^{1}H Nuclear Magnetic Resonance Investigation of Pain Processing in the Human Somatosensory Cortex

by

Steven Jon Nichols

A dissertation submitted to the Graduate Faculty of
Auburn University
In partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Auburn, Alabama
August 3, 2024

Keywords: pain, acute, functional magnetic resonance spectroscopy (fMRS),
Functional magnetic resonance imaging (fMRI),
Primary somatosensory cortex (SI)

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

Abstract

The perception of pain is an adaptive trait critical to survival. Prolonged painful stimulation in the form of chronic pain, however, can be maladaptive and cause significant physical and emotional distress. Indeed, chronic pain is linked to substantial health and economic burdens. To improve pain-related outcomes and to advance the field's understanding of the neurochemical mechanisms of normative pain processing, the current study used functional magnetic resonance spectroscopy (fMRS) to investigate glutamate and γ-Aminobutyric acid (GABA) levels in the primary somatosensory cortex (SI) in response to acute, pressure-based pain. Additionally, the current study investigated the link between pain physiology and perception by assessing the relationship between pain-related neurometabolites and subjective pain ratings. Data suggest that pain was not associated with changes in either glutamate or GABA. Further, linear regressions did not reveal any predictive relationship between glutamate or GABA and subjective pain ratings. Finally, statistical analysis revealed a significant reduction in total creatine levels pre- versus post-task. These data add to the growing corpus of spectroscopic pain literature and to the gap in current literature regarding the role of SI in normative pain processing.

Acknowledgments

I would like to thank Dr. Tom Denny and Dr. Meredith Reid for their MATLAB code, as well as the volunteers who participated in the study.

Table of Contents

List of Tables

List of Figures

Introduction

The perception of pain is a critical survival mechanism for many species including human and non-human animals (Diatchenko et al., 2007). Although infrequent occurrences of acute pain are an adaptive trait, frequent occurrences of pain sustained over long periods can become problematic. Recent estimates indicate approximately 51.6 million people in the United States are suffering from chronic pain, 17.1 million of whom are classified as high-impact chronic pain (daily activities are greatly restricted due to pain) (Rikard, 2023). This is particularly concerning given that chronic pain has been shown to have a negative impact on brain function, cardiovascular health, mood, and sleep (Fine, 2011). Furthermore, expenses related to chronic pain (e.g., healthcare costs, lost wages, medication use/misuse, etc.) have resulted in an economic burden between $560 and $635 billion annually in the United States (Henschke et al., 2015). 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 pressure-based pain.

Pain Network

In the past, researchers sought to identify an area of central processing for pain within the brain, similar to the areas responsible for primary visual and auditory processing (Mano & Seymour, 2015). Early efforts to find a ‘pain cortex,’ like those made by Penfield and Jasper (1954), failed to reveal a primary processing center for the

sensation or perception of pain. Subsequent failures to find a single pain cortex and improved knowledge of neurofunctional circuitry have led to the contemporary understanding that pain processing is the result of a widespread network within the brain. Meta-analytic data indicate that this network encompasses both cortical brain structures such as the anterior cingulate cortex (ACC), prefrontal cortex (PFC), and the primary and supplementary somatosensory cortices (SI & SII), as well as subcortical structures within the basal ganglia, thalamus, and brainstem (Coghill et al., 1999; 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, others have received less investigative inquiry. One such node within the pain network is the primary somatosensory cortex. To my knowledge, only one study (Nichols et al., 2023) has examined the neurochemical underpinnings of acute pain processing within SI. However, the methodological limitations of this study, in tandem with the overall lack of human pain research in SI, highlight the need for further investigation into the role of SI in pain processing, as well as its place in the pain network as a whole.

Primary Somatosensory Cortex

The primary somatosensory cortex (SI) consists of Brodmann areas 1-3 located in the postcentral gyrus. SI has been implicated as a key node in the processing of sensory signaling related to tactile stimulation (Raju & Tadi, 2023). Aided by supplementary processing nodes such as the secondary somatosensory area (SII) and parietal-ventral (PV) area (Disbrow et al., 2003), SI is the destination for multiple afferent pathways that transmit unique aspects of physical sensation, including texture,

temperature, composition, and pain (Hashim et al., 2017; Raju & Tadi, 2023). This sensory information ascends the spinal cord via thousands of afferent fibers connecting sensory receptors in the skin to the brain (Jenkins & Lumpkin, 2017). Although the precise number is currently unknown, recent estimates place the number of afferent tactile fibers between 200,000 and 270,000 in total (Corniani & Saal, 2020). Interestingly, sensory receptors are not distributed uniformly around the body. Rather, certain body parts, particularly those covered in glabrous skin (e.g., lips and fingertips), have a much higher relative innervation density than other regions (Corniani & Saal, 2020). As a result, the amount of cortical tissue in SI devoted to a particular region of skin is not determined by the surface area of the region but rather is associated with the degree to which it is innervated by afferent tactile fibers in a phenomenon known as cortical magnification (Catani, 2017; Gandhoke et al., 2019). For example, in humans, the amount of cortical space devoted to processing information from a single thumb is roughly equivalent to the entire forearm (Goldstein, 2009).

These inputs are processed contralaterally, such that tactile stimulation transduced on one side of the body is processed in SI of the opposing brain hemisphere (Raju & Tadi, 2023). Extensive neurofunctional mapping of these sensory pathways has resulted in a detailed topographic map of SI known as the sensory homunculus. This map highlights corresponding body-part brain processing areas (i.e., localization of function) and is scaled to approximate the amount of cortical tissue devoted to certain body parts, as represented by the enlarged hands and facial features. The use of the homunculus map can aid in precise investigation of sensory processing related to specific regions of the body (e.g., lips, limbs, and digits) (Puckett & Sanchez Panchuelo,

2023).This project made use of this topographic map by examining neurochemical responses to tactile pain stimulation of the nondominant hand in corresponding cortical tissue.

Transmission of tactile stimulation from sensory receptors to SI occurs along two major pathways: the dorsal column medial lemniscus pathway (DCML) and the spinothalamic pathway (Watson et al., 2010). The former is responsible for communicating information about fine touch and proprioception (Al-Chalabi, Reddy, & Alsalman, 2024), while the latter contains fibers related to temperature and pain information (Al-Chalabi, Reddy, & Gupta, 2024). These pathways are structurally similar, as both are composed of triple-neuron chains that stretch from receptor to brain (this structure varies slightly between information traveling through the spinal cord and cranial nerves) (Watson et al., 2010). The cell bodies of the sensory neurons that begin this chain are located in spinal and/or cranial nerve ganglions; the second neuron in this chain has its cell body in the gray matter of the spinal cord or the hindbrain; and the third neuronal cell body in the chain is housed in the thalamus (Watson et al., 2010). The level at which they cross the midline represents a major difference between these pathways, as the spinothalamic pathway crosses at the spinal cord, while the DCML pathway does not cross until it reaches the brain (Al-Chalabi, Reddy, & Alsalman, 2024; Al-Chalabi, Reddy, & Gupta, 2024). These accounts of somatosensory anatomy reveal the similar, yet distinct, organization of painful and nonpainful pathways in the nervous system.

The projection of the spinothalamic pathway to SI highlights its involvement in nociceptive signaling, in addition to nonpainful tactile stimulation. In a review of

nociceptive neurofunctional mapping in SI, Vierck et al. (2013) corroborates the ascending pathways for pain signaling in response to environmental stimuli. In their review, they note that, similar to non-painful sensation, afferent pain signals travel through the dorsal horn of the spinal cord, projecting to thalamic nuclei and eventually Brodmann areas 1 and 3a/b in SI. Diversity exists within these subregions in terms of their responses to nociceptive stimulation. In a study by Tommerdahl and colleagues (1996) examining SI in squirrel monkeys, area 3a demonstrated consistent, prolonged activation following pain onset, while suppression of areas 1 and 3b, which persisted after stimulus removal, was observed (Tommerdahl et al., 1996). Interestingly, researchers in an earlier study observed peak activation in area 3a to occur *after* stimulus removal (Tommerdahl et al., 1993). This suggests that area 3a is involved in detection of both stimulus onset and cessation. This response diversity is unsurprising, given Vierck and colleagues' (2013) explanation that SI acts as a site for the integration of various afferent sources, including a combination of myelinated and unmyelinated axon tracts originating in the peripheral nervous system that coalesce in SI to encode for a variety of stimulus properties including presence, location, temperature, intensity, and modality (Kenshalo & Willis, 1991; Vierck et al., 2013).

These findings highlight the unique role of SI compared to other cortical regions within the pain network. As SI plays a more objective role related to sensation, whereas in addition to sensory processing, regions such as the ACC and prefrontal cortex (PFC) are believed to be heavily involved in the affective experience of pain (Xiao & Zhang, 2018) and cognitive appraisal/antinociceptive modulation of pain (Ong et al., 2019), respectively. Previous work to map and summarize the neurofunctional circuitry of pain

has created a strong foundation for future studies to examine nociception under specific contexts with new methodologies as they become available. As indicated by the above literature, SI is a critical node in the body's somatosensory and nociceptive systems and served as the primary region of interest in the current study.

Functional Magnetic Resonance Spectroscopy

One emerging methodology to study the neurometabolic correlates of pain is the use of functional magnetic resonance spectroscopy (fMRS). Traditional MRS characterizes metabolites within the brain by collecting a single spectrum while the subject is at rest in the scanner (i.e., a no-task condition). FMRS is an alternative technique that captures neurometabolite level changes in both task and rest conditions by collecting multiple spectra over time (Duarte et al., 2012). In this way, researchers can compare averages of neurochemical changes across task conditions within a single participant, allowing the dynamic shifts between baseline and task-related conditions to be captured. FMRS is a robust and reliable technique for the *in vivo* characterization of neurometabolites (Prichard et al., 1991) and serves as a pivotal tool for investigating the underlying neurochemical systems within the brain. Here, I used fMRS to characterize the neurometabolic changes in SI in response to acute, pressure-based pain stimulation.

Imaging sensitivity is a key challenge facing fMRS researchers. Lower magnitudes of imaging sensitivity have often led to a decrease in spectral reliability, particularly in subcortical brain regions. Thus, applications of fMRS techniques have been limited in the past (Stanley & Raz, 2018). Recent improvements in imaging technology and accessibility, however, have led to advancements in fMRS

methodologies. Utilization of high (≥3 Tesla (3T)) and ultra-high field (≥7 Tesla (7T)) magnetic resonance imaging (MRI) machines have provided advancements across the entire neuroimaging community and have been especially beneficial for spectroscopic research. Compared to lower field strengths (≤ 4.5T), the increased field strength of 7T spectroscopy offers improved data quality metrics such as signal-to-noise ratio (SNR; ensuring greater sensitivity to detect a true signal), metabolite line widths (narrower line widths lead to improved metabolite estimation), and Cramer-Rao Lower Bounds (CRLB; a measure of fit for metabolites) (Reid et al., 2019). The enhanced imaging sensitivity afforded by these key benefits of 7T allows for more precise and replicable measurement 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., SI) represents a unique opportunity to characterize neurometabolic changes during acute pain administration. Such data could lead to advancements in the field of pain research and improved pain-related outcomes by advancing our understanding of the neurochemical systems of the pain network within the brain, which may contribute to novel therapeutic interventions for pain-related illness.

Glutamate

The proposed study catalyzed the aforementioned strengths of 7T fMRS to measure SI glutamate levels during the acute administration of pain. Glutamate is an abundant metabolite within the vertebrate nervous system (Meldrum, 2000), can be detected reliably via MRS (Graaf, 2019), and has been heavily implicated in pain research (Archibald, MacMillan, Graf, et al., 2020). Increases in glutamate have been

reported in multiple pain network nodes during experimental pain. In one seminal study, Mullins et al. (2005) noted 9.3% and 11.4% increases in ACC glutamate and Glx (the combined signal of glutamate and glutamine). Here, researchers utilized a modified cold pressor task to demonstrate a significant increase in neurochemical excitation in response to acute pain. Since then, several key studies have corroborated these results in the ACC using thermal and pressure pain (Archibald et al., 2020; Nichols et al., 2023), while others have subsequently reported comparable increases in glutamate in other brain regions (e.g., occipital cortex and brainstem nuclear complex) (Cleve et al., 2015; de Matos et al., 2017). These data suggest that glutamate plays a critical role in nociceptive processes throughout the brain's pain network and demonstrate the efficacy with which fMRS can be used to study these phenomena *in vivo.*

Regarding SI, I am aware of only one study that has reported acute pain-related glutamate changes. Nichols et al. (2023) investigated the effect of experimentally administered pressure-pain on SI glutamate, reporting a null effect of pain on glutamate. Given the medium effect size observed in our study (Cohen's d = 0.467), we argued that methodological limitations, specifically small sample size and the lack of a true baseline condition, hindered the statistical power of the study, and that future studies with improved designs would likely be able to detect the extant relationship between pain and glutamate. Although there is a shortage of available fMRS research concerning glutamate and acute pain, there have been several functional MRI (fMRI) studies that offer some insight into the role of SI in pain processing. Indeed, multiple groups have reported significant blood-oxygen-level-dependent (BOLD) changes in response to experimental pain in the contralateral (Chen et al., 2002; Henderson et al., 2006;

Macefield et al., 2007; Takahashi et al., 2011) and bilateral SI (Nash et al., 2010; Niddam et al., 2002). These data, combined with reports of correlated excitatory neurochemical and BOLD responses in SI and other brain regions (Ip et al., 2019; Kiemes et al., 2021; Moon et al., 2021), may point toward an elevated glutamatergic response in SI following acute pain. For these reasons, glutamate was a primary metabolite of interest in the proposed study.

GABA

The proposed study also examined acute pain-related changes in γ-Aminobutyric acid (GABA). Similar to glutamate, GABA is a key neurometabolite in the vertebrate nervous system (Watanabe et al., 2002) and can be detected reliably via fMRS (Reid et al., 2022; Wijtenburg et al., 2014). Although GABA has been implicated in pain literature, it is less frequently the subject of spectroscopic research, and even less so for acute pain-related research, relative to glutamate (Archibald, MacMillan, Enzler, et al., 2020). This gap in literature is due, in part, to increased difficulty in the characterization of GABA, as it exists in relatively small amounts in the human nervous system (Graaf, 2019) and can be difficult to detect at lower field strengths due to signal overlap with metabolites of greater concentrations (Graaf, 2019; Mullins et al., 2014). For these reasons, additional data processing steps known as “GABA editing” are often necessary to obtain accurate GABA measurements with fMRS. Indeed, common GABA editing methodologies, such as Meshcher-Garwood point resolved spectroscopy (MEGA-PRESS), often involve additional spectral processing to boost the data quality metrics for GABA (e.g., SNR and CRLB). However, given that recent 7T STEAM (stimulated echo acquisition mode) sequences have demonstrated high reproducibility for GABA

(Wijtenburg et al., 2013), the proposed study did not utilize such GABA-editing techniques.

