Protocol

1. Project Title.

Neuroimaging Age-related versus Pain-related Changes in Pain Modulation (**N**euromodulatory **E**xamination of **P**ain and Mobility **A**cross the **L**ifespan – **NEPAL** Study)

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3. Abstract:

Recent studies show that older adults exhibit dysfunctional pain modulation characterized by increased facilitation and reduced inhibition of pain, which increases the risk for experiencing clinical pain. However, knowledge of the supraspinal mechanisms contributing to age-related dysfunctional pain modulation is lacking, specifically relating to brain structure and function. Thus, our goal is to identify the neurobiological mechanisms contributing to dysfunctional pain modulation in older adults. We will examine the structural and functional connectivity (aim 1) and biochemical concentrations (aim 2) of cortico-striatal regions in older adults with and without pain compared to younger adults and determine their associations with an individual's pain modulatory capacity and physical performance (aim 3). The proposed research addresses a significant gap in the literature and is the first to evaluate the age-related versus pain-related changes in the neural substrates of endogenous pain modulation and mobility. The identification of key top-down modulatory brain networks will increase our understanding of age versus pain-related changes in pain processing that may ultimately lead to personalized, targeted therapies. Given the expected increase and burden of chronic pain in older adults in the coming decades, effective strategies to reduce the risk of older adults experiencing chronic pain are urgently needed. We will compare young individuals to older individuals with and without chronic musculoskeletal pain since it represents the leading cause of disability worldwide in our aging population.

4. Background and Specific Aims:

Older adults experience significantly greater clinical pain and disability than younger individuals[1-2]. The experience of pain is a complex phenomenon under the control of endogenous modulatory systems that both facilitate and inhibit pain. Accumulating evidence using quantitative sensory testing (**QST**) indicates that older adults exhibit aberrant pain modulation. *These studies suggest that aging is associated with reduced endogenous pain inhibition*[3-7] *and increased pain facilitation*[6,8-9] *similar to chronic pain patients*. Such an imbalance between endogenous pain facilitation and inhibition may place older adults at an increased risk for clinical pain and disability compared to younger cohorts[10-11].

The biological bases for these age-related changes in pain modulation are still unclear, although changes in brain structure and function are likely important contributors[12]. Both functional and structural connectivity studies have shown age-related changes in cingulo/frontal, insular and striatal regions[13-14]. Similarly, imaging pain studies suggest that cingulo/frontal regions represent a major pathway of descending pain modulation with the prefrontal cortex initiating top-down control[15]. Cingulo/frontal regions receive pain-specific information from the insula and have direct projections to brainstem areas that modulate nociceptive transmission at the spinal cord. The prefrontal cortex also sends direct projections to the striatum, which studies in animals and humans implicate in pain modulation[15-18]. The striatum receives sensory input from cortical (i.e., prefrontal, cingulate, insula), thalamic and spinal structures while its descending projections reach the modulatory pain network. Although historically the striatum has been linked to pain because of motor processing (i.e., we feel pain and quickly move to avoid or reduce it), increasing evidence implicates the striatum directly with the sensory aspects of pain. Indeed, a recent study found that cortico-striatal functional connectivity predicted the transition from acute to chronic pain[19]. In light of recent evidence and given the striatum's connectivity with brain regions involved in pain processing, differences in pain modulation between young and old individuals may be accounted in part, by age-related changes in striatal structure and function. However, no study to date has examined the relationship between age-related changes in brain structure and function to the age-related decline in endogenous pain modulation documented with QST. Furthermore, it is not known, whether this decline in pain modulation is pain versus age-dependent. ***Our central hypothesis is that disrupted brain connectivity and biochemistry of cingulo/frontal and striatal regions will be significantly associated with age-related decreased pain inhibition and increased pain facilitation and will account for increased musculoskeletal pain and disability progression overtime.*** We will assess 200 older adults with and without musculoskeletal pain to 50 younger adults without chronic pain to determine the relative contribution of age versus clinical pain to the dysfunctional pain modulation profile in older adults.

