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Sofia Heras April 1st, 2024

Psychophysical and fMRI Temporal Pain Dynamics Assessment of Task-based Variability

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An abstract submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment of the requirements of the degree of Bachelor of Science with Honors

Neuroscience and Behavioral Biology

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Abstract

Introduction and Background: In 2019, approximately 20.9% of Americans suffered from chronic pain. The mechanisms underlying the pain experience are critical to understanding this condition and its biomarkers, but this characterization is challenging due to the dynamic nature of pain. Functional magnetic resonance imaging (fMRI), combined with quantitative sensory testing (QST), can unveil the relationship between painful sensations and neurobiological underpinnings of the pain experience. Current pain measurement in fMRI experiments assess perceived pain following each fMRI run, or trial, using a discrete estimate (e.g., 40/100) of pain, and then model the brain activations based on when the stimulus was being delivered. Given the dynamic nature of the pain experience and the delays between stimulus onsets and experiencing pain from a stimulus, we expected fMRI signals to be better visualized when the temporal course of the pain experience is considered.

Materials and Methods: Twenty-seven pain-free individuals (14 females, mean age = 23.2 yrs) underwent 20-second, moderately painful (i.e., 40/100) noxious heat stimuli on each leg interleaved with 20-seconds of no stimulation. In the lab, participants indicated perceived pain of these stimuli continuously for varying intensities to determine the temporal profile of the pain experience. Then, participants were scanned in a Siemens 3T Magnetom Prisma Fit Scanner during the application of identical stimuli to record pain-evoked brain responses.

Results: Results revealed an average delay of 8 seconds between the onset of a thermal stimulus and perceptible pain, suggesting that modeling brain activations using stimulus onsets is not ideal. Incorporating this delay in the significantly enhanced BOLD signals in key pain-processing areas, including the thalamus, insula, anterior cingulate cortex, and somatosensory cortices. This evidence suggests that incorporating the dynamics of the pain experience in the analysis of pain-evoked BOLD could increase the signal in these types of experiments. Different pain intensities unveiled brain changes directly correlating to the level of intensity, with higher intensity stimuli resulting in more significant activations. These results speak to the sensitivity of the pain experience, as subtle changes in stimuli can cause significant enhancement in activations of pain-processing regions.

Conclusion: Distinguishing the onset of stimulus to that of pain can uncover the mechanisms underpinning the pain experience, helping advance the diagnosis and treatment of chronic pain. Understanding how brain responses differ in accordance with noxious stimuli changes is a useful tool in characterizing the temporal dynamics of perceived pain as well.

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Introduction and Background

The mechanisms underpinning the pain experience have been a captivating topic of research for many years in the fields of neuroscience, biology, chemistry, and physics. This is greatly due to the diverse ways in which individuals undergo and process pain. In particular, the isolation of pain from confounding factors, such as emotional and cognitive components, has been a well sought-after objective for much of this research, as it provides a precise means to characterize the pain experience. Pain can be categorized into two main types: acute pain, which typically stems from specific injury, illness or inflammation, and chronic pain, which persists over a long period of time and can be detrimental to a person's well-being (NINDS, 2023). Acute pain functions as a protective mechanism signaling the body to address and heal underlying issues. In contrast, chronic pain, persisting beyond the expected recovery period, lacks this protective function and poses long-term challenges to overall health. This prolongation of pain is believed to be caused by peripheral and central sensitization of the nervous system, where there is an increased responsiveness to pain and an impairment of pain inhibitors (Courtney et al., 2017), and descending pain modulation, where cortical and subcortical sites can influence nociception (Ossipov et al., 2014). Incidences of chronic pain have been reported to increase each year, with an estimate of 20.9% of adults experiencing chronic pain in the US in 2019 (Rikard et al., 2023). Some of the long-term consequences of this pain include alterations in sleep, impairment in cognitive processes and brain function, detriment to cardiovascular health, and a negative impact in the overall quality of life (Fine, 2011). A comprehensive understanding of the underlying mechanisms of pain can contribute to the development of innovative treatment for chronic pain patients and enhance our overall knowledge of the pain experience.

Quantitative Sensory Testing (QST)

There are various methods that can be employed to unravel the underlying mechanisms of pain, one of which is quantitative sensory testing (QST). QST involves systematic assessments to measure an individual's response to various sensory stimuli. These stimuli, including noxious inputs like tactile, thermal and pain-inducing measures, activate specialized sensory nerve cells called nociceptors. These nociceptors relay information about sensation through electrical impulses and the activation of thin myelinated Aδ and unmyelinated C fibers (Garland, 2012). Nociceptors also receive signals from damaged tissue (Dafny, 1997), thus playing a crucial role in the detection of pain and in the assessment of sensory impairments in both clinical and research settings (Shy et al., 2003). These specialized sensory nerve cells relay sensory information up to the dorsal horn of the spine, which then allows for the information to move up the spinal cord through the spinothalamic tract until it reaches the cerebral cortex (Garland, 2012), a system known as the ascending pain pathway. The perception of pain occurs when Aδ are sufficiently activated to create a sensation of sharp pain. The activation of these fibers also allows for the cutaneous localization of pricking pain, greatly due to the sensory-discriminative component of pain mediated by primary somatosensory cortices (S1) (Treede, 1999). In this way, then, the brain is then able to identify the intensity and location of the noxious stimulus (Yam et al., 2018).

QST allows for the measurement and quantification of different aspects of sensory experiences, including intensity, quality, extent, and duration, in response to a specific stimulus. Techniques such as the method of limits can be applied to understand how these sensory components manifest (Gescheider, 1976). This technique involves the presentation of stimuli above or below pain threshold and averaging transition points from ascending and descending series to determine absolute thresholds personalized to each individual's sensitivity (Gescheider,

1976). This adaptability makes QST a versatile and malleable method, providing a comprehensive approach to testing and describing the intricacies of the sensory component of the pain experience.

Functional Magnetic Resonance Imaging (fMRI)

In addition to QST, functional magnetic resonance imaging (fMRI) is a powerful tool to unravel the underlying mechanisms of pain. Task-based fMRI scans in particular offer a unique method to measure neural processes associated with pain perception (Buckner, 1998). During task-based fMRI, also referred to as event-related fMRI, individuals may engage in certain stimuli such as heat of varying intensities designed to elicit pain responses. The resulting scans capture changes in blood flow and oxygenation levels, commonly referred to as blood oxygenation level dependent (BOLD) signals (Amaro & Barker, 2006), providing insights into brain regions activated during these tasks. This method not only identifies where pain is processed in the brain, but also offers insight into the intricate interplay between regions during various pain conditions such as changes in pain intensity (Buckner, 1998). It also allows for the exploration of whether different types of pain stimuli activate different brain regions (Amaro & Barker, 2006). A commonly adopted design for event-related fMRI experiments is the fixed inter-stimulus-interval (ISI) (Dale, 1999), often modeled as ON/OFF block intervals of painful and non-painful stimuli (Figure 1). However, QST by our lab and others, has shown that in these designs, there is a significant amount of aftersensations (pain persisting during the OFF block) and delays in the pain response (no pain during the first seconds of the ON block) (Gottrup et al., 2003; Schott, 2001; Staud et al., 2007). Moreover, other studies have found poor trait stability in task-based fMRI measures in children, with low reliability and stability values across all brain regions of interest (Kennedy et al., 2022). Therefore, using a strict ON/OFF block based on the ISI may not be the most effective design to measure brain activity in response to pain. Instead, this design may benefit from insights offered

by QST which can model an ON/OFF block based on a personalized individual response to the stimuli. Indeed, in a study with fibromyalgia patients, it was reported that individuals described pronounced painful aftersensations up to 15 seconds after the removal of cuff stimulation (Schreiber et al., 2017). Furthermore, another study utilizing pinprick stimulation in nerve injury patients with allodynia and capsaicin suggests that aftersensations may be a useful parameter in the assessment of central sensitization, as both control and test subjects reported aftersensations following high pain scores (Gottrup et al., 2003). Lastly, a review looking at observations of the delayed onset of pain after disease or trauma concluded that slow anatomical and physiological changes may underlie late-onset pains (Schott, 2001). Therefore, the integration of QST with task-based fMRI in this project aims to enhance the characterization of the pain experience, considering pain onset and offset differences, and providing a more accurate representation of pain processing.

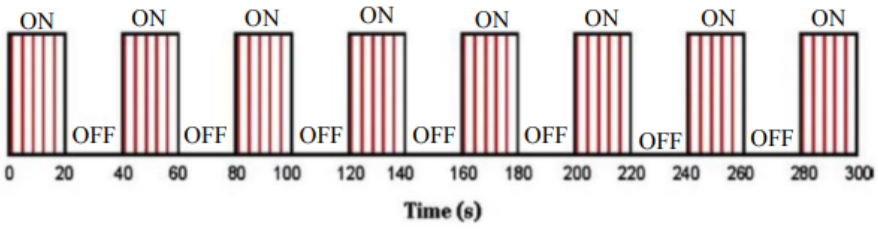

Figure 1. Adapted from (Tie et al., 2009). Figure reflects typical block design used in fMRI experiments. In figure above, each "ON" block lasts 20 secs and is followed by 20-sec "OFF" block.

During the application of a noxious pain stimulus the ascending pain pathway is responsible for transferring the signal to the higher brain area. First, the signal is sent via the dorsal horn of the spine, which then allows for the information to move up the spinal cord through the spinothalamic tract until it reaches the thalamus (Garland, 2012). The thalamus acts as the brain's main "relay station" for newly received sensory information, which is then finally sent into the cerebral cortex. Activation of brain regions such as the primary somatosensory cortex (S1), insular

regions, anterior cingulate cortex (ACC), and thalamus are expected as a response to painful stimuli (Bushnell & Duncan, 1989; NINDS, 2023; Peyron et al., 2000; Porro et al., 1998). These regions have been found to have an increased regional cerebral blood flow (rCBF) in response to noxious stimuli and are thus thought to be related to the sensory-discriminative aspects of the pain experience (Peyron et al., 2000). As previously mentioned, the thalamus is the brain's main "relay center" of sensory information and is a critical component in pain processing, as it receives information from multiple ascending pathways (Ab Aziz & Ahmad, 2006). Furthermore, studies suggest that pain intensity coding occurs in parallel cortical channels, with information about pain intensity being conveyed to the ACC by medial thalamic neurons (Bushnell & Duncan, 1989; Porro et al., 1998). The ACC is commonly known to modulate the affective pain experience, which may dictate the aversive behavior often noted in the anticipation of noxious painful stimuli (Fuchs et al., 2014; Sun et al., 2023). Recent studies have identified that the ACC, along with S1, also plays a crucial role in temporal pain processing (Sun et al., 2023). It is believed that both regions have a similar time course in the processing of temporal thermal pain (Sun et al., 2023), making them key regions in studying subjective pain experience. The S1 is also responsible for discriminative aspects of somatic sensation, meaning that it is crucial for segregating sensations pertaining to pain (Bushnell et al., 1999). Moreover, studies have shown that the human pain pathway includes a somatotopic representation in S1 in the contralateral hemisphere to stimulation (Ogino, 2005), and that this region responds to noxious stimuli using a large body surface map (Omori, 2013). Lastly, other studies suggest that the insular cortex may play a role in prompting cortical regions to use previous cognitive information during pain processing (Starr et al., 2009), and that it may help in the emotional processing of pain (Labrakakis, 2023).

