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Assessment of Differences in Temporal Pain Dynamics in Pain-Evoked fMRI Experiments

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An abstract of a thesis 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 Sciences with Honors

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Abstract

Pain-evoked functional magnetic resonance imaging (fMRI) experiments have helped uncover numerous features of the brain's neural underpinnings of the pain experience. These analyses commonly contrast blood oxygenation level dependent (BOLD) responses to noxious stimuli applied in ON blocks (i.e., during application of a noxious stimulus) with the BOLD response during OFF blocks (i.e. during periods of no, or innocuous stimulation). In most studies, it is generally assumed that pain is experienced primarily during the ON blocks, with minimal pain experienced during OFF blocks. However, C fibers transmit nociceptive signals to the brain slowly (as slow as 0.5 m/s), leading to delays in the onset of pain, and painful after-sensations (AS) are sometimes experienced after noxious stimulation has ended. Previous research has shown that AS is greater in individuals with chronic pain (e.g. fibromyalgia), meaning that pain experienced during off blocks could add noise to the analysis pipeline by comparing groups who are not experiencing pain similarly over time. In many fMRI studies of pain, one to several discrete numeric rating scale (NRS; e.g. 0-100) pain ratings are obtained after the presentation of a noxious stimulus, capturing the overall rather than the moment-to-moment experience of the pain. Given that the temporal experience of pain may differ depending on the different modalities (e.g., thermal vs. mechanical) that are utilized in a study, the need to characterize the temporal nature of the pain experience of stimuli that are used in pain-evoked fMRI experiments is also essential. Therefore, in the present study we evaluated pain using a continuous visual analog scale (VAS) that recorded the experience of both a noxious mechanical and a noxious thermal stimulus on a moment-to-moment basis (every 0.1 sec). We assessed how stimulus type (thermal or mechanical), change in intensity (slowly increasing, constant, or slowly decreasing), and stimulus location (left vs. right calf) may influence the temporal progression of the pain

experience during a typical fMRI block design experiment of 20 secs ON interleaved with 20 secs OFF. On average, participants took 8.63 secs (± 3.10) from the onset of the ON block to perceive the thermal stimuli as painful (i.e. > 5/100) and 5.12 secs (± 2.53) to perceive pain from the mechanical pressure stimuli. Stimulus intensity was not a predictor of when the stimulus would be perceived as painful, but rather stimulus type (whether noxious heat or noxious pressure) was the only predictor, with noxious heat taking longest to be perceived at all intensities. On aftersensations, participants reported noxious heat eliciting longer lasting painful sensations after the stimuli had been removed (time for pain to disappear was measured as VAS<5/100)—with the increasing and constant conditions taking longest to do so: 6.67 ± 2.09 secs and 6.62 ± 2.09 secs, respectively. Significantly, as many as 75% of subjects reported for the sensation of pain to reach its peak after stimulus had been turned off during the blocks where the increasing thermal stimulus was applied.

Conclusions: These results provide additional evidence that the temporal dynamics of the pain experience are not entirely in line with the onset and offset of the eliciting stimulus. Taking these nuances in the temporal dynamics of pain into account during analysis of pain-evoked BOLD could improve in the signal to noise ratio of the neural correlates of the pain experience. Modified blocks of time considering when subjects begin and stop perceiving a painful sensation, which varies dependent upon the intensity of a stimuli and the type of stimuli being applied, may ultimately lead to more precise assessment of this objective marker of pain. Our future analyses of the fMRI data from these same participants will use modified “perceived pain ON” and “perceived pain OFF” blocks accounting for these temporal differences.

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Acknowledgements

I would like to thank Dr. Daniel Harper for his endless support throughout the development and carrying out of this project. I would like to also thank Dr. Ali Alsouhibani for his guidance and expertise in conducting data analysis as well as the design of the study. Lastly, I would like to thank Sofia Heras, Justin Thomas and Senna Kim for their assistance in subject recruitment and data collection and preprocessing throughout the study.

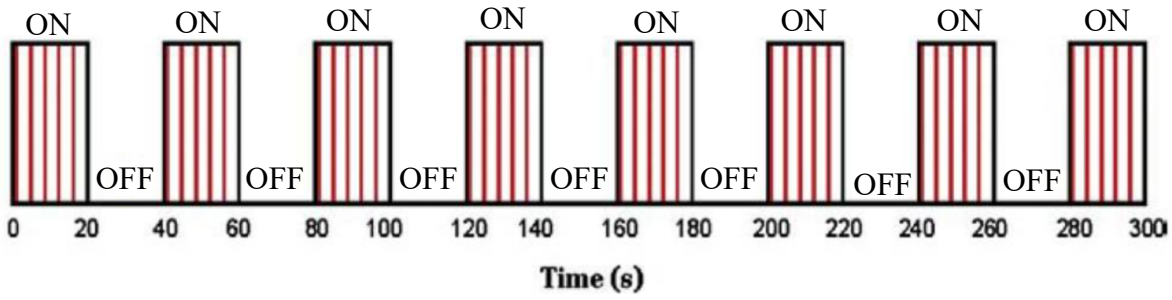
Table of Contents

Introduction

In 2016, approximately 50 million adults–nearly twenty percent of the US population–suffered from chronic pain. It is estimated that approximately eight percent of U.S. living adults suffer from high-impact chronic pain[1]. Given the increasing prevalence of chronic pain, researchers from all fields of neuroscience, biology, chemistry, and physics continue to investigate the mechanisms behind the experience of pain—which is not yet clear-cut. When a stimulus reaches an intensity that becomes noxious or suggestive of damage, peripheral nerve fibers (nociceptors) become activated to further relay information to the central nervous system. These nociceptors are found in the skin, bones, and muscles, and may be activated separately or together depending upon the type and intensity of stimulation. Nociceptors can be classified in two major groups: fast, thinly myelinated Aδ fibers which can conduct signals at a velocity of 5 m/s; and slow, unmyelinated C fibers, which can conduct nociceptive signals as slow as 0.5-2 m/s[2]. Scientists typically rely upon the use of several research tools such as Quantitative Sensory Testing (QST) and functional magnetic resonance imaging (fMRI) for assessing and quantifying their understanding of pain in humans. Specifically, functional neuroimaging has been used among the pain community for investigating the functionality and connectivity of key brain regions associated with the experience of pain. Biomarkers most frequently studied include regions such as the primary (S1) and secondary (S2) somatosensory cortex, the anterior cingulate cortex (ACC), anterior and posterior regions of the insula, prefrontal cortices (PFC), limbic structures, cerebellum, and striatum[3-9]. As fMRI studies rely upon changes in blood-oxygen-level-dependent (BOLD) signal as indicative of activation in brain regions, they are designed with the goal of allowing to isolate or highlight these changes in pain-evoked activity in various brain regions[10-11]. In pain research, one common way for researchers to highlight these differences is by using the traditional “block” or “boxcar” design (Figure 1), which uses changes in BOLD signal during the ON blocks (set periods of time where a noxious stimulus is presented, or a task is performed)

as representative of pain, while the OFF blocks (which can be resting state or periods of innocuous stimuli) may serve as a baseline for comparison[10-11]. Nevertheless, it is not clear whether this typical model used for designing fMRI experiments is an accurate representation of the temporal dynamics of the pain experience. Considering the varying conduction velocities of different nociceptors and the brain's processing time (approximately 500-800ms), which includes perception, decision, and execution[12-13], it may be difficult for studies to accurately determine when a stimulus has become painful or stopped being so as the traditional model would suggest. As a matter of fact, some studies already suggest that these blocks may be leaving out portions of the experience of pain. For example, a study in patients with nerve injury found that patients frequently reported intense pain during the moments after a stimulus had been removed[14]. Another study in patients suffering from chronic pain (e.g.,fibromyalgia) found that some patients experience painful after sensations in the immediate fifteen seconds upon removal of a stimulus[15-16]. Such evidence then raises multiple questions about the characterization of the pain experience: for instance, is this traditional boxcar model (with defined on versus off epochs) representative of the experience of pain? How does the experience of pain change upon the use of different modalities (i.e., thermal versus mechanical noxious stimuli) and lastly, are there other factors such as overall intensity of the stimuli and location (i.e., side where the stimuli is applied) that play a role in the experience of pain?

Figure 1)



Adapted from Tie, Yanmei et al., 2008. Figure reflects typical block design used in fMRI experiments. In figure above, each "ON" block lasts 20 secs and is followed by 20 secs "OFF" block.

In the present study, we investigate these questions by evaluating the subjective experience of pain using a continuous visual analog scale (VAS) that recorded the experience of both a noxious mechanical and a noxious thermal stimulus on a moment-to-moment basis (every 0.1 secs). We assessed how stimulus type (thermal or mechanical), changes in intensity (slowly increasing, constant, or slowly decreasing), and stimulus location (left versus right calf area), may influence the temporal progression of the pain experienced during a typical block design experiment of 20 secs ON interleaved with 20 secs OFF. The second portion of this working study will focus on utilizing modified blocks of time that may be more representative of the pain experience for the analysis of collected fMRI data.

As a secondary aim, the study assessed whether the side where a stimulus is being applied may influence the subjective experience of pain. In specific, previous studies have demonstrated that pain may exhibit a hemispheric right-bias, meaning that some regions of the right hemisphere will respond either solely or more strongly to pain regardless of which side the stimuli may be applied[17-18]. The data on these studies has often been limited to using one type of noxious stimuli in their results, thus leaving the possibility that this effect may be unique to their specific

stimulus and location being applied. Given the application of two different stimuli and at a different location, the present study allowed us to assess this question.

Methods

Participants:

Fourteen healthy volunteers from the Emory University community and its surroundings were recruited, 6 males and 8 females, age 22 ±7.35 yrs; the demographics consisted of 9 individuals identifying as White, 3 Asian, and 2 as other; 10 were right-handed, 2 left-handed, and 2 ambidextrous. Participants signed a written consent form, and all study-related procedures were explained prior to completion of any tasks. All procedures were approved by the Emory Institutional Review Board.

Preparation Procedure:

After undergoing the consent process, participants were provided with questionnaires aiming to assess eligibility upon additional variables—anxiety, initial pain prior to study session—as well as collect additional information such as demographics and handedness. For anxiety and initial pain, the short-form McGill Pain Questionnaire was used[19]; a short form of the Edinburgh Handedness questionnaire was used to assess handedness[20]. Questionnaires were used for pre-screening of participants prior to any study-related procedures and were not utilized during any part of the data analysis. Additionally, women of premenopausal age were required to undergo a pregnancy test given that the effects of MRI on a developing fetus have not yet been determined.

