7 Tesla Functional Magnetic Resonance Spectroscopy Analysis of Pain Processing in Humans

by

Steven Jon Nichols

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Approved By

Jennifer L. Robinson, Ph.D., Chair, Professor of Psychological Sciences
Richard J. Macatee, Ph.D., Assistant Professor of Psychological Sciences
Meredith A. Reid, Ph.D., Assistant Professor of Electrical and Computer Engineering

Abstract

Globally, one in five people will experience some form of chronic pain in their life. In the United States, an estimated 50 million people manage chronic pain daily, nearly 20 million of which are categorized as high-impact chronic pain. Unfortunately, our understanding of the basic physiological aspects of pain is limited yet necessary to advance our understanding of pain processes as well as develop effective therapeutic interventions. Previous neuroimaging research has identified a network of interrelated brain regions that seem to be implicated in the processing and experience of pain. Among these, the anterior cingulate cortex (ACC) plays an important role in the affective experience of pain signals. The current study leveraged functional magnetic resonance spectroscopy (fMRS), a robust and sensitive measurement of neurometabolites, to investigate the underlying dynamic shifts in the neurometabolic signature of the human ACC at rest and during acute pain. Results provide support for increased glutamate levels following acute pain administration. Specifically, a 4.6% increase in glutamate was observed during moderate pressure pain compared to baseline. These data contribute toward the characterization of neurometabolic shifts which lend insight to the role of the ACC in the pain network. Further research in this area with larger sample sizes could contribute to the development of novel therapeutics or other advances in pain-related outcomes.

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Introduction

20% of the world's population will experience a form of chronic pain at some point in their lives. An estimated 50 million people in the United States suffer from chronic pain on a daily basis, 20 million of whom are classified as high-impact chronic pain (Dahlhamer, 2018). The prevalence of chronic pain in the United States has created a substantial economic burden. A variety of pain-related expenses, including things such as healthcare costs, medication use/misuse, and lost wages, cost an estimated $560 billion to $635 billion annually in the United States alone (Henschke et al., 2015). Moreover, the impacts of pain are not exclusively monetary; mood, sleep, cardiovascular health, and brain function have all been shown to be negatively affected by chronic pain (Fine, 2011). While a comprehensive understanding of pain at its most basic level remains elusive to both research and medical fields alike, chronic pain continues to have an enormous impact on those affected.

Pain serves as a key survival mechanism fundamental to the human experience (Diatchenko et al., 2007). While infrequent occurrences of acute pain may be an adaptive trait, frequent occurrences of pain sustained over long periods become problematic. Indeed, the importance of identifying and characterizing a functional pain network cannot be overstated, and yet we still do not have a complete understanding of the underlying physiological mechanisms subserving pain within the human nervous system, and specifically within the brain. Here, we aimed to contribute to the knowledge of normative pain processing by examining the neurometabolic underpinnings of acutely administered pain.

Pain Network

Previous research has sought to identify a 'pain cortex' within the brain, similar to the cortices seen in primary visual and auditory areas (Mano & Seymour, 2015). Efforts to find this putative pain cortex, like those by Penfield and Jasper (1954), have not reliably revealed a primary processing center for pain. Failures to find a single pain cortex, and an increased understanding of neurofunctional circuitry, have led contemporary researchers to explain pain processing as a widespread network within the brain. Meta-analytic data indicate that this network encompasses brain structures such as the anterior cingulate cortex (ACC), the primary and supplementary somatosensory cortices (SI & SII), and subcortical structures within the basal ganglia and thalamus (Coghill et al., 1999) (see also Hutchison et al., 1999; Jones et al., 1991; Vierck et al., 2013). While many of the neural nodes identified within this pain network, such as the ACC, have been studied extensively, questions still remain regarding the neurochemical underpinnings of these structures.

Anterior Cingulate Cortex

The anterior cingulate cortex (ACC) consists of the rostral portion of the cingulate cortex which surrounds the rostrum and genu of corpus callosum. Previous research has implicated the ACC in a variety of processes such as response selection, affective cognition, and pain (Devinsky et al., 1995a). With regard to response selection, functional magnetic resonance imaging (fMRI) data have highlighted the role of the ACC in high-level error monitoring and reward prediction processes. In one fMRI study by Marsh et al. (2007), participants were trained to associate unique reward values with visual stimuli. Later, when given the choice between several of these stimuli,

participants were asked to select the stimulus associated with the greatest respective reward amount. Blood-oxygen-level-dependent (BOLD) activation in the dorsal ACC (dACC) was positively associated with increases in reward choice options. Additionally, BOLD activation in the rostral ACC (rACC) was positively associated with increases in reward potential. As part of the limbic system, it is unsurprising that ACC activation was associated with these response selection processes, given the limbic system's role in reward processing (Rajmohan & Mohandas, 2007). Additional fMRI results published by Taylor et al. (2006) corroborate rACC function in error monitoring. In this study, Taylor and colleagues used a modified Eriksen flanker task to measure BOLD responses following selection errors. Researchers randomized these selection errors such that some were tied to monetary penalties, while others were not. FMRI data indicated that rACC hemodynamic activity was significantly higher in response to errors that resulted in monetary loss. This study further supports the ACC's role in error monitoring and response selection. Taken together, these results implicate the ACC in various aspects of reward-based decision making.

Meta-analytic data implicate the rACC is involved in processing the salience of emotional information as well as regulating emotional responses (Bush et al., 2000a). This subdivision of the ACC has direct connections to the amygdala, hypothalamus, and insula (Devinsky et al., 1995a). In light of the contributions made by these structures to emotional processing (Maren, 1999; Phan et al., 2002; Swanson, 2000), these connections support the ACC's involvement in this system. Fourie et al. (2014) found evidence substantiating the ACC's affective function specific to negative emotional processing. Researchers used fMRI to examine activation in emotion-specific brain

regions in response to a social prejudice task. While being scanned, 22 low-prejudice individuals completed a social prejudice questionnaire. The task induced guilt by using preprogrammed feedback to suggest their responses to the questionnaire items were indicative of extreme prejudice. Resultant neuroimaging data indicated activity increases in both the ACC and anterior insula following the preprogrammed feedback. Interestingly, considerable personality changes have been observed across several species following lesions to the ACC including apathy and emotional instability (Kennard, 1955; Tow & Whitty, 1953). Kennard (1955) observed increased aggression in cats following surgical ablation of the bilateral cingulate gyrus. Researchers noted that following surgery, the cats displayed excessively aggressive behavior in response to caretaker interaction that had not been observed previously. Tow & Whitty (1953) observed distinct personality changes following cingulotomy in a small sample of men and women. Researchers noted that following the procedures, patients displayed noticeable decreases in depth and variety of general interests, as well as a marked lack of energy with regard to those interests, both of which appear symptomatic of increased apathy. Indeed, the convergent data produced by this collection of studies has elucidated the ACC's role in multiple aspects of affective processing.

With regard to pain, evidence from studies across domains suggest that the ACC is involved in the affective experience of pain. More specifically, the ACC has been implicated in pain aversiveness (Cottam et al., 2016a; Navratilova, Xie, et al., 2015; Navratilova, Atcherley, et al., 2015b; Navratilova & Porreca, 2014). These findings are unsurprising given the above evidence concerning the affective processing associated with the ACC. The use of high-field fMRI has been particularly useful in uncovering the

pain-specific functions of the ACC. In one such fMRI study, Cottam and colleagues (2016) sought to map the neurocorrelates of osteoarthritis (OA) -related knee pain. The study recruited 26 patients with painful knee OA. Researchers observed a positive correlation between pain severity and cerebral blood flow in several key nodes within the pain network such as the ACC, insula, and brainstem, indicating that these regions are likely involved in the processing of aversive pain signals. While these results bear particular clinical significance, it should be noted that ACC activation has been observed in response to experimental pain as well. This was demonstrated by Christmann et al. (2007) with electrical pain stimulation. Researchers attached an electrode to participants' right thumb while undergoing both electroencephalogram (EEG) and fMRI recording. The resultant data revealed significant ACC activation in response to this form of experimental pain. Here again, increasing activation was positively correlated with increasing nociceptive intensity. Moreover, Yanes et al. (2020) demonstrated a similar effect using a pressure-pain design. In this study, participants experienced pressure-based pain between the first and second knuckles on the nondominant hand during scanning. Neuroimaging data implicated dACC activity in response to yet another pain paradigm. This study is particularly relevant in that both BOLD responses and neurometabolite levels were recorded. Indeed, researchers observed changes in glutamate concentration across pain conditions within the dACC. Perhaps due, in part, to the sustained pain design, the effect of glutamate concentration diminished over time. This finding presents an opportunity to investigate pain-related dACC glutamate using alternative designs. Given the abundance of supporting evidence, the ACC, specifically

the dACC, appears to be a candidate for further investigation into the underlying neurocorrelates of pain processing.

Functional Magnetic Resonance Spectroscopy

Here, we used functional magnetic resonance spectroscopy (fMRS) to characterize the neurometabolic changes in the dACC during rest and acute pain administration. Traditional static MRS, a common neuroimaging technique, characterizes metabolites within the brain. This method involves collecting a single spectrum while the subject is at rest in the scanner (i.e., a no-task condition). In contrast, fMRS capitalizes on neurometabolite level changes in both task and rest conditions to collect multiple spectra over time (Duarte et al., 2012). In this way, researchers can compare averages of neurochemical changes in different conditions within the same participant allowing for characterization of dynamic shifts from baseline to task-related conditions. FMRS is a robust and reliable application of spectroscopy-based imaging (Prichard et al., 1991) and represents an important contribution to our understanding of neurometabolite shifts during task engagement.

In the past, sensitivity has been a key challenge facing MRS and fMRS research. Lack of imaging sensitivity needed to collect reliable spectra in subcortical regions limited the applications of this imaging technique (Stanley & Raz, 2018). Subsequent improvements in imaging technology and accessibility, however, have afforded advancements in MRS and fMRS methodologies. Utilization of 3 Tesla (3T) and, more recently, 7 Tesla (7T) magnetic resonance imaging (MRI) machines have propelled the neuroimaging community as a whole, but have been particularly transformative for the spectroscopy field. Compared to lower field strengths (generally defined as ≤ 4.5T), the

increased field strength of 7T spectroscopy offers improved signal-to-noise ratio and spectral resolution (Reid et al., 2019). The bolstered imaging sensitivity afforded by these key benefits of 7T allow for better and more reliable quantification of neurometabolites (Oeltzschner et al., 2019; Pradhan et al., 2015). Applying fMRS at ultra-high field strength, thus leveraging the increased sensitivity, to a critical brain region within the pain network (e.g., the dACC) represents a unique opportunity to characterize neurometabolic changes during acute pain administration. Such data could lead to advancements in the field of pain research.

Glutamate

The current study leveraged the aforementioned strengths of 7T fMRS to measure glutamate levels in the dACC during the acute administration of pain. Glutamate is abundant in the vertebrate nervous system (Meldrum, 2000), can be detected reliably via MRS (Graaf, 2019), and has been implicated in pain research (Archibald, MacMillan, Graf, et al., 2020). Increases in glutamate levels within multiple brain regions have been noted during experimental pain. For example, previous research has implicated glutamate release in the periaqueductal gray in the reduction of nociceptive effects in rats and mice (Rossi et al., 1994; Samineni et al., 2017). Similar research studies focusing on other brain regions (e.g., occipital cortex and brainstem nuclear complex) have reported comparable glutamate concentration changes in response to pain stimulation (Cleve et al., 2015; de Matos et al., 2017). With regard to the ACC, several studies have supported pain related changes in glutamate concentration. Mullins et al. 2005) observed a substantial increase (9.3% from baseline) in ACC glutamate concentration in response to cold pressor pain. Archibald et al. (2020)

further demonstrated increases in glutamate concentration in response to pain. In their study, Archibald and colleagues (2020) utilized a thermal pain devise to administer sustained experimental pain over several minutes. FMRS data collected at 3T from this study revealed an increase in ACC glutamate during pain administration relative to baseline. Of particular relevance to the present study, Jelen et al. (2021) observed significant increases in Glx (the combined signal of glutamate + glutamine) in the dACC in response to acute, pressure-based pain at 3T. Taken together, these data support the role of glutamate in nociceptive processes in both the ACC as well as other pain network nodes as well. Additionally, these studies demonstrate the efficacy with which fMRS can be used to study these phenomena *in vivo*. For these reasons, glutamate served as the primary neurometabolite of interest in the present study.

Present Study

This project sought to fill several gaps in current neuroimaging and pain literature. First, this project contributes to the dearth of literature in pain fMRS. Relative to fMRI, fMRS as a technique, is underrepresented in the literature, even more so with regard to fMRS studies that examine pain processing. Furthermore, the use of 7T fMRS is particularly novel given the increased sensitivity to detect neurometabolites. Second, this research applied ultra-high field fMRS to the dACC, a critical node within the pain network. Due to the relative scarcity of fMRS studies focusing on the pain network, many questions remain regarding the neurometabolic function of pain network nodes. Previous research has reliably demonstrated changes in dACC glutamate concentrations in response to painful stimulation (Archibald, MacMillan, Graf, et al., 2020); however, to my knowledge, no study to date has documented these underlying

neurochemical processes using an on/off stimulation design at 7T. We used fMRS to investigate acute pain-related changes in ACC glutamate levels among female participants. Specifically, we used a pressure-based pain device to administer pain in three conditions (i.e., baseline, low, and moderate pain). Each pain trial was accompanied by a subjective pain rating (0-10 rating: 0 = 'no pain', 10 = 'most pain possible'). Participants' evaluations of each stimulus allowed for comparisons between ACC glutamate levels and perceived pain severity.

Given the relationship between pain, glutamate, and the dACC, I hypothesized that:

1. dACC glutamate levels would be greater during (1A) low pain versus baseline, (1B) moderate pain versus baseline, and (1C) moderate pain versus low pain. To test this hypothesis, I conducted a repeated-measures, within-subjects analysis of variance (ANOVA) with the effect of condition (i.e., baseline, low, and moderate pain) as the main independent variable, and glutamate level as the dependent variable.
2. Evaluations of pain stimuli (i.e., subjective pain ratings) would be associated with changes in dACC glutamate levels. Specifically, I expected that as subjective pain ratings increase, dACC glutamate would also increase. To test this hypothesis, I conducted a simple linear regression to evaluate the relationship between dACC glutamate levels and subjective pain ratings.

Methods

Overview

Based on the literature reviewed above, I hypothesized that (i) increasing levels of acute, pressure-based pain would result in corresponding increases in dACC glutamate levels and (ii) participant ratings of the pain stimuli would predict dACC glutamate levels. A dual timepoint study was designed to assess these hypothesized changes in glutamate. To identify eligible participants, demographic, mental health, and substance-use data were collected through online screening materials during Timepoint 1. Based on similar MR studies conducted at Auburn University (Yanes, 2020), we expected to recruit 1,250 participants during Timepoint 1. We collected fMRS data during Timepoint 2 to evaluate neurochemical changes associated with pain processing. We aimed to recruit approximately 31 healthy controls based on our power analyses for Timepoint 2. A more detailed description of each timepoint is provided below. Before data collection, the current study was preregistered through the Open Science Framework (OSF; https://osf.io/9vqy4/). OSF is a free, open platform used to preregister and share projects, data, and materials. Study procedures were approved by the Auburn University Institutional Review Board (IRB), protocol #21-073 MR 2102. All participants provided written informed consent.