Trends in current pain literature remain unclear as both increases and decreases in GABA have been reported across brain regions. To date, only four MRS studies have been published that report acute pain-related GABA levels (excluding Nichols et al. (2023), which reported no GABA changes, attributable to the previously mentioned methodological limitations). Several researchers have reported decreased GABA concentration in regions such as the ACC, occipital cortex (OC), and trigeminal brainstem nuclear complex (TBSNC) (Cleve et al., 2015; de Matos et al., 2017). Cleve and colleagues (2015) were among the first to report acute pain-related changes in glutamate and GABA. They observed 15.2% (ACC) and 15.7% (OC) decreases in GABA/total creatine (GABA/tCr) ratios in response to thermal pain stimulation administered to the inner forearm. Similarly, de Matos and colleagues (2017) observed an 11% decrease in TBSNC GABA following acute electric-dental pain administered to the upper-right canine. Conversely, Kupers et al. (2009) observed an *increase* in GABA concentrations in the rostral portion of the ACC (rACC). In this study, researchers observed a 15% increase in rACC GABA during acute thermal pain stimulation to the upper-right thigh. It should be noted, however, that this study was the only one among the four not to use GABA editing. Given the relative difficulty of quantifying GABA at lower field strengths without the use of editing, this raises concerns regarding their reported increase in GABA, as the two studies reporting GABA decreases did use editing. Finally, Cleve and colleagues (2017), the fourth study to report acute pain-related GABA, administered thermal pain to the back of the hand but found no resultant

differences in GABA concentration. Taken together, these results highlight the opacity of experimental pain-related GABA dynamics within the brain and the need for further research to elucidate these neurochemical relationships.

The variety of pain modalities and administration locations represented within neuroimaging pain research must be considered. Among the studies cited here, four different modalities were used (i.e., cold pressor, thermal, electrical, and pressure) in five separate locations (i.e., hand, foot, forearm, thigh, and teeth). The variety of methodologies seen here is especially problematic given that pain processing is a relatively small field compared to other neuroimaging domains. As a comprehensive MRS analysis of various pain modalities and locations has yet to be published (to my knowledge), we cannot be certain whether pain modality has a differential effect on neurometabolic responses. Considering the relatively small body of research in this area, more work is needed to uncover the role of the various metabolite systems in the brain during pain processing. The current study contributes to a growing literary base examining the effect of acute pain on glutamate. Furthermore, it is one of the few to characterize the relationship between GABA and experimentally induced pain. The present study also represents a continuation of one of the first lines of research to investigate these changes at 7T (Nichols et al., 2023). This simultaneous characterization of GABA and glutamate allows for a more complete understanding of the excitatory and inhibitory neurotransmitter contributions to pain systems in the brain and particularly within SI.

Present Study

The current study advances the pain and magnetic resonance spectroscopy literature bases in several ways. First, the use of 7T fMRS to study normative pain processing is particularly novel given that the majority of previous research has been done at lower field strength (i.e., ≤3T) (Archibald, MacMillan, Enzler, et al., 2020). The use of ultra-high field technology allows for more precise and reproducible characterization of neurometabolites (Reid et al., 2022). Second, the proposed study is among the first to report acute pain-related changes in neurometabolites in the primary somatosensory cortex (SI). Although previous research has used fMRI to examine event-related BOLD changes in pain processing, little is known about the underlying neurochemical systems that support normative acute pain processing in SI. Third, this project is among the few to report pain-related changes in GABA in the brain. By leveraging the strength of 7T fMRS, we were able to overcome challenges faced by previous research regarding the detection and measurement of GABA at lower field strengths.

Here, we used 7T fMRS to investigate acute pain-related changes in SI glutamate and GABA in healthy adults. We used a pressure-based pain device to administer acute pain across three conditions (no pain, low pain, and moderate pain). Based on the relationship between glutamate, GABA, and pain, the current study had the following hypotheses:

1. SI glutamate levels would be higher during low pain versus no pain (1A), moderate pain versus no pain (1B), and moderate pain versus low pain (1C).

2. SI GABA levels would be lower during low pain versus baseline (2A), moderate pain versus baseline (2B), and moderate pain versus low pain (2C).
3. Subjective pain evaluations would significantly predict changes in SI glutamate.
4. Subjective pain evaluations would significantly predict changes in SI GABA.

Methods

Overview

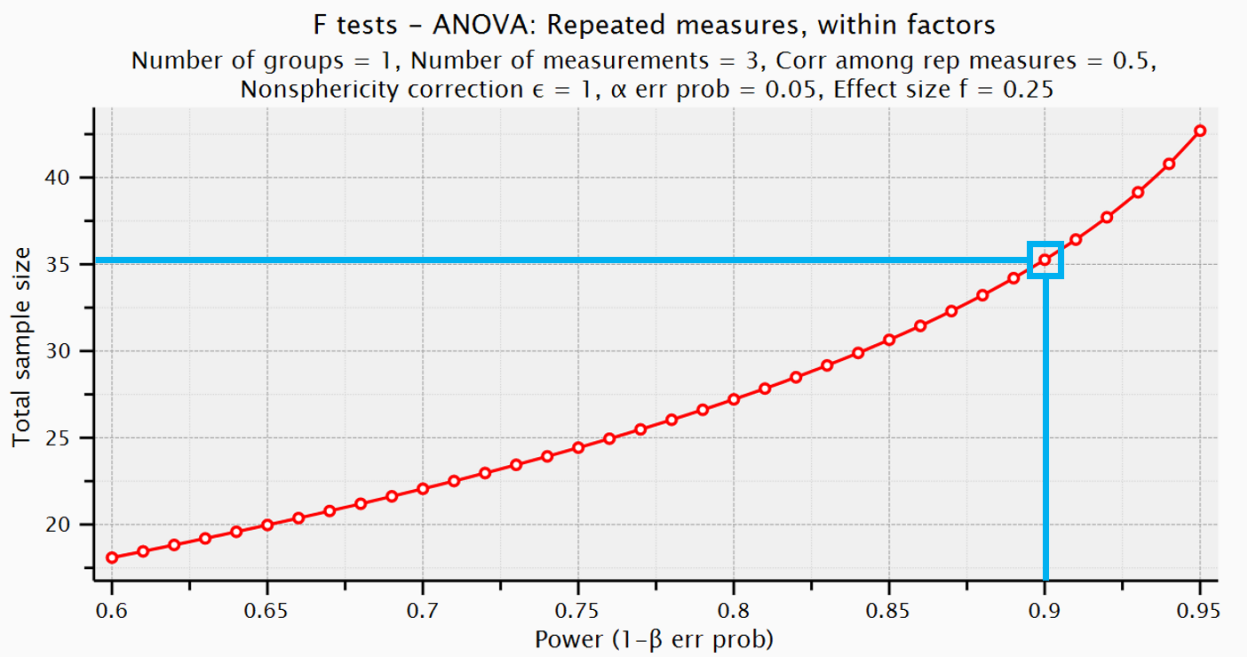
Based on the background literature reviewed above, I hypothesized that increasing amounts of nociceptive stimulation would result in 1) higher levels of SI glutamate and 2) lower levels of SI GABA. Further, I hypothesized that changes in subjective pain evaluations would be predictive of changes in 1) glutamate and 2) GABA levels. A dual timepoint study was used to assess these hypothesized neurometabolite changes. Timepoint 1 consisted of an online pre-screen survey to collect demographic information and identify eligible participants. Respondent eligibility was determined based on mental health status, substance use, and chronic pain history. Based on similar research done at Auburn University, I expected to recruit 500 participants for Timepoint 1. Timepoint 2 consisted of a single neuroimaging session to evaluate acute pain-related neurochemical changes in SI. We aimed to recruit approximately 36 participants for Timepoint 2 based on an *a priori* power analysis. A detailed description of each timepoint is provided below. All study procedures were approved by the Auburn University Institutional Review Board (protocol # 21-073 MR 2102). All participants provided written informed consent.

Participants

Adult participants between the ages of 19-50 were recruited from Auburn University and the greater Auburn-Opelika community (see Appendix A for recruitment materials). An *a priori* power analysis for repeated measures ANOVAs with an alpha = 0.05, a medium-large effect size, and 90% power recommended a sample size of n = 36 (Figure 1). Power analysis parameters were chosen based on findings from similar studies which observed moderate and large effect sizes of pressure-based pain on SI glutamate and GABA, respectively (Nichols et al., 2023). Only volunteers who met the following criteria were allowed to participate: 1) were not taking any over-the-counter or prescription medication that may cause or increase bleeding (i.e., blood thinners), 2) did not have a history of seizures, nor taking medication to treat seizures, 3) had not consumed drugs (including alcohol) in the 24 hours prior to the research study session, 4) had not consumed pain relievers in the 8 hours prior to the research study session, 5) had not consumed food, drinks (except water), caffeine, and/or nicotine in the 3 hours prior to the research study session, and 6) 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 (see appendix C). Examples included but were not limited to implanted cardiac pacemakers, embedded metal objects/fragments, and claustrophobia. Although MR is not associated with harmful effects on pregnancy, pregnant volunteers were not included as a precaution.

Figure 1

A Priori Power Analysis



A priori power analysis performed for a one-way repeated measures ANOVA (effect size f = 0.25, alpha = 0.05, 90% power).

Participant eligibility was also determined via pre-screen questionnaires inquiring about mental health status, substance use history, and chronic pain history (see Appendix B). These additional inclusion criteria included: 1) no dependence for alcohol and nicotine as determined by the Alcohol Dependence Scale (Doyle & Donovan, 2009) and a modified version of the Nicotine Dependence Scale for Adolescents (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) (as indicated by the Severity of Dependence Scale), and 3) must not have exceeded established “severe” cut-off scores

for anxiety (as indicated by the Generalized Anxiety Disorder 7, score > 15) (Spitzer et al., 2006), depression (as indicated by the Patient Health Questionnaire 9, score > 20, (Kroenke et al., 2001), and/or psychosis (Prodromal Questionnaire Brief Version, score > 6) (Loewy et al., 2011) (see Appendix B for questionnaires and survey materials used in Timepoint 1).

Menstrual Cycle Variance

Meta-analytic data indicate that current menstrual cycle phase may influence tolerance to experimentally induced pain (Riley et al., 1999). These data suggest that pain tolerance is lower during participants' follicular (pre-ovulation) phase. With this in mind, neuroimaging data were collected during the luteal phase (post-ovulation) of participants who experience menstrual 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), imaging data were 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 mitigate potential extraneous influences on pain perception, not due to the study procedures. Additionally, the within-subject design also minimized confounding factors with regard to individual differences in pain tolerance.

Materials

<u>Timepoint 1</u>

The following scales and inventories were used to characterize participants and determine eligibility during Timepoint 1: 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, depression, and psychosis. 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 were 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 >20 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 (see Appendix A). Neuroimaging data collection was carried out on the Siemens Terra.X 7T scanner at the Auburn University Neuroimaging Center. The scanner was outfitted with an 8-channel transmit, 32-channel receive head coil (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 ranged from 1-10, such that 1 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 were administered, as sleep quality has been shown to impact neuroimaging outcomes (Chee & Chuah, 2008; Ma et al., 2015). As a quality assurance measure, I 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 (outlier bounds were created by multiplying the IQR of the dataset by a step value of 1.5), thus I determined sleep patterns did not impact the fMRS data collected in the study. 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 provided written informed consent (Appendix C). After 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 were swept for metal using a hand-held metal detector. Participants laid on the scanner table and were given earplugs for hearing protection, an MR-compatible computer mouse that was used to provide subjective pain ratings, and a squeeze ball 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 several pain tolerance trials (described below) were administered to determine each participant's individual pressure levels to be used during the task.

Pain Stimulation

During fMRS data acquisition, participants underwent 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; Nichols et al., 2023) (Figure 2). The apparatus was fastened to participants' non-dominant hand as part of the scanner preparation procedure prior to entering the scanner (this placement was selected so that participants could interact with the MR-friendly computer mouse using their dominant hand). The pressures to be used for each participant's pain conditions were determined via several pain tolerance trials. Tolerance trials involved delivering increasing pressure to the apparatus until the participant indicated that 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 25% and 75% of each participant's maximum pain tolerance, respectively. These values were based on previous research using the same apparatus in similar conditions (Nichols et al., 2023). For example, if a participant's pain tolerance was measured to be 100 mm/Hg, their low and moderate pain pressure amounts would have been 25 mm/Hg and 75 mm/Hg, respectively (note: all participants' 'no pain' conditions were 0 mm/Hg). If any participant's pain tolerance exceeded the maximum pressure of the device, they would have been excluded from fMRS data collection, as accurate pressure levels for

low and moderate pain conditions could not have been calculated. No participants were excluded from the study for this reason.

Figure 2

Images of Pain Device

The device consists of a sphygmomanometer surrounding a small plastic disk with a rounded point positioned between participants' first and second knuckles on the nondominant hand.

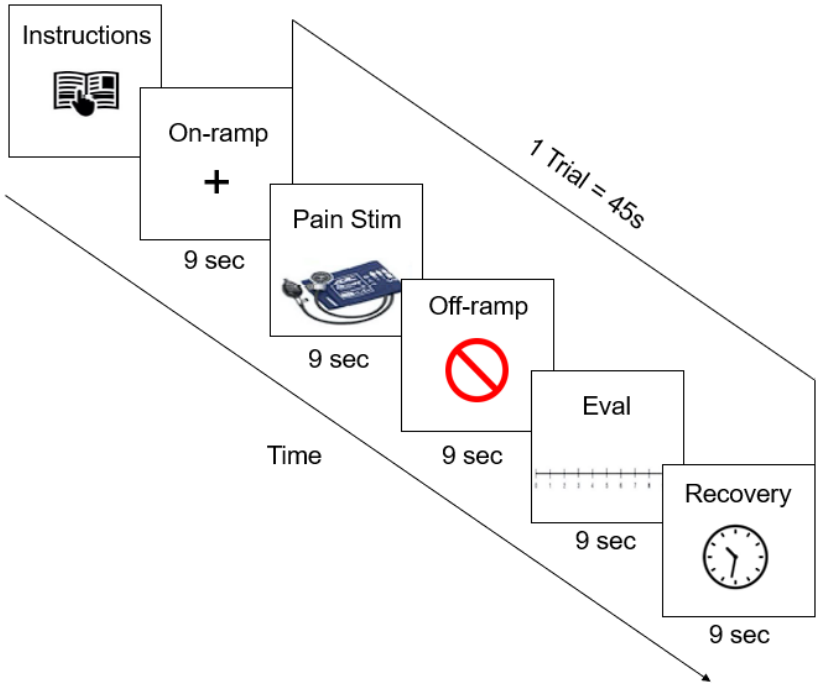
fMRS Data Collection

To characterize pain-related metabolite levels, fMRS data were collected during a single block of pseudo-randomized trials. These trials correspond to three separate pain conditions: 'no pain,' 'low pain,' and 'moderate pain.' Scanning during the pain task

consisted of 21 45-second trials for a total duration of approximately sixteen minutes (945 seconds; 315 averages). Each trial was divided into five 9-second phases: 1) the ‘ramp up’ phase (pressure increases to target level), 2) the ‘pain’ phase (pressure persists at target level), 3) the ‘off ramp’ phase (pressure decreases to 0), 4) the ‘eval’ phase (participants provide subjective pain ratings), and 5) the ‘recovery’ phase (a 9-second period of no pain stimulation) (Figure 3). 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) moderate pain, (2) no pain, and (3) low pain. During the evaluation phase, participants provided subjective pain ratings on a scale from 1-10 (1 = “no pain”; 10 = “most pain possible”) regarding the previous trial. Baseline MRS scans immediately preceded and followed the pain task. These scans consisted of 100 spectral averages collected at rest with no stimulation other than a visual crosshair (total duration: 5.5 minutes each). Data from these scans were collected to compare resting-state metabolite activity pre- and post-pain administration in a series of exploratory analyses.