The combination of QST with an fMRI research design can be a powerful way to significantly advance our understanding of the mechanisms underlying pain. This integrated approach of quantification of sensory responses to diverse stimuli and capturing real-time neural activity during pain-induced tasks bridges the gap between behavioral responses and neural mechanisms. A QST session first performed outside of the scanner provides individual and group level information on the delayed onset of pain sensations contrasted with the onset of a painful stimulus. This information is then taken to create three fMRI models: one comparing the traditional ON/OFF ISI design, another accounting for the individual delays of the pain intensity based on QST reports of pain, and a final model taking the average of the individual delays. Following the evidence listed above and results from QST data, it is expected to observe more activation in areas like S1 and ACC when modeled to the onset and offset of each subject's reported pain rather than when modeled to the onset and offset of stimuli. A delay in the processing of the pain response is also expected with more pronounced activations a couple seconds into the ON block rather than at the beginning, both at the individual and group levels. Previous evidence suggests a correlation between mean and peak responses of real-time intensity ratings to the pain experience (Koyama et al., 2004). This strongly suggests, along with the QST data, that there will be a delay in the activation of these pain-responding regions as subjects did not report intense pain until a few seconds into each trial.

Materials and Methods

The study was conducted at Emory University. All protocols were approved by the Emory University Institutional Review Board. Written and informed consent was obtained from all participants.

Participants

A total of 32 participants (15 females; 21.6 ± 5.2 years old [mean ± SD]) enrolled in the study from the Emory University campus and surrounding areas. While there were no age specifications to participate, subjects were screened to assess multiple variables such as existing levels of anxiety and pain, demographics, and handedness (Table 1). This was done through the McGill Pain Questionnaire (Melzack, 1987) and an abridged version of the Edinburgh Handedness questionnaire. Participants were excluded for existing pain up to 2 weeks prior to the study visit and conditions of chronic pain. The original enrolled number of participants was reduced to 27 once data analysis began. This was due to factors such as too much motion inside of the scanner, failure to follow instructions properly throughout the study visit, or not completing the scanner session due to unremovable metal jewelry.

	Male	Female
n	12	15
Age	25.3±9.4	21.6±5.2
Chronic Pain	0	0
Previous Pain	3	0
Anxiety and/or Depression	1	4
Medication	1	3
Race		
White	5	10
Asian	4	3
African American	2	1
Other	1	1
Hispanic	2	3
Handedness		
Right-handed	9	12
Left-handed	2	1
Ambidextrous	1	2

Table 1. Subject demographics showing the total number of subjects divided into male or female participants, mean age, any pain previously experienced for two weeks prior to the study visit, anxiety and/or depression, medication, race, Hispanic, and handedness.

QST Behavioral Visit

All subjects participated in a QST behavioral visit, which included the determination of a moderately painful stimuli (described below; referred to as Pain40), followed by a pseudorandomized series of thermal stimuli where temperatures would increase, decrease, or remain constant from Pain40. For stimuli application, the Thermal Sensory Analyzer (TSA) 2 (Medoc, Israel) machine was utilized, placing a 30x30mm thermode on a midpoint between the patella and talus measured for each subject. The calibration of a moderately painful stimulus was achieved by applying an ascending and descending series of thermal stimuli on each subject's leg (following the model of method of limits) and then calculating which temperature would correspond to a pain rating of 40/100 using a linear regression. Once Pain40 was calculated, it was utilized as the baseline for further testing. Subjects were then instructed to continuously rate their pain in a visual analog scale (VAS) as a pseudorandomized series of 12 trials was administered, where 4 trials were classified as increasing, 4 as decreasing, and 4 as constant in a 20-sec ON/OFF design (Figure 2). For every trial, the first 10 sec of the ON block was set to each subject's Pain40, and the last 10 sec increased or decreased by 1^{o}C or remained constant. Implementing different intensities tests participant's responsiveness and sensitivity to varying magnitudes of pain and allows for better characterization of the pain experience. Additionally, the collection of pain ratings from the VAS allowed for the collection of data pertaining to the continuous pain experience of subjects rather than just during the ON blocks.

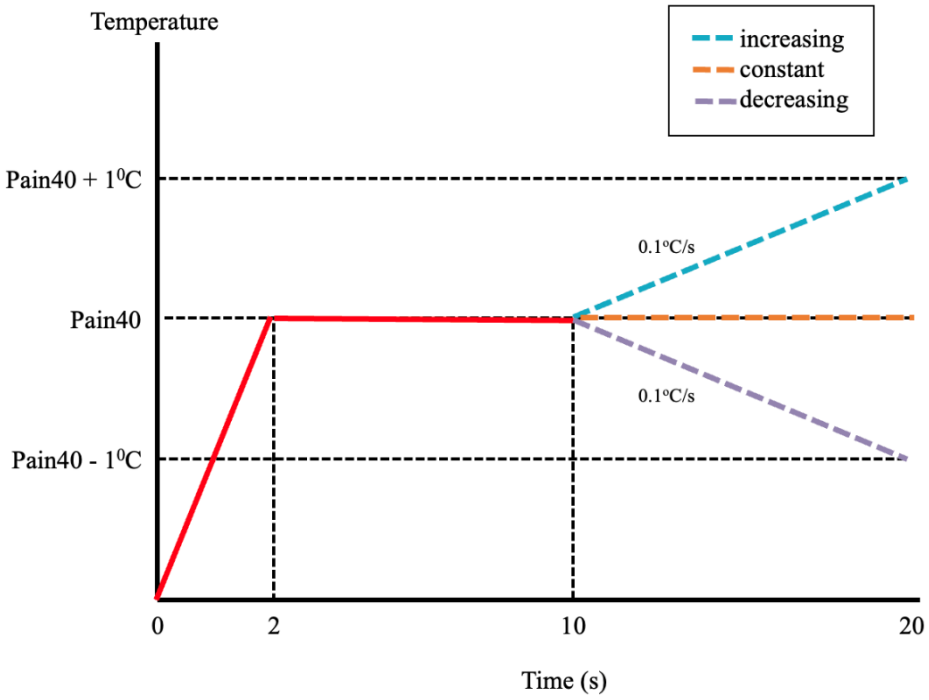

Figure 2. Study design showing the increasing rate of stimuli to Pain40 from baseline, followed by the different trial types in the latter 10 sec of the interval. It took 2 sec for the thermode to reach the target temperature of Pain40 once the ON block began, and 2 sec to return to baseline at the end of the 20-sec ON block.

Imaging Visit

The same participants described above were escorted to the Center for Systems Imaging (CSI) Core MRI scanner located in the Emory University Hospital. Participants were pre-screened to ensure eligibility to undergo an fMRI scan. The QST protocol sequence of 12 pseudorandomized trials described above was applied in the same area for both left and right legs of each subject. However, subjects were not instructed to rate their pain or complete any tasks, they were asked to just feel the sensation. This was carried out inside of a Siemens 3T Magnetom Prisma Fit Scanner. An 8 minute and 20 sec BOLD functional MRI scan was acquired for each thermal stimuli application. For each series, 250 echo-planar images were collected with a single-shot, gradient-echo echoplanar (EPI) pulse sequence [TR = 2000 ms; TE = 27 ms; flip angle = 80°; FOV = 220 mm]. For anatomical localization and spatial normalization, a multi-echo MPRAGE

(T1-weighted structural MRI) volume was collected at the beginning of each trial using a 32 channel receiver head coil [TE (echo time) = 2.96 ms; TR (repetition time) = 2.53s; flip angle = 7^{o}; slice thickness = 1 mm; FOV (field of view) = 256 mm; resolution = 256 x 256].

Data preprocessing

BOLD scans were pre-processed using a combination of tools from FSL and FreeSurfer software packages. Data were corrected for slice-timing, head motion and frame displacement-based motion outliers. Correcting for these factors is crucial to the higher-level analysis performed later. For instance, slices obtained from the scanner are naturally misaligned due to the inability of fMRI acquisition protocols to obtain slices simultaneously (Parker & Razlighi, 2019), making slice-timing correction a key component in the data preprocessing. To ensure maintenance of the data integrity, head motion must be minimized as well, and individuals with excessive in-scanner motion (outliers) were excluded from analysis (Hausman et al., 2022). Moreover, head motion correction is needed as motion during the scanner session reduces statistical significance of BOLD signals and enables greater false activations (Zaitsev et al., 2017). Data also underwent brain extraction (Institute; Smith, 2013) (Figure 3), co-registration (Jahn, 2022) to MPRAGE, spatial smoothing (Jahn, 2022) with a 6mm Gaussian Kernel and high-pass temporal filtering (cut-off frequency = 0.008 Hz). Non-linear transformation to MNI space was used to spatially normalize the contrast of parameter estimates and associate variance images.

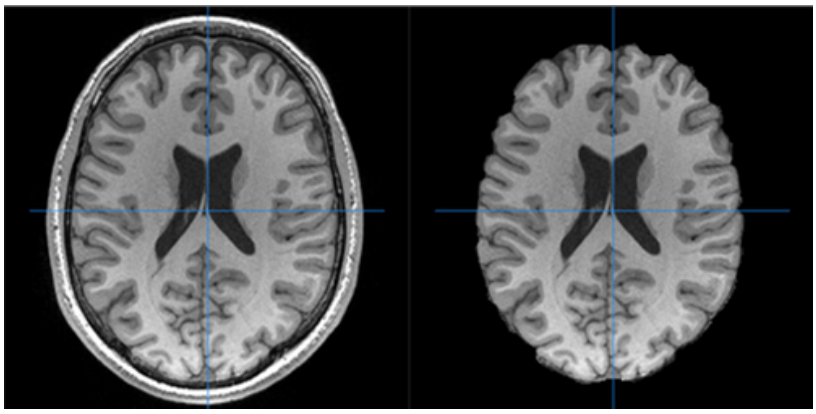

Figure 3. from University of Texas Health Science Center, Mango Viewer, 2006-2024. Image showing comparison between no brain extraction (left) and brain tissue extraction (right). Highlights tool's efficacy in separating brain and non-brain tissue in fMRI images.

Statistical Analysis

To estimate brain responses to stimuli, general linear modeling was performed on the preprocessed fMRI data. The stimulation period and the OFF blocks were modeled as explanatory variables in first-level analyses, including the six motion parameters (three rotations and three transformations) and frames flagged as motion outliers as covariates of no interest. Resultant outputs such as parameter estimates and their variances, spatially normalized to MNI152, were then passed up to a randomized analysis using a threshold free cluster enhancement. All these analyses were performed with FSL's FEAT GLM tool (Hanayik, 2019; Webster, 2018) (Figure 4).