Participants

Female participants between 19-30 years of age were recruited from the university and Auburn-Opelika community (please see Appendix A for recruitment materials). An *a priori* power analysis for a repeated-measures ANOVA with an α = 0.05, a medium-large effect size based on meta-analytic data on the effects of acute

pain administration on glutamate (Cohen's f = 0.25) (Mullins, 2018), and 85% power recommended a sample size of N = 31. However, due to cost restrictions, recruitment issues, and the scope of this thesis, a total N of 15 was collected. Only participants who met the following criteria were allowed to participate: 1) assigned biologically female at birth, 2) were not taking any over-the-counter or prescription medication which may cause or increase bleeding (i.e., blood thinners), 3) did not have a history of seizures, nor were they taking medication to treat seizures, 4) had not consumed drugs (including alcohol) in the 24 hours prior to the research study session, 5) had not consumed pain relievers in the 8 hours prior to the research study session, 6) had not consumed food, drinks (except water), caffeine, and/or nicotine in the 3 hours prior to the research study session, and 7) had not exercised in the 30 minutes prior to the research study session. In addition to these inclusion criteria, we also excluded participants with standard MR contraindications as determined by the MRI Pre-Entry Screening Form. Examples include, but are not limited to: implanted cardiac pacemakers, embedded metal objects/fragments, and claustrophobia. Although MR is not associated with harmful effects on pregnant women, we excluded pregnant women as a precaution. Participant eligibility was also determined from pre-screen questionnaires inquiring about mental health and substance use history (please see Appendix B). Additional eligibility criteria included 1) no dependence for alcohol and nicotine as determined by the Alcohol Dependence Scale (Doyle & Donovan, 2009) and the Nicotine Dependence Scale for Adolescents (modified to include vaporizer/e-cigarette use) (Nonnemaker et al., 2004), 2) must not have had more than three use-episodes across major drug classes in the 12-month period prior to data collection (i.e., amphetamine, benzodiazepine, cannabis,

cocaine, and heroin), and 3) must not exceed established cut-off scores for anxiety (as indicated by the Generalized Anxiety Disorder 7, score > 10) (Spitzer et al., 2006), depression (as indicated by the Patient Health Questionnaire 9, score > 10, (Kroenke et al., 2001), and/or psychosis (Prodromal Questionnaire Brief Version, score > 3) (Loewy et al., 2011). Please see Appendix B for a full list of questionnaires and survey materials used in Timepoint 1.

Demographic, mental health, and substance-use data were collected through online screening materials during Timepoint 1. Based on similar MR studies conducted at Auburn University (Yanes, 2020), we expected to recruit 1,250 participants during Timepoint 1. In total, 359 respondents completed the online Timepoint 1 pre-screen survey. We collected fMRS data during Timepoint 2 to evaluate neurochemical changes associated with pain processing. We aimed to recruit approximately 31 healthy controls based on our power analyses to Timepoint 2. In total, 16 participants were recruited and completed Timepoint 2.

Rationale for Male Exclusion

Previous research has identified differences in experimentally induced pain tolerance across sexes (Riley et al., 1998). Specifically, meta-analytic data indicate that biological females demonstrate lower pain tolerances to experimentally induced pain than their male counterparts (Wiesenfeld-Hallin, 2005). These results seem to be consistent across acute pain modalities. To reduce sample variability and maximize our power, we included only one sex in the current study. Due to greater rates of chronic pain among females (Bartley & Fillingim, 2013), as well as an existing female majority

represented in Auburn University MR research samples, we chose to include females in the present study.

Menstrual Cycle Variance

In addition to cross-sex differences in pain tolerance, meta-analytic data indicate that pain-tolerance may fluctuate as a function of the current menstrual phase in female participants (Riley et al., 1999). These data suggest that pain tolerance is lowest during participants' follicular, or pre-ovulation, phase. With the previous findings in mind, all data in the current study were collected from participants during the luteal phase of their cycles, as determined by estimations from self-report data. Given that the luteal phase is the least variable menstrual phase by duration across individuals (Reed & Carr, 2000), data was collected between days 19 and 25 of their cycles (this window is well within the luteal phase that is observed in typical cycle length) (Lenton et al., 1984). These procedures were implemented to reduce any extraneous influences on pain perception not due to the study procedures. Additionally, our within-subject design also minimized confounding factors.

Materials

Timepoint 1. During Timepoint 1, the following scales and inventories were used to characterize participants and determine eligibility (please see Appendix B): demographics questionnaire, Warwick-Edinburgh Well-Being Scale (WEMWBS) (Tennant et al., 2007), Perceived Stress Scale (PSS) (Lee, 2012), Generalized Anxiety Disorder – 7 (GAD7), Patient Health Questionnaire – 9 (PHQ9), Prodromal Questionnaire - Brief Version (PQ-B), Graded Chronic Pain Scale (Von Korff et al., 1992), Neuropathic Pain Scale (Galer & Jensen, 1997), modified versions of the

Severity of Dependence Scale (SDS) (Gossop et al., 1995), the Alcohol Dependence Scale (Doyle & Donovan, 2009), and the Nicotine Dependence Scale for Adolescents (Nonnemaker et al., 2004). These scales cover several major drug classes including cannabis, opioids, cocaine, amphetamine, and prescription psychomotor stimulants (e.g., Adderall). Additionally, the mental health inventories were used to screen and/or characterize participants with regard to a number of disorders including anxiety, psychosis, and depression. Participants who met inclusion criteria at Timepoint 1 were invited to complete a neuroimaging data collection session during Timepoint 2. Below are brief descriptions of the inventories that we used during Timepoint 1:

a. Warwick-Edinburgh Well-Being Scale (WEWBS; Tennant et al., 2007) – This scale uses positively-worded items to determine mental well-being. The WEMWBS uses items on a 1-5 Likert scale, with a minimum score of 14 and a maximum of 70 (scores <42 indicate generally poor mental health). The scale was validated in student and general populations, as well as focus groups.
b. Perceived Stress Scale (PSS; Lee, 2012) – This scale includes questions regarding how unpredictable or uncontrollable respondents find events in their lives to be on a 0-4 Likert scale. With a minimum score of 0 and a maximum score of 40, the PSS measures current and past stress levels over the last 30 days. A score of >20 indicates high levels of stress.
c. Generalized Anxiety Disorder – 7 (Spitzer et al., 2006) – This scale contains 7 questions related to symptomology associated with anxiety. GAD7 questions use a 0-3 Likert scale to assess feelings of worry, stress, and anxiety. Scoring

>15 is indicative of high anxiety. Spitzer and colleagues (2006) validated this scale in primary care, community, and acute psychiatric settings.

d. Patient Health Questionnaire – 9 (PHQ9; Kroenke et al., 2001) – This scale is used to gather data about depression levels in respondents. The PHQ9 uses 9 items of established depression criteria on 0-3 Likert scales. Scoring >15 is indicative of depression. Kroenke and colleagues (2001) showed this scale to have comparable sensitivity to lengthier depression inventories.

e. Prodromal Questionnaire - Brief Version (Loewy et al., 2011) – The PQ-B is used to identify symptoms and risk of psychosis in respondents. This scale uses 21 yes/no questions relating to abnormal perceptual experiences and thought processes. If the answer to an original question was “yes,” each question has a 5-point Likert scale follow up question to determine the extent to which respondents find a particular experience to be frightening. Scores >6 indicate higher likelihood of psychosis.

f. Graded Chronic Pain Scale (GCPS; Von Korff et al., 1992) – This scale is used to assess the recency and severity of chronic pain in general populations as well as primary care settings. There are 6 questions in total, all of which focus on the degree to which chronic pain interferes with everyday life. All questions are answered on a 0-10 scale (“0” = no pain/change/interference, “10” = extreme pain/change/interference). Respondents are categorized into one of five pain categories (0-4). Placement in any category other than 0 indicates some degree of chronic pain.

g. Neuropathic Pain Scale (NPS; Galer & Jensen, 1997) – This scale was developed to assess pain characteristics distinctly associated with neuropathic pain. The NPS asks 5 questions relating to several potential characteristics of respondents' pain including intensity, temperature, and sharpness/dullness. These questions are answered on a 0-10 scale ("0" = pain is not intense/sharp/dull/hot/cold, "10" = pain is extremely intense/sharp/dull/hot/cold). Scores >0 indicate some degree of chronic pain.
h. Severity of Dependence Scale (SDS; Gossop et al., 1995) – This scale provides a short, flexible way of assessing potential dependence across drug classes. The questions are written such that any drug class may be substituted for another (e.g., "have you consumed 'x' drug more than 3 times in the last 12 months?"). Given that the current study did not have a Certificate of Confidentiality, we only used this scale to determine if participants used a particular substance more than 3 times in the last year. This yes/no style question afforded us the ability to determine participant eligibility without inquiring about specific information regarding illicit substance use behavior. This modification of the SDS was approved by the Auburn University IRB.
i. Alcohol Dependence Scale (Doyle & Donovan, 2009) – This 25 question scale was used to characterize respondent's alcohol consumption. The ADS assesses signs of alcohol use disorder as well as measures the extent to which a respondent is dependent on alcohol. The scale consists of a series of yes/no and 3-point Likert scale (1 = "no", 2 = "sometimes", 3 = "almost every

time I drink") questions regarding alcohol use behavior. Scores >13 indicate intermediate alcohol dependence.

j. Nicotine Dependence Scale for Adolescents (Nonnemaker et al., 2004) – This 6-item scale is used to assess the extent to which respondents are dependent on nicotine. I modified this scale to include vaporizer/e-cigarette use, as the original scale did not include them. The NDSA uses a series of Likert-type questions to determine frequency and duration of nicotine use. Scores >6 indicate moderate nicotine dependence.

Timepoint 2. Individuals who met inclusion criteria, as determined by their responses to Timepoint 1 questionnaires, were invited via email to participate in a neuroimaging session (please see Appendix A). Neuroimaging data collection was carried out on the Siemens 7T MAGNETOM at the Auburn University MRI Research Center. The scanner was outfitted with a 32-channel head coil provided by Nova Medical (Wilmington, MA). The fMRS procedure is detailed below. In brief, in-scanner pain testing involved an MR-compatible pressure-based pain apparatus. Pain ratings were collected via a numeric rating scale, and answered via an MR-compatible device. The rating scale ranges from 0-10, such that 0 indicates "no pain" and 10 indicates "most pain possible." A post-experiment questionnaire and a shortened St. Mary's Hospital Sleep Questionnaire (SMHSQ) (Ellis et al., 1981) to assess sleep the previous night was administered, as sleep quality has been shown to impact neuroimaging outcomes (Chee & Chuah, 2008; Ma et al., 2015). As a quality assurance measure, we performed outlier analysis on SMHSQ data to determine if any participants reported poor or no sleep the night prior to Timepoint 2. No outliers were identified in the SMHSQ data, thus we determined sleep

patterns did not impact our fMRS outcomes. Please see Appendix C for a full list of materials used in Timepoint 2.

Procedure

Scanner Preparation

Upon arrival at the Auburn University MRI Research Center, participants completed the MRI Pre-Screen Entry Form and written, informed consent form was obtained (Appendix C). Once participants consented to the study procedures, they changed into surgical scrubs, were weighed (a common safety practice in MR studies related to the Specific Absorption Rate (SAR) of radio frequencies (Baker et al., 2004)), and swept for metal using a hand-held metal detector. Participants laid on the scanner table and were given earplugs that function as speakers through which verbal task instructions were delivered, an MR-compatible mouse which was used to provide subjective pain ratings, and a squeeze ball which was used in the event of an emergency if the participant needed to talk to the investigator immediately at any point during data collection. Once all other scanner preparation steps had been completed, the pain device (described below) was fastened to participants' non-dominant hand at which point researchers proceeded with several practice pain trials (described below) to determine each participants' individual pressure levels to be used during the task.

Pain Stimulation

During fMRS data acquisition, we asked participants to undergo pain stimulation across three conditions (no pain, low pain, and moderate pain) using an MR-compatible, pressure-based pain apparatus. This apparatus has been validated and used in other Auburn University research (Davis et al., 2016; Yanes, 2020) (Figure 1). The apparatus

was fastened to participants' non-dominant hand as part of the scanner preparation procedure prior to entering the scanner. The pressures to be used for each participants' pain conditions were determined via several 'practice' trials. Practice trials involved delivering increasing pressure to the apparatus until the participant indicated the pain was too uncomfortable to continue, at which point the device was deflated immediately. This process was performed three times to calculate an average maximum pain tolerance. The pressure amount for 'low' and 'moderate' pain conditions was calculated as 33% and 66% of each participant's maximum pain tolerance, respectively. These values are based on previous research using the same apparatus in similar conditions (Yanes, 2020). For example, if a participant's pain tolerance was measured to be 100 mm/Hg, their low and moderate pain pressure amounts would be 33 mm/Hg and 66 mm/Hg respectively (note: all participants 'baseline' conditions were 0 mm/Hg). At any time during the actual fMRS experiment, if participants indicated that the pain had become "too uncomfortable to continue" via the squeeze ball, we relieved pressure immediately. Additionally, any participant whose pain tolerance exceeded the maximum pressure of the device would have been considered an outlier and excluded from fMRS data collection. No participant enrolled in the study exceeded this limit or stopped data collection early due to discomfort caused by the pain device.

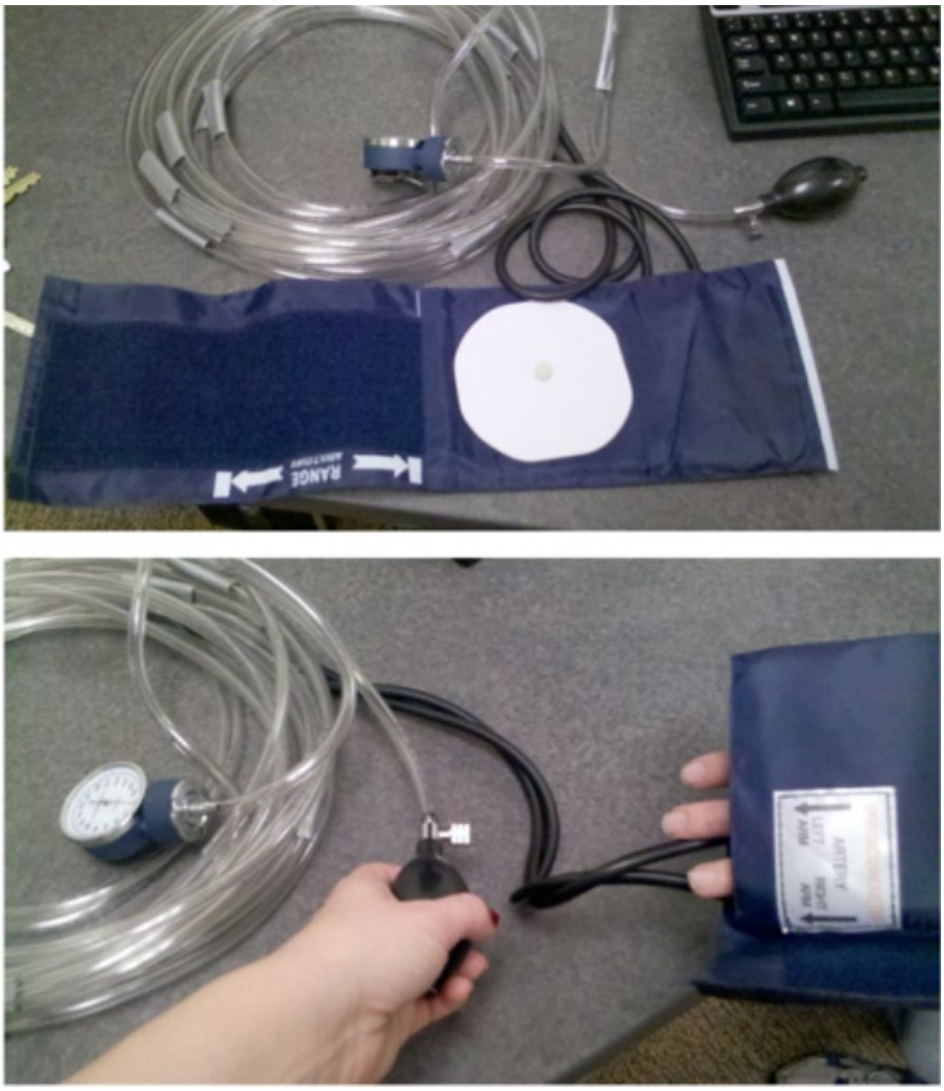

Figure 1. Picture of the pain apparatus. The pain apparatus consists of a standard blood pressure cuff surrounding a small plastic disc with a small point that fits between participants' first and second fingers.