Figure 3

Pain Task Trial Diagram



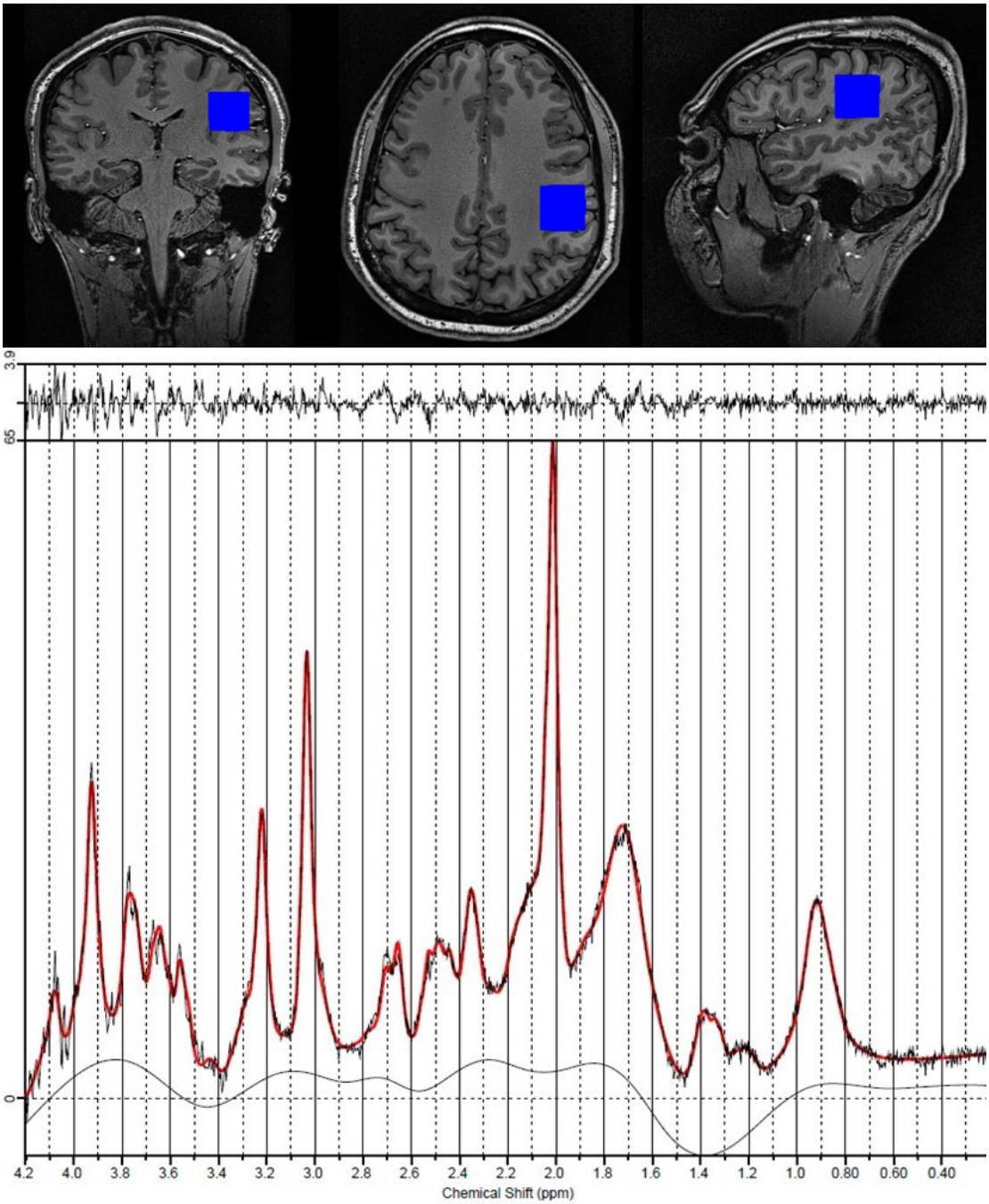
Trial structure was identical for no-, low-, and moderate pain trials. Instruction screen appeared before task onset and was not a part of the individual trial structure.

I collected fMRS data from one voxel (25 × 25 × 25 mm) centered around the right SI, contralateral to the hand on which the pain device was placed (Figure 4). Precise voxel placement was determined based on similar research (Nichols et al., 2023). Spectral averages were collected using a Siemens product STEAM sequence (TR/TE = 3000/20 ms, 4kHz spectral bandwidth, 2048 points). Three dimensional structural images were acquired to aid in fMRS voxel placement (MPRAGE, TR/TE/TI = 4000/3.15/1500 ms, CS acceleration factor = 2.9, FOV = 240 × 240 mm, base/phase resolution = 416/100%, 256 slices, 0.65 mm slice thickness, sagittal acquisition). The central sulcus served as a major landmark for voxel placement. The voxel was placed

around the lateral region of the postcentral gyrus, corresponding to left-hand digit representation in SI. The topographical map of SI detailed above provided further reference for voxel placement.

Figure 4

Voxel Placement and Sample Spectrum



Participant-level high-resolution structural images were used to guide placement of the 25 × 25 × 25 mm voxel in the right primary somatosensory cortex contralateral to participants' nondominant hand.

fMRS Data Preprocessing

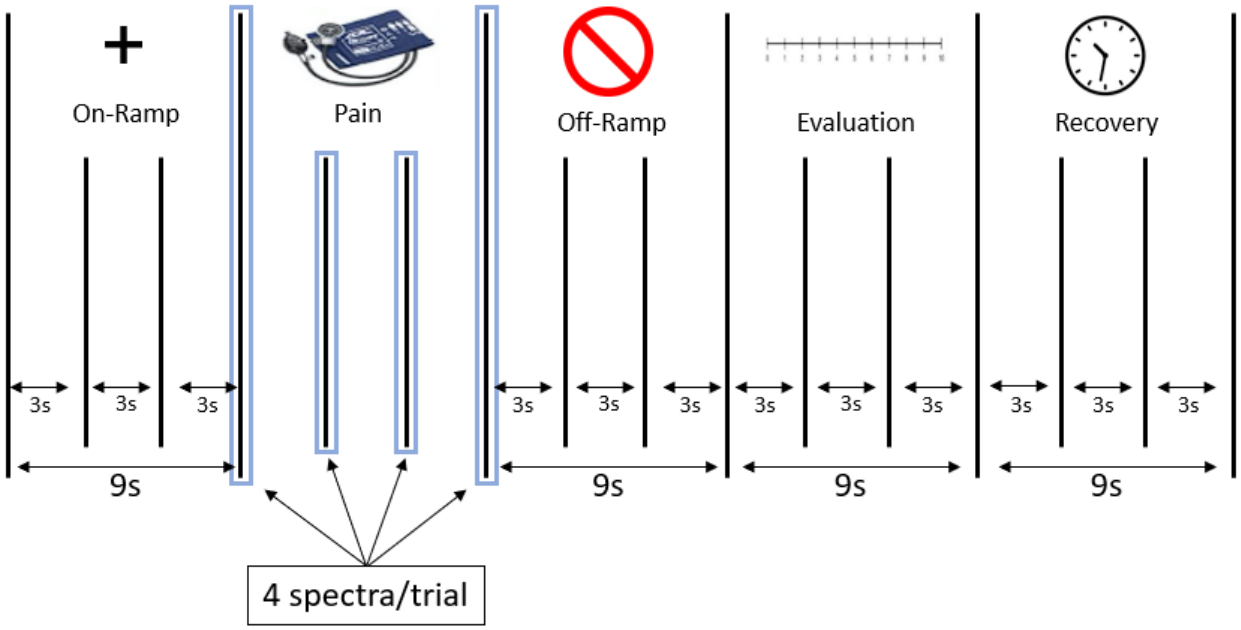
FMRS data were processed using LCModel software, a program used to fit *in vivo* proton MR spectra (Provencher, 2001). LCModel estimates observed spectra based on known values from simulations or from aqueous metabolite solutions (i.e., phantom solutions with known metabolite levels) (Provencher, 1993). Basis sets are created within LCModel using these 'model' spectra. This study used a basis set related to metabolites detectable via proton ^{1}H MR Spectroscopy with matching sequence parameters (i.e., field strength and TE). The following metabolites from this basis set are reported in the current study: total creatine (tCr), GABA, glutamine, glutamate, glutamate + glutamine (Glx), total *N*-acetylaspartate (tNAA), total choline (tCho), *myo*-inositol (Ins), and glutathione (GSH). The water-suppressed spectra were eddy current-corrected and quantified using an unsuppressed water signal (4 averages).

The following inclusion criteria were used for metabolite data: a signal-to-noise ratio (SNR) greater than or equal to 20, Cramer-Rao lower bounds (CRLB) less than or equal to 30%, and mean metabolite linewidths less than or equal to 0.07 ppm. These data quality thresholds were based on recommendations from recent expert consensus papers (Near et al., 2021a; Öz et al., 2021). Prior to metabolite level estimation, spectra were averaged over several trials and across conditions such that individual spectra corresponding to each participant's no-, low-, and moderate pain trials were averaged into a single value for each condition (i.e., singular mean values for no-, low-, and moderate pain-related metabolites were calculated for each participant). Specifically, the four spectral averages collected during all the 'pain' phases of the task were grouped by condition to create a single mean value (Figure 5). Ultimately, these single mean

values for each pain condition consisted of 28 spectral averages each (7 trials per condition * 4 spectral averages per trial = 28). Following metabolite estimation through LCModel, metabolite data underwent partial volume-correction prior to statistical analysis, a standard practice when using water-scaling (Near et al., 2021). This was facilitated by voxel tissue segmentation performed in MATLAB using the Gannet Toolbox (Edden et al., 2014). Gannet uses SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) to segment the structural image before calculating tissue ratios within the voxel (i.e., gray matter, white matter, and cerebrospinal fluid). Metabolite data were then analyzed via repeated-measures ANOVAs, linear regressions, and paired t-tests as described below.

Figure 5

Visual depiction of MATLAB Preprocessing Steps



Vertical lines represent spectral averages separated by the 3000ms repetition time of the MRS sequence. Highlighted are the 4 averages per trial that were selected and combined to create mean metabolite values per pain condition.

Analytic Plan

Hypothesis 1 (1A-C)

To test whether SI glutamate levels increased with respect to pain levels, I conducted a repeated measures ANOVA with pain level as the within-subjects variable of interest.

Hypothesis 2 (2A-C)

To test whether SI GABA levels decreased with respect to pain levels, I conducted a repeated-measures ANOVA with pain level as the within-subjects variable of interest.

Hypotheses 3 & 4

To test whether changes in SI glutamate and GABA were significantly predicted by participant pain ratings, I performed linear regressions to determine the relationship between pain ratings (predictor variable) and metabolite levels (outcome variable).

Exploratory Analyses

To examine potential differences in resting-state metabolite levels before and after acute pain administration, I performed a series of paired-samples t-tests with condition as the independent variable (i.e., pre- vs. post-pain) and metabolite levels as the dependent variable.

Post-Hoc and Outliers

Tukey's post-hoc tests were to be used to determine group differences in the event of a significant omnibus ANOVA for Hypotheses 1 and 2. Outliers were defined as any data point which falls outside the upper and lower bounds created by multiplying the

interquartile range (IQR) of the dataset by a step value of 1.5. Normality was assessed using the Shapiro-Wilk test (Shapiro & Wilk, 1965).

Results

Participants

During recruitment (Timepoint 1), 139 respondents completed the online pre-screen materials. Of those, 38 respondents met the eligibility criteria and were invited to participate in part two of the study. Ultimately, 24 healthy participants (mean age = 23.63 ± 3.09 years (M ± SD)) were enrolled in Timepoint 2 of the study and completed data collection. Due to poor data quality, and controlling for univariate outliers, data from several participants were excluded from data analysis. Thus, sample sizes for the final analyses were n = 21 for glutamate and n = 19 for GABA (see Table 1 for a complete demographic summary).