Session Protocol:

The study involved the application of two moderately painful stimuli, noxious heat as applied using the Thermal Sensory Analyzer (TSA) 2 (Medoc, Israel), and noxious deep pressure using a rapidly inflatable blood pressure cuff device (Hokanson, Bellevue, WA), both of which are regularly used in academic and clinical research. For the safety of participants, temperatures

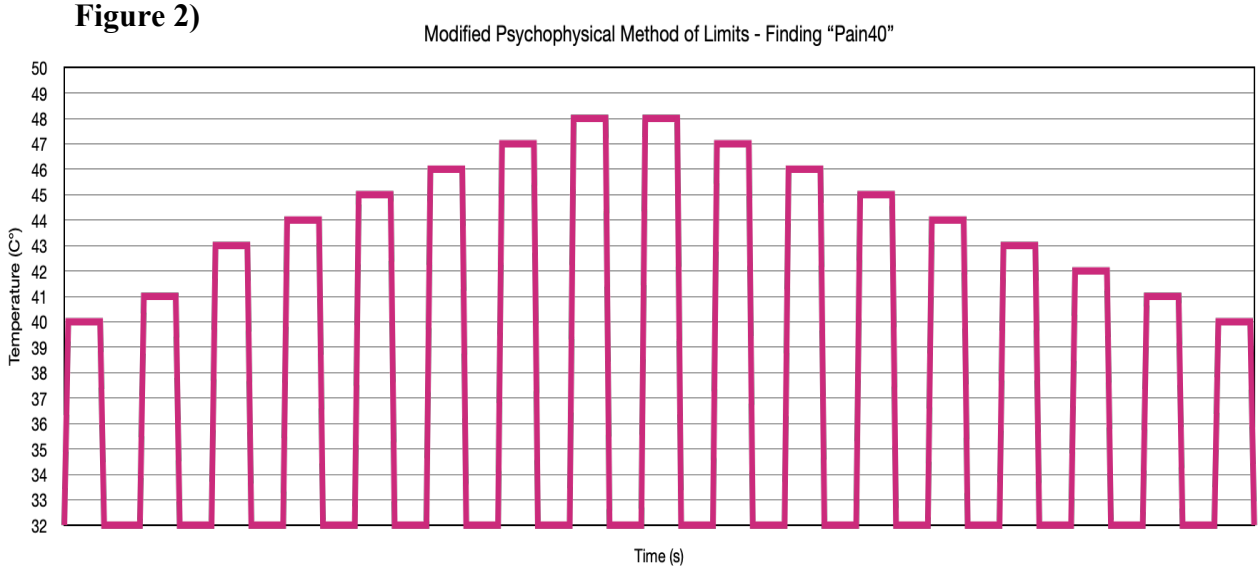
above 50°C and pressure above 300 mmHg were not applied. All study-related interventions were completed over one 3-hr session, which involved part one of solely QST and a second portion that included experiencing the stimuli while undergoing a functional magnetic resonance imaging (fMRI) scan.

Initial calibration of a moderately painful stimulus:

Seated in a reclined chair and extending their leg on another chair placed directly in front of them, a mark was made on participants' tibialis anterior muscle. To ensure continuity on testing location, a mark was assessed by measuring the midpoint between participants' patella and talus, then moving 3 cm laterally. Though somewhat arbitrary, this location would ensure the tibialis anterior muscle would be the exact location being targeted and where the thermode head used for applying heat stimuli (30x30 mm) was attached. Additionally, it allowed for repetition of testing area and avoiding any confounding results that may be due to differences in testing location. The calf area was used for the testing of noxious pressure. Participants were asked before the study session to wear clothing that would make this region accessible without causing any discomfort that could interfere with study goals (i.e., additional pressure from rolling up pants). Thermal and pressure stimuli were calibrated to take ~2 secs to ramp up to maximum physical intensity at the beginning of the ON blocks. Using a modified method of limits (Figure 2), participants were presented stimuli in an ascending series, increasing 1°C or 20 mmHg each time, lasting 10 secs followed by a 10 secs interval. At the end of each block, subjects were asked to provide a verbal rating on a scale of 0-100—where 0 represents "no pain" while 100 represents "the most painful sensation imaginable" from the stimulus. Ascending sequences were carried out by presenting the lowest stimuli first, either 40°C or 100mmHg, followed by increases in intensity until a rating of 60 or the highest available stimulus was reached (48°C or 300mmHg), whichever occurred

first. Upon reaching the target value, then a descending sequence would start, decreasing 1°C or 20 mmHg each time, until a rating of 30 or the lowest available stimulus was reached. Using a linear regression analysis, the values corresponding to a pain intensity level of 40 (Pain 40) were calculated and used for the main experiment (or closest available value). As used in previous studies, 40 is a value that would correspond to a moderately painful stimulus[21-25]. In cases where Pain40 was a decimal value, values were rounded up or down to the next available integer following standard logic rules (value>0.5, round up; value<0.5 round down) due to equipment limitations; the Medoc device does not allow for values with more than one decimal spot for rate of change and Hokanson cuff only allows for integer pressure values. This procedure was repeated on each leg for each stimulus while randomizing which leg was tested first, as to avoid any possible order effects.

Figure 2)



Participants were presented stimuli in an ascending series, increasing 1°C each time, lasting 10 secs followed by a 10 secs interval. At the end of each block, subjects were asked to provide a verbal rating on a scale of 0-100—where 0 represents no pain while 100 represents the most painful sensation imaginable from the stimulus. Ascending sequences were carried out by presenting the lowest stimuli followed by increases in intensity until a rating of 60 or the highest available stimulus was reached (48°C or 300mmHg), whichever occurred first. Upon reaching the target value, then a descending sequence would start, decreasing 1°C or 20 mmHg each time, until a rating of 30 or the lowest available stimulus was reached. Using a linear regression analysis, the values corresponding to a pain intensity level of 40 (Pain 40) were calculated and used for the main experiment (or closest available value).

Main experiment:

Quantitative sensory testing (outside of MRI scanner):

Seated in the same position, each stimulus was applied 12 times in blocks consisting of 20 secs on and 20 secs off. Stimuli were applied using a pseudorandomized sequence consisting of 1) 4 *constant* trials where the stimuli were constant at pain 40; 2) 4 *increasing* trials where the stimuli reached pain 40, was maintained for the first 10 secs, and then increased at a rate of 0.1°C/2mmHg per second above pain 40; and 3) a *decreasing* series of 4 decreasing sequences at a rate of 0.1°C/2mmHg per second in a similar manner (pain 40 for 10 secs, then 10 secs decreasing below pain 40 (Figure 3). The pseudorandomized sequence maintaining at pain 40 and then either increasing, decreasing, or remaining constant was designed to discourage subjects from trying to guess the intensity of each following sequence and thus focus more on how each individual stimulus felt. The time of the blocks, 20 secs, was designated to induce tonic acute pain, which is mediated primarily by C fibers[26]. During this period, participants were asked to rate their sensation of pain continuously as it was applied using a computerized visual analog scale (VAS) that was placed directly in front of them. The scale relied upon the movement of a mouse to record the rated sensation of pain, and the range was similar to the verbal—where 0 (left-far end) represented “no pain” and 100 (far-right end) represented “the most painful sensation imaginable” from the stimulus. This VAS recorded participants’ subjective experience of pain every 0.1 secs and saved it as a numeric file that would be later available for analysis.

Figure 3)

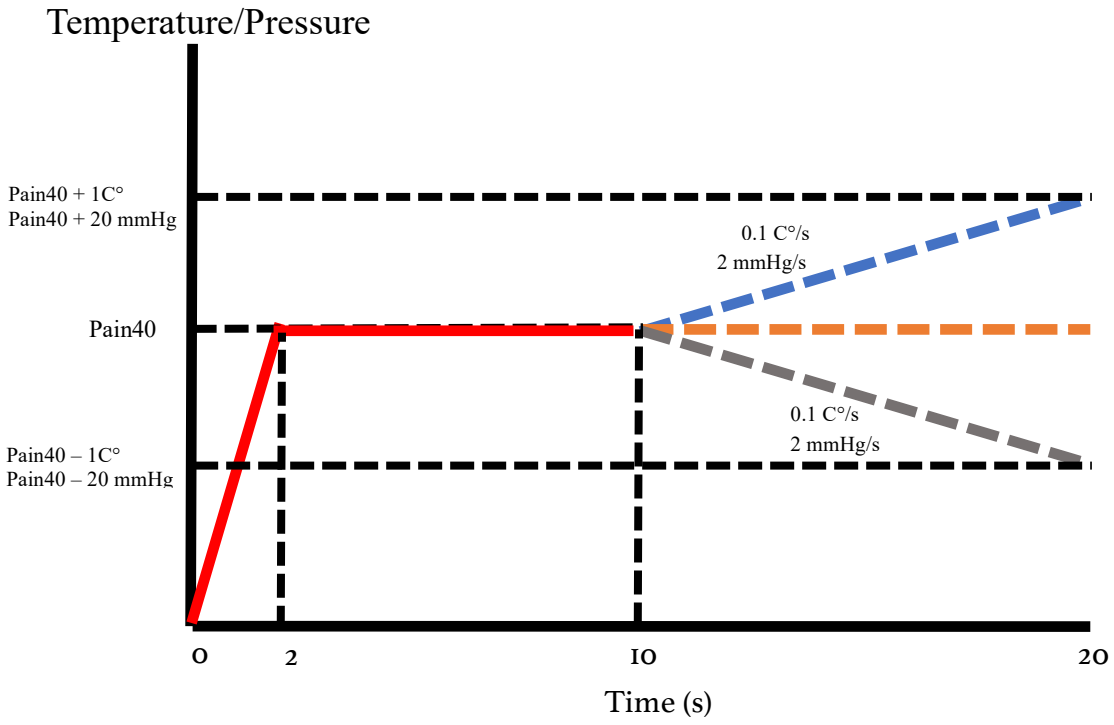


Figure above illustrates the experimental paradigm

Functional Magnetic Resonance Imaging:

Upon completion of all QST-related tasks, participants were escorted by a study team member to the Center for Systems Imaging (CSI) Core located in the Emory University Hospital where the experiment sequence was repeated using the previously determined ratings inside of a Siemens 3T Magnetom Prisma Fit scanner. Using a 32 channel receiver head coil, high-resolution multi-echo T1 [TE (echo time) = 2.96 ms; TR (repetition time) = 2.53s; 7° flip angle; slice thickness = 1 mm; FOV (field of view) = 256 mm; resolution = 256 × 256] anatomic images were collected at the beginning of each run. For the four different blocks series, 250 echo-planar images were collected using a single-shot, gradient-echo echoplanar (EPI) pulse sequence [TR = 2000 ms; TE = 27 ms; flip angle = 80°; FOV = 220 mm]. Seventy-two contiguous transversal 2.0-mm thick slices were selected to provide whole-brain coverage (voxel size: 2.0 mm x 2.00 mm x 2.00 mm).

Data Analysis:

Repeated-measures analyses of variance confirmed there were no differences in between trials; thus, sequences of same modality were pooled together to yield average increasing, decreasing and constant ratings of each stimulus for each subject. Unlike more commonly designed experiments trying to assess the subjectivity of pain, the continuous nature of the VAS allowed for the assessment of pain sensation throughout the interval rather than collecting ratings at a specific point in time during the interval— i.e: ratings at 1 sec were ten successive 0.1 secs time periods rather than one of the points during the block. Ratings were consequently averaged to yield a 30-secs interval from the beginning of the onset of the stimulus. Additionally, all participants' data was checked for normalization and verified to meet all the assumptions necessary for analysis using general linear model (GLM). Both procedures were conducted prior to any analysis of the data. All statistical analysis of QST data was conducted using IBM's Statistical Package for the Social Sciences (SPSS). Due to time constraints, one subject did not complete QST portion of the study. Additionally, one subject's data was removed from analysis due to noncompliance (subject failed to concentrate on the task and therefore did not provide ratings for analysis in multiple runs).

Multivariate analysis revealed there were no significant differences mediated by side, thus both left and right were pooled across sides per stimulus per run for both conditions being tested: time to first perceive the sensation as painful (VAS>5%) and time for sensation to reach a non-painful threshold (VAS<5%). Aftersensations were measured as the area under the curve remaining after 20 secs, when the noxious stimulus was removed.