fMRS Data Collection

To characterize pain-related metabolite levels, we collected fMRS data during scanning blocks consisting of pseudo-randomized trials (Figure 2). These trials corresponded to three separate pain conditions: 'no pain', 'low pain', and 'moderate pain.' There was also a baseline fMRS scan prior to the pain task that consisted of 260 seconds of data collection at rest with no stimulation. Data from this scan was used for participants' baseline metabolite levels in statistical analysis (i.e., their 'no pain'

condition). Scanning during the pain task blocks consisted of 15 40-second trials for a total duration of approximately ten minutes (600 seconds). Each trial was divided further into four 10-second phases (Figure 3). The trials consisted of the following phases: 1) the 'ramp up' phase (pressure increased to target level), 2) the 'pain' phase (pressure persisted at target level), 3) the 'off ramp' phase (pressure decreased to 0), 4) and the 'eval' phase (participants gave subjective pain ratings). The trial order was predetermined such that an equal number of trials of each pain condition were interspersed among the total number of trials. Trials proceeded in the following order in repetition for all participants: (1) high pain, (2) no pain, and (3) low pain. Inclusion of the 'off-ramp' and 'eval' phases, as well as 'no pain' trials, allowed participants to recover from painful stimulation. During the evaluation ('eval') phase, participants completed subjective pain ratings (0 = "no pain"; 10 = "most pain possible") regarding the previous trial. We collected fMRS data from one voxel (40 × 25 × 15 mm), centered around the dACC, using a standard STEAM sequence (TR/TE = 10000/5 ms) consisting of three slice-selective 90° pulses (Zhu & Barker, 2011) (Figure 3). We also acquired structural images to aid in fMRS voxel placement (MPRAGE, TR/TE = 2200/2.96ms, flip angle = 7°, GRAPPA acceleration factor = 2, FOV = 224 × 224 mm, base/phase resolution = 384/100%, voxel size = 0.7 mm^3 isotropic resolution, slices = 256, sagittal acquisition). Following shimming with FASTESTMAP (fast, automatic shim technique using echo-planar signal readout for mapping along projections) to enhance magnetic field homogeneity, we acquired single spectra continuously every 10 seconds across trials.

Of note, this project also used a similar task design to collect fMRS data in the primary somatosensory cortex (SI) for the purposes of an exploratory assessment. This

sequence used a 20 × 20 × 20 mm voxel centered around the right SI contralateral to the nondominant hand on which the pain apparatus was placed (Figure 4). Precise voxel placement was determined via comparison to similar previous research (Bhattacharyya et al., 2011) as well as known organization of human sensory and motor areas. This task did not include low pain trials, only moderate pain and baseline due to the exploratory nature and time constraints. Aside from voxel size and placement, the fMRS sequence details were the same as those detailed above for the dACC.

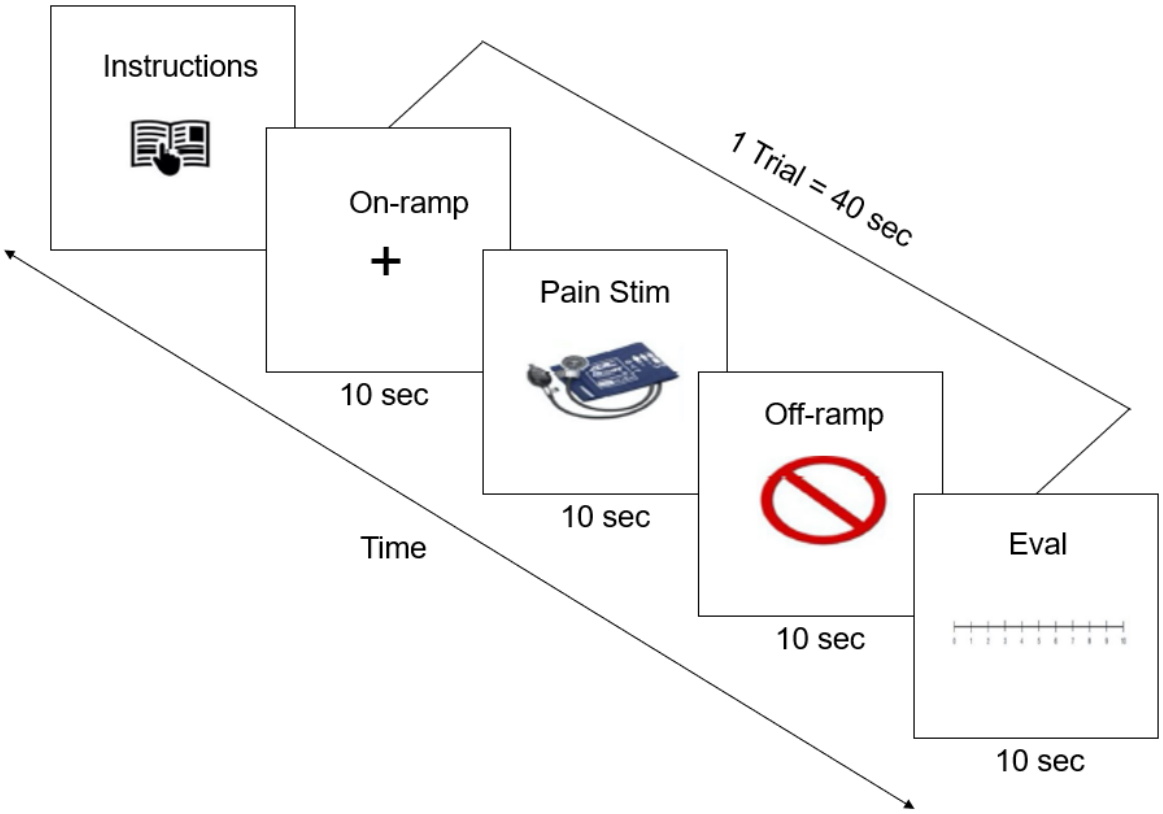

Figure 2. FMRS pain trial. Trial structure is the same in low, moderate, and no pain conditions.

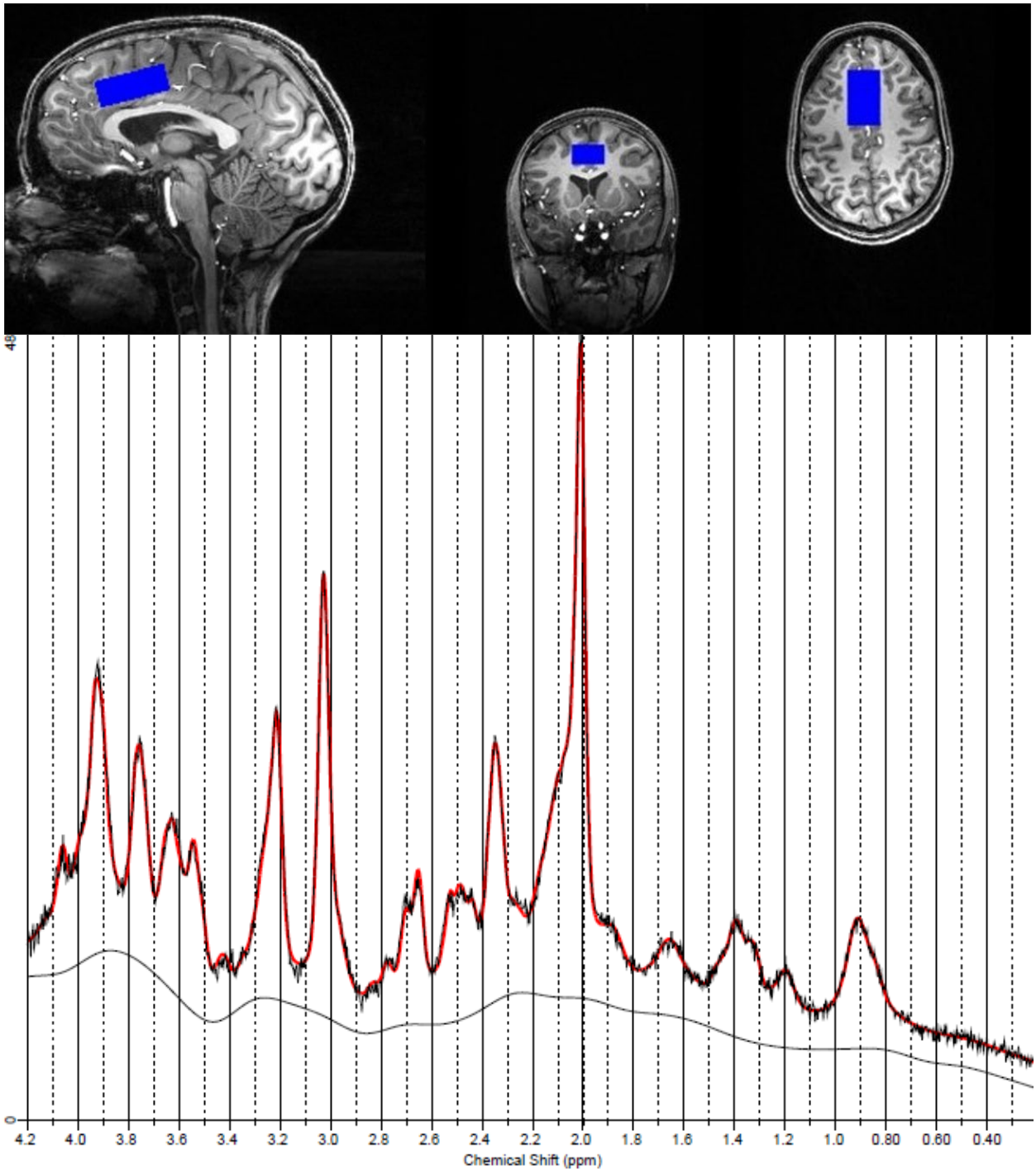

Figure 3. Anatomically informed dACC fMRS voxel placement and LCModel sample spectrum. Participant-level high-resolution structural images were used to guide placement of the 40 × 25 × 15 mm voxel around the bilateral dACC.

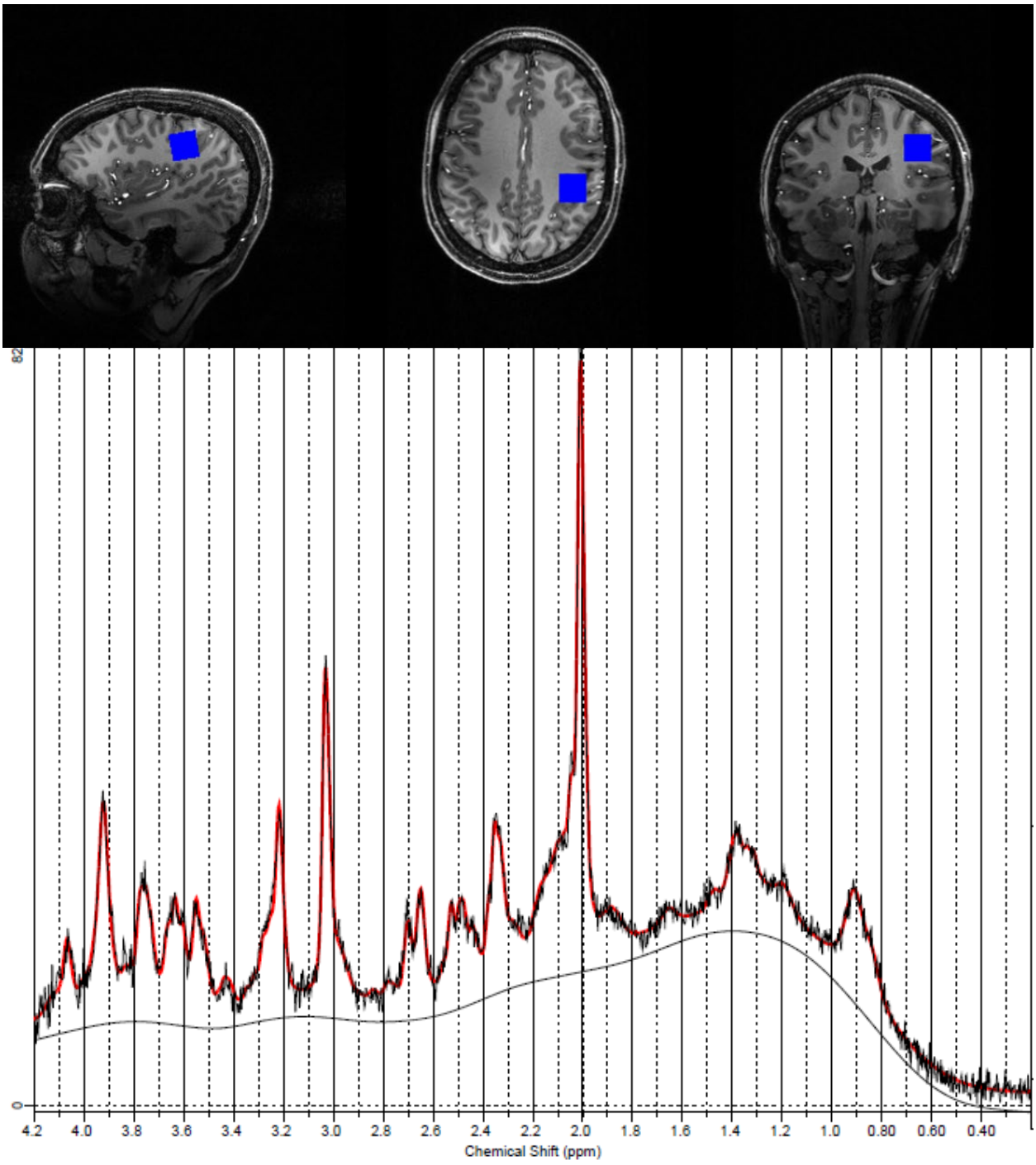

Figure 4. Anatomically informed SI fMRS voxel placement and LCModel sample spectrum. Participant-level high-resolution structural images were used to guide placement of the 20 × 20 × 20 mm voxel in the right SI contralateral to participants' nondominant hands.

Analytic Plan

Below, I outline the analytic plan for my hypotheses:

Hypothesis 1 (1A-C): To test whether dACC glutamate levels increased with respect to pain levels, I conducted a repeated-measures, within-subjects ANOVA with pain level as the within-subjects variable of interest. Including pain condition as a three-level factor produced three estimates: (1) mean glutamate level under 'baseline' conditions, (2) change from 'baseline' to 'low pain', and (3) change from 'baseline' to 'moderate pain.' Outliers were defined as any data point which fell outside the upper and lower bounds created by multiplying the interquartile range (IQR) of the dataset by a step value of 1.5. No outliers were identified using this method.

Hypothesis 2: To test whether subjective pain ratings were predictive of changes in dACC glutamate levels, I performed a simple linear regression to evaluate the relationship between ratings of pain stimuli (predictor variable) and dACC glutamate levels (outcome variable).

fMRS Data Preprocessing

FMRS data was processed using LCModel software (version 6.3-1R), a program used to quantify proton MR spectra *in vivo* (Provencher, 2001). LCModel estimates observed spectra based on known values, either from simulations or from aqueous metabolite solutions (i.e., phantom solutions with known metabolite levels) (Provencher, 1993). These 'model' spectra form basis sets within LCModel. Our study utilized an existing basis set from Meredith Reid at the Auburn University MRI Research Center related to metabolites detectable via proton ^{1}H MR Spectroscopy (Reid et al., 2022).