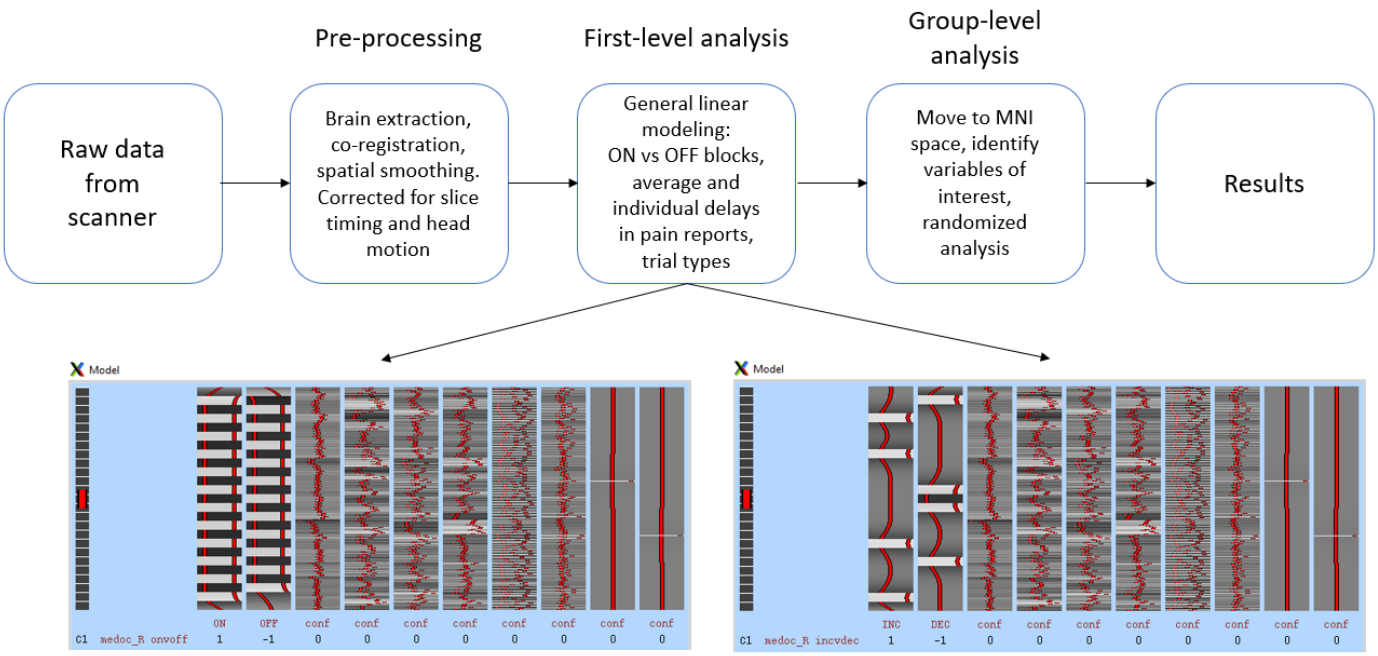

Figure 4. Imaging pipeline showing the pre-processing, first-level, and group-level analyses undergone by fMRI images, as well as examples of FEAT's general linear modeling showing some of the models created for the analysis of the obtained data.

The first-level statistical analysis included a variety of modelling with the ON and OFF blocks (Figure 5). The first model followed only the onset and offset of the stimulus, not accounting for any delays that may have been reported by subjects using the VAS in the QST portion of the visit (Figure 5A). Then, individual delays in the onset of the pain sensation as reported with the VAS were included, modifying the ON block to reflect the timing of these delays as the onset of the stimulus (Figure 5B). The mean of all individual delays was calculated to obtain the average group delay for the report of painful stimuli, and this value was then included in the modelling of the ON block to act as the onset of the stimulus for the analysis (Figure 4C). All three models were created from data obtained from right leg and left leg stimulation.

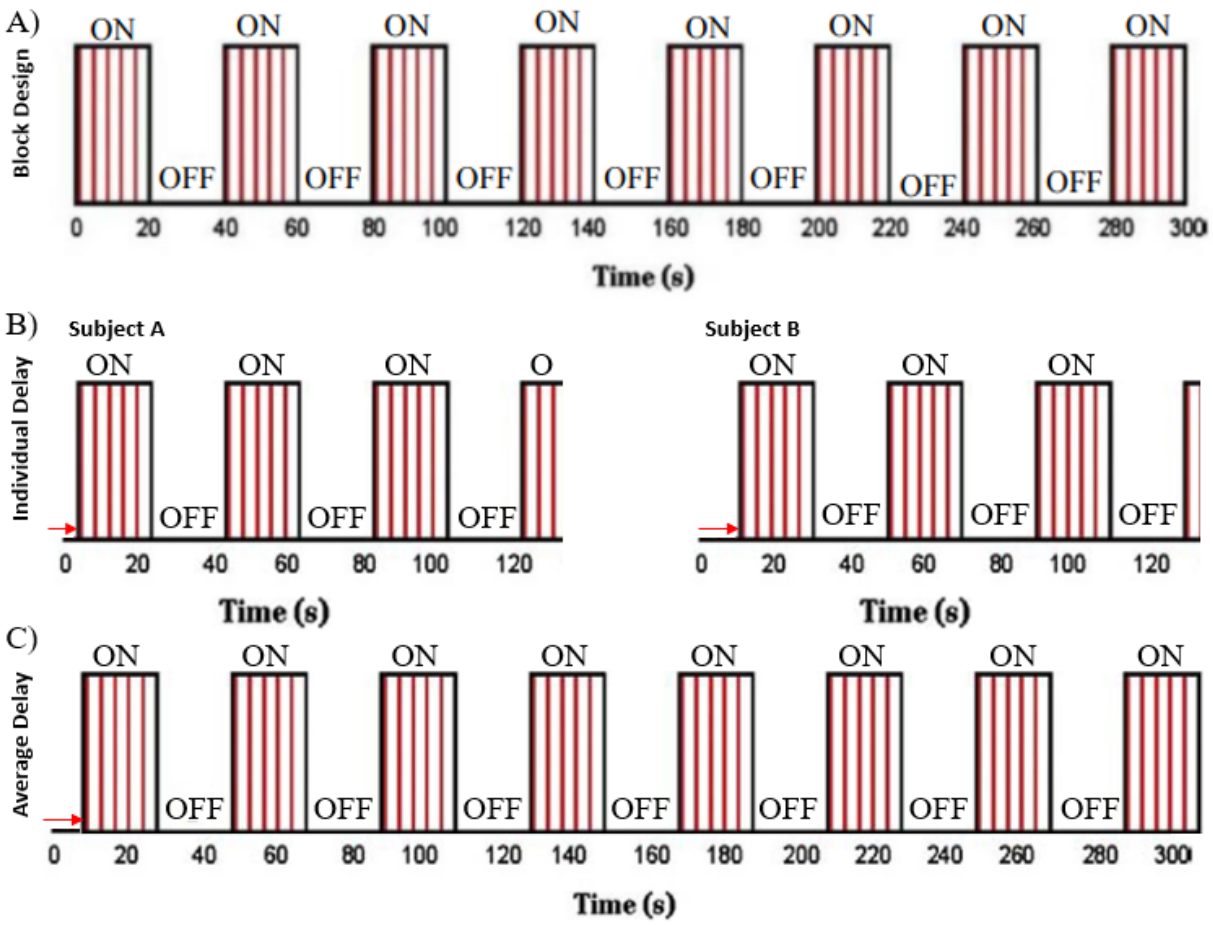

Figure 5. Block designs showing different ON/OFF block timings for linear modelling in first-level analysis of fMRI data. The first row shows the block design as it was created for this protocol. The second shows different delays in the report of pain by subjects, and the third shows the average delay. Red arrows indicate the difference in time when considering delays based on VAS reports versus the block design.

Further first-level analyses were performed to assess the effect of different trial types (Figure 6). One model included the comparison of increasing versus decreasing trials, first with the onset and offset of the stimuli only, and then including the individual and average group delays (Figure 6A). This was done to determine the magnitude at which the intensity of the stimulus affects the pain experience and thus BOLD signal intensity in pain-processing regions. The next model compared increasing versus constant trials, again including the different onset and offset timings of the stimulus (Figure 6B). Lastly, the final model analyzed constant versus decreasing trials for the ON/OFF block timing, the individual delays, and the average group delay (Figure 6C).

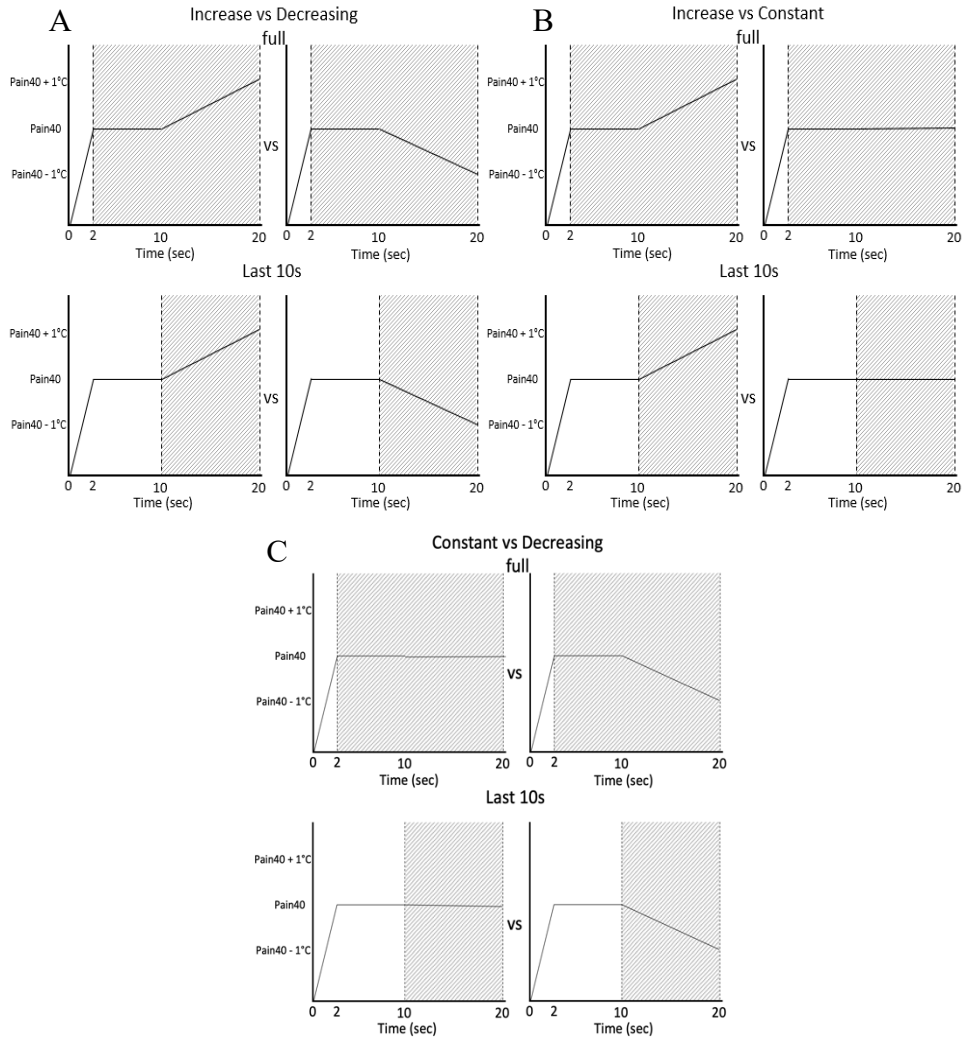

Figure 6. Models for the comparison of different trial types. A) shows the model comparing increasing versus decreasing trials, B) for increasing versus constant trials, and C) constant versus decreasing trials. The grey area shows the time of the ON block considered for the stimulus: first, the whole 20 sec ON block was analyzed, followed by the analysis of the last 10 sec where the stimulus changed.