Results

Average pain 40s were 45.03 C° ± 2.28 and 45.05 C° ± 2.83 for the thermal pain stimuli, on left side and right side, respectively, while cuff pressures were 231 mmHg ± 63 and 227 ± 58, for left and right, accordingly. These differences in ratings between sides were not statistically significant. On average, participants took 8.63 secs (±3.10) from the onset of the ON block to perceive the thermal stimuli as painful (i.e. > 5/100). Similarly, it took participants 5.12 secs (±2.53) to perceive pain from the mechanical pressure stimuli (Figure 5). Within subject multivariate analysis showed that neither side where the stimulus was being applied (0.008, 1, p=0.931) nor overall intensity (.277, 1, p=0.615) were predictors of these delays. Stimulus type was the only significant predictor (15.215, 1, p=0.006), with the noxious thermal stimuli taking the longest to be perceived regardless of intensity (6.103,11, p<0.001)(Table 2).

Figure 4)

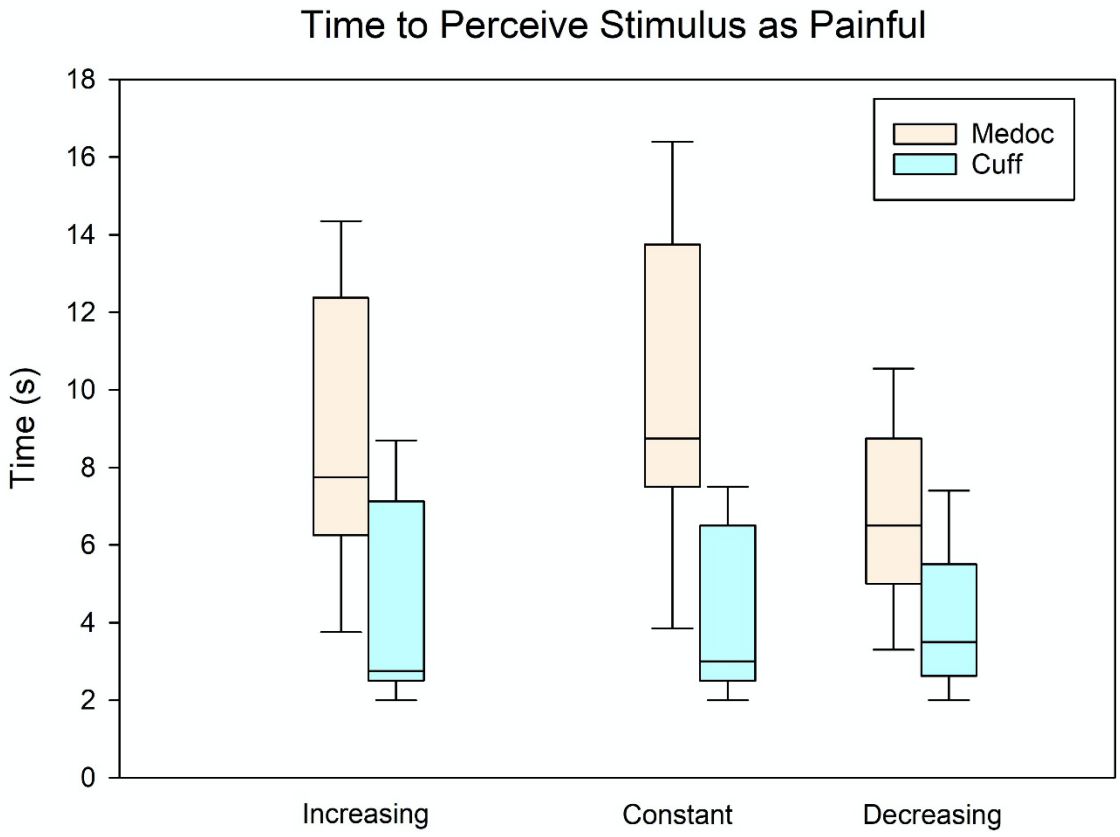


Table 1)

Time to Perceive Stimulus as Painful[a]

	Heat Increasing	Heat Constant	Heat Decreasing	Pressure Increasing	Pressure Constant	Pressure Decreasing
Mean	8.9167	9.8750	6.7500	4.3333	4.0000	4.0833
Std. Deviation	3.59819	4.06272	2.40738	2.59662	2.13307	1.85660
Minimum	3.00	2.50	3.00	2.00	2.00	2.00
Maximum	14.50	17.00	11.00	9.00	7.50	8.00

a. Painful defined as achieving a VAS rating greater than 5

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Figure 5)

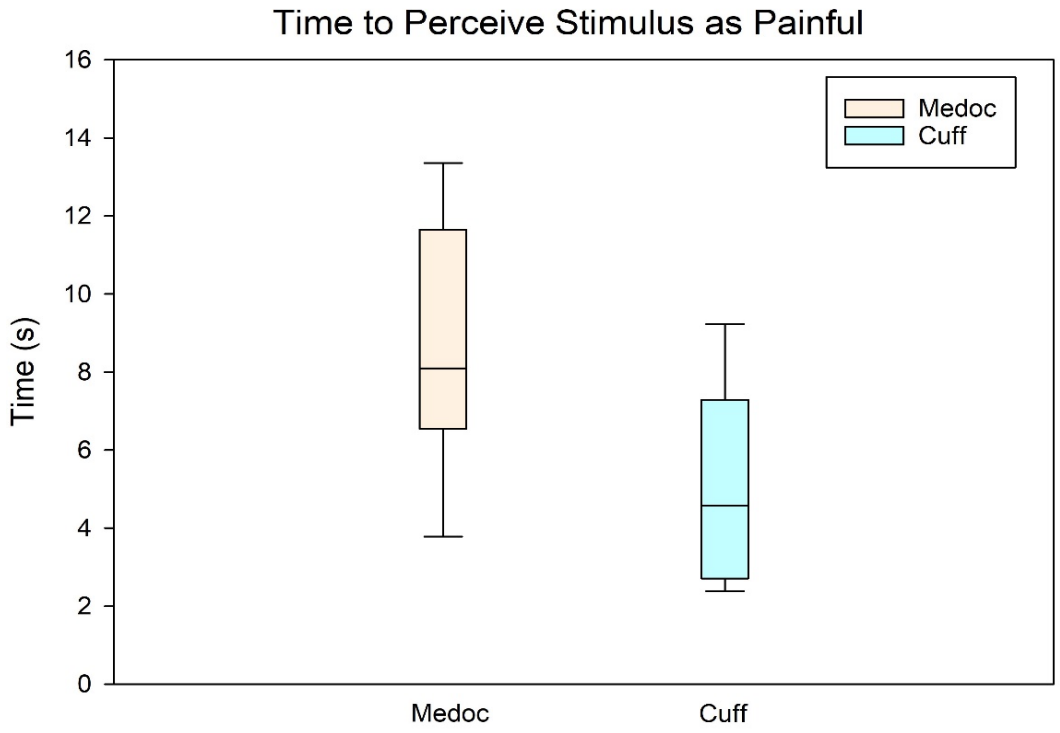


Table 2)

Time to Perceive Stimulus as Painful[a]

	Heat	Pressure
Mean	8.6333	5.1250
Std. Deviation	3.10204	2.53374
Minimum	2.83	2.33
Maximum	13.80	9.33

a. Given the multivariate analysis showed that intensity was not a contributing factor in time to perceive pain, this table reflects the times of each stimulus to be perceived as painful--regardless of overall trial intensity.

Regarding aftersensations, the temporal course of pain during the pressure stimulus was more so aligned with the temporal course of the physical stimulation compared with the perception of the thermal stimulus. Participants reported noxious heat eliciting longer lasting painful sensations after the stimuli had been removed (time for pain to disappear was measured as VAS<5/100)—with the increasing and constant conditions taking longest to do so: 6.67 ± 2.09 secs and 6.62±2.09 secs, respectively (Figure 6). Side was not a contributing factor to the duration of after sensations at a significance of $p<0.05$ (3.513, 1, $p=0.088$). Instead, stimulus type, heat versus pressure (19.110, 1, $p=0.001$) and the intensity of the trial (39.205, 1, $p<0.001$) were the predictors of duration. Paired t-test comparison showed the effect of intensity was most relevant in the increasing (5.252, 11, $p<0.001$) and constant conditions (3.621, 11, $p=0.004$) and not significant during the decreasing trials (1.713, 11, $p=0.115$). At all intensities, nonetheless, noxious heat elicited longer lasting aftersensations (Table 3).

Figure 6)

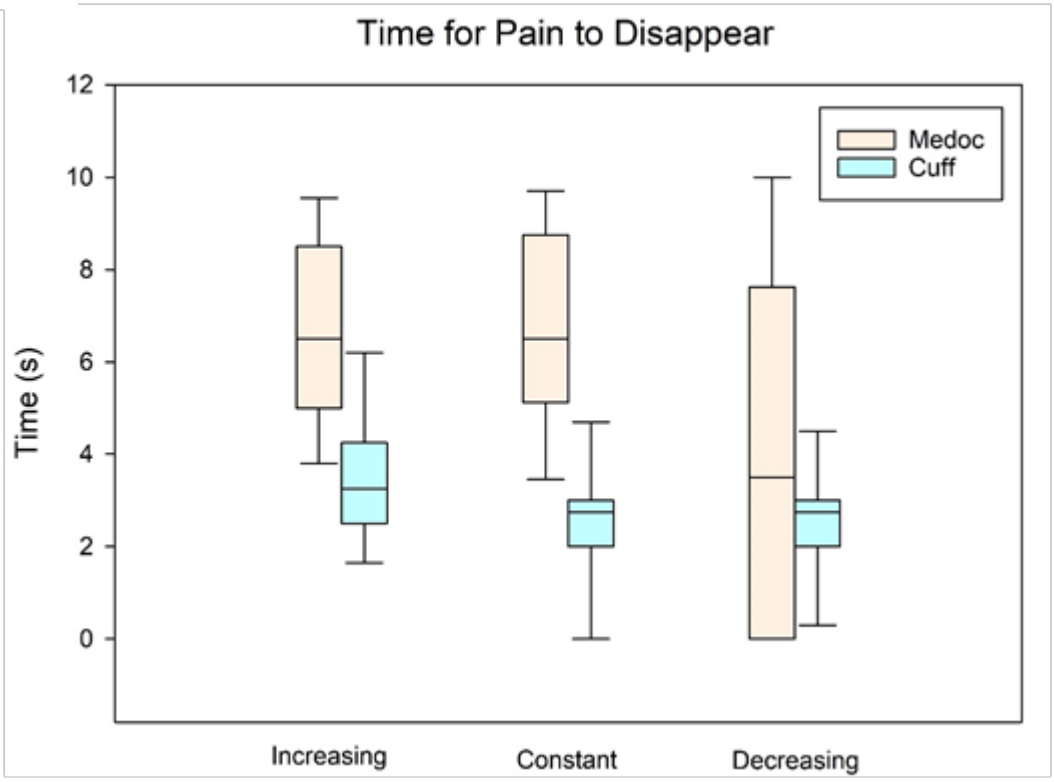


Table 3)

Time for Pain to Disappear[a]

	Heat Increasing	Heat Constant	Heat Decreasing	Pressure Increasing	Pressure Constant	Pressure Decreasing
Mean	6.6667	6.6250	4.1250	3.4583	2.4583	2.5833
Std. Deviation	2.09256	2.09029	3.89711	1.43746	1.43746	1.27624
Minimum	3.50	3.00	.00	1.50	.00	.00
Maximum	10.00	10.00	10.00	6.50	5.00	4.50

a. Measured as the time to reach a VAS rating of 5 or less.