These metabolites include, but are not limited to, aspartate, creatine, GABA, glucose, glutamine, glutamate, lactate, *N*-acetyl aspartate (NAA), phosphocholine, taurine, and glycine. The water-suppressed spectra were eddy current-corrected and quantified using the unsuppressed water signal. Cramer-Rao lower bounds (CRLB) were used as a measure of fit. All glutamate data fell below CRLB = 20 , a commonly accepted threshold for spectral quality (Robinson et al., 2021) (see Table 2 for a full summary of MRS quality measures). Prior to metabolite quantitation, spectra were averaged over several trials and across conditions such that individual spectra corresponding to each participants' baseline, low, and moderate pain trials were averaged into a single value for each condition (i.e., participants had one mean value for baseline, low, and moderate pain-related glutamate). Following metabolite quantitation, these data were then analyzed via repeated-measures within-subjects ANOVA. Neurometabolite data are presented as institutional units (IU).

Results

Participants

During recruitment (Timepoint 1), 359 respondents completed the online pre-screen materials. Of those, 17 participants were enrolled in Timepoint 2 of the study. One participant did not complete data collection due to discomfort inside the scanner, another participant's data was excluded due to a technical issue related to task timing within the experimental software, and one participant's subjective pain ratings could not be recorded due to a technical issue with the MR-friendly computer mouse. Thus, the final analyses included 15 healthy participants (mean age = 23.6 ±0.68 (M ± SD); 15 White (0 Hispanic/Latino)) for the Hypothesis 1 ANOVA and 14 for the Hypothesis

2 regression. Challenges associated with the novelty of the exploratory somatosensory cortex (SI) voxel resulted in an additional participant's data being unusable due to poor signal-to-noise ratio. Thus, the SI-related analyses represent a sample size of *N* = 14 (see Table 1 for a complete demographic summary).

Sample Characteristics	**Column1**
n	15
Women (%Women)	15 (100%)
Age (years)	23.6 ± 0.68
% Race (A/B/H/I/W)	(0/0/0/0/100)
% Hispanic	0
% Left Handed	0
Health	
WEWBS	54.47 ± 5.07
PSS	13.47 ± 5.11
GAD-7	4.67 ± 5.18
PHQ-9	4.67 ± 3.18
PQB	1.13 ± 1.6
Pain	54.47 ± 5.12
GCPS	0
NPS	0
Substance Use	
SDS - Amphetamines	0
SDS - Cannabis	0
SDS - Cocaine	0
SDS - Opiods	0
SDS - Stimulants	0
ADS	4.53 ± 3.85
NDSA	0
Experimental Pain	
Tolerance (mmHg)	192.1 ± 76.98
Low (mm/Hg)	63.39 ± 25.4
Moderate (mm/Hg)	126.78 ± 750.8

Table 1. Demographic information. All data are presented as M ± SD. Race and ethnicity data are Native American/Black/Asian/Hispanic or Latino/White. Health scales: Warwick-Edinburgh Well-Being Scale, Perceived Stress Scale, Generalized Anxiety Disorder 7, Patient Health Questionnaire 9, Prodromal Questionnaire Brief Version. Pain scales: Graded Chronic Pain Scale and Neuropathic Pain Scale. Substance use scales are Severity of Dependence Scale,

Alcohol Dependence Scale, and Nicotine Dependence Scale for Adolescents. Pain data: mmHg, millimeters of mercury.

MRS Quality Measures		
ACC Variables		
SNR		
Baseline	54.87	± 10.85
Low Pain	37.53	± 9.06
Moderate Pain	35.4	± 12.54
CRLB		
Baseline	1.8	± 0.41
Low Pain	2	± 0.0
Moderate Pain	2	± 0.0
FWHM		
Baseline	0.03	± 0.006
Low Pain	0.031	± 0.008
Moderate Pain	0.031	± 0.007
SI Variables		
SNR		
Baseline	20.92	± 3.82
Moderate Pain	23.17	± 4.3
CRLB		
Baseline	3.58	± 0.67
Moderate Pain	3.33	± 0.49
FWHM		
Baseline	0.035	± 0.011
Moderate Pain	0.034	± 0.006

Table 2. MRS quality measures for anterior cingulate and somatosensory cortex voxels. Variables are signal-to-noise ratio, Cramer-Rao lower bound, and full-width at half-maximum. All data are presented as M ± SD.

Glutamate (Hypothesis 1)

A repeated-measures ANOVA was conducted with glutamate levels at each condition as the within-subjects factor. There was a significant effect of condition ($F(2, 28) = 6.53$, $p = 0.005$, partial $\eta^2 = 0.318$; $Glu_{baseline} = 11.06 \pm 0.68$, $Glu_{low} = 11.51 \pm 0.90$, and $Glu_{moderate} = 11.57 \pm 0.89$ (all values M ± SD; Figure 5). Post-hoc pairwise comparisons with Bonferroni correction for multiple comparisons revealed glutamate concentrations to be significantly higher under moderate pain conditions compared to baseline ($t(14) = 3.06$, $p_{Bonferroni} = 0.026$). There was no significant difference between baseline and low-pain glutamate ($t(14) = 2.23$, $p_{Bonferroni} = 0.128$). Additionally, there was no significant difference between low- and moderate-pain glutamate ($t(14) = 1.17$, $p_{Bonferroni} = 0.784$). These changes in glutamate represent a 3.6% increase from baseline to low pain, a 4.6% increase from baseline to moderate pain, and a 0.9% increase from low to moderate pain.

Subjective Pain Rating Regression (Hypothesis 2)

Participants provided subjective pain ratings during the task for each pain stimulus at the end of every trial. These pain ratings were measured on a 1-10 scale such that 1 = ‘no pain’ and 10 = ‘most pain possible.’ Mean subjective pain ratings were $Pain_{baseline} = 1.40 \pm 0.19$, $Pain_{low} = 4.15 \pm 0.41$, and $Pain_{moderate} = 6.97 \pm 0.45$. A simple linear regression was run to predict dACC glutamate concentrations based on subjective pain ratings, however this was not significant ($F(1,40) = 0.179$, $p = 0.675$; $R^2 = 0.0045$) (Figure 6).

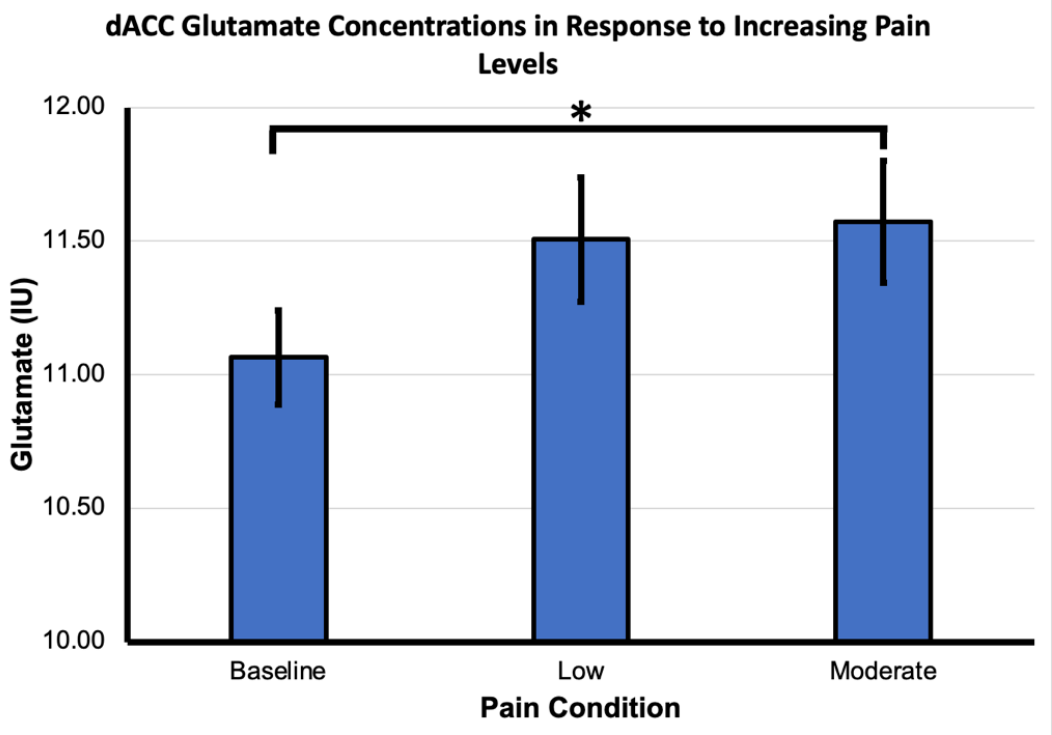

Figure 5. Mean dACC glutamate concentrations by pain condition. Error bars represent standard error. * = significant at $p_{Bonferroni} < 0.05$. IU = Institutional Units.

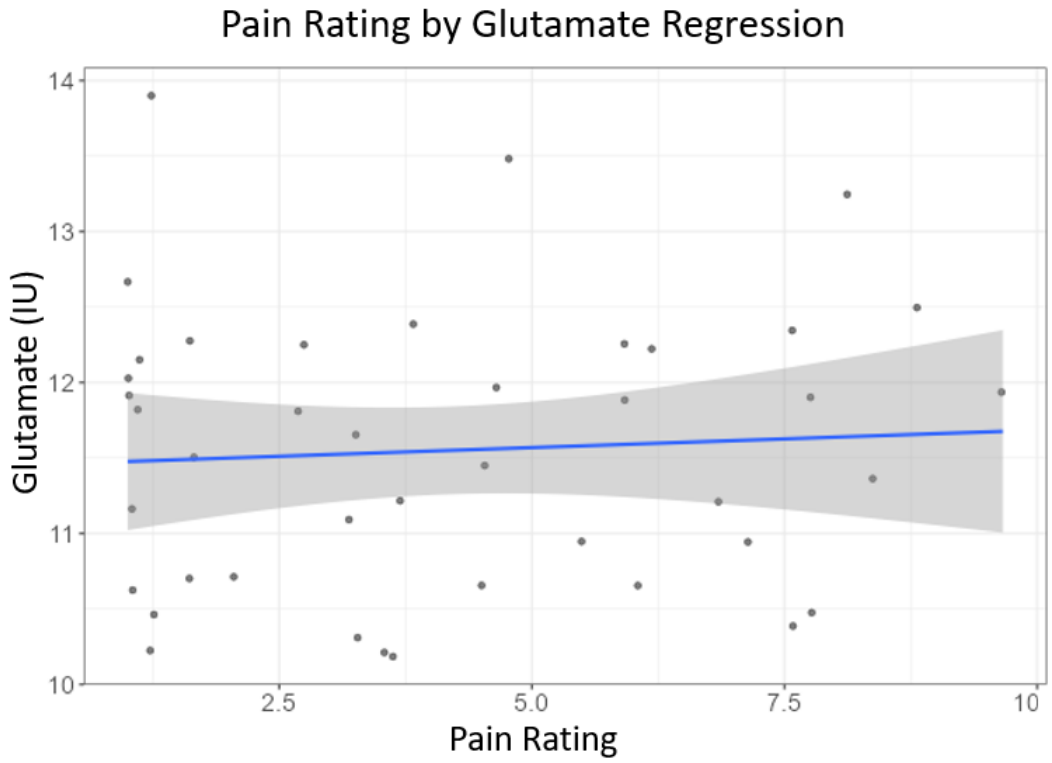

Figure 6. Subjective pain ratings regressed onto dACC glutamate concentration. $F(1,40) = 0.179$, $p = 0.675$; $R^2 = 0.0045$.

Exploratory Analyses

A two-tailed paired samples t-test was run to assess the impact of acute pain on S1 glutamate levels. Statistical analysis did not reveal a significant difference between moderate pain glutamate and no pain glutamate levels ($t(13) = 1.50$, $p = 0.158$, $d = 0.401$) ($S1\text{-}Glu_{baseline} = 9.90 \pm 1.48$ and $S1\text{-}Glu_{moderate} = 10.35 \pm 1.47$ (Figure 7). This represents a 4.3% increase from baseline to moderate pain.

Subjective pain ratings were also collected and compared to S1 glutamate concentrations following acute pain administration. Descriptive analyses revealed mean subjective pain ratings to be $S1\text{-}Pain_{baseline} = 1.42 \pm 0.09$ and $S1\text{-}Pain_{moderate} = 6.80 \pm 0.26$. We ran a linear regression to determine if S1 glutamate concentrations predicted subjective pain ratings. The regression model did not reach statistical significance ($F(1,26) = 0.441$, $p = 0.513$) with an $R^2 = 0.0167$ (Figure 8).

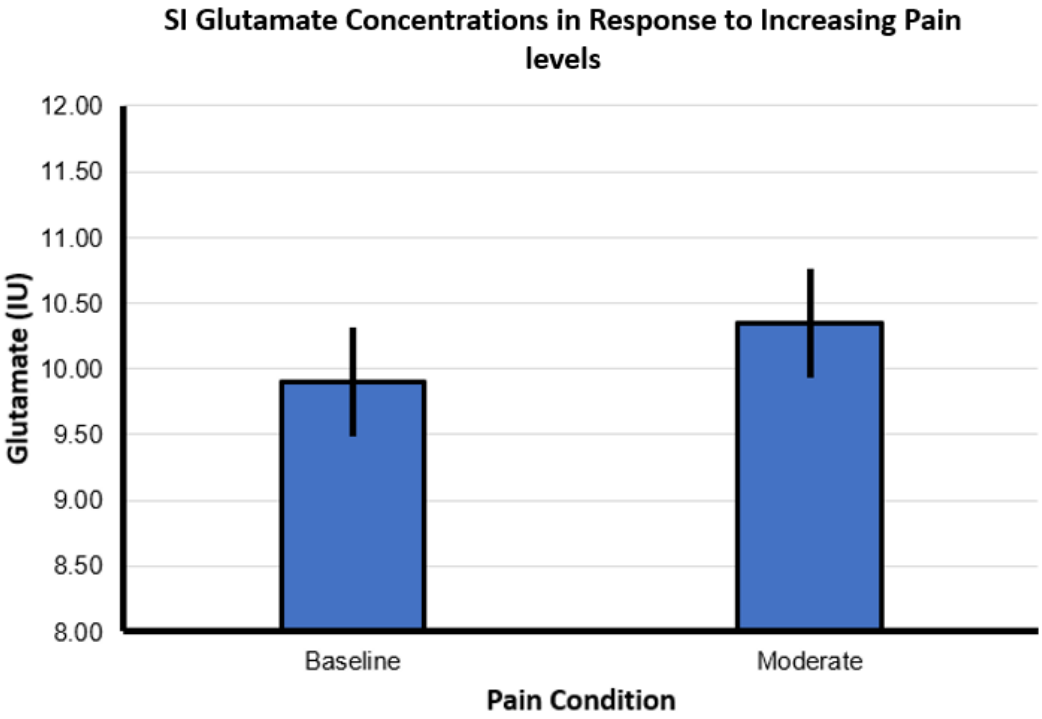

Figure 7. Mean SI glutamate concentrations by pain condition. Error bars represent standard error.