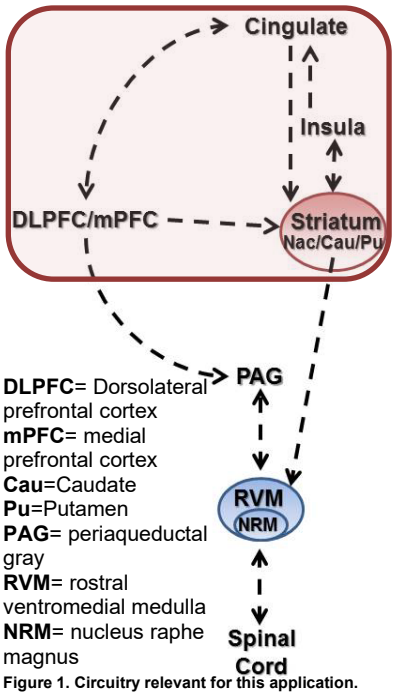

Figure 1. Circuitry relevant for this application.

Specific Aim 1: To characterize the functional and structural connectivity of cingulo/frontal, striatal and insular regions among older adults with and without chronic musculoskeletal pain and younger adults. We will use resting state (RS-fMRI) and diffusion tensor neuroimaging (dMRI) to quantify the functional and structural connectivity among cingulo/frontal, striatal and insular areas. We test the hypothesis that older adults with chronic musculoskeletal pain will exhibit decreased functional and structural connectivity between the cingulo/frontal-striatal and insula-striatal circuits compared to older adults without chronic pain as well as compared to younger adults.

Specific Aim 2: To measure the biochemistry of cingulo/frontal, striatal and insular regions among older adults with and without chronic musculoskeletal pain and younger adults. We will use Magnetic Resonance Spectroscopy (MRS), a non-invasive method to measure biochemical concentrations in the brain, to quantify the concentrations of GABA, the

main inhibitory neurotransmitter of the nervous system in cingulo/frontal, striatal and insular regions. We test the *hypothesis* that older adults with chronic musculoskeletal pain will exhibit decreased GABA concentrations in cingulo/frontal, striatal and insular regions compared to older adults without chronic pain as well as compared to younger adults.

Specific Aim 3: To characterize baseline associations between cingulo/frontal, striatal and insular region connectivity and biochemistry with endogenous pain modulatory capacity and physical performance among older adults with and without chronic musculoskeletal pain and younger adults. We will perform a QST battery to characterize altered endogenous pain modulation in older and younger adults. We *hypothesize* that brain connectivity and biochemistry will be associated with pain modulation, such that altered connectivity/biochemistry will predict greater pain facilitation versus pain inhibition as tested with QST at baseline. Similarly, we *hypothesize* that brain connectivity and biochemistry will be associated with physical performance at baseline. Finally, we hypothesize that baseline brain connectivity and biochemistry will predict clinical musculoskeletal pain changes over a one-year period.

The proposed research addresses a significant gap in the literature and is the first to evaluate age-related versus pain-related changes in the neural substrates of endogenous pain modulation and their associations with physical function. The identification of key top-down modulatory brain networks will increase our understanding of age-related changes in pain processing that may ultimately lead to personalized, targeted therapies. The work proposed will also provide the preliminary data necessary for several competitive R01 applications that will be the first to ***directly*** investigate cortico-striatal circuits in relation to pain modulation and to prospectively account for mechanisms underlying increased clinical pain and decreased physical function in older age.

6. Research Plan:

6.1. Participants, Recruitment and Compensation. We propose to enroll and screen 350 persons, a total of 250 (i.e., 50 younger adults (18 to 28 years old) and 200 older adults over 60 years of age: two-thirds older adults with chronic musculoskeletal pain and one-third age and BMI-matched controls without chronic pain). Potential participants will be asked about subjective musculoskeletal complaints in the neck, shoulders, hands, lower back, knees and feet during the preceding 12 months and 7 days. We will attempt to recruit equivalent numbers of males and females and include minority adults. Subjects will be tested in the Clinical Research Center (CRC), the Institute on Aging (IOA) clinical research laboratories, the Pain Clinical Research Unit in the Dental tower, and clinical research laboratories in the Florida Gym. Neuroimaging will take place at the Clinical Translational Research Institute (CTSI) human imaging core at the McKnight Brain Institute. Recruitment of participants will be accomplished using flyers at UF/VA/Shands as well as using the Consent2Share program and the PRICE Registry. Participants will be compensated in the amount of $35 for each session attended, for a total of up to $175 Interested individuals will call our laboratory to learn about the study and complete a telephone screening to establish preliminary eligibility.