Table 4 contains a summary of the percentage of pain that was experienced following the removal of stimuli—as measured by the portion of AUC after the stimulus had been turned off. Generally, the noxious heat stimuli elicited a greater magnitude of pain after the stimulus had been turned off. Remarkably, as many as 75% of subjects reported for the sensation of pain to reach its peak after stimulus had been turned off during the blocks where the increasing thermal stimulus was applied. Within subject multivariate analysis showed that the type of stimulus and whether the run was increasing, decreasing or constant was highly correlated with the painfulness of after sensations ($p<0.001$) with the noxious heat increasing, constant and decreasing trials eliciting the greatest percentages of pain after stimulus had been turned off (Figure 7). The overall temporal dynamics are further represented in the supplementary figures.

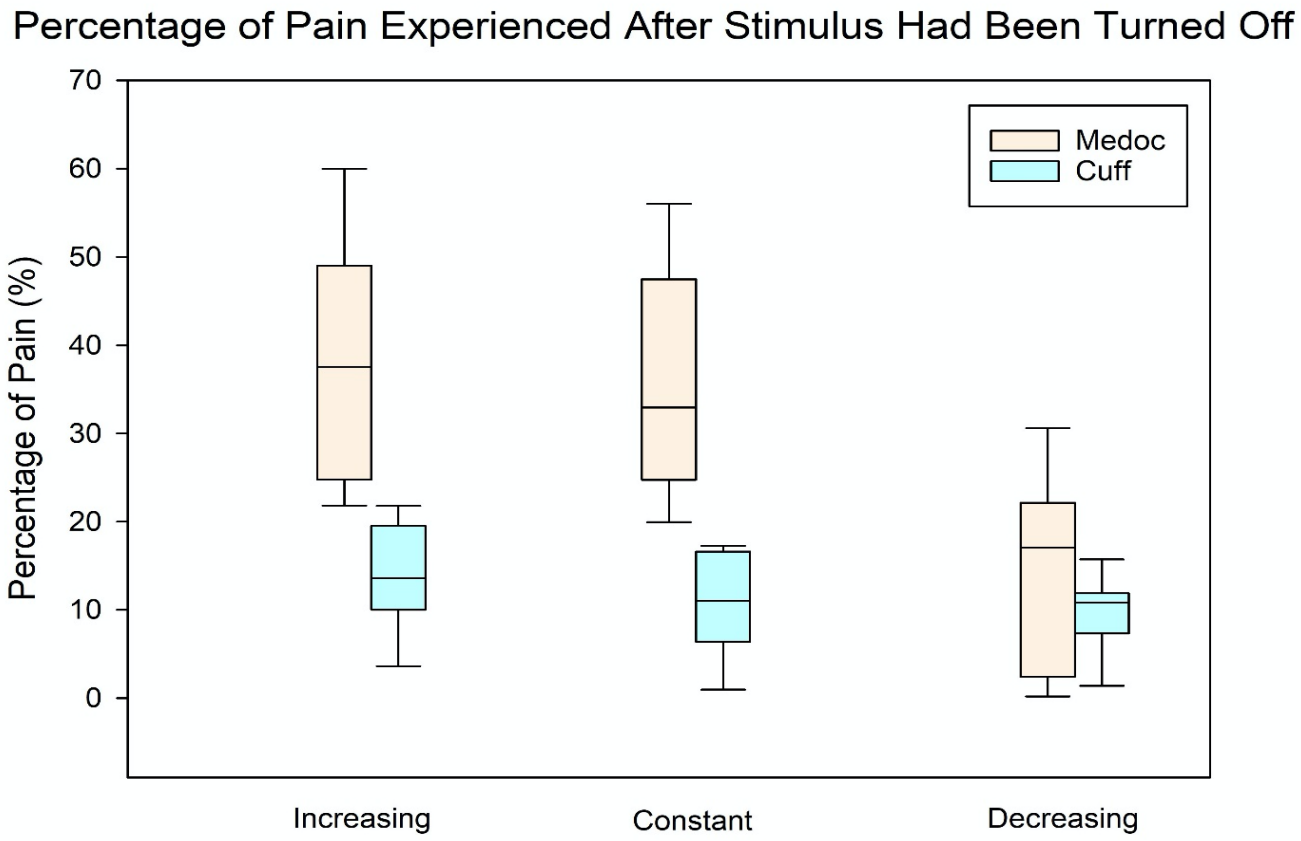
Table 4)

Percentage of Pain Experienced After Stimulus Had Been Turned Off[a]

	Heat Increasing	Heat Constant	Heat Decreasing	Pressure Increasing	Pressure Constant	Pressure Decreasing
Mean	37.9461	35.4134	14.0303	13.9329	10.4477	9.7767
Std. Deviation	13.35785	12.84462	11.16337	6.18581	5.89559	4.50596
Minimum	21.64	18.25	.17	3.16	.65	1.03
Maximum	63.17	57.52	33.26	22.34	17.32	16.09

a. Measured as a percentage of the total area under the curve

Figure 7)


Percentage of Pain Experienced After Stimulus Had Been Turned Off
Percentage of Pain (%)
70
60
50
40
30
20
10
0
Medoc
Cuff
Increasing
Constant
Decreasing

Discussion

In the present study, we focused on determining stimuli perception time courses as well as factors that may play an influential role in the time to perceive onset and offset of noxious stimuli. Consistent with previous literature suggesting that pain intensity is correlated to the duration of aftersensations in chronic pain patients[16], the present study found a direct relationship between stimuli type and intensity and the percentage of AUC remaining after stimuli had been removed ($p<0.001$), with the effect being of greater intensity for thermal stimuli in increasing and constant sequences.

Use of the traditional block designs has been challenged since the earliest days of fMRI. A 1992 study for example displayed a flashing checkerboard for distinct durations and assessed at finding whether the stimuli evoked the expected box-car shape used in block design. They found that stimuli of longer duration were more in line with this shape, while short duration stimuli elicited a sharp yet delayed BOLD response from when the stimulus was first presented. Significantly, this study came to yield what is now known as event-related design[27-28] (figure 8). The main takeaway from this evidence though is that there is room for improvement in current block design methodology, and that the traditional boxcar shape may not be a suitable method for analysis for all research fields. As it pertains to pain, multiple research studies in chronic pain patients have previously hinted that the experience of pain may not follow this traditional model—such as the documenting of after sensations, for example, where patients report continuing to perceive pain even after the stimuli has been removed[16, 29]. Nonetheless, to our knowledge, this is the first study that aims at characterizing the experience of pain by explicitly defining time thresholds at which pain is first perceived, determining when it first vanishes, and assessing which factors are most relevant to the onset and duration of these sensations. The present data serves as evidence that the pain experience does not follow the traditional boxcar

model that is commonly utilized for the analysis of data and could serve as a guideline for future studies aiming to isolate the subjective experience of pain.

Figure 8)

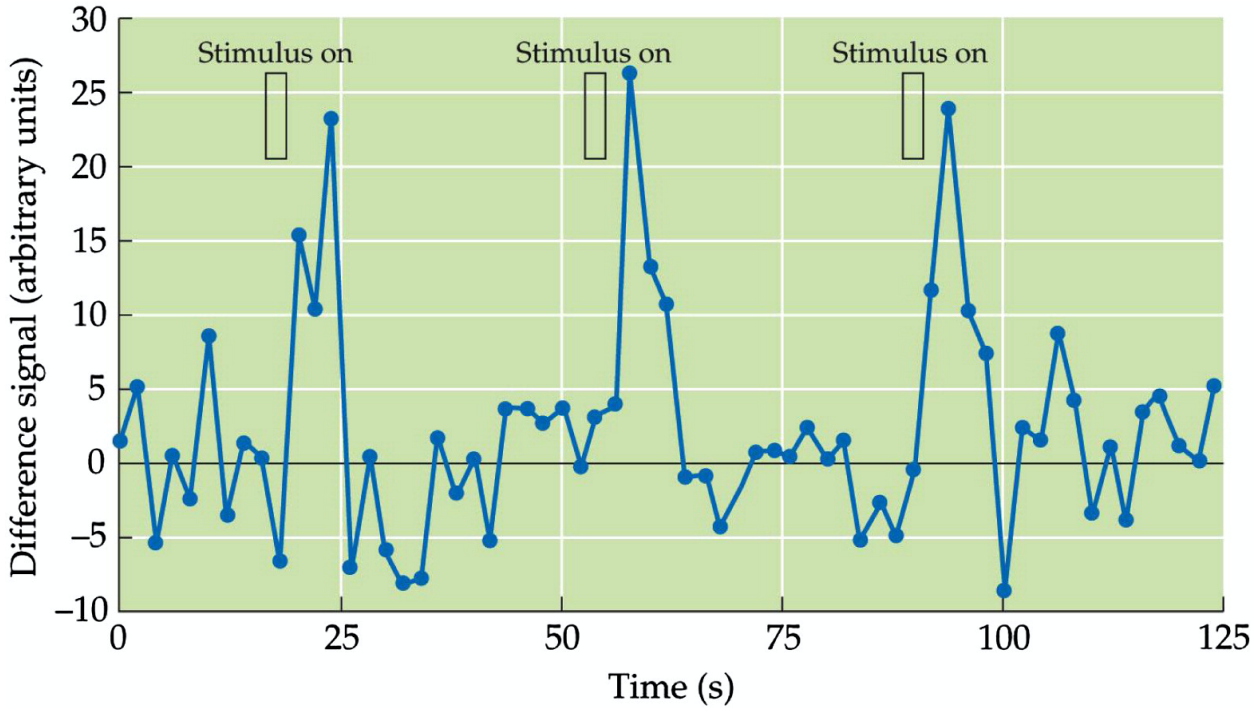


Figure adapted from Huettel et al 2009—using data from Blamire et al 1992. Figure above shows how short duration stimuli elicited a change in the BOLD hemodynamic response, typically referred to as fMRI signal, that was not representative of the boxcar model normally assumed.[27-28]

Traditionally, research studies have argued the experience of pain to be tridimensional: sensory-discriminative, affective-motivational, and cognitive-evaluative. The sensory-discriminative dimension is what characterizes the physical stimulus and other factors like location, compared to the affective-motivational where one assesses factors like emotion and the feeling of unpleasantness. Lastly, the cognitive-evaluative dimension refers to the gauging of the significance or meaning of pain (i.e, state or consequences as a result of injury)[30]. Studies have demonstrated the significance of emotional state (affective-motivational dimension) on the

overall perception of pain: a study on healthy dental patients found that negative emotions elicited stronger sensations of pain, whereas positive thoughts or emotions had the opposite effect[31-32]. Other studies in fibromyalgia patients have found that catastrophizing, or magnifying the fear of pain, and even low self-esteem have been correlated with augmenting the experience of pain[33-35]. Given the importance of each dimension, the pain research community continues to increase their efforts to separate these dimensions from each other—like isolating the subjective sensation of pain versus other factors like emotion, cognition and motivation that may also influence the overall experience,[16,36]. Our evidence suggests that traditional data analysis models where ON and OFF blocks are the designated cutoff points may be magnifying the difficulty of isolating these dimensions, given that analysis blocks include time where subjects are not perceiving stimuli as painful and leave out portions where subjects may still be perceiving pain. Our findings suggest current research methodology may be leaving out an average of 29% or more of the subjective experience of pain from ON blocks in the analysis of fMRI data when using thermal noxious stimuli, for example. Previous studies in the pain community have established the existence of strong temporal non-linearities in the relationship between the pattern of a stimulus and the reported ratings by participants[37], thus assessing how the experience of pain is translated into concrete time thresholds like time to perception and time for pain to vanish is essential for reducing the gap between theory and the phenomenon that actually occurs. Further pertaining to our results, it is worth noting that there is a significant difference in perception of pain as well as aftersensations in between both the cuff and the Medoc device. Although both may stimulate Aδ and C-fibers alike[2, 26], the cuff device through its rapid inflation mechanism also induces a period of innocuous pressure, which may have led subjects to begin rating the stimulus as soon as it was turned on. Innocuous pressure, as initially induced by the cuff, is also mediated primarily by the thickly myelinated Aβ fibers, which can conduct signals