Table 1

Demographics

Sample Characteristics	
N	24
Sex	16 F, 8 M
Age (years)	23.63 ± 3.09
Race (A/B/H/W)	(1/3/0/20)
Hispanic	1
Left-Handed	0
Mental Health	
WEWBS	52.67 ± 6.20
PSS	16 ± 6.21
GAD-7	5 ± 3.71
PHQ-9	3.76 ± 4.14
PQB	0.62 ± 1.29
Pain History	
GCPS	0
NPS	0
Substance Use	
SDS - Amphetamines	0
SDS - Cannabis	0
SDS - Cocaine	0
SDS - Opioids	0
SDS - Stimulants	0
ADS	3.14 ± 2.99
NDSA	0
Experimental Pain	
Tolerance (mmHg)	195.2 ± 73.83
Low (mm/Hg)	48.8 ± 18.46
Moderate (mm/Hg)	146.4 ± 55.37

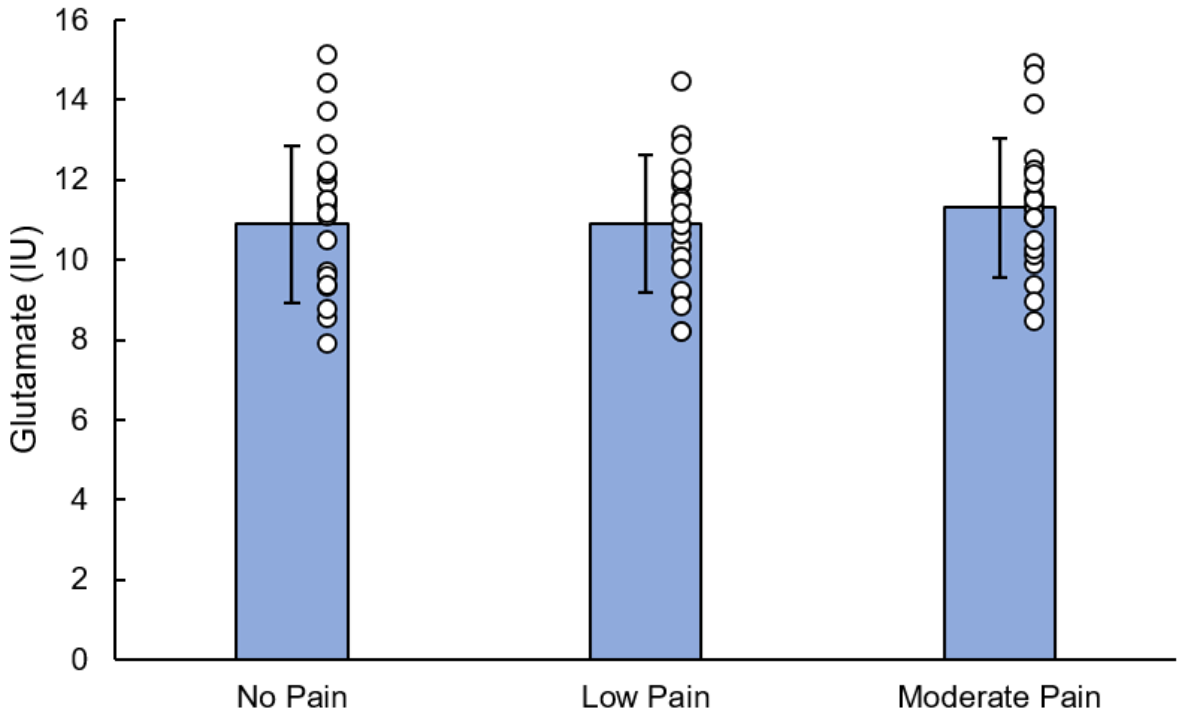
All data are presented as M (SD). Race data are Native American/Black/Asian/White. Mental health scales are Warwick-Edinburgh Mental Wellbeing Scale (WEWBS), Perceived Stress Scale (PSS), Generalized Anxiety Disorder 7 (GAD-7), Patient Health Questionnaire 9 (PHQ-9), Prodromal Questionnaire Brief Version (PQB), Pain scales are Graded Chronic Pain Scale (GCPS) and Neuropathic Pain Scale (NPC). Substance use scales are Severity of Dependence Scale (SDS), Alcohol Dependence Scale (ADS), and Nicotine Dependence Scale for Adolescents (NDSA). Pain data are millimeters of mercury (mmHg).

Pain and SI Glutamate (Hypothesis 1)

A repeated measures ANOVA was conducted with glutamate as the dependent variable and pain condition as the within-subjects factor. There was no significant effect of pain condition on SI glutamate: $F(2, 36) = 0.885$, $p = 0.422$, partial $\eta^2 = 0.047$; $Glu_{no\text{-}pain} = 11.1 \pm 1.97$, $Glu_{low\text{-}pain} = 10.9 \pm 1.72$, and $Glu_{moderate\text{-}pain} = 11.4 \pm 1.73$ (all values are M ± SD; Figure 6). After controlling for univariate outliers, the final sample size was $n = 21$. Shapiro-Wilk tests indicated the data were normally distributed ($p_{no} = 0.40$, $p_{low} = 0.37$, $p_{moderate} = 0.72$).

Figure 6

SI Glutamate by Pain Condition



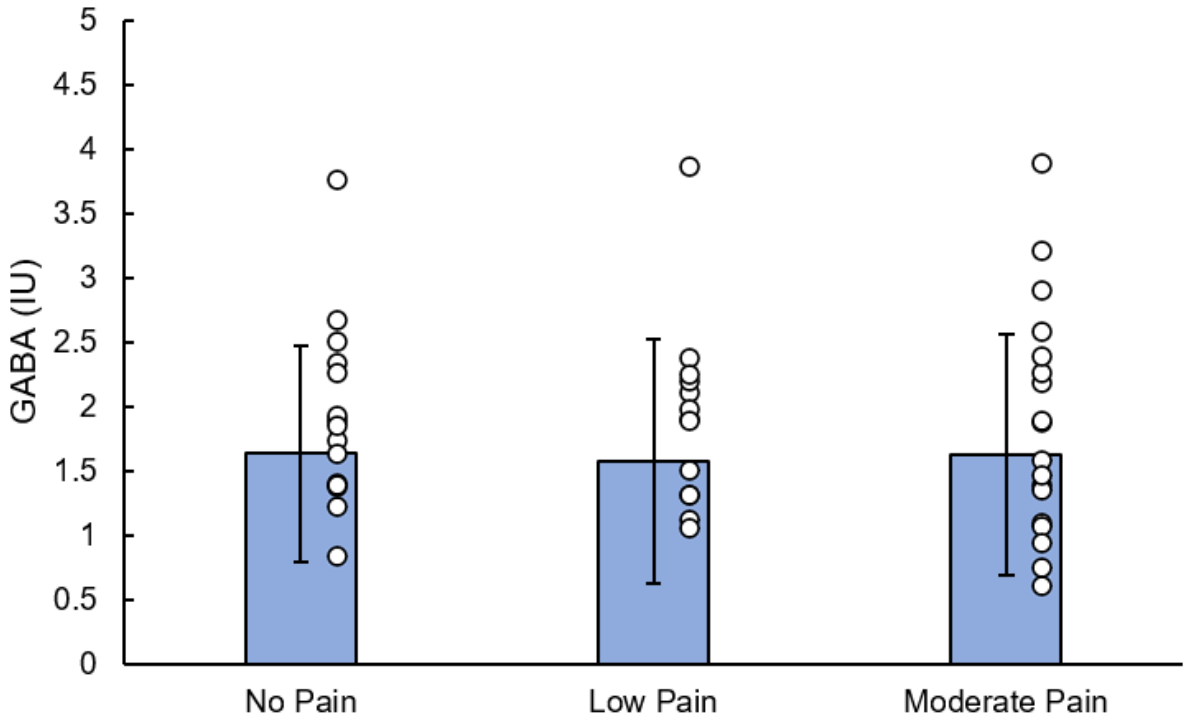
Data are presented in institutional units (IU). Error bars are standard deviation.

Pain and SI GABA (Hypothesis 2)

A repeated measures ANOVA was conducted with GABA as the dependent variable and pain condition as the within-subjects factor. There was no significant effect of pain condition on SI GABA: $F(2, 32) = 1.06$, $p = 0.358$, partial $\eta^2 = 0.062$; $GABA_{no\text{-}pain} = 1.61 \pm 0.84$, $GABA_{low\text{-}pain} = 1.58 \pm 0.95$, and $GABA_{moderate\text{-}pain} = 1.63 \pm 0.94$ (data are M ± SD; Figure 7). After controlling for univariate outliers, the final sample size was $n = 19$. Shapiro-Wilk tests indicated the data were normally distributed ($p_{no} = 0.33$, $p_{low} = 0.30$, $p_{moderate} = 0.31$). See Table 2 for a full list of SI metabolites.

Figure 7

SI GABA by Pain Condition



Data are presented in institutional units (IU). Error bars are standard deviation.

Table 2

Pain ANOVAs & Data Quality Metrics

Measure	No Pain	Low Pain	Mod. Pain	Statistic	*p*	η^2_p
GABA	1.61 ± 0.84	1.58 ± 0.95	1.63 ± 0.94	*F*(2,32) = 1.06	0.358	0.062
CRLB	15.45%	16.70%	14.80%			
Glutamate	11.1 ± 1.97	10.9 ± 1.72	11.4 ± 1.73	*F*(2,36) = 0.885	0.422	0.047
CRLB	3.41%	3.35%	3.46%			
Glutamine	2.04 ± 0.68	1.97 ± 0.65	1.96 ± 0.58	*F*(2,36) = 0.314	0.732	0.017
CRLB	10.50%	10.61%	11.13%			
Glu + Gln	13.2 ± 2.34	12.8 ± 2.10	13.3 ± 2.05	*F*(2,36) = 0.403	0.671	0.022
CLRB	2%	2.40%	2.40%			
Glu/GABA	5.51 ± 1.91	5.77 ± 2.04	5.39 ± 1.53	*F*(2,32) = 0.554	0.580	0.032
CRLB	--	--	--			
GSH	2.38 ± 0.72	2.38 ± 0.73	2.60 ± 0.65	*F*(2,32) = 1.01	0.374	0.053
CLRB	8.64%	9.52%	9.17%			
***myo*-Inositol**	7.21 ± 1.77	7.08 ± 1.68	7.23 ± 1.08	*F*(2,34) = 2.49	0.101	0.151
CRLB	6.45%	7.52%	5.30%			
Total Choline	1.66 ± 0.34	1.61 ± 0.33	1.67 ± 0.33	*F*(2,36) = 0.298	0.744	0.016
CRLB	5.64%	5.04%	5.80%			
Total Creatine	8.95 ± 0.91	8.97 ± 0.90	9.13 ± 0.83	*F*(2,40) = 0.554	0.580	0.030
CRLB	2.50%	2.26%	2.33%			
Total NAA	13.8 ± 2.28	13.7 ± 2.34	14.4 ± 1.16	*F*(2,38) = 0.946	0.398	0.050
CRLB	2.18%	2.13%	2.16%			
SNR	32.0 ± 10.1	32.1 ± 10.2	32.6 ± 10.7	*F*(2,40) = 0.243	0.785	0.012
Metabolite LW	0.038 ± 0.01	0.037 ± 0.01	0.038 ± 0.01	*F*(2,40) = 0.371	0.693	0.018
GM, %	47.4 ± 7.1	--	--	--	--	--
WM, %	49.9 ± 7.9	--	--	--	--	--
CSF, %	2.8 ± 1.4	--	--	--	--	--

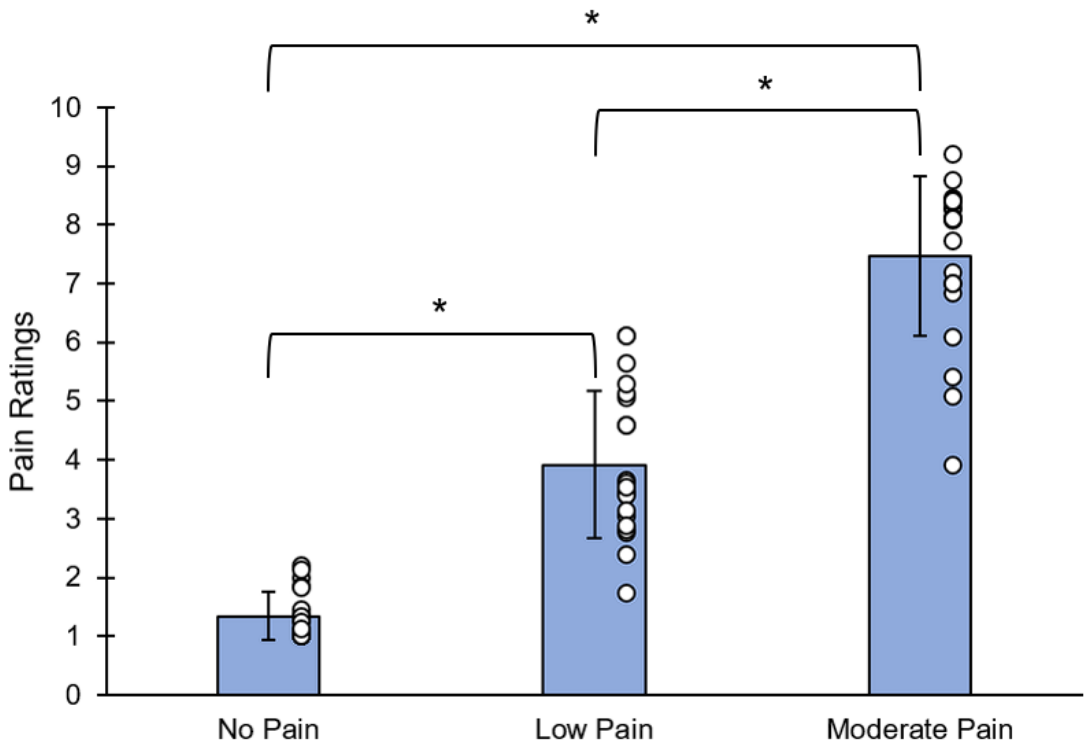
Data are presented in institutional units as M ± SD. Effect sizes are partial eta squared. Metabolite line-widths are ppm. Metabolite abbreviations are γ-Aminobutyric Acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (GSH), and total N-acetylaspartate (Total NAA). Data quality metrics are Cramer-Rao Lower Bounds (CRLB), signal-to-noise ratio (SNR), and metabolite line width (LW). Voxel tissue composition is broken down into percentage of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF).

Subjective Pain Rating Regressions (Hypotheses 3 & 4)

Participants provided subjective pain ratings during the task for each pain stimulus for 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 for each condition were $Pain_{no}$ = 1.34 ± 0.40, $Pain_{low}$ = 3.92 ± 1.22, and $Pain_{moderate}$ = 7.47 ± 1.32 (data are M ± SD) (Figure 8). A repeated measures ANOVA revealed a significant difference between pain ratings ($F(2, 40) = 326$, $p < 0.001$, partial $\eta^2 = 0.942$), indicating that the pain manipulation was effective in producing perceptually different amounts of stimulation. Post-hoc comparisons with Tukey's correction for multiple comparisons revealed subjective ratings in the no-pain condition to be significantly lower than the low-pain condition ($t(20) = 11.5$, $p_{Tukey} < 0.001$) and the moderate pain condition ($t(20) = 23.8$, $p_{Tukey} < 0.001$). Subjective pain ratings were also significantly lower in the low-pain condition than moderate pain ($t(20) = 14.8$, $p_{Tukey} < 0.001$). Linear regressions were run to predict SI glutamate and GABA levels based on subjective pain ratings. The glutamate regression model was not statistically significant ($F(1,57) = 0.998$, $p = 0.322$; $R^2 = 0.017$) (Figure 9), nor was the GABA model ($F(1,56) = 0.412$, $p = 0.523$; $R^2 = 0.007$) (Figure 10). The regression models indicate that pain ratings were not predictive of either SI glutamate or GABA levels. See Table 3 for a full list of metabolite-by-pain rating regressions.

