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Understanding the neural mechanisms of aversive processing using fNIRS

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Thesis

UNDERSTANDING THE NEURAL MECHANISMS OF AVERSIVE PROCESSING USING FNIRS

by

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I would like to dedicate this work to my mom and dad. Additionally, I want to thank Dr. Keerthana Deepti Karunakaran for allowing me to work on her research. Lastly, thank you to my friends who helped me through this program.

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ABSTRACT

Functional near-infrared spectroscopy (fNIRS) provides a non-invasive approach to investigate autonomic responses to various types of stimuli. The aim of this thesis is to use fNIRS to evaluate the hemodynamic and physiological changes in response to aversive and positive visual stimuli. The study suggests that aversive vs. positive stimuli share a neural network composed of frontopolar cortex, prefrontal cortex (PFC), primary and secondary somatosensory areas, and visual cortex. Physiological measure markers such as heart rate variability (HRV), respiration, partial pressure of oxygen are expected to yield differential outcomes in provide differential results in response to negative and positive visual stimuli.

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LIST OF ABBREVIATIONS

ACC ..... Anterior cingulate cortex

Cpz ..... Posterior to central sulcus corresponding to somatosensory cortex

DMPFC ..... Medial dorsal prefrontal cortex

EEG ..... Evoked electroencephalography

ERSP ..... Event-related spectral perturbation

fMRI ..... Functional magnetic resonance imaging

HbO ..... Oxy hemoglobin/oxygenated (concentration)

HbR ..... Deoxygenated hemoglobin (concentration)

HDR ..... Hemodynamic response

IAPS ..... International Affective Picture System

LED ..... Light-emitting diode

MBLL ..... Modified Beer-Lambert Law

NIRS ..... Near-infrared spectroscopy

NIRS ..... Near-infrared spectroscopy

Oz ..... Primary visual cortex

Fpz ..... Frontopolar cortex

PFC ..... Prefrontal cortex

PPG ..... Photoplethysmography

S1 ..... Somatosensory cortices

INTRODUCTION

An aversive stimulus is a noxious or an unpleasant event that elicits withdrawal, avoidance or escape behavior. These stimuli may be auditory, visual, tactile such as pain, or olfactory. An individual's ability to detect and respond to potentially aversive events in the environment is thought to be an evolutionary mechanism of self-preservation during potential danger. Much of our understanding of human aversive processing comes from neuroimaging techniques (Hayes & Northoff, 2011). There are multiple methods to measure brain structure and function, namely, magnetic resonance imaging (MRI), electroencephalography (EEG), positron emission tomography (PET), etc. These have provided for advancements in neurosciences, driven by measurements of brain structure and morphology, brain activity in response to various stimuli (Xu et al., 2021; Yücel et al., 2019), at rest (known as the resting-state brain) (Biswal et al., 1995), and following pharmacological agents.

Commonly, aversive emotional processing is known to recruit subcortical areas such as the amygdala, thalamus, striatum and periaqueductal grey areas (Carlezon & Thomas, 2009; Michely et al., 2020). However, more recent studies on humans suggests a core network involving prefrontal cortex, anterior cingulate cortex, and insula, is likely common to various aversive modalities viz. tactile pain, visual, and so on (Hayes & Northoff, 2011). Although, some studies also suggest the involvement of secondary motor area, orbitofrontal cortex, and temporal gyrus regions (Hayes & Northoff, 2012).

The complexity of measuring *state aversion* — characterized by an acute emotional response to a stimulus — may explain the discrepancies between studies. Aversive processing is a subjective response that is based on an individual's emotional reactivity, perception, experiences, and emotional expression. Electrophysiological, and neuroimaging tools are one-way to obtain objective surrogates of *state aversion*, since these techniques can recognize the brain state with greater specificity and sensitivity (Xu et al., 2021).

The advantages of techniques such as functional MRI or EEG is also that these techniques have enabled investigation of aversive reactions, particularly to noxious stimuli, in developmental neonates, children, adolescents and adults (Crawford et al., 2023; Li et al., 2016). Though interpretation of pediatric data is more challenging than adults, the ability to measure alterations in aversive and reward processing can help recognize developmental abnormalities, and/or dysregulation in mood processing (Sava et al., 2009; van der Vaart et al., 2022). Studies investigating attention deficit hyperactivity disorder (Van Dessel et al., 2018), autism spectrum disorders (Corona et al., 1998; S. A. Green et al., 2015), and anxiety (Grillon et al., 2009; Robinson et al., 2014), have reported dysregulation in various aspects of aversive processing.

Not to mention, aversive stimuli also result in autonomic arousal, seen in the form of physiological changes in skin conductance, heart rate, and breathing (Kyle & McNeil, 2014; Mériau et al., 2009), that may further influence neurovascular activity. For instance,

noxious tactile stimuli induction is associated with a sympathetic mediated cardiac response, increased heart rate, muscle tension, pupil dilation amongst others (Kyle & McNeil, 2014). The challenge, however, is that conventional imaging techniques such as MRI and PET are often impractical, expensive, and only accessible in urban areas. They are also not conducive for routine concurrent physiological monitoring. EEG, despite having high temporal resolution and being portable, and cost-effective suffers from poor spatial resolution and are easily altered due to physical movement leading to global artifacts that are difficult to decipher (van der Vaart et al., 2022, Xu et al., 2021). Thus, there is a need for a reliable alternative to fMRI that can (i) provide spatial specificity, and (ii) be easily deployed in naturalistic settings to study the brain (iii) in parallel with autonomic arousal during aversive states. The ability to objectively quantify brain state related to *state aversion* could be useful both as a neuroinvestigative tool as well as potentially guide in the diagnosis, prognosis and treatment of disorders involving affective-sensory dysregulation.

Functional near-infrared spectroscopy (fNIRS) is a non-invasive brain imaging technique based on the optical properties of hemoglobin molecules in the blood. Unlike other neuroimaging methods in place, such a fMRI, or even PET, there are distinct advantages of fNIRS. The method of fNIRS maps the neuronal activity changes depicting cerebral blood flow and oxygenation levels. These responses are specifically known as hemodynamic responses, which indirectly measure brain activity. The technique of using near-infrared light through the scalp and skull, can measure the intensity of the light

absorbed by brain tissue. This absorption will infer with changes in blood oxygenation levels, distinguishing neuronal activity in specific underlying brain regions (Gervain et al., 2023).

FNIRS allows for high temporal resolution, allowing for real-time monitoring of brain activity. The precision of this technique and feedback can be beneficial is measuring cognitive process, such as comprehension, motor control, along with emotional processes as well. Even though spatial resolution may not be as high as fMRI, it is sufficient for many experimental and clinical paradigms (Karunakaran et al., 2021). Unlike fMRI, fNIRS technology is portable, and can be used in a wide range of environments. The technology aids to its versatility, along with the inexpensive financial cost (Yen et al., 2023). The non-invasive approach is suitable for difficult populations to obtain data from, such as children and patients with mobility issue. Unlike fNIRS however, fNIRS is limited to superficial brain tissue for monitoring hemodynamic changes. For this reason, artifact secondary to participant movement must be accounted for. Even with these limitations, fNIRS remains a valuable tool for studying brain function (Yücel et al., 2015).

FNIRS is a rapidly expanding utility in all domains of neuroscience and clinical research. There is an extensive record of its use in all age groups particularly infants, and pediatric population not limiting to neurological conditions such as ADHD, Autism, Depression, and mood disorders. Its widespread acceptability among researchers is likely due to its (a) practical nature, (b) low susceptibility to head motion, (c) moderate spatial-resolution, and

(d) easy use and interpretation. Owing to these reasons, fNIRS could be a suitable modality for understanding *state aversion* in both healthy and clinical cohorts (Gallagher et al., 2023; Gervain et al., 2023; Gu et al., 2017; Ho et al., 2020; Husain et al., 2021; Lloyd-Fox et al., 2014; Zhang & Roeyers, 2019).