as fast as 14 m/s[38]. Additionally, we believe the application of each stimulus may have played a difference as well; whereas the thermal device forced participants to solely rely on the experience of the thermal noxious stimuli to identify the perception of pain and vanishing of aftersensations, though non-intended, the cuff device provided an additional cue of sound where a noticeable "snap-like" sound inadvertently may have alerted participants whether the stimulus was being turned on or off. Although there is no certainty whether this was a determining factor, previous pain research studies analyzing demand characteristics have found that participants may augment their perceived painfulness of a stimulus when given cues or directions significant to their expected performance[39-40]. In this case, the sound of the cuff being turned on may have alerted participants of "having to" begin to report sensation given the stimulus was on. To avoid this effect in the first place, beyond procedure explanations during consent process, subjects were not explicitly notified when either stimulus would be turned on or off. Additionally, to further discourage participants from being cued or guessing, all runs started with the same stimulus intensity for the first 10 secs (Pain 40) and then diverged to their respective target sequence—whether increasing, decreasing, or remaining constant (figure 3). Though unmeasured, it is also worth noting that, post-fMRI session, participants often accounted for the stimuli being significantly more painful while undergoing the scan—though intensities were the same as the QST session. Despite being narrative-based, pain-catastrophizing has been well-documented in the literature—where the participants may perceive a stimulus as more painful based on the "dread" or expectation of knowing that a noxious stimulus is coming at a future time and feeling unable to address it[19,41-44]. Inside the scanner room, participants were blinded as to whether the stimulus was on or off, or when the next sequence would begin given the separation between scanner control room and participant undergoing scan. This cue could be an explanatory variable for the temporal course of pain in thermal versus mechanical pain being

significantly different under the same perceived intensity; participants may have had a more difficult time predicting the beginning and ending of thermal stimuli relying solely on perception while compared to the cuff.

One of the limitations of this study was the use of a numeric rating to collect initial rating and assess pain 40. Upon visual evidence, it became clear that subjects often struggled thoroughly grasping the concepts of no pain (0) versus the most painful sensation imaginable from the stimuli (100). This was evidenced by either extreme VAS movements either too high, or not all, upon perception of the stimuli. Given the aim at calibrating a moderately painful stimulus, these movements were not expected. Upon first observation of this limitation (first 2 subjects), we modified our protocol to present the stimuli to be used (pain 40) three times before asking for continuous ratings to verify that it indeed was moderately painful. Ultimately, this limitation speaks to the efficacy of using alternate scales such as a continuous VAS and attests how there is information NRSs may leave out. Studies analyzing the use of NRSs, in the clinical setting for example, suggest that these scales miss nearly a third of patients who experience pain considered to be clinically relevant[45]. As it pertains to the study of pain, given the impactful role that research plays in the advancement of the field, future studies should reassess whether these scales are the most efficient and appropriate for the desired measures.

Lastly, there is the possibility these results may be localized to the area being tested. Given the size of the leg area in the sensory distribution of the cerebral cortex, also known as sensory homunculus, there is the possibility these delays in perception may be unique to the stimulus location[46]. Future studies should address the temporal course of pain in other areas that may be more densely innervated. Nevertheless, the leg area is a location used for quantitative sensory testing in numerous pain-related studies and the relevance of the present study is not hindered. The leg was used specifically in this study given it permitted for access to switching between

sides per stimuli while the participant remained in the scanner, and it is likely a reason why it may continue to be used as a testing site in other fMRI studies involving pain.

Conclusion and Significance for Clinical Research

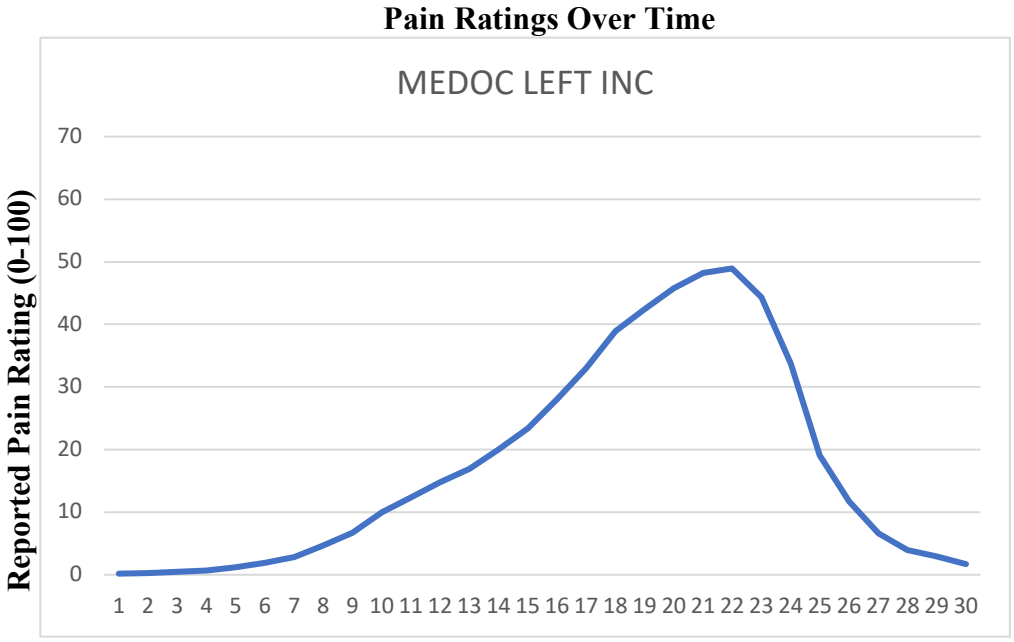
Upon completion of this preliminary study, the next steps will include a two-way analysis of the collected fMRI data comparing the standard predetermined blocks consistently used in standard research procedures versus the proposed new block. As it stands, this study may serve as a guideline for the improvement in accuracy for identifying biomarkers of acute and neuropathic pain where investigators account for this delay in the subjective experience of pain and continuous effects even after the stimuli is removed. The results in this study indicate that there is room for improvement in reducing the influence of other factors that may be associated with the experience of pain, like fear/anxiety, or even noise and opens to the possibility of increasing the collection of more accurate data as the field of pain research continuous to advance. Ultimately, this proposed method for data analysis may help identify other biomarkers of the subjective pain experience that may otherwise be left out by using the standardized blocks for data collection. In the future, these modified blocks considering the perceived pain experience may be useful for emerging approaches toward the study of pain that rely upon already available data—such as machine learning algorithms and neural nets.

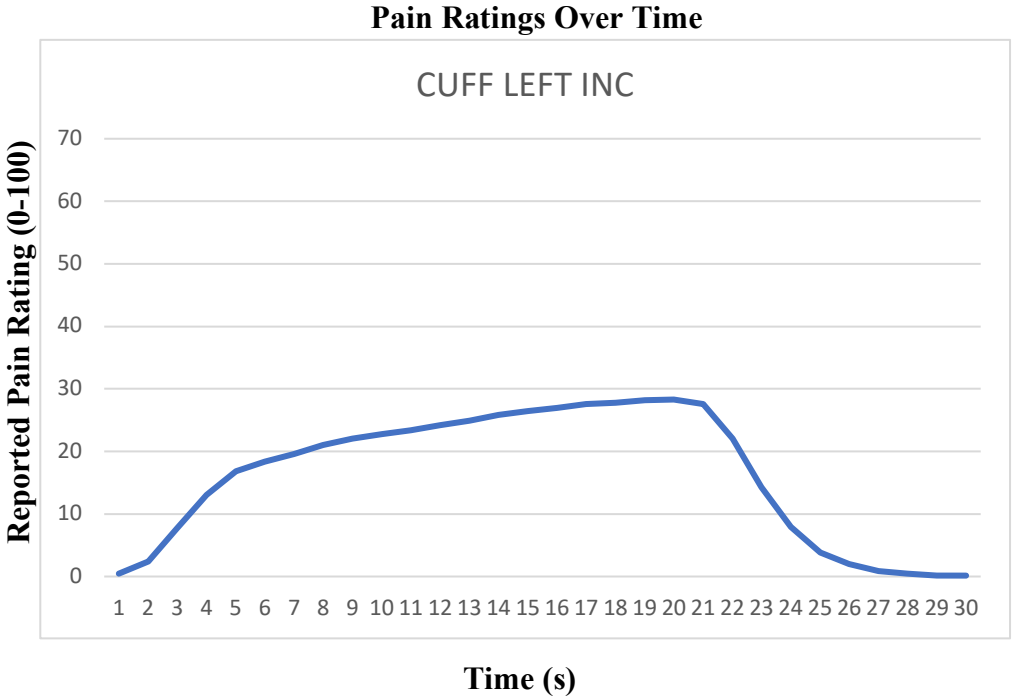
Supplementary Figures:

Figures below (A-F) are average runs from all participants for each stimulus, intensity, and side.

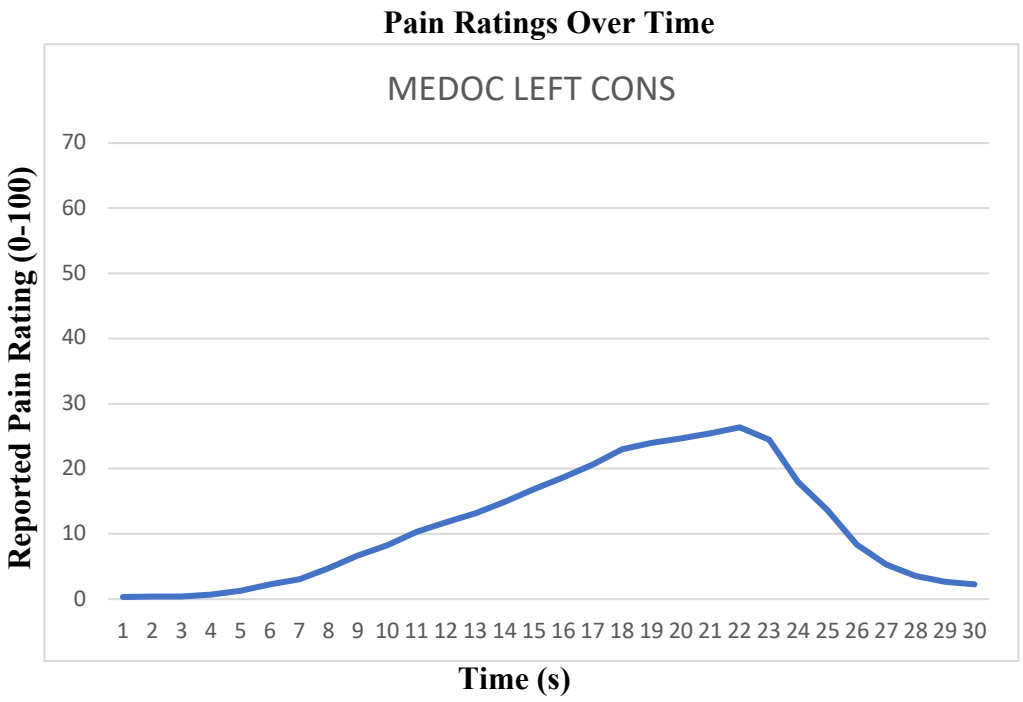
It is worth emphasizing that stimulus began being applied at 0 secs and was turned off at 20 secs.

