment response. We will also assess physical, cognitive, and emotional functioning. Informed by our pilot data, we hypothesize that compared to P, OT treatment will result in significant reduction in: **H1a**) self-reported pain intensity; **H1b**) experimental pain sensitivity; and increase in **H1c**) physical, **H1d**) cognitive, and **H1e**) emotional functioning assessed with self-reported and performance-based measures.

Specific Aim 2: To characterize inflammatory mechanisms contributing to the inter-individual variability in analgesic response to OT. We will assess central and peripheral inflammatory processes using proton MRS in frontal brain regions and plasma immune markers, respectively. Based on our preliminary data, we hypothesize that compared to P, OT administration will result in significant: **H2a**) decrease in plasma levels of IL-6, TNF-α, and IL-10; and **H2b**) decrease in brain metabolite concentrations associated with inflammation (i.e., *MI*, Cr, Cho). Further, **H2c**) decrease in brain metabolites and systemic cytokines will be significantly associated with decrease in clinical pain intensity. Other markers of brain structure and function will also be significantly associated with changes in clinical pain intensity (**H2d**).

Exploratory Aim 3: To characterize effects of acute intranasal OT administration on clinical and experimental pain as well as underlying neurobiological mechanisms in younger and older controls. We will perform similar experiments as above in control participants to further understand OT's analgesic effects across the lifespan in the absence of knee OA pain. However, acute OT administration will be used in healthy controls versus chronic administration in the presence of Knee OA.

6. Research Plan

We propose a double-blinded, randomized cross-over design that will randomize older adults with symptomatic knee OA pain and/or back pain to four weeks of intranasal self-administration of either OT or P (48 IUs daily) followed by a four-week washout period and a second four weeks of intervention (either OT or P). Our study design was guided by recommendations by the IMMPACT group for clinical pain trials.[74-77] After initial screening, eligible participants will undergo up to four baseline sessions (health, sensory, and neuroimaging) for collection of clinical, functional (physical, cognitive, emotional), QST, and brain imaging data lasting 2-3 hours. At some point during the baseline, intervention, and post-intervention periods, participants may wear a Samsung Gear S® or Apple smartwatch or Oura ring to obtain daily pain, mood, and social activity assessments and to objectively measure physical activity levels and sleep quality. During the intervention, participants will self-administer twice daily 24 IUs

intranasal OT or P and will be contacted once a week for assessment of adverse effects and for download of the smartwatch/ring data. As in our pilot study, during the last week of each intervention period, up to four assessment sessions will follow that will be identical to the baseline sessions. On the mornings of assessment days, participants will not apply the OT/P nasal spray, to avoid confounding of acute and chronic effects.

To increase flexibility and reduce participant burden, portions of the study (including the ICF) may be conducted remotely via phone calls, UF Zoom and/or online (RedCap). Parts of the study that cannot be conducted remotely, will be conducted in a single visit pre- and post- the intervention period and as needed study portions may be dropped. These portions include but are not limited to: blood draw and urine sample, Neuroimaging, sensory testing, and questionnaires. Questionnaires and /or surveys with sensitive questions, such as childhood trauma or suicidal ideations, will continue to be administered in person.

Participants: Based on power-analytic considerations, we plan to enroll a total of 160 individuals of both sexes (approx. 50% male) from various ethnicities. This sample size includes a 20% attrition rate. Older adults over 45 years of age who meet American College of Rheumatology clinical criteria for knee OA[78] and/ or back pain of at least six months duration, experience pain on more days than not, with moderate pain at baseline (i.e., > 3/6 in the VDS), and who have elevated levels of plasma IL-6 (>2.5 pg/ml) will be considered for participation. We will also recruit 160 control individuals (80 older adults over 45 years of age without knee osteoarthritis pain and 80 younger controls 18 to 30 years of age). Both community-based and clinic-based recruitment methods established in our labs will be utilized. We will also utilize IRB approved registries to contact potential participants.

Exclusion criteria will align with study and safety requirements related to: **1) OT application** [hypersensitivity to OT or vasopressin, history of hyponatremia, syndrome of inappropriate antidiuretic hormone secretion, or psychogenic polydipsia, on vasoconstrictors such as desmopressin, pseudoephedrine, or antidiuretic medication, or anti-inflammatory drugs, or muscle relaxants, low sodium and high osmolality levels, blurred vision caused by a medical condition, excessive smoking, excessive drinking, muscle pain as a result of systemic disease, significant nasal pathology, previous or concurrent use of narcotics delivered intranasally (e.g., cocaine), or gastroparesis. If a participant has both low sodium (<134mEq/L) and high osmolality (>1200L), we will conduct a second blood test. If the sodium is again low (<134mEq/L) in the repeat blood draw, the participant will be excluded. Given that myocardial OT receptors,[79] when bound to, inhibit cardiovascular activity,[80] individuals with heart problems (e.g., cardiomyopathy, history of myocardial infarction, arrhythmias, prolonged QT interval) , and any form of cardiac surgery will be excluded]. Results of blood or/and urine testing may indicate that the participant should not participate in the study (e.g., certain test results could be out of normal range). If this is the case, we will give the participant a copy of your lab results, so that the participant may share them with the participant's own medical provider and seek care if appropriate.

2) Pain testing [participants will be excluded if they have concurrent medical or arthritic conditions that could confound symptomatic knee OA-related outcomes or coexisting disease that could preclude successful completion of the protocol including: systemic rheumatic condition (e.g. rheumatoid arthritis, systemic lupus erythematosus, fibromyalgia); a history of clinically significant surgery to the index knee; uncontrolled hypertension (>150/95); poorly controlled diabetes (HbA1c>7%); cardiovascular or peripheral arterial disease; serious psychiatric disorder requiring hospitalization within the past twelve months or characterized by active suicidal ideation; diminished cognitive function that would interfere with completion of study procedures (i.e., unable to understand instructions)];[81] and **3) MRI/MRS** [large pieces of metal in the body or metal in the face or neck, claustrophobia, major medical surgery in the past two months, history of brain surgery or any serious brain condition like aneurysm, stroke, or

seizures]; and **4) pregnant individuals will be excluded; and 5) enrolled in another interventional research study**.

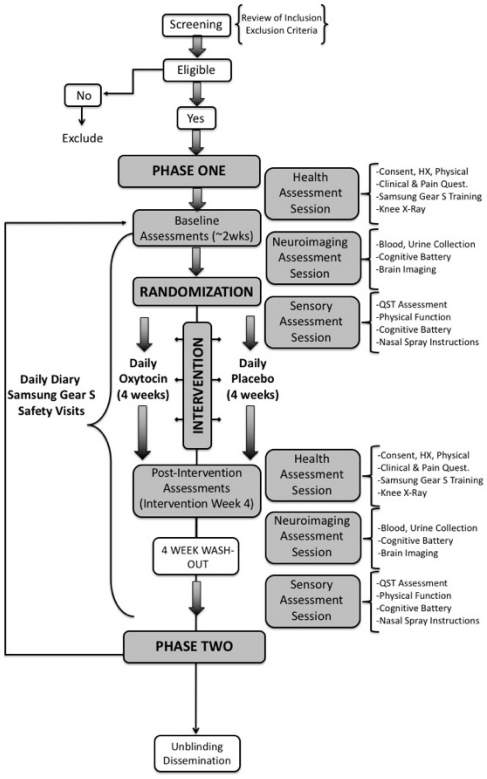
Initial screening: After phone consent, all potential participants will undergo an initial screening interview, via telephone or in-person depending on the recruitment setting (**Figure 7**). This screening procedure will include a knee OA screening interview that has shown 87% specificity and 92% sensitivity for detecting knee OA.[82] This interview includes four questions regarding knee pain and swelling and previous diagnosis of knee OA. The initial screening will also assess MRI eligibility, age, gender, handedness, and additional health history information, and demographic information such as education level, household size and race/ethnicity for sample descriptive purposes. The Telephone Interview for Cognitive Status (**TICS**) will be given to participants to assess cognitive status. Eligible individuals will be scheduled for the Health Assessment Session. All participants will undergo the same procedures as described below, but control subjects will have the option to participate in acute (i.e., same day) oxytocin/placebo administration. If a control participant does not wish to undergo the spray administration, they would complete all other aspects of the visits. If control participants wish to undergo the spray administration, this will occur the same day as the neuroimaging visit (i.e., acute administration).

Phase One Procedures for Knee OA Participants:

Health Assessment Visits (Pre/Post)

Saliva, Urine and blood samples. After written informed consent, participants will provide a urine sample to determine osmolality and a fasting blood sample to evaluate standard clinical parameters (Comprehensive Metabolic Panel (CMP-14) Blood Test), which will be reviewed by Co-I Staud to determine study continuation. Following established pilot study procedures, blood samples will also be used for determination of plasma neuropeptide (e.g., OT, arginine vasopressin (AVP)) concentrations and inflammatory markers (e.g., IL-6, TNF-α, C-reactive protein (CRP)); as well as genetic markers associated with OT. This session will take place in the mornings to accommodate for fasting and to control for circadian fluctuations. For women under the age of 62, pregnancy tests will be conducted at study start. Samples may be collected during any session that is most convenient for the participants as long as this is collected before randomization. Saliva samples will be collected for additional DNA testing on genetic markers associated with OT. Saliva collection has been done previously for the study of OT and other hormones in the body.

Figure 7. Study Procedures



Physical examination. Height and weight will be measured and Body Mass Index (BMI) will be calculated as it is significantly related to pain symptoms in OA.[83] Participants will complete a thorough Pain and Medical History Questionnaire, including a review of bodily systems assessing the reported duration of OA, current and past treatments for OA, comorbid conditions, baseline adverse events reporting, and current medication use as in our previous knee OA studies.[5,57] Information regarding hormone replacement therapy will be obtained for both sexes, and for women, information regarding menopausal status, as this may interact with baseline OT levels and OT intervention effects.[84] If there are concerns with eligibility, participants will undergo a Physical

Examination by the study rheumatologist (Co-I Staud) to: 1) confirm the diagnosis of knee OA according to ACR clinical and radiographic criteria;[78] 2) rule out any exclusion criteria; and 3) identify the most symptomatic knee. Manual examination of joint tenderness may be performed bilaterally for hands, hips, and knees. Symptomatic hip OA is common in the knee OA population[85-87] and is associated with disability[87] and thus will be included as a covariate.

Clinical pain will be assessed according to location, overall pain severity, and pain interference with daily activities. These measurements will encompass the key dimensions for pain assessment as recommended by the American Geriatrics Society[88] and according to the pain taxonomy by the Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks and the American Pain Society group (**AAPT**).[89,90] A Verbal Descriptor Scale (**VDS**) will assess changes in pain intensity before and after treatment. The VDS has anchors of "*No pain*" (scored as 0) and "*The most intense pain imaginable*" (scored as 6) and is easier to understand and preferred over numerical rating scales by older individuals,[67] and has been effectively utilized in our pilot study. We will also administer the Western Ontario and McMaster Universities Osteoarthritis Index (**WOMAC**),[91] a reliable, well-validated measure of lower extremity pain and function in individuals with knee OA. Summary scores of the WOMAC range from 0 (*No pain or disability*) to 96 (*Extreme pain or disability*).

Knee x-ray. Weight-bearing radiographs will be taken for all participants. Co-I Staud will determine the Kellgren-Lawrence (**KL**) score and indicate which compartment(s) are affected in each knee (medial, lateral, patellofemoral) to determine study continuation. KL scores will be assessed in a blinded manner. This is only done at the baseline visit.

Real-time assessment of pain and mobility. At some point during the baseline sessions, participants may be trained by study staff in the use of the Samsung Gear S® or Apple smartwatch or Oura Ring. Participants will also be given a guidebook explaining how to use the smartwatch/ring. Traditional self-report measures of pain cannot capture real-time data under naturalistic conditions or measure fluctuations on a daily or hourly basis. The UF Institute of Aging Data Science and Applied Technology (**DSAT**) Core has recently validated the use of electronic data capture by the Samsung Gear S® or Apple smartwatch to measure pain, mood, mobility, and other outcomes in older adults.[92,93] The DSAT Core infrastructure includes a secure smart watch app which summarizes sensor-monitored data (e.g., tri-axial accelerometer, GPS location) and a graphical interface allowing ecological momentary pain, mood and social activity assessments through a turn dial bezel (**Figure 8**). Data collection on smart watches offers several advantages including capability of long-term continuous data collection, ecological validity, cost effectiveness, and convenience and low participant burden. "Momentary" pain intensity will be captured randomly four times per day throughout the study. Data will be downloaded remotely or when the individual reports to the clinic during the intervention phase. Participants will start wearing the device during the baseline weeks and at each visit their proficiency and use will be ascertained. Data extraction and analysis will be performed by the DSAT Core.