Figure 8

Subjective Pain Ratings

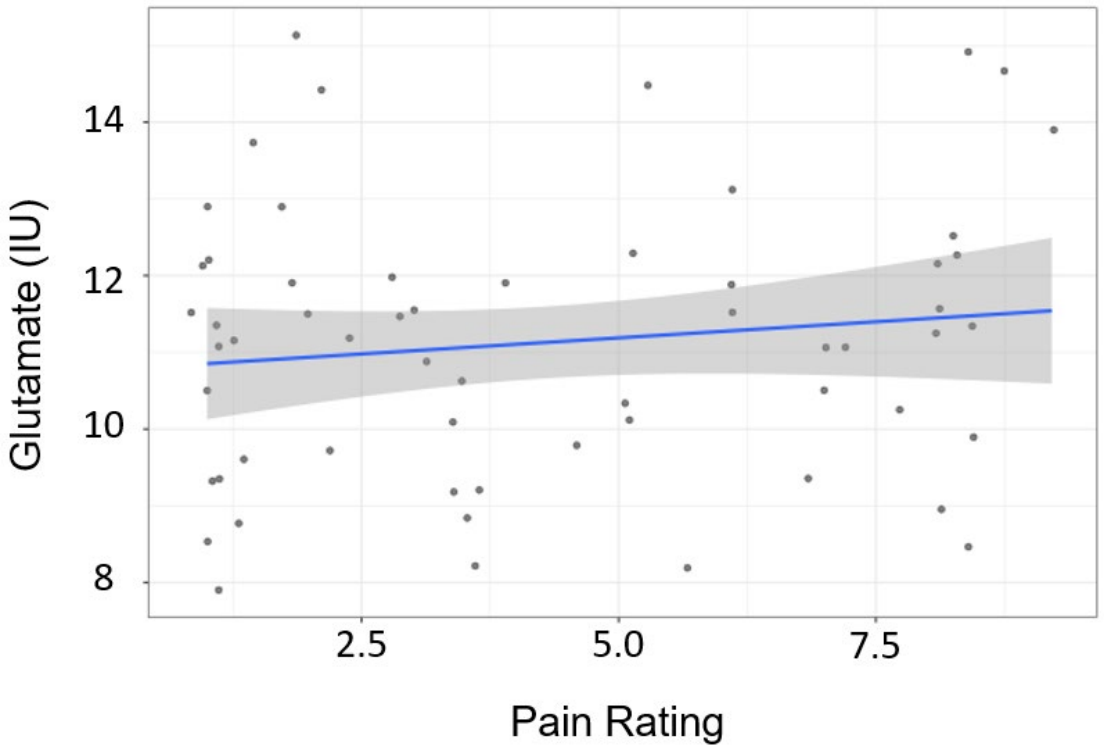


Data are on a scale from 1-10 where 1 = 'no pain' and 10 = 'most pain possible. Error bars are standard deviation.

* = significant at $p < 0.001$.

Figure 9

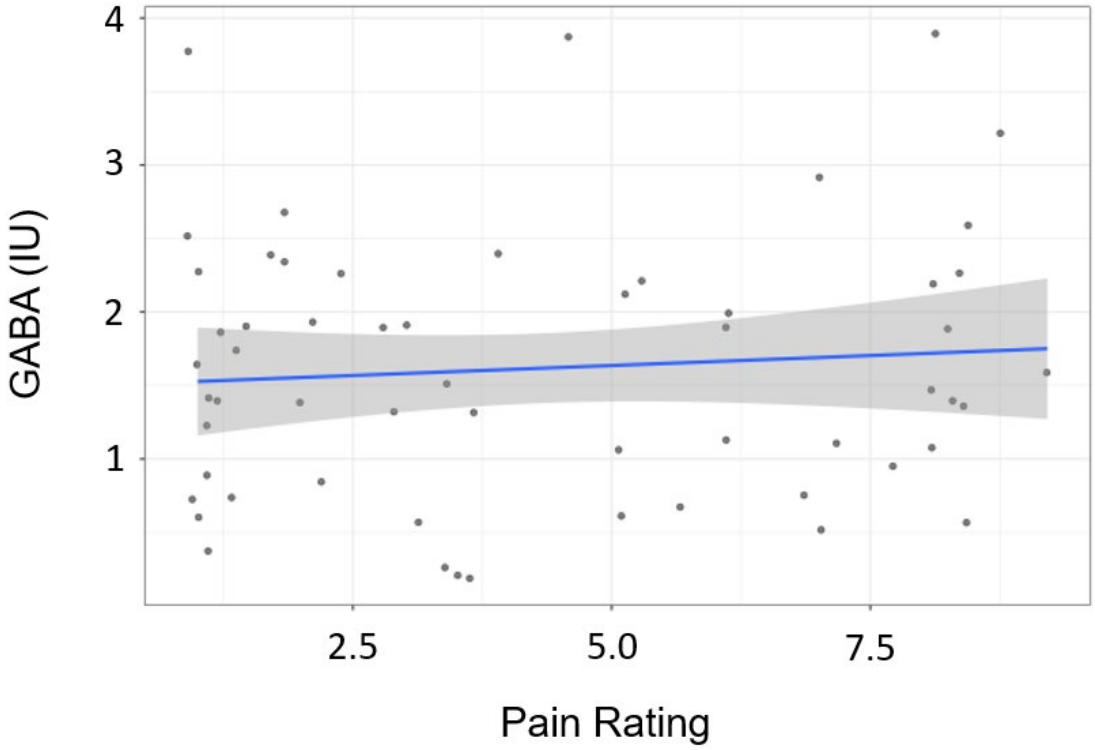
Pain Rating by Glutamate Regression



Subjective pain rating by SI glutamate linear regression. The fit line and 95% confidence bands are represented by the blue line and shaded area, respectively.

Figure 10

Pain Rating by GABA Regression



Subjective pain ratings by SI GABA linear regression. The fit line and 95% confidence bands are represented by the blue line and shaded area, respectively.

Table 3

Metabolite Regressions & Mean Pain Ratings

Measure	Statistic	*p*	R^2
GABA	$F(1,56) = 0.412$	0.523	0.007
Glutamate	$F(1,57) = 0.99$	0.322	0.017
Glutamine	$F(1,57) = 0.254$	0.616	0.004
Glu + Gln	$F(1,57) = 0.968$	0.329	0.017
Glu/GABA	$F(1,56) = 0.01$	0.919	0.0002
GSH	$F(1,57) = 0.954$	0.333	0.017
***myo*-Inositol**	$F(1,56) = 0.152$	0.698	0.003
Total Choline	$F(1,57) = 0.148$	0.702	0.003
Total Creatine	$F(1,57) = 0.744$	0.392	0.013
Total NAA	$F(1,57) = 1.13$	0.292	0.020

Measure	No Pain	Low Pain	Moderate Pain
Mean Rating	1.34 ± 0.4	3.90 ± 1.2	7.47 ± 1.3

Complete results of pain rating-by-metabolite regressions and mean pain condition ratings. Pain ratings are M ± SD Metabolite abbreviations are γ-Aminobutyric Acid (GABA), glutamate + glutamine (Glx), glutathione (GSH), and total N-acetylaspartate (Total NAA).

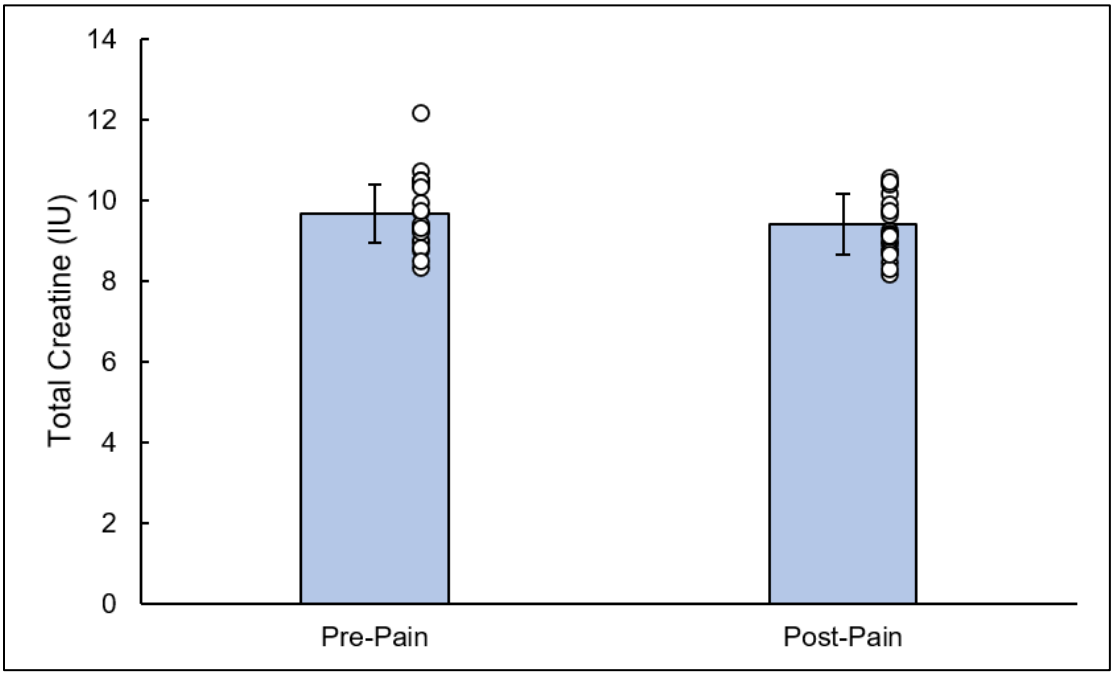
Exploratory Analyses

Two-tailed, paired samples t-tests were run to investigate potential differences in resting-state neurometabolite data (i.e., no-task conditions) collected pre- and post-experimental pain administration. Among these, total creatine was statistically significant ($t(20) = 2.65$, $p = 0.015$, $d = 0.578$) ($tCr_{pre\text{-}pain} = 9.56 \pm 0.73$ and $tCr_{post\text{-}pain} = 9.33 \pm 0.75$ (data are M ± SD) (Figure 11). After controlling for univariate outliers, the final sample size was $n = 19$. Shapiro-Wilk tests indicated the data were normally distributed ($p_{pre} = 0.82$, $p_{post} = 0.57$). No statistically significant relationships were observed among the

other metabolites. See Table 4 for a full comparison of pre-pain versus post-pain metabolite levels.

Figure 11

Total Creatine Pre- vs. Post-Pain



Data are presented in institutional units (IU). Error bars are standard deviation.

* = significant at $p = 0.05$.

Table 4

Pre-Pain Versus Post-Pain MRS

Measure	Pre-Pain	Post-Pain	Statistic	*p*	Cohen's *d*
GABA	1.96 ± 0.80	1.79 ± 0.55	t(18) = 1.01	0.327	0.238
CRLB	11.18%	11.87%			
Glutamate	12.36 ± 1.48	12.10 ± 1.35	t(20) = 1.04	0.311	0.227
CRLB	2.46%	2.42%			
Glutamine	3.02 ± 0.76	2.93 ± 0.63	t(19) = 0.21	0.836	0.05
CRLB	6.83%	6.46%			
Glu + Gln	11.41 ± 1.22	11.03 ± 1.22	t(20) = 0.71	0.485	0.156
CLRB	4.29%	4.93%			
Glu/GABA	5.94 ± 1.27	5.05 ± 2.13	t(18) = 1.36	0.197	0.364
CRLB	--	--			
GSH	2.15 ± 0.26	2.13 ± 0.29	t(20) = 0.91	0.371	0.200
CRLB	5.54%	5.54%			
myo-Inositol	5.67 ± 0.50	5.65 ± 0.46	t(20) = 0.09	0.927	0.020
CRLB	4.54%	4.17%			
Total Choline	1.83 ± 0.14	1.81 ± 0.18	t(20) = 1.56	0.135	0.340
CRLB	3.71%	4.13%			
Total Creatine *	9.56 ± 0.73	9.33 ± 0.75	t(20) = 2.65	0.015	0.578
CRLB	1.88%	1.88%			
Total NAA	14.5 ± 1.01	14.23 ± 1.17	t(20) = 1.40	0.179	0.304
CRLB	1.83%	1.88%			
SNR	51.1 ± 16.8	52.7 ± 14.3	t(20) = 0.78	0.441	0.170
Metabolite LW	0.04 ± 0.01	0.04 ± 0.01	t(20) = 0.29	0.769	0.065
GM, %	47.4 ± 6.9	--	--	--	--
WM, %	49.9 ± 7.6	--	--	--	--
CSF, %	2.8 1.5	--	--	--	--

All data are presented in institutional units as M ± SD. Metabolite abbreviations are γ-Aminobutyric Acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (GSH), and total N-acetylaspartate (Total NAA). Data quality metrics are Cramer-Rao Lower Bounds (CRLB), signal-to-noise ratio (SNR), and metabolite line width (LW). Voxel tissue composition is broken down into percentage of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Metabolite linewidths are ppm.

* = significant at $p = 0.05$.

Discussion

To advance the field's understanding of normative pain processing in the brain, the current study examined the underlying neurometabolic signatures of SI in response to experimental pain using ultra-high field fMRS. Here, a pressure-based pain task was utilized to assess SI glutamate and GABA, thus capturing pain-evoked changes in neurochemical excitation/inhibition. Data from the present study suggest that neither low nor moderate levels of acute pressure-based pain were associated with significant changes in SI glutamate or GABA. Indeed, null statistical results from pain-related glutamate and GABA ANOVAs indicate a lack of support for Hypotheses 1 & 2, respectively. The current study also investigated a potentially predictive relationship between glutamate and GABA levels and subjective pain ratings. This was accomplished through a series of linear regressions to model the relationship between these variables. Regression models failed to establish a statistically significant link between pain ratings and metabolites, such that pain-related glutamate and GABA levels were not predictive of subsequence subjective pain ratings. Thus, I did not find evidence to support Hypotheses 3 & 4.

The Somatosensory Cortex within the Pain Network

A large body of current literature has identified major nodes throughout the brain involved in pain processing. These nodes collectively form what is known as the 'pain network' that collaboratively inform the conscious perception of pain. This network spans major cortical regions such as the ACC, PFC, and SI while also including subcortical structures such as the brainstem, thalamus, and basal ganglia (Coghill et al., 1999; Hutchison et al., 1999; Jones et al., 1991; Vierck et al., 2013). Among the cortical

regions in this network, SI's role in pain processing is unique. While the ACC and PFC appear to be predominantly involved in affective and cognitive aspects of pain, consensus exists that SI is primarily involved in encoding information related to pain stimulus itself (Vierck et al., 2013). Indeed, SI has been shown to be responsible for processing a variety of nociceptive information including fast-moving signals arriving in area 3a/1 via myelinated axons, and slow-moving signals arriving in area 3a via unmyelinated axons (Kenshalo et al., 2000; Oshiro et al., 2009). SI acts as an integration site for these signals, interpreting the sensory information projected from thalamic nuclei to inform our perception of stimulus presence, modality, and intensity (Vierck et al., 2013). These findings highlight the diversity of function within SI regarding pain processing.