Study Exclusions: 1) pregnant women; 2) history of alcohol/drug abuse in the past; 3) known intra-cerebral pathology or epilepsy; 4) significant cognitive impairment as evidenced by the 3MSE); 5) hospitalizations for mental health reasons in the past year; 6) not meeting MRI screening requirements (e.g., implants, prosthesis, artificial limb/joint, shunt, metal rods, hearing aid, claustrophobia or anxiety); 7) chronic/current use of narcotic medications; 8) serious systemic (uncontrolled diabetes self-reported HA1C >7), neurological, or cardiovascular disease (uncontrolled hypertension > 155/90 mm Hg); and 9) liver or kidney disease. 9) Inability to

consent for study participation. Over the counter medications (e.g., NSAIDS, topical pain relievers) will be allowed, but we will ask that participants do not take them any days of testing.

Initial Phone Pre-Screening. Interested individuals will call in using provided phone number and one of the study team members will obtain a verbal consent for the telephone interview. This will provide the individuals information about the study and if they are interested a pre-screening will be conducted. After the phone pre-screening, if participants are eligible and still interested in participation, a baseline orientation visit will be scheduled. Given there are 4 study visits participants will be allowed to complete any of the assessments during extra visits or in any desired order to reduce their burden and avoid participant fatigue. Visits may be consolidated if participants request it as long as it does not exceed 3 hours per visit.

Baseline Orientation Visit. Persons who express interest in participating in the study will be told about the study in details and informed about HIPAA regulations and be asked to review and sign an Informed Consent Form to agree for study participation and to grant authorization for collection of health data needed to determine eligibility. Those individuals who grant authorization by signing the Informed Consent Form will receive a health assessment consisting of a health questionnaire, supplemented by interview if any clarification is necessary, and a blood pressure measurement to determine study eligibility. We will further screen for MRI-specific exclusions using a contraindications checklist. Individuals will be exposed to receive 2 or 3 sample pain stimuli before making the final decision whether or not to enroll as a subject. Those individuals who decline at this point will receive compensation for that session. Those who accept will be enrolled in the study as subjects. Participants will have the option to decline participation in the longitudinal portion of the study or the MRI or venipuncture procedure. Some of the measures listed below will be administered electronically, via an iPad.

After informed consent, subjects will be administered the Modified Mini-Mental State Examination (3MS) and The Montreal Cognitive Assessment (MoCA) (15 minutes) to screen for dementia. The Center for Epidemiologic Studies Depression Scale (CES-D) (10 minutes) and in older adults the Geriatric Depression Scale (GDS, 5 minutes) will be administered to screen for depression. Individuals who are found to have dementia and/or severe depression will be referred to their primary care physician in order to obtain a referral for a mental health professional for follow-up. If the individual does not have a primary care physician, will be referred to the on-site Senior Care clinic (for older participants) or the UFHealth Psychology Service (for younger participants) for follow-up. At this point, female participants between 9 and 62 years of age will take a pregnancy test. If participants are still eligible for participation, a detailed health review will consist of a medical, pain and hospital admission history, pain questionnaires (i.e., SF-MPQ-2, WOMAC, Pain-DETECT, Coping Strategies Questionnaire-Revised), a Minimal Dataset questionnaire, a medication inventory, demographic and anthropometric data including waist circumference (approximately 30-45 minutes). A standardized paper and pencil cognitive battery will be administered to assess cognitive functions: HVLT-R, Trail Making A&B, Boston Naming Test, COWA, Stroop Interference test and the Ruff Figural Fluency test. The Edinburgh Handedness Inventory (5 minutes) will also be administered. At the end of the session, subjects will be provided a copy of the following questionnaires:

- the Ten-Item Personality Inventory (TIPI),
- the Pittsburgh Sleep Quality Index (PSQI),
- the trait version of the State-Trait Anxiety Inventory (STAI),
- the trait version of the Positive and Negative Affect Schedule (PANAS)
- the Raven's Progressive Matrices Test

for them to fill out at their convenience and bring back to their next visit.

Before every session, subjects will complete the following questionnaires: The state version of PANAS, the state version of the STAI, the Graded Chronic Pain Scale (GCPS) as well as listing of all the current medications taken and food eaten within the past 2-4 hours.

Finally, the older participants will get a knee x-ray to determine OA severity and this may be done at any study visit when is most convenient for each older participant.