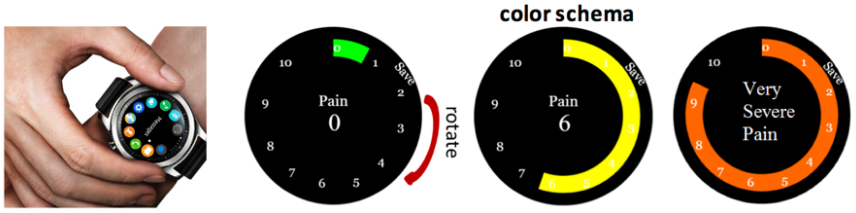


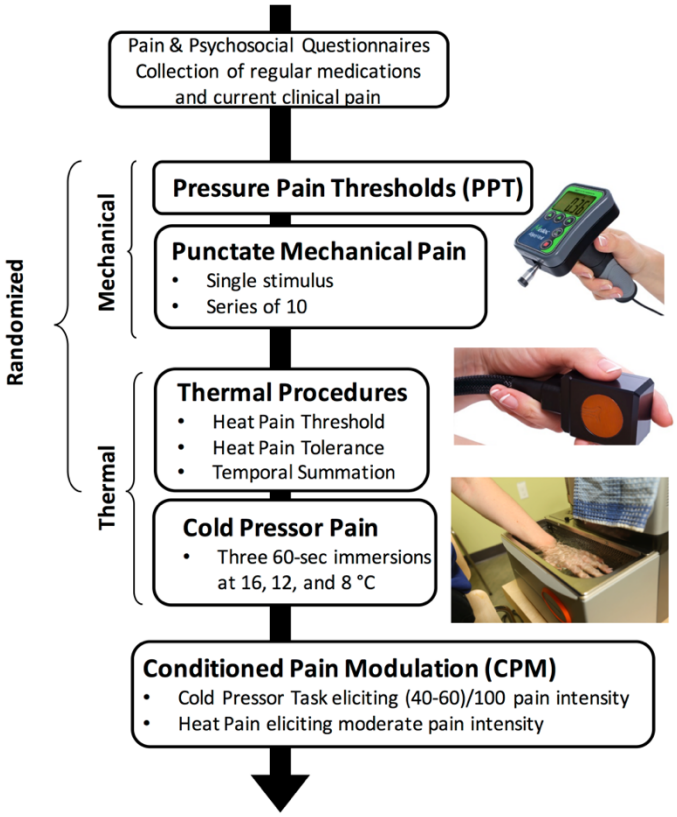
Figure 8. (**a**) The Samsung Gear S smartwatch is used by rotating the bezel to select pain ratings; (**b & c**) the color schema changes from green to reddish-orange as ratings are elevated toward 10; (**d**) ratings are then saved by pressing a physical, top-right hand button located on top of the bezel.

<u>Sensory Assessment Visits (Pre/Post)</u>

Pain and psychosocial questionnaires. We will also administer the West Haven Yale Multidimensional Pain Inventory providing well-validated measures of several important dimensions of the chronic pain experience. Multiple psychosocial factors have been related to chronic pain. We will assess psychosocial factors across the following broad domains. **1) Pain coping:** The Coping Strategies Questionnaire-Revised (**CSQ-R**)[94] consists of 27 items relating to how individuals cope with pain. It comprises six subscales based on pain coping strategies that individuals report (i.e., diverting attention, catastrophizing, praying and hoping, ignoring pain sensations, reinterpreting pain sensations, and coping self-statements). **2) Affective distress:** The Beck Depression Inventory, 2nd Edition[95] is a widely used depression scale that assesses affective (e.g., sadness, loss of interest), cognitive (e.g., worthlessness, guilty feelings), and somatic (e.g., changes in sleep, tiredness or fatigue) symptoms common amongst depressed individuals. It contains 21 self-report items assessing the frequency and severity of depressive symptoms over the previous two weeks.

Since item # 9 from the Beck Depression Inventory refers to suicidality, the experimenter will, before the end of the test session, review the participant's response to this item, and if the participant has chosen response option 2 = "I would like to kill myself "or 3 = "I would kill myself if I had the chance", the experimenter will contact the PIs immediately and the PIs or their designee will contact Dr. Dawn Bowers (or her designee) at the University of Florida Psychology Clinic (352-265-0294) who will make recommendations. In the unexpected event that the clinic personnel are not available, the Alachua County Crisis Center will be contacted. The same procedure will be implemented for the post-intervention assessment. The Positive and Negative Affect Scale (**PANAS**) is a 20-item scale that assesses positive and negative affect.[96] The PANAS has demonstrated adequate reliability and validity.[97] For this study, participants will be requested to provide "state" information by responding to items "at the present moment." **3) Satisfaction/quality of life:** We will assess self-reported quality of life using the 36-Item SF Survey Quality of Life[98] consistent with our pilot study, while the Satisfaction with Life Scale (**SWLS**)[99] will assess satisfaction with people's lives as a whole. **4) Sleep and Food Recall:** Self-reported sleep quality during the past month will be measured using the Pittsburgh Sleep Quality Index (**PSQI**).[100] We will also ask participants to report what they ate the previous 24 hours. **5) Interoceptive awareness:** We will assess self-reported interoceptive awareness using the multidimensional assessment of interoceptive awareness (MAIA)[114] **6) Chronic Stress:** Using the **Daily Stress Inventory**, we will collect participants' self-reported daily assessment of the sources and individualized impact of relatively minor stressful events.[11520] **7) Perceived Stress Scale**: Participants fill out a self-report assessment of their thoughts and feelings during the last month.[116] **8) Impulsivity:** We will assess self-reported behavioral inhibition using the Behavioral Inhibition System and Behavioral Activation System (BIS/BAS) scales.[117] **9) Neuropathy**: Individuals will respond to the PainDetect questionnaire to assess neuropathic pain detection. If an individual scores greater than 12, their clinical pain for each area of testing will be assess during the

Figure 9. Sensory assessment session protocol



quantitative sensory testing session.[118] **10) Empathy**: We will assess empathy using the Affective and Cognitive Measure of Empathy (ACME) questionnaire.[119] **11) Back Pain:** If an individual indicates they experience back pain, we will assess such pain using the Chronic Low-Back Pain (Minimal Dataset)[120], Oswestry Low Back Pain Disability Questionnaire[121], and Brief Pain Inventory[122] questionnaires. **12) Early Trauma:** Individuals will respond to the Childhood trauma questionnaire-short form (CTQ-SF)[123], the Adverse and Traumatic Experiences Scale[124] and the Brief Resilient Coping Scale.[125] **13) Aggression**: Individuals will respond to the State-trait Anger Expression Inventory-2 (STAXI-2)[126], Cohen-Mansgield Agitation Inventory (CMAI) aggressive behavior subscale (CMAI-ABS)[127] and the Ryden Aggression Scale (RAS) physically aggressive behavior subscale (RAS-PABS)[127].

Quantitative Sensory Testing (QST). Participants will undergo QST to determine responses to mechanical and thermal stimuli and conditioned pain modulation (CPM) (**Figure 9**; see also[5,12,101]). We will randomize the order of thermal and mechanical testing. Cold pressor assessment, including conditioned pain modulation, will always occur last to avoid carryover effects. Patients' medications and current clinical pain will also be confirmed.

Mechanical testing procedures. Pressure pain threshold (**PPT**) will be assessed at the medial and lateral joint lines of the index knee, and at the ipsilateral quadriceps and trapezius muscles. For all PPT measurements, after an initial practice trial, three trials will be conducted and their average will be computed for data analysis. Using a digital, handheld, clinical grade pressure algometer (Algomed, Medoc, Ramat Yishai, Israel), the examiner will apply a constant rate (30 kPa/second) of pressure and the participant will press a button when the sensation first becomes painful, at which time the device records the pressure. Punctate Mechanical Pain will be assessed at the patella of the index knee and the dorsal aspect of the ipsilateral hand using a nylon monofilament (Touchtest Sensory Evaluator 6.65) calibrated to bend at 300g of pressure. As in our previous studies,[5,12,57] participants will provide a pain rating following a single contact, after which they will provide another pain rating following a series of ten contacts at a rate of one contact per second. The difference between the pain rating for the single versus multiple contacts reflects temporal summation of mechanical pain.

Thermal testing procedures. Thermal stimuli will be delivered to the (most) affected knee and/or to the ipsilateral forearm and/or thenar eminence, in randomized order, using a Medoc Pathway Thermal Sensory Analyzer (Ramat Yishai, Israel). Heat pain threshold will be assessed at each site, followed by heat pain tolerance and temporal summation. Heat pain thresholds and tolerances will be assessed using an ascending method of limits as in previous studies.[5,12,57] Temporal summation of thermal pain will be assessed using the same stimulus parameters as previously reported by us and others.[5,12,57] To measure Cold Pressor Pain, a modified cold-pressor procedure will be conducted. Each participant will complete a series of 60-second immersions in cold water baths at 10ºC, with water temperature maintained (±0.1ºC) by a refrigeration unit. Participants will immerse their left hand up to the wrist with their fingers spread apart. Participants will report when they first feel pain, and this time point will be recorded as the pain threshold. Then, they will continue for one minute or until they report intolerable pain. If a participant terminates early, the time at which they terminate will be recorded as pain tolerance. Participants will be prompted to rate the intensity of the cold-pressor pain at 30-sec intervals.

Conditioned Pain Modulation (CPM) will be used to assess pain-inhibitory function. The conditioning stimulus will be the cold pressor task applied to the left hand, which will be tailored for each participant to achieve a stimulus that produces moderate pain (i.e., a rating of 40-60 on the 0-100 scale) and can be tolerated for a 60-second period. The test stimulus will be heat pain applied to the opposite ventral forearm, at a stimulus intensity which produces moderate but tolerable pain. First, baseline heat pain responses will be assessed, after which the participant will immerse their hand in the cold water bath for 60 seconds. Immediately afterwards the heat pain will again be applied to the opposite arm and pain ratings will be obtained.

Short Physical Performance Battery (SPPB) and Mobility Assessment. As a performance-based indicator of OT's effect on function we will use this brief assessment consisting of four lower-extremity function measures: standing, balance, walking speed, and ability to rise from a chair. These measures have been standardized and are widely used in older populations and have been used in our own labs.[102,103] We will also measure movement-evoked pain during each task which is associated with central pain processing in knee OA.[103] Participants will also be asked to undergo a number of mobility assessments. Participants will be asked to walk over a mat without any obstacles, as well as stepping over an obstacle, and while performing a cognitive task. Some tests will be performed while walking over an instrumented walkway, which measures spatiotemporal gait parameters.

Neuroimaging Assessment Visits (Pre/Post)

Cognitive battery. Previous studies including those from PI Ebner support OT's role in cognition,[65,84], while those from PI Cruz-Almeida implicates decreased cognitive function in older adults with chronic pain.[128-130] Given our preliminary findings of changes in metabolites in the frontal cortex, assessment of executive function is particularly warranted. Cognitive performance will be assessed via the NIH Toolbox (www.nihtoolbox.org).[104] This battery comprises measures of executive function, attention, episodic memory, language, processing speed, and working memory. **Following NIH scoring guidelines,** administering this battery will yield the Cognitive Function Composite Score and the Crystallized Cognition Composite Score (e.g., Picture Vocabulary and Reading Recognition), in addition to subtest scores. We will also administer the Montreal Cognitive Assessment (MoCA) as in our previous work.[128]

Neuroimaging (up to 2 visits each time). Participants will lay supine in the MRI magnet for up to 2 hours and fill out a post event questionnaire at the conclusion of the scan. We will use a state-of-the-art 3T Siemens Prisma MR system at the McKnight Brain Institute (MBI) for collecting anatomical, structural, functional, and biochemical scans. Anatomical image acquisition will involve a T1-weighted structural scan using a magnetization-prepared rapid gradient echo (MPRAGE) sequence, and a T2 FLAIR scan, which provides good contrast between gray and white matter, and cerebrospinal fluid based on tissue T1 and T2 differences. Functional scans (fMRI) will be taken while the participant is resting quietly, completing a cognitive task and while painful stimulation is being applied using a (MRI-compatible) heat thermode routinely used in neurological examinations. We will also acquire single-voxel MEGA-PRESS water-suppressed 1H spectra with water T2 measurement. A simple visual task will also be performed to control for non-specific cognitive influences. Brain MRI data will be used to measure the structural and functional integrity of the brain including, but not limited to, descending sensory and motor tract integrity (fiber tractography), intracranial volume, whole brain volume, lacunae as well as metabolite concentrations.

fMRI data analysis. We will use the same stages for functional connectivity analysis as prior work[12,13] using Freesurfer (http://surfer.nmr.mgh.harvard.edu/) and AFNI (http://afni.nimh.nih.gov/afni). Seed regions will be placed in areas that include prefrontal, striatal and insular regions. To calculate the time series of the region of interest (ROI), a sphere with a radius of 3.5mm will be placed in the structure of interest (based on an MNI template). An inverse transform derived from the anatomical scan will be applied to transform the ROI from MNI common space into subject space. The mean signal of non-zero voxels within the ROI will be calculated. Pearson correlation maps will be created by correlating time series in a seed region to residual time series in each voxel of the brain. The maps are then converted to a z-score via a Fisher tanh-1 transform in order to perform a group analysis and z-score maps are transformed into MNI space via transforms derived from warping the anatomical scans.

dMRI data analysis. We will use FSL (http://www.fmrib.ox.ac.uk/fsl). A 3D affine registration will be applied to each diffusion weighted scan to correct for head motion and image distortions and a binary mask will be created to mask the brain and avoid the skull. Bayesian estimation will be used to calculate

a probability distribution of fiber directions for each voxel[21], allowing estimates of two directions per voxel[12]. Using the preprocessed data, probabilistic tractography will be used to identify probable fiber tracts between a seed region and a waypoint. Each voxel within the seed will have a fiber leaving it, and the probable fiber tracts will be traced between the seed voxel and waypoint. From all voxels within the seed mask, the tractography algorithm generates 5000 streamline samples, with a step length of 0.5mm and a curvature threshold of 0.2. These samples are computed through the probability estimate on fiber direction at each voxel. The conjunction of these 5000 paths will then calculated. Higher values reflect a greater probability that the voxel is part of the tract between the seed and waypoint with a distance correction. The voxel with the highest value will be identified in each tract and 1% of this value will be used as the threshold value to remove voxels. Analysis will be restricted to voxels within the white matter using individual's gray matter masks derived from each T1-weighted image. Masks will be generated with FreeSurfer's automated tools for segmentation. Integrity measures will then be extracted from each fiber tract mask. For spatial normalization we will use DTI-TK. DTI-TK is a non-parametric, diffeomorphic deformable image registration that incrementally estimates its displacement field using a tensor-based registration formulation. It is designed to take advantage of similarity measures comparing whole tensors via explicit optimization of tensor reorientation[13].