Results

QST Previous Data

As it is essential to this thesis, results from unpublished data previously collected by the Harper PaIN Lab will be included. The results aimed at studying delays in pain onset and aftersensations does in fact suggest the presence of both phenomena based on QST: on average, it took subjects 8.29±4.11 to report thermal stimuli as painful when applied to the right calf area, and 9.21±4.13 sec when the stimuli was applied on the left calf area (Figure 7). Furthermore, subjects reported sensations of pain 6.18±3.16 sec after the stimulus had been removed on the right calf area, and 5.10±3.02 sec after stimulus removal on the left calf area (Harper PaIN Lab, unpublished data, Figure 8). The average Pain40 temperature was of 47ºC±9.27 ºC.

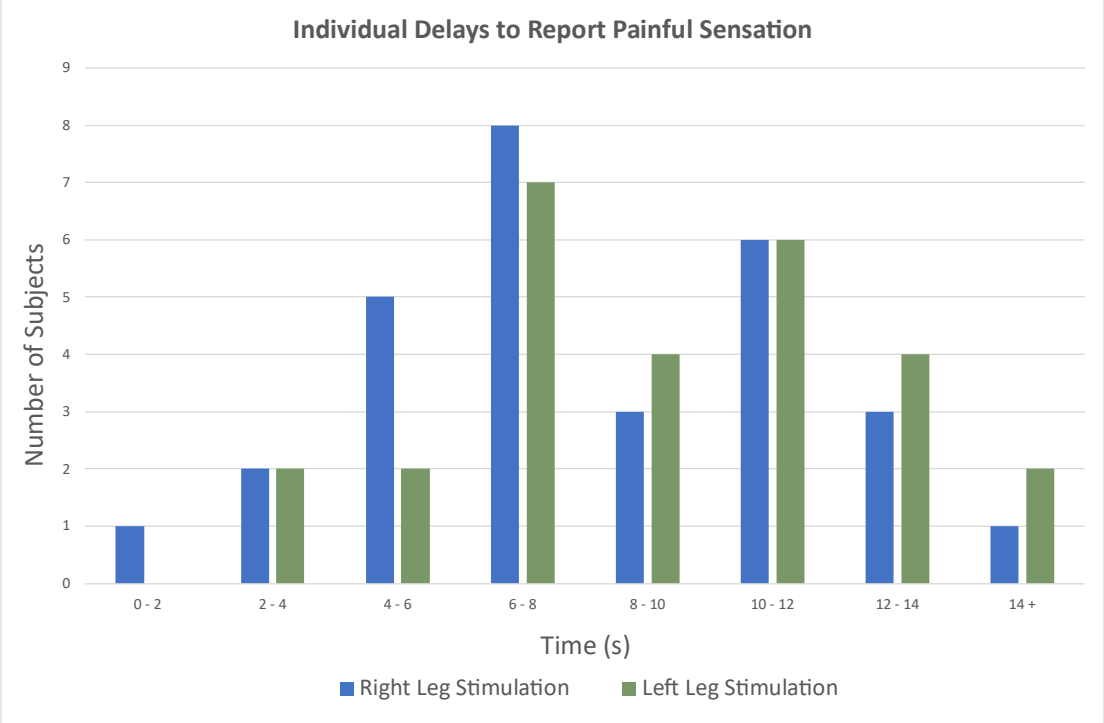

Figure 7. Frequency histogram showing how long it took individual subjects to report the thermal stimuli as painful. On average, it took most subjects between 6 and 8 seconds to report the sensation as painful once the ON block began.

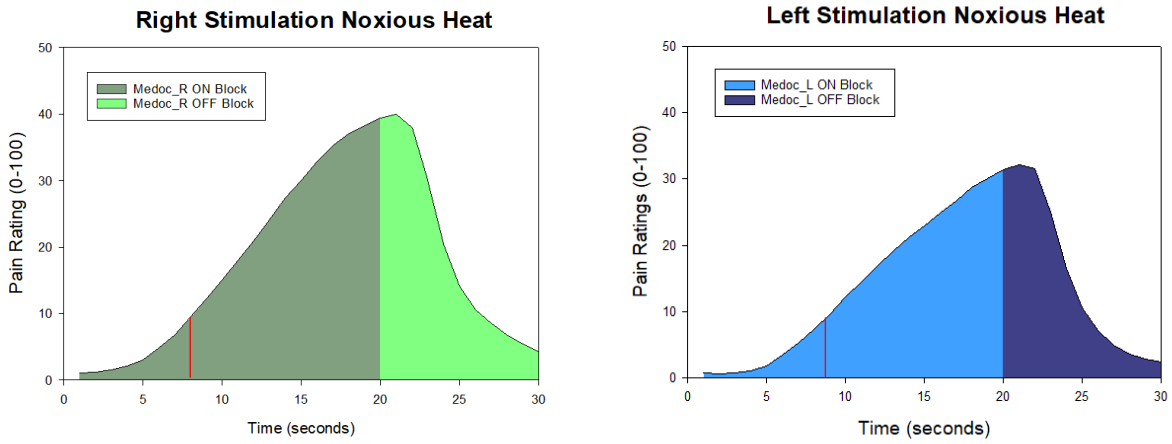

Figure 8. Area graphs showing the progression of subject's pain ratings (0-100) during the ON block and for 10 sec of the OFF block. Red line indicates the average time it took for subjects to report the sensation as painful: 8.29±4.11 sec for the right calf area and 9.21±4.13 sec for the left calf area. Light green and dark blue areas after the 20 sec marks illustrate the amount of pain reported by subjects even after the stimuli had been turned off.

Moreover, increasing trials for thermal stimuli applied in both sites had the highest pain ratings. For the right side, increasing trials had an average pain rating of 22.4±19.5, constant trials of 17.1±13.3, and decreasing trials of 14.14±9.7. For the left side, increasing trials had an average pain rating of 18.3±16.5, constant trials of 12.5±10.2, and decreasing trials of 10.7±8.1. Accordingly, increasing trials also had the most aftersensations, as subjects reported painful sensations for up to 7±2.4 sec for increasing trials on the right side, 6.7±2.5 sec for constant trials, and 4.9±3.9 for decreasing trials (Figure 9). Similarly, aftersensations on the left side were reported, on average, for 6.5±1.9 sec for increasing trials, 5.7±3.0 sec for constant trials, and

3.1±2.9 sec for decreasing trials (Figure 9). This serves as further evidence to support the idea of a dynamic pain experience rather than a clear-cut model often used in fMRI designs.

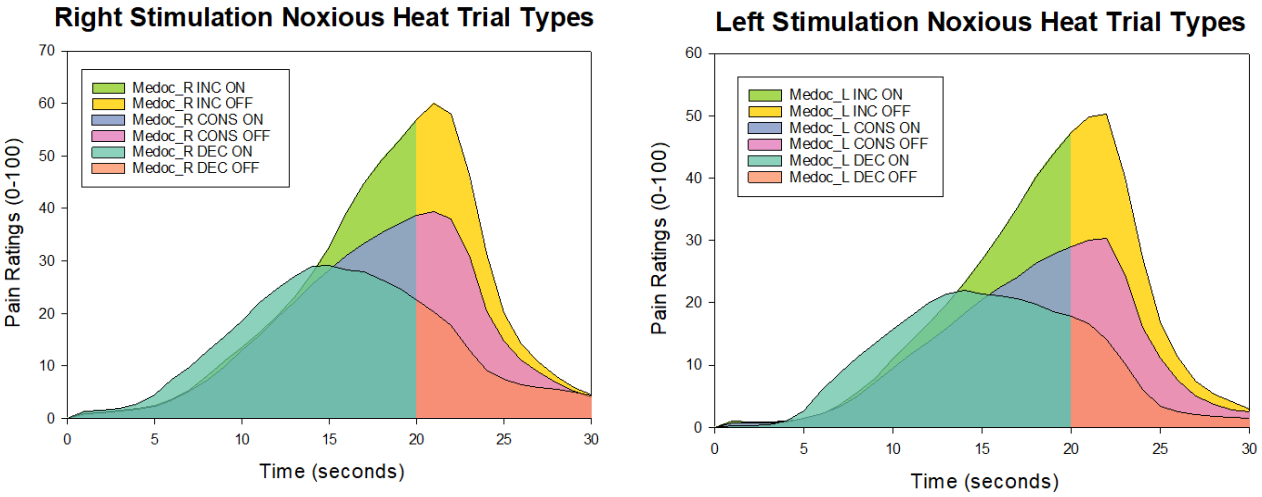

Figure 9. Area graphs showing all subject's pain ratings based on increasing, constant, and decreasing trials. Yellow, pink, and orange areas illustrate the amount of pain reported by subjects even when the thermal stimuli had been turned off.

This marks the end of the previously collected and analyzed data. The following is data collected and analyzed by the author of this thesis.

ON vs OFF Block Comparison

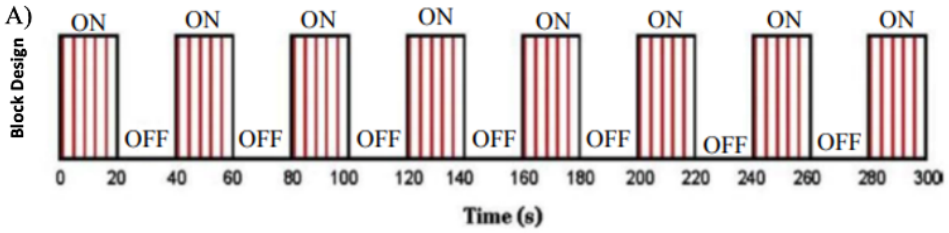

Figure 10. Traditional ON/OFF block model based on the ISI.

Analysis from the traditional ON/OFF block model, not accounting for any temporal delays in the pain sensation (Figure 10), showed little to no BOLD signal in key pain-processing regions, such as ACC, insular cortex, primary somatosensory regions (S1), and thalamus. More specifically,

right leg stimulation showed activations in brain stem and cerebellum areas, while left leg stimulation exhibited no BOLD activations (Figure 11).