A)





B)


Pain Ratings Over Time
MEDOC LEFT CONS
Reported Pain Rating (0-100)
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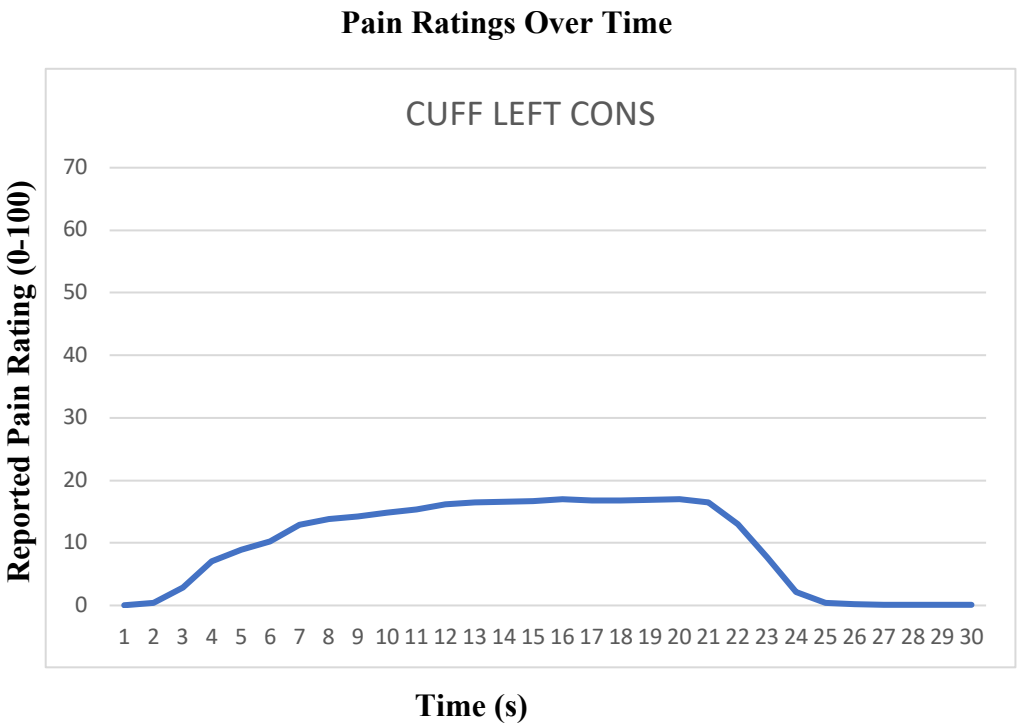
Time (s)


Pain Ratings Over Time
CUFF LEFT CONS
Reported Pain Rating (0-100)
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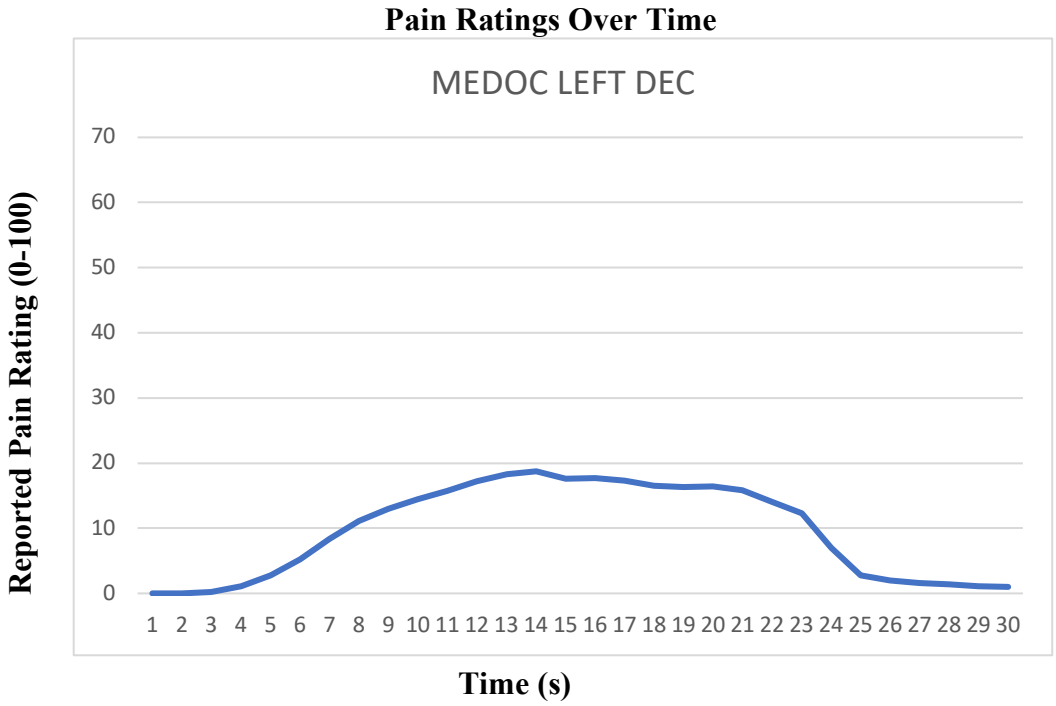
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (s)

C)


Pain Ratings Over Time
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Reported Pain Rating (0-100)
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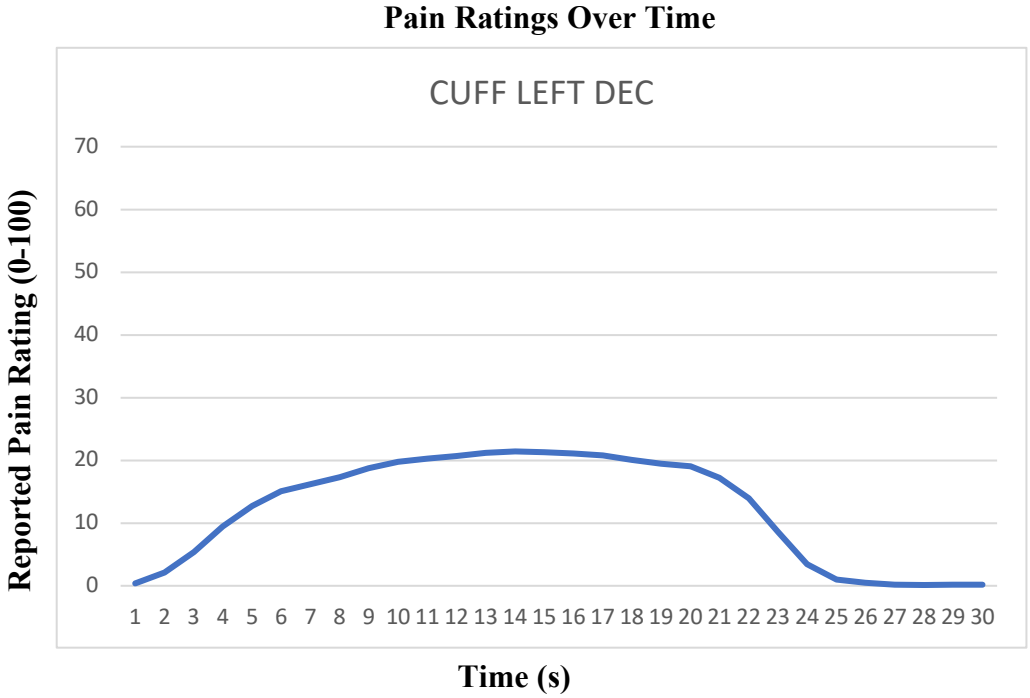
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (s)


Pain Ratings Over Time
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Reported Pain Rating (0-100)
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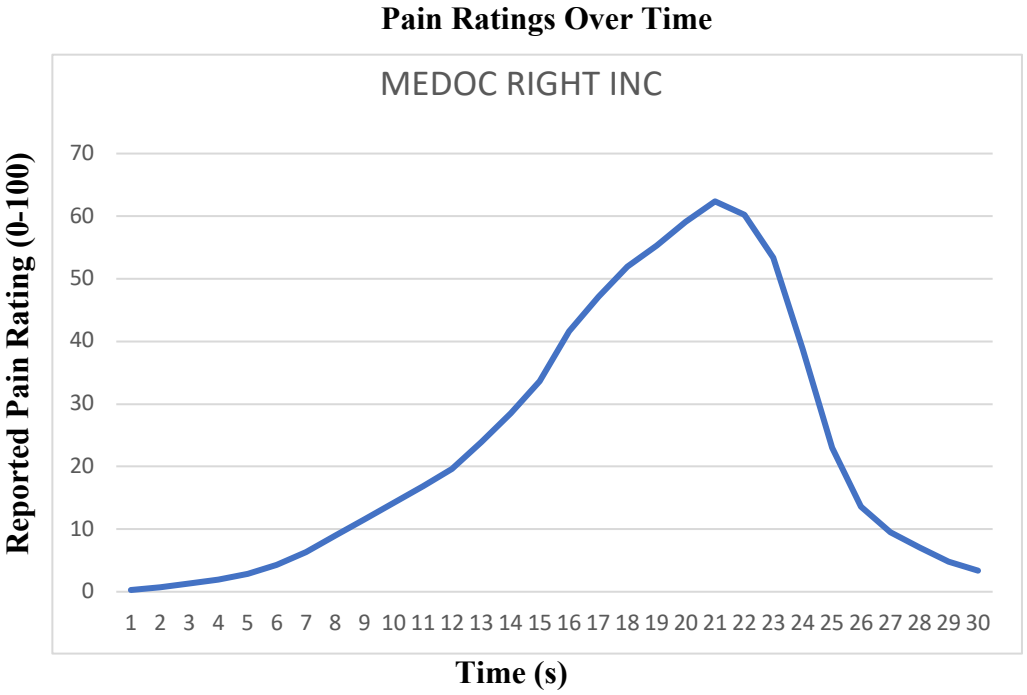
Time (s)

D)


Pain Ratings Over Time
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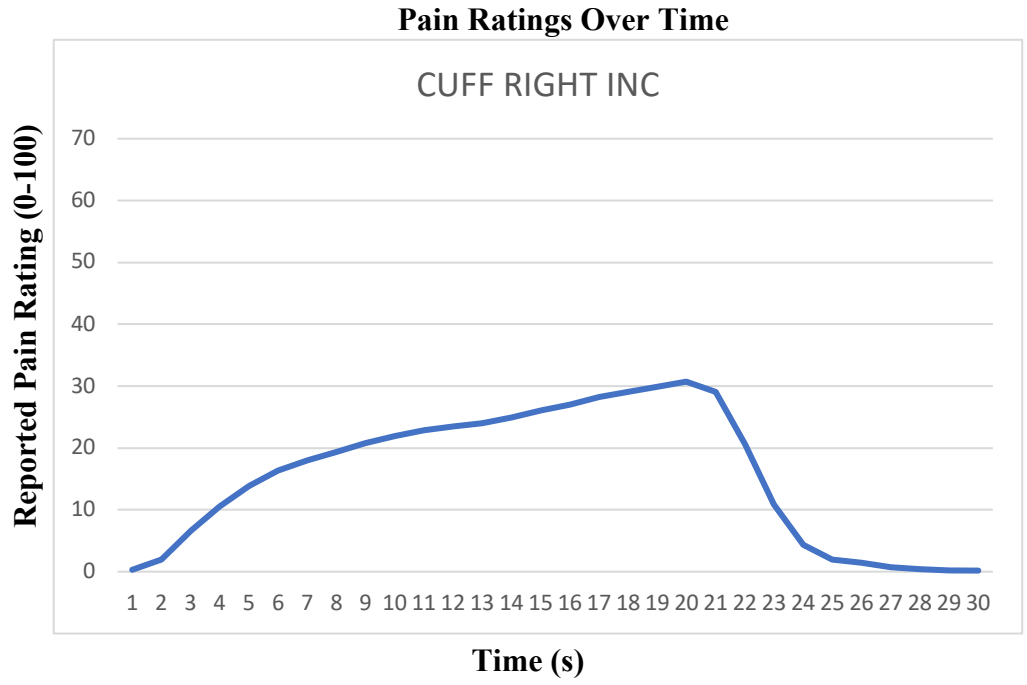
Time (s)