We will also take vital signs including temperature, blood pressure and heart rate at the beginning and end of each session.

Subjects will participate in 3 separate experimental sessions: 1) an imaging (**MRI**) session, 2) a **QST** session to assess basal pain sensitivity and pain modulation and 3) a physical and cognitive function session. A fourth experimental session may be held if any study visit is incomplete. This is to ensure all data needed is captured and to reduce their burden and avoid participant fatigue.

MRI Neuroimaging. Participants will lay supine in the MRI magnet for up to 1.5 hours. Scans will be taken while the participant is resting quietly, completing cognitive tasks and while painful stimulation is being applied using a plastic (MRI-compatible) MediPin routinely used in neurological examination. Additionally, an MRI compatible thermode will be placed on one or both forearms, and a series of heat stimulation will be administered. A simple visual task will also be performed to control for non-specific cognitive influences. MRI data will be used to measure the structural and functional integrity of the brain. Specific items of interest include, but are not limited to, descending sensory and motor tract integrity (fiber tractography), intracranial volume, whole brain volume, lacunae as well as metabolite concentrations.

At the end of the neuroimaging session, participants will be taken to the research laboratory where after a 10-minute rest period, subjects will undergo the conditioned pain modulation (CPM) procedure. They will be given brief heat pain pulses followed by immersion of their hand in a cold-water bath for up to 1 minute. At this point, they will be instructed to take their hands out of the water and the brief heat pulses will be repeated. The difference between the post-immersion and pre-immersion will be calculated as the amount of pain inhibition achieved.

Quantitative Sensory Testing (QST). Participants will come for the study visit to our laboratory at the CTRB for up to 2 hours. We will make sure that ample time is scheduled for the first session, so the participant does not feel rushed and has time to get to know the lab environment and investigators. In addition, we allow the participant to experience a few sample stimuli before deciding whether or not to go ahead with the actual testing. QST will be performed in standardized sites using anatomical landmarks. All tests will be demonstrated and explained prior to being performed. All participants will be tested on the hands and feet. For participants with pain, additional standardized testing sites will be chosen to include painful areas. The “TSA Thermal Sensory and Vibratory Sensory Analyzer (Model TSA II, VSA 3000) (Medoc. LTD)” will be used in this study to quantify nerve fiber dysfunction with measurements of vibratory, pinprick and thermal sensory thresholds (warm, cold, heat-induced pain, and cold-induced pain). A hand-held algometer (FDX, Wagner Instruments and/or Algomed, Medoc.LTD) will be used to assess pressure pain sensitivity. Prior to testing we will obtain skin temperature readings at all testing sites by using the DT1001 DermaTemp infrared scanner (Exergen). Similar procedures have been used by us and other investigators in older adults with and without pain.

a. Vibratory Detection Thresholds: Vibratory threshold is tested with a vibratory pin, which presses the measured area with a consistent pressure of 50g. The vibratory sense analysis will be performed using upward-moving stimulus (increasing in intensity until a sensation is

perceived). Several vibrations will be given sequentially and the mean end variance will be determined to verify the consistency of the test.

b. Tactile Detection Thresholds: Thresholds for light touch will be assessed with von Frey monofilaments, using two ascending and two descending stimulus series, according to the method of limits. Detection threshold at each test site will be determined by the obtaining the geometric mean across these four test series.

c. Thermal Detection Thresholds, Pain Thresholds and Temporal Summation: Following a brief introduction familiarizing each subject with the procedure, several trials will be performed for each sensory modality and a mean threshold will be calculated. For threshold determination we will use a "reaction time-inclusive" method, the method of limits, consisting of continuously changing intensities of stimuli halted automatically by the subject at the moment that the requested sensation is perceived. The following thresholds will be evaluated using the TSA II: (1) cool sensation; (2) warm sensation; (3) cold pain; and (4) heat pain. Subjects will also be asked to rate the painful sensation. Temporal summation of heat pain may be assessed by administering brief repetitive suprathreshold heat stimuli to the hand.

d. Allodynia and Temporal Summation: Dynamic mechanical allodynia will be investigated using a soft brush and lightly brushing the skin of the hands and feet as well as any reported painful areas. If pain is evoked in the test area, the participant will be asked to rate the intensity of the pain. If an allodynic area is detected, temporal summation will be evoked by repetitively tapping the skin of the allodynic area with von Frey hairs (100g) at 1 Hz for 10 seconds. If temporal summation is evoked in the allodynic test area the participant will be asked to rate the intensity of the pain.