Magnetic Resonance Spectroscopy (MRS): A non-invasive, non-ionizing radiation technique that uses proton signals to determine the relative concentrations of target brain metabolites. The GABA-edited spectra will be acquired using a MEGA-PRESS sequence, TE 68ms; TR 2s; 14ms sinc-Gaussian editing pulses applied at 1.9 ppm (ON) and 7.56 (OFF); VAPOR water suppression; 10 min duration). Spectra will be analyzed using the Gannet package for the batch analysis of GABA-edited MR spectra, quantifying GABA concentration relative to water, correcting for voxel CSF fraction. OFF spectra will be analyzed using LCModel to give concentrations for additional metabolites NAA, Glx, Cho, MI, and Cr. Assurance: GABA-edited MR spectra will be visually screened, and spectra with a fitting residual of over 10% will be accepted for further analysis. The concentration measures for the additional metabolites will be accepted with an LCModel Cramer-Rao lower bounds (%SD) of less than 20%, a reliable estimate for a given metabolite for group comparisons.

In general, questionnaires and testing may be collected during any session that is most convenient for the participants as long as all the data is collected before randomization to intervention. Participants will also be allowed to complete any of the assessments during extra visits to reduce their burden and avoid participant fatigue. All sessions will begin with an intake interview regarding events that occurred during the past 24 hours.

Intervention period for Knee OA Participants

During the 4-week intervention, participants will self-administer via intranasal spray 24 IUs of OT or P twice a day at home, at 8-9AM and again at 5-6PM. Participants that take any other nasal spray, will be asked to separate sprays by 20 minutes and this will be recorded in the diary. Missed doses will not be a deviation and participants are instructed to log each day whether they took the spray or not. The utilized synthetic OT is a formulation developed in Dr. Feifel's lab at UCSD (IND #100,860; see letter of support from Dr. Feifel and IND letter). Dr. Ebner has been sub-investigator on this IND for several years.[49,105] The drug (i.e., synthetic OT) and the P (i.e., containing all of the inert ingredients except for the OT) will be compounded at UF's Investigational Drug Services (IDS). Dr. Ebner has established this compounding route together with Dr. Susan Beltz, chief pharmacist at UF IDS under Dr. Feifel's protocol and successfully and safely implemented procedures in our pilot study. Compliance will be monitored by measuring the fluid left in the spray bottle after the treatment period and using a log that participants fill in each day during the intervention. If any time during the treatment it is determined that the participant should not continue due to adverse events, the participant will be discontinued. Furthermore, at baseline and at the end of the intervention, blood and urine samples will be checked

(e.g., for osmolality levels, sodium levels, and any other blood markers out of range) to ensure that no adverse changes have occurred during the study intervention. Additionally, before and after the intervention period participant will fill out the Goal Attainment Scale (**GAS).** For the majority of the 4-week intervention periods, participants may complete daily assessments via their Samsung Gear S® or Apple smartwatch or Oura Ring. We will be able to assess pain, mood, and activities at random times throughout the day. Participants will be contacted once a week to assess side effects and asked if they have been completing their diaries. Any symptom reported as moderate or severe will be brought to the PIs' attention and will be discussed with the study MD as needed on a case by case basis. These weekly calls will also ensure regimen compliance and will assist participants in download of the Samsung Gear S® or Apple smartwatch or Oura Ring data to a secure research server. We have successfully established a similar approach in other projects within our institute, and the DSAT Core has validated pipelines for data analysis.[92,106]

Post-Intervention Assessments for Knee OA Participants

In the last week of the intervention, participants will be scheduled to return for up to four (or 6 with the MRI) post-intervention visits, which will be identical (with minor exceptions) to the baseline visits. Importantly, at the follow-up Health Assessment we will assess changes in health status, and their global impression of change (**PGIC/MPIC**) since starting the intervention.

Wash-out Period for Knee OA Participants

Participants will undergo a four-week wash-out period in between phases one and two. The wash-out period will serve to prevent confounding variables between both drug administrations. Four weeks is a sufficient period of time for participants to return to baseline levels prior to drug administration.

Phase Two Procedures for Knee OA Participants

After the four-week wash-out period, participants will enter phase two of the study. Phase Two is completely identical to Phase One: up to 4 (or 6 with the MRI) Baseline Visits, Intervention Period, and up to 4 (or 6 with the MRI) Post-Intervention Visits. Participants will be given the other drug from the first intervention period per randomization.

At study closure, participants will also be asked to guess what study medication they were taking in order to assess the effectiveness of blinding, followed by a full debriefing regarding the study aims and a study post event questionnaire. Our pilot data suggest that participants are unaware of their assigned treatment condition. We will implement several strategies to enhance retention, and based on our pilot data, we anticipate high success in completing all follow-up visits in at least 90% of our sample. Finally, one week, three months and at 6 months after the last treatment phase, participants will receive a follow-up phone call to determine if any side effects occurred, as well as answer the WOMAC and PANAS to inquire about pain. All study participants will be financially compensated for study participation. During these calls, if symptoms that indicate imminent danger to the participant are reported such as light headedness, fainting, heart changes, palpitations, shortness of breath, and abdominal pain, staff will immediately advise participants to contact emergency medical services. Simultaneously, this matter will be immediately escalated to study physician and PIs and the physician or his proxy will be in immediate contact with the participant to determine appropriate steps for follow-up.

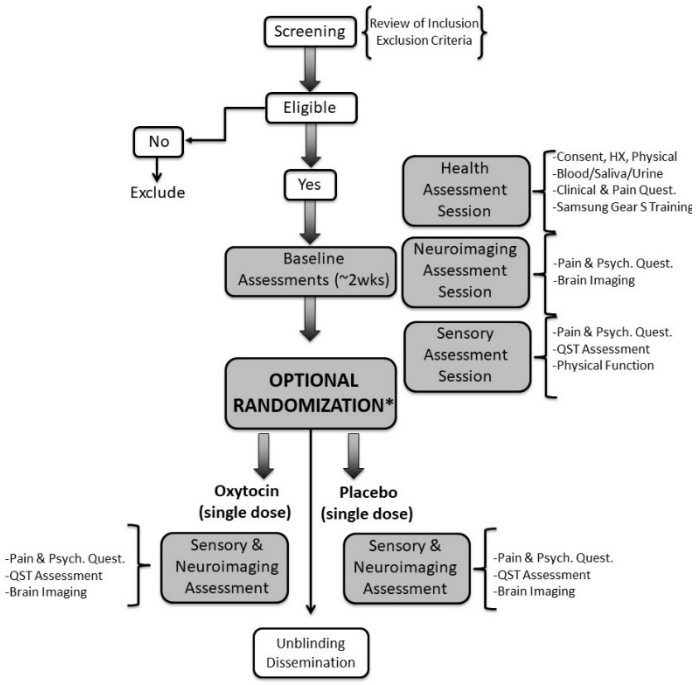
Compensation

We will compensate participants in the form of a pre-paid, reloadable VISA card issued by the University of Florida or by cash payments. Payments are broken down by visits ($75 per visit), the four week interventions ($75 for each intervention) and additional compensation for returning all study

materials ($75). The MRI portion of the study is optional, which would decrease the number of visits needed to attend for the entire study.

Procedures for Control Participants:

Figure 10. Study Methodology for Controls



*Note: Participants will only undergo two neuroimaging sessions if they go through the acute OT/Placebo administration. If they decline OT/Placebo administration, they will undergo one neuroimaging session only.

Health Assessment Visits

Urine and blood samples. After written informed consent, if participants choose to take the OT/Placebo administration, control participants will provide a urine sample to determine osmolality and a fasting blood sample to evaluate standard clinical parameters (Comprehensive Metabolic Panel (CMP-14) Blood Test), which will be reviewed by Co-I Staud to determine study continuation. All control participants, regardless whether they will receive acute OT/Placebo will provide blood samples for determination of plasma neuropeptide (e.g., OT, arginine vasopressin (AVP)) concentrations and inflammatory markers (e.g., IL-6, TNF-α, C-reactive protein (CRP)); as well as epigenetic markers associated with OT. This session will take place in the mornings to accommodate for fasting and to control for circadian fluctuations. For women under the age of 62, pregnancy tests will be conducted at study start.

Physical examination. Height and weight will be measured and Body Mass Index (BMI) will be calculated. Participants will complete a thorough Pain and Medical History Questionnaire, including current medication use. Information regarding hormone replacement therapy will be obtained for both sexes, and for women, information regarding menopausal status, as this may interact with OT levels and effects.[84]

Cognitive battery. Participants will undergo the same cognitive testing procedures as the knee OA participants.

Real-time assessment of pain and mobility. At some point during the baseline sessions, participants may be trained by study staff in the use of the Samsung Gear S® or Apple smartwatch or Oura Ring. Participants will also be given a guidebook explaining how to use the smartwatch/ring. Participants will wear the device during the baseline weeks.

Sensory Assessment Visits

Pain and psychosocial questionnaires. We will also administer the same questionnaires as in the knee OA participants to ascertain daily pain and emotional functioning. If control participants agree and consent to the OT/Placebo acute administration, they will also undergo these measures after the OT/Placebo administration.

Quantitative Sensory Testing (QST). Participants will undergo the same QST procedures as the knee OA participants (**Figure 9**; see also[5,12,101]). If control participants agree and consent to the OT/Placebo acute administration, they will also undergo these measures after the OT/Placebo administration.

Short Physical Performance Battery (SPPB) and Mobility Assessment. Participants will undergo the same performance-based measures of function as the participants with knee OA. If control participants agree and consent to the OT/Placebo acute administration, they will also undergo these measures after the OT/Placebo administration.

Neuroimaging. Participants will undergo the same procedures as the knee OA participants. Control participants that agree to take part in the OT/Placebo administration will undergo 2 MRIs, before and after OT/Placebo administration.

OT/Placebo Acute Administration for Control Participants

Control participants will self-administer one acute administration of OT or P, at 8-9AM if they have previously consented to this part of the study. At study closure, participants will also be asked to guess what study medication they took in order to assess the effectiveness of blinding, followed by a full debriefing regarding the study aims and a study post event questionnaire. Control participants will also be financially compensated for study participation.

Sample assays: The **OT neuropeptide assay** will be conducted in Dr. Carter's lab, using standard enzyme-linked immunosorbent assay (EIA) and LC Mass Spectrometry methods; in collaboration with the biochemical research company Martin Protean LLC. Although EIAs are typically highly sensitive, with minimum detection levels of 12pg/mL OT and very little antibody cross-reactivity with other neuropeptides, all samples will be run in duplicate. Dr. Carter and PI Ebner have been collaborating on sample assays over the past years.[65,107] The **immune assay** will include inflammatory biomarkers (e.g., CRP, TNFa, IL-6) via immunoassay kits as in our previous studies.[60,61,108] All samples will be measured in triplicate and the average of the three measures will be used for data analysis. Samples will be analyzed by the Institute on Aging Metabolism and Translational Science Core. The samples collected in the present study will only be used to answer questions about this study and will not be banked for future studies.

Compensation for Control Participants

We will compensate control participants with up to $330 upon study completion in the form of a pre-paid, reloadable VISA card issued by the University of Florida or by cash payments. In particular, for completion of

- Screening Visit, Baseline Visit: $75
- Sensory Visit: $75
- Neuroimaging Visits (up to 2 visits): $75 per visit
- (Optional) extra $30 if you choose to participate in the nasal spray treatment.

Power and sample size considerations: Based on our pilot study estimates, we plan to recruit 160 participants, i.e., 80 participants in each of the randomly assigned treatment groups for the baseline visits. Assuming an attrition rate of 20% at the follow-up visits, we will have 128 participants (64 in the OT and 64 in the P group) at the 4-week post-intervention follow-up visits. This sample size yields a power over 0.80, even based on a very conservative Bonferroni correction for multiple comparisons (i.e., testing twelve hypotheses), which lowers the alpha level from α=0.05 to α=0.006. However, our proposed data-analytic strategy will use the Benjamini and Hochberg procedure to control for multiple comparisons, which is less stringent than the Bonferroni correction, and thus we expect the actual power to be larger than 0.80.