Current literature on aversive stimuli using fNIRS is relatively limited. An olfaction study reports deactivation of medial prefrontal cortex during exposure to unpleasant odor in infants (Bartocci et al., 2001),whereas another fNIRS study evaluating bitter taste reports (Gallagher et al., 2023; Gervain et al., 2023; Gu et al., 2017; Ho et al., 2020; Husain et al., 2021; Lloyd-Fox et al., 2014; Zhang & Roeyers, 2019)activation of the ventral portion of the anterior PFC (Minematsu et al., 2018).This is inconsistent with another study reporting deactivation of PFC during unpleasant food taste when compared to pleasant food taste (Hu et al., 2014). In contrast, fNIRS studies during aversive tactile stimuli such as experimental pain are well reported. These studies have mapped areas of pain related processing during experimental, clinical as well as chronic pain (Aasted et al., 2016; De Oliveira Franco et al., 2022; Gracz, 1976; Komatsu et al., 2007; Kussman et al., 2016; Peng, Deepti Karunakaran, et al., 2021; Peng, Karunakaran, et al., 2021; Zeng et al., 2023), reporting consistent activation in both the PFC and the regions of the somatosensory cortex during various types of pain stimulation (Yücel et al., 2019, Crawford et al., 2023; Karunakaran et al., 2022). The primary somatosensory cortex (S1) region of the brain can sense pain, locate pain, and encode the quality of pain, whereas the regions of the PFC together with anterior cingulate cortex (ACC) is involved in the cognitive and emotional

appraisal of the pain stimuli, its conscious perception and modulation via descending pain inhibitory pathways (Li et al., 2016).

Sensitivity arises in perception of pain intensity as well, as there are variations depending on acute or repeated stimuli (Crawford et al., 2023). Sub portions of PFC are associated with memory and attentiveness, along with physiological changes noted in these regions due to various stimuli (Li et al., 2016). In response to painful stimuli specifically, increased activity was shown in certain regions of PFC (dorsolateral PFC) (Crawford et al., 2023) while deactivation is reported in others, such as frontopolar cortex (Blin et al., 1983). These findings underscore the potential of fNIRS in providing objective surrogates of pain for diagnosis and treatment as (i) it is consistent with fMRI studies describing the pain network (Wager et al., 2013), (ii) it is attenuated following opioids (Blin et al., 1983), and (iii) have been reproduced across multiple studies, age groups, experimental conditions (Gracz, 1976; Karunakaran et al., 2023; Mannerfelt, 1968; Peng, Deepti Karunakaran, et al., 2021).

Even with rising studies and research on this topic, pain continues to be a subjective phenomenon with no gold standard for objective assessments. Therefore, targeted pain treatment is still in its infancy. Addressing the gaps in neuroimaging literature of portable brain-based assessment of pain would first require a thorough investigation of the specificity of these brain measures during pain vs. non-painful stimuli, which includes understanding the common and distinct neural substrates of aversive noxious (pain) vs.

aversive non-sensory stimuli (visual, olfactory or another modality that may induce unpleasant emotions and avoidance behavior).

Existing studies on aversive processing are primarily performed using fMRI during viewing of unpleasant images with various levels of hedonic valence, emotional arousal, and dominance scores. fMRI studies indicate that exposure to aversive stimuli, unpleasant vs. pleasant, results with different activation patterns across various brain regions. Firstly, it has shown an elevated deactivation of PFC during aversive stimuli. This could imply diminished cognitive processing, or regulation of emotions (Aldhafeeri et al., 2012). On contrary, there is an increased activation in response to positive visual stimuli, signifying increased cognitive processing, or positive emotional appraisal (Hayes & Northoff, 2012; Wilson et al., 2020). In addition to the PFC, the somatosensory cortex resulted in elevated activation during aversive stimuli, indicating increased somatosensory processing associated with perception of pain (Becerra et al., 2009; Wilson et al., 2020). In conjunction to this findings, neuronal processing is prioritized, or enhanced, during aversive stimuli rather than positive stimuli. Other regions, such as supplementary motor area and visual cortex, demonstrated elevated and reduced activation in response to emotionally unpleasant vs. pleasant stimuli, respectfully. Visual cortex perhaps indicated increased or decreased attentive engagement (Čeko et al., 2022; Wilson et al., 2020). In conclusion, fMRI results help explain neural mechanisms distinguishing differential responses of the PFC, somatosensory cortex, supplementary motor visual area, and visual cortex to both

aversive versus pleasant visual stimuli (Aldhafeeri et al., 2012; Čeko et al., 2022; Wilson et al., 2020).

Collectively, these studies suggest both common and unique brain networks in the subjective experience of aversive unpleasant visual stimuli in relation to other modalities such as tactile or olfaction. However, there has not been any formal investigation of these models using fNIRS, that are required to produce fNIRS-based predictive markers of pain, emotion, or sensory dysfunction. Thus, in this study we propose to use multimodal fNIRS to investigate the neurovascular and physiological substrates of aversive stimuli, particularly visual stimuli, in comparison with non-aversive visual stimuli. Though fMRI studies also report the involvement of subcortical areas, the fNIRS technique is limited to the superficial layers of the cortex (<1cm). Therefore, the objectives of this thesis is to (i) establish the feasibility of fNIRS augmented with systemic physiology measures for the study of pleasant and unpleasant visual processing, (ii) determine whether fNIRS-based cortical measures of visual aversion are consistent with fMRI-based cortical measures of visual aversion, and (iii) describe the differences in cortical activity during aversive stimuli vs. cortical activity during aversive pain stimuli reported in the literature.

SPECIFIC AIMS

The objectives of this thesis are to,

Aim 1: Quantify the neurovascular response to aversive visual vs. non-aversive visual stimulation in a pilot sample of healthy adults using multimodal fNIRS.

Aim 2: Investigate the autonomic changes during the viewing of aversive visual vs. non-aversive visual stimulation using multimodal fNIRS.

Our working hypotheses have many elements. (a) We hypothesize that there exists a common neural network involving the regions of the frontopolar cortex, dorsal and ventral prefrontal cortex, the somatosensory cortex and secondary somatosensory areas, among other regions, that form the basis for processing aversive stimuli of tactile, visual, auditory or other origin when compared to non-aversive/neutral stimuli. (b) Specific spatial and temporal characteristics of these brain regions may encode the stimulus-specific aversive response. (c) Dissociation between medial frontopolar cortex and S1 regions is anticipated to correspond with negative affect. (d) Physiological indicators such as heart rate variability, respiration, and partial pressure of oxygen increase in response to negative stimuli.

Primary Outcome Measures: (a) Relative changes in oxy-hemoglobin concentration following task (hemodynamic response) between painful aversive vs. non-painful aversive

stimuli. (b) Heart rate variability measured using R-R tachogram, respiration rate and heart rate between painful aversive vs. non-painful aversive stimuli.

Secondary Outcome Measures: Patterns of fNIRS co-activation between PFC and S1.

METHODS

PARTICIPANTS

Healthy volunteers were recruited by postings via bulletin boards in the hospital testing campus, email, and word of mouth. Inclusion criteria included individuals between 18-70 years of age, males and females, and right-handed. Individuals were excluded if they had significant medical disorders, such as diabetes, cardiac disease, autism, developmental delay, or cancer (Karunakaran et al., 2023). Individuals with structural brain disorders or neurological conditions, such as seizure disorders, prior brain or spinal cord surgery, brain tumors, or genetic disorders affecting brain structure were also excluded. Subjects who could not provide reliable NIRS measures in the initial signal test and were unable to keep their head still for a period of 200 consecutive seconds were also excluded. Each participant was debriefed on the nature of the study, its experimental paradigms, and associated risks prior to obtaining written informed consent from participants. All study procedures were approved by the Institutional Review Board (IRB) of the Massachusetts General Hospital, Boston, Massachusetts.

