DISSERTATION

RESIDUAL EFFECTS OF CANNABIS ON ATTENTION TOWARD AND AWARENESS OF EMOTIONAL FACIAL EXPRESSIONS: EVENT-RELATED POTENTIAL STUDIES

Submitted by

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ABSTRACT

RESIDUAL EFFECTS OF CANNABIS ON ATTENTION TOWARD AND AWARENESS OF EMOTIONAL FACIAL EXPRESSIONS: EVENT-RELATED POTENTIAL STUDIES

Cannabis use has increased since legalization in various states within the United States of America. Although much of the research on the neurological and psychological effects of cannabis has been on non-human animals, the current research suggests that it can have anxiolytic effects but also decrease some cognitive functioning (e.g. memory, emotional processing, etc.). Individuals with high anxiety has been suggested to have increased attentional bias towards threat-related stimuli. The purpose of the current two studies was to examine the residual effects cannabis has on attentional bias towards and awareness of emotional facial expressions. Both experiments used event-related potential (ERP) to measure brain activity related to attentional processing. Experiment 1 used a dot-probe task with fearful and neutral facial expression to examine attentional bias. The second experiment used a backward masking paradigm to restrict awareness of facial expressions (i.e. fearful, happy, and neutral). The results indicated that cannabis use was associated with differences in attentional processing. Specifically, experiment 1 suggested cannabis users had reduced attentional bias towards fearful facial expressions as compared to non-users. The results from experiment 2 suggested an opposite effect, cannabis users had increased processing of emotional facial expressions. An explanation of the difference in results is the cannabis users in experiment 1 used less frequently than users in experiment 2. The results of both studies suggested cannabis use has an inverse relationship with anxiety related attentional processing of emotional expressions.

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Chapter 1 – Introduction

The legalization of cannabis is no longer just a topic of debate and is becoming a reality. Canada recently legalized cannabis use federally and will take effect in October 2018 (Canadian Department of Justice, 2018). In the United States of America cannabis is federally illegal, although, 29 states and Washington D.C. have legalized cannabis use for medical purposes and eight states along with Washington D.C. legalized cannabis for recreational use (National Conference of State Legislatures, 2018). There is a body of research which suggests cannabis can have anxiolytic effects (Berrendero & Maldonado, 2002; Patel & Hillard, 2006; Rubino et al., 2007; Viveros, Marco, & File, 2005), however, cannabis can have anxiogenic effects (Viveros et al., 2005). Not surprisingly, some cannabis users report using to self-medicate their anxiety, depression, and posttraumatic stress disorder (PTSD) (Crippa et al., 2009; Troup, Andrzejewski, Braunwalder, & Torrence, 2016). Previous researchers have indicated that cannabis is associated with cognitive deficits including memory, attention, and emotional processing (Broyd, Van Hell, Beale, Yücel, & Solowij, 2016; Lovell et al., 2018; Troup, Bastidas, et al., 2016; Troup, Torrence, Andrzejewski, & Braunwalder, 2017). Long term cannabis use has also been correlated with neuroanatomical differences areas associated with the aforementioned cognitive deficits (Lorenzetti, Solowij, & Yücel, 2016). However, the neurocognitive mechanisms of cannabis's anxiolytic effects are unknown. Individuals with anxiety typically display a hypervigilance towards threat-related stimuli (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Two experiments were developed to test the residual effects of cannabis (i.e. the effects of regular use and not the acute effects) on attention to emotional stimuli. In the first experiment, a dot-probe task was used with fear and neutral facial

expression and the second experiment awareness of emotional facial expressions were measured in a backward masking paradigm. Both experiments used event related potentials (ERPs) to measure the neural correlates of attention. In general, it was hypothesized that cannabis users would have reduced attentional bias and emotional processing of emotional facial expressions.

Emotional Faces and Attentional Bias

Attentional bias happens in three stages: orientation, engagement, and then disengagement (Posner, Snyder, & Davidson, 1980). One widely used method of measuring attentional bias has been the dot-probe task (MacLeod, Mathews, & Tata, 1986). The dot-probe task typically consists of two stimuli (one salient and one neutral) presented simultaneously either horizontal or vertical to each other (see Figure 1). Researchers have used various stimuli types (e.g. emotional facial expressions, International Affective Picture System [IAPS] images, etc.) to measure what type of stimuli captures visual spatial attention (for review see van Rooijen et al., 2017). A target dot, or probe, is then presented in place of one of the stimuli. The participant's objective is to either indicate the location if the target (e.g. left or right) or the orientation of the target (e.g. vertical bar or horizontal bar; Mogg & Bradley, 1999; Salemink et al., 2007). When the target appears behind the salient stimuli it is a congruent trial. That is, the target is spatially congruent with the salient image. If the target is in place of the neutral stimuli, then that is considered incongruent. Typically, attention is allocated to the location of the salient stimuli and reaction time (RT) to the congruent target is faster than RT to incongruent targets (e.g., attentional bias). Previous research introduced a neutral-neutral and/or fearful-fearful trial as a type of baseline or control trial (Carlson & Reinke, 2008; Carlson, Reinke, & Habib, 2009; Carlson, Reinke, LaMontagne, & Habib, 2011). The neutral-neutral trial can be used to examine differences between orientation and delayed disengagement. Faster RT for congruent compared

to neutral-neutral that would indicate rapid orientation, whereas slower RT for incongruent compared to neutral-neutral would indicate delayed disengagement.

Using various stimulus onset asynchrony (SOA) is another method used to examine engagement and disengagement. Torrence, Wylie, and Carlson (2017) varied the amount of time between face offset and dot onset (i.e. SOA) in two experiments using either fear-neutral or neutral-neutral face pairs, and in a separate experiment they used happy-neutral and neutral-neutral face pairs. That is, in two experiment the faces were displayed for 51 ms and had either a 33, 117, 285, or 621 ms delay from face offset to dot onset. Their results suggested that attention rapidly orients towards fearful faces, significant difference in RT at 84 ms, but attentional bias to the fearful face location does not last longer than 300 ms. However, happy faces had a delayed orienting effect (i.e. 168 ms) but attention was engaged in that location for longer (i.e. 336 ms) than fearful faces. These results were consistent with other research that suggest attentional bias is rapid (i.e. < 500 ms) in non-clinical samples (Cooper & Langton, 2006; Koster, Verschuere, Crombez, & Van Damme, 2005).

The dot-probe task can also be used with backward masking to restrict awareness. Backward masking is accomplished by having a target stimulus (e.g. fearful face) displayed for a short duration (e.g. 16 or 33 ms) and is immediately replaced with a neutral stimulus (e.g. neutral face). Carlson and Reinke (2008) presented one fearful face and one neutral face for 33 ms and then immediately replaced them with two neutral facial expressions for 100 ms. They found that there was an attentional bias toward the fearful face even when awareness of the fearful face was limited. Similarly, Carlson, Torrence, and Vander Hyde (2016) cropped out all non-eye facial features and used a backward masking paradigm. Their results suggest that the eyes of the fearful face capture spatial attention even when awareness was restricted. Pessoa, Japee, and

Ungerleider (2005) manipulated the duration of the target face in a backward masking paradigm to test at which duration the target face is below the level of awareness. They found that when the target face was displayed for 17 ms and replaced with a masking face, most of the participants were unable to report emotional expression of the target face (fear, happy, or neutral). When the target face was displayed for more than 33 ms, most of the participants were able to identify the emotion. The key difference between the masked dot-probe task in Carlson and Reinke (2008) and the awareness task in Pessoa et al. (2005) is that the faces in the dot-probe task are not centered in the screen and requires orienting of spatial attention, whereas the awareness task does not require spatial attention. However, recent research has suggested that using RT in the dot-probe task has been unreliable (Price et al., 2015; Puls & Rothermund, 2017). Other research indicated that using neuroimaging methods like functional magnetic resonance imaging (fMRI; White et al., 2016) and ERP (Kappenman, Farrens, Luck, & Proudfit, 2014) were more reliable than RT.

Neural Correlates of Attention to Fearful Facial Expressions

Attention towards threat-related stimuli requires the amygdala, as indicated by human lesion studies (Anderson & Phelps, 2001; Bach, Hurlemann, & Dolan, 2015). Similarly, fMRI research has indicated the amygdala is more active when attending towards positive and negative emotionally salient stimuli (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Hamann & Mao, 2002; Yang, Dong, Chen, & Zheng, 2012). Additionally, the amygdala is correlated activity in the visual system. Patients with amygdala damage also have less activation in the visual cortex when viewing fearful facial expression (Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). The control of attention seems to involve the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), and the anterior insula (Carlson, Cha, & Mujica-parodi, 2013;

Carlson, Reinke, & Habib, 2009; Fu, Taber-Thomas, & Pérez-Edgar, 2015; Liddell et al., 2005; Price et al., 2014; White et al., 2016a). Therefore, the amygdala is necessary to orient attention to a salient stimulus which enhances visual processing in the visual cortex for engagement, and then the frontal areas modulate when to disengage.

Although fMRI research has afforded understanding of the brain areas correlated with attentional bias, electroencephalography (EEG) and ERP research has been used to understand the time-course of attentional bias. EEG and ERP signals originate in thousands of pyramidal cells with similar orientation firing together. Time-frequency analysis has been used to study frequency oscillations in the time domain. Theta oscillations (5-7 Hz) has been related to cholinergic projections from the basal forebrain being synchronized with the cortex (Lee, Hassani, Alonso, & Jones, 2005). Other research has suggested that cholinergic projections from the basal forebrain to the visual cortex modulates visuospatial attention (Chaves-Coira, Rodrigo-Angulo, & Nuñez, 2018; Pinto et al., 2013). ERPs are obtained from EEG recordings that are time-locked to a stimulus onset (e.g. face) and averaged across multiple trials. Although ERP methodology ignores the frequency domain, ERPs do have greater temporal resolution. Multiple ERP components have been related to attending towards salient stimuli.

One of the earliest ERP components related to attention is P1 which typically peaks around 100 -130 ms after stimulus onset and found in lateral occipital electrodes. P1 amplitude is typically greater in response to salient stimuli (Morel, George, Foucher, Chammat, & Dubal, 2014; Pourtois, Grandjean, Sander, & Vuilleumier, 2004; Torrence & Troup, 2017; Patrik Vuilleumier & Pourtois, 2007), especially in individuals with high anxiety (Harrewijn, Schmidt, Westenberg, Tang, & Van der Molen, 2017; Helfinstein, White, Bar-Haim, & Fox, 2008; Holmes, Bradley, Kragh Nielsen, & Mogg, 2009; Mueller et al., 2009). The N170, however, is

more related to face specific processing and is modulated by emotional expression (Hinojosa, Mercado, & Carretié, 2015). Typically, the N170 has right hemisphere lateralization for faces as opposed to left for words (Maurer, Rossion, & McCandliss, 2008). Source analysis indicated that N170 was correlated with activity in the superior temporal sulcus (STS), occipital face area (OFA), and the fusiform face area (FFA) (Deffke et al., 2007; Herrmann, Ehlis, Muehlberger, & Fallgatter, 2005; Itier & Taylor, 2004). Previous dot-probe literature observed and enhanced N170 in electrodes posterior contralateral to a threat-related facial expression (Carlson & Reinke, 2010; Rossignol, Campanella, Bissot, & Philippot, 2013). Similarly, the posterior contralateral N2 (N2pc) is the second negative peak occurring around 200 – 300 ms in the lateral occipital electrodes. N2pc is defined as greater amplitude posterior contralateral to the attended stimulus and is thought to represent initial orientation of spatial attention (Diao, Qi, Xu, Fan, & Yang, 2017; Dowdall, Luczak, & Tata, 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015). Previous research has found that there is enhanced N2pc towards threating images (Kappenman et al., 2014; Kappenman, MacNamara, & Proudfit, 2015). Some facial dot-probe studies found enhanced N2pc for negative facial expressions (Holmes et al., 2009; Holmes, Mogg, de Fockert, Nielsen, & Bradley, 2014). Fox, Derakshan, and Shoker (2008) found that high trait anxiety participants also had enhanced N2pc towards angry facial expressions, whereas low trait anxiety participants had no difference in N2pc.

The N2pc is associated with orienting spatial attention whereas anterior N2 is associated with cognitive control (Folstein & Van Petten, 2008). The anterior N2 peaks around 250 – 300 ms and is thought to originate in medial frontal cortex (e.g. ACC) and the right inferior frontal cortex (Aron, Robbins, & Poldrack, 2016; Folstein & Van Petten, 2008; Ridderinkhof & Ullsperger, 2004). Some backward masking studies found that backward masking had no effect

on N2 (Liddell, Williams, Rathjen, Shevrin, & Gordon, 2004; Vukusic, Ciorciari, & Crewther, 2017). That is, there were differences between emotional expression in the unmasked condition as well as within the masked condition. Whereas, Pegna, Landis, and Khateb (2008) only found differences in N2 in the unmasked condition. Lastly, the late P3 component (400 – 600 ms) can be found in central posterior electrodes (e.g. PZ) (Kiss & Eimer, 2008). The P3 has been suggested to be enhanced for higher level emotional and attentional processing (Johnston, Miller, & Burleson, 1986; Polich, 2007). Kiss and Eimer (2008) and Liddell et al. (2004) found that the P3 was modulated by emotional facial expressions only in unmasked conditions, no such effects were found in the masked condition. Their results suggested the P3 is only affected by consciously perceived emotional expressions.

Neurological Effects of Cannabis

The main psychoactive cannabinoid, Δ9-tetrahydrocannabinol (THC), binds to the Cannabinoid Receptor-1 (CB1). CB1 is part of the endocannabinoid system (ECS) and is one of the most abundant G-protein coupled receptors in the human brain, found in areas such as the cingulate cortex, amygdala, hippocampus, and the prefrontal cortex (Burns et al., 2007). Given these brain regions, the ECS has been suggested to be involved in regulation of fear and anxiety (Ruehle, Rey, Remmers, & Lutz, 2012). The retrograde transmitters, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are agonists to the CB1 receptor which is located on the presynaptic cell and inhibits the release of gamma-aminobutyric acid (GABA) and glutamate (Kano, Ohno-shosaku, Hashimotodani, & Uchigashima, 2009). THC is a partial agonist to CB1 and has similar effects on AEA and 2-AG. Previous research has indicated that increasing AEA levels in the amygdala by decreasing fatty acid amide hydrolase (FAAH), the catabolic enzyme

that degrades AEA, was related to decreased stress and increased amygdala habituation (Gunduz-Cinar et al., 2013; Gunduz-Cinar, Hill, Mcewen, & Holmes, 2013).

Given that THC acts as a partial agonist to the CB1 receptor, like AEA, researchers have used non-human animals to better understand how THC effects anxiety. A recent review by Patel, Hill, and Hillard (2014) discussed how THC is dose-level dependent. That is, repeated administration of high doses of THC result in anxiogenic effects, whereas, low dose of THC resulted in anxiolytic effects in rats. Research suggested that the in low doses of THC, cortical glutamatergic neurons are inhibited and thereby reducing anxiety, however, in high doses, cortical GABAergic neurons are inhibited (reducing the inhibitory effects) which results in increased anxiety (Lutz, Marsicano, Maldonado, & Hillard, 2015). There are individual differences on the effects cannabis has on anxiety. Green, Kavanagh, and Young (2003) discussed how across multiple studies, there were variances in reported anxiety. However, they also discussed that method and environment could modulate anxiety symptoms following cannabis use. Specifically, oral administration of cannabis was more likely to increase anxiety and smoking in a small group was related to reduction of anxiety. Schubart et al. (2011) found that different ratios of CBD to THC have different effect on psychosis. CBD seems to minimize THC induced psychosis. THC has increased from 4% in 1995 to 12% in 2014 where CBD has deceased from 0.28% in 2001 to 0.15% in 2014 (ElSohly et al., 2017). The effects of cannabis on anxiety have been largely studied on non-human animals and given the difficulty conducting controlled experimental studies on humans, the results have been mixed.

A recent review of literature (Lorenzetti et al., 2016) examined neuroanatomical alterations related to cannabis use. Cannabis use was strongly correlated with decreased grey matter volume in the hippocampus, amygdala, insula, and orbital frontal cortex. Differences in

grey matter volume, as measured using voxel-based morphometry, indicate differences in dendritic spine density which could relate to afferent connectivity (Keifer et al., 2015). That is, decreased grey matter volume might be related to decreased input connectivity. Additionally, cannabis use has been associated with reduction of right ACC grey matter volume (Hill, Sharma, & Jones, 2016). Synthetic cannabis (a full CB1 agonist) use was related to reduction in the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, and cingulum– hippocampus (Zorlu et al., 2016). The longitudinal fasciculus mediates the communication between the amygdala and the visual cortex (Fischer et al., 2016). However, these reductions were related to reported long term cannabis use, whereas amount of cannabis use in the last 30 days was not associated with grey nor white matter differences (Thayer et al., 2017). Pietrzak et al. (2014) suggested that in trauma survivors, reduced CB1 availability in the amygdala was associated with attentional bias toward trauma related words. That is, less inhibition of GABA related to less CB1 receptors in the amygdala was associated with greater attentional bias towards threat related words. The differences in neural networks and brain areas that relate to cannabis use are the same areas involved in attentional bias toward and awareness of fearful facial expressions.

Purpose and Hypotheses

Cannabis has been suggested to have anxiolytic effects, although there are individual differences and environmental factors that relate to cannabis having anxiogenic effect (Green et al., 2003; Viveros et al., 2005). Cannabis use has also been related to neuroanatomical differences in the amygdala, PFC, and insula (Hill, Sharma, & Jones, 2016; Lorenzetti et al., 2016). These same brain areas are also part of the neural network involved in attentional bias towards and awareness of threat-related stimuli (Carlson, Cha, & Mujica-parodi, 2013; Carlson, Reinke, & Habib, 2009; Fu, Taber-Thomas, & Pérez-Edgar, 2015; Liddell et al., 2005; Price et

al., 2014; White et al., 2016a). Individuals with high anxiety symptoms typically have a hypervigilance towards salient stimuli (Bar-Haim et al., 2007; Morel et al., 2014). The residual effects of cannabis use on anxiety have been understudied. Two ERP studies were used to examine anxiety related neurocognitive effects of cannabis use. The first study used a dot-probe task with fearful and neutral faces to explore differences in attentional bias towards threat-related stimuli between cannabis users and non-users. The second study used a backward masking paradigm to explore differences in perceptual awareness of emotional facial expressions. It was hypnotized that cannabis users would have reduced ERP amplitudes related to attention compared to non-users.

Chapter 2 – Experiment 1

Introduction

Although Cannabis sativa (cannabis, marijuana, weed, etc.) is currently federally illegal in the United States of America, 29 states and the District of Columbia have legalized medical use and 8 states and District of Columbia have legalized cannabis for both recreational and medical use (National Conference of State Legislatures, 2018). Cannabis use among individuals older than 12 years old has increased from 6.2% in 2002 to 8.3% in 2015 (Substance Abuse and Mental Health Services Administration, 2016). Cannabis has been suggested to have negative effects on memory, attention, executive function, and emotional processing (Broyd et al., 2016; Lovell et al., 2018; Troup, Bastidas, et al., 2016; Troup et al., 2017). and has also been correlated with differences in grey matter volume in areas of the brain involved in attention, emotion, and memory (Lorenzetti et al., 2016). Although there are some deficits correlated with cannabis use, some researchers have suggested that cannabinoids have anxiolytic effects (Berrendero & Maldonado, 2002; Patel & Hillard, 2006; Rubino et al., 2007; Viveros et al., 2005). Additionally, previous research has found that many cannabis users, use cannabis to self-medicate for anxiety, depression, and PTSD (Crippa et al., 2009; Troup, Andrzejewski, et al., 2016). Anxiety symptoms, particularly social anxiety, has been related to an over attentional bias towards threat-related stimuli (Bar-Haim et al., 2007). Given the potential effect of cannabis on anxiety and that anxiety is associated with differences in attentional bias, this study examined attentional bias towards fearful faces in cannabis users using event-related potentials (ERPs).

The dot-probe task is a widely used method of studying attentional bias toward various stimuli (for review see van Rooijen et al., 2017) and differences between low and high anxiety

(Bar-Haim et al., 2007). Typically, the dot-probe task displays a salient stimulus (fearful face) and a neutral stimulus (neutral face) simultaneously. Comparing the reaction time (RT) in congruent trials (dot is spatially congruent with salient stimuli) and incongruent trials (dot is spatially incongruent with salient stimuli) allows an analysis of a general attentional bias. However, Posner, Snyder, and Davidson (1980) suggested that there are three facets of attentional bias: orienting, engaging, and disengaging. Therefore, previous research included a baseline trial (two neutral stimuli or two salient stimuli) to examine the differences between orienting and delayed disengagement (Carlson & Reinke, 2008; Carlson, Reinke, & Habib, 2009; Carlson et al., 2011). Torrence, Wylie, and Carlson (2017) suggested that attention towards fearful faces is rapid but fleeting. That is, there is a rapid orientation towards the fearful face, but engagement to that location does not last long (< 300 ms). Despite the plethora of dot-probe research, current research has brought up serious, and valid, concerns of reliability of the dot-probe task when using RT (Price et al., 2015; Puls & Rothermund, 2017; Schmukle 2017; Staugaard, 2009). However, other research has suggested that alternative measures of attentional bias might be more reliable than RT, such as eye-tracking (Price et al., 2015; Waechter, Nelson, Wright, Hyatt, & Oakman, 2014), functional magnetic resonance imaging (fMRI) (White et al., 2016), and ERPs (Kappenman et al., 2015).

The amygdala is necessary for allocating attentional resources towards threat-related stimuli, as indicated by human lesion studies (Anderson & Phelps, 2001; Bach, Hurlemann, & Dolan, 2015). Similarly, fMRI research has indicated that amygdala is activated in attending towards positive and negative stimuli (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Hamann & Mao, 2002; Yang, Dong, Chen, & Zheng, 2012). Attentional bias has been associated with a network of brain areas including the anterior cingulate cortex (ACC), medial prefrontal

cortex (mPFC) and anterior insula (Carlson, Cha, & Mujica-parodi, 2013; Carlson, Reinke, & Habib, 2009; Fu, Taber-Thomas, & Pérez-Edgar, 2015; Liddell et al., 2005; Price et al., 2014; White et al., 2016a). In the dot-probe task amygdala activity has been correlated with activation in the visual cortex (Carlson et al., 2009) suggesting that the amygdala is involved in enhancing visual processing towards the salient stimuli.

To obtain timing information on attentional bias, researchers have used ERPs in the dot-probe task. Although there were some inconstancies in the results, research using facial expression in the dot-probe task found that ERP components can detect attentional bias towards emotional facial expressions (Torrence & Troup, 2017). There are different ways to design the dot-probe task for ERP research. One way is to examine ERP components time-locked to the dot onset and there for there is no need to delay the dot after face offset. The second way is to examine the ERP components time-locked to face onset and therefore delaying the dot after face offset as to avoid stimulus overlap in the ERP signal. This study used the latter and therefore will discuss the ERP components time-locked to face onset.

The P1 component has a positive peak occurring around 80-120 ms after stimulus onset in lateral occipital electrodes. Previous research indicated that P1 was enhanced when viewing a negative facial expression and has been suggested to originate in the posterior fusiform gyrus (Mueller et al., 2009; Pourtois, Thut, De Peralta, Michel, & Vuilleumier, 2005). Enhancement of the P1 is thought indicate increased attention to threat (Vuilleumier & Pourtois, 2007). The N170 component has a negative peak around 170 ms from the lateral posterior electrodes (Bentin, Allison, Puce, Perez, & Mccarthy, 1996). It was previously thought that the N170 was more enhanced for face vs non-face objects and emotional expression did not influence the N170 amplitude. However, a recent meta-analysis found that the N170 was more enhanced for

emotional facial expressions than for neutral facial expressions (Hinojosa et al., 2015). Dot-probe studies have found that posterior-contralateral electrode to the negative facial expression had more negative N170 than the electrode ipsilateral to the negative face (Carlson & Reinke, 2010; Rossignol et al., 2013). Given that the N170 might reflect activation of the superior temporal sulcus (STS), occipital face area (OFA), and the fusiform face area (FFA) (Deffke et al., 2007; Herrmann et al., 2005; Itier & Taylor, 2004), the enhancement posterior-contralateral would indicate increased facial processing in that visual field. The N2 posterior-contralateral (N2pc) is a negative component peaking around 150-250 ms after stimulus onset in electrodes posterior-contralateral to the salient stimulus. Previous research suggested that the N2pc reflects initial orientation of spatial attention (Diao et al., 2017; Dowdall et al., 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015).

ERP methods have been used in the dot-probe task to study differences between various forms of high anxiety and low anxiety (i.e. trait, social, and panic). Participants with high social anxiety had greater overall P1 amplitudes compared to participants with low social anxiety (Helfinstein et al., 2008). Additionally, Mueller et al. (2009) found that participants with social anxiety disorder had increased P1 amplitudes for angry-neutral trials compared to happy-neutral trials, which was not significant in low social anxiety participants. Another study found no group differences between high and low social anxiety in the N170 (Rossignol et al., 2013). However, enhanced N2pc towards angry faces was found in high social anxiety (Reutter, Hewig, Wieser, & Osinsky, 2017) and trait anxiety (Fox et al., 2008).

Attentional bias towards threat-related stimuli has been suggested to be enhanced in high anxiety participants (Bar-Haim et al., 2007). A recent review found that ERP components can be used to measure the increased attentional bias (Torrence & Troup, 2017). In addition, previous

research suggested that cannabis use might be associated self-medicating for anxiety (Crippa et al., 2009; Rubino et al., 2007) and may have anxiolytic effects (Berrendero & Maldonado, 2002; Patel & Hillard, 2006) but has also been associated with anxiogenic effects (Genn, Tucci, Marco, Viveros, & File, 2004; Viveros et al., 2005). The purpose of this study was to examine if the residual effects of cannabis use was associated with a reduced attentional bias to fearful facial expressions using ERP. We predicted that cannabis users would exhibit reduced biases to fearful faces as measured by the P1, N170, and N2pc ERP components. A reduction in attentional bias could potentially be a mechanism of the anxiolytic effects of cannabis use or increased attentional bias as a mechanism for anxiogenic effects.

Method

Participants

Forty undergraduate students (24 females; ages 18 – 27, $M = 19.66$, $SD = 2.18$) from Colorado State University participated in this study were recruited from Introduction to Psychology and Research Methods courses. Thirty-nine participants were right handed, and all had normal or corrected to normal vision in both eyes and no history of neurological and development disorders. The experiment was approved by Colorado State University Institutional Review Board.

Questionnaires

A personal inventory (Appendix D) was used to determine age, vision, history of disorders, history of medicines used, etc. The Recreational Cannabis Use Questionnaire (RCUE; Appendix E; Troup et al., 2016) was used to measure cannabis use. The RCUE was developed to better understand cannabis use among residents of Colorado and contains questions related to type of use (medical or recreational), method of use (inhalants, edibles, concentrates,

transdermal, etc), duration of use, frequency of use, and past use. The participants were divided into two groups, users and non-users. Non-users having never used or have not used in the last two years, whereas cannabis users were defined as using cannabis at least monthly for more than one year. The RCUE was giving to the participants after giving consent but before the task. This was to screen out participants that did not fall into user or non-user groups. The participants also completed the Center for Epidemiological Studies Depression (CESD; Radloff, 1977), the state portion of the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), and PTSD Checklist for DSM-5 (PCL-5; Weathers et al., 2013).

Dot-probe task

The task was programed in Stim2 (Compumedics USA, Inc., Charlotte, NC, USA) and the stimuli were displayed on a 20-inch PC monitor with a refresh rate of 60 Hz and a screen resolution of 1600 × 900. The stimuli used in the dot-probe task were obtained from Gur et al (2002): two female faces and two male faces. Each face had a fearful expression and a neutral expression. The facial expressions were grey scaled, and a custom cropping tool was used to remove non-face stimuli (e.g. hair, background, etc.). The facial expressions were displayed on the left and right side of the computer screen. The faces subtended 5º x 7º and were separated by 14º of the visual angle (7º from center of monitor) at 59 cm from the screen. The task was programed for the presentation of faces at 50 ms, a delay of 500 ms, and the dot (1 cm in diameter) appearing until response (see Figure 1). There was a 2,000 ms inter trial interval (Figure 1). The task consisted of three trial types, congruent (dot on same side of fearful face), incongruent (dot on opposite side of fearful face) and neutral-neutral (two neutral faces). There was a total of five blocks with 144 trials in each block (48 trials of each trial type), for a total of 720 trials. All trials were randomly presented within each block.

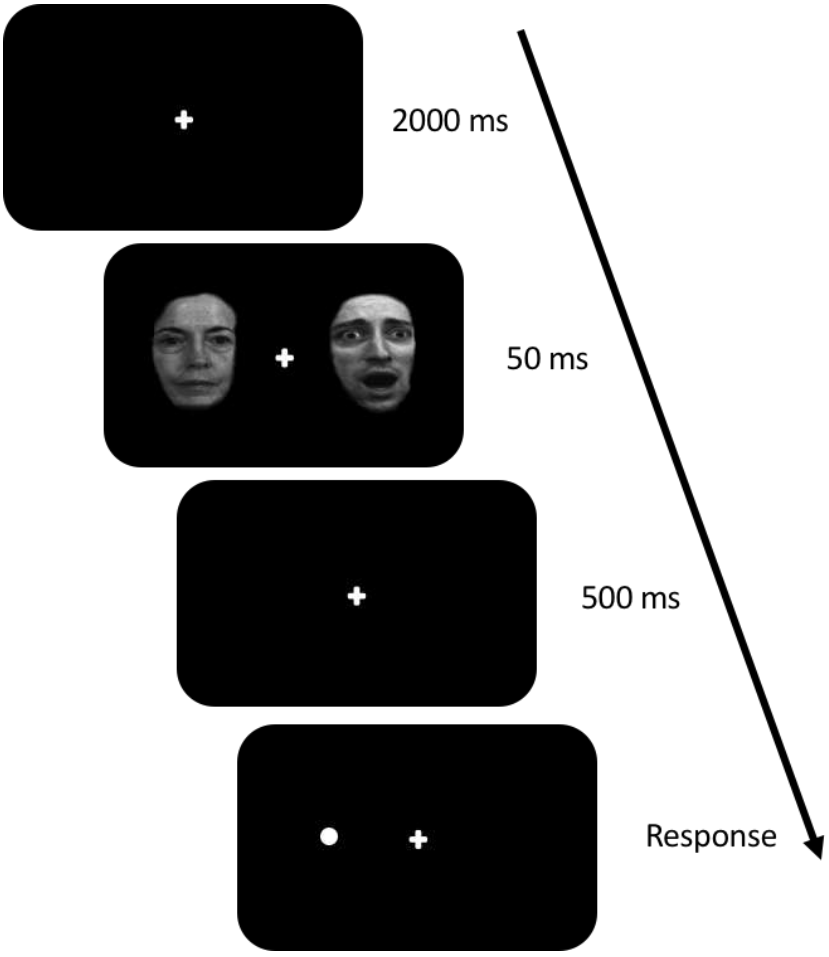

Figure 1: Dot-probe task

EEG collection

The EEG data were acquired using Curry 7 using 33 Ag/AgCl electrodes from a SynAmpsRT 64-channel QuickCap (Compumedics USA, Inc., Charlotte, NC, USA) using the 10-20 system. Ground was located between FZ and FPZ on the midline. The right mastoid was a reference during acquisition and the following electrodes were used for recording: FP1, FP2, F7, F3, FZ, F4, F8, FC5, FC1, FC2, FC6, T7, C3, CZ, C4, T8, CP5, CP1, CP2, CP6, P7, P3, PZ, P4, P8, PO7, PO3, POZ, PO4, PO8, O1, and O2. Horizontal electro-oculogram (HEO) electrodes were placed on the outer canthi of the left and right eye to detect saccades and eye blinks.

Impedances were kept below 10 kΩ using electrolyte gel. Sampling rate was 500 Hz. The default recording bandwidth was from DC to 250 Hz.

ERP analysis

The EEG data was converted from Curry 7 format to EEGLAB format using the EEGLAB toolbox (Delorme & Makeig, 2004). ERPLAB (Lopez-Calderon & Luck, 2014) was used for preprocessing and analyzing the data. The EEG data was re-referenced to the common average reference. The data was filtered with a bandpass of 0.1 to 40 Hz and epochs were extracted from -200 ms to 1000 ms (0 ms being face onset). A simple voltage threshold of -100 and 100 μV were used to remove artifacts. The data was also visually inspected for motion artifacts. Participants were removed if there was greater than 40% rejected trials of one or more trial type. Mean amplitudes were analyzed for C1 (50 – 80 ms) and P1 (80 – 120 ms) components were analyzed from O1 and O2 electrodes. Posterior-contralateral N170 (150 – 190 ms) and N2pc (250 – 320 ms) from the P7 and P8 electrodes. All components were time-locked to face onset.

Data analysis

C1 and P1 amplitudes for trials that included fearful faces were analyzed between groups using an independent t-test. Electrodes O1 and O2 were averaged for analyses using independent t-tests. For posterior-contralateral N170 and N2pc, contralateral was defined as P7 fear right trials and P8 fear left trials averaged together and ipsilateral P7 fear left and P8 fear right trials averaged together. For the N170 and N2pc components, a mixed-factor ANOVA, 2 (users and non-users) × 2 (contralateral and ipsilateral) was used to determine differences in mean amplitude between groups and conditions. Bonferroni corrections were used when appropriate and for planned within group differences.

Results

One participant was removed because of too many rejected trials (70% rejected) leaving 39 participants. There were 20 non-users (15 females) and 19 users (9 females) (Table 1). There was no difference between groups in age, STAI, CES-D, or the PCL-5. The PCL-5 difference approached significance, however, $t(36) = -1.67$, $p = .104$, $d = .459$. Cannabis users (one cannabis user did not complete the PCL-5) had a mean PCL-5 of 21.22 ($SD = 17.12$) and the non-users had a mean of 13.2 ($SD = 12.37$).

Table 1

Mean scores and standard deviations of age, STAI, CES-D, PCL-5, age of onset, and monthly use

	Cannabis users ($n = 19$)		Non-users ($n = 20$)	
	M	*SD*	*M*	*SD*
Age	19.84	2.34	19.5	2.06
STAI-State	40.78	7.99	37.68	11.25
CES-D	19.26	9.52	18	11.26
PCL-5	21.22	17.12	13.2	12.37
Age Onset	15.84	1.98	-	-
Monthly use	12.79	17.11	-	-

Note: There were no statistical significances between groups in age and questionnaires. PCL-5 was approaching significance, $p = .104$.

C1

There was no significant difference between users ($M = -0.23$, $SD = 0.81$) and non-users ($M = -0.11$, $SD = 0.97$) in C1, $t(37) = 0.40$, $p = .690$, $d = .134$ (Figure 2).

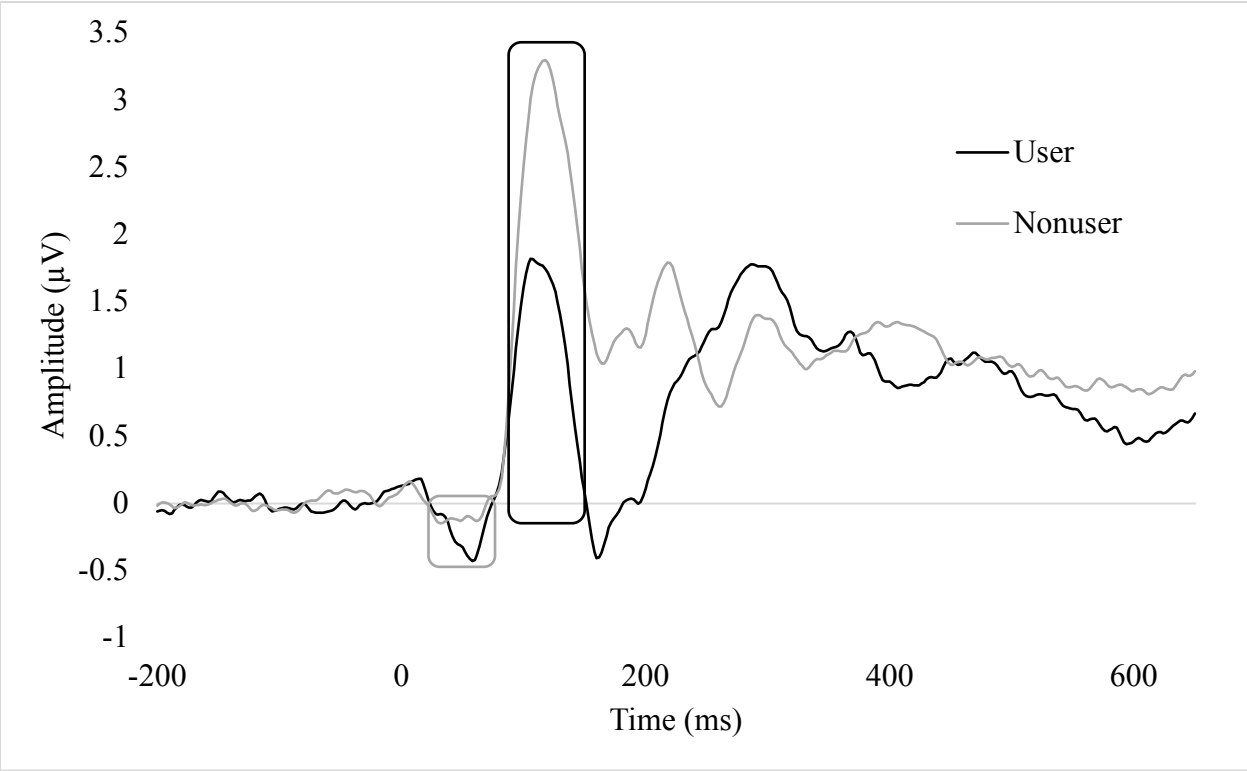

Figure 2: Event-related potentials from O1 and O2

P1

There was a significant difference in P1 amplitude between cannabis users and non-users, $t(37) = 2.08$, $p = .044$, $d = .672$. Non-users ($M = 2.71$, $SE = 0.55$) had significantly greater P1 amplitude than cannabis users ($M = 1.36$, $SE = 0.33$) (Figure 2).

N170

There was a significant main effect for condition, $F(1, 37) = 17.11$, $p = .000$, $\eta_p^2 = .316$. Bonferroni post hoc comparisons indicated that contralateral electrodes ($M = -1.99$, $SE = 0.70$) had more negative amplitude than ipsilateral electrodes ($M = -1.71$, $SE = 0.70$, $p = .000$). There was not a significant interaction, $F(1, 37) = 0.68$, $p = .417$, $\eta_p^2 = .018$, indicating that there were no significant differences between users and non-users in contralateral and ipsilateral ($p > .200$). However, Bonferroni corrected planned comparisons within both groups suggested that in non-

users, contralateral (M = -1.53, SE = 0.54) was more negative compared to ipsilateral (M = -1.30, SE = 0.50, p = .023). Within cannabis users, contralateral (M = -2.46, SE = 0.55) was also more negative compared to ipsilateral (M = -2.11, SE = 0.51. p = .001) (Figure 3).

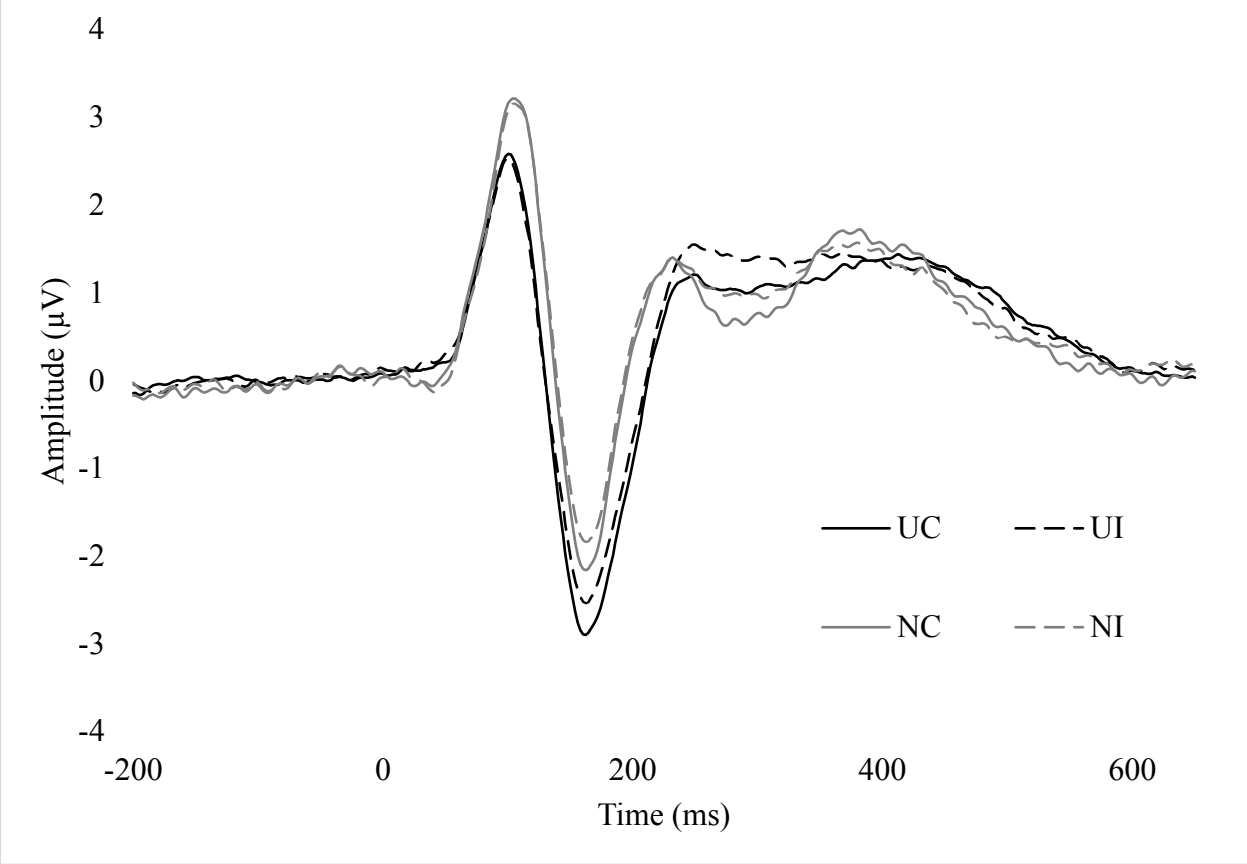

Figure 3: Event-related potentials from P7 and P8

N2pc

There was a significant main effect for condition, $F(1, 37) = 8.17$, $p = .007$, $\eta_p^2 = .181$. Bonferroni post hoc comparisons indicated that contralateral (M = 0.94, SE = 0.28) was more negative than ipsilateral (M = 1.23, SE = 0.28. p = .007). There was no significant interaction between groups and conditions, $F(1, 37) = 0.36$, $p = .553$, $\eta_p^2 = .010$, suggesting the neither condition was different between users and non-users. Within group Bonferroni corrected planned analysis suggested that there was no significant difference between contralateral (M = 0.81, SE =

0.39) and ipsilateral (M = 1.05, SE = 0.35, p = .114) in non-users, however, contralateral (M = 1.07, SE = 0.40) was more negative than ipsilateral (M = 1.45, SE = 0.36, p = .021) in cannabis users (Figure 3). However, an independent t-test was used to explore group differences in difference score (contralateral – ipsilateral). Cannabis users (M = -0.36, SD = 0.47) were not different than non-users (M = -0.23, SD = 0.77), $t(37) = 0.60$, p = .553, d = .204.

Exploratory Analysis

An exploratory correlation analysis was conducted examining the relationship between N170 difference (contralateral minus ipsilateral) and reported monthly use. There was a significant positive correlation between N170 difference and monthly use, R^2 = .236, $F(1, 17) = 5.26$, p = .035 (Figure 4). No other ERP component was correlated with monthly use. Additionally, uncorrected for multiple comparisons STAI was neither correlated with monthly use nor the ERP components (within groups and overall). We also conducted an exploratory analysis to examine sex differences. The same analyses for cannabis use was used between males and females (ignoring cannabis use) and the results yielded no significant differences between sex in the ERP components.

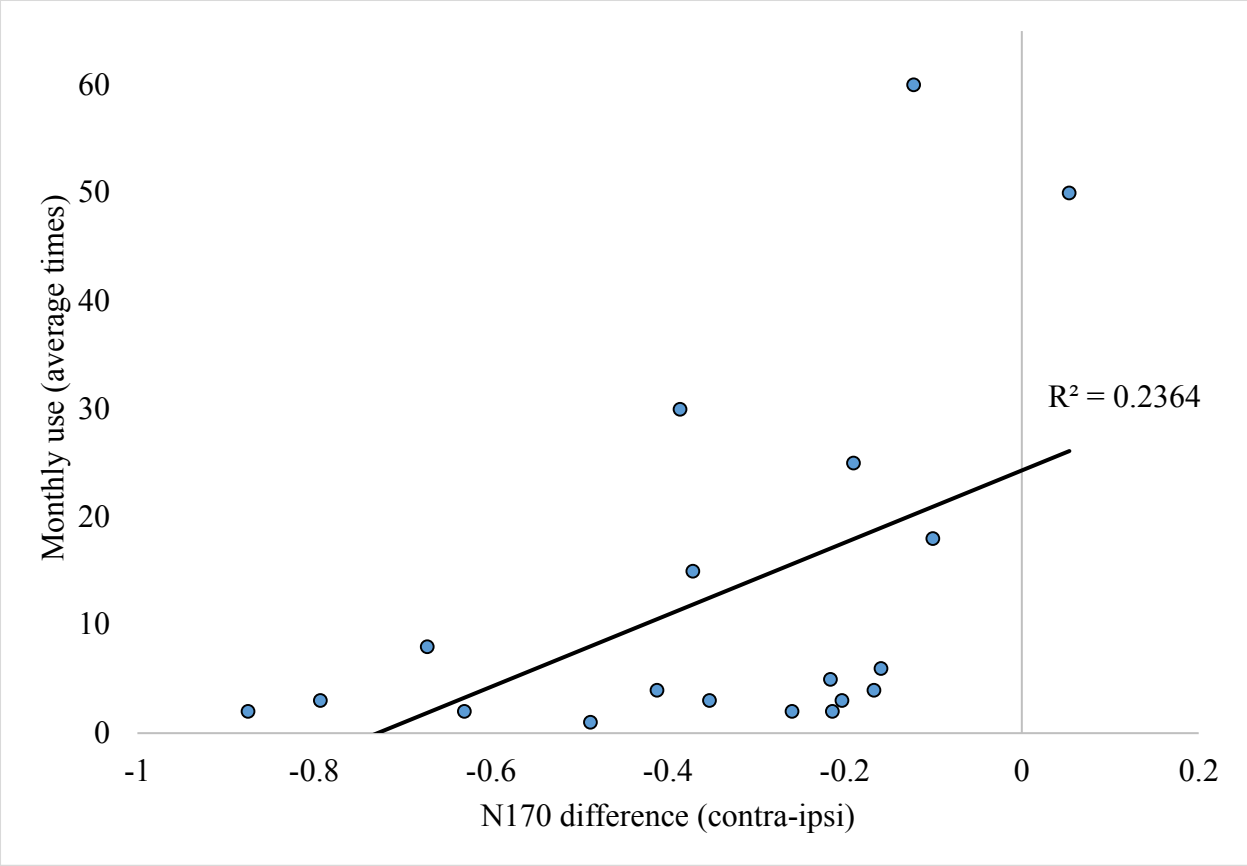

Figure 4: Monthly Use and N170 Differences

Discussion

The ERP results indicated that there were differences between cannabis user and non-users in their response to fearful faces in the dot probe task. Specifically, non-users had greater P1 amplitudes than users in response to fearful faces. Greater P1 amplitude towards threat-related facial expressions has been associated with anxiety (Mueller et al., 2009). Both groups had differences in between contralateral and ipsilateral N170 indicating that both groups had enhanced face processing to the fearful facial expression. However, there was a correlation between monthly cannabis use and N170 difference. That is, the more frequent cannabis users had less difference between contralateral and ipsilateral N170. Only cannabis users had

differences in N2pc, which would suggest cannabis users had greater orientation towards the fearful facial expression.

Although N2pc showed an attentional bias towards fearful faces, the P1 data suggested that cannabis users had reduced attention towards fearful faces compared to non-users. Previous research found that individuals with low social anxiety had reduced P1 amplitudes when a negative facial expression was present as compared to high social anxiety individuals (Helfinstein et al., 2008). Enhanced P1 amplitude towards threat-related facial expressions is thought to reflect increased processing in the visual cortex modulated by the amygdala (Carlson et al., 2009; Pourtois et al., 2004). Even though this study found no correlations with state anxiety, the results did suggest that residual effects of cannabis were related to decreased in initial attention to fearful faces and may be a mechanism of how cannabis can have anxiolytic effects.

The significant main effect for condition is consistent with previous research that suggested enhanced N170 amplitudes posterior-contralateral to the treat-related facial expression (Carlson & Reinke, 2010; Rossignol et al., 2013). There were no group differences in N170 amplitude, but each group had differences between contralateral and ipsilateral. The appeared group differences in N170 could be driven by the difference in P1. Given that both groups had attentional bias towards the fearful face indicates that there was no difference between cannabis users and non-users in processing the fearful facial expression. However, a within cannabis group correlation revealed that the more cannabis used in a month was related to less difference in contralateral and ipsilateral N170 amplitudes. That is, heavier users had less processing of the fearful facial expressions compared to neutral. A recent review suggested that the N170 was not different between social anxiety and controls when the participants viewed emotional facial

expression (Harrewijn et al., 2017). Similarly, in the dot-probe task, Rossignol et al. (2013)suggested that fear of negative evaluation, a component of social anxiety, had no effect on N170 amplitude in contralateral electrodes. The enhanced contralateral N170 may reflect increased processing of the fearful facial expression in the contralateral STS but may not reflect the hypervigilance seen in anxiety (Harrewijn et al., 2017).

Since the N170 may not be related to increased attentional bias in anxiety, an alternative explanation is that increased cannabis use is associated with decreased emotional processing of facial expression. A recent study by Brooks and Brenner (2017) suggested non-users had enhanced N170 amplitudes to faces compared to heavy cannabis users (more than once a week) and moderate users (once or twice a month), there were no statistical differences between cannabis groups. Additionally, these researchers found that the attenuated N170 in cannabis users was similar individuals with high schizotypal personality traits in that there was reduced N170 amplitudes to faces compared to controls. The results of this study build upon the results of Brooks and Brenner (2017) that heavy cannabis use is associated with attenuated N170 towards fearful facial expressions.

Unlike posterior-contralateral N170, this study only found differences in N2pc within cannabis users, although there was a trend in non-users. The N2pc is thought to reflect an initial shift in orientation towards a salient stimuli (Diao et al., 2017; Dowdall et al., 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015). Furthermore, the N2pc has been correlated with increased theta oscillations towards angry facial expressions in a dot-probe task (Diao et al., 2017). Maratos, Mogg, Bradley, Rippon, and Senior (2009) used magnetoencephalography (MEG) to study theta oscillations in the amygdala while the participants examined blurry and normal emotional facial expression. The researchers suggested that there were increased theta

oscillations in the amygdala in blurry threat-related faces compared to neutral. Additionally, theta oscillations were found in the primary visual cortex and frontal cortex 50 to 250 ms after stimulus onset. Reutter et al. (2017) found enhanced N2pc correlated with increased social anxiety. Assuming the N2pc reflects theta oscillations in the visual cortex which is mediated by theta oscillations in the amygdala, it is no surprising that fMRI research has found hyperactivation of the amygdala when viewing negative facial expression in patients with generalized social phobia (Phan, Fitzgerald, Nathan, & Tancer, 2006). In this current study, only cannabis users had enhanced N2pc amplitudes towards fearful faces suggesting that, unlike P1, cannabis users had increased attentional bias which resembles what would be found in anxiety. However, frequency of use had no relationship with N2pc difference scores (contralateral minus ipsilateral).

Alterations in attentional bias may be a mechanism of how cannabis can have anxiolytic effects. Previous research suggested that individuals with low anxiety have less attentional bias towards threat-related stimuli compared to individuals with high anxiety (Bar-Haim et al., 2007). Attention bias modification training, train attention away from threat, could be a potential route to reducing anxiety related symptoms (Mogg, Waters, & Bradley, 2017). Cannabis may also reduce initial attentional response and face processing but does no effect spatial orientation of attention. Although, selecting anxiety groups within cannabis users and non-users was outside the scope of this study, future research could select individuals with high or low anxiety that use cannabis to determine if a reduction in attentional bias is associated with reduced anxiety.

Although this study found that cannabis use was associated with reduced attention towards fearful facial expression, there were limitations. Examining ERPs time-locked to the probe would not have been reliable since there was a 500 ms delay from face offset to dot onset.

Behavioral research has suggested that 300 ms after face onset, attentional is no longer allocated to the location of the fearful face (Torrence et al., 2017). P1 time-locked to the probe has been used to examine engagement to an attended location. In congruent trials, the amplitude for P1 is more than for incongruent. Pourtois et al. (2005) conducted a source analysis for P1 and they suggested that for congruent trials P1 was associated with posterior parietal and inferior temporal cortices, whereas incongruent trials were associated with anterior cingulate cortex. Given that some research has suggested anxiety is also related to a delayed disengagement and not only rapid orientation (Fox, Russo, & Dutton, 2002), examining P1 time-locked to the probe would add to these current findings. Additionally, we did not find a wide range of frequency of cannabis use in our sample. Future research could select different groups of heavy, moderate, and seldom cannabis use. Given that high cannabis use frequency has been associated with CB1 desensitization and downregulation (Lazenka & Selley, 2013), it is possible that heavy, long-term users would experience more anxiety and have greater attentional bias towards threat-related stimuli. Another limitation was that the ratio of men to women were different between cannabis users and non-users in our sample. Previous research has suggested that there are behavioral and ERP related differences in attentional bias between males and females (see Torrence & Troup, 2017).

This study was the first to examine the effects of residual cannabis use on attentional bias towards facial expressions using ERPs. The results suggested that there was a reduction in early attention response to fearful faces, as measured by P1, in cannabis users. Frequency of cannabis use was also associated with reduced processing of fearful faces, measured by N170. However, cannabis use was associated with increased attentional bias towards fearful facial expression in N2pc whereas non-users had no difference. Given the neuroanatomical differences, within the

attentional bias network, correlated with cannabis use (Lorenzetti et al., 2016), the results here suggest that there are also functional differences in attentional bias towards threat-related stimuli within cannabis users.

Chapter 3 – Experiment 2

Introduction

There has been a significant increase in cannabis use among adolescents between 2002 and 2015 (Substance Abuse and Mental Health Services Administration, 2016). In the United States, cannabis is currently federally illegal, but 29 states and Washington D.C. have legalized cannabis for medical use and eight of those states and Washington D.C. legalized it for recreational use (National Conference of State Legislatures, 2018). Given that cannabis use has increased in availability and use, it is important to understand the effects cannabis use has on brain and behavior. Previous research has suggested that cannabis use was correlated with decreased in memory, attention, and emotional processing (Broyd et al., 2016; Lovell et al., 2018; Troup, Bastidas, et al., 2016; Troup et al., 2017). Neuroanatomical differences have also been found between cannabis users and non-users, specifically in the amygdala, prefrontal cortex (PFC), and insula (Lorenzetti et al., 2016). The endocannabinoid system has been a target for treatment of anxiety related disorders (Korem, Zer-Aviv, Ganon-Elazar, Abush, & Akirav, 2016; C. Rabinak & Phan, 2014), however, how cannabis might affect emotion processing disorders is unclear. The main phytocannabinoid found in cannabis, Δ9-tetrahydrocannabinol (THC), has been suggested to have anxiolytic effects (Berrendero & Maldonado, 2002; Patel & Hillard, 2006; Rubino et al., 2007; Viveros et al., 2005), although other research has indicated that excessive cannabis use has anxiogenic effects (Viveros et al., 2005). Individuals with anxiety tend to have enhanced attention and processing of threat-related (Bar-Haim et al., 2007) and positive (Morel et al., 2014) stimuli. These differences in attentional processing can be measured using event-related potentials (ERPs) (Harrewijn et al., 2017). The aim of this study was to

examine the residual effects of cannabis use on attention to emotional facial expressions when awareness was restricted versus when awareness was not restricted using a backward masking paradigm.

Backward masking occurs when a target face is displayed for a short duration and then is immediately replaced by a mask stimulus (neutral face or scrambled face). Pessoa, Japee, and Ungerleider (2005) conducted a behavioral study in which they varied the target face (fearful, happy, or neutral face) and target duration (17, 33, and 83 ms) to test the awareness threshold duration for the target face. The researchers found that a target face duration of 17 ms was below the awareness threshold, at least for most of the participants (nine out of 11); at 33 ms, seven out of 11 participants scored above chance level in detecting the target face, and all of the participants were aware of the target face displayed for 83 ms. These results suggested there are individual differences in perceptual awareness and establish that backward masking is most effective when the target faces are displayed for 17 ms or less.

Backward masking fMRI studies suggested the amygdala was more active for negative facial expressions compared to neutral or happy faces, even when awareness was restricted (Morris, 1998; Suslow et al., 2006; Whalen et al., 1998). Similarly, dot-probe task fMRI research found that the visual cortex had increased activity when attending toward fearful faces (Carlson et al., 2011; Pourtois, Schwartz, Seghier, Lazeyras, & Vuilleumier, 2006) and visual cortex activity was correlated with amygdala activity (Carlson et al., 2009). Researchers have used event-related potentials (ERPs) to measure the time-course of processing facial expressions. A number of studies indicated that even when awareness of emotional facial expressions was restricted using backward masking, multiple ERP components were modulated by negative target faces (fear and anger) compared to non-negative faces (happy and neutral) (Del Zotto & Pegna,

2015; Pegna, Darque, Berrut, & Khateb, 2011; Pegna et al., 2008; Vukusic et al., 2017). The P1 ERP component has a positive peak around 80 to 120 ms in the lateral occipital electrodes. Participants with high trait anxiety had more enhanced P1 amplitude to happy faces compared to neutral and there was no difference between fear and neutral (Morel et al., 2014) whereas other research suggested that P1 was more enhanced in high anxiety towards negative stimuli (Harrewijn et al., 2017; Helfinstein et al., 2008; Holmes, Nielsen, & Green, 2008; Mueller et al., 2009; Torrence & Troup, 2017). The N170 component is a negative deflection in the ERP waveform which peaks around 170 ms after stimulus onset and is found in lateral posterior electrodes, which typically has a right hemisphere lateralization (Bentin et al., 1996). A meta-analysis indicated that the N170 is sensitive to facial expression, especially to anger, fear, and happiness expressions (Hinojosa et al., 2015). In addition, these studies found that the overall amplitude of N170 was more negative for faces displayed for a long duration compared to a short duration. That is, when awareness of a fearful face was restricted, the N170 was enhanced compared to neutral. The same was found when awareness was not restricted, but the amplitude in the aware condition was more negative overall. Source localization of the N170 was found to originate in the right extrastriata visual cortex (Pegna et al., 2008). However, another study indicated that emotional expression did not influence the N170 (Kiss & Eimer, 2008).

In addition to P1 and N170, the N2 component has also been suggested to indicate orientation to salient facial expressions regardless of awareness (Liddell et al., 2004; Vukusic et al., 2017). The N2 ERP component the second negative peak occurring 180 to 300 ms and can be found in central electrodes (i.e. FZ, CZ, and PZ). Contrary to Liddell et al. (2004) and Vukusic et al. (2017), Pegna et al. (2008) only found N2 differences in unmasked conditions as opposed to masked. Other research suggested the N2 component involves cognitive control, or controlling

actions (Folstein & Van Petten, 2008). Although the source of anterior N2 is debated, it is thought to originate in the medial frontal cortex (e.g. ACC) and the right inferior frontal cortex (Aron et al., 2016; Folstein & Van Petten, 2008; Ridderinkhof & Ullsperger, 2004). Lastly, the P3 ERP component has multiple subcomponents, but this article focuses on the later P3 between 400 and 600 ms and found in central, posterior electrodes (Kiss & Eimer, 2008). The enhanced P3 amplitude reflects higher level emotional and attentional processing (Johnston, Miller, & Burleson, 1986; Polich, 2007). Previous research suggested that cannabis use modulates the P3 amplitude towards emotional facial expressions, particularly in implicitly processed (Troup, Bastidas, et al., 2016; Troup et al., 2017).

The main purpose of this study was to examine the residual effects of cannabis use on processing facial under restricted awareness. Given the neural networks involved in attending to and being aware of emotional faces, as well as the neural networks that are affected by cannabis (Lorenzetti et al., 2016), we hypothesized that individuals that use cannabis would have residual attenuation in their ERPs in responses to emotional faces. More specifically, cannabis users would have reduced (less enhanced) P1, N170, N2, and P3 amplitudes to fearful and happy facial expression.

Method

Participants

Forty adults from Colorado State University and members of the Fort Collins community participated in this study (23 females, 1 non-binary, age $M = 23.75$, $SD = 3.94$). Participants were recruited from students enrolled in summer courses and received extra credit. The students also received extra credit for each person they recruited from the community. Thirty-three reported they were right handed and four indicated they were ambidextrous. The participants

reported normal or corrected vision and no history of brain injury or psychiatric disorders. All participants provided informed consent before participating. This experiment was approved by the Colorado State University Institutional Review Board.

Questionnaires

A custom personal inventory was used to assess recent use of caffeine, tobacco, cannabis, and alcohol, as well as age, vision, history of disorders, and medicines used. To divide the participants into cannabis users and non-users the Recreational Cannabis Use Questionnaire (RCUE; Troup et al., 2016) was used. The RCUE asked the participants about their history of cannabis use, including average monthly use and preferred method (e.g. smoking, edibles, dabs, etc). Cannabis users were defined as using more than monthly for at least one year. Non-users were defined as never using or not using in the last two years. The Center for Epidemiological Studies Depression scale (CESD; Radloff, 1997), the state portion of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983), and PTSD Checklist for DSM-5 (PCL-5; Weathers et al., 2013). were collected for exploratory analyses.

Awareness task

The facial stimuli were neutral, happy, and fearful facial expressions from the Karolinska Directed Emotional Faces database (Lundqvist, Flykt, & Öhman, 1998). All non-face stimuli (i.e. background, hair, neck and ears) were cropped and the faces were greyscale. The task was programed and displayed using Stim2 software (Compumedics USA, Inc., Charlotte, NC, USA). This task displayed one face at a time in the center of the screen at 3° × 4.4° of the visual angle on a 20-inch, 144 Hz LCD monitor. Trials started with a white fixation cue (+) for 1000 ms on a black screen, followed by the target face (fearful, happy, or neutral expression) and immediately replaced with the masking face (neutral face with open mouth). In the restricted awareness

(masked) condition the target face was displayed for either 16.66 ms followed by 150 ms mask. The aware condition (unmasked) displayed the target face for 133.33 ms and the mask was displayed for 33.33 ms. In both conditions there was a stimulus present for the same amount of time (166.66 ms). However, after all the data was collected, we tested the actual stimulus duration using a photodiode (AMS Technologies, model TSL257) and Arduino Micro microcontroller. We found that the 16.66 ms duration was actually 30 ms, 150 ms was 151 ms, 133 ms was 135 ms, and 33 ms was 44 ms. After the masking face a fixation cue was present for 500 ms followed by a question. The question asked the participants whether the target face was fearful, happy, or neutral, to which the participants responded using the number pad on a keyboard (1, 2, or 3) (see Figure 5). The participants were told before the task to use their gut instinct and respond as quickly as possible. The task was divided into 13 blocks with 72 trials in each block for a total of 936 trials. Within each block, there were equal number of trial types (duration and emotion) presented randomly. The task took between 35 min and 45 min, depending on how long the participant took to respond and how long they took between blocks.

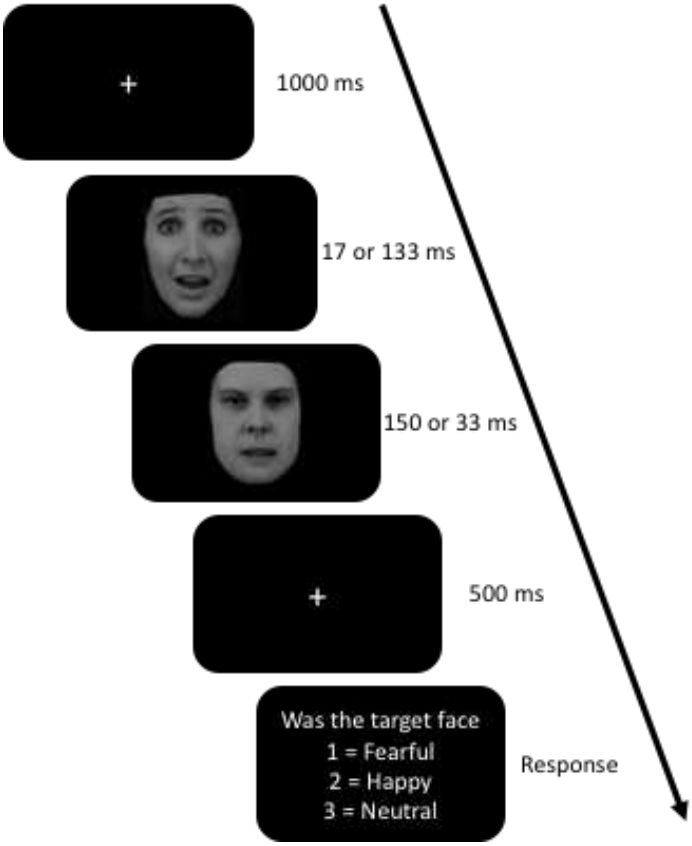

Figure 5: Awareness Task

EEG data collection

The acquisition software used to collect the EEG data was Curry 7 using 33 Ag/AgCl electrodes from a SynAmpsRT 64-channel QuickCap (Compumedics USA, Inc., Charlotte, NC, USA). The following electrodes were used for recording: FP1, FP2, F7, F3, FZ, F4, F8, FC5, FC1, FC2, FC6, T7, C3, CZ, C4, T8, CP5, CP1, CP2, CP6, P7, P3, PZ, P4, P8, PO7, PO3, POZ, PO4, PO8, O1, and O2 with the right mastoid was a reference during acquisition and ground located between FCZ and FZ. Neurocompumedics Quick Gel was used to reduce impedances which were kept below 10 kΩ. Horizontal electro-oculogram (HEO) electrodes were placed on the outer canthi of the left and right eye. The sampling rate was 500 Hz and the recording bandwidth was DC to 250 Hz.

Mean amplitudes were calculated for each ERP component time locked to target face onset for all trials (correct and incorrect trials). P1 (80 – 120 ms) was taken from the O2 electrode, N170 (150 – 190 ms) was taken from P7 and P8 electrodes, N2 (180 – 300 ms) was examine using FZ, CZ, and PZ (Vukusic et al., 2017), and P3 (400 – 600 ms) from PZ electrode (Kiss & Eimer, 2008).

Data analysis

We used uncorrected *t*-tests to examine group differences in questionnaires and age. The behavioral data was calculated as a percent correct for each emotion in each duration (masked or unmasked). A 3 (emotion) × 2 (duration) × 2 (group) ANOVA was used for the behavioral data, P1, and P3. For N170 we used a 3 (emotion) × 2 (duration) × 2 (hemisphere) × 2 (group) ANOVA with P7 and P8 electrodes for hemisphere. N2 was examined using a 3 (emotion) × 2 (duration) × 3 (electrode) × 2 (group) ANOVA. Greenhouse-Geisser and Bonferroni corrected comparisons were used when appropriate.

Results

Four participants were removed from the study, one had 73% of their trials rejected, two reported vision problems (one had an under developed left optic nerve and the other had nystagmus), and the fourth was stopped early due to all the electrodes going over the impendence threshold. This left 18 cannabis users (10 females) and 18 non-users (11 females, 1 non-binary) (Table 1). Two cannabis users did not complete the STAI and one non-user did not complete the CES-D, their data were excluded from any analyses involving those questionnaires. There were no significant differences between age, STAI, CES-D, or PCL5.

Table 2

Mean scores and standard deviations of age, STAI, CES-D, PCL-5, age of onset, and monthly use

	Cannabis users (n = 18)		Non-users (n = 18)	
	M	*SD*	*M*	*SD*
Age	23.94	4.19	23.56	3.78
STAI-State	37.13	11.62	33.83	8.48
CES-D	17.06	9.60	13.12	6.87
PCL-5	16.83	19.59	14.28	14.12
Age Onset	15.88	2.03	-	-
Monthly use	27.33	31.08	-	-

Note: There were no significant differences between groups in any of the measures

Behavioral

There was a significant main effect emotion $F(1.84, 62.52) = 4.42$, $p = .018$, $\eta_p^2 = .115$. Bonferroni post hoc comparisons indicated that participants had less accuracy to fearful (M = 0.73, SE = 0.02) compared to happy (M = 0.81, SE = 0.02). There was also a significant main effect for duration $F(1, 34) = 401.70$, $p < .001$, $\eta_p^2 = .922$. Subjects were less accurate in the masked condition (M = 0.62, SE = 0.02) had less accuracy compared to unmasked condition (M = 0.93, SE = 0.01). The interaction between group and duration approached significance, $F(1, 34) = 3.75$, $p = .061$, $\eta_p^2 = .099$. While there were no differences between groups, both groups had within group differences between masked and unmasked. Even though there were no group effects there was variability in accuracy, especially in the masked condition (see Figure 6).

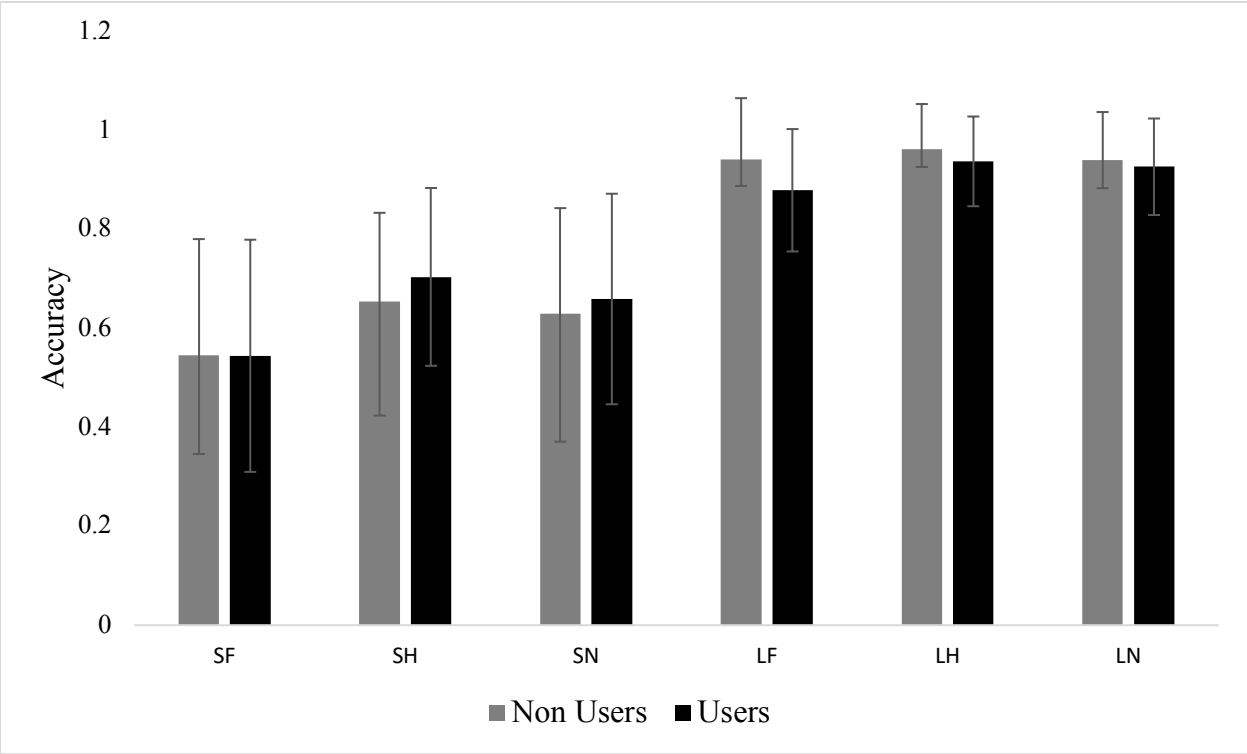

Figure 6: Mean accuracy for each group in each condition

P1

We found a significant main effect for emotion, $F(1.43, 48.64) = 8.87$, $p = .002$, $\eta_p^2 = .207$. Happy ($M = 4.36$, $SE = 0.49$) was significantly greater than fearful ($M = 3.93$, $SE = 0.48$, $p = .004$) and neutral ($M = 3.95$, $SE = 0.47$, $p = .018$). No difference was found between fear and neutral. There was also a significant main effect for duration, $F(1, 34) = 14.88$, $p < .001$, $\eta_p^2 = .304$. The masked condition ($M = 4.29$, $SE = 0.47$) was significantly greater than unmasked ($M = 3.87$, $SE = 0.49$, $p < .001$).

No significant group interactions were observed. However, there was a trend in emotion by group, $F(1.43, 48.64) = 3.03$, $p = .074$, $\eta_p^2 = .081$. There were no differences between emotions within non-users, but within cannabis users, happy ($M = 4.58$, $SE = 0.70$) was greater than fear ($M = 3.99$, $SE = 0.68$, $p = .005$) and neutral ($M = 3.90$, $SE = 0.67$, $p = .004$) (Figure 7).

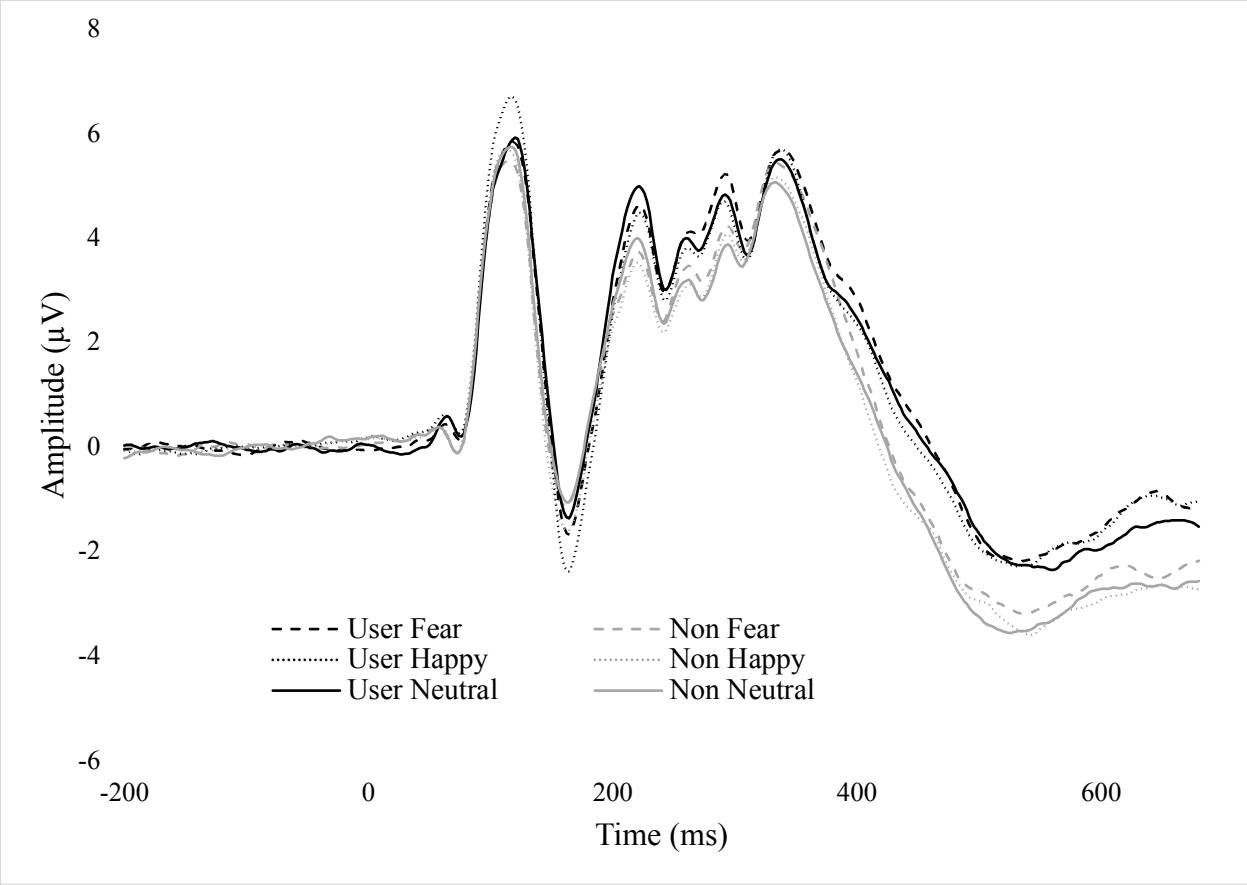

Figure 7: ERP wave from O2

N170

There was a significant main effect for emotion, $F(2, 68) = 24.31$, $p < .001$, $\eta_p^2 = .417$. All three emotions were significantly different from each other, happy ($M = -3.19$, $SE = 0.45$) was more enhanced than fearful ($M = -2.89$, $SE = 0.45$) and both were more enhanced than neutral ($M = -2.50$, $SE = 0.41$), $ps < .002$. There was also a significant main effect for duration, $F(1, 34) = 10.90$, $p = .002$, $\eta_p^2 = .423$. The unmasked faces ($M = -2.99$, $SE = 0.44$) elicited an enhanced N170 compared to masked ($M = -2.73$, $SE = 0.43$), $p = .002$. Hemisphere also a had a significant main effect, $F(1, 34) = 7.47$, $p = .010$, $\eta_p^2 = .180$. Overall the P8 electrode ($M = -$

3.56, *SE* = 0.56) was more negative than P7 (*M* = -2.16, *SE* = 0.44), *p* = .010. No other main effects or interactions were significant.

However, the interaction between group, emotion, duration, and hemisphere was approached significance, *F* (2, 68) = 2.76, *p* = .071, η_p^2 = .075. An exploratory Bonferroni post hoc comparisons indicated that there was hemisphere lateralization (i.e. enhanced N170 in P8 compared to P7) in non-users for masked happy (P7 *M* = -1.94, *SE* = 0.63; P8 *M* = -3.85, *SE* = 0.83, *p* = .017), unmasked happy (P7 *M* = -2.34, *SE* = 0.68; P8 *M* = -4.12, *SE* = 0.84, *p* = .034), masked neutral (P7 *M* = -1.72, *SE* = 0.59; P8 *M* = -3.22, *SE* =0.76, *p* = .038), and unmasked neutral (P7 *M* = -1.53, *SE* = 0.63; P8 *M* = -3.39, *SE* = 0.76, *p* = .015). Cannabis users, however, did not have any significant differences in hemisphere lateralization (Figure 8).

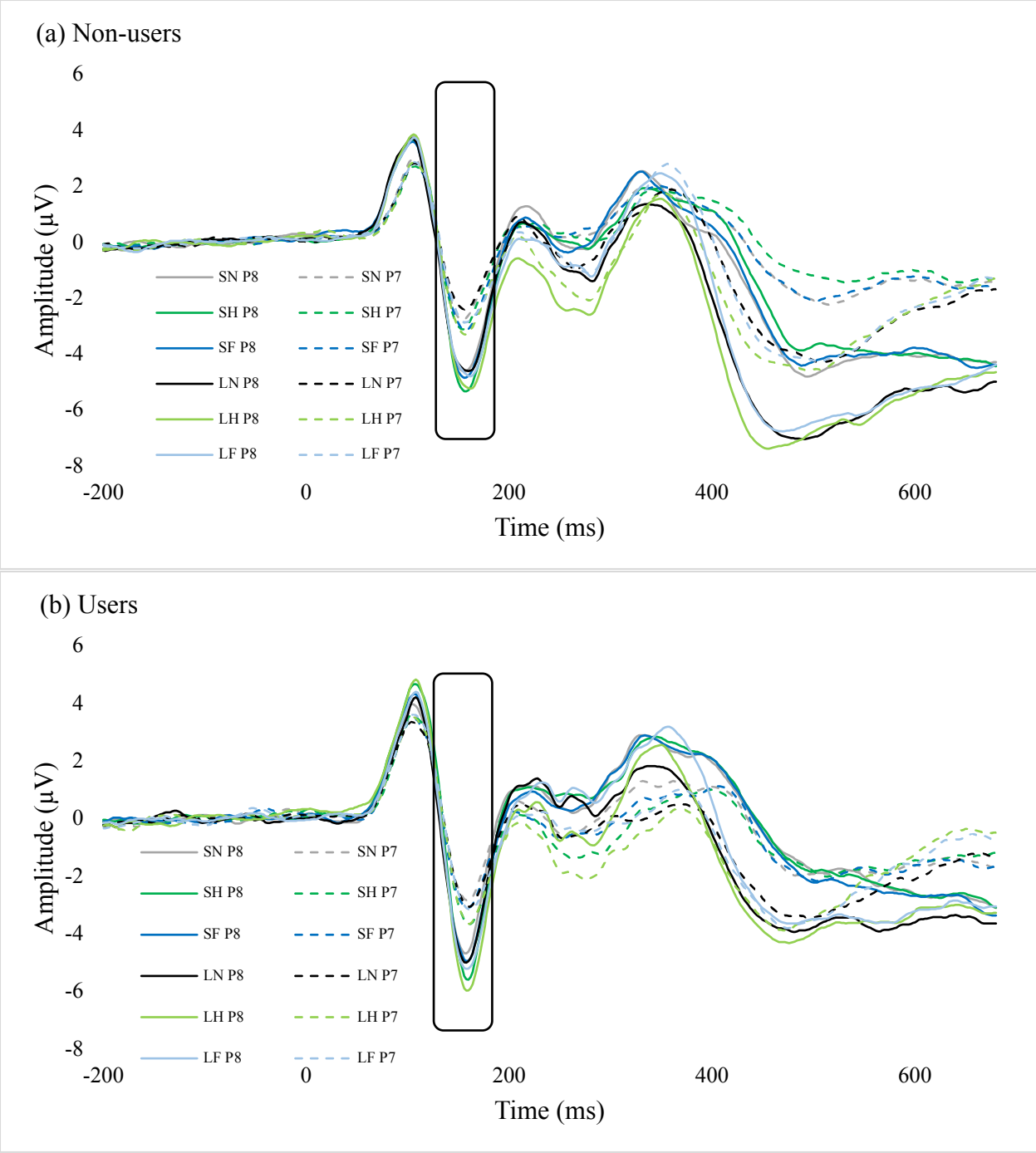

Figure 8: ERP waves from P7 and P8 for each group

N2

There was a significant main effect for emotion, $F(2, 68) = 15.59$, $p < .001$, $\eta_p^2 = .314$. Fear ($M = -0.67$, $SE = 0.28$) and neutral ($M = -0.62$, $SE = 0.26$) were more negative than happy

(*M* = -0.39, *SE* = 0.28, *p*s < .001), no difference between fear and neutral. There was also a significant main effect for electrode, $F(1.12, 37.68) = 35.20$, $p < .001$, $\eta_p^2 = .509$. FZ (*M* = -2.30, *SE* = 0.47) was more negative than CZ (*M* = -1.15, *SE* = 0.36) which both were more negative than PZ (*M* = 1.78, *SE* = 0.35, *p*s < .001). There was a significant interaction between emotion and duration, $F(2, 68) = 2.60$, $p = .002$, $\eta_p^2 = .167$. Post hoc comparisons revealed no differences within the masked condition but in the unmasked condition, happy (*M* = -0.22, *SE* = 0.30) was greater than fear (*M* = -0.65, *SE* = 0.30, *p* < .001) and neutral (*M* = -0.61, *SE* = 0.28, *p* < .001), no difference between fear and neutral. There was a significant interaction between emotion, duration, and electrode, $F(2.53, 85.98) = 9.49$, $p < .001$, $\eta_p^2 = .218$. Within FZ, there were no differences between masked emotional expression, however in the unmasked condition, happy (*M* = -1.54, *SE* = 0.56) was greater than fear (*M* = -2.52, *SE* = 0.49, *p* < .001) and neutral (*M* = -2.47, *SE* = 0.49, *p* < .001). Similarly, in CZ no differences in masked faces but in unmasked, happy (*M* = -0.88, *SE* = 0.40) was greater than fear (*M* = -1.44, *SE* = 0.39, *p* < .001) and neutral (*M* = -1.29, *SE* = 0.35, *p* < .001). No differences were found within PZ.

There was also a significant interaction between group and duration, $F(1, 34) = 4.66$, $p = .038$, $\eta_p^2 = .120$. There were no between group differences in duration. However, within non-users masked faces (*M* = -0.72, *SE* = 0.37) had more negative amplitudes than unmasked