Hypotheses and data-analytic strategy: We summarize all hypotheses and planned analyses in **Table 3 for aims 1 and 2**. The statistical analyses will be conducted by Co-I Huo (Biostatistician) in

collaboration with Dr. Wu (Consultant, Institute on Aging Biostatistics and Bioinformatics Core Leader, see letter of support) using R software. For studying the relationship between pre- to post-intervention (longitudinal) outcomes and predictors, we will fit linear mixed effects models (**LMEMs**) [1] with subject specific random effects to account for the within-subject correlation, by R function "lmer".[109] Baseline outcomes and covariates will be adjusted to take account for confounding effects. Below is the formula for the linear mixed effect model:

$$y_{ik} = \mu + \alpha_i + \beta\mathbb{I}(z_i = 1) + \sum_{l=0}^{L} \gamma_l x_{il} + \eta\mathbb{I}(k = 2) + \theta\mathbb{I}(z_i = 1, k = 2) + \varepsilon_{ik}, \alpha_i \sim N(0, \sigma_0^2), \varepsilon_{ik} \sim N(0, \sigma^2) \quad [1]$$

where y_{ik} is the outcome variable (e.g., pain intensity/sensitivity/function for Aim 1) for subject i and visit k ($k = 1$ for intervention and $k = 2$ for post-intervention); μ is the intercept term; $\alpha_i \sim N(0, \sigma_0^2)$ is the random effect with variance term σ_0^2 for subject i; z_i is the treatment indicator with $z_i = 0$ for P and $z_i = 1$ for OT, β is the treatment effect (OT effect compared to P effect) and $\mathbb{I}(.)$ is the indicator function which equals 1 if the argument inside $(.)$ is true and equals 0 otherwise; x_{il} is the l^{th} covariates for subject i with $l = 0$ for baseline level (e.g. baseline pain intensity before treatment for Aim 1) and $l = 1,2, \dots, L$ for L covariates (i.e., age, sex, race…); η is the time effect of post-intervention visit compared to pre-intervention visit; θ is the interaction effect between post-intervention and OT; $\varepsilon_{ik} \sim N(0, \sigma^2)$ is the error term with variance σ^2. From equation [1], we will test whether there is a significant OT effect for both intervention and post-intervention. LMEMs allow for missing data if missing-at-random (MAR).

Table 3. Proposed hypotheses and planned analyses.

Specific Aims	Hypotheses	Planned Analyses
#1: Clinical and experimental pain	***H1a*:** OT vs. P administration will reduce self-reported pain intensity	**LMEMs***; **DV:** Clinical pain **IV:** Treatment (OT/P)
	***H1b*:** OT vs. P administration will decrease experimental pain sensitivity	**LMEMs***; **DV:** QST **IV:** Treatment (OT/P)
	***H1c-1e*:** OT vs. P administration will increase physical, cognitive and emotional function	**LMEMs***; **DV:** SPPB, Exe. Function, Catastrophizing **IV:** Treatment (OT/P)
#2: Inflammatory mechanisms	***H2a*:** OT vs. P will decrease systemic inflammation	**LMEMs***; **DV:** Plasma cytokines (IL6, TNFα, IL10) **IV:** Treatment (OT/P)
	***H2b*:** OT vs. P will decrease brain metabolite concentrations associated with neuroinflammation	**LMEMs***; **DV:** Brain metabolites (tCr, MI, Cho) **IV:** Treatment (OT/P)
	***H2c*:** Decrease in brain metabolites and systemic cytokines will be associated with decrease in clinical pain	**Correlations/ Regressions** **IV:** Decrease in brain metabolites and systemic cytokines **DV:** Decrease in clinical pain

Note. **IV** = Independent variable; **DV** = Dependent variable; **LMEMs**: linear mixed effects models; *****Adjusted for baseline outcomes and covariates (age, sex, race, BMI, KL score, hip OA, plasma biomarkers).

For **Aim 1**, the primary outcome will be self-reported pain, and plasma levels of OT as a modifier of a significant treatment (OT vs. P) response, using an intent-to-treat approach. Secondary outcomes include a standardized, multimodal experimental pain battery and physical, cognitive, and emotional functioning measures. For hypotheses **H1a** to **H1e**, we will use equation [1] to evaluate and test the effect of OT at 4-weeks post-intervention compared to P. Baseline outcomes and covariates (age, sex, race, hip OA, and inflammatory biomarkers) will be properly adjusted. A 50% reduction in self-reported pain intensity will demonstrate a clinically significant effect of OT compared to P. For **Aim 2**, the primary outcomes are proton MRS metabolites in frontal brain regions (tCr, MI, Cho) and plasma immune markers (IL-6, TNF-α, IL-10). For hypotheses **H2a** and **H2b**, we will use equation (1) to evaluate changes from baseline to post-intervention. Covariates (age, sex, race, hip OA) will be properly adjusted. For hypothesis **H2c**, we will use correlational tests and regression methods to evaluate the association between brain metabolites, systemic cytokines, and clinical pain to consider the inter-individual variability in treatment response. Consistent with IMMPACT recommendations we will perform a secondary analysis considering total number and amount of rescue medication doses in a matrix alongside pain relief, with higher scores assigned to those patients with high levels of relief and minimum rescue, and lower scores assigned to patients who have rescued the most and have lower pain relief scores.[77] For exploratory aim 3, similar strategies will be employed.

7. Possible Discomforts and Risks

Equipment used in this research meet all current safety standards, and all research staff will be highly trained in the use of all equipment. This study may include risks that are unknown at this time. Potential risks for this study are related to use of study product, neuroimaging, blood draw procedure, blood pressure measurement, the physical performance tests, the cognitive function tests, the sensory testing, and potential loss of confidentiality related to study participation, as discussed in detail below.

All assessment will be conducted at the Clinical Translational Research Building, the Pain Clinical Research Unit (Dental Tower), the McKnight Brain Institute, and the Department of Psychology and all sessions will be conducted by trained and certified research staff, which will monitor potential adverse experiences and symptoms. We will do safety labs within 48 hours if participant withdraws due to potential adverse effects to study drug (feeling weak/tired/low energy or changes in mental status). During the testing sessions, a fully equipped defibrillator is available along with a semi-automated ECG cart. All personnel associated with the study have received CPR training and training on management of acute events including syncope, chest pain, acute dyspnea, focal neurological symptoms and abnormal vital signs. Portable defibrillators are available at the assessment site. Additionally, a study physician is available on call and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If participants develop chest pain, shortness of breath, or dizziness at any point during a screening or assessment visit, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur.

Additionally, the study physician is available on call and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If participants develop chest pain, shortness of breath, or dizziness at any point during a screening or assessment visit, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur.

Participants will be instructed to talk with the investigators about any discomforts that occur during the study. If for any reason the participant reports an injury, chest pain, leg swelling or pain, excessive shortness of breath, abnormal heart rhythm or dizziness, they will be referred to their doctor, or the PIs

will call the doctor or other health care provider.

Prior to beginning data collection, all study coordinators undergo standardized training on all study procedures. A detailed checklist for each data collection element is in place to ensure all study coordinators are appropriately trained and able to conduct study procedures. Research coordinators will be certified in each element of the study visit including obtaining informed consent, administering questionnaires, protecting confidentiality of collected data, performing the test batteries. Any study coordinator who is found to not adhere to all aspects of the protocol will be required to complete additional training followed by re-certification.

All research staff complete human subjects training required by The University of Florida institutional review board (IRB) and National Institutes of Health (NIH). This training includes education about the importance of maintaining confidentiality of personal health information. The study principal investigators or a co-investigator will be available to answer questions that arise during the informed consent process as needed. More information about specific protective measures against risk is outlined below.

OT Nasal Spray Application

We have been applying the identical drug compound in our current Oxytocin Aging Trial (IRB 201300801) over the last 3 years; we have currently completed over 100 older participants from a comparable age range and at the same dose and frequency and have no evidence of consistent or adverse reported side effects**.**

Physiological Effects of OT. The physiological effects of OT include stimulating uterine contraction during the second and third stages of labor, facilitating the milk-ejection reflex in mammary glands of lactating (breastfeeding) women, contributing to aspects of the sexual response cycle (erection, ejaculation, and orgasm), and attenuating cytokine-mediated inflammation to promote wound healing (Uvnas-Moberg, 2003). OT, at high doses, can bind vasopressin receptors in the kidney reducing the excretion of urine and can stimulate sodium excretion from the kidneys (Gimpl, & Fahrenholz, 2001). OT can also indirectly inhibit release of adrenocorticotropic hormone and cortisol in response to stress (Bao et al., 2008).

Safety of OT in Humans. The participant age group targeted in the present study is comparable with those tested in other studies, including a comprehensive meta-analysis that focused on potential risks and side effects associated with synthetic OT (given in the form of Syntocinon (Novartis), Pitocin, or our compound with the IND # 100,860; see MacDonald et al., 2011). This meta-analysis supports evidence that the use of synthetic OT nasal spray administered in the tested doses produces no consistent side effects or adverse events. In particular, MacDonald and colleagues collected data from n = 1,529 people treated with intranasal OT since 1990. In this review, participants did not report notable subjective side effects. Even though 18% of patients reported mild side effects, there was essentially a 1:1 ratio of frequency among placebo and OT recipients. Additionally, the majority of participants (93%) were not able to discern drug effects from placebo. The reported side effects include increased calmness/euphoria, lightheadedness, drowsiness, headache, nasal irritation, and dry mouth/throat. In one of the few long-term studies, patients with constipation received intranasal OT (40 IU twice a day) for 13 weeks. Participants in this trial were queried about side effects weekly throughout the trial. Three female participants (2 OT, 1 placebo) reported severe vertigo on the first day of treatment (Ohlsson et al., 2002). Five participants in this trial reported menstrual disturbance, though four of them were on placebo. Additionally, in a recent clinical trial in older adults (mean age 80 years) by Barraza and colleagues (2013) OT (Pitocin) was administered over 10 days once a day at a dose of 40 IUs and no

significant adverse events were reported throughout the entirety of the study, indicating that OT can be safely used within this age group.

There have been three reported cases of significant adverse reactions related to intranasal OT, all linked to OT's potential to exert antidiuretic effects in high doses (by binding vasopressin receptors in the kidney; Joo et al., 2004). The first two of these reports involved the excessive use of intranasal OT and excessive IV hydration (Mayer-Hubner, 1996; Seifer et al., 1985), and the third was the result of long-term intranasal OT use (2.8-5.6 IU thrice-daily for 4 weeks; Ansseau et al., 1987). All three cases noted a decrease in the patients' plasma sodium and osmolality levels. OT has a very small -- 0.25 % -- affinity for the renal AVP system (Liggins, 1963). Therefore, it can theoretically cause a syndrome similar to inappropriate ADH/AVP secretion -- SIADH, although carefully controlled human studies without water intoxication have not substantiated a natriuretic or antidiuretic effect in humans (Rasmussen et al., 2004). In none of Dr. Feifel's studies were changes in sodium levels or osmolality noted, including those administering up to 168 IU of intranasal OT for 6 weeks. Additionally, cardiovascular effects have not been noted in intranasal human trials with non-pregnant patients, including longer-term trials of higher dose (den Boer & Westenberg, 1992; Epperson et al., 1996).

The dose given in our research will be lower than in most other studies. For example, Epperson and colleagues (1996) gave five participants with OCD 160 IU of intransal OT daily for a week and another two patients 320 IU/day for a week and found no adverse effects with either dose including measures of blood pressure and osmolality. Dr. Feifel's team has substantial experience with participants in a number of different studies receiving intranasal OT in various doses up to 168 IU (84 IU twice a day). They have administered 80 IU / day (40 IU twice a day) to patients with GAD for 3 weeks and found it was very well tolerated. In another small study looking at the effects of OT in individuals with schizophrenia, ten participants received 84 IU twice a day for 3 weeks. In another study eight schizophrenia participants received 6-weeks of 84 IU twice a day intranasal OT, while approximately 24 participants so far have received 48 IU twice a day for 12 weeks. In none of these participants was there any evidence of either subjective side effects or objective evidence of physiological adverse effects (including blood work and measurement of urine osmolality).

In the event of severe side effects while taking either OT or the P, the blind for that participant will be broken. In this case, we will have the participant contact his or her primary care physician.

Excluded Medical Conditions. As a precaution, we will exclude participants who take vasoconstrictors such as vasopressin (e.g., desmopressin) or pseudoephedrine at time of study enrollment. Also, since OT can potentially produce antidiuretic effects (via activating vasopressin receptors on the kidney) we will exclude patients taking antidiuretic medication. As hypersensitivity to OT is a contraindication (other than certain cases of abnormal labor), we will exclude participants with a hypersensitivity to OT or vasopressin, given OT's structural similarity to vasopressin.

Drug-Interactions. Severe hypertension has been reported when OT is administered via IV during labor for 3-4 hours following prophylactic administration of a vasoconstrictor in conjunction with caudal block anesthesia. Cyclopropane anesthesia may modify OT's cardiovascular effects, producing unexpected results such as hypotension. There have not been any reports of drug-interactions with chronic intranasal OT administration in the many published studies in which patients took concomitant medications (Feifel et al., 2010; MacDonald et al., 2011; Pedersen et al., 2011).