FNIRS SETUP AND ACQUISITION

After providing written informed consent, subjects were asked to fill out a Pain Catastrophizing and Fear of Pain (FPQ-III) survey (Di Tella et al., 2019; Leung, 2012; Sullivan et al., 1995). Following which, the fNIRS device was setup starting with positioning of the probe on the subject's head (using an elastic cap similar to an electroencephalography/EEG cap) holding the optical sensors ("optodes"). A state of the

art, portable fNIRS system, NIRSport2 (NIRx Inc), was used to collect concurrent changes in cortical hemodynamics and physiology. The NIRSport2 by NIRx is a wearable, wireless, light-emitting diode (LED) based continuous wave fNIRS device with the ability to obtain concurrent changes in physiological measures such as heart rate, respiration etc. It has 16 sources and 16 detectors, combined weighing less than one kg. The system emits non-ionizing light at wavelengths of 760 and 850 nm. Optodes were affixed to the subject's scalp using an elastic cap such that the sensors were positioned at the front (just above the eyebrows) covering the medial frontopolar cortex (Brodmann area 10), while those at the rear were positioned atop the head (over the somatosensory cortex) and the back of the head (covering the visual cortex). **Figure 1** shows the locations of the optodes (sources in red and detectors in blue) on a 10-20 EEG system. The black lines denote the 47 channels measured (pair of source-detector). Additionally, the system incorporates 8 short-distance detectors for measuring extra-cerebral signals and 9-axis accelerometers to monitor head movement. Short-separation detectors are placed at close proximity to the source (~ 0.8cm) and are indicated by the blue circles surrounding the sources (**Figure 1**). Variable tension spring holders were utilized to ensure optimal scalp coupling, effectively minimizing noise artifacts arising from differences in scalp hair density. Furthermore, it includes an auxiliary module called NIRxWINGS, which enables the measurement of physiological changes via pulse oximetry, and respiration via two electrodes placed below the clavicle and a ground sensor to the elbow. Each subject's physiological data included photoplethysmography (PPG), electrocardiography (ECG), heart rate, partial pressure of oxygen, and respiration rate. The pulse oximeter was placed on the subject's distal finger, measuring oxygen

saturation, heart rate, and photoplethysmography. Bioimpedance-based respiration sensors (like ECG) were placed bilaterally on the chest, measuring respirations and ECG as well.

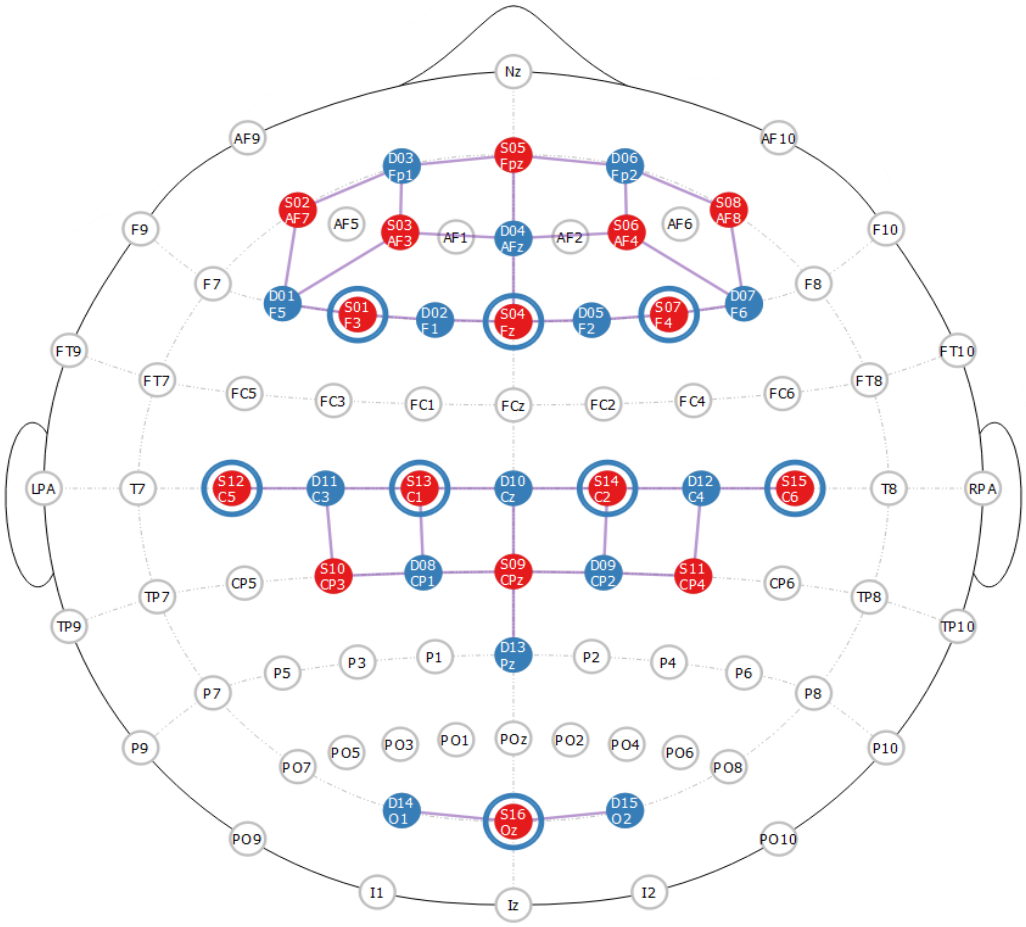

Figure 1. Optode Configuration showing the locations of the sources (Red), detectors (blue), channels i.e., pairs of source and detectors (black) and short-separation detectors (blue circle) on a 10-20 EEG system. The cortical regions are shown by the 10-20 EEG labels, where the anterior most source is placed on Fpz (frontopolar cortex), superior

most source on Cpz (posterior to central sulcus corresponding to somatosensory cortex) and posterior most source is placed on Oz (primary visual cortex).

TASK PARADIGM

All healthy participants completed the same experimental procedure as follows: 600 seconds of Resting-state sessions (first five minutes with eyes open and next five minutes with eyes close randomized between the participants) and 660 seconds of aversive [i]visual paradigm. The study was conducted in a dark room, to reduce any interference in the NIRS signal emerging from ambient lighting of the room. A key aspect of studying aversive processing using fNIRS is the selection of experimental stimuli. Emotional stimuli present with emotional valence, positive or negative, type of stimuli, which an arousal score can reveal the level of activation in specific regions being processed (Xu et al., 2021; Yücel et al., 2015). Thus, during the aversive task, the subjects were presented with visual images from the International Affective Picture System (IAPS) that were classified as either aversive or positive based on their emotional valence and arousal scores. The paradigm is shown in **Figure 2**, where the scan started with 5 seconds of checkerboard image, then 5 blocks of 21 seconds of visual images (comprised of 7 images each presented for 3 second duration). This was followed by a second checkerboard image, and another 5 blocks of 21 seconds of visual images (comprised of 7 images each presented for 3 second duration). All visual stimuli (either checkboard or images) were alternated with 15 seconds of rest. The order of the visual blocks (5 blocks of aversive followed by 5 blocks of positive or vice versa) were randomized across participants to account for order effects. Images

were presented using Psychopy toolbox, and a stimuli marker timestamping each trial was sent to the NIRS acquisition software (Aurora) using a lab stream layer. Aversive images were characterized as images having an emotional valence > 5 & emotional arousal > 5. Whereas positive images were characterized as having emotional valence <3 and emotional arousal > 5.