Given the nuanced nature of pain integration in SI, it is possible that the pain stimulus induced fluctuating neurometabolic changes throughout the task. This proposed variation in neurochemical response behavior may have contributed to the current study's null glutamate and GABA findings, as these data were averaged across the duration of the task for statistical analysis. Follow up time course analyses may reveal significant changes in pain-related metabolite levels over time that were masked by averaging the data. Further, the distribution of various neuronal subtypes involved in nociceptive and nonpainful tactile stimulation in SI is not fully understood in humans. Given the relatively large voxel size (25x25x25mm) used in the current study, it is likely that the multiple SI neuronal subtypes were all represented within the voxel-tissue. This may have confounded results from the current study, as differential effects of stimulus modality on neurochemical excitation/inhibition have yet to be reported. The questions

that remain surrounding these phenomena stem from the overall lack of current SI-specific neuroimaging pain research and highlight the need for further research.

Glutamate and Acute Pain

Regarding Hypothesis 1, data from the current study indicate that increasing levels of nociceptive stimulation did not lead to a subsequent rise in glutamate level. As this was only the second reported observation (to my knowledge) of acute pain-related SI glutamate levels, there is little current research to which direct comparisons can be made. In a similar study, we (Nichols et al. (2023)) also reported a null relationship between acute pain and glutamate. However, we did report a moderate effect size of glutamate (d = 0.467), attributing the nonsignificant result to insufficient power. This effect exceeds the one seen here (η^2_p = 0.047), despite the improved sample size in the current study.

Given the ubiquity of pain-related glutamate increases that have been reported throughout the brain, results from the current study are also inconsistent with previous research overall. Indeed, trends in current spectroscopic pain literature suggest that experimental pain is associated with increased glutamate levels in multiple brain regions including subregions of the anterior cingulate (dACC (Nichols et al., 2023) and rACC (Kupers et al., 2009)); multiple insular subdivisions (bilateral insular cortex (Gutzeit et al., 2011, 2013), anterior and posterior insula (Gussew et al., 2010; Gutzeit et al., 2013)); the occipital cortex (Cleve et al., 2015); and the brainstem (de Matos et al., 2017). Differences in pain modality may have contributed to these inconsistencies. While pressure-based pain has been shown to be an effective manipulation, thermal pain is more commonly seen among neuroimaging pain research. It is possible that

different pain modalities (e.g., mechanical, thermal, electrical, etc.) may elicit differential neurometabolic responses in the pain network. Unfortunately, to my knowledge, there has been no published study examining the potential effects of pain modality. Without such a study to address this question specifically, we cannot say for certain whether neurochemical systems in the pain network are differentially affected by these various modalities. Furthermore, although data from this project are inconsistent with previous work, they represent a singular study among an overall body of literature that overwhelmingly supports a positive relationship between experimental pain administration and glutamate levels/concentrations.

GABA and Acute Pain

Regarding Hypothesis 2, data from the current study did not indicate that rising levels of nociceptive stimulation resulted in subsequent decreases in GABA levels. Similar to glutamate, this study is only the second to report acute pain-related GABA levels in SI. We (Nichols et al. (2023)) reported a narrowly insignificant decrease in GABA during moderate pain conditions with a large effect size ($p = 0.058$; $d = 0.739$). Once again, present results were inconsistent with our previous study, observing both a nonsignificant *p*-value (0.840) and a negligible effect size ($\eta^2_p = 0.01$).

Results from this study contribute to a growing body of pain-related GABA literature from which clear trends have yet to emerge. Due to challenges associated with using MRS to investigate GABA in the brain (i.e., lack of ultra-high field imaging, weak metabolite signal, additional processing steps, etc.), the collection of studies that report GABA levels remains small, particularly in the field of pain. Among this small group, positive, negative, and null relationships have all been reported among other

brain regions. In support of an inverse relationship between GABA and pain, Cleve et al. (2015) and de Matos et al. (2017) both reported decreases in GABA following experimental pain administration. These results fit logically into our current knowledge of underlying neurochemical systems. As increases in both neurochemical excitation and blood-oxygen-level-dependent (BOLD) activation across brain regions have been consistently reported in previous research (Ip et al., 2019; Kiemes et al., 2021; Moon et al., 2021), a corresponding decrease in GABA would be a reasonable expectation, given the shift away from inhibition. Contradicting these results, Kupers et al. (2009) reported an increase in rACC GABA concentrations following pain administration. Despite its contradiction to other studies, an increase in GABA may also fit logically into our understanding of neurochemistry. Given the ACC's role in affective pain processing (Xiao & Zhang, 2018), an increase in neurochemical inhibition of the area to modulate its response to prolonged pain stimulation (four minutes of constant stimulation in their study) is plausible. Taken together, these findings may suggest a more nuanced response to pain in the brain, rather than one that elicits purely increased excitation *or* inhibition.

Finally, in support of a null relationship between GABA and pain, Cleve et al. (2017) reported no changes in GABA concentration from baseline to pain-on conditions. It is possible that in this study, as well as our own, a phenomenon similar to the one described above occurred in which increased neurochemical inhibition in response to a prolonged pain stimulus produced a null result when averaging the data across the duration of the task. This point is given further consideration below. Overall, it is unclear why the current study failed to observe significant relationships between pain, and

glutamate or GABA. In addition to the contextualization of these results among previous research provided above, several possible explanations for our findings are given further consideration below.

A Neuromodulatory Model of Pain Processing

Our current understanding of neurological pain processing informs us that the brain's response to painful stimuli is a multifaceted system. Indeed, the underlying mechanisms that support pain processing are influenced by more than simply raw nociceptive input; the descending pathway of pain modulation is influenced by cognitive and emotional factors as well (Gebhart, 2004). With this in mind, neurological and behavioral responses to pain aversiveness may provide insight into the findings of the current study.

The brain's response to, and ongoing communication of, pain signals is the result of a complex system of interrelated chemical pathways (Bingel & Tracey, 2008). While these pathways are subject to influence from objective factors such as stimulation intensity, they may also be influenced by cognitive and emotional factors associated with the perception of the stimulus (Benedetti et al., 2005; Khera & Rangasamy, 2021). Given that these modulatory systems are primarily inhibitory (Gebhart, 2004), the current study's failure to observe differences in GABA levels between pain conditions may be the result of a uniform increase in neurochemical inhibition across the pain matrix in response to prolonged stimulation. If such a phenomenon was occurring, the similarity in GABA levels among task conditions observed in the current study could be due to heightened levels of the metabolite throughout the task as part of a reactionary response to the unpleasant stimulation, thus resulting in downstream increases in

inhibition. Previous research demonstrating increased activation of pain network nodes associated with stimulus aversiveness may support this proposed explanation (ACC (Fuchs et al., 2014); PFC (Ong et al., 2019); insula (Labrakakis, 2023)). Participant evaluations of the pain stimulus complement this proposal, as many volunteers reported pain mitigation strategies that highlight the aversive nature of the stimulus such as “bracing for the pain,” “clenching my teeth,” and “taking deep breaths.” In short, the similarity in GABA levels seen in SI throughout the task may be the result of a larger affective response mechanism within the pain network. Although this theory is informed by previous research, it represents a single proposed explanation in an extremely novel area of inquiry (SI pain MRS). Follow-up studies are needed to learn more about the neurochemical systems within the pain network, specifically the somatosensory cortex.

Neurochemical Limitations During Prolonged Stimulation

The above model of descending pain modulation provides a plausible explanation for the GABA findings in the current study. Similarly, neurochemical energy demands during a prolonged stimulation task (15+ minutes in the present study) may offer some insight into the lack of hypothesized glutamate increase. The brain requires large portions of the body’s energy supply to perform its multitude of functions. Commonly accepted measurements estimate that the brain represents ~20% of the body’s metabolic load, while representing only ~2% of its weight (Mallik & Frank, 2023; Rolfe & Brown, 1997). Previous research has demonstrated that creatine plays a crucial role in managing the brain’s energy supply by aiding the recycling of ATP (adenosine triphosphate) (Brosnan & Brosnan, 2007; Wallimann et al., 2011; Wyss & Kaddurah-Daouk, 2000). Therefore, increased demand placed on a particular brain region, such

as the demand placed on SI in the pain task, may result in a temporary reduction in energy supply. This may explain the statistically significant decrease in total creatine from pre- to post-stimulation resting state observed in the current study ($p = 0.047$, $d = 0.489$). This proposed decrease in energy supply may lead to a reduction in overall glutamate neurotransmission. This would be supported by previous research that has shown the particularly high energy demands of excitatory synaptic neurotransmission (Harris et al., 2012; Sengupta et al., 2010). Additionally, if the previously considered pain modulation explanation is correct, the resultant elevation in GABA levels may exacerbate the effect of low energy supply on glutamate. To summarize, increased energy demands placed on SI throughout the duration of the ~15.5-minute pain task may contribute to an overall decrease in glutamatergic neurotransmission.

Although this explanation is empirically supported and fits eloquently with the previously discussed neuromodulation effect, it is by no means a certainty. Once again, more data collected in various populations under multiple pain paradigms are needed to form a more complete understanding of the neurochemical dynamics that support pain processing in the somatosensory cortex. Therefore, consideration of the discussion points provided above should be done cautiously and within the context of previously published peer-reviewed research in the field.

Limitations and Future Directions

Results from the current study should be evaluated with several methodological limitations in mind. First, the study was likely underpowered. A prospective power analysis indicated that a sample size of $n = 36$ would be ideal to detect a medium effect of pain on glutamate and GABA. While it is possible that experimental pain has no effect

on SI glutamate and GABA, it is also possible that the current sample was insufficient to detect a relationship between variables if it exists. A post-hoc power analysis indicated the current study achieved 75% power. Although the current sample was comparable to previous work, the current study was still underpowered. However, given the extremely novel nature of the research, in addition to reaching a comparable sample size seen in similar studies (*n* = 27 in Cleve et al. (2017); *n* = 26 in de Matos et al. (2017); *n* = 18 in Archibald 2020), I felt a sample of *n* = 24 was appropriate and represented a meaningful contribution to the field. Future work attempting to further this line of research should make efforts to ensure a sufficient sample size is recruited. Second, participants in the current study are not representative of the general population. The overrepresentation of young, white participants with secondary education may lead to a decrease in overall generalizability of the results. Future work should emphasize recruiting a more diverse sample. Third, shortcomings of the pressure-based pain apparatus may have interfered with our ability to detect differences in pain-related neurometabolites. While the device only received pressure during pain-on phases of the task, the plastic component of the apparatus remained in constant contact with the participant. Future work with similar devices may consider collecting data while the device is not equipped and/or including a sub-threshold condition of nonpainful tactile stimulation. Additionally, future work directly comparing neurochemical responses to multiple pain modalities is needed. Finally, the current study did not use functional motor mapping for precise voxel placement. Rather, voxel placement relied on previous research (Nichols et al., 2023) and known organization of SI. Future studies may consider utilizing motor mapping to increase specificity of voxel placement.

Conclusions

The data presented here contribute to a growing body of spectroscopic pain literature. Although we did not find evidence to support Hypotheses 1-4, the data collected here advance the field's understanding of the neurobiological mechanisms that subserve pain processing by reporting pain-related neurometabolite data in an understudied node of the pain matrix. This is particularly true as the use of ultra-high field imaging machines (i.e., 7T) remains uncommon among pain research. Further, this study supports the continued use of MRS and fMRS as effective techniques to investigate pain processing in humans. Data produced by this study may aid in the development of novel therapeutic interventions for pain-related illnesses, which remain a prevalent issue around the world.

References

Al-Chalabi, M., Reddy, V., & Alsalman, I. (2024). Neuroanatomy, Posterior Column (Dorsal Column). In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK507888/

Al-Chalabi, M., Reddy, V., & Gupta, S. (2024). Neuroanatomy, Spinothalamic Tract. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK507824/

Archibald, J., MacMillan, E. L., Enzler, A., Jutzeler, C. R., Schweinhardt, P., & Kramer, J. L. K. (2020). Excitatory and inhibitory responses in the brain to experimental pain: A systematic review of MR spectroscopy studies. *NeuroImage*, *215*, 116794. https://doi.org/10.1016/j.neuroimage.2020.116794

Archibald, J., MacMillan, E. L., Graf, C., Kozlowski, P., Laule, C., & Kramer, J. L. K. (2020). Metabolite activity in the anterior cingulate cortex during a painful stimulus using functional MRS. *Scientific Reports*, *10*(1), 19218. https://doi.org/10.1038/s41598-020-76263-3

Baker, K. B., Tkach, J. A., Nyenhuis, J. A., Phillips, M., Shellock, F. G., Gonzalez-Martinez, J., & Rezai, A. R. (2004). Evaluation of specific absorption rate as a dosimeter of MRI-related implant heating. *Journal of Magnetic Resonance Imaging*, *20*(2), 315–320. https://doi.org/10.1002/jmri.20103

Benedetti, F., Mayberg, H. S., Wager, T. D., Stohler, C. S., & Zubieta, J.-K. (2005). Neurobiological Mechanisms of the Placebo Effect. *Journal of Neuroscience*, *25*(45), 10390–10402. https://doi.org/10.1523/JNEUROSCI.3458-05.2005

Bingel, U., & Tracey, I. (2008). Imaging CNS Modulation of Pain in Humans. *Physiology*, *23*(6), 371–380. https://doi.org/10.1152/physiol.00024.2008

Brosnan, J. T., & Brosnan, M. E. (2007). Creatine: Endogenous Metabolite, Dietary, and Therapeutic Supplement. *Annual Review of Nutrition*, *27*([Volume 27, 2007, Volume 27,]), 241–261. https://doi.org/10.1146/annurev.nutr.27.061406.093621

Catani, M. (2017). A little man of some importance. *Brain*, *140*(11), 3055–3061. https://doi.org/10.1093/brain/awx270

Chee, M. W., & Chuah, L. Y. (2008). Functional neuroimaging insights into how sleep and sleep deprivation affect memory and cognition. *Current Opinion in Neurology*, *21*(4), 417–423. https://doi.org/10.1097/WCO.0b013e3283052cf7

Chen, J.-I., Ha, B., Bushnell, M. C., Pike, B., & Duncan, G. H. (2002). Differentiating Noxious- and Innocuous-Related Activation of Human Somatosensory Cortices Using Temporal Analysis of fMRI. *Journal of Neurophysiology*, *88*(1), 464–474. https://doi.org/10.1152/jn.2002.88.1.464

Cleve, M., Gussew, A., & Reichenbach, J. R. (2015). In vivo detection of acute pain-induced changes of GABA+ and Glx in the human brain by using functional 1H MEGA-PRESS MR spectroscopy. *NeuroImage*, *105*, 67–75. https://doi.org/10.1016/j.neuroimage.2014.10.042

Coghill, R. C., Sang, C. N., Maisog, J. Ma., & Iadarola, M. J. (1999). Pain Intensity Processing Within the Human Brain: A Bilateral, Distributed Mechanism. *Journal of Neurophysiology*, *82*(4), 1934–1943. https://doi.org/10.1152/jn.1999.82.4.1934

Corniani, G., & Saal, H. P. (2020). Tactile innervation densities across the whole body. *Journal of Neurophysiology*, *124*(4), 1229–1240. https://doi.org/10.1152/jn.00313.2020

Davis, M. T., Daniel, T. A., Witte, T. K., Beyers, R. J., Willis, J. Z., Wang, Y., Denney, T. S., Katz, J. S., Salibi, N., & Deshpande, G. (2016). Demonstration and validation of a new pressure-based MRI-safe pain tolerance device. *Journal of Neuroscience Methods*, *271*, 160–168. https://doi.org/10.1016/j.jneumeth.2016.07.001

de Matos, N. M. P., Hock, A., Wyss, M., Ettlin, D. A., & Brügger, M. (2017). Neurochemical dynamics of acute orofacial pain in the human trigeminal brainstem nuclear complex. *NeuroImage*, *162*, 162–172. https://doi.org/10.1016/j.neuroimage.2017.08.078

Diatchenko, L., Nackley, A. G., Tchivileva, I. E., Shabalina, S. A., & Maixner, W. (2007). Genetic architecture of human pain perception. *Trends in Genetics*, *23*(12), Article 12. https://doi.org/10.1016/j.tig.2007.09.004

Disbrow, E., Litinas, E., Recanzone, G. H., Padberg, J., & Krubitzer, L. (2003). Cortical connections of the second somatosensory area and the parietal ventral area in macaque monkeys. *Journal of Comparative Neurology*, *462*(4), 382–399. https://doi.org/10.1002/cne.10731

Doyle, S. R., & Donovan, D. M. (2009). A Validation Study of the Alcohol Dependence Scale. *Journal of Studies on Alcohol and Drugs*, *70*(5), 689–699.