e. Punctate Pain Testing and Temporal Summation: We will apply punctate mechanical stimuli to the test sites with a series of weighted probes. Probes of different weights will be applied to participants' skin to determine the level that produces slight discomfort or pain. Two measures are obtained: 1) Pain threshold is determined by applying probes of different weights in ascending and descending sequences and participants are asked to tell the examiner which probes produce pain; 2) A weighted probe is applied either once or several times in a row and participants are asked to rate the pain they experience from the probe. In addition, a standardized plastic MediPin commonly used in neurological examinations will be applied to testing areas and participants will be asked to rate the intensity of any pain that is experienced.

f. Pressure Pain Thresholds: Pressure is delivered by a hand-held algometer (spring-controlled device delivering calibrated pressure via a flat 10mm diameter rubber tip). Pressure is delivered at an approximate rate of 1 kg/sec. Participants respond when they first feel pain, at which time the pressure is removed.

Physical and Cognitive Function Testing. Participants will complete a physical and cognitive performance battery and a number of validated self-report questionnaires.

a. Upper Limb Isometric Strength. Upon arrival subjects will undergo a handgrip strength test using a dynamometer. The subject will be instructed to squeeze the dynamometer with maximum isometric effort for about 5s. No other body movement will be allowed.

b. Short physical performance battery (SPPB). The SPPB is based on a timed short distance walk, repeated chair stands and a balance test, which will be administered by a trained study team member.

c. Mobility assessments: Participants will 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. Changes in participants' skin conductance may be measured (Biograph® ProComp5™ Infinity) during mobility assessments. Conductive electrodes will be attached to participants' fingers to measure any emotional arousal elicited by obstacles and/or cognitive tasks.

d. Physical Function Questionnaire. At this point we will ask participants to complete a modified version of a disability instrument called the Pepper Assessment Tool for Disability (PAT-D). The validated questionnaire consists of 5 subscales: mobility, transferring, upper extremity, instrumental and basic ADLs.

e. Knee Extension Isokinetic Strength. An isokinetic dynamometer modified with increased sensitivity force transducers will be used to record Total work (Joules) and maximal force production (Biodex System 3, Shirley, NY). Participants will move through a full range of motion (if no contraindications are present), performing contractions at 60, 90 and 120 degrees/second to document potential effects on the velocity of movement.

f. Neurocognitive battery. During this visit participants will complete the NIH-Toolbox: cognitive module, assessing a number of cognitive domains as detailed in the table. Depending on the participant's preference, the cognitive battery may also be completed during the QST session or another visit (with separate payment).

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.

Power and statistical analysis. Our study is powered on aim 1 and aim 2- due to the lack of data to directly inform aim 3- using effects sizes derived from a previous study measuring cortico-striatal functional connectivity. A sample size of 200 per older group will provide evidence with at least 80% power to detect associations in behavioral measures of pain modulation and predicting clinical pain progression and physical function among older and 50 younger adults.

Other methodology.

BioMarker Detection. In order to simultaneously measure plasma levels of 4 Neuropeptides (β-endorphin, Substance P, αMSH) and 9 cytokines and chemokines (IFNγ, IL1β, IL4, IL5, IL6, IL8, IL10, IL12(p70), MCP1, and TNFα), Milliplex® multi-plex kits (Millipore) will be used according to the manufacturer's instructions and analyzed on a Luminex 100. Here all mediators can be measured simultaneously in a 50 µL sample of plasma. After which, data will be analyzed using MilliplexQT® software using standard curves and five-parameter logistics. The Luminex 100 analyzes Milliplex® beads with varying ratios of two fluorescent dyes, and can resolve 100 different dye sets. The Milliplex® beads are coupled to antibodies specific for each mediator of interest such that each particular bead's fluorescent 'address' is associated with a known mediator. Therefore when captured biotinylated mediators are detected with strepavidin-phycoerythrin (SA-PE), and the samples are analyzed on the Luminex 100, it is capable of identifying the fluorescent bead address and measuring the SA-PE intensity. Here the SA-PE intensity is relative to the concentration of a given mediator assigned that particular bead's 'address'. Alternatively, mediator concentrations can be determined using standard ELISA technology (Biosource) where single mediators are measured in individual assays.