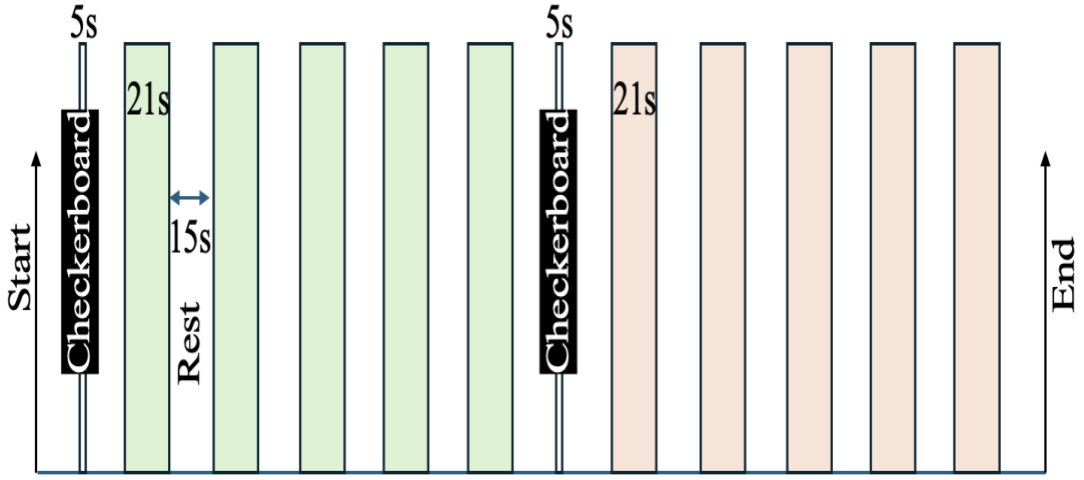

Figure 2. Experimental task paradigm. Green/Red blocks indicate periods of visual stimuli each lasting for 21 seconds comprising of seven 3 second images. If aversive images were presented first (green blocks), then the positive images were presented in the second half (red blocks), vice versa. The blocks were alternated with 15 seconds of rest durations.

FNIRS PREPROCESSING

The fNIRS data from the brain was collected at a sampling frequency of 5.08 Hz (sampling period of 0.1966 seconds), whereas the physiological data were collected at a sampling

frequency of 500 Hz. Quality control evaluation and preprocessing were conducted using the publicly available MATLAB platform (Mathworks, Natick, MA), along with the HOMER2 toolbox. Initially, the raw NIRS signals were converted into changes in optical density. High frequency motion artifacts and physiological noise were limited by performing band-pass filtering at 0.01-0.2 Hz using a third order Butterworth filter. The filtered optical density data was further transformed into alterations in oxygenated hemoglobin (ΔHbO) and deoxygenated hemoglobin (Δ HbR) using the Modified Beer-Lambert Law (MBLL) (Yücel et al., 2014) . Following which, the average signal from all short-separation channels were regression from each of the 47 channels measuring hemodynamic activity from the cortex. A third order polynomial regression was finally performed to reduce linear and non-linear trends in the data.

FNIRS ANALYSIS

The acute response to stimuli was accessed by analyzing block-averaged hemodynamic responses by analyzing block-averaged HDRs. A block was defined as the 4.5 seconds prior to a stimulus, 21 seconds of the stimulus and the 20 seconds following the stimulus (total duration of 46.5 seconds). Average changes in Δ HbO were calculated for aversive vs. positive visual stimuli in each participant. Characteristics of HDR, including peak amplitude for S1, precuneus and V1, and nadir (i.e. decrease in magnitude) for PFC regions, were quantified from these responses. Physiological data such as HRV and respiration, collected concurrently with fNIRS data, will undergo analysis using MATLAB for all participants. The alterations in the heart rate variability derived from the PPG signal

were analyzed using the HRVTool (Vollmer, 2019). The resulting R-R tachogram was compared between the 160 seconds of aversive vs. positive visual blocks. Additional measures of HRV in the time domain included RMSSD, pNN50, and SDNN (see **Table 3**). Respiration rate during aversive vs. positive visual tasks was determined by plotting the power spectral density of the respiration signal in the frequency domain.

STATISTICAL ANALYSIS

Maximum increase in ΔHbO concentration of S1, precuneus and visual cortex in the 5 to 20 seconds of task stimuli were compared between positive vs. aversive stimuli. Similarly, the maximum decrease in ΔHbO concentration of medial PFC, DMPFC and other PFC regions were compared between positive vs. aversive stimuli. Additionally, the difference in magnitude of S1 response with dorsomedial PFC within each hemisphere was compared between the two task conditions. All within-subject comparisons were performed using a Paired sample t-test with a significance level of $p<0.05$.

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RESULTS

PARTICIPANTS

A total of three healthy right-handed adults (42.6±4.7 years, 1 female) completed the study. The participants completed a fear of pain and pain catastrophizing questionnaire, 6 minutes of resting-state scan and 10 minutes of affective visual paradigm. Only the brain hemodynamic activity was recorded for all three participants. The brain hemodynamic changes and the concurrent changes in cardiac and respiratory activity was collected for one participant. Table 1 shows a summary of the demographics of the subjects.

SUBJECT	AGE (YEARS)	SEX	ETHNICITY	FEAR OF PAIN (OUT OF 100)	PAIN CATASTROPHIZING (0-52)
001	39	F	Asian	49	3
002	41	M	Caucasian	45	23
003	48	M	Caucasian		

Table 1. Subject characteristics

AVERSIVE/NEGATIVE VISUAL STIMULI

The average hemodynamic response defined as the oxy-hemoglobin concentration change following a stimulus was calculated by averaging the DHbO timeseries across all trials/blocks of a task condition (aversive/positive) in each subject. A block was defined as

the 4.5 seconds prior to a stimulus, 21 seconds of the stimulus and the 20 seconds following the stimulus (total duration of 46.5 seconds). There was a total of 5 blocks for each aversive and positive visual conditions. The average HDR to aversive visual stimulation was quantified from four cortical areas, bilateral medial PFC (S5-D3, S5-D6), bilateral S1 (S10-D08 and S11-D09), medial precuneus (S09-D13) and bilateral V1 (S16-D14 and S16-D15). The average HDR (**Figure 3**) to aversive stimuli was both anatomically and temporally distinct across the brain regions. Bilateral medial PFC channels corresponding to the frontopolar cortex/Brodmann Area 10 exhibited a decrease in DHbO concentration during the viewing of emotionally arousing unpleasant images. The HDR begins to approach baseline levels DHbO after the completion of the task (at ~21 secs). In contrast, bilateral S1 channels corresponding to the medial primary and secondary somatosensory cortices activated i.e., increased during the viewing of painful unpleasant images. Medial precuneus, corresponding to the medial parietal cortex (Pz on the EEG 10-10) also showed a sustained activation to images portraying painful, unpleasant events. Lastly, as expected, bilateral primary visual cortices activated during the visual task.