Duarte, J. M. N., Lei, H., Mlynárik, V., & Gruetter, R. (2012). The neurochemical profile quantified by in vivo 1H NMR spectroscopy. *NeuroImage*, *61*(2), 342–362. https://doi.org/10.1016/j.neuroimage.2011.12.038

Edden, R. A. E., Puts, N. A. J., Harris, A. D., Barker, P. B., & Evans, C. J. (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid–edited MR spectroscopy spectra. *Journal of Magnetic Resonance Imaging: JMRI*, *40*(6), 1445–1452. https://doi.org/10.1002/jmri.24478

Ellis, B. W., Johns, M. W., Lancaster, R., Raptopoulos, P., Angelopoulos, N., & Priest, R. G. (1981). The St. Mary's Hospital sleep questionnaire: A study of reliability. *Sleep*, *4*(1), 93–97. https://doi.org/10.1093/sleep/4.1.93

Fine, P. G. (2011). Long-term consequences of chronic pain: Mounting evidence for pain as a neurological disease and parallels with other chronic disease states. *Pain Medicine (Malden, Mass.)*, *12*(7), 996–1004. https://doi.org/10.1111/j.1526-4637.2011.01187.x

Fuchs, P. N., Peng, Y. B., Boyette-Davis, J. A., & Uhelski, M. L. (2014). The anterior cingulate cortex and pain processing. *Frontiers in Integrative Neuroscience*, *8*, 35. https://doi.org/10.3389/fnint.2014.00035

Galer, B. S., & Jensen, M. P. (1997). Development and preliminary validation of a pain measure specific to neuropathic pain: The Neuropathic Pain Scale. *Neurology*, *48*(2), 332–338. https://doi.org/10.1212/wnl.48.2.332

Gandhoke, G. S., Belykh, E., Zhao, X., Leblanc, R., & Preul, M. C. (2019). Edwin Boldrey and Wilder Penfield's Homunculus: A Life Given by Mrs. Cantlie (In and Out of Realism). *World Neurosurgery*, *132*, 377–388. https://doi.org/10.1016/j.wneu.2019.08.116

Gebhart, G. F. (2004). Descending modulation of pain. *Neuroscience & Biobehavioral Reviews*, *27*(8), 729–737. https://doi.org/10.1016/j.neubiorev.2003.11.008

Gossop, M., Darke, S., Griffiths, P., Hando, J., Powis, B., Hall, W., & Strang, J. (1995). The Severity of Dependence Scale (SDS): Psychometric properties of the SDS in English and Australian samples of heroin, cocaine and amphetamine users. *Addiction (Abingdon, England)*, *90*(5), 607–614. https://doi.org/10.1046/j.1360-0443.1995.9056072.x

Graaf, R. A. de. (2019). *In Vivo NMR Spectroscopy: Principles and Techniques*. John Wiley & Sons.

Harris, J. J., Jolivet, R., & Attwell, D. (2012). Synaptic Energy Use and Supply. *Neuron*, *75*(5), 762–777. https://doi.org/10.1016/j.neuron.2012.08.019

Hashim, I. H. M., Kumamoto, S., Takemura, K., Maeno, T., Okuda, S., & Mori, Y. (2017). Tactile Evaluation Feedback System for Multi-Layered Structure Inspired by Human Tactile Perception Mechanism. *Sensors*, *17*(11), Article 11. https://doi.org/10.3390/s17112601

Henderson, L. A., Bandler, R., Gandevia, S. C., & Macefield, V. G. (2006). Distinct forebrain activity patterns during deep versus superficial pain. *Pain*, *120*(3), 286–296. https://doi.org/10.1016/j.pain.2005.11.003

Henschke, N., Kamper, S. J., & Maher, C. G. (2015). The Epidemiology and Economic Consequences of Pain. *Mayo Clinic Proceedings*, *90*(1), Article 1. https://doi.org/10.1016/j.mayocp.2014.09.010

Hutchison, W. D., Davis, K. D., Lozano, A. M., Tasker, R. R., & Dostrovsky, J. O. (1999). Pain-related neurons in the human cingulate cortex. *Nature Neuroscience*, *2*(5), Article 5. https://doi.org/10.1038/8065

Ip, I. B., Emir, U. E., Parker, A. J., Campbell, J., & Bridge, H. (2019). Comparison of Neurochemical and BOLD Signal Contrast Response Functions in the Human Visual Cortex. *Journal of Neuroscience*, *39*(40), 7968–7975. https://doi.org/10.1523/JNEUROSCI.3021-18.2019

Jenkins, B. A., & Lumpkin, E. A. (2017). Developing a sense of touch. *Development (Cambridge, England)*, *144*(22), 4078–4090. https://doi.org/10.1242/dev.120402

Jones, A. K. P., Brown, W. D., Friston, K. J., Qi, L. Y., & Frackowiak, R. S. J. (1991). Cortical and subcortical localization of response to pain in man using positron emission tomography. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *244*(1309), 39–44. https://doi.org/10.1098/rspb.1991.0048

Kenshalo, D. R., Iwata, K., Sholas, M., & Thomas, D. A. (2000). Response properties and organization of nociceptive neurons in area 1 of monkey primary somatosensory cortex. *Journal of Neurophysiology*, *84*(2), 719–729. https://doi.org/10.1152/jn.2000.84.2.719

Kenshalo, D. R., & Willis, W. D. (1991). The Role of the Cerebral Cortex in Pain Sensation. In A. Peters & E. G. Jones (Eds.), *Normal and Altered States of Function* (pp. 153–212). Springer US. https://doi.org/10.1007/978-1-4615-6622-9_5

Khera, T., & Rangasamy, V. (2021). Cognition and Pain: A Review. *Frontiers in Psychology*, *12*. https://doi.org/10.3389/fpsyg.2021.673962

Kiemes, A., Davies, C., Kempton, M. J., Lukow, P. B., Bennallick, C., Stone, J. M., & Modinos, G. (2021). GABA, Glutamate and Neural Activity: A Systematic Review With Meta-Analysis of Multimodal 1H-MRS-fMRI Studies. *Frontiers in Psychiatry*, *12*, 644315. https://doi.org/10.3389/fpsyt.2021.644315

Kreis, R. (2016). The trouble with quality filtering based on relative Cramér-Rao lower bounds. *Magnetic Resonance in Medicine*, *75*(1), 15–18. https://doi.org/10.1002/mrm.25568

Kroenke, K., Spitzer, R. L., & Williams, J. B. W. (2001). The PHQ-9. *Journal of General Internal Medicine*, *16*(9), 606–613. https://doi.org/10.1046/j.1525-1497.2001.016009606.x

Kupers, R., Danielsen, E. R., Kehlet, H., Christensen, R., & Thomsen, C. (2009). Painful tonic heat stimulation induces GABA accumulation in the prefrontal cortex in man. *Pain*, *142*(1), 89–93. https://doi.org/10.1016/j.pain.2008.12.008

Labrakakis, C. (2023). The Role of the Insular Cortex in Pain. *International Journal of Molecular Sciences*, *24*(6), 5736. https://doi.org/10.3390/ijms24065736

Lee, E.-H. (2012). Review of the Psychometric Evidence of the Perceived Stress Scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Lenton, E. A., Landgren, B.-M., & Sexton, L. (1984). Normal variation in the length of the luteal phase of the menstrual cycle: Identification of the short luteal phase. *BJOG: An International Journal of Obstetrics & Gynaecology*, *91*(7), 685–689. https://doi.org/10.1111/j.1471-0528.1984.tb04831.x

Loewy, R. L., Pearson, R., Vinogradov, S., Bearden, C. E., & Cannon, T. D. (2011). Psychosis risk screening with the Prodromal Questionnaire—Brief version (PQ-B). *Schizophrenia Research*, *129*(1), 42–46. https://doi.org/10.1016/j.schres.2011.03.029

Ma, N., Dinges, D. F., Basner, M., & Rao, H. (2015). How Acute Total Sleep Loss Affects the Attending Brain: A Meta-Analysis of Neuroimaging Studies. *Sleep*, *38*(2), 233–240. https://doi.org/10.5665/sleep.4404

Macefield, V. G., Gandevia, S., & Henderson, L. A. (2007). Discrete Changes in Cortical Activation during Experimentally Induced Referred Muscle Pain: A Single-Trial fMRI Study. *Cerebral Cortex*, *17*(9), 2050–2059. https://doi.org/10.1093/cercor/bhl113

Mallik, B., & Frank, C. A. (n.d.). Is creatine a CNS neurotransmitter? *eLife*, *12*, e91824. https://doi.org/10.7554/eLife.91824

Mano, H., & Seymour, B. (2015). Pain: A Distributed Brain Information Network? *PLoS Biology*, *13*(1), Article 1. https://doi.org/10.1371/journal.pbio.1002037

Meldrum, B. S. (2000). Glutamate as a Neurotransmitter in the Brain: Review of Physiology and Pathology. *The Journal of Nutrition*, *130*(4), 1007S-1015S. https://doi.org/10.1093/jn/130.4.1007S

Moon, H. S., Jiang, H., Vo, T. T., Jung, W. B., Vazquez, A. L., & Kim, S.-G. (2021). Contribution of Excitatory and Inhibitory Neuronal Activity to BOLD fMRI. *Cerebral Cortex*, *31*(9), 4053–4067. https://doi.org/10.1093/cercor/bhab068

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A. J., Vidyasagar, R., Evans, C. J., & Edden, R. A. E. (2014). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *NeuroImage*, *86*, 43–52. https://doi.org/10.1016/j.neuroimage.2012.12.004

Nash, P. G., Macefield, V. G., Klineberg, I. J., Gustin, S. M., Murray, G. M., & Henderson, L. A. (2010). Changes in human primary motor cortex activity during acute cutaneous and muscle orofacial pain. *Journal of Orofacial Pain*, *24*(4), 379–390.

Near, J., Harris, A. D., Juchem, C., Kreis, R., Marjańska, M., Öz, G., Slotboom, J., Wilson, M., & Gasparovic, C. (2021). Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: Experts' consensus recommendations. *NMR in Biomedicine*, *34*(5), e4257. https://doi.org/10.1002/nbm.4257

Nichols, S. J., Yanes, J. A., Reid, M. A., & Robinson, J. L. (2024). 7 T characterization of excitatory and inhibitory systems of acute pain in healthy female participants. *NMR in Biomedicine*, *37*(4), e5088. https://doi.org/10.1002/nbm.5088

Niddam, D. M., Yeh, T.-C., Wu, Y.-T., Lee, P.-L., Ho, L.-T., Arendt-Nielsen, L., Chen, A. C. N., & Hsieh, J.-C. (2002). Event-Related Functional MRI Study on Central Representation of Acute Muscle Pain Induced by Electrical Stimulation. *NeuroImage*, *17*(3), 1437–1450. https://doi.org/10.1006/nimg.2002.1270

Nonnemaker, J., Mowery, P., Hersey, J., Messeri, P., Dr.P.H, M. L. H., Nimsch, C., & Farrelly, M. (2004). Measurement properties of a nicotine dependence scale for adolescents. *Nicotine & Tobacco Research*, *6*(2), 295–301. https://doi.org/10.1080/14622200410001676413

Oeltzschner, G., Wijtenburg, S. A., Mikkelsen, M., Edden, R. A. E., Barker, P. B., Joo, J. H., Leoutsakos, J.-M. S., Rowland, L. M., Workman, C. I., & Smith, G. S. (2019). Neurometabolites and associations with cognitive deficits in mild cognitive impairment: A magnetic resonance spectroscopy study at 7 Tesla. *Neurobiology of Aging*, *73*, 211–218. https://doi.org/10.1016/j.neurobiolaging.2018.09.027

Ong, W.-Y., Stohler, C. S., & Herr, D. R. (2019). Role of the Prefrontal Cortex in Pain Processing. *Molecular Neurobiology*, *56*(2), 1137–1166. https://doi.org/10.1007/s12035-018-1130-9

Oshiro, Y., Quevedo, A. S., McHaffie, J. G., Kraft, R. A., & Coghill, R. C. (2009). Brain mechanisms supporting discrimination of sensory features of pain: A new model. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(47), 14924–14931. https://doi.org/10.1523/JNEUROSCI.5538-08.2009

Öz, G., Deelchand, D. K., Wijnen, J. P., Mlynárik, V., Xin, L., Mekle, R., Noeske, R., Scheenen, T. W. J., Tkáč, I., & Mrs, the E. W. G. on A. S. V. 1H. (2021). Advanced single voxel 1H magnetic resonance spectroscopy techniques in humans: Experts' consensus recommendations. *NMR in Biomedicine*, *34*(5), e4236. https://doi.org/10.1002/nbm.4236

Penfield, W., & Jasper, H. (1954). *Epilepsy and the functional anatomy of the human brain* (pp. xv, 896). Little, Brown & Co.