Pain Ratings Over Time
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Reported Pain Rating (0-100)
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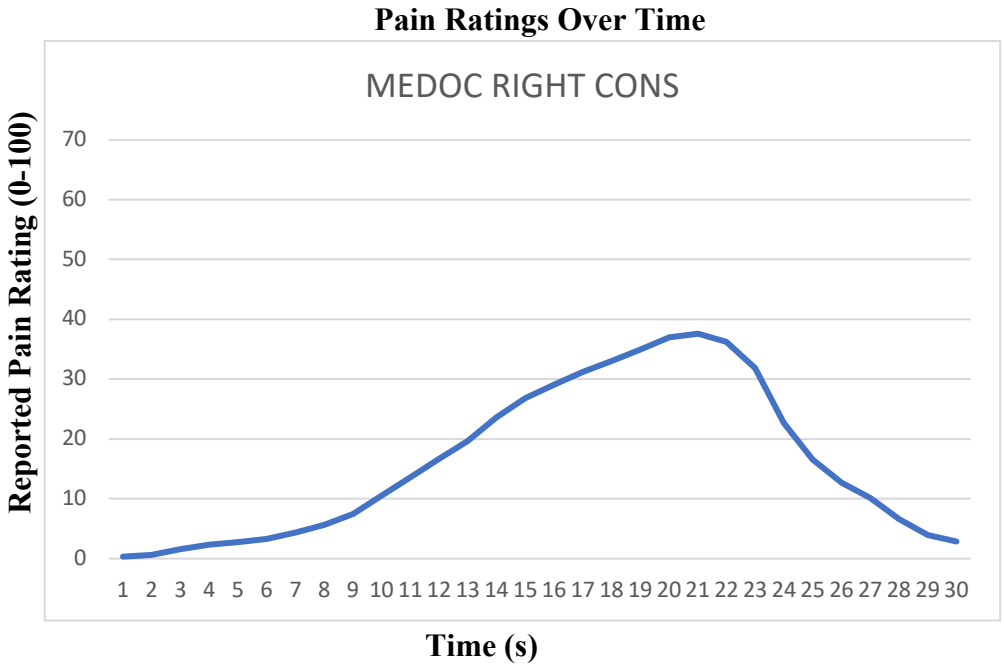
Time (s)

E)


Pain Ratings Over Time
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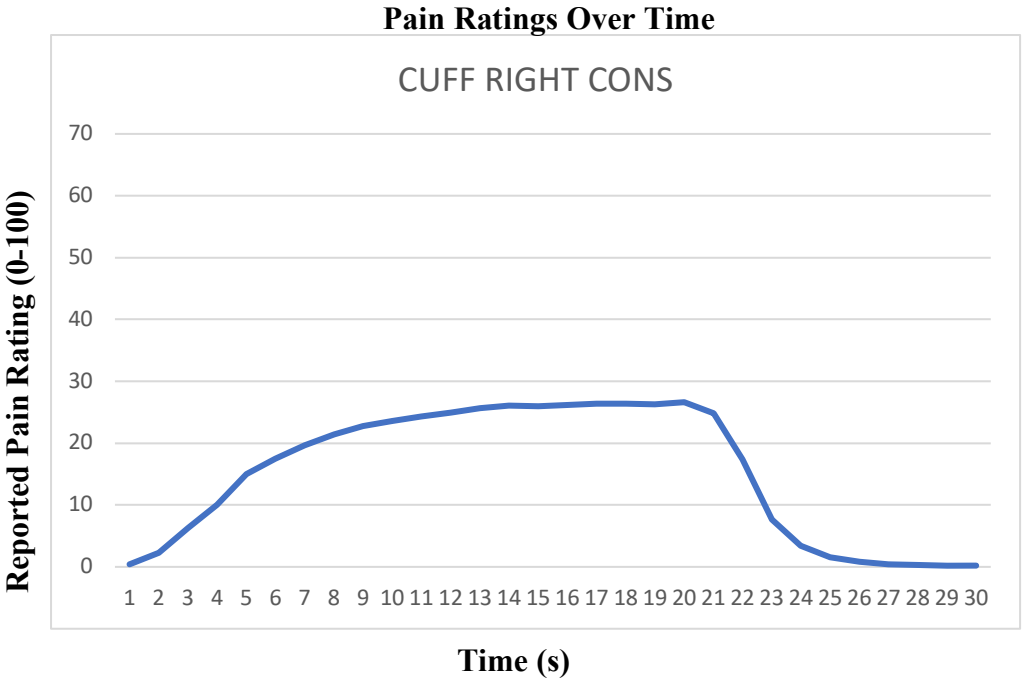
Time (s)


Pain Ratings Over Time
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Reported Pain Rating (0-100)
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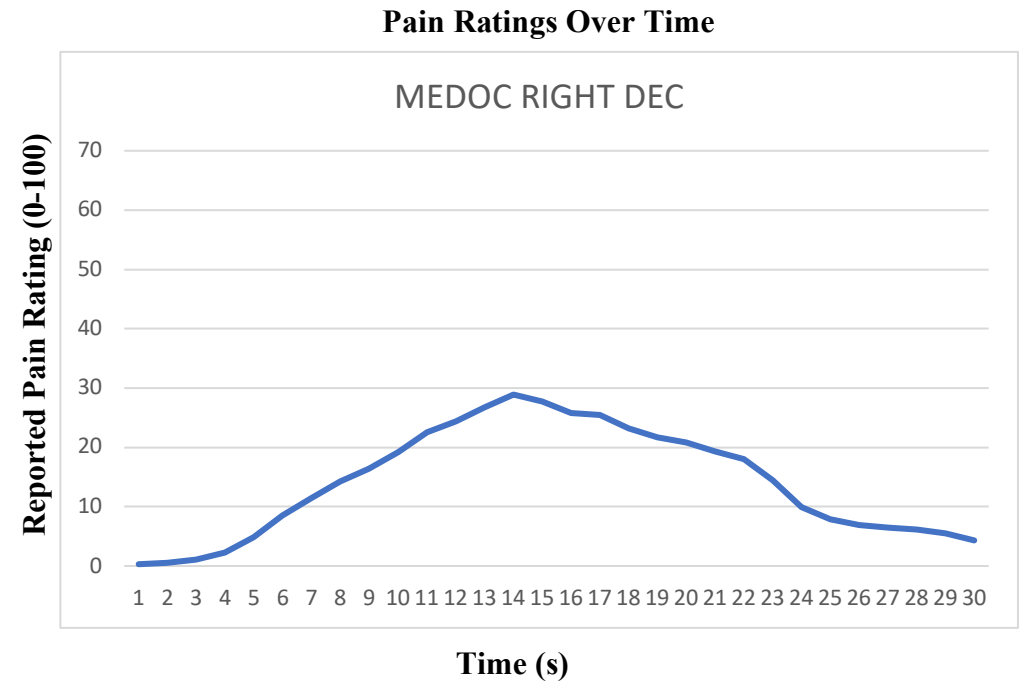
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (s)

F)


Pain Ratings Over Time
MEDOC RIGHT DEC
Reported Pain Rating (0-100)
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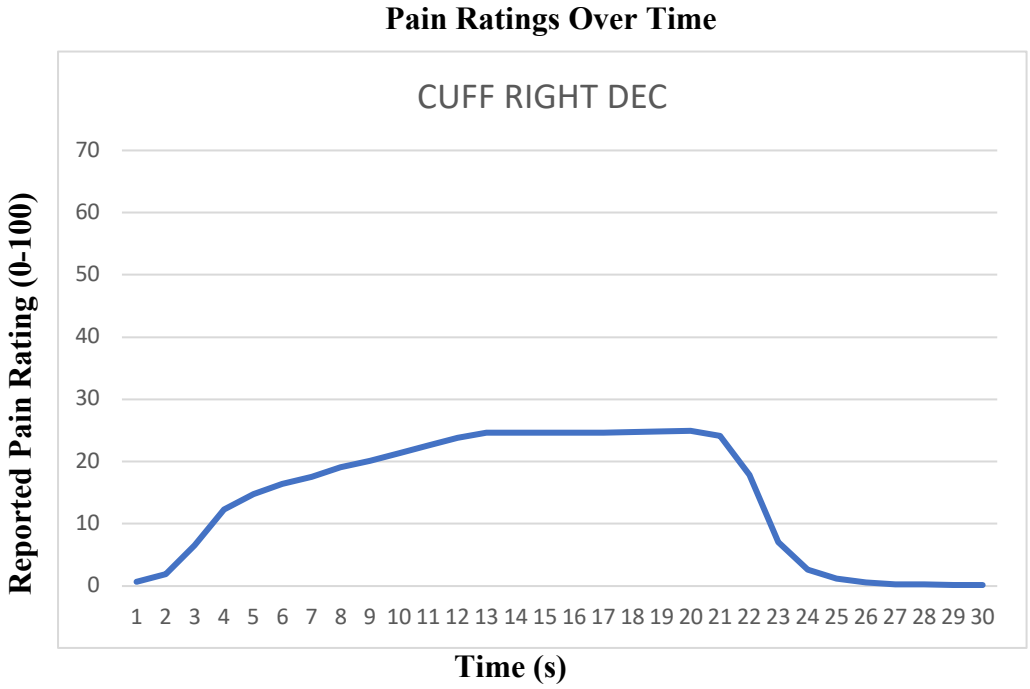
Time (s)


Pain Ratings Over Time
CUFF RIGHT DEC
Reported Pain Rating (0-100)
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (s)

References

1. Centers for Disease Control and Prevention. (2019, September 16). *Prevalence of chronic pain and high-impact chronic pain among adults - United States, 2016*. Centers for Disease Control and Prevention. Retrieved March 22, 2022, from https://www.cdc.gov/mmwr/volumes/67/wr/mm6736a2.html
2. Armstrong SA, Herr MJ. Physiology, Nociception. [Updated 2021 May 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551562/HHS Vulnerability Disclosurehttps://www.ncbi.nlm.nih.gov/books/NBK551562/
3. Wager TD, Atlas LY, Lindquist MA, Roy M, Woo CW, Kross E. An fMRI-based neurologic signature of physical pain. N Engl J Med. 2013;368:1388–97.
4. Porro CA, Cettolo V, Francescato MP, Baraldi P. Temporal and intensity coding of pain in human cortex. J Neurophysiol. 1998;80:3312–20.
5. Davis KD, Moayedi M. Central mechanisms of pain revealed through functional and structural MRI. J Neuroimmune Pharmacol. 2012;8:518–34.
6. Porro CA, Baraldi P, Pagnoni G, Serafini M, Facchin P, Maieron M, et al. Does anticipation of pain affect cortical nociceptive systems? J Neurosci. 2002;22:3206–14.
7. Tracey I. Imaging pain. Br J Anaesth. 2008;101:32–9.
8. Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000) Neurophysiol Clin. 2000;30:263–88.
9. Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci. 2002;22:2748–52.