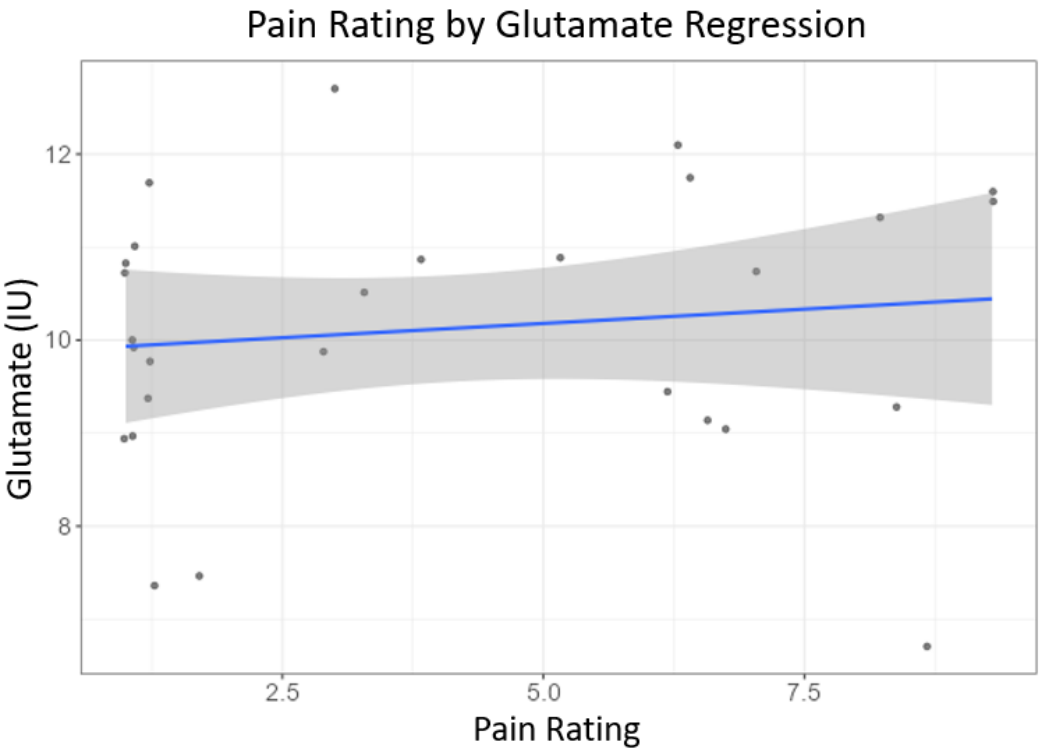

Figure 8. Subjective pain ratings regressed onto SI glutamate concentration. ($F(1,26) = 0.179$, $p = 0.675$; $R^2 = 0.0045$).

Discussion

To further our understanding of the pain processing systems within the brain, the current study used 7T fMRS to characterize the underlying neurometabolite systems of the dACC in response to acute pain. Specifically, we designed a pressure-based pain task to assess the impact of varying intensities of nociceptive stimulation on dACC glutamate. Our findings indicate that moderate levels of acute, pressure-based pain appear to increase glutamate concentration. In support of Hypothesis 1(b), a one-way repeated measures ANOVA indicated a strong effect of pain condition on subsequent glutamate concentration such that moderate levels of pain resulted in significantly higher levels of glutamate compared to baseline. Conversely, we did *not* find evidence that low levels of pain resulted in increased glutamate relative to baseline. Further, moderate and low levels of pain were not different in their effects on dACC glutamate. Thus, Hypotheses 1(a) and 1(c) were not supported. The current study also sought to assess the degree to which subjective pain ratings were predictive of changes in dACC glutamate. This was accomplished through a simple linear model which regressed glutamate concentration onto subjective pain rating which indicated that pain ratings were not predictive of changes in glutamate. Thus, we did not find support for Hypothesis 2.

The Relationship Between Glutamate and Pain

Our findings indicate that acute pain can produce meaningful increases in dACC glutamate. This is consistent with previous MRS research in neuronal pain processing according to a recent systematic review by Archibald (2020). Results from this review of fMRS studies suggest that the ACC is one of several brain regions in which acute

pain produces elevated glutamate-related metabolites (i.e., glutamate, glutamine, and glutamate + glutamine (Glx)) (Archibald, 2020). Relevant to the present study, Mullins et al. (2005) and Cleve et al. (2015) observed a 9% increase in dACC glutamate and a 22% increase in dACC Glx, respectively, when administering thermal pain. Of note, Glx represents a combination of the glutamate and glutamine signals detected via neurometabolite quantitation. Due to the difficulty of distinguishing between these two signals at lower field strengths (Ford & Crewther, 2016), some researchers simply report the combined Glx signal. Given that there is modest evidence that glutamate is the primary contributor to this signal (Ford & Crewther, 2016), researchers often opt to interpret it as glutamate. Interestingly, these researchers demonstrated greater pain-related glutamate increases than what was observed in our own data. Although the methodologies of the current and aforementioned studies were similar, they were not identical. Special note should be made of the differences in pain modalities (i.e., thermal vs. pressure pain) and administration (i.e., sustained vs transient stimulation), as these represent important differences in experimental design. Thus, direct comparisons of findings across studies must be made cautiously. Overall, our observations are in line with neuronal pain processing literature and demonstrate that current models for experimental pain-related modulation of glutamate hold true in on/off-style stimulation task designs measured at 7T field strength.

The relationship between nociception and glutamate has long been documented. Pain-related increases in glutamatergic neurotransmission have been reported as early as 1992 (Dougherty et al., 1992). This glutamatergic signaling occurs rapidly within the central nervous system’s ascending nociceptive pathway, originating

from sensory neurons in the spinal cord and spreading to multiple nodes within the CNS including the thalamus and sensory cortices (Bleakman et al., 2006). Results from a systematic review suggest strengthened glutamate neurotransmission by both ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors appears to be the driving mechanism behind the glutamatergic response to pain (Pereira & Goudet, 2019). While iGluRs seem to play a fast and facilitative role in overall neurotransmission and are expressed both pre- and postsynaptically, mGluRs, primarily subtypes II & III, are involved in the slow neuromodulatory response to glutamate and are predominantly expressed on presynaptic terminals (Mazzitelli et al., 2018). Agonists of these mGluRs subtypes throughout the pain neuraxis have been shown to produce analgesic effects (Chiechio & Nicoletti, 2012). In response to acute pain, these changes occur transiently (Bleakman et al., 2006). Conversely, these changes in relation to chronic pain occur slowly and with greater longevity due to upregulation of glutamate receptors in a process called central sensitization (Huang et al., 2006). Taken together, these findings explain our observation of acute pain-related modulation of dACC glutamate as it has been shown to play an active role in the brain's pain network (Cottam et al., 2016b; Navratilova, Atcherley, et al., 2015a), and is therefore likely involved in this elevated glutamatergic neurotransmission within the pain neuraxis.

ACC Task Engagement

Interestingly, the current study did not detect meaningful changes in dACC glutamate among the different pain conditions within the task (i.e., no-, low-, and moderate pain. Not to be confused with the baseline data in the statistical analysis of

Hypothesis 1). While we cannot be certain of the mechanism(s) that underly this observation, previous research on ACC pain processing and general task engagement may offer some insight. The current literature on neuronal pain processing suggests that the ACC is involved in several aspects of pain. Namely, the dACC appears to be heavily involved in affective and cognitive components of pain such as empathy, emotion, and aversion (Bush et al., 2000b; Devinsky et al., 1995b; Wiech & Tracey, 2009). These trends in pain-related ACC research were summarized and supported in a recent systematic review by Xiao & Zhang (2018). Among the central conclusions made by this review were (i) that the ACC is involved in both noxious and affective pain processing and (ii) the ACC is specifically involved in processing pain-related negative emotions (Xiao & Zhang, 2018). Thus, the specialized role in higher-order aspects of pain highlighted by these studies, coupled with the more generalized role in physiological pain processing, suggest an acute pain response behavior that is not driven purely by nociception.

To further this line of reasoning, modulation of ACC neurometabolite systems outside the world of pain research may be considered. Although task-based modulation of neurometabolites (i.e., fMRS) in this region is seldom reported, there is limited evidence of elevated glutamate concentrations in several non-pain related paradigms. For instance, (Taylor et al., 2015) observed significantly higher glutamate levels during a Stroop task among their healthy control group while investigating potential differences in ACC neurometabolites among participants with schizophrenia and major depressive disorder. Furthermore, (Kühn et al., 2016) also observed increases in glutamate among healthy controls during their own Stroop task,

suggesting the ACC is engaged during interference-based cognitive control tasks. Additionally, elevated glutamate levels have been reported during working memory paradigms. In a recent study to assess neurochemical differences between patients with schizophrenia and bipolar affective disorder, Jelen et al. (2019) observed significantly elevated glutamate and Glx levels during an N-back task among healthy controls. Taken together, these findings indicate that multiple behaviors other than the physiological response to pain are driving ACC activity during the task, suggesting an overall degree of general task engagement regardless of paradigm. This would explain, at least in part, why we observed elevated glutamate concentrations throughout the task, not just during pain-on conditions. This also explains the lack of support for Hypothesis 2. Despite the clear differences in pain ratings for the varying device pressures used throughout the task, these differences lacked explanatory power over subsequent glutamate levels, as these levels were consistently elevated throughout the task. It should also be noted that our sample size was relatively small. Although the within-subject design likely offset some of the power concerns associated with similar sized samples in between-subjects designs, additional participants would have ideally been recruited. Thus, general task engagement by the ACC and small sample size should both be considered when postulating explanations of the findings from the current study.

Exploratory Analyses

Our findings indicate that moderate levels of acute pain did not elicit meaningful changes in SI glutamate. Interestingly, I was unable to identify any published MRS studies which reported changes in SI glutamate in response to acute pain, and only

one study that reported glutamate changes in chronic pain (Sharma et al., 2011). On the other hand, more research has been published using fMRI to assess SI blood-oxygen-level-dependent (BOLD) changes in response to acute pain (Burns et al., 2016). Indeed, several studies have reported increased SI activation in response to experimental muscle pain both bilaterally (Nash et al., 2010a, 2010b; Niddam et al., 2002) and contralaterally (Henderson et al., 2006; Macefield et al., 2007; Takahashi et al., 2011). Additionally, and particularly relevant to the current study, Loggia et al. (2002) reported an increased contralateral BOLD response to mechanically induced acute pain. These findings, in tandem with reports of correlated excitatory-neurochemical and BOLD responses in SI as well as other cortical areas in non-pain research (Ip et al., 2019; Kiemes et al., 2021; Moon et al., 2021) may point toward an elevated glutamatergic response in SI following acute pain. If such a relationship does exist, it is possible that the current study was underpowered to detect it given the small sample size, and the study design which did not include a true baseline scan for the SI voxel prior to pain administration. We elected not to include such a baseline scan as true baseline conditions could not be achieved given that participants had already been exposed to the pain stimulus. Further, inclusion of this true SI baseline scan would have created unnecessary complications to the protocol due to the required scan order (i.e., (1) dACC baseline, (2) SI baseline, (3) dACC pain task, (4) SI pain task). The highlighted methodological differences between the present and aforementioned studies mean that any comparisons drawn between the two should be considered indirect. Therefore, any resultant interpretations and/or conclusions should be made cautiously.

Limitations & Future Directions

Results from the current study should be considered with respect to several methodological limitations. First, this study was likely underpowered based on the *a priori* power analysis. Initial estimates suggested that a sample size of $N = 31$ would be ideal to find a medium effect of pain on glutamate, if such an effect existed. While a sample size of $N = 15$ was sufficient to find a meaningful difference between moderate pain and baseline, it is possible that it was insufficient to detect extant differences between baseline to low pain, and low pain to moderate pain, in support of hypotheses 1(b) and 1(c) respectively. Future work attempting to replicate the present findings should focus on recruiting larger samples. Second, participants in the current study represent a convenience sample. Given the overrepresentation of white, secondary education students aged 19-27, our sample was not demographically representative of the general population. Future work should focus on recruiting more diverse samples to bolster generalizability. Third, and related to the previous point, the current sample consisted entirely of female participants that reported no biological sex or gender transitions. Due to reports from previous research of differences among genders in pain perception, a homogenous sample with regard to sex and gender was recruited to minimize variability. Future work may consider recruiting a more heterogeneous sample to examine sex-based differences and further increase generalizability. This would also help mitigate limitation one with regard to small sample size. Fourth, the current study employed a ‘clean sample’ recruiting strategy such that all participants enrolled in the study fell below threshold scores across mental health status. As this severely limited enrollment in the study (only 7.3% enrollment rate of screened female

applicants), and was likely a further detriment to generalizability, future work may consider allowing for select mental health diagnoses. One final future direction proposed by researchers from the current study would be to implement a multimodal acute pain design. This would allow for direct and systematic assessment of differences between acute pain modalities.

Conclusions

These data advance our understanding of the underlying neurobiological mechanisms that support normative acute pain processing in the female human brain. Specifically, we found support for elevated glutamate levels elicited by acute, pressure-based pain. Further, this phenomenon was characterized for the first time using a transient on/off stimulation design under ultra-high field strength (i.e., 7T). The present work adds to a growing corpus of literature utilizing the strengths of MRS and fMRS to investigate nociceptive processing in the human brain. By advancing our understanding of how neurometabolites respond to acute pain, the data produced by this study may contribute to the development of novel therapies for chronic pain, which remains a ubiquitous issue around the world.

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Appendix A – Materials

Appendix A – Recruitment Flyer

Earn SONA Credit, $20, and Get a Picture of Your Brain!

Auburn University MRI Research Center
Department of Psychological Sciences

Announcement – Females ages 19-30 may be eligible to participate in an MRI research study about how the brain processes pain. Participants will undergo a 75-min MRI scan while completing short tasks that involve some pain. Participants will feel some dull pain on their hand for less than 10 seconds at a time.

Get Paid!!!- Participants earn up to $20 plus 3 hours of SONA Credit. All participants can see and take a picture of their brain!

Exclusions – You may not be eligible for the study depending on the following criteria:

- Metal in your body
- Breathing, motion, or inner-ear disorders
- Claustrophobia
- Tattoos containing metal
- Non-removable piercings
- Substance use
- Mental health status

THE ACTIVTIES IN THIS STUDY ARE FOR RESARCH PURPOSES ONLY

Investigator: Steven J. Nichols (sjn0016@auburn.edu) for more info

Neuroimaging and Pain AUneuroscienceResearch@gmail.com

Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
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Neuroimaging and Pain AUneuroscienceResearch@gmail.com

Appendix A – Recruitment Email Scripts

To: RESPONDENT
From: auneuroscienceresearch@gmail.com
Subject: Participation – Neuroimaging and Pain Project, Auburn University

Hello,

Thanks very much for expressing interest in the Neuroimaging and Pain Project. The project is coordinated by investigators in the Auburn University MRI Research Center and Department of Psychological Sciences. Your participation is voluntary and you may withdraw at any time.

To determine whether you are eligible, we invite you to complete a series of online questionnaires. The total time commitment is approximately 30 minutes (or less). Once we have scored your data, an investigator will contact you about your participation. Participants enrolled in undergraduate psychology courses receive up to one research hour via Sona Systems.

Questionnaires: (shared link to online screening materials [Appendix C])

Should you have questions, please do not hesitate to contact the project's lead investigators, Steven J. Nichols (sjn0016@auburn.edu) and Dr. Jennifer L. Robinson (jrobinson@auburn.edu).

Thanks

Cognitive & Affective Neuroscience (CAN) Laboratory
Auburn University

Website: www.aucanlab.com
Email: auneuroscienceresearch@gmail.com

To: RESPONDENT
From: auneuroscienceresearch@gmail.com
Subject: Participation – Neuroimaging and Pain Project, Auburn University

Hello,

You are receiving this email because you have recently completed Phase 1 of the Neuroimaging and Pain Project. After reviewing your data, we have determined that you are eligible to complete Phase 2. As a participant, you would undergo one 75-minute MRI scan while completing short tasks that involve some pain. Your total time commitment would be approximately two hours.

Participants earn up to $20. Also, participants enrolled in undergraduate psychology courses receive up to three research hours via Sona Systems. Everyone receives a picture from their brain scan.