Laboratory tests. Participants will have blood drawn at screening and during the post-intervention visit to measure their blood chemistry and will provide urine samples for osmolality tests. Abnormal results will be brought to the principal investigator's attention immediately. The results will be reviewed and

signed by the study clinician and Drs. Cruz-Almeida or Ebner. If a participant has both low sodium (<134mEq/L) and high osmolality (>1200L), we will conduct a second blood test. If the sodium is again low (<134mEq/L) in the repeat blood draw, the participant will be excluded.

Assessment of Adverse Events. A standard operating procedure will be established which is followed to collect and deal with adverse events during the research project. In particular, adverse events will be systematically monitored both during the treatment phases, as well as 3 and 6 months following study completion. In particular, participants will respond to a side effect check list to detect any possible physiological reactions related to the drug administration. This will include series of open-ended questions asked by trained research staff regarding any adverse events that have occurred since the last visit or phone contact. Aside from open questions about pain experienced in various parts of the body, dizziness or light headedness, research staff will inquire about changes in thirst or urination, as OT can alter water concentration in the kidneys in rare cases. Additionally, during the 3 and 6 months follow up, participants will be asked to the WOMAC and PANAS questionnaires to assess pain and feelings. This report will be reviewed by the principal investigator and study physician in a timely fashion. Any serious untoward event, whether related or unrelated to the study drug or procedures, will be reported to the UF IRB and FDA immediately as per their regulations.

Adverse events will be defined as any physical symptoms or side effects that began following administration of the study drug. All reported and observed adverse events will be tracked in a running adverse events participant log, which will contain information regarding dates, description, and severity. All adverse events will be coded using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA). The number and percentage of participants reporting treatment-emergent adverse events will be tabulated by treatment, system organ class and MedDRA-preferred term. Treatment-emergent adverse events will be those adverse events that begin or worsened following the first dose of study drug. Adverse events will be further classified by severity and investigator-assigned relationship to study drug. All reported and observed adverse events will be tracked on a running adverse events log (specific to each subject and maintained in his/her study file) and will contain information regarding onset/offset dates, time course (i.e., single, sporadic), severity, action taken regarding study drug, relationship to study drug, and whether it meets FDA criteria for “Serious.” Each reported event is reviewed by the principal investigator and the study physician in a timely fashion. Additionally, the principal investigator reviews all adverse events recorded in each participant’s log at the conclusion of study participation.

In Case of Emergency. Participants will have 24-hour access to emergency care and will be provided with a means of contacting the on-call study doctor and research staff. Participants will be informed and encouraged to contact the study physician and study staff immediately with medical concerns or unusual reactions. Participants will also be informed of the closest Emergency Department location to their residence.

Data and Safety Monitoring Plan. A Data Safety Monitoring Plan will be in place. In particular, the following procedures will be implemented to ensure data and participant safety. Study progress and safety will be reviewed by the principal investigators in collaboration with Dr. Roland Staud, M.D., the study MD, and Dr. Feifel, M.D., Ph.D., the sponsor of the IND and consultant on the project.

We will stop the intervention if 30% or more of the participants in the OT condition report 10 or more (out of forty-four) of the side effects at moderate to severe levels over more than 2 weeks as acquired during the weekly check in phone calls during the intervention phase. Data collected prior to stop of

intervention will be used for analysis. All participants who went through the intervention phase of the study will be followed up by phone calls at multiple time points to ensure safety; every 7 days during the intervention, 7 days post intervention, 3 months post intervention, and 6 months post intervention. Progress reports, including participant recruitment, retention/attrition, and adverse events will be provided to an Independent Safety Monitor(s) for reviews on a bi-annual basis. The Independent Safety Monitor(s) for this study will consist of an established senior investigator not associated with our study. A study end report will be compiled and will include a list and summary of adverse events. In addition, this report will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The annual report will be signed by the Independent Safety Monitor and will be forwarded to the IRB, NIH, and FDA. The Independent Data Safety Monitoring Plan will also require that all significant serious adverse events that may be possibly related to the study participation will be reported to the IRB, FDA, and NIH within 48 hours of the principal investigators learning of the event. In addition, any unanticipated serious adverse events that may increase the risk of the research for participants or potential participants will be reported to the IRB and the NIH within 48 hours of the principal investigators being informed about the event. This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio, (2) study recruitment or retention becomes futile, (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) if independent safety monitor indicates that stopping the trial is necessary due to adverse event frequency or severity.

ClinicalTrials.gov Requirements. In accordance with Public Law 110-85 and NIH guidelines, the proposed research will be registered with ClinicalTrials.gov prior to commencement of study procedures involving participants.

Magnetic Resonance Imaging (MRI)/ Magnetic Resonance Spectroscopy (MRS)

MRI and MRS are used routinely for medical care and are very safe for most people, but participants will be monitored during the entire scan in case any problems occur. The risks of MRI/MRS are:

- The scanner contains a very strong magnet. Participants may not be able to have the MRI/MRS if they have any type of metal implanted in their body, for example, any pacing device (such as a heart pacer), any metal in their eyes, or certain types of heart valves or brain aneurysm clips. Therefore, participants will be extensively screened. Participants who would normally be excluded from getting an MRI/MRS for safety reasons will be excluded, applying criteria for exclusion that are more stringent than those imposed for standard clinical MRI/MRS scans.
- There is not much room inside the scanner. Participants may be uncomfortable if they do not like to be in close spaces ("claustrophobia"). Participants will be screened for known claustrophobia.
- The scanner produces a loud hammering noise, which has produced hearing loss in a very small number of people. Participants will be given earplugs to reduce this risk. In addition, they will be informed of this possibility in the consent form and that they may immediately withdraw from the study at any time. During the procedure, participants will be able to talk with the staff through a speaker system, and, in the event of an emergency, participants can tell them to stop the scan.

During neuroimaging, participants will be situated comfortably on the scanner table, with a head mounted, MRI compatible eye tracker system attached. Visual stimuli will be presented using an LCD screen inside the scanner. Brain image acquisition will take place on a 3T Siemens Prisma human imaging system at AMRIS, at the McKnight Brain Institute, employing pulse sequences and hardware

that have been approved by the FDA for human clinical use. All MRI/MRS safety guidelines and precautionary steps established by the AMRIS will be strictly followed. At the end of the session, a post-scan debriefing questionnaire will be administered that examines discomfort during and after the scanning.

Blood Draw

The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

Blood Pressure Measurement

The risks of placing a blood pressure cuff on a participant's arms are that it may cause pinching or slight bruising.

Physical Tests

There is a risk of losing balance and falling or development of chest discomfort due to coronary ischemia or dyspnea due to heart failure or lung disease associated with the physical performance-based testing (e.g., the ¼ mile walk test, balance tests, rising from a chair). We will minimize this risk by: (1) safely escorting participants to chairs located along the walking course should they become unsteady; (2) following participants at a close distance; and, (3) being at participants' side should they need assistance. Research staff members will be trained in the conduct of the physical performance tests before they work with study participants. Study staff members are trained not to perform these tests if they feel that testing is unsafe. In addition, participants will be asked whether they feel the test is safe. Those who state it may be unsafe will not be allowed to complete the test. Staff members will be trained to protect against falling and will be trained in CPR. They will be trained in activating the emergency response system at the research facility.

Sensory Testing

The sensory testing procedures may be uncomfortable or unpleasant, in that participants may experience some temporary discomfort from the thermal, pressure, and mechanical stimulation. However, if the participants feel the sensation is greater than they wish to tolerate, they can stop any of the procedures at any time.

Personal Information

Some of the questions participants will be asked may be personal in nature and may make participants feel uncomfortable. Time will be made to talk with the participant if they appear to be distressed. We will be asking for information about sensitive issues, such as mood and thoughts of suicide which may make participants feel uncomfortable. If they are uncomfortable answering these questions, they can choose not to answer and discontinue participation in the study. Some people, when asked such questions, experience strong emotional reactions that may require counseling. If they do, they are strongly encouraged to tell the Principal Investigator, who can make an appropriate referral to the Psychology Clinic or to the UF Counseling & Wellness Center at (352) 392-1575. If we find that the participant is suicidal, he/she will be excluded from further participation in the study and an appropriate referral will be made. If we should discover, based on the questionnaires or formal clinical interview, that the participant experiences marked depression or suffer from another psychiatric condition, we will offer to make an appropriate medical, psychiatric, and/or psychological referral.

Confidentiality

Data that will be collected from human subjects for this project will include behavioral performance measures, rating scales assessed through interviews and patients' responses to self-rated scales, physiological, and neuroimaging data. Data will be collected specifically for the proposed research project. Access to individually identifiable private information about human subjects will be limited to research staff affiliated with the project. Research records will be kept confidential to the extent provided by law. The collection and submission of medical information will be accomplished with strict adherence to professional standards of confidentiality. Participants' medical information will be kept in a secure location accessible only to research staff associated with this study. Participants will only be identifiable by ID number, and any published findings resulting from the study will not refer to or identify individuals. A number of methods will be employed to maintain confidentiality of participants. First, data will be collected in secure spaces where the session cannot be overheard. Secondly, only study investigators and key research staff (i.e. data manager and study programmers) will have access to the study database. Third, participants will be assigned a unique study identifier. Individual names are not linked with collected data in the password-protected study database and only the unique study identifier will be used. A file linking the participant name and contact information and their specific study identifier will be kept separately and will be password protected and only accessible to study team members. Fourth, collected data will be maintained in locked computer files and file cabinets to which only study team members have access. Collected data will be used only for research purposes. Published data will not contain any individual identifiers. Finally, all research staff members have to retake refresher course certification exams every year.

Informed Consent

Prior to entry in the trial, the investigator(s) or study coordinator(s) will explain to potential participants the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that their participation in the study is entirely voluntary and that they may withdraw consent to participate at any time. They will be told that competent physicians/lab personnel will examine their records but that personal information will be treated as strictly confidential and will not be publicly available. Subjects will be given the opportunity to ask questions and will be given sufficient time to review all of the relevant information associated with the study. After this explanation and before entry to the trial, the subject's consent will be obtained and documented via signature of an informed consent form by both the subject and the person conducting the informed consent discussion. If signed informed consent cannot be obtained, the subject will not be included in the trial.

Inclusion of Women and Minorities/ Inclusion of Children

Men and women will be approximately equally represented in the sample. No clear evidence exists yet that OT levels vary between cultures. However, prosocial behavior associated with the OT system, have been shown to vary by culture (Kim et al., 2010). In addition, there is evidence that OT-related polymorphisms may vary across culture (Liu et al., 2010). Based on these empirically supported considerations we will enter race/ethnicity as covariate into our statistical models.

The proposed research will address effects of OT on pain, cognition, inflammation, and physical health in older men and women. Children are not the focus of this project, and by design, children will not be included in the study.

8. Possible Benefits

There are no direct benefits for study participation. There may be benefits that we are not yet aware of. The report about the results of the study will be made available to study participants as well as others in the community. However, the greatest benefit will be to society as a whole. Scientific knowledge advances slowly, but study results will increase our understanding of pain and cognition in aging. Such knowledge may be implemented in intervention programs that may have the potential to increase function and personal well-being.

9. Conflict of Interest

There are no conflicts of interest with this project.

Protocol

1. Project Title

Pain and Nutrition in Dementia and Alzheimer's (PANDA); ancillary to the UCOPE Study: **U**nderstanding **C**ognition, **O**xytocin, and **P**ain in **E**lders)

2. Investigator(s)

Dr. Larissa Strath (PI)
Dr. Yenisel Cruz-Almeida (co-I)

3. Abstract

Growing evidence suggests the presence of dysregulated pain modulation in older adults, and affect which may heighten age-associated risk for chronic pain. Additionally, chronic pain and Alzheimer's Disease and related dementias (AD/ADRD) are highly prevalent and comorbid in older adults, and research suggests that they may have overlapping etiologies and pathologies. Chronic pain may a predictor for the development of AD, and almost half of AD patients report having pain. Thus, understanding of the shared mechanisms underlying both is critical in order to develop effective treatment and prevention modalities. Recently, epigenetics has been implicated in both disease states, with many modifications of the epigenome that may go on to result in immune system dysfunction, of which is a hallmark of both chronic pain and AD. While there are many environmental factors that can influence the epigenome, nutrition status has been shown to be one of the most common and modifiable factors therein. Thus, it may be efficacious to understand dietary interactions with the epigenome to target epigenetic regulation of the development and maintenance of chronic pain and AD. Therefore, the overall goal for this mentored career development proposal is to fill this knowledge gap and determine the influence of overall diet pattern as well as Vitamins A and D specifically on the epigenetic environment as it relates to chronic pain and AD/ADRD. This study will assess dietary differences and their associations with differences in epigenetic aging, pain, and cognition in individuals with and without chronic pain. This proposed career development plan extends from the PIs prior work in dietary and immune system modulation of pain, and will forge a path towards understanding and investigating side-effect free nutrigenomic targets that improve pain and AD/ADRD outcomes in older adults.

4. Background

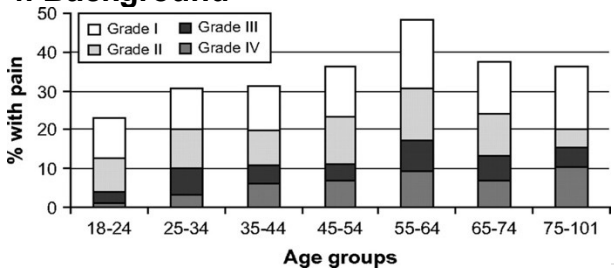


Figure 1. Prevalence of chronic pain by age and chronic pain grade (N = 2497); adapted from Parsons et al., *Fam Pract*, Volume 24, Issue 4, August 2007, Pages 308–316.