Pradhan, S., Bonekamp, S., Gillen, J. S., Rowland, L. M., Wijtenburg, S. A., Edden, R. A. E., & Barker, P. B. (2015). Comparison of single voxel brain MRS AT 3T and 7T using 32-channel head coils. *Magnetic Resonance Imaging*, *33*(8), 1013–1018. https://doi.org/10.1016/j.mri.2015.06.003

Prichard, J., Rothman, D., Novotny, E., Petroff, O., Kuwabara, T., Avison, M., Howseman, A., Hanstock, C., & Shulman, R. (1991). Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. *Proceedings of the National Academy of Sciences*, *88*(13), 5829–5831. https://doi.org/10.1073/pnas.88.13.5829

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, *30*(6), 672–679. https://doi.org/10.1002/mrm.1910300604

Provencher, S. W. (2001). Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR in Biomedicine*, *14*(4), 260–264. https://doi.org/10.1002/nbm.698

Puckett, A. M., & Sanchez Panchuelo, R. M. (2023). Imaging Somatosensory Cortex: Human Functional Magnetic Resonance Imaging (fMRI). In N. P. Holmes (Ed.), *Somatosensory Research Methods* (pp. 397–430). Springer US. https://doi.org/10.1007/978-1-0716-3068-6_18

Raju, H., & Tadi, P. (2023). Neuroanatomy, Somatosensory Cortex. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK555915/

Reed, B. G., & Carr, B. R. (2000). The Normal Menstrual Cycle and the Control of Ovulation. In K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, W. W. de Herder, K. Dungan, A. Grossman, J. M. Hershman, H. J. Hofland, G. Kaltsas, C. Koch, P. Kopp, M. Korbonits, R. McLachlan, J. E. Morley, M. New, J. Purnell, F. Singer, C. A. Stratakis, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK279054/

Reid, M. A., Forloines, M. R., & Salibi, N. (2022). Reproducibility of 7-T brain spectroscopy using an ultrashort echo time STimulated Echo Acquisition Mode sequence and automated voxel repositioning. *NMR in Biomedicine*, *35*(2), e4631. https://doi.org/10.1002/nbm.4631

Reid, M. A., Salibi, N., White, D. M., Gawne, T. J., Denney, T. S., & Lahti, A. C. (2019). 7T Proton Magnetic Resonance Spectroscopy of the Anterior Cingulate Cortex in First-Episode Schizophrenia. *Schizophrenia Bulletin*, *45*(1), 180–189. https://doi.org/10.1093/schbul/sbx190

Rikard, S. M. (2023). Chronic Pain Among Adults—United States, 2019–2021. *MMWR. Morbidity and Mortality Weekly Report*, *72*. https://doi.org/10.15585/mmwr.mm7215a1

Riley, J. L., Robinson, M. E., Wise, E. A., & Price, D. (1999). A meta-analytic review of pain perception across the menstrual cycle. *Pain*, *81*(3), 225–235. https://doi.org/10.1016/S0304-3959(98)00258-9

Rolfe, D. F., & Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological Reviews*, *77*(3), 731–758. https://doi.org/10.1152/physrev.1997.77.3.731

Sengupta, B., Stemmler, M., Laughlin, S. B., & Niven, J. E. (2010). Action Potential Energy Efficiency Varies Among Neuron Types in Vertebrates and Invertebrates. *PLOS Computational Biology*, *6*(7), e1000840. https://doi.org/10.1371/journal.pcbi.1000840

SHAPIRO, S. S., & WILK, M. B. (1965). An analysis of variance test for normality (complete samples)†. *Biometrika*, *52*(3–4), 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Spitzer, R. L., Kroenke, K., Williams, J. B. W., & Löwe, B. (2006). A Brief Measure for Assessing Generalized Anxiety Disorder: The GAD-7. *Archives of Internal Medicine*, *166*(10), 1092–1097. https://doi.org/10.1001/archinte.166.10.1092

Stanley, J. A., & Raz, N. (2018). Functional Magnetic Resonance Spectroscopy: The "New" MRS for Cognitive Neuroscience and Psychiatry Research. *Frontiers in Psychiatry*, *9*. https://doi.org/10.3389/fpsyt.2018.00076

Takahashi, K., Taguchi, T., Tanaka, S., Sadato, N., Qiu, Y., Kakigi, R., & Mizumura, K. (2011). Painful muscle stimulation preferentially activates emotion-related brain regions compared to painful skin stimulation. *Neuroscience Research*, *70*(3), 285–293. https://doi.org/10.1016/j.neures.2011.04.001

Tennant, R., Hiller, L., Fishwick, R., Platt, S., Joseph, S., Weich, S., Parkinson, J., Secker, J., & Stewart-Brown, S. (2007). The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): Development and UK validation. *Health and Quality of Life Outcomes*, *5*(1), 63. https://doi.org/10.1186/1477-7525-5-63

Tommerdahl, M., Delemos, K. A., Vierck, C. J., Favorov, O. V., & Whitsel, B. L. (1996). Anterior parietal cortical response to tactile and skin-heating stimuli applied to the same skin site. *Journal of Neurophysiology*, *75*(6), 2662–2670. https://doi.org/10.1152/jn.1996.75.6.2662

Tommerdahl, M., Favorov, O., Whitsel, B. L., Nakhle, B., & Gonchar, Y. A. (1993). Minicolumnar activation patterns in cat and monkey SI cortex. *Cerebral Cortex (New York, N.Y.: 1991)*, *3*(5), 399–411. https://doi.org/10.1093/cercor/3.5.399

Vierck, C. J., Whitsel, B. L., Favorov, O. V., Brown, A. W., & Tommerdahl, M. (2013). Role of primary somatosensory cortex in the coding of pain. *PAIN*®, *154*(3), 334–344. https://doi.org/10.1016/j.pain.2012.10.021

Von Korff, M., Ormel, J., Keefe, F. J., & Dworkin, S. F. (1992). Grading the severity of chronic pain. *Pain*, *50*(2), 133–149. https://doi.org/10.1016/0304-3959(92)90154-4

Wallimann, T., Tokarska-Schlattner, M., & Schlattner, U. (2011). The creatine kinase system and pleiotropic effects of creatine. *Amino Acids*, *40*(5), 1271–1296. https://doi.org/10.1007/s00726-011-0877-3

Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., & Hayasaki, H. (2002). GABA and GABA Receptors in the Central Nervous System and Other Organs. In K. W. Jeon (Ed.), *International Review of Cytology* (Vol. 213, pp. 1–47). Academic Press. https://doi.org/10.1016/S0074-7696(02)13011-7

Watson, C., Kirkcaldie, M., & Paxinos, G. (2010). Chapter 6—Gathering information–the sensory systems. In C. Watson, M. Kirkcaldie, & G. Paxinos (Eds.), *The Brain* (pp. 75–96). Academic Press. https://doi.org/10.1016/B978-0-12-373889-9.50006-1

Wijtenburg, S. A., Gaston, F. E., Spieker, E. A., Korenic, S. A., Kochunov, P., Hong, L. E., & Rowland, L. M. (2014). Reproducibility of phase rotation STEAM at 3T: Focus on glutathione. *Magnetic Resonance in Medicine*, *72*(3), 603–609. https://doi.org/10.1002/mrm.24959

Wijtenburg, S. A., Rowland, L. M., Edden, R. A. E., & Barker, P. B. (2013). Reproducibility of brain spectroscopy at 7T using conventional localization and spectral editing techniques. *Journal of Magnetic Resonance Imaging*, *38*(2), 460–467. https://doi.org/10.1002/jmri.23997

Wyss, M., & Kaddurah-Daouk, R. (2000). Creatine and Creatinine Metabolism. *Physiological Reviews*, *80*(3), 1107–1213. https://doi.org/10.1152/physrev.2000.80.3.1107

Xiao, X., & Zhang, Y.-Q. (2018). A new perspective on the anterior cingulate cortex and affective pain. *Neuroscience & Biobehavioral Reviews*, *90*, 200–211. https://doi.org/10.1016/j.neubiorev.2018.03.022

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

The Auburn University Institutional Review Board has approved this Document for use from 02/06/2024 to 02/05/2025 Protocol # 21-073 MR 2102

Announcement – Adults ages 19-50 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
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com
Neuroimaging and Pain AUneuroscienceResearch@gmail.com

Appendix A – Recruitment Email and Presentation 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

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 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: auneuroscienceresearch@gmail.com

"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 adults, ages 19-50, 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 is eligible to receive 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: auneurosciencesearch@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: auneurosciencesearch@gmail.com

Appendix B – Timepoint 1 Materials

Appendix B – Timepoint 1 Prescreen Survey

Neuroimaging and Pain Project, Pre-Scan

Start of Block: Information Letter

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 Student at Auburn University, and Dr. Jennifer L. Robinson, 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.

End of Block: Information Letter

Start of Block: Email

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.

_____

End of Block: Email

Start of Block: Demographics

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	○	○	○	○	○

End of Block: Demographics

Start of Block: 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

End of Block: Well-Being Scale

Start of Block: Perceived Stress Scale (PSS) 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

End of Block: Perceived Stress Scale (PSS) 4

Start of Block: Generalized Anxiety (GAD) 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

End of Block: Generalized Anxiety (GAD) 7

Start of Block: PHQ-SADS

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

End of Block: PHQ-SADS

Start of Block: Prodromal Questionnaire Brief Version (PQB)

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

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

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

- ○ Yes
- ○ No

Regarding the previous question, when this happens, I feel frightened, concerned, or it causes problems for me.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

End of Block: Prodromal Questionnaire Brief Version (PQB)

Start of Block: Graded Chronic Pain Scale (GCPS)

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?

_____

End of Block: Graded Chronic Pain Scale (GCPS)

Start of Block: Neuropathic Pain Scale (NPS)

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	

End of Block: Neuropathic Pain Scale (NPS)

Start of Block: Severity of Dependence Scale (SDS) - Alcohol

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

Did you ever think your use of marijuana/cannabis was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on using marijuana/cannabis make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of marijuana/cannabis?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop using marijuana/cannabis ?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without marijuana/cannabis?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Alcohol

Start of Block: Severity of Dependence Scale (SDS) - 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

Did you ever think your use of amphetamines was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on amphetamines make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of amphetamines?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop using amphetamines?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without amphetamines?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Amphetamines

Start of Block: Severity of Dependence Scale (SDS) - 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

Did you ever think your use of marijuana/cannabis was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on using marijuana/cannabis make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of marijuana/cannabis?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop using marijuana/cannabis ?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without marijuana/cannabis?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Cannabis

Start of Block: Severity of Dependence Scale (SDS) - 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

Did you ever think your use of cocaine was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on cocaine make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of cocaine?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop using cocaine?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without cocaine?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Cocaine

Start of Block: Severity of Dependence Scale (SDS) - 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

Did you ever think your use of opioids was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on opioids make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of opioids?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without opioids?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Opioids

Start of Block: Severity of Dependence Scale (SDS) - 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

Did you ever think your use of psychomotor stimulants was out of control?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

Did the prospect of missing out on psychomotor stimulants make you very anxious or worried?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How much did you worry about your use of psychomotor stimulants?

- ○ Not at all
- ○ A little
- ○ Often
- ○ Always or almost always

Did you wish you could stop using psychomotor stimulants?

- ○ Never or almost never
- ○ Sometimes
- ○ Often
- ○ Always

How difficult would you find it to stop or go without psychomotor stimulants?

- ○ Not difficult at all
- ○ Quite difficult
- ○ Very difficult
- ○ Impossible

End of Block: Severity of Dependence Scale (SDS) - Psychomotor Stimulants

---

Start of Block: Nicotine Dependence Scale for Adolescents (NDSA)

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

End of Block: Nicotine Dependence Scale for Adolescents (NDSA)

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)

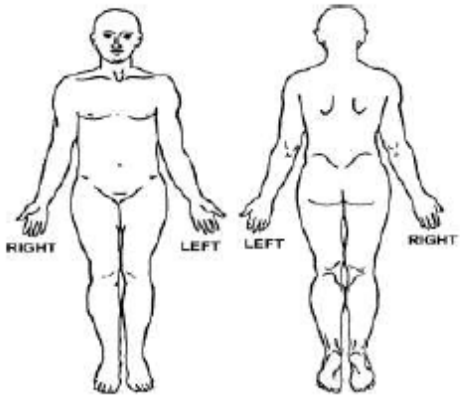
9.	☐Yes ☐No	Do you have a history of cardiovascular disease?
10.	☐Yes ☐No	Do you have a breathing problem or motion disorder?
11.	☐Yes ☐No	Are you claustrophobic?
12.	☐Yes ☐No	Do you have inner ear disorders or experience vertigo or dizziness?
13.	☐Yes ☐No	Do you have tattoos or permanent makeup that 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 to 50.

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 three 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 5 minutes to complete. **In another experiment**, the device will continue to administer pressure until it reaches one of three pre-determined stopping points based on your responses to the previous tolerance experiment. These stopping points are referred to as "low," "moderate," and "baseline." When the device reaches these points, 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.

Risk & Precautions for COVID-19
Due to the need for your physical presence at the research site, face to face interaction with the researcher or others, etc., there is a risk that you may be exposed to COVID-19 and the possibility that you may contract the virus. For most people, COVID-19 causes only mild or moderate symptoms. For some, especially older adults and people with existing health problems, it can cause more severe illness. Current information suggests that about 2% of people who are infected with COVID-19 might die as a result. You will need to review the Information on COVID-19 for Research Participants that is attached to this consent document. To minimize your risk of exposure we will screen you for symptoms of COVID-19 and risk factors for COVID-19 prior to admitting you to the MRI suite. Anyone with symptoms of COVID-19 or risk factors for COVID-19 will be excused from the scanning session and escorted out of the MRI suite. Upon arrival at the Auburn University MRI Research Center, but before admitting you to the MRI suite, we will take your temperature with a touchless forehead thermometer. If your temperature is 99.0 degrees or higher, we will inform you that you cannot complete your scanning session. At that time, we may reschedule the scanning session for another date no less than 14 days from the initial scanning session. Researchers will remain, at minimum, 6 feet away from you and other protocol personnel when possible. We will provide you with a surgical mask. Also, we will ask you to wear the surgical mask at all times while in the MRI suite including the time you spend inside the scanner. We will adhere to these procedures for all participants/visitors.

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

Participant's Initials _____

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

The Auburn University Institutional Review Board has approved this Document for use from 02/06/2024 to 02/05/2025
Protocol # 21-073 MR 2102

Appendix C – 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.	○	○	○	○	○

→

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