To schedule your appointment, click the link provided below and select the date/time that works best. Once you have selected a date/time, scroll down to click "Submit and Sign Up." Note, it is very important that you just select times that you can make.

Appointments: (shared link to anonymous scheduler with available appointment dates/times)

Should you have questions, please do not hesitate to contact the project's lead investigators, Steven J. Nichols (sjn0016@auburn.edu) and Dr. Jennifer L. Robinson (jrobinson@auburn.edu).

Thanks,

Cognitive & Affective Neuroscience (CAN) Laboratory
Auburn University

Website: www.aucanlab.com
Email: auneuroscienceresearch@gmail.com

B4. SCRIPT – RESPONSE, EXCLUDE

To: RESPONDENT
From: auneurosciencesearch@gmail.com
Subject: Participation – Neuroimaging and Pain Project, Auburn University

Hello,

You are receiving this email because you have recently completed Phase 1 of the Neuroimaging and Pain Project. After reviewing your data, we have determined that you are not eligible to complete Phase 2. We appreciate your time.

Should you have questions, please do not hesitate to contact the project's lead investigators, Steven J. Nichols (sjn0016@auburn.edu) and Dr. Jennifer L. Robinson (jrobinson@auburn.edu).

Thanks

Cognitive & Affective Neuroscience (CAN) Laboratory
Auburn University

Website: www.aucanlab.com
Email: auneurosciencesearch@gmail.com

B5. SCRIPT – IN-CLASS PRESENTATION

"Hello!"

"My name is Steven, and I am a graduate student in the Department of Psychological Sciences here at Auburn University. Today, I am here to tell you about the Neuroimaging and Pain Project."

"We are recruiting females, ages 19-30, to participate in a neuroimaging research project on pain. As a participant, you'd undergo one 75-minute MRI scan (7 Tesla), while completing short tasks that involve some pain. Your total time commitment would be approximately two hours."

"Participants earn up to $20. Also, participants currently enrolled in undergraduate psychology courses receive up to three research hours via Sona Systems. Finally, everyone receives a picture from their brain scan."

"Participants cannot participate if they have: metal in their body (except for dental work), breathing or motion disorders, inner-ear disorders, claustrophobia, tattoos that contain metal, piercings that cannot be removed, and current pregnancy."

"If you would like to participate, please use the contact information shown on the screen."

"Thanks for your attention!"

B6. SCRIPT – CONFIRMATION

To: RESPONDENT
From: auneuroscienceresearch@gmail.com
Subject: Participation – Neuroimaging and Pain Project, Auburn University

Hello,

This is to confirm your scanning session on [DATE] from [START TIME] to [END TIME] at the Auburn University MRI Research Center [560 Devall Drive, Auburn, Alabama, 36849], in associated with the Neuroimaging and Pain Project.

As a reminder, participants earn up to $20. Also, participants enrolled in undergraduate psychology courses receive up to three research hours via Sona Systems. Everyone receives a picture from their brain scan.

Please review the following pre-scan checklist. Only participants who meet the following criteria are allowed to scan: (2) are not taking any over-the-counter or prescription medication which may cause or increase bleeding, (3) have no history of seizure, (4) are not taking medication to treat seizure, (5) have not consumed drugs (including alcohol) in the 24-hour period prior to the research study session, (6) have not consumed pain relievers in the 8-hour period prior to the research study session, (7) have not have consumed food, drinks (except water), caffeine, and/or nicotine in the 30-minute period prior to the research study session, and (8) have not have exercised in the 30-minute period prior to the research study session. We will review this checklist again before your scan.

Should you have questions, please do not hesitate to contact the project's lead investigators, Steven J. Nichols (sjn0016@auburn.edu) and Dr. Jennifer L. Robinson (jrobinson@auburn.edu).

Thanks

Cognitive & Affective Neuroscience (CAN) Laboratory
Auburn University

Website: www.aucanlab.com
Email: auneuroscienceresearch@gmail.com

Appendix B – Timepoint 1 Materials

Appendix B – Timepoint 1 Pre-Screen Survey

You are invited to participate in a research study examining how the human brain processes pain. This research study is being conducted by Steven J. Nichols, Graduate Research Assistant at Auburn University, and Dr. Jennifer L. Robinson, Associate Professor at Auburn University. You were selected as a possible participant because you expressed interest via email or Sona Systems.

What will be involved if you participate? If you decide to participate in Part 1 of this research study, you will be asked to complete online questionnaires. The . questionnaires will relate to mental health, physical health, and substance use. Completing these questionnaires should take 30 minutes. Based on their responses to specific questions, some participants may be eligible to participate in Phase 2 of this research study, which involves an MRI scanning session.

Are there risks or discomforts? The risks associated with participating in Phase 1 of this research study are that you experience emotional distress that could result from thinking about certain topics (e.g., mental health, pain). If you find yourself experiencing distress, you may discontinue participation at any time. Should you decide to discontinue, you would receive research hours via Sona Systems that correspond to time spent completing the questionnaires. If you wish to speak with someone about your distress, a reference list of resources in the Auburn-Opelika area will be available following the questionnaires. Also, you can request of copy of the reference list by contacting the investigators listed on this letter.

There are also risks associated with confidentiality breaches. To minimize this risk, only investigators have access to data obtained in connection with the research study that can be identified as belonging to you. If you decide to withdraw, you may withdraw any data that has been collected as long as it is identifiable. You will be assigned a participant number so that your name and other pieces of identifying information are not directly associated with data collected. All data, including your responses to these questionnaires, will be associated with that participant number. Following data collection completing, any/all links to identifiable information will be

destroyed. The results of this study may be presented in a professional venue, such as a journal or conference. In such an event, group data will be presented.

Are there benefits to yourself or others? If you participate in Phase 1 of this research study, you can expect to receive no direct personal benefits.

Will you receive compensation? During Phase 1, you will be compensated for participation with one research hour via Sona Systems. Your instructors should assign specific values of course credit to these hours. Please check with your instructors for more information. During Phase 2, you will be compensated for participation with three research hours via Sona Systems. Moreover, during Phase 2, you will be compensated $5 for showing up to your MRI scanning session. Furthermore, you will receive $5 for every 30-minute block you are inside the scanner. The total compensation will be $10 for 0-30 minutes of scanning, $15 for 30-60 minutes of scanning, and $20 for 60-90 minutes of scanning. If you volunteered through Sona Systems, you will be compensated for participating with three research hours.

Are there costs? If you decide to participate in this research study, you will not incur any costs. If you require medical attention, you will be responsible for all costs for medical attention/treatment.

If you change your mind about participating, you can withdraw from the research study at any time. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate will not jeopardize your relationship with Auburn University, or any associated/affiliated department, center, or office.

If you have questions about this research study, please ask them now. Alternatively, you can contact Steven J. Nichols, at sjn0016@auburn.edu, or Dr. Jennifer L. Robinson, jrobinson@auburn.edu, who are the research study investigators. A copy of this document will be given to you for your records at your request.

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board by phone (334)844-5966 or email at hsubjec@auburn.edu or IRBchair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY.

You may print a copy of this information letter to keep for your records.

● I have reviewed the information letter and would like to continue with Phase 1.

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Again, your privacy will be protected. Before completing Phase 1, you will be asked to provide your email address. We will use provided email addresses to contact participants about Phase 2. Investigators that oversee this project are required by the Auburn University Institutional Review Board not to disclose any information you provide in this study that could identify you as a participant, or any other personal information you may reveal. This protects you, as well as the investigators, from legal action(s) that could be associated with reporting illicit activities (e.g., substance use, etc.).

Please provide your email address in the space below so that we may contact you about Phase 2.

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How old are you? Use the slider below to indicate your age.

0 10 20 30 40 50 60 70 80 90 100

Age

Which of the following best describes your sex?

- Male
- Female

Which of the following best describes your gender?

- Male
- Female
- Non-Binary
- Non-Conforming

How would you describe yourself? Please select one that best describes you.

- American Indian or Alaska Native
- Asian or Asian American
- Black or African American
- Hawaiian or Pacific Islander
- White

How would you describe yourself? Please select one that best describes you.

- ○ Hispanic or Latino
- ● Non-Hispanic or Non-Latino

How would you describe yourself? Please select one that best describes you.

- ○ Student
- ○ Full-time employed
- ○ Part-time employed
- ○ Out of work for more than one (1) year
- ○ Out of work for less than one (1) year
- ○ Retired
- ○ Unable to work

What is the highest education level that you've achieved?

- ○ Never attended school or only attended kindergarten
- ○ 1st - 8th grade (i.e., elementary school)
- ○ 9th - 11th grade (i.e., some high school)
- ○ 12th grade of GED (i.e., high school graduate)
- ○ Currently enrolled in undergraduate program
- ○ Completed undergraduate program
- ○ Currently enrolled in graduate program
- ○ Completed graduate program

What is the primary language you speak at home?

- ○ English
- ○ Spanish
- ○ Other

Are you currently taking medication for psychological/psychiatric conditions (e.g., Xanax, Adderall, etc.)?

- ○ Yes
- ○ No

Do you have a current diagnosis of a psychological/psychiatric condition (e.g., Anxiety, ADHD, etc.)

- ○ Definitely yes
- ○ Probably yes
- ○ Might or might not
- ○ Probably not
- ○ Definitely not

Have you previously received counseling or psychotherapy?

- ○ Yes
- ○ No

Have you ever been hospitalized for psychological/psychiatric reasons?

- ○ Yes
- ○ No

Has someone from your family (i.e., parents, grandparents, siblings, other relatives) been diagnosed and/or treated for psychological/psychiatric conditions?

- ○ Yes
- ○ No

Please indicate your preferences in the use of hands in the following activities or objects

	Always left	Usually Left	Both Equally	Usually Right	Always Right
Writing	○	○	○	○	○
Throwing	○	○	○	○	○
Toothbrush	○	○	○	○	○
Spoon	○	○	○	○	○

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Warwick-Edinburgh Well-Being Scale

The following questions relate to your sense of well-being. Please select the response that best describes your experience in the last two weeks.

I've been feeling optimistic about the future.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling useful.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling relaxed.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling interested in other people.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've had energy to spare.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been dealing with problems well.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been thinking clearly.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling good about myself.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling close to other people.

- ○ None of the time
- ○ Rarely
- ○ Some of the time
- ○ Often
- ○ All of the time

I've been feeling confident.

- None of the time
- Rarely
- Some of the time
- Often
- All of the time

I've been able to make up my own mind about things.

- None of the time
- Rarely
- Some of the time
- Often
- All of the time

I've been feeling loved.

- None of the time
- Rarely
- Some of the time
- Often
- All of the time

I've been I've been interested in new things.

- None of the time
- Rarely
- Some of the time
- Often
- All of the time

I've been feeling cheerful.

- None of the time
- Rarely
- Some of the time
- Often
- All of the time

Perceived Stress Scale – 4

The following questions ask about your thoughts/feelings. In each case, please select the answer that best describes how you've felt during the last month (i.e., approximately the last 30 days).

In the last month, how often have you felt that you were unable to control the important things in your life?

- ○ Never
- ○ Almost Never
- ○ Sometimes
- ○ Fairly Often
- ○ Very Often

In the last month, how often have you felt confident about your ability to handle your personal problems?

- ○ Never
- ○ Almost Never
- ○ Sometimes
- ○ Fairly Often
- ○ Very Often

In the last month, how often have you felt that things were going your way?

- ○ Never
- ○ Almost Never
- ○ Sometimes
- ○ Fairly Often
- ○ Very Often

In the last month, how often have you felt that difficulties were piling up so high that you could not overcome them?

- ○ Never
- ○ Almost Never
- ○ Sometimes
- ○ Fairly Often
- ○ Very Often

Generalized Anxiety Disorder – 7

Over the last 2 weeks, how often have you been bothered by the following problems?

Feeling nervous, anxious, or on edge

- ○ 0. Not at all
- ○ 1. Several days
- ○ 2. More than half the days
- ○ 3. Nearly every day

Not being able to stop or control worrying

- ○ 0. Not at all
- ○ 1. Several days
- ○ 2. More than half the days
- ○ 3. Nearly every day

Worrying too much about different things

- ○ 0. Not at all
- ○ 1. Several Days
- ○ 2. More than half the days
- ○ 3. Nearly ever day

Trouble relaxing

- ○ 0. Not at all
- ○ 1. Several days
- ○ 2. More than half the days
- ○ 3. Nearly every day

Being so restless that it is hard to sit still

- ○ 0. Not at all
- ○ 1. Several days
- ○ 2. More than half the days
- ○ 3. Nearly every day

Becoming easily annoyed or irritable

- ○ 0. Not at all
- ○ 1. Several days
- ○ 2. More than half the days
- ○ 3. Nearly every day

Feeling afraid as if something awful might happen

- ○ 0. Not at all
- ○ 1, Several days
- ○ More than half the days
- ○ Nearly every day

Patient Health Questionnaire

Your answers to the questions below will help the research understand any health-related problems you may have. Please answer each question to the best of your abilities.

During the last four (4) weeks, how much have you been bothered by any of the following problems?

	Not bothered	Bothered a little	Bothered a lot
Stomach pain	○	○	○
Back pain	○	○	○
Pain in your arms, legs, or joints (knees, hips, etc.)...	○	○	○
Feeling tired or having little energy	○	○	○
Trouble falling or staying asleep, or sleeping too much	○	○	○
Menstrual cramps or other problems with your period	○	○	○
Pain or problems during sexual intercourse	○	○	○
Headaches	○	○	○
Chest pain	○	○	○
Dizziness	○	○	○
Fainting spells	○	○	○
Feeling your heart pound or race	○	○	○
Shortness of breath	○	○	○
Constipation, loose bowels, or diarrhea	○	○	○
Nausea, gas, or indigestion	○	○	○

Over the last two (2) weeks, how often have you been bothered by any of the following problems?

	Not at all	Several days	More than half the days	Nearly every day
Feeling nervous, anxiety, or on edge	○	○	○	○
Not being able to stop or control worrying	○	○	○	○
Worrying too much about different things	○	○	○	○
Trouble relaxing	○	○	○	○
Bring so restless that it is hard to sit still	○	○	○	○
Becoming easily annoyed or irritable	○	○	○	○
Feeling afraid as if something awful might happen	○	○	○	○

Questions about anxiety attacks. If you've never had anxiety attacks, you should select "no" for every answer.

	No	Yes
A. In the last four (4) weeks, have you had an anxiety attack - suddenly feeling fear or panic?	○	○
Has this ever happened before.	○	○
Do some of these attacks come suddenly out of the blue - that is, is situations where you don't expect to be nervous or uncomfortable.	○	○
Do these attacks bother you a lot or are you worried about having another attack.	○	○
During your last bad anxiety attack, did you have symptoms like shortness of breath, sweating, or your heart racing, pounding, or skipping?	○	○

Over the last two (2) weeks, how often have you been bothered by any of the following problems?

	Not at all	Several days	More than half the days	Nearly every day
Little interest or pleasure in doing things.	○	○	○	○
Feeling down, depressed, or hopeless.	○	○	○	○
Trouble falling or staying asleep, or sleeping too much.	○	○	○	○
Feeling tired or having little energy.	○	○	○	○
Poor appetite or overeating.	○	○	○	○
Feeling bad about yourself - or that you are a failure or have let yourself or your family down.	○	○	○	○
Trouble concentrating on things, such as reading the newspaper or watching television.	○	○	○	○
Moving or speaking so slowly that other people could have noticed? Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual.	○	○	○	○
Thoughts that you would be better off dead a lot more than usual.	○	○	○	○

If you said that you've experienced any of the problems in this questionnaire, describe how difficult these problems have made it for you to do your work, take care of things at home, or get along with other people.