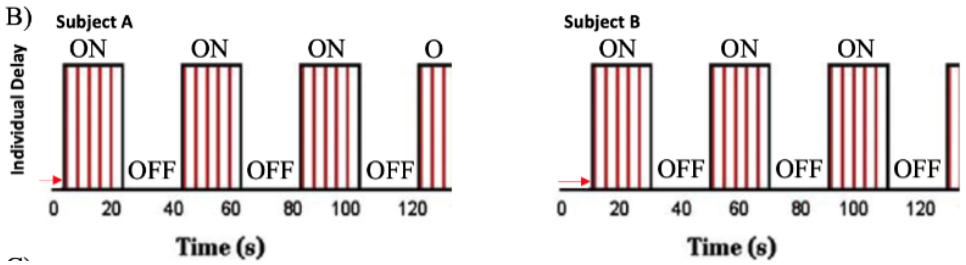

Figure 11. Modified ON/OFF block design incorporating individual temporal delays to pain response. Red arrows indicate the difference in time when considering delays based on VAS reports versus the block design.

Incorporating subject's individual temporal delayed response to pain into the model (Figure 11) significantly enhanced BOLD activations in key pain-processing regions, with peak p-values nearing 0.01. However, the same trend regarding left and right leg stimulation were observed, with right leg stimulation showing more activations than left leg stimulation. Left leg stimulation did result in activations in other areas, though, such as putamen (Figure 14).

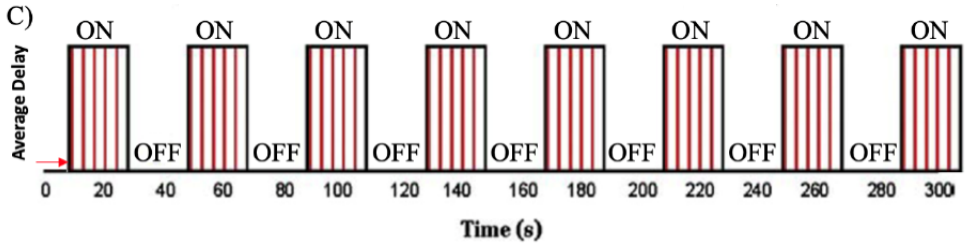

Figure 12. Modified ON/OFF block design incorporating the group average delay in pain reports recorded by VAS. Red arrows indicate the difference in time when considering delays based on VAS reports versus the block design.

Furthermore, employing the temporal group average delay from VAS pain reports (Figure 12, 13) of 8 sec for right leg stimulation and 9 sec for left leg stimulation resulted in even greater enhancement, with peak p-values in key pain regions closer to 0.001. While right leg stimulation again resulted in activation of cerebellum regions, both right and left leg stimulation exhibited strong activations in regions of interest, including ACC, thalamus, and insular cortex (Figure 14).

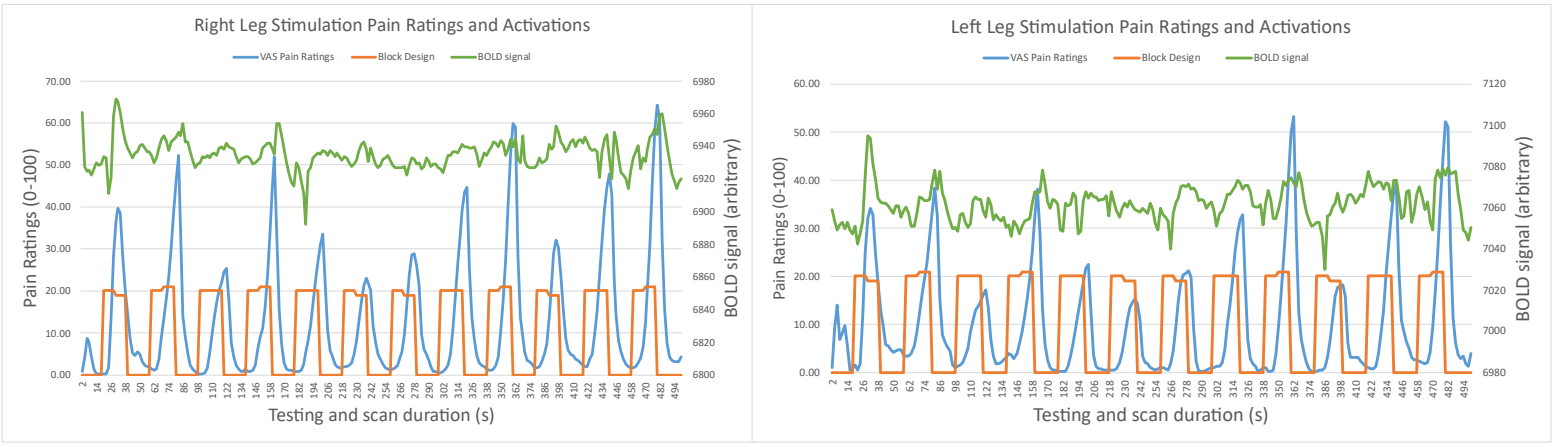

Figure 13. Thermal stimuli VAS pain ratings and activations. The green line indicates BOLD activity during the duration of the scan, while the blue line shows the variation in VAS pain ratings, both during the application of the pseudorandomized trials. Orange columns indicate the onset and offset of OFF blocks accounting for increasing, constant, or decreasing trials. Delays in the report of painful sensations is evident for both measures, with peaks rising about halfway through the ON blocks.

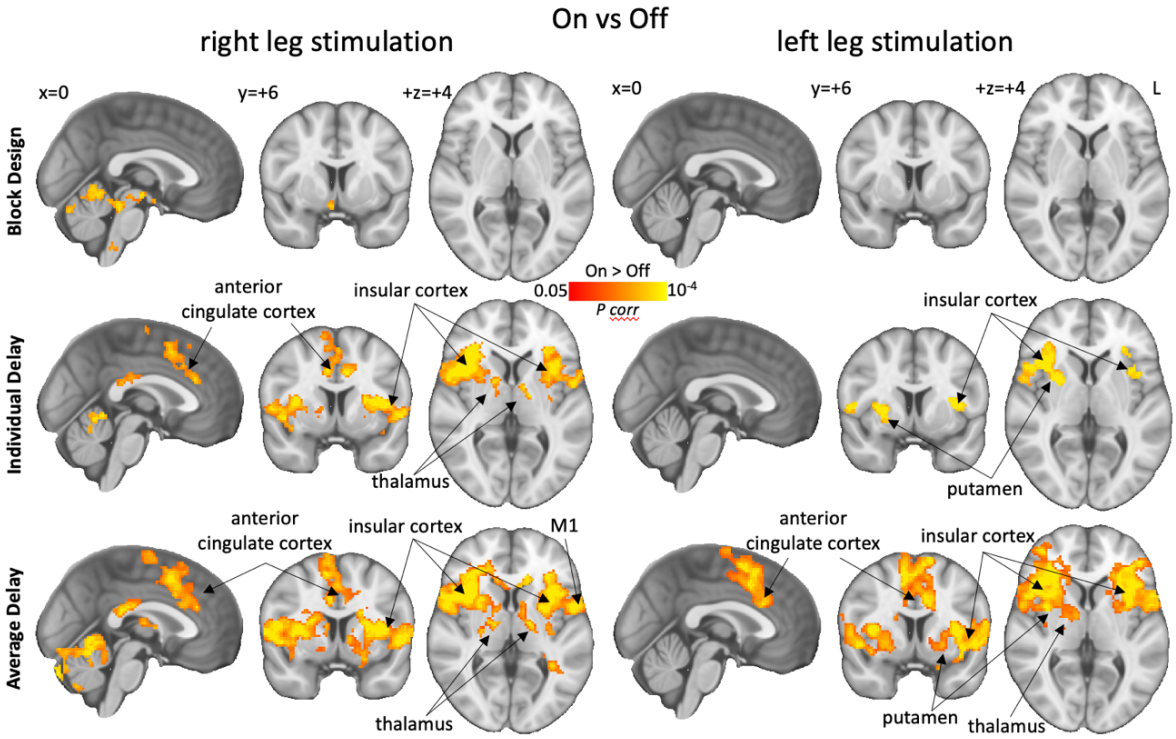

Figure 14. Different BOLD activations in key pain-processing regions. Top row shows activations based on the onset and offset of the stimulus alone, middle row shows activations when taking individual delays of pain reports into consideration, and the bottom row shows activations when noting the average group delay.

Increasing vs Decreasing Trials Comparison

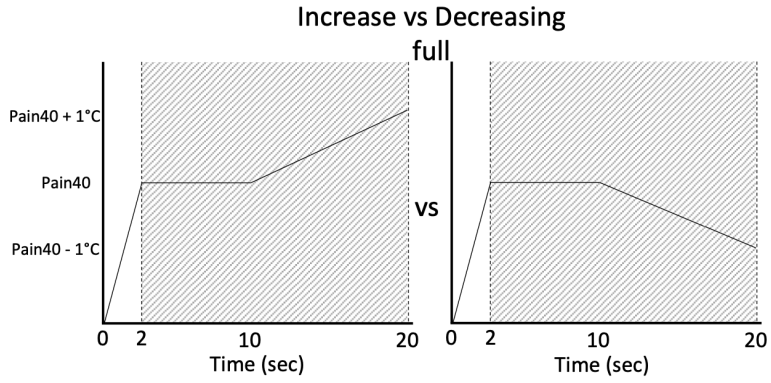

Figure 15. Increasing versus Decreasing trials comparison. Grey area indicates the analysis of the full 20 sec ON block.

For this analysis, the comparison between the increasing and decreasing trials was explored. As described earlier, the stimuli would change temperature or remain constant for the last 10 sec of the ON block, so the magnitude at which BOLD signals would differ depending on stimuli intensity was explored (Figure 15). For the entire 20-sec ON block of this comparison, there were no significant BOLD activations when looking at the onset and offset of stimuli for either right or left leg stimulation. Incorporating individual delays into the analysis, right leg stimulation resulted in BOLD signals in S1 and the mid ACC, with peak p-values just below 0.05. Adding the group delay, activations in these areas strengthened and activations in other areas became evident, including precuneus, primary motor cortex (M1), and insular cortex. Peak p-values neared the 0.01 to 0.001 range for right leg stimulation of this analysis (Figure 16). It is important to note, however, that left leg stimulation did not result in any significant activations for either of the analysis. Possible reasons for this observation will be visited in the discussion section.