Chronic pain is highly prevalent starting in middle age. Globally, approximately 1.5 billion people experience chronic pain, and in the United States (US) alone, 1 in 3 individuals will have chronic pain at any given time[1]. It has significant negative physical, psychosocial, and economic costs[1-3]. In the US, chronic pain represents a major public health issue, as it is the most common reason for seeking medical care, increases physical and cognitive disability, increases risk of opioid abuse[4]. Since chronic pain is more prevalent in older adults **(Figure 1)** and the number of adults over the age of 65 is expected to increase from 40 to 88 million by 2050 [5], the health concerns related to chronic pain for both older individuals and society at large will significantly increase in the coming decades. Like chronic pain, the number of individuals living with Alzheimer's Disease (AD) and Alzheimer's Disease

Related Dementias (ADRD) in the US is expected to almost triple to 14 million in the next 30-40 years[4]. Dementia and AD symptoms vary at the early stages of the disease, increase in prevalence and severity as the disease progresses, and include cognitive, behavioral, psychological, and physical (i.e., falling, jumbled speech, inability to coordinate movement) symptoms[6]. *Health concerns related to AD/ADRDs will also significantly increase in the near future, especially if research distinguishing the etiologies, as well as successful interventions/prevention strategies are not of upmost importance.*

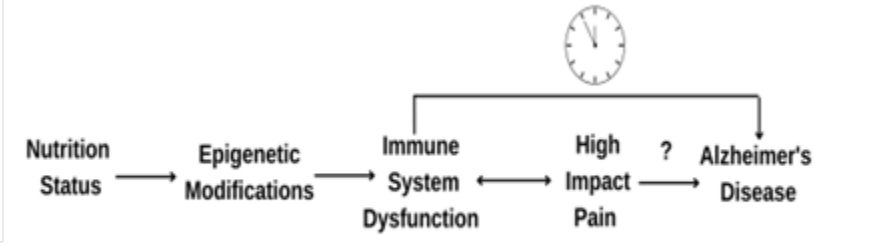


Figure 2. Conceptual mechanistic model of the relationship of nutrition, high impact chronic pain, and AD/ADRD.

Chronic pain and AD/ADRD are both characterized by neuroinflammatory processes that are controlled by the epigenome. Throughout the literature, a bidirectional association is present between chronic pain and AD, though a clear mechanistic link remains to be elucidated. Approximately 48.5% of AD patients report having chronic pain, and pain intensity is also positively correlated with dementia severity[7]. During the preclinical phase of AD and related dementias, there is a 20 to 30-year span where cognitive deficits are not present, but pathological changes may already be occurring [8, 9]. It is during this age range prior to AD diagnosis that we see the peak prevalence of multiple chronic pain conditions. Recent reports suggest that chronic widespread pain predicts cognitive decline, as chronic pain was associated with a 43% increase in all-cause dementia, and a 47% increase in AD/ADRD risk[10]. Both disease states are characterized by activated microglia[11] and central nervous system (CNS) neuroinflammation. The relationship of chronic pain and neuroinflammation is well established, with the presence of activated microglia being a hallmark of chronic pain conditions[12]. Recently, inflammation-associated PET-MRI studies have shown microglial activation in the brains of AD patients. Microglial activation has been found to be present before cognitive decline in AD patients, suggesting its early participation in AD pathology[13]. There is evidence to suggest that such immune dysfunction seen in both chronic pain and AD is regulated by the epigenome, which may serve as a reasonable target of intervention in order to control the progression of both disease states[12, 14]. Epigenetic modifiers, such as DNA methylation, histone modification, and microRNA expression, are important modulators of gene expression. These modifiers, influenced by external environmental factors, are what aid and abet microglia to transform themselves into their various phenotypes in neurodegenerative disease[14] and have been found to be differentially methylated in both patients with chronic pain and patients with AD[15, 16]. *Epigenetic modifications that lead to immune system dysfunction may be a key player in the similar etiologies and pathologies seen in both chronic pain and AD/ADRD. Because of the epigenome's readiness to respond to changes in its environment, interventions targeting it may help to prevent and treat subsequent AD/ADRD and chronic pain.*

Studies examining the relationship between chronic pain and AD/ADRD have lacked rigor and not taken into account pain impact, likely leading to mixed results. Historically, studies exploring the relationship between pain and AD/ADRD have simply used a binary pain/no pain variable potentially leading to studies showing significant relationships between pain and cognitive outcomes and those showing no significant differences[17-33]. However, recent evidence from the pain field suggests that experiments must also take pain impact into account, not just its prevalence[34]. Pain impact not only serves as a proxy for pain intensity and severity, but also takes into account the amount of interference in daily-life tasks the pain causes. Typically, chronic pain measures are used to derive at least three impact groups: pain-free controls, low-impact pain, meaning there is pain present but it does not significantly modify daily living; and high-impact pain, or pain that significantly disrupts daily life-related tasks. We (Strath et al., in process; Peterson & Strath et al, under review) and others [34] have suggested that individuals experiencing high-impact pain show the greatest declines across a variety of health related outcomes, including cognition. *Thus, it is imperative to stratify chronic pain groups if we are to truly understand the relationship between pain and cognitive diseases such as AD/ADRD.*

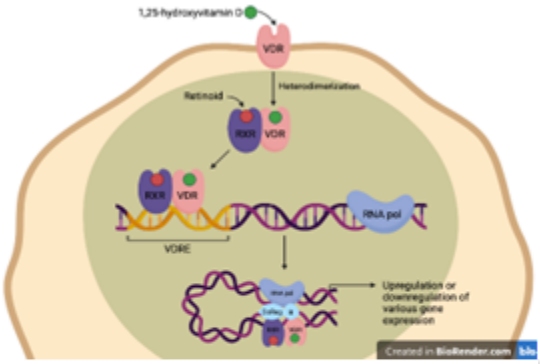


Figure 3. Vitamin D Receptor (VDR) exists as a heterodimer with the retinoid X receptor (RXR), and this VDR/RXR complex binds to specific genomic sequences on the promotor regions of target genes (vitamin D response elements, or VDREs) and recruits transcription factors (B) and other co-regulatory molecules to activate or suppress gene transcription.

Diet pattern and nutrition status have been previously implicated in chronic immune system activation and inflammation and epigenetic regulation. The relationship between inflammation and diet is well-documented, and many elements of diet have been reported to activate immune cells and trigger an inflammatory response[35] [36]. Excess amounts of dietary oils and fatty acids have been shown to participate in lipid oxidation, leading to reactive oxygen species (ROS) and subsequent inflammation[37]. Omega-6 polyunsaturated fatty acids (PUFAs), such as linoleic and arachidonic acid, are precursors for inflammatory prostaglandins[38], and are consumed in much higher amounts in the typical American diet than anti-inflammatory omega-3 fatty acids[39]. Excess carbohydrates increase the presence of Advanced Glycation End Products (AGEs) that bind to immune cells and stimulate a pro-inflammatory response[40]. In addition to pro-inflammatory effects, a diet can have anti-inflammatory effects as well. A Mediterranean diet can reduce markers of inflammation in obese individuals [41] and in patients with osteoarthritis [42] and rheumatoid arthritis[43] [44]. Interestingly, one study found that the patients on a Mediterranean diet showed significant reductions in inflammatory markers prior to weight loss, suggesting that the diet itself produced anti-inflammatory effects[45]. Studies have shown that specific micronutrients, such as Vitamins A and D, may have the ability to control gene expression by interacting with the epigenome. Both Vitamins A and D have their own receptors (Vitamin D Receptor or VDR, and the Retinoid X Receptor or RXR) are expressed both on cells as well as DNA[46, 47] **(Figure 3)**. These receptors can act as transcription factors participating in DNA transcription or physically block transcription from occurring, depending on the scenario. Additionally, there is also evidence to support that both vitamins and their receptors may participate in DNA methylation mechanisms, furthering their potential ability to influence the epigenome and downstream protein expression[48], many genes of which function to regulate the immune system and inflammation[49-52]. Evidence suggests that not only are many chronic pain and/or AD patients deficient in these vitamins, but have differential DNA methylation status on these immune/inflammation-related genes influenced by Vitamins A and D (Strath et al, 2022, in process)[53-55]; and the subsequent differentially expressed genes may contribute to pain and cognitive decline[56]. Additionally, there is evidence that Vitamin A deficiency may be associated with dysfunctional microglia[57]. Vitamin D deficiency is a risk factor for the development of AD and chronic pain, and low levels of this vitamin are related to increased cognitive decline[58, 59]. Vitamin D has also been shown to mitigate neuroinflammation present in the brain[60]. Both basic science and clinical models also show that diets low in these micronutrients are linked to increased pain severity and disability in chronic pain, as well as increased Aβ and tau proteins in serum and PET scans, as well as more severe cognitive deficits in AD [58]. In addition to being more cost-effective than most pharmaceutical interventions, nutrition based-interventions carry an incredibly positive side-effect profile. *Finally, nutrition interventions are highly adaptable, and can be adjusted to accommodate cost, flavor profiles, as well as be inclusive to cultural and religious practices, making them it a suitable strategy for all regardless of background.*

Rigor of the prior research. There are noted gaps and weaknesses of the prior research that need to be addressed in order to move this field forward that this application plans to address. Historically, the consideration of pain impact has been overlooked, likely leading to inconsistent results among the literature observing the

relationship between AD/ADRD and chronic pain. Additionally, studies implicating lifestyle factors such as nutrition as a possible mechanism influencing the relationship between pain and cognitive disease have not been completed, despite evidence of nutrition status influencing on both disease states independently. Finally, the examination of epigenetics (influenced by nutrition) such as DNA methylation and epigenetic/cellular aging as a common mechanism driving both pain and AD/ADRD in tandem has not been appreciated. This proposal not only will provide the candidate with the opportunity to train in an outstanding research environment, but also allow her to contribute to filling the gaps in the literature that will allow the field to progress towards solutions to solve the complex problems seen in chronic pain and AD/ADRD.

5. Specific Aims

Specific Aim 1: Determine the association among DII, epigenetic clocks/cellular aging and cognitive function middle-age and older adults with low-impact pain, high-impact pain and pain free controls.

Hypotheses 1a-d: Individuals with chronic pain will (H1a) have a more positive/inflammatory DII, which will be associated with (H1b) accelerated epigenetic aging and (H1c) greater cognitive impairment. These associations will be more significant in the high-impact pain group compared to those with (H1d) low-impact pain and pain-free controls.

6. Research Plan

We propose a cross-sectional, observational study design that will assess the relationship between pain, cognition and dietary pattern in older adults with and without symptomatic knee OA pain. This will be completed in 2 experimental sessions approximately one week apart. After initial screening, eligible participants will undergo the sessions (health and sensory sessions) for collection of clinical, functional (physical, cognitive, emotional), QST, and blood-based biomarker data lasting 2-3 hours.

Participants: *This study will be ancillary to one of primary mentor Dr. Cruz-Almeida's ongoing studies, significantly increasing feasibility.* Thus, participant data for this phase will be collected from **previously obtained** experimental pain outcomes (QST), cognitive outcomes, self-reported demographic variables (age, sex/gender, race/ethnicity, education, household income), other covariate (depression, anxiety, smoking, body mass index) information due to their known influence on pain outcomes, as well as previously collected and stored blood samples (n=50). Additionally, **we plan on enrolling 40 more participants** into the PANDA study that do not meet the inclusion/exclusion criteria of the parent UCOPE study. Based on power-analytic considerations, we plan to obtain data for a total of 90 individuals of both sexes (approx. 50% male) from various ethnicities. This sample size includes a 20% attrition rate. Older adults over 55 years of age who meet American College of Rheumatology clinical criteria for knee OA[78] of at least six months duration, experience pain on more days than not, with moderate pain at baseline (i.e., > 3/6 in the VDS), and who have elevated levels of plasma IL-6 (>2.5 pg/ml) will be considered for participation. Both community-based and clinic-based recruitment methods established in our labs will be utilized. We will also utilize IRB approved registries to contact potential participants.

Exclusion criteria will align with study and safety requirements related to 1) **Pain testing:** participants will be excluded if they have concurrent medical or arthritic conditions that could confound symptomatic knee OA-related outcomes or coexisting disease that could preclude successful completion of the protocol including: systemic rheumatic condition (e.g. rheumatoid arthritis, systemic lupus erythematosus, fibromyalgia); a history of clinically significant surgery to the index knee; uncontrolled hypertension (>150/95); poorly controlled diabetes (HbA1c>7%);; cardiovascular or peripheral arterial disease; serious psychiatric disorder requiring hospitalization within the past twelve months or characterized by active suicidal ideation; diminished cognitive function that would interfere with completion of study procedures (i.e., MoCA score < 25)];[81] and **2) pregnant individuals will be excluded; and 3) enrolled in another interventional research study**.