- ○ Not difficult at all
- ○ Somewhat difficult
- ○ Very difficult
- ○ Extremely difficult
- ○ I haven't experienced any of the problems in this questionnaire

Prodromal Questionnaire

Please indicate whether you have had the following thoughts, feelings, and experiences in the past month by checking "yes" or "no" for each item. Do not include experiences that occur only while under the influence of alcohol, drugs, or medications that were not prescribed to you. If you answer "yes" to an item, also indicate how distressing that experience has been for you.

Do familiar surroundings sometimes seem strange, confusing, threatening or unreal to you?

- ○ Yes
- ○ No

Have you heard unusual sounds like banging, clicking, hissing, clapping, or ringing in your ears?

- ○ Yes
- ○ No

Do things that you see appear different from the way they usually do (brighter or duller, larger or smaller, or changed in some other way)?

- ○ Yes
- ○ No

Have you had experiences with telepathy, psychic forces, or fortune telling?

- ○ Yes
- ○ No

Have you felt that you are not in control of your own ideas or thoughts?

- ○ Yes
- ○ No

Do you have difficulty getting your point across, because you ramble or go off the track a lot when you talk?

- ○ Yes
- ○ No

Do you have strong feelings or beliefs about being unusually gifted or talented in some way?

- ○ Yes
- ○ No

Do you feel that other people are watching you or talking about you?

- ○ Yes
- ○ No

Do you sometimes get strange feelings on or just beneath your skin, like bugs crawling?

- ○ Yes
- ○ No

Do you sometimes feel suddenly distracted by distant sounds that you are not normally aware of?

- ○ Yes
- ○ No

Have you had the sense that some person or force is around you, although you couldn't see anyone?

- ○ Yes
- ○ No

Do you worry at times that something may be wrong with your mind?

- ○ Yes
- ○ No

Have you ever felt that you don't exist, the word does not exist, or that you are dead?

- ○ Yes
- ○ No

Have you been confused at times whether something you experienced was real or imaginary?

- ○ Yes
- ○ No

Do you hold beliefs that other people would find unusual or bizarre?

- ○ Yes
- ○ No

Do you feel that parts of your body have changed in some way, or that parts of your body are working differently?

- ○ Yes
- ○ No

Are your thoughts sometimes so strong that you can almost hear them?

- ○ Yes
- ○ No

Do you find yourself feeling mistrustful or suspicious of other people?

- ○ Yes
- ○ No

Have you seen unusual things like flashes, flames, blinding light, or geometric figures?

- ○ Yes
- ○ No

Have you seen things that other people can't see or don't seem to see?

- ○ Yes
- ○ No

Do people sometimes find it hard to understand what you are saying?

- ○ Yes
- ○ No

Graded Chronic Pain Scale

Throughout our lives, most of us experience pain from time-to-time (e.g., such as minor headaches, sprains, and toothaches). Have you experienced pain other than these time-to-time pains? If so, the following questions pertain to that pain.

How would you rate your pain on a 0-10 scale at the present time, that is right now, where 0 is "no pain" and 10 is "pain as bad as can be?"

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

In the past six months, how intense was your worst pain, rated on a 0-10 scale, where 0 is "no pain" and 10 is "pain as bad as can be?"

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

In the past six months, on the average, how intense was your pain rated on a 0-10 scale, where 0 is "no pain" and 10 is "pain as bad as can be?" (That is, what was your usual pain at times when you were experiencing pain?)

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

In the past six months, how much has pain interfered with your daily activities rated on a 0-10 scale, where 0 is "no interference" and 10 is "unable to carry out daily activities?"

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

In the past six months, how much has pain changed your ability to take part in recreational, social and family activities rated on a 0-10 scale, where 0 is “no change” and 10 is “extreme change?”

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

In the past six months, how much has pain changed your ability to do housework rated on a 0-10 scale, where 0 is “no change” and 10 is “extreme change?”

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

About how many days in the last six months have you been kept from your usual activities (work, school or housework) because of pain?

Neuropathic Pain Scale

Throughout our lives, most of us experience pain from time-to-time (e.g., such as minor headaches, sprains, and toothaches). Have you experienced pain other than these time-to-time pains? If so, the following questions pertain to that pain.

Please tell us how intense your pain feels. Using the slider below, please choose the number that best describes the intensity of your pain.

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

Tell us how sharp your pain feels. Words used to describe "sharp" feelings include "like a knife," "like a spike," "jabbing," or "like jolts."

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

Please tell us how hot your pain feels. Words used to describe very hot pain include "burning" and "on fire."

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

Please tell us how dull your pain feels. Words used to describe very dull pain include "like a dull toothache," "dull pain," "aching," and "like a bruise."

0 1 2 3 4 5 6 7 8 9 10

Use the slider to provide your answer

Please tell us how cold your pain feels. Words used to describe very cold pain include "like ice" and "freezing."

0 1 2 3 4 5 6 7 8 9 10

Like a great deal

Alcohol Dependence Scale

The following questions relate to alcohol. For each question, enter the answer choice which best describes your alcohol use over the last 12 months.

How much did you drink the last time you drank?

- ○ Enough to get high or less
- ○ Enough to get drunk
- ○ Enough to pass out
- ○ I don't drink

Do you often have hangovers on Sunday or Monday mornings?

- ○ No
- ○ Yes

Have you had the "shakes" when sobering up (hands tremble, shake inside)?

- ○ No
- ○ Sometimes
- ○ Often

Do you get physically sick (e.g., vomit, stomach cramps) as a result of drinking?

- ○ No
- ○ Sometimes
- ○ Almost every time I drink

Have you had the "DTs" (delirium tremens) - that is, seen, felt or heard things not really there; felt very
anxious, restless, and over excited?

- ○ No
- ○ Sometimes
- ○ Several times

When you drink, do you stumble about, stagger, and weave?

- ○ No
- ○ Sometimes
- ○ Often

As a result of drinking, have you felt overly hot and sweaty (feverish)

- ○ No
- ○ Once
- ○ Several times

As a result of drinking, have you seen things that were not really there?

- [x] No
- [ ] Once
- [ ] Several times

Do you panic because you fear you may not have a drink when you need it?

- [x] No
- [ ] Yes

Have you had blackouts ("loss of memory" without passing out) as a result of drinking?

- [ ] No, never
- [ ] Somestimes
- [x] Often
- [ ] Almost every time I drink

Do you carry a bottle with you or keep one close at hand?

- [x] No
- [ ] Some of the time
- [ ] Most of the time

After a period of abstinence (not drinking), do you end up drinking heavily again?

- [x] No
- [ ] Sometimes
- [ ] Almost every time I drink

In the past 12 months, have you passed out as a result of drinking?

- [x] No
- [ ] Once
- [ ] More than once

Have you had a convulsion (fit) following a period of drinking?

- [x] No
- [ ] Yes
- [ ] Several times

Do you drink throughout the day?

- [x] No
- [ ] Yes

After drinking heavily, has your thinking been fuzzy or unclear?

- [ ] No
- [x] Yes, but only for a few hours
- [ ] Yes, for once or two days
- [ ] Yes, for many days

As a result of drinking, have you felt your heart beating rapidly?

- [x] No
- [ ] Yes
- [ ] Several times

Do you almost constantly think about drinking and alcohol?

- [x] No
- [ ] Yes

As a result of drinking, have you heard "things" that were not really there?

- [x] No
- [ ] Yes
- [ ] Several times

Have you had weird and frightening sensations when drinking?

- [x] No
- [ ] Once or twice
- [ ] Often

As a result of drinking have you "felt things"crawling on you that were not really there (e.g., bugs,
spiders)?

- ○ No
- ○ Yes
- ○ Several times

With respect to blackouts (loss; of memory):

- ○ Have never had a blackout
- ○ Have had blackouts that last less than an hour
- ○ Have had blackouts that last for several hours
- ○ Have had blackouts that last a day or more

Have you tried to cut down on your drinking failed?

- ○ No
- ○ Yes
- ○ Several times

Do you gulp drinks (drink quickly?)

- ○ No
- ○ Yes

After taking one or two drinks, can you usually stop?

- ○ No
- ○ Yes

Severity of Dependence Scale – Amphetamines

The following questions relate to amphetamines. For each question, enter the answer choice which best describes your amphetamines use over the last 12 months.

Have you consumed amphetamines more than three times in the last 12 months?

- ○ Yes
- ○ No

Severity of Dependence Scale – Marijuana/Cannabis

The following questions relate to marijuana/cannabis. For each question, enter the answer choice which best describes your marijuana/cannabis use over the last 12 months.

Have you consumed marijuana/cannabis more than three times in the last 12 months?

- ○ Yes
- ○ No

Severity of Dependence Scale – Cocaine

The following questions relate to cocaine. For each question, enter the answer choice which best describes your cocaine use over the last 12 months.

Have you consumed cocaine more than three times in the last 12 months?

- ○ Yes
- ○ No

Severity of Dependence Scale – Opioids

The following questions relate to opioids, including prescription and non-prescription treatments (e.g., heroin). For each question, enter the answer choice which best describes your opioids sue over the last 12 months.

Have you consumed opioids, including prescription and non-prescription treatments (e.g., heroin) more than three times in the last 12 months?

- ○ Yes
- ○ No

Severity of Dependence Scale – Psychomotor Stimulants

The following questions relate to psychomotor stimulants commonly used to treat ADHD (e.g., Adderall, Vyvanse). For each question, enter the answer choice which best describes your psychomotor stimulant use over the last 12 months. Note, this includes prescription and non-prescription (e.g., recreational) use.

Have you consumed psychomotor stimulants more than three times in the last 12 months?

- ○ Yes
- ○ No

Nicotine Dependence Scale for Adolescents

1. Do you think you would be able to quit smoking cigarettes or using a vaporizer/ e-cig if you wanted to?

- ○ I don't smoke now
- ○ Definitely yes
- ○ Probably yes
- ○ Probably not
- ○ Definitely not

How soon after you wake up do you usually smoke your first cigarette or first use your vaporizer/e-cig on a weekday (Monday to Friday)?

- ○ I don't smoke now
- ○ Less than 15 minutes
- ○ 15 to 30 minutes
- ○ More than 30 but less than 60 minutes
- ○ 1 to 2 hours
- ○ More than 2 hours but less than half a day
- ○ More than half a day
- ○ I don't smoke during the weekdays

How soon after you wake up do you usually smoke your first cigarette or first use your vaporizer/ e-cig during the weekend (Saturday to Sunday)?

- ○ I don't smoke now
- ○ Less than 15 minutes
- ○ 15 to 30 minutes
- ○ More than 30 but less than 60 minutes
- ○ 1-2 hours
- ○ More than 2 hours but less than half a day
- ○ More than half a day
- ○ I don't smoke during the weekend

3. If you are sick with a bad cold or sore throat, do you smoke cigarettes or use a vaporizer/ e-cig?

- ○ I don't smoke now
- ○ No, I stop smoking when I am sick
- ○ Yes, but i cut down on the amount I smoke
- ○ Yes, I smoke the same amount as when I'm not sick

How true is this statement for you?

When I go without a smoke/vape for a few hours, I experience craving.

- ○ I don't smoke now
- ○ Not at all true
- ○ Not very true
- ○ Fairly true
- ○ Very true

How true is this statement for you?

I sometimes have strong cravings where it feels like I'm in the grip of a force that I can't control.

- ○ I don't smoke now
- ○ Not at all true
- ○ Not very true
- ○ Fairly true
- ○ Very true

We thank you for your time spent taking this survey.
Your response has been recorded.

Appendix C – Timepoint 2 Materials

Appendix C – MRI Pre-Entry Screening Form

MRI Pre-Entry Screening Form Revised 9/19/2019	Auburn University MRI Research Center 560 Devall Drive Suite 202 Auburn, AL 36849 Tel: (334) 844-6747 Fax: (334) 844-0214
This form to be used for: Screening of research subjects immediately prior to an MRI study (File completed form with Principal Investigator) *Instructions for completing this form available at http://www.eng.auburn.edu/research/centers/mri/forms*	

Name _____
Last First MI

Address _____ City _____

State _____ Zip Code _____ Male/Female_____

Phone (___)_____ (___)_____ (___)_____
Home Work Cell

Birthdate _____ Email Address _____

Primary Physician (Optional):

Name_____ Phone (___)_____

AUMRIRC Use Only

Principal Investigator: _____

IRB Protocol # _____

Subject # _____

Date/Time of MRI study ___/___/_____ ___:___

Subject Weight (lbs) _____

Height(ft/in) _____

1.	☐Yes ☐No	Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind? If yes, give date and type of surgery, and indicate where on your body using the diagram. Date: __/__/____ Type of surgery: _____ Date: __/__/____ Type of surgery: _____ Date: __/__/____ Type of surgery: _____
2.	☐Yes ☐No	Have you had any medical condition that prevented you completing an MRI exam in the past or had any related to a previous MRI examination or procedure? If yes, please describe: _____
3.	☐Yes ☐No	Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)? If yes, please describe: _____

WARNING: Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). Do not enter the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the AU MRI Research Center staff BEFORE entering the MR system room. **The MR system magnet is ALWAYS on.**

Answering "Yes" to any of the following questions excludes you from the study

4.	☐Yes ☐No	Do you have a cardiac pacemaker or implanted cardioverter defibrillator (ICD)?
5.	☐Yes ☐No	Is there a possibility of metal in your head (for example aneurysm clips, do not include dental work)? If yes, please describe: _____
6.	☐Yes ☐No	Have you had an injury to the eye involving a metallic object or fragment (for example, metallic slivers, shavings, foreign body), or have you ever needed an eyewash having worked with metals? If yes, please describe: _____
7.	☐Yes ☐No	Do you have an implanted medical device that is electrically, magnetically, or mechanically controlled or activated? If yes, please describe: _____
8.	☐Yes ☐No	**Females Only**: Are you pregnant or is there any possibility that you may be pregnant?

Protocol-Specific Questions (Answering "Yes" to any of the following questions may exclude you from the study)

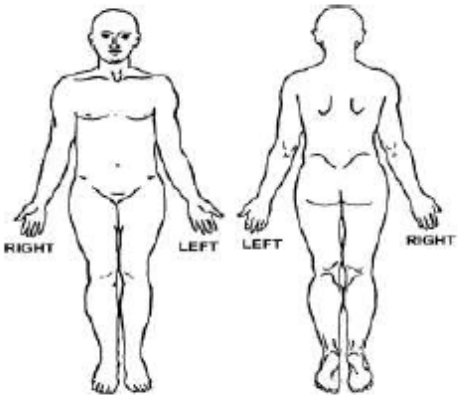
9.	☐Yes ☐No	Do you have a history of cardiovascular disease?
10.	☐Yes ☐No	Do you have a breathing problem or motion disorder?
11.	☐Yes ☐No	Are you claustrophobic?
12.	☐Yes ☐No	Do you have inner ear disorders or experience vertigo or dizziness?
13.	☐Yes ☐No	Do you have tattoos or permanent makeup that contains metal?
14.	☐Yes ☐No	Do you have body piercing jewelry that cannot be removed?
15.	☐Yes ☐No	Do you have braces?