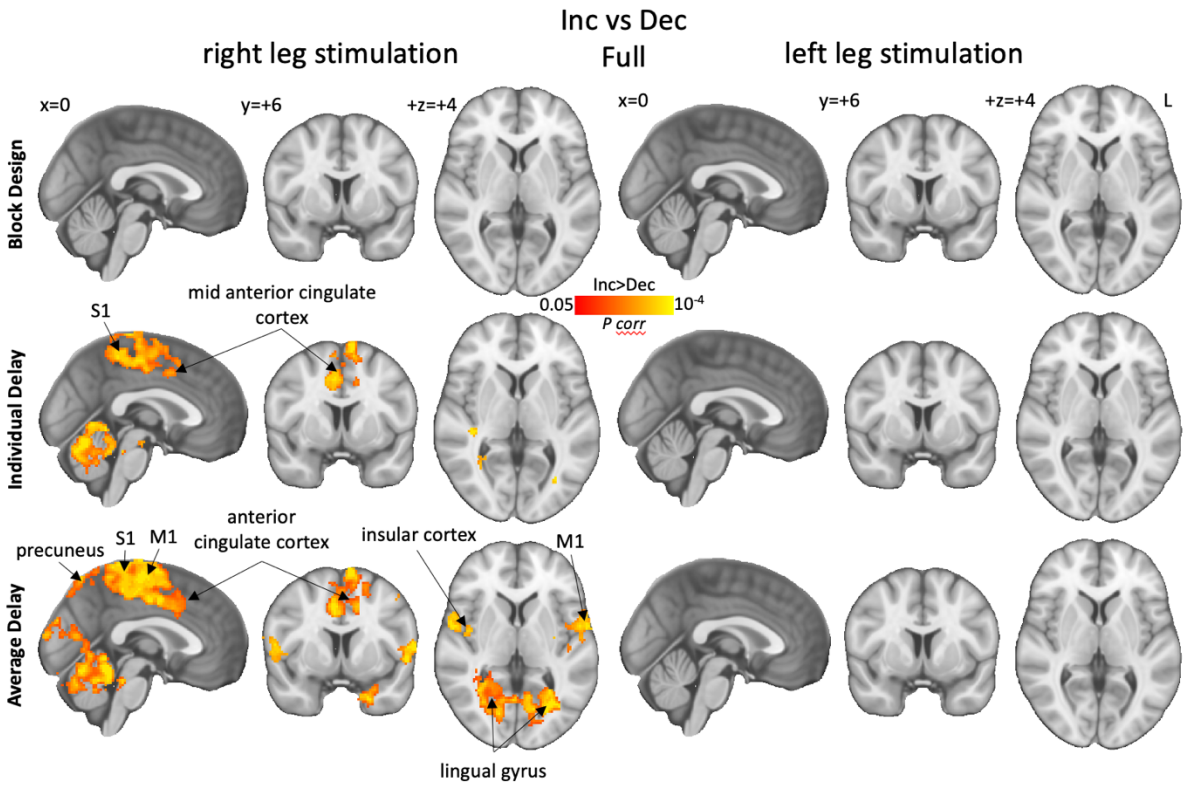

Figure 16. BOLD signals for the different comparisons of increasing versus decreasing trials for the entire duration of the ON block. First row shows activations based on the onset and offset of the stimulus alone, sec row shows activations when taking individual delays of pain reports into consideration, and the third row shows activations when noting the average group delay. Left leg stimulation resulted in no activation for either of the analysis, while right leg stimulation significantly enhanced BOLD signals as the delays were incorporated.

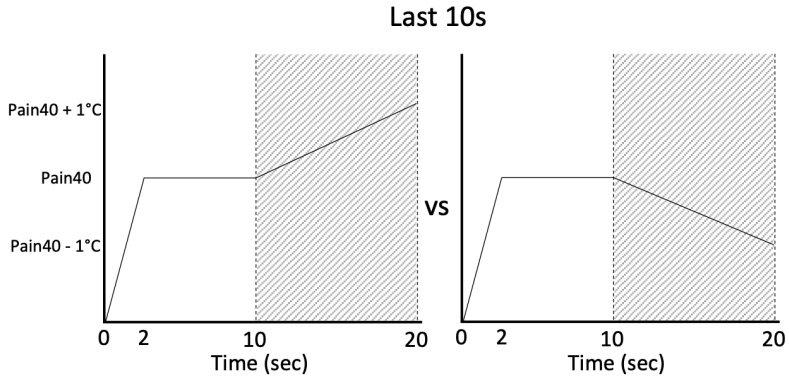

Figure 17. Increasing vs Decreasing trial type comparisons. Grey area indicates the analysis of the latter 10 sec of the ON block.

Following these results, the timing of the ON block was modified to only reflect the last 10 sec of stimulus application (Figure 17). This way, the magnitude at which the BOLD signals differ based on stimulus intensity could be more meticulously analyzed. For the block design without any temporal delay consideration, right leg stimulation showed no significant activations, while left leg stimulation showed activations during the OFF block in regions of interest, such as ACC and putamen. Employing the individual delays, right leg stimulation showed strong activations in many key pain-processing regions, such as S1, M1, ACC, insular cortex, and thalamus. Left leg stimulation showed activations primarily in M1 and the insular cortex. Furthermore, the group average delay for right leg stimulation showed even greater activations in these areas, with peak p-values nearing 0.001 significance. For left leg stimulation, strong activations were also observed in ACC, M1, and the central opercular cortex, with peak p-value also near 0.001 significance (Figure 18). Considering the last 10 sec of the ON block resulted in increased BOLD signals all around, except for right leg stimulation following the onset and offset of stimulus. This implies that there may be a correlation with the intensity and change in a painful stimulus rather than just the sensation itself, expanding insights into the pain experience.

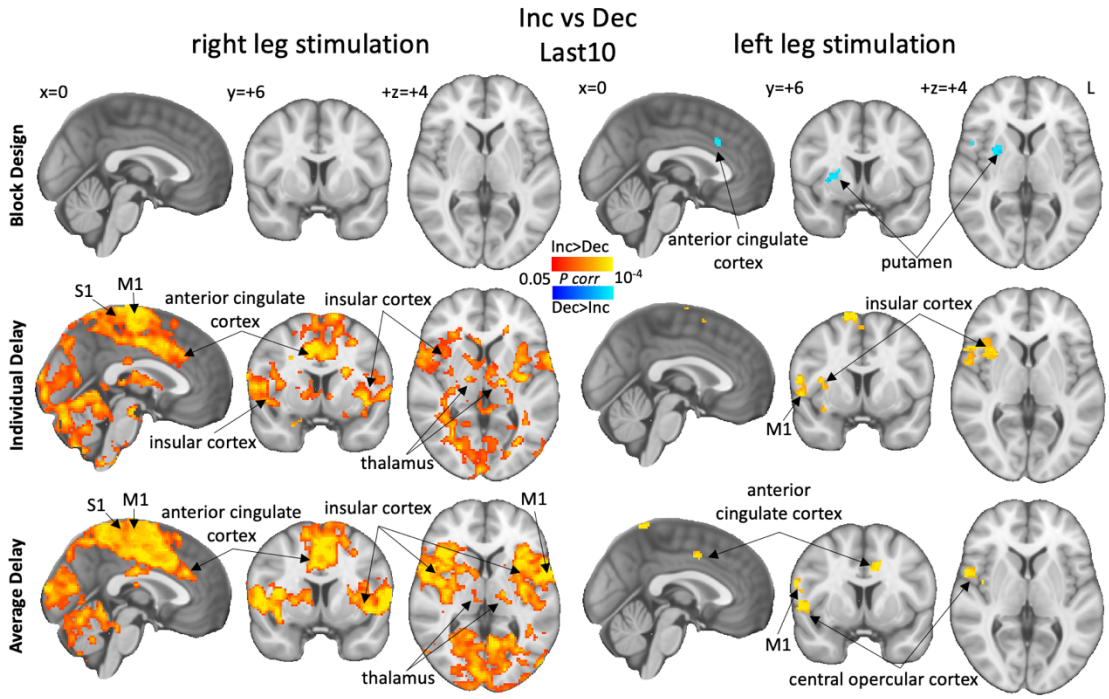

Figure 18. Brain activations for the different models of increasing versus decreasing trials for the last 10 sec of the ON block. BOLD signals progressively and significantly increased once delays were incorporated into the analysis, showing strong activations in key pain-processing regions for right leg stimulation primarily.

Increasing vs Constant Trials Comparison

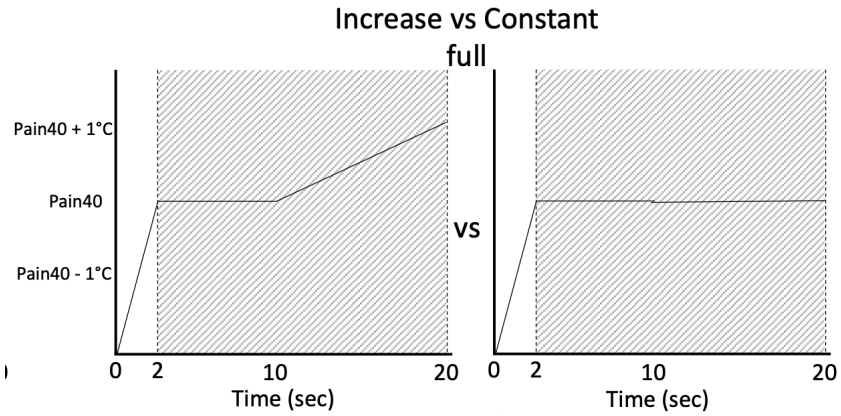

Figure 19. Increasing versus Constant trials comparison. Grey area indicates the analysis of the full 20 sec ON block.

Moving on to the comparison of increasing versus constant trials (Figure 19), the temperature would increase or remain the same for the entire 20-sec duration of the ON block, respectively. This contrast further explored the different BOLD responses based on intensity of the stimuli. The analysis revealed that right leg stimulation did not result in the activation of pain regions of interest, with activations in more posterior areas of the brain for the traditional ON/OFF block design. The incorporation of individual delays as reported by subjects resulted in more significant activation of areas of interest, including S1, M1, ACC, and insular cortex for right leg stimulation. The group average delay yielded even stronger BOLD signals for these areas, also including the thalamus, with peak p-values near 0.0001. Unfortunately, left leg stimulation did not result in any significant activations ($p>0.05$) for neither of the three models (Figure 20).