7. Possible Discomforts and Risks:

Thermal injury. The risk of burn injury as a result of overheating contact thermode due to technical malfunction is very unlikely because the subject: (1) is free to withdraw from the thermode; (2) has the option to stop the stimulus at any point. In addition, the stimulator incorporates automatic safety features that do not depend on actions of the investigator or subject: (3) a safe range (max 52^0C) is programmed into the system preventing accidental use of potentially harmful temperature set-points; (4) the software continuously monitors thermode temperature and automatically interrupts thermode contact with the skin when the process value exceeds the set-point by >1.0^0 C.

Psychological discomfort. The prospect of being subjected to painful stimulation in an unfamiliar location, surrounded by unfamiliar investigators and equipment that may look intimidating may lead to anxiety. We try to minimize this anxiety by thoroughly explaining all

procedures and taking the time to answer all questions the participant might have. We make sure that ample time is scheduled for the first session, so the participant does not feel rushed and has time to get to know the lab environment and investigators. In addition, we allow the participant to experience a few sample stimuli before deciding whether or not to go ahead with the actual testing. Our tests always start with a few non-nociceptive stimuli before the temperature rises gradually. In spite of these measures we cannot completely rule out that some participants may feel some anxiety during the first session. However, based upon experience in other studies using similar protocols, we are confident that most subjects will not experience psychological stress during the second and subsequent sessions, when they have become familiar with the experimental setting.

Muscle strength testing: It is possible that muscle soreness may result from the exercise testing. If this does occur it will peak within 24-48 hours following the testing session and then gradually subside.

Physical Function testing: There is a risk of losing one's balance and falling during the physical performance-based testing (e.g., the ¼ mile walk test, balance tests, rising from a chair) and participation in physical activity. Falling also places participants at risk for a bone fracture. We will minimize this risk by: (1) safely escorting them to chairs located along the walking course should you become unsteady; (2) following them at a close distance; and, (3) being at their side should they need assistance. Study staff will also omit any tests that they feel would be unsafe for a participant to complete.

Neuroimaging: Although unlikely, MRI protocols pose risks because of the strength of the clinical magnet. One of the main risks is for those with metal implanted in their body. For that reason, these individuals are excluded from the present project. Other risks include feelings of claustrophobia and potential hearing loss due to exposure to the noise of the MRI equipment. Participants will be provided with earplugs and a speaker system through which they can ask to stop the procedure if they wish.

Cognitive tests: There is a risk that participants will find memory and concentration tests stressful and might feel tired or sad because it may be difficult to remember things that they are asked to remember. Participants may skip any question you do not wish to answer. Research staff will explain what to do and answer participants' questions they might have during cognitive testing.

To maximize the participants' safety, we will follow a standardized screening protocol. All potential participants will undergo screening for cardiovascular and other major diseases by means of a screening health questionnaire. Those with overt cardiovascular diseases (or other severe diseases) that meet the exclusion criteria are excluded.

Other Risks:

The experimental pain procedures described in this application are widely used and safe procedures. Confidentiality will be maintained by assigning each participant a number, which will be used in all data tabulation. All findings from the survey will be reported in aggregate and in a manner that precludes identification of any individual respondent. Disclosure of the respondents' answers outside the research could not reasonably be thought to place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, or reputation.

If participants experience an injury that is directly caused by this study they will receive professional medical, dental and consultative care at the University of Florida Health Science Center without charge. However, hospital expenses will have to be paid by the participant if the participant's insurance provider does not cover them. The participant will

not be charged for any of the procedures performed as part of the study (e.g., pain tests, surveys, blood pressure measurement). None of the procedures performed as part of the study should be considered part of the treatment for any conditions.8.Possible Benefits:

There is no direct benefit to participants in this study. Information gained in this study may assist researchers in understanding how changes in the nervous system contribute to physical disability in older adults. This information may help with developing new methods to prevent disability. Given the low level of risks and the substantial long-term benefits to society, the benefits outweigh the risks.

9. Conflict of Interest:

The study investigators do not have any conflict of interest to report.

10. Procedures for data and safety monitoring

PI will review adverse events on a weekly basis with study staff and the IRB will review progress of this study on an annual basis.

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