WARNING: Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). Do not enter the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the AU MRI Research Center staff BEFORE entering the MR system room. **The MR system magnet is ALWAYS on.**

Please indicate if you have any of the following:

16.	☐Yes ☐No	Neurostimulation system
17.	☐Yes ☐No	Spinal cord stimulator
18.	☐Yes ☐No	Internal electrodes or wires
19.	☐Yes ☐No	Bone growth/bone fusion stimulator
20.	☐Yes ☐No	Cochlear, otologic, or other ear implant
21.	☐Yes ☐No	Insulin or other infusion pump
22.	☐Yes ☐No	Implanted drug infusion device
23.	☐Yes ☐No	Any type of prosthesis (eye, penile, etc.)
24.	☐Yes ☐No	Heart valve prosthesis
25.	☐Yes ☐No	Eyelid spring or wire
26.	☐Yes ☐No	Artificial or prosthetic limb
27.	☐Yes ☐No	Metallic stent, filter, or coil
28.	☐Yes ☐No	Shunt (spinal or intraventricular)
29.	☐Yes ☐No	Vascular access port and/or catheter
30.	☐Yes ☐No	Radiation seeds or implants
31.	☐Yes ☐No	Swan-Ganz or thermodilution catheter
32.	☐Yes ☐No	Medication patch (Nicotine, Nitroglycerine)
33.	☐Yes ☐No	Any metallic fragment or foreign body
34.	☐Yes ☐No	Wire mesh implant
35.	☐Yes ☐No	Tissue expander (e.g., breast)
36.	☐Yes ☐No	Surgical staples, clips, or metallic sutures
37.	☐Yes ☐No	Joint replacement (hip, knee, etc.)
38.	☐Yes ☐No	Bone/joint pin, screw, nail, wire, plate, etc.
39.	☐Yes ☐No	IUD, diaphragm, or pessary
40.	☐Yes ☐No	Dentures or partial plates
41.	☐Yes ☐No	Permanent retainer
42.	☐Yes ☐No	Braces
43.	☐Yes ☐No	Tattoo or permanent makeup
44.	☐Yes ☐No	Body piercing jewelry
45.	☐Yes ☐No	Hearing aid *(Remove before entering MRI scanner room)*
46.	☐Yes ☐No	Other implant_____

Please mark on the figure(s) below the location of any implant or metal inside of or on your body.



IMPORTANT INSTRUCTIONS

Before entering the MR scanner room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry, body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clippers, tools, clothing with metal fasteners, & clothing with metallic threads.

Please consult the research staff if you have any question or concern BEFORE you enter the MR scanner room.

NOTE: You may be advised or required to wear earplugs or other hearing protection during the MR procedure to prevent possible problems or hazards related to acoustic noise.

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

This form is valid only on the day it is completed.

Signature of Person Completing Form: _____ _____
Signature Date

Form Completed By: ☐ Subject ☐ Relative _____ _____
Print Name Relationship to Subject

Form Information Reviewed By: _____ _____
Print Name Signature

Form Information Reviewed By: _____ _____
Print Name Signature

Appendix C – Consent Form

DEPARTMENT OF
PSYCHOLOGICAL SCIENCES

AUBURN UNIVERSITY

COLLEGE OF LIBERAL ARTS

NOTE: DO NOT SIGN THIS DOCUMENT UNESS AN IRB APPROVAL STAMP WITH CURRENT DATES HAS BEEN APPLIED.

INFORMED CONSENT
for a research study, entitled

"7T fMRS Analysis of Pain Processing in Human Controls"

You are invited to participate in a research study to examine how the human brain processes pain. Please note that the biomedical imaging scans acquired during this research study are for research purposes only and are not suitable in any way for clinical diagnosis. This research study is being conducted by Steven J. Nichols, Graduate Research Assistant, under the direction of Dr. Jennifer L. Robinson, Associate Professor in the Auburn University Department of Psychological Sciences. You were selected as a possible participant based on your responses to the questionnaires you completed during Phase 1 and are age 19 or older.

What will be involved if you participate? If you decide to participate in Phase 2 of this research study, you will be asked to undergo MRI (7T) scanning while completing short tasks that involve some pain.

The following takes place outside the scanner. Before commencing with MRI data collection, you will be asked to complete an MRI screening form in the MRI research center waiting area. The MRI screening form is designed to make sure it is safe for you to undergo MRI scanning. Common contraindications for MRI scanning are pacemakers, implanted cardioverter defibrillators, implanted medical devices or non-removable devices or objects, breathing problems or disorders, claustrophobia, inner ear disorders, vertigo or dizziness, tattoos or permanent makeup containing metal, or body piercing or jewelry that cannot be removed.

The following takes place inside the scanner. For the scanning session, you will be asked to lie on a bed that slides into the long tube of the scanner. You will be asked to place your head in a helmet-like device mounted on the scanner bed. The scanner is a magnet with a small enclosed space. Radio waves and strong, changing magnetic fields are used to make images of your body.

You will be asked to remain very still at times throughout the scanning session. To help you keep as still as possible, we will put cushions around your head/neck/shoulders. We will check in with you throughout the scan. If you have discomforts, please notify the operator.

The following takes places inside the scanner. Several scans will be performed during the scanning session with approximately one minute of rest between scans. Individual scans last approximately between 5 and 10 minutes depending on the scan. However, individual scans never last more than 20 minutes. Your expected total time in the scanner is approximately 75 minutes. After the scanning session, you will be asked to complete a short questionnaire about your experience during the scanning session and another short questionnaire about your sleep pattern the night before the scanning session. Your expected total time commitment, including pre-scan preparation, scanning, and post-scan questionnaires, is approximately two hours.

Participant's Initials _____

226 Thach Hall, Auburn, AL 36849-5214; Telephone: 334-844-4412; Fax: 334-844-4447
www.cla.auburn.edu

The following takes place inside the scanner. While inside the scanner, you will be asked to complete short tasks that involve some pain. This will involve the researcher using a device to press down on your hand, administering pressure to the point at which you experience some pain. This will cause mild, temporary discomfort. In addition, there is a small chance of temporary swelling and/or bruising. You will be free to stop this portion of the study at any time if you find it to be too uncomfortable. **In one experiment**, when you indicate that the pain becomes too uncomfortable to continue, or when the pressure reaches a maximum of 2,000Kpa (which we found to be an appropriate upper-limit in other studies), the researcher will immediately stop and any/all pressure being applied to your hand will immediately discontinue. The pressure at the time the test was stopped will be recorded. This process will repeat ten times so that the investigator can obtain an average rating. You are free to stop this portion of the research study at any time. In total, the pain *tolerance* measurements should take approximately 10 minutes to complete. **In another experiment**, the device will continue to administer pressure until it reaches a pre-determined stop point. When the device reaches that point, the investigator will immediately stop and any/all pressure being applied to your hand will immediately discontinue. Then, you will be asked to evaluate the pain using a number scale. This process will repeat ten times so that the investigator can obtain an average rating. You are free to stop this portion of the research study at any time. In total, the pain *rating* measurements should take approximately 10 minutes to complete.

The following takes places inside the scanner. In addition, you will be asked to complete galvanic skin response measurements during the scanning session. This involves the investigator using two electrodes, secured to your first and second fingers on your non-dominant hand, to record sympathetic nervous system activation without contamination from parasympathetic nervous system activation. Electrodes are small conductors used to detect electrical signals coming, in this case, coming from the skin surface. Again, you are free to stop this portion of the research study at any time.

Of note, only participants who meet the following criteria are allowed to participate: (1) are right-handed, (2) are not taking any over-the-counter or prescription medication which may cause or increase bleeding, (3) have no history of seizure, (4) are not taking medication to treat seizure, (5) have not consumed drugs (including alcohol) in the 24-hour period prior to the research study session, (6) have not consumed pain relievers in the 8-hour period prior to the research study session, (7) have not have consumed food, drinks (except water), caffeine, and/or nicotine in the 3-hour period prior to the research study session, and (8) have not have exercised in the 30-minute period prior to the research study session.

None of the scans done during this scanning session are appropriate for clinical interpretation. This means that they are not designed to assess any medical condition that you may have. They are not designed to reveal any existing disease pathology. Rather, they are intended solely for research purposes.
Your total time commitment will be approximately 75 minutes.

Participant's Initials _____

Are there risks or discomforts? The risks or discomforts associated with participation in this research study are:

1. The most obvious personal risk associated with an MRI scanning session is blunt trauma due to metallic objects being brought into the magnetic field. As such, all necessary steps will be taken to make sure neither you nor anyone else who enters the MRI scanning room is in possession of an unrestrained metal object, and no unauthorized person will be allowed to enter the MRI scanner room.
2. Participants who have iron or steel implants or clips from surgery within their body or metallic objects such as shrapnel or metal slivers in their body should not participate in this research study as the magnetic field may pull these objects and cause injury.
3. The scanner makes intermittent noises which some participants have find annoying.
4. Some participants may feel uncomfortable being in an enclosed place (claustrophobia), and others may find it difficult to remain still.
5. Some participants experience dizziness or a metallic taste in their mouth if they move their head rapidly in the scanner.
6. Some participants experience brief nausea when being put into or taken out of the scanner. This is more prominent in 7 Tesla MRI due to the increased magnetic-field strength and shielding effects.
7. Interaction with MRI Center personnel during the scanning session carries some risk of exposure to COVID-19.
8. The pressure-based algometer device is associated with risk of temporary, minor discomfort, swelling and/or potential bruising. For this reason, no one taking blood thinners or other anticoagulants like aspirin, coumadin, etc. should participate in this study due to increased risk of bruising.

Although long-term risk of exposure to the magnet is not known, the possibility of and long-term risk is extremely low based on information accumulated over the last 30 years of routine clinical use of MRI. In addition, research over the last 12 years has not suggested any long-term risk of exposure to 7 Tesla MRI.

To minimize these risks, we will:

1. We will have you complete a screening form to determine if you have iron or steel implants, clips from surgery, or other metallic objects in your body. If you have implants, clips, or objects in your body, you will not be able to undergo MRI scanning.
2. We will ask you to change into surgical scrubs supplied by the Center and remove any watches, rings, earrings, or other jewelry and metallic objects. You will be provided a private place to change and you may retain your undergarments. If you are wearing undergarments that contain underwire and/or metal fasteners, you will be asked to remove them prior to scanning.
3. We will scan you with a handheld metal detector to detect any unknown metallic objects.
4. We will provide you with either earplugs specifically designed to work in an MRI scanner.

5. We will maintain visual contact and audio contact with you during the scan and check with you frequently to determine if you are having any negative feelings or sensations. Please inform the investigator if you have negative feelings or sensations (e.g., nausea, claustrophobia).

Participant's Initials _____

6. If some unknown risk becomes a safety issue, the investigator will immediately stop the scan and remove you from the scanner.
7. You can stop the scan at any time and be immediately removed from the scanner. You can notify the investigator verbally or by using the squeeze ball provided.
8. To protect the confidentiality of all information, forms will be coded with your study-specific unique participant number and will be stored in a private office in a locked filing cabinet. Electronic data will be stripped of identifiable information, coded with your participant number, and stored on password-protected computers and servers with access limited to investigators on this research study.
9. Although MR is not associated with harmful effects on pregnant women, we will exclude pregnant woman as a precaution.
    I. Pregnancy status will be determined by a simple yes/no response provided by the participant. No pregnancy test will be administered.

You are responsible for any costs associated with medical treatment due to any injuries incurred.

Are there benefits to yourself or others? If you participate in the research study, you can expect to receive no direct personal benefits. However, we hope that the results of this research study will provide better understanding that may lead to improved pain-related outcomes. We hope that this leads to better medications and therapeutic targets to manage and treat pain. We/I cannot promise you that you will receive any or all of the benefits described.

Will you receive compensation? To thank you for your time you will be offered $5 for showing up today. Additionally, you will receive $5 for every 30-minute block you are inside the scanner. The total compensation will be $10 for 0-30 minutes of scanning, $15 for 30-60 minutes of scanning, and $20 for 60-90 minutes of scanning. If you are a student at Auburn University, and if you volunteered through Sona Systems, you will be compensated for participating with three research hours. Your instructors should assign specific values of course credit to these hours. Please check with your instructors for more information.

Are there costs? If you decide to participate in this research study, you will not incur any costs. If you require medical attention, you will be responsible for all costs for medical attention/treatment.

If you change your mind about participating, you can withdraw from the research study at any time. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate will not jeopardize your relationship with Auburn University, the Department of Psychological Sciences, or any other center, or office.

Your privacy will be protected. Any information obtained in connection with this research study will remain confidential. At the end of the research study, all links to identifiable information will be destroyed. Data obtained through your participation may be published in a professional journal or presented at a professional meeting.

Participant's Initials _____

Incidental findings. These procedures are carried out purely for experimental purposes. The MRI scans that are acquired in this research study are not the same as those acquired during a clinical examination as requested by a medical doctor. Therefore, they are not useful to investigate any abnormalities or medical condition you may have. Furthermore, the investigators who will analyze these images are not medical doctors and are not trained to evaluate these scans.

It is possible, however, that an abnormality may be noticed. If this happens, a brief diagnostic scan will be performed and referred to a radiologist for reading. If you choose to provide the name and contact information of your primary physician on the MRI screening form, the results of the scan will be provided to them. If you do not have a primary physician or do not provide contact information for your primary physician, the results will be provided to Dr. Fred Kam, MD, at the Auburn University Medical Clinic, who will discuss the results of the scan with you at your expense.

If you have questions about this research study, please ask them now. Alternatively, you can contact Steven J. Nichols, at sjn0016@auburn.edu, or Dr. Jennifer L. Robinson, jrobinson@auburn.edu, who are the research study investigators. A copy of this document will be given to you for your records at your request.

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board by phone (334)844-5966 or email at hsubjec@auburn.edu or IRBchair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES YOUR WILLINGNESS TO PARTICIPATE.

Participant's Signature Date	Signature, Investigator Obtaining Consent Date
Printed Participant Name	Name, Investigator Obtaining Consent
	Co-Investigator Signature Date
	Printed Name

The Auburn University Institutional Review Board has approved this Document for use from 02/17/2021 to 02/16/2022
Protocol # 21-073 MR 2102

Appendix C – Post-Scan Survey

Post-Scan Survey

Thanks for completing the Neuroimaging and Cannabis Project. These are some post-scan questions to help us better understand your data. Please answer them to the best of your abilities.

To begin, please provide your participant number.

Women: please enter the date of your last menstrual period. If you do not know the exact date, please provide your best estimate. Use the format mm/dd/yy.

- ☐ mm
- ☐ dd
- ☐ yy

During the short tasks that involved some pain, please describe in a few words any strategies used to deal with the pain.

We are interested in the thoughts and feelings that you experienced during the scans, particularly when you weren't engaged in a task (e.g., structural scans, pain-task scans). Please indicate the extent to which each of the following statements characterized your thoughts and feelings during the scans.

	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
I thought about my feelings.	○	○	○	○	○
I felt restless.	○	○	○	○	○
I felt anxious.	○	○	○	○	○
I felt tired.	○	○	○	○	○
I felt sleepy.	○	○	○	○	○
I felt comfortable.	○	○	○	○	○
I felt relaxed.	○	○	○	○	○
I felt happy.	○	○	○	○	○
I enjoyed the session.	○	○	○	○	○

→

St. Mary's Sleep Questionnaire

This questionnaire refers to your sleep over the past 24 hours. Please try to answer each question to the best of your abilities.

Last night, at what time did you settle down?

☐ hours

☐ minutes

Last night, at what time did you finally fall asleep?

☐ hours

☐ minutes

This morning, at what time did you wake up?

☐ hours

☐ minutes

This morning, at what time did finally get out of bed?

☐ hours

☐ minutes

Please use the slider to describe how light/deep your sleep was , where 0 = "very light" and 10 = "very deep."

0 1 2 3 4 5 6 7 8 9 10

Please use the slider to describe how badly/well you slept , where 0 = "very badly" and 10 = "very well."

0 1 2 3 4 5 6 7 8 9 10

Please use the slider to describe how unsatisfied/satisfied you were with your sleep , where 0 = "very unsatisfied" and 10 = "very satisfied."

0 1 2 3 4 5 6 7 8 9 10

Please use the slider below to indicate how many times you woke up, where 0 = "zero times" and 10 = "ten times or more."

0 1 2 3 4 5 6 7 8 9 10

We thank you for your time spent taking this survey.
Your response has been recorded.