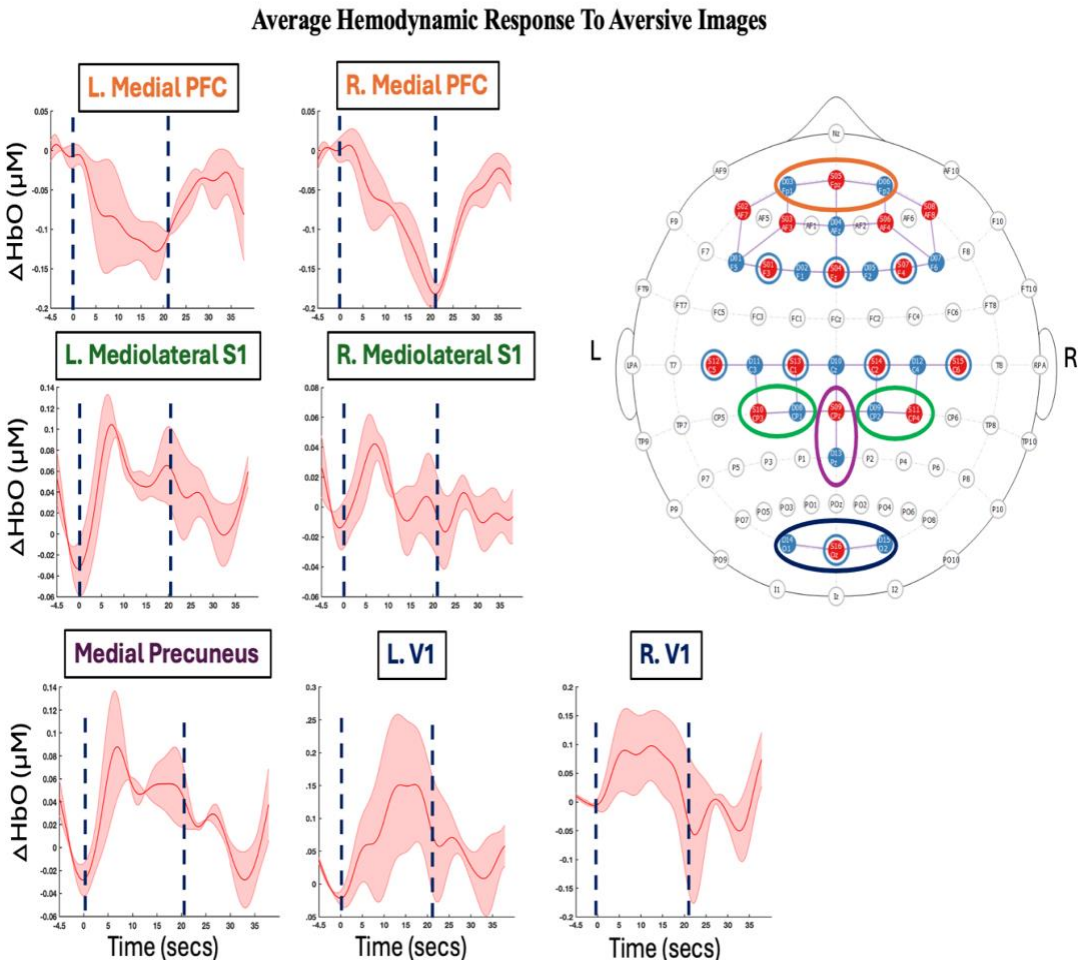

Figure 3: Average cortical hemodynamic response to aversive visual stimulation in 3 healthy adults. The regions shown include bilateral medial prefrontal cortex (medial PFC in orange), bilateral mediolateral primary somatosensory cortex (Mediolateral S1 in green), Medial Precuneus (violet), and bilateral primary visual cortex (V1 in blue). The corresponding cortical regions are highlight on the adjacent optode brain map. The start and end of the visual stimuli, presented as a series of seven images for 3 second durations, is indicated by the dashed lines (total of 21 secs).

POSITIVE VISUAL STIMULI

Similar to the aversive visual stimuli, a block-averaging technique was repeated to quantify the HDR to emotionally positive images from four cortical areas, bilateral medial PFC (S5-D3, S5-D6), bilateral S1 (S10-D08 and S11-D09), medial precuneus (S09-D13) and bilateral V1 (S16-D14 and S16-D15). The average HDR to positive stimuli (**Figure 4**) was comparable to aversive stimuli but at a lower amplitude. Bilateral medial PFC channels corresponding to the frontopolar cortex/Brodmann Area 10 exhibited a decrease in DHbO concentration, whereas the bilateral S1 channels exhibited an increase during the viewing of positive images. Medial precuneus, corresponding to the medial parietal cortex (Pz on the EEG 10-10) as well as the bilateral V1 regions showed a sustained activation to the visual task.

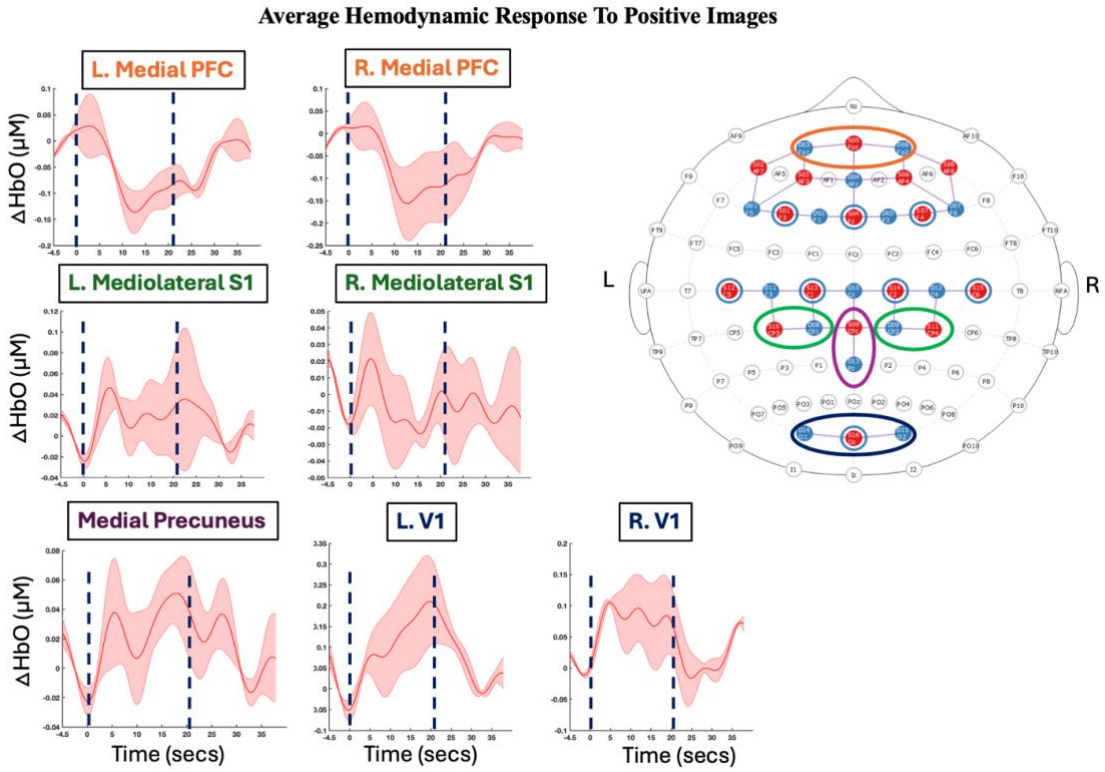

Figure 4: Average cortical hemodynamic response to positive visual stimulation in 3 healthy adults. The regions shown include bilateral medial prefrontal cortex (medial PFC in orange), bilateral mediolateral primary somatosensory cortex (Mediolateral S1 in green), Medial Precuneus (violet), and bilateral primary visual cortex (V1 in blue). The corresponding cortical regions are highlight on the adjacent optode brain map. The start and end of the visual stimuli, presented as a series of seven images for 3 second durations, is indicated by the dashed lines (total of 21 secs).

DISTINCT AVERSIVE VS. POSITIVE VISUAL PROCESSING

Comparing the HDR between the two visual tasks (aversive vs. positive) showed amplitude differences in the bilateral medial dorsal PFC (DMPFC) and bilateral mediolateral S1.

Specifically, the aversive stimuli depicting painful events were associated with a greater activation in S1 (for e.g., max DHbO concentration of 0.1 mM during aversive stimuli as opposed to 0.04 mM during positive stimuli in the left S1 region, see **Figure 5A**). A paired sample t-test comparing the maximum DHbO concentration in the 10 to 20 seconds of aversive stimulation in the left S1 when compared to left S1 response during positive stimulation showed a statistically greater response to aversive images ($p=0.023$, Cohen's $d=3.7$, large effect). Statistical results of other regions are summarized in Table 2. Though not significant at $p<0.05$, the activity in the DMPFC (more dorsal to the medial PFC described in Figure 2 and 3) showed greater deactivation during aversive images as opposed to positive images (**Figure 4A**).

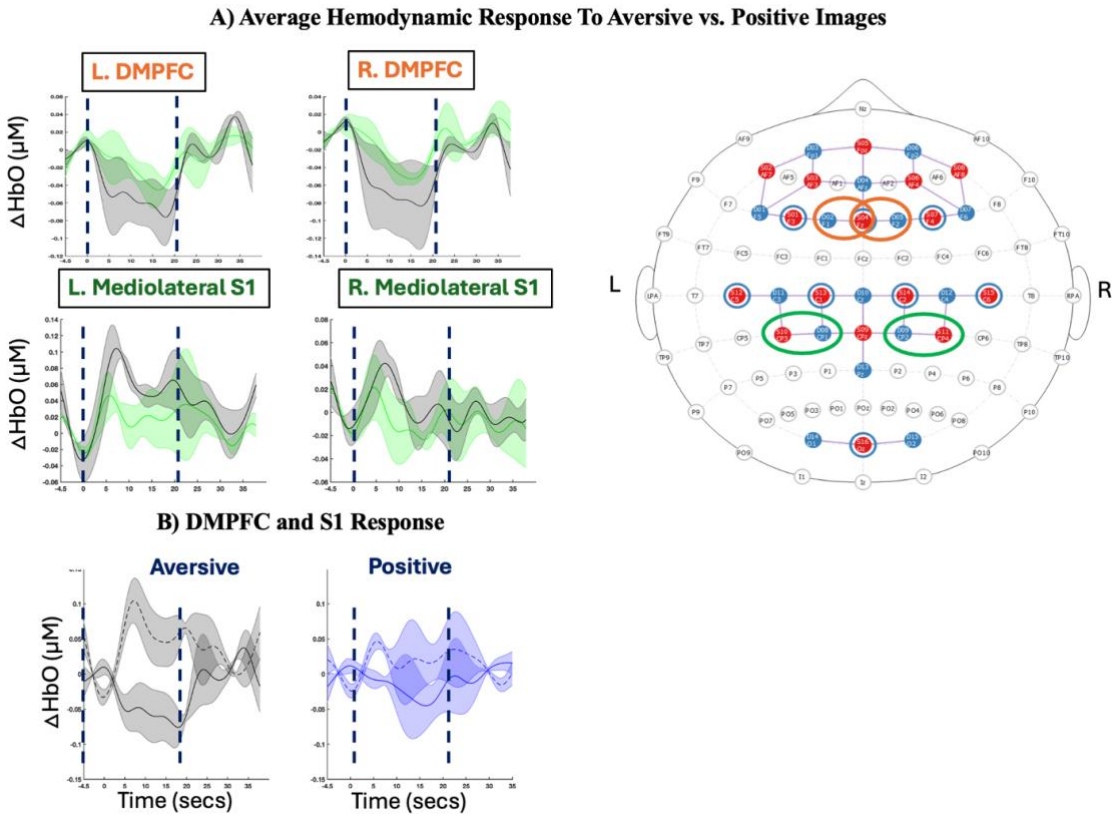

Figure 5. (A) Average cortical hemodynamic response to aversive vs. positive visual stimulation in 3 healthy adults. The regions shown include bilateral dorsomedial PFC (DMPFC in orange), and bilateral mediolateral primary somatosensory cortex (Mediolateral S1 in green). The corresponding cortical regions are highlighted on the adjacent optode brain map. The start and end of the visual stimuli, presented as a series of seven images for 3 second durations, is indicated by the dashed lines (total of 21 secs). B) Comparing the relationship between left DMPFC and left S1 response during aversive (black) vs. positive visual stimulation (blue). The solid line represents DMPFC, and dashed lines represent S1 regions, respectively.

Further comparing the relationship between the two regions (DMPFC and S1) showed a greater dissociation or difference between them during aversive condition that was absent

or negligible in the positive condition (**Figure 5B**). Therefore, emotionally unpleasant images may not only result in greater activity in the cortical areas associated with affective-pain processing but may also affect their interaction. Although, paired sample t-tests comparing the D (S1-DMPFC) didn't show any significant effects at $p<0.05$, the Cohen's d measure indicated a large effect (**Table 2**), suggesting a large sample may capture these effects.

Metric	T-Stat	P-Value	Cohen's d Effect Size
Min DHbO: L.DMPFC	-1.144	0.371	0.660
Min DHbO: R.DMPFC	-0.904	0.461	0.522
Max DHbO: L.S1	6.414	0.023	3.703
Max DHbO: R.S1	1.416	0.279	0.847
D (L.S1-L.DMPFC)	2.39	0.1393	0.847
D (R.S1-R.DMPFC)	1.106	0.383	0.847

Table 2. Statistical summary comparing hemodynamic features during aversive vs. positive visual tasks using a paired sample t-test (n=3)

PHYSIOLOGICAL CHANGES DURING AVERSIVE PROCESSING: A CASE STUDY

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Due to technical errors in the device, concurrent changes in physiological and cortical states were record in only one out of the three subjects (S003). These measures included photoplethysmography (PPG), electrocardiography, heart rate, partial pressure of oxygen, and respiration. The average heart rate during the aversive task period (~160 seconds) was compared with the average heart rate during the positive task period (~160 seconds). As seen in **Figure 6**, both tasks were associated with a similar mean heart rate of 61 to 62.5 beats per minute. However, using the PPG signal, the R-R tachogram, which is the time interval between the R-to-R peaks in the QRS response, was quantified. R-R tachogram represents the heart rate variability (HRV) in seconds (**Figure 6B**). Interestingly, the HRV measures were much greater during the aversive task period as opposed to the positive task condition, suggesting the heart rhythm was more variable and perhaps erratic during the viewing of emotional triggering images.

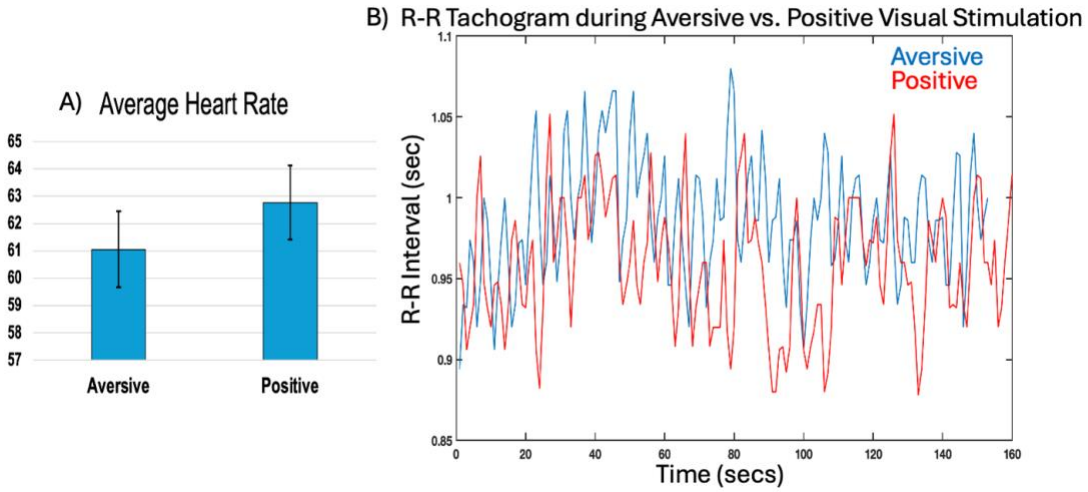

Figure 6. (A) Average heart rate during ~160 seconds of aversive vs. positive visual stimulation in a healthy 48-year-old male. The error bar represents the standard deviation of heart rate during the 160 seconds of task. (B) R-R Tachogram representing the R-R interval during the 160 seconds is shown for aversive (blue) and positive (red) visual tasks.

Additionally, time-domain measures of HRV were quantified for each of the task phases. These included the standard deviation of the normal sinus R-R timeseries (SDNN), root mean square of successive differences between heart beats (RMSSD), and percentage of adjacent NN (i.e., RR intervals) that differ from each other by more than 50 secs (See **Table 3**). Lastly comparing the respiration rate between the two task conditions using a power spectrum analysis showed that the respiration rate was consistent between the two tasks (at approx. 0.2 Hz, which is 12 breaths per minute shown in **Figure 7**).

METRIC	AVERSIVE	POSITIVE	DESCRIPTION
SDNN (msec)	36.890	39.309	Standard deviation of the normal sinus-initiated R-R inter beat interval.
RMSSD (msec)	36.472	33.835	The beat-to-beat variance in HR reflecting vagally mediated changes.
pNN50 (%)	16.107	16.774	Percentage of adjacent NN intervals that differ from each other by more than 50 msec.

Table 3. Time-domain HRV metrics during aversive and positive visual stimulation

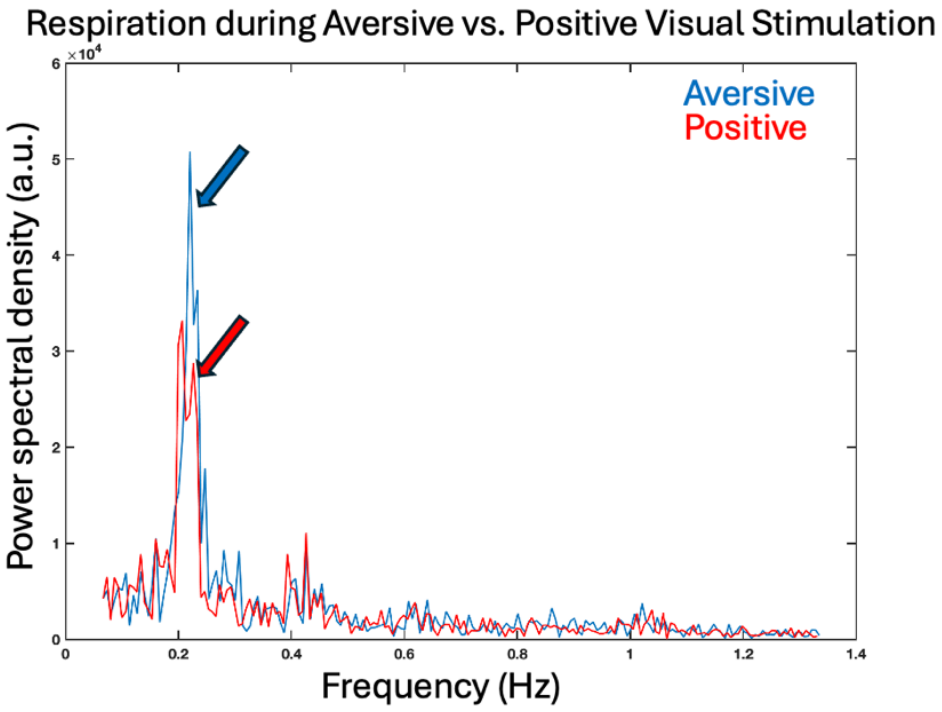

Figure 7. Power spectral density of the respiration signal. The error bar represents the standard deviation of heart rate during the 160 seconds of task. (B) R-R Tachogram

representing the R-R interval during the 160 seconds is shown for aversive (blue) and positive (red) visual tasks.

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DISCUSSION

In this pilot sample, aversive stimuli depicting unpleasant, gory and painful images, triggered heightened activation in bilateral medial primary and secondary somatosensory (S1), medial precuneus and visual cortex (Gordon et al., 1985; Pessoa & Ungerleider, 2004). The outcomes of this investigation corroborate prior research, indicating the involvement of cortical regions during viewing of unpleasant images (Gerdes et al., 2010; Hayes & Northoff, 2011). Both positive and aversive visual stimulation recruit common cortical areas that are related to emotional-pain processing as well as visual processing (Aasted et al., 2016; Yücel et al., 2015). The PFC areas likely process visual images based on its emotional valence, negative or positive, and levels of arousal low to high (Li et al., 2016). Subregions within the PFC, for example dorsomedial PFC and frontopolar cortex, including Brodmann Area 10, are thought to play a role in subjective appraisal, cognitive control, empathy and anticipation (Macpherson, 1965). The primary visual cortex processes visual information particularly sensitive to arousing information independent of the emotional valence (pleasant or unpleasant), as seen through our results (Becerra et al., 2009; Gerdes et al., 2010). The precuneus is thought to deploy attention to and away from images depending on its interaction with amygdala and prefrontal cortex. This explains the greater mean oxy-hemoglobin concentration change we observe during unpleasant images relative to pleasant images due to greater number of arousing regions in unpleasant images- Interestingly, aversive when compared to positive stimuli resulted in increased activation in S1, depicting differential neural responses to varying emotional valences. The channels in the primary somatosensory cortex intersecting with the secondary somatosensory cortex

and parietal cortex, has also demonstrated involvement in emotional (Kropf et al., 2019). Certain regions are recognized for their significant role in each stage of emotional processing and regulation (Kropf et al., 2019) including the identification, generation and regulation of emtoional states.

DISSOCIATION BETWEEN PFC AND S1 DURING AVERSIVE STIMULI

Further processing of results revealed differences in the DMPFC and mediolateral S1 regions between aversive and positive visual stimuli. Both S1 and mPFC regions presented with greater activation to aversive vs. positive stimuli. Specifically, a greater dissociation or contrast was observed between the two regions during the viewing of unpleasant visual images portraying painful events emphasizing distinct cortical interactions to different emotional stimuli. The findings revealed heightened deactivation in the dorsomedial PFC during the appraisal of negative images, coupled with activation of the S1 and precuneus, which are linked to sensory-emotional regulation and attention (Peng, Deepti Karunakaran, et al., 2021). This pattern may suggest increased attention directed towards negatively charged images compared to positive or neutral ones. This effect correlates with previous research displaying the processing of other modalities of aversive stimuli viz. pain in PFC, dorsolateral prefrontal cortex (DLPFC) and S1 (Becerra et al., 2009; S. Green et al., 2022; Kenney & Ganta, 2014). This observation aligns with our hypothesis that aversive stimuli, irrespective of the modality, recruit a core network of brain regions.

These activation patterns within the DMPFC and precuneus offer valuable insights into the neural fundamental mechanisms within the evaluation and processing of emotional stimuli. Furthermore, they highlight the significance of these cortical areas in modulating attentional mechanisms and executive functions within the context of emotional perception. The collaboration of findings from different studies enhances our understanding of the complete neural network involved in emotional processing and cognitive regulation, depicting key regions in experimental and regulatory aspects of visual emotional responses.

SOMATOSENSORY CORTEX AND EMOTIONAL PROCESSINGAdditionally, considering the aversive images were painful events, the increased somatosensory activation (associated with sensory discriminative processing) in combination with greater deactivation of medial PFC (Figure 2, associated with cognitive modulation) may be a result of increased pain perceived during the viewing of images (Crawford et al., 2023). Aversive images, especially depicting painful, bloody, and gruesome events, are expected to induce feelings of perceived pain or threat, as well as increased attention (Branco et al., 2023). The observed simultaneous increase in somatosensory activation and decrease in medial PFC activity could indicate an intensified perception of pain or threat while viewing emotionally challenging images, especially those portraying gruesome or distressing events. (Kropf et al., 2019; Branco et al., 2023). This suggests a complex interaction between sensory processing and emotional evaluation, demonstrating the brain's tendency to prioritize the processing of significant and potentially threatening stimuli.

The findings suggest a profound integration between emotional and sensory processing mechanisms in response to aversive stimuli, particularly those depicting painful events. The heightened activation observed in the somatosensory cortex implies an intensified sensory discriminative processing, indicating a heightened sensitivity to the emotional content of the images (Kropf et al., 2019; Crawford et al., 2023). This heightened activation is coupled with a simultaneous deactivation of the medial PFC, a region associated with cognitive modulation, suggesting a shift towards more primitive, instinctual responses to the perceived threat or pain (Kropf et al., 2019; Crawford et al., 2023).

The heightened engagement induced by aversive stimuli offers significant emotional context in sensory experiences, providing valuable insights into the neural mechanisms underlying pain perception and emotional processing (Kropf et al., 2019; Branco et al., 2023). These findings contribute to a deeper understanding of how the brain integrates sensory and emotional information to generate subjective experiences of pain and threat, highlighting the intricate interplay between cortical regions involved in sensory discrimination and emotional regulation.

HEART RATE VARIABILITY

Heart rate variability is higher in aversive condition compared to positive visual condition. It is likely that emotionally aversive images trigger a parasympathetic response in the autonomic system: emotional arousal and physiological activity as a sequel to the stimuli.

It also describes the subject's affective perception: cognitive and physiological states' correspondence (Loggia et al., 2011; Van Der Vaart et al., 2022). Further work is required to understand how the cortical state relates to the autonomic state. Perhaps emphasis on a coupling analysis between the two is necessary, in order to distinguish the underlying dynamics of emotional processing with aversive stimuli.

The presence of visible differences in heart rate variability during aversive and positive conditions suggests a more complex autonomic response to emotionally striking images. Specifically, the presentation of aversive stimuli involves a parasympathetic response of the autonomic nervous system, which can reflect an increase in emotional arousal and physiological reactivity to the stimuli (Loggia et al., 2011; Van Der Vaart et al., 2022). Heart rate exhibits strong correlation to affective valence among autonomic responses. Previously, studies has shown a reduced HRV in response to aversive stimuli, while positive stimuli resulted in reduced heart rate initially, with a slight increase followed by decrease (Wilson et al., 2020). Our results exhibited greater HRV for positive stimuli, therefore indicating a greater correlation to emotional valence during positive stimuli.

While the clarification of the autonomic basis for emotional perception contributes to the understanding of the complicated relationships between cognitive and physiological processes involved in the experience, the implications of these findings are not limited to this observation. Thus, based on the results of the pilot study, one can claim that the cortical states need to be studied further in their relationship to the autonomic responses. Further

developments should create multiple levels of assessment in the emotional process (Loggia et al., 2011; Van Der Vaart et al., 2022).

IMPLICATIONS OF FINDINGS

The findings of this study carry significant implications for our understanding of emotional processing mechanisms, particularly in the area of pain perception and emotional regulation (Loggia et al., 2011; Kropf et al., 2019; Branco et al., 2023). By clarifying the neural responses involved in emotional visual processing, these results could provide valuable insights for developing targeted interventions aimed at addressing conditions characterized by abnormal emotional responses or heightened sensitivity to pain. (Loggia et al., 2011; Kropf et al., 2019; Branco et al., 2023). Understanding the complex interaction between the somatosensory cortex and emotional processing regions such as the medial prefrontal cortex, provide a deepened understanding into potential therapeutic targets for modulating pain perception and emotional distress (Kropf et al., 2019; Branco et al., 2023).

Furthermore, the varying responses seen in the brain and autonomic nervous system when exposed to negative versus positive stimuli highlight the importance of taking emotional context into account when evaluating and treating emotional disorders and conditions related to pain. Furthermore, these findings highlight the importance of adopting a comprehensive approach that integrates neural and physiological markers to advance our understanding and management of emotional experiences and pain perception (Loggia et al., 2011; Branco et al., 2023; Di Tella et al., 2019).

LIMITATIONS

Several limitations should be acknowledged in our study. Firstly, the small sample size restricts the generalizability of our findings and necessitates caution in interpreting the results (Sen et al., 2020). Secondly, technical limitations, such as the limited penetration depth of fNIRS, restrict our ability to capture deeper brain structures involved in emotional processing, such as the amygdala (Aasted et al., 2015). Thirdly, our study was limited to superficial layers of the cortex, particularly the anterior regions, potentially overlooking deeper cortical structures crucial for emotional processing including insula and anterior cingulate cortex.

Furthermore, the use of randomly selected images depicting situations of emotional valence and arousal >5 introduces variability in the perception of aversiveness across participants, which may confound the results (Branco et al., 2023). Additionally, reflective differences in individual perceptions of aversiveness were not systematically accounted for, potentially influencing the observed cortical and physiological responses (Sullivan et al., 1995). Nevertheless, it is crucial to acknowledge the limitations of our study, including the small sample size and the lack of diversity in participant characteristics. Future research with larger and more diverse samples is necessary to validate and expand upon our findings (Van Der Vaart et al., 2022). Moreover, incorporating advanced analytical and statistical techniques may further elucidate the complex relationships between cortical and physiological responses to emotional stimuli (Butler et al., 2018; Stuart et al., 2019).

CONCLUSION

The outcomes of this study unveil the intricate neural and physiological mechanisms involved in processing aversive and positive visual stimuli. Through the integration of fNIRS with measures of cardiac and respiratory activity, we sought to elucidate the complex interplay between cortical and autonomic responses to emotional stimuli.

Our investigation uncovered distinct cortical activation patterns in response to aversive and positive stimuli across various brain regions, consistent with prior research (Aasted et al., 2016; Branco et al., 2023). Aversive stimuli evoked heightened activation in regions associated with emotional processing and pain perception, such as the bilateral medial primary and secondary somatosensory cortices (S1) and the medial precuneus (Crawford et al., 2023; Dowling, 1983). On the contrary, positive stimuli were linked to lower negative activation in the medial prefrontal cortex (PFC) and lower positive activation in S1, indicating differential neural responses to varying emotional valences and degrees of arousal (Kropf et al., 2019).

Moreover, our analysis revealed significant amplitude differences in the dorsomedial PFC (DMPFC) and mediolateral S1 between aversive and positive stimuli, underscoring the distinct role of cortical regions with overlapping functional states (Peng et al., 2018). The heightened activation in S1 during aversive stimuli suggests its pivotal role in processing

aversive emotional experiences, while the differential activity in DMPFC suggests its involvement in emotion regulation processes (Li et al., 2016).

Additionally, our investigation explored physiological changes during aversive processing in a case study. We observed increased heart rate variability (HRV) during aversive tasks, indicating heightened physiological arousal during exposure to emotionally triggering stimuli (Loggia et al., 2011). This highlights the intricate interplay between cortical and autonomic responses to emotional stimuli, emphasizing the holistic nature of emotional processing. (Loggia et al., 2011).

Nevertheless, it is crucial to acknowledge the limitations of our study, including the small sample size and the lack of diversity in participant characteristics. Future research with larger and more diverse samples is necessary to validate and expand upon our findings (Van Der Vaart et al., 2022). Moreover, incorporating advanced statistical techniques and machine learning algorithms may further elucidate the complex relationships between cortical and physiological responses to emotional stimuli (Butler et al., 2018; Stuart et al., 2019).

In conclusion, our study offers valuable insights into the neural and physiological substrates of emotional processing, laying the foundation for further investigations into the neural basis of emotion regulation and the development of targeted interventions for emotional well-being and pain management (Sen et al., 2020; Zhou et al., 2021). By

advancing our understanding of these mechanisms, we may ultimately contribute to improving the assessment and treatment of emotional disorders and chronic pain conditions.

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