10. Amaro, E., & Barker, G. J. (2006, January 19). *Study design in fmri: Basic principles*. Brain and Cognition. Retrieved March 22, 2022, from https://www.sciencedirect.com/science/article/abs/pii/S0278262605001752
11. U.S. Department of Health and Human Services. (n.d.). *Background: General Information FMRI Experimental Design* . National Institute of Mental Health. Retrieved March 22, 2022, from https://afni.nimh.nih.gov/pub/dist/HOWTO/howto/ht03_stim/html/stim_background.html
12. Konrad A, Vucurevic G, Musso F, Stoeter P, Winterer G. Correlation of brain white matter diffusion anisotropy and mean diffusivity with reaction time in an oddball task. Neuropsychobiology. 2009;60(2):55-66. doi: 10.1159/000236445. Epub 2009 Sep 10. PMID: 19752579.
13. Aarts E, Roelofs A, van Turennout M. Attentional control of task and response in lateral and medial frontal cortex: brain activity and reaction time distributions. Neuropsychologia. 2009 Aug;47(10):2089-99. doi: 10.1016/j.neuropsychologia.2009.03.019. Epub 2009 Apr 5. PMID: 19467359.
14. Gottrup H, Kristensen AD, Bach FW, Jensen TS. Aftersensations in experimental and clinical hypersensitivity. Pain. 2003 May;103(1-2):57-64. doi: 10.1016/s0304-3959(02)00415-3. PMID: 12749959.
15. Schreiber KL;Loggia ML;Kim J;Cahalan CM;Napadow V;Edwards RR; (n.d.). *Painful after-sensations in fibromyalgia are linked to catastrophizing and differences in brain response in the medial temporal lobe*. The journal of pain. Retrieved March 22, 2022, from https://pubmed.ncbi.nlm.nih.gov/28300650/
16. Wilcox, C. E., Mayer, A. R., Teshiba, T. M., Ling, J., Smith, B. W., Wilcox, G. L., & Mullins, P. G. (2015, November). *The subjective experience of pain: An FMRI study of*

percept-related models and functional connectivity. Pain medicine (Malden, Mass.). Retrieved March 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4653099/#R26

17. Symonds LL, Gordon NS, Bixby JC, Mande MM. Right-lateralized pain processing in the human cortex: an FMRI study. J Neurophysiol. 2006 Jun;95(6):3823-30. doi: 10.1152/jn.01162.2005. Epub 2006 Mar 22. PMID: 16554508. https://journals.physiology.org/doi/full/10.1152/jn.01162.2005

18. Brügger M, Ettlin DA, Meier M, Keller T, Luechinger R, Barlow A, Palla S, Jäncke L, Lutz K. Taking Sides with Pain - Lateralization aspects Related to Cerebral Processing of Dental Pain. Front Hum Neurosci. 2011 Feb 7;5:12. doi: 10.3389/fnhum.2011.00012. PMID: 21344018; PMCID: PMC3036976. https://www.frontiersin.org/articles/10.3389/fnhum.2011.00012/full

19. Melzack R. The short-form McGill Pain Questionnaire. Pain. 1987 Aug;30(2):191-197. doi: 10.1016/0304-3959(87)91074-8. PMID: 3670870.

20. Veale JF. Edinburgh Handedness Inventory - Short Form: a revised version based on confirmatory factor analysis. Laterality. 2014;19(2):164-77. doi: 10.1080/1357650X.2013.783045. Epub 2013 May 10. PMID: 23659650.

21. Cardoso JS, Riley JL 3rd, Glover T, Sibille KT, Bartley EJ, Goodin BR, Bulls HW, Herbert M, Addison AS, Staud R, Redden DT, Bradley LA, Fillingim RB, Cruz-Almeida Y. Experimental pain phenotyping in community-dwelling individuals with knee osteoarthritis. Pain. 2016 Sep;157(9):2104-2114. doi: 10.1097/j.pain.0000000000000625. PMID: 27340911; PMCID: PMC4988907. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4988907/

22. Harper DE, Ichesco E, Schrepf A, Hampson JP, Clauw DJ, Schmidt-Wilcke T, Harris RE, Harte SE. Resting Functional Connectivity of the Periaqueductal Gray Is Associated With Normal Inhibition and Pathological Facilitation in Conditioned Pain Modulation. J Pain. 2018 Jun;19(6):635.e1-635.e15. doi: 10.1016/j.jpain.2018.01.001. Epub 2018 Jan 31. PMID: 29360608; PMCID: PMC5972067. https://www.sciencedirect.com/science/article/pii/S1526590018300245

23. Riley JL 3rd, Cruz-Almeida Y, Staud R, Fillingim RB. Age does not affect sex effect of conditioned pain modulation of pressure and thermal pain across 2 conditioning stimuli. Pain Rep. 2019 Dec 24;5(1):e796. doi: 10.1097/PR9.0000000000000796. PMID: 32072094; PMCID: PMC7004505. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7004505/

24. Abrecht CR, Cornelius M, Wu A, Jamison RN, Janfaza D, Urman RD, Campbell C, Smith M, Haythornthwaite J, Edwards RR, Schreiber KL. Prediction of Pain and Opioid Utilization in the Perioperative Period in Patients Undergoing Primary Knee Arthroplasty: Psychophysical and Psychosocial Factors. Pain Med. 2019 Jan 1;20(1):161-

171. doi: 10.1093/pm/pny020. PMID: 29522115; PMCID: PMC6329440. https://academic.oup.com/painmedicine/article/20/1/161/4924619

25. Geuze E, Westenberg HGM, Jochims A, et al. Altered Pain Processing in Veterans With Posttraumatic Stress Disorder. Arch Gen Psychiatry. 2007;64(1):76–85. doi:10.1001/archpsyc.64.1.76 https://jamanetwork.com/journals/jamapsychiatry/fullarticle/209980

26. BISHOP GH, LANDAU WM, JONES MH. Evidence for a double peripheral pathway for pain. Science. 1958 Sep 26;128(3326):712-4. doi: 10.1126/science.128.3326.712. PMID: 13580241. https://pubmed.ncbi.nlm.nih.gov/13580241/

27. Blamire AM, Ogawa S, Ugurbil K, Rothman D, McCarthy G, Ellermann JM, Hyder F, Rattner Z, Shulman RG. Dynamic mapping of the human visual cortex by high-speed magnetic resonance imaging. Proc Natl Acad Sci U S A. 1992 Nov 15;89(22):11069-73. doi: 10.1073/pnas.89.22.11069. PMID: 1438317; PMCID: PMC50485.

28. Huettel SA. Event-related fMRI in cognition. Neuroimage. 2012 Aug 15;62(2):1152-6. doi: 10.1016/j.neuroimage.2011.08.113. Epub 2011 Sep 22. PMID: 21963919; PMCID: PMC3277683.

29. Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD. Abnormal sensitization and temporal summation of second pain (wind

30. Melzack R, Casey KL. Sensory, motivational and central control determinants of pain. In: Kenshalo DR, editor. The Skin Senses. Springfield, Ill: Thomas; 1968. pp. 423–443.

31. Lumley MA, Cohen JL, Borszcz GS, Cano A, Radcliffe AM, Porter LS, Schubiner H, Keefe FJ. Pain and emotion: a biopsychosocial review of recent research. J Clin Psychol. 2011 Sep;67(9):942-68. doi: 10.1002/jclp.20816. Epub 2011 Jun 6. PMID: 21647882; PMCID: PMC3152687.

32. Loggia ML, Schweinhardt P, Villemure C, Bushnell MC. Effects of psychological state on pain perception in the dental environment. J Can Dent Assoc. 2008 Sep;74(7):651-6. PMID: 18789200.

33. Ellingson LD, Stegner AJ, Schwabacher IJ, Lindheimer JB, Cook DB. Catastrophizing Interferes with Cognitive Modulation of Pain in Women with Fibromyalgia. Pain Med. 2018 Dec 1;19(12):2408-2422. doi: 10.1093/pm/pny008. PMID: 29474665; PMCID: PMC6659027.

34. Galvez-Sánchez CM, Reyes Del Paso GA, Duschek S. Cognitive Impairments in Fibromyalgia Syndrome: Associations With Positive and Negative Affect, Alexithymia, Pain Catastrophizing and Self-Esteem. Front Psychol. 2018 Mar 22;9:377. doi: 10.3389/fpsyg.2018.00377. PMID: 29623059; PMCID: PMC5874325.

35. Severeijns R, Van den Hout MA, Vlaeyen JWS, Picavet SJ. Pain catastrophizing and general health status in a large Dutch community sample. Pain 99, 367–376. (2002). doi: 10.1037/0278-6133.23.1.49

36. Henderson LA, Di Pietro F, Youssef AM, Lee S, Tam S, Akhter R, Mills EP, Murray GM, Peck CC, Macey PM. Effect of Expectation on Pain Processing: A Psychophysics and Functional MRI Analysis. Front Neurosci. 2020 Jan 31;14:6. doi: 10.3389/fnins.2020.00006. PMID: 32082106; PMCID: PMC7004959. https://www.frontiersin.org/articles/10.3389/fnins.2020.00006/full

37. Yelle MD, Rogers JM, Coghill RC (2008) Offset analgesia: a temporal contrast mechanism for nociceptive information. Pain 134: 174–186.

38. Staud R, Koo E, Robinson ME, Price DD. Spatial summation of mechanically evoked muscle pain and painful aftersensations in normal subjects and fibromyalgia patients. Pain. 2007 Jul;130(1-2):177-87. doi: 10.1016/j.pain.2007.03.015. Epub 2007 Apr 24. PMID: 17459587; PMCID: PMC2041939. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2041939/

39. Fernandez E, Turk DC. Demand characteristics underlying differential ratings of sensory versus affective components of pain. J Behav Med. 1994 Aug;17(4):375-90. doi: 10.1007/BF01858009. PMID: 7966259. https://link.springer.com/content/pdf/10.1007/BF01858009.pdf

40. McCambridge J, de Bruin M, Witton J. The effects of demand characteristics on research participant behaviours in non-laboratory settings: a systematic review. PLoS One. 2012;7(6):e39116. doi: 10.1371/journal.pone.0039116. Epub 2012 Jun 19. PMID: 22723942; PMCID: PMC3378517. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3378517/

41. Leung L. Pain catastrophizing: an updated review. Indian J Psychol Med. 2012 Jul;34(3):204-17. doi: 10.4103/0253-7176.106012. PMID: 23441031; PMCID: PMC3573569. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3573569/

42. Story GW, Vlaev I, Seymour B, Winston JS, Darzi A, Dolan RJ. Dread and the disvalue of future pain. PLoS Comput Biol. 2013;9(11):e1003335. doi: 10.1371/journal.pcbi.1003335. Epub 2013 Nov 21. PMID: 24277999; PMCID: PMC3836706. https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1003335#:~:text=People%20often%20prefer%20to%20'get,disadvantageous%2C%20rather%20like%20pain%20itself.

43. Huang Y, Shang Q, Dai S, Ma Q. Dread of uncertain pain: An event-related potential study. PLoS One. 2017 Aug 23;12(8):e0182489. doi: 10.1371/journal.pone.0182489. PMID: 28832607; PMCID: PMC5568389. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5568389/

44. Quartana PJ, Campbell CM, Edwards RR. Pain catastrophizing: a critical review. Expert Rev Neurother. 2009 May;9(5):745-58. doi: 10.1586/ern.09.34. PMID: 19402782; PMCID: PMC2696024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2696024/

45. Krebs EE, Carey TS, Weinberger M. Accuracy of the pain numeric rating scale as a screening test in primary care. J Gen Intern Med. 2007 Oct;22(10):1453-8. doi: 10.1007/s11606-007-0321-2. Epub 2007 Aug 1. PMID: 17668269; PMCID: PMC2305860.

46. Nguyen JD, Duong H. Neurosurgery, Sensory Homunculus. [Updated 2021 Jul 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549841/