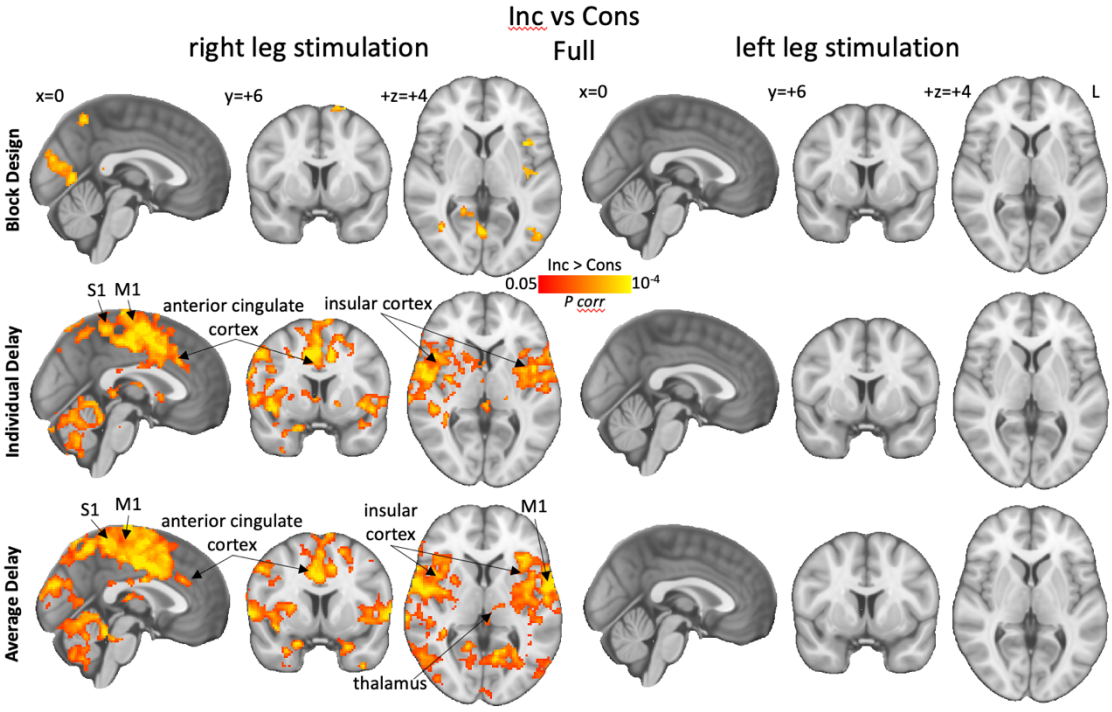

Figure 20. BOLD signals for the different comparisons of increasing versus constant trials for the entire duration of the ON block. First row shows activations based on the onset and offset of the stimulus alone, sec row shows activations when taking individual delays of pain reports into consideration, and the third row shows activations when noting the average group delay. Left leg stimulation resulted in no activation for either of the analysis, while right leg stimulation significantly enhanced BOLD signals as the delays were incorporated.

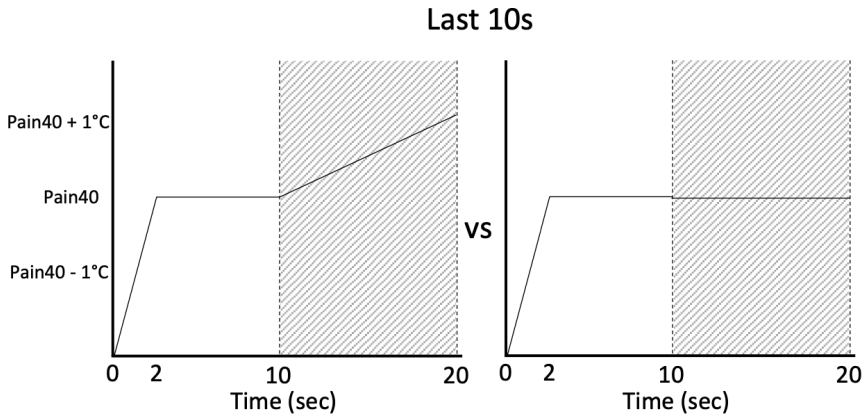

Figure 21. Increasing vs Constant trial type comparisons. Grey area indicates the analysis of the latter 10 sec of the ON block.

Once the model was modified to observe only the last 10 sec of the ON block (Figure 21), right and left leg stimulation resulted in no significant BOLD signals for the traditional ON/OFF block design ($p>0.05$). Incorporating the individual delays, however, showed enhanced activations of M1, ACC, thalamus, and precuneus for right leg stimulation, with peak p-values close to 0.01. Differing from the previous results, left leg stimulation showed BOLD signals in the putamen. The average group delay yielded even more significant activation of these areas, also including S1 and insular cortex for right leg stimulation. The ventral medial prefrontal cortex (vmPFC) was activated for left leg stimulation when considering this delay (Figure 22).

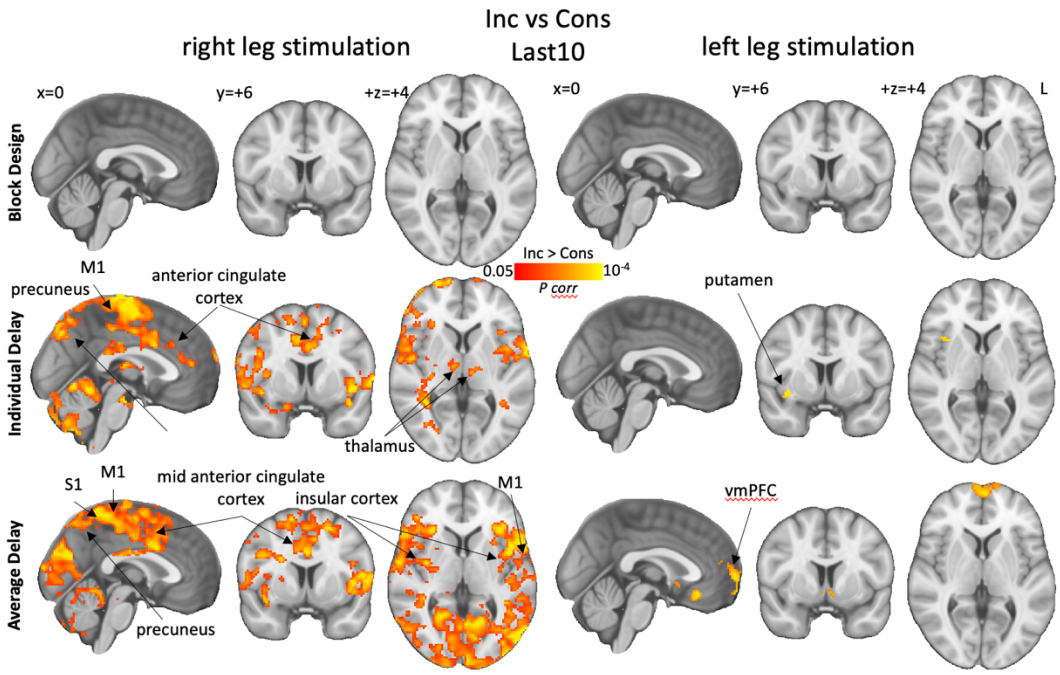

Figure 22. Brain activations for the different models of increasing versus constant trials for the last 10 sec of the ON block. BOLD signals progressively and significantly increased once delays were incorporated into the analysis, showing strong activations in key pain-processing regions for right leg stimulation primarily.

Constant vs Decreasing Trials

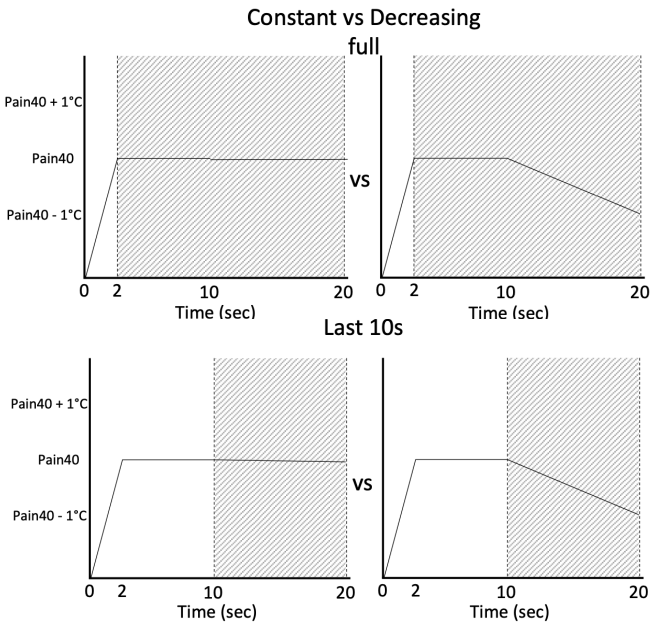

Figure 23. Top panel: Constant versus decreasing trials comparison. Grey area indicates the analysis of the full 20 sec ON block. Bottom panel: Constant versus Decreasing trial type comparisons. Grey area indicates the analysis of the latter 10 seconds of the ON block.

For the final analysis, constant versus decreasing trial types were compared for the full duration of the ON block and the last 10 sec (Figure 23), where the stimulus remained constant or decreased. The analysis resulted in no BOLD signals for either of the three models previously described: ON/OFF block design, individual, and group average delay. This was the case for both left and right leg stimulation. Possibilities for these results will be explored in the discussion section.

Discussion

The present study provides valuable insights into the multifaceted nature of pain perception and its neural correlates. Analysis of unpublished data from the Harper PaIN Lab revealed significant delays in pain onset and aftersensations following thermal stimuli application, highlighting the temporal dynamics inherent in the pain experience. Particularly noteworthy was the variability observed in response times across different stimulus sites, with longer delays recorded in reporting pain sensations on the left calf area compared to the right calf area. Moreover, our neuroimaging analyses uncovered intriguing patterns in blood-oxygen-level dependent (BOLD) signals during thermal stimulation paradigms. The incorporation of individual and group average delays in pain perception significantly altered BOLD activations in key pain-processing regions, including the anterior cingulate cortex (ACC), insular cortex, and

thalamus. This enhancement suggests a more comprehensive representation of the neural processes underlying pain perception when temporal delays are accounted for. Notably, right leg stimulation consistently elicited stronger BOLD activations compared to left leg stimulation across various trial types, underscoring potential hemispheric differences in pain processing.

Incorporating average and individual delays in this experimental design produced stronger signal output compared to the traditional ON/OFF block design for several reasons. Based on QST data, the traditional block design lacks temporal accuracy, as it presumed instantaneous perception and response to stimuli. However, the incorporation of the delays accounted for the temporal delays of pain perception, hence, enhancing its validity. Moreover, by accounting for the delays, the experimental design is more sensitive to subtle variations in neural activity associated with different phases of pain perception. Studies have shown low reliability in fMRI results for pain-processing regions when compared to individual's ratings using VAS (Letzen et al., 2016). Others have also stressed that adjustments to the modelling of the BOLD signals to match the perception of the stimuli can increase fMRI testing reliability for major depressive disorder (MDD) (Compère et al., 2021). This highlights the importance of measuring psychometry before and/or during fMRI study sessions, as it provides essential information required to adjust fMRI statistical modelling to better resemble the perception of a stimulus. Thus, in our study, the increased sensitivity enabled by the incorporation of the delays allowed for a more precise detection of neural responses and increased the reliability of the BOLD activations observed in the brain. Furthermore, the enhancement in BOLD activations observed with the average delay can be attributed to greater statistical power; the significance of the signal may have been enhanced based on the consideration of all subject's delays rather than each individual subject's (Suresh & Chandrashekara, 2012).