Initial screening: After phone consent, all potential participants will undergo an initial screening interview, via telephone or in-person depending on the recruitment setting (**Figure 7**). This screening

procedure will include a knee OA screening interview that has shown 87% specificity and 92% sensitivity for detecting knee OA.[82] This interview includes four questions regarding knee pain and swelling and previous diagnosis of knee OA. The initial screening will also assess MRI eligibility, age, gender, handedness, and additional health history information, and demographic information such as education level, household size and race/ethnicity for sample descriptive purposes. The Telephone Interview for Cognitive Status (**TICS**) will be given to participants to assess cognitive status. Eligible individuals will be scheduled for the Health Assessment Session.

Health Assessment

Urine and blood samples. After written informed consent, participants will provide a urine sample to determine osmolality and a fasting blood sample to evaluate standard clinical parameters (Comprehensive Metabolic Panel (CMP-14) Blood Test), which will be reviewed by Co-I Staud to determine study continuation. Following established pilot study procedures, blood samples will also be used for determination of plasma neuropeptide (e.g., OT, arginine vasopressin (AVP)) concentrations and inflammatory markers (e.g., IL-6, TNF-α, C-reactive protein (CRP)); as well as genetic markers associated with OT. This session will take place in the mornings to accommodate for fasting and to control for circadian fluctuations. For women under the age of 62, pregnancy tests will be conducted at study start. Samples may be collected during any session that is most convenient for the participants as long as this is collected before randomization.

Dietary Inflammatory Index. The Dietary Inflammatory Index (DII) was developed to provide a quantitative means for assessing the role of diet in relation to health outcomes ranging from blood concentrations of inflammatory cytokines to chronic diseases. **For the K99 phase**, diet data has been previously collected in the form of a 24-hour food recall. To collect diet data, participants were asked to document food and beverages consumed, as well as supplements taken 24 hours prior to their visit. Assessments were conducted interview-style by a trained research assistant in order to probe deeper into the details of meals. Visual aids such as standard measuring cups, plates, and drink cups in various sizes etc. were provided to aid in accuracy. These data will then be entered into the Nutrition Data System for Research (NDSR) for nutrient analysis. Following quantification, nutrient values will be used to calculate DII score according to the instructions laid out by Hebert & Shivappa et al[65].

Vitamin A and D Analyses. In order to quantify serum Vitamin D levels, the Quantikine® Human Vitamin D BP immunoassay (R&D Systems) will be employed. To quantify Vitamin A levels, a Colormetric Human Vitamin A ELISA Kit (Novus Biologicals) will be used. Kit-specific protocols will be used during quantification.

Epigenetic Analyses. Details regarding the protocol for processing and analyzing epigenetic data published elsewhere [66, 67]. In short, the raw data generated by Illumina EPIC array (.idat files) will undergo quality control and normalization prior to the calculation of DNAmGrimAge via an online calculator (dnamage.genetics.ucla.edu). The age-adjusted AgeAccelGrim variable will be calculated as the difference between chronological age and DNAmGrimAge and used throughout the analyses.

Physical examination. Height and weight will be measured and Body Mass Index (BMI) will be calculated as it is significantly related to pain symptoms in OA.[83] Participants will complete a thorough Pain and Medical History Questionnaire, including a review of bodily systems assessing the reported duration of OA, current and past treatments for OA, comorbid conditions, and current medication use as in our previous knee OA studies.[5,57] Information regarding hormone replacement therapy will be obtained for both sexes, and for women, information regarding menopausal status, as this may interact with baseline OT levels and OT intervention effects.[84] If there are concerns with eligibility, participants will undergo a Physical Examination by the study rheumatologist (Co-I Staud) to: 1) confirm the diagnosis of knee OA according to ACR clinical and radiographic criteria;[78] 2) rule out any exclusion criteria; and 3) identify the most symptomatic knee. Manual examination of joint tenderness may be

performed bilaterally for hands, hips, and knees. Symptomatic hip OA is common in the knee OA population[85-87] and is associated with disability[87] and thus will be included as a covariate.

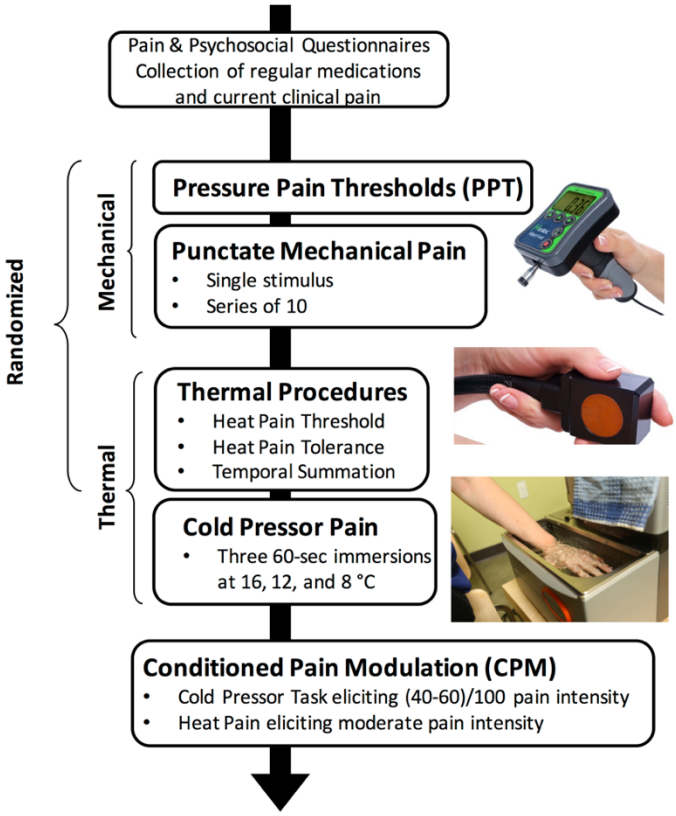
Clinical pain will be assessed according to location, overall pain severity, and pain interference with daily activities. These measurements will encompass the key dimensions for pain assessment as recommended by the American Geriatrics Society[88] and according to the pain taxonomy by the Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks and the American Pain Society group (**AAPT**).[89,90] A Verbal Descriptor Scale (**VDS**) will assess changes in pain intensity before and after treatment. The VDS has anchors of "*No pain*" (scored as 0) and "*The most intense pain imaginable*" (scored as 6) and is easier to understand and preferred over numerical rating scales by older individuals,[67] and has been effectively utilized in our pilot study. We will also administer the Western Ontario and McMaster Universities Osteoarthritis Index (**WOMAC**),[91] a reliable, well-validated measure of lower extremity pain and function in individuals with knee OA. Summary scores of the WOMAC range from 0 (*No pain or disability*) to 96 (*Extreme pain or disability*).

Knee x-ray. Weight-bearing radiographs will be taken for all participants. Co-I Staud will determine the Kellgren-Lawrence (**KL**) score and indicate which compartment(s) are affected in each knee (medial, lateral, patellofemoral) to determine study continuation. KL scores will be assessed in a blinded manner. This is only done at the baseline visit.

Sensory Assessment Visit

Pain and psychosocial questionnaires. Multiple psychosocial factors have been related to chronic pain. We will assess psychosocial factors across the following broad domains. **1) Pain coping:** The Coping Strategies Questionnaire-Revised (**CSQ-R**)[94] consists of 27 items relating to how individuals cope with pain. It comprises six subscales based on pain coping strategies that individuals report (i.e., diverting attention, catastrophizing, praying and hoping, ignoring pain sensations, reinterpreting pain sensations, and coping self-statements). **2) Affective distress:** The Beck Depression Inventory, 2nd Edition[95] is a widely used depression scale that assesses affective (e.g., sadness, loss of interest), cognitive (e.g., worthlessness, guilty feelings), and somatic (e.g., changes in sleep, tiredness or fatigue) symptoms common amongst depressed individuals. It contains 21 self-report items assessing the frequency and severity of depressive symptoms over the previous two weeks.

Figure 9. Sensory assessment session protocol



Since item # 9 from the Beck Depression Inventory refers to suicidality, the experimenter will, before the end of the test session, review the participant's response to this item, and if the participant has chosen response option 2 = "I would like to kill myself "or 3 = "I would kill myself if I had the chance", the experimenter will contact the PIs immediately and the PIs or their designee will contact Dr. Dawn Bowers (or her designee) at the University of Florida Psychology Clinic (352-265-0294) who will make recommendations. In the unexpected event that the clinic personnel are not available, the Alachua County Crisis Center will be contacted. The same procedure will be implemented for the post-intervention assessment. The Positive and Negative Affect Scale (**PANAS**) is a 20-item scale that assesses positive and negative affect.[96] The PANAS has demonstrated adequate reliability and validity.[97] For this study, participants will be requested to provide "state" information by responding to items "at the present moment." **3) Satisfaction/quality of life:** We will assess self-reported quality of life using the 36-Item SF Survey Quality of Life[98] consistent with our pilot study, while the Satisfaction with Life Scale (**SWLS**)[99] will assess satisfaction with people's lives as a whole. **4) Sleep:** Self-reported sleep quality during the past month will be measured using the Pittsburgh Sleep Quality Index (**PSQI**).[100] **5) Interoceptive awareness:** We will assess self-reported interoceptive awareness using the multidimensional assessment of interoceptive awareness (MAIA)[114] **6) Chronic Stress:** Using the **Daily Stress Inventory**, we will collect participants' self-reported daily assessment of the sources and individualized impact of relatively minor stressful events.[11520] **7) Perceived Stress Scale**: Participants fill out a self-report assessment of their thoughts and feelings during the last month.[116] **8) Impulsivity:** We will assess self-reported behavioral inhibition using the Behavioral Inhibition System and Behavioral Activation System (BIS/BAS) scales.[117] **9) Neuropathy**: Individuals will respond to the PainDetect questionnaire to assess neuropathic pain detection. If an individual scores greater than 12, their clinical pain for each area of testing will be assess during the quantitative sensory testing session.[118] **10) Empathy**: We will assess empathy using the Affective and Cognitive Measure of Empathy (ACME) questionnaire.[119] **11)**

Back Pain: If an individual indicates they experience back pain, we will assess such pain using the Chronic Low-Back Pain (Minimal Dataset)[120], Oswestry Low Back Pain Disability Questionnaire[121], and Brief Pain Inventory[122] questionnaires.

Quantitative Sensory Testing (QST). Participants will undergo QST to determine responses to mechanical and thermal stimuli and conditioned pain modulation (CPM) (**Figure 9**; see also[5,12,101]). We will randomize the order of thermal and mechanical testing. Cold pressor assessment, including conditioned pain modulation, will always occur last to avoid carryover effects. Patients' medications and current clinical pain will also be confirmed.

Mechanical testing procedures. Pressure pain threshold (**PPT**) will be assessed at the medial and lateral joint lines of the index knee, and at the ipsilateral quadriceps and trapezius muscles. For all PPT measurements, after an initial practice trial, three trials will be conducted and their average will be computed for data analysis. Using a digital, handheld, clinical grade pressure algometer (Algomed, Medoc, Ramat Yishai, Israel), the examiner will apply a constant rate (30 kPa/second) of pressure and the participant will press a button when the sensation first becomes painful, at which time the device records the pressure. Punctate Mechanical Pain will be assessed at the patella of the index knee and the dorsal aspect of the ipsilateral hand using a nylon monofilament (Touchtest Sensory Evaluator 6.65) calibrated to bend at 300g of pressure. As in our previous studies,[5,12,57] participants will provide a pain rating following a single contact, after which they will provide another pain rating following a series of ten contacts at a rate of one contact per second. The difference between the pain rating for the single versus multiple contacts reflects temporal summation of mechanical pain.

Thermal testing procedures. Thermal stimuli will be delivered to the (most) affected knee and/or to the ipsilateral forearm and/or thenar eminence, in randomized order, using a Medoc Pathway Thermal Sensory Analyzer (Ramat Yishai, Israel). Heat pain threshold will be assessed at each site, followed by heat pain tolerance and temporal summation. Heat pain thresholds and tolerances will be assessed using an ascending method of limits as in previous studies.[5,12,57] Temporal summation of thermal pain will be assessed using the same stimulus parameters as previously reported by us and others.[5,12,57] To measure Cold Pressor Pain, a modified cold-pressor procedure will be conducted. Each participant will complete a series of 60-second immersions in cold water baths at 8ºC, with water temperature maintained (+0.1ºC) by a refrigeration unit. Participants will immerse their left hand up to the wrist with their fingers spread apart. Participants will report when they first feel pain, and this time point will be recorded as the pain threshold. Then, they will continue for one minute or until they report intolerable pain. If a participant terminates early, the time at which they terminate will be recorded as pain tolerance. Participants will be prompted to rate the intensity of the cold-pressor pain at 30-sec intervals.

Conditioned Pain Modulation (CPM) will be used to assess pain-inhibitory function. The conditioning stimulus will be the cold pressor task applied to the left hand, which will be tailored for each participant to achieve a stimulus that produces moderate pain (i.e., a rating of 40-60 on the 0-100 scale) and can be tolerated for a 60-second period. The test stimulus will be heat pain applied to the opposite ventral forearm, at a stimulus intensity which produces moderate but tolerable pain. First, baseline heat pain responses will be assessed, after which the participant will immerse their hand in the cold water bath for 60 seconds. Immediately afterwards the heat pain will again be applied to the opposite arm and pain ratings will be obtained.

Short Physical Performance Battery (SPPB) and Mobility Assessment. As a performance-based indicator of OT's effect on function we will use this brief assessment consisting of four lower-extremity function measures: standing, balance, walking speed, and ability to rise from a chair. These measures have been standardized and are widely used in older populations and have been used in our own labs.[102,103] We will also measure movement-evoked pain during each task which is associated with central pain processing in knee OA.[103] Participants will also be asked to undergo a number of mobility

assessments. Participants will be asked to walk over a mat without any obstacles, as well as stepping over an obstacle, and while performing a cognitive task. Some tests will be performed while walking over an instrumented walkway, which measures spatiotemporal gait parameters.

Hypotheses and data-analytic strategy: Prior to the testing of proposed hypotheses, each variable will be carefully examined to identify missing values, statistical outliers, and violations of relevant assumptions (i.e., Durbin-Watson, Shapiro-Wilk). Sensitivity analyses would then be completed to examine differences in results between variables with missing data and those same variables with data imputation. A corrected threshold will be set to 0.05, two-tailed. Descriptive statistics will be computed and represented as percentages or means (standard deviations). Group differences (pain impact groups; Vitamin A and D clinical cutoff differences) among potential covariates of interest will be examined using a series of one-way analysis of variance tests (ANOVAs) with follow-up/post hoc Bonferroni corrections. All participants will be matched on race and sex due to their relationships with pain and cognitive outcomes. All statistical analyses employed in this study were performed using SPSS (IBM) version 28.0 (IBM; Armonk, NY). Specific to hypotheses 1a-d: Sequential hierarchical multiple regression models will be employed to investigate the extent to which the continuous DII variable is associated with (H1a) greater severity, duration, and frequency of experimental and clinical pain outcomes, (H1b) accelerated epigenetic aging, and (H1c) worse cognitive decline between individuals with and without pain. Analyses of Variance (ANOVAs) will be performed in order to examine differences in variables of interest between groups: high-impact chronic pain, low-impact pain, and pain free controls. Additionally, three separate sequential hierarchical regression models within each group will be used to examine the extent to which higher scores DII scores are associated with epigenetic aging and cognitive outcomes. Variables of interest (DII, epigenetic age, cognitive status) will be entered into *step 1* of the analysis, relevant clinical covariates (e.g., BMI, smoking) will be entered in *step 2* of the hierarchical regression models, and relevant demographic variables (i.e., age, race, sex, etc.) in *step 3* of the regression models. Finally, a modified Poisson-regression analysis will be performed to assess the relative risk of clinical cognitive impairment between pain impact groups.

7. Possible Discomforts and Risks

Equipment used in this research meet all current safety standards, and all research staff will be highly trained in the use of all equipment. This study may include risks that are unknown at this time. Potential risks for this study are related to use of study product, neuroimaging, blood draw procedure, blood pressure measurement, the physical performance tests, the cognitive function tests, the sensory testing, and potential loss of confidentiality related to study participation, as discussed in detail below.

All assessment will be conducted at the Pain Clinical Research Unit (Dental Tower) and all sessions will be conducted by trained and certified research staff, which will monitor potential adverse experiences and symptoms. We will do safety labs within 48 hours if participant withdraws due to potential adverse effects to study drug (feeling weak/tired/low energy or changes in mental status). During the testing sessions, a fully equipped defibrillator is available along with a semi-automated ECG cart. All personnel associated with the study have received CPR training and training on management of acute events including syncope, chest pain, acute dyspnea, focal neurological symptoms and abnormal vital signs. Portable defibrillators are available at the assessment site. Additionally, a study physician is available on call and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If participants develop chest pain, shortness of breath, or dizziness at any point during a screening or assessment visit, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur.

Additionally, the study physician is available on call and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If participants develop chest pain, shortness of breath, or dizziness at any point during a screening or assessment visit, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur.

Participants will be instructed to talk with the investigators about any discomforts that occur during the study. If for any reason the participant reports an injury, chest pain, leg swelling or pain, excessive shortness of breath, abnormal heart rhythm or dizziness, they will be referred to their doctor, or the PIs will call the doctor or other health care provider.

Prior to beginning data collection, all study coordinators undergo standardized training on all study procedures. A detailed checklist for each data collection element is in place to ensure all study coordinators are appropriately trained and able to conduct study procedures. Research coordinators will be certified in each element of the study visit including obtaining informed consent, administering questionnaires, protecting confidentiality of collected data, performing the test batteries. Any study coordinator who is found to not adhere to all aspects of the protocol will be required to complete additional training followed by re-certification.

All research staff complete human subjects training required by The University of Florida institutional review board (IRB) and National Institutes of Health (NIH). This training includes education about the importance of maintaining confidentiality of personal health information. The study principal investigators or a co-investigator will be available to answer questions that arise during the informed consent process as needed. More information about specific protective measures against risk is outlined below.

Laboratory tests. Participants will have blood drawn at screening and during the post-intervention visit to measure their blood chemistry and will provide urine samples for osmolality tests. Abnormal results will be brought to the principal investigator's attention immediately. The results will be reviewed and signed by the study clinician and Drs. Strath and Cruz-Almeida. If a participant has both low sodium (<134mEq/L) and high osmolality (>1200L), we will conduct a second blood test. If the sodium is again low (<134mEq/L) in the repeat blood draw, the participant will be excluded.

Assessment of Adverse Events. A standard operating procedure will be established which is followed to collect and deal with adverse events during the research project. In particular, adverse events will be systematically monitored both during the sessions. In particular, participants will respond to a side effect check list to detect any possible physiological reactions related to the drug administration. This will include series of open-ended questions asked by trained research staff regarding any adverse events that have occurred since the last visit or phone contact. Open questions about pain experienced in various parts of the body will be delivered by study staff. Additionally, participants will be asked to the WOMAC and PANAS questionnaires to assess pain and feelings. This report will be reviewed by the principal investigator and study physician in a timely fashion. Any serious untoward event, whether related or unrelated to the study drug or procedures, will be reported to the UF IRB immediately as per their regulations.

Adverse events will be defined as any physical symptoms or side effects that began following session commencement. All reported and observed adverse events will be tracked in a running adverse events participant log, which will contain information regarding dates, description, and severity. All adverse events will be coded using the most current version of the Medical Dictionary for Regulatory

Activities (MedDRA). The number and percentage of participants reporting treatment-emergent adverse events will be tabulated by treatment, system organ class and MedDRA-preferred term. Treatment-emergent adverse events will be those adverse events that begin or worsened following the first dose of study drug. Adverse events will be further classified by severity and investigator-assigned relationship to the study variable. All reported and observed adverse events will be tracked on a running adverse events log (specific to each subject and maintained in his/her study file) and will contain information regarding onset/offset dates, time course (i.e., single, sporadic), severity, action taken regarding study drug, relationship to study drug, and whether it meets FDA criteria for "Serious." Each reported event is reviewed by the principal investigator and the study physician in a timely fashion. Additionally, the principal investigator reviews all adverse events recorded in each participant's log at the conclusion of study participation.

In Case of Emergency. Participants will have 24-hour access to emergency care and will be provided with a means of contacting the on-call study doctor and research staff. Participants will be informed and encouraged to contact the study physician and study staff immediately with medical concerns or unusual reactions. Participants will also be informed of the closest Emergency Department location to their residence.

Data and Safety Monitoring Plan. A Data Safety Monitoring Plan will be in place. In particular, the following procedures will be implemented to ensure data and participant safety. Study progress and safety will be reviewed by the principal investigators in collaboration with Dr. Roland Staud, M.D., the study MD, and Dr. Feifel, M.D., Ph.D., the sponsor of the IND and consultant on the project. The Independent Safety Monitor for this study will consist of an established senior investigator not associated with our study. A study end report will be compiled and will include a list and summary of adverse events. In addition, this report will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The annual report will be signed by the Independent Safety Monitor and will be forwarded to the IRB, NIH, and FDA. The Data Safety Monitoring Plan will also require that all significant serious adverse events that may be possibly related to the study participation will be reported to the IRB, FDA, and NIH within 48 hours of the principal investigators learning of the event. In addition, any unanticipated serious adverse events that may increase the risk of the research for participants or potential participants will be reported to the IRB and the NIH within 48 hours of the principal investigators being informed about the event. This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio, (2) study recruitment or retention becomes futile, (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) if independent safety monitor indicates stopping trial is necessary due to adverse event frequency or severity.

ClinicalTrials.gov Requirements. In accordance with Public Law 110-85 and NIH guidelines, the proposed research will be registered with ClinicalTrials.gov prior to commencement of study procedures involving participants.

Blood Draw

The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

Blood Pressure Measurement

The risks of placing a blood pressure cuff on a participant's arms are that it may cause pinching or slight bruising.

Physical Tests
There is a risk of losing balance and falling or development of chest discomfort due to coronary ischemia or dyspnea due to heart failure or lung disease associated with the physical performance-based testing (e.g., the ¼ mile walk test, balance tests, rising from a chair). We will minimize this risk by: (1) safely escorting participants to chairs located along the walking course should they become unsteady; (2) following participants at a close distance; and, (3) being at participants' side should they need assistance. Research staff members will be trained in the conduct of the physical performance tests before they work with study participants. Study staff members are trained not to perform these tests if they feel that testing is unsafe. In addition, participants will be asked whether they feel the test is safe. Those who state it may be unsafe will not be allowed to complete the test. Staff members will be trained to protect against falling and will be trained in CPR. They will be trained in activating the emergency response system at the research facility.

Sensory Testing
The sensory testing procedures may be uncomfortable or unpleasant, in that participants may experience some temporary discomfort from the thermal, pressure, and mechanical stimulation. However, if the participants feel the sensation is greater than they wish to tolerate, they can stop any of the procedures at any time.

Personal Information
Some of the questions participants will be asked may be personal in nature and may make participants feel uncomfortable. Time will be made to talk with the participant if they appear to be distressed. We will be asking for information about sensitive issues, such as mood and thoughts of suicide which may make participants feel uncomfortable. If they are uncomfortable answering these questions, they can choose not to answer and discontinue participation in the study. Some people, when asked such questions, experience strong emotional reactions that may require counseling. If they do, they are strongly encouraged to tell the Principal Investigator, who can make an appropriate referral to the Psychology Clinic or to the UF Counseling & Wellness Center at (352) 392-1575. If we find that the participant is suicidal, he/she will be excluded from further participation in the study and an appropriate referral will be made. If we should discover, based on the questionnaires or formal clinical interview, that the participant experiences marked depression or suffer from another psychiatric condition, we will offer to make an appropriate medical, psychiatric, and/or psychological referral.

Confidentiality
Data that will be collected from human subjects for this project will include behavioral performance measures, rating scales assessed through interviews and patients' responses to self-rated scales, physiological, and neuroimaging data. Data will be collected specifically for the proposed research project. Access to individually identifiable private information about human subjects will be limited to research staff affiliated with the project. Research records will be kept confidential to the extent provided by law. The collection and submission of medical information will be accomplished with strict adherence to professional standards of confidentiality. Participants' medical information will be kept in a secure location accessible only to research staff associated with this study. Participants will only be identifiable by ID number, and any published findings resulting from the study will not refer to or identify individuals. A number of methods will be employed to maintain confidentiality of participants. First, data will be collected in secure spaces where the session cannot be overheard. Secondly, only study investigators and key research staff (i.e. data manager and study programmers) will have access to the study database. Third, participants will be assigned a unique study identifier. Individual names are not linked with collected data in the password-protected study database and only the unique study identifier will

be used. A file linking the participant name and contact information and their specific study identifier will be kept separately and will be password protected and only accessible to study team members. Fourth, collected data will be maintained in locked computer files and file cabinets to which only study team members have access. Collected data will be used only for research purposes. Published data will not contain any individual identifiers. Finally, all research staff members have to retake refresher course certification exams every year.

Informed Consent

Prior to entry in the trial, the investigator(s) or study coordinator(s) will explain to potential participants the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that their participation in the study is entirely voluntary and that they may withdraw consent to participate at any time. They will be told that competent physicians/lab personnel will examine their records but that personal information will be treated as strictly confidential and will not be publicly available. Subjects will be given the opportunity to ask questions and will be given sufficient time to review all of the relevant information associated with the study. After this explanation and before entry to the trial, the subject's consent will be obtained and documented via signature of an informed consent form by both the subject and the person conducting the informed consent discussion. If signed informed consent cannot be obtained, the subject will not be included in the trial.

Inclusion of Women and Minorities/ Inclusion of Children

Men and women will be approximately equally represented in the sample. No clear evidence exists yet that OT levels vary between cultures. However, prosocial behavior associated with the OT system, have been shown to vary by culture (Kim et al., 2010). In addition, there is evidence that OT-related polymorphisms may vary across culture (Liu et al., 2010). Based on these empirically supported considerations we will enter race/ethnicity as covariate into our statistical models.

The proposed research will address effects of OT on pain, cognition, inflammation, and physical health in older men and women. Children are not the focus of this project, and by design, children will not be included in the study.

8. Possible Benefits

There are no direct benefits for study participation. There may be benefits that we are not yet aware of. The report about the results of the study will be made available to study participants as well as others in the community. However, the greatest benefit will be to society as a whole. Scientific knowledge advances slowly, but study results will increase our understanding of pain and cognition in aging. Such knowledge may be implemented in intervention programs that may have the potential to increase function and personal well-being.

9. Conflict of Interest

There are no conflicts of interest with this project.

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