Parallel to VAS pain ratings in the QST session, increasing trials resulted in the strongest BOLD activations. Opposite to these, decreasing trials provided not only the lowest VAS ratings but the least BOLD signals as well. Consequently, the comparison between increasing and decreasing trials yielded significant BOLD activations. These results reflect the magnitude in which brain responses can change based on slight stimuli fluctuations (1^{o}C), stressing the sensitivity of pain processing and the subjective pain experience. These results could be explained by a comparative lesser pain perceived by subjects in contrast to a greater pain elicited by increasing trials, as pain intensity is directly correlated to temperature (Hollins et al., 2011). Studies have shown that there is a close relationship between pain ratings during the application of heat induced pain and post-stimulus ratings, with temporal pain summation when temperatures are high (Koyama et al., 2004; Staud et al., 2007). Temporal pain summation refers to an increased perception or experience of pain caused by repeated presentation of noxious stimuli of a specific threshold (Kong et al., 2021; Medoc). More specifically, noxious heat stimuli is perceived as increasingly painful when the stimulus duration is extended beyond 5 seconds, and this is reflected in increased activation volumes in areas including somatosensory cortices (S1), left lateral thalamus, and posterior insula (Tran et al., 2010). This evidence aligns with the increasing trials presented in this study, as these accounted for the highest intensity of pain and accordingly the highest VAS ratings during and after stimulus presentation, as well as the most significant BOLD signals.

Opposite to this, habituation may account for the low response to decreasing trials, as studies have suggested that repeated stimulations in the same testing area results in temperature-dependent habituation (Jepma et al., 2014). This is applicable to the protocol presented here, as all testing was done in the same site on each leg. Furthermore, VAS ratings collected during real-time

stimulus presentation have indicated adaptation when temperatures are low to moderate (Koyama et al., 2004), and other studies have also noted a plateau in cortical activation volumes after sustained pain duration (Tran et al., 2010). Although the lowest temperatures employed for decreasing trials still fall within the noxious heat range (Hoffstaetter et al., 2018), these temperatures were comparatively less intense than those utilized for other more intense trial types (increasing and constant). Subjects may have been habituated or de-sensitized to the decreasing trials during the QST and scanner sessions due to the least intense nature of this trial type.

Following increasing versus decreasing trials, the comparison of increasing versus constant trials also showed significant BOLD activations. We hypothesize this may be due to habituation of the testing site to the stimuli during the constant trials, as the temperature remained the same for the entire 20 sec ON block. Hence, the testing site's response to this unvarying stimulus weakens, as the brain and body adjust to the constant presence of the stimulus and cease to react as strongly. As a result, even though we aimed to measure the difference between increasing and constant stimuli, the constant condition, over time, almost acts as a baseline or control condition—akin to an "OFF" block—because the participant's physiological response has habituated to the unchanging stimulus, thereby diminishing the observed reaction. This habituation underlines the brain's tendency to respond more robustly to changing conditions or stimuli while gradually reducing its response to constant, unvarying inputs. This increases the difference in signal between the increasing trial and the constant trial. Indeed, studies have shown that a decreased perception of pain is reflected in weaker BOLD responses to noxious stimuli in areas including thalamus, insula, and putamen (Bingel et. al, 2007). This may have been the case for constant trials as there was no stimuli change to prompt a response.

Constant versus decreasing trials, however, did not result in any significant activations for either left or right leg stimulation. Reasons for this could be the habituation mentioned above, as participant's physiological response adapts to an unvarying stimulus, or, in this case, gradually decreases as the temperature of the stimulus slowly lowers back to baseline as well. Further evidence also suggests that the extent of habituation to a stimulus is mediated by repetition and can increase if the process is repeatedly engaged (Hollins et al., 2011). Therefore, the sustained application of constant and decreasing trials could've ultimately de-sensitized subjects to the thermal stimuli, resulting in lack of BOLD signals in the key pain-processing regions regardless of side of stimulation.

Considering different pain intensities can be a useful tool in the measure of neural correlates pertaining to the pain experience, as brain responses are mediated by pain intensities correspondingly. Studies have asserted that high intensity noxious heat stimuli, similar to the increasing trials presented here, evoke robust increases in areas associated with nociceptive processing and pain (Hoeppli et al., 2022). The same study also confirmed the brain's ability to detect slight changes in thermal stimuli, with BOLD signals reflecting these changes accordingly (Hoeppli et al., 2022). Notably, our study reported low perceived pain responses and BOLD activations for decreasing trials and the opposite for increasing trials. The resulting BOLD activations may have also been mediated by the affective aspect of the pain experience, as pain is commonly mediated by previous experience (Yoo et al., 2023). Experiencing a higher intensity pain sensation from increasing trials may elicit emotional stress (Lumley et al., 2011), but following this with a less intense sensation in constant or decreasing trials may lessen the emotional response to the stimulus and thus the perception of it in both QST and fMRI measures.

This ultimately helps better understand the psychophysical response to pain, which can be applied to better understanding chronic pain and other pain conditions (Fillingim et al., 2016).

Furthermore, the account of the full 20-sec ON block resulted in overall less BOLD activations than the last 10 sec, where the stimulus increased, decreased, or remained constant. This was expected, as VAS pain ratings not only revealed delays in the onset of pain sensations, but ratings corresponded to trial types accordingly. These results suggest that responsiveness to noxious stimuli is proportionally prompted by new or unexpected changes, regardless of whether a moderately painful stimulus is already present or not. Indeed, studies testing brain sensitivity to differential stimulus intensity using electroencephalography (EEG) have concluded that neural responses are most sensitive to sensory changes that represent new objects or events in an environment (Somervail et al., 2020). Each trial's ability and extent to which it can cause changes in brain responses is supported not only by the analysis of the last 10 sec of the ON block, but by the analysis of the entire 20-sec as well. For the latter, participants endured Pain40 for 10 sec before the thermode temperature changed or remained constant. Thus, the BOLD signals observed for this analysis suggest that different pain intensities can result in significant activations of key pain-processing regions, even if not gradually changing intensities for the entire duration of an ON block. A transcranial magnetic stimulation (TMS) study measuring brain responses with EEG demonstrated that stimulus intensity dictated response amplitude in neuronal activity (Komssi et al., 2004). In accordance, increasing trials with the highest intensities resulted in the greatest activations for both the 20-sec and last 10 analyses, with activations lowering as intensities lowered as well. Accounting for the period of stimulus change gives precise insight into the temporal dynamics of distinct intensities in responses to pain. This can be a useful consideration for the fMRI analysis of pain in an effort to reduce noise produced by this process. Whereas, in addition

to accounting for the period of stimulus change, also accounting for the period *before* stimulus change reveals the extent to which the change in intensity can activate regions of interest, even if only for the latter half of stimulus presentation. The combination of both analyses can provide a dual perspective in the characterization of the pain experience and may help refine fMRI study designs of pain and the accuracy of pain investigation.

An important observation from the results obtained is the lack of signal/activations produced by left leg stimulation. A study in 2021 revealed that brain responses can be significantly larger when nociceptive stimuli is delivered to the non-dominant hand of individuals due to greater sensitivity (Zhang et al., 2021). Moreover, other studies have relied on the contralateral projection of nociceptors to explain pain lateralization processes (Allen et al., 2021). Taking this evidence into account and the fact that most participants were right-handed (n=21), it was expected for left leg stimulation to result in greater activations or enhanced signals. Also, pain40 values differences within each subject were subtle, with temperatures being the same or varying at most one to two degrees between the left and right legs. Surprisingly, the opposite was observed, with right leg stimulation yielding the most responses to noxious stimuli. Interestingly, cerebral processing of dental pain research has confirmed that the S1, thalamus, and posterior insula are contralaterally activated for the processing of pain, and it's hypothesized this might be due to protective motor action from the side of stimulation (Brügger et al., 2011). These are the some of the same regions largely observed in this study, and this hypothesis could suggest evasive behaviors from the more sensitive side of stimulation (left leg) resulting in less activations. The QST analysis, however, revealed no significant difference observed in VAS pain ratings between stimulation sites. Studies investigating the lateralization of pain have stated that both hemispheres of the brain play a role in the processing of pain, with the left hemisphere being more efficient than the right in processing

cutaneous sensory input (Merskey & Watson, 1979). The same study also claimed that the right hemisphere is dominant in the emotional experience of pain (called affective-motivational), determining the left hemisphere's prevalence of pain processing (Merskey & Watson, 1979). More recent studies have also recognized the right hemisphere lateralization of pain's affective-motivational components (Roza & Martinez-Padilla, 2021). This right hemisphere characteristic would support the notable activation of pain regions such as the ACC, which has been associated with affective aspects of pain (Yang & Chang, 2019). However, other studies have challenged these findings by revealing that sensory information is processed by right lateralized systems (Coghill et al., 2001). This evidence, although contradictory, suggests that both hemispheres can be involved in the processing of pain, aligning with the bilateral activations yielded by right leg stimulation. Further investigation should be employed to clarify the underlying differences of contralateral stimulation in cerebral pain processing.

Some limitations of this study include the determination of Pain40, the anticipation of a painful stimulus, and the lack of pain rating reports in the scanner session. Partial unclearness was noted on the scale of 0-100 presented to participants at the time of determining this value for each individual. More specifically, participants seemed to confuse hot versus painful sensations, and seemed sometimes overwhelmed by the large range of numbers they could choose from. Therefore, a visual representation, like the VAS or a scale of this nature, could've been employed to help guide participants in the determination of Pain40 or some sort of other visual aid. Moreover, the anticipation of a painful stimuli could've affected the brain responses reported by the results, especially for the increasing trials. Studies have shown that the anticipation of the painful stimuli, also known as pain-catastrophizing, can result in amplified activations of neural regions involved in pain processing (Quartana et al., 2009). Others attest that activations can be seen in midcingulate

and anterior insula cortices during anticipation periods of a painful stimuli (Palermo, 2015). This suggests that the participant's anticipation of the noxious thermal stimuli may have manifested in increased BOLD signals at the time of the analysis. Controlling for these anticipation periods in pain study designs may help obtain more accurate psychophysical responses pertaining to the pain experience. However, the enhanced activations observed upon the incorporation of delays in pain reports challenges this possibility, as stronger BOLD signals would've been observed by the ON/OFF block analysis otherwise. Lastly, pain ratings could've been recorded during the collection of BOLD signals in the scanner. This would've further confirmed the results reported here and relate the volume of activations to subjective sensations more directly.

The present study aimed to better characterize the subjective pain experience by applying QST-reported delays in the onset of pain sensations to fMRI models of analysis of a task-based study design. The analysis presented here revealed that incorporating these delays, both at the individual and group level, significantly enhanced BOLD signals representing the activation of key pain processing regions during the stimulation periods. This suggests that the pain experience is not as clear-cut as fMRI inter-stimulus-interval designs have long modeled it to be, and that the delay in onset of pain is a critical component to consider for the treatment and management of pain. Importantly, accounting for these delays can be a useful tool to advance drug testing and the development of chronic pain treatment (Reddy et al., 2012). Accounting for these delays also reinforces the psychophysical aspect of the pain experience, as they resulted in activations significantly different from just the onset and offset of stimuli, giving a better measure of the subjective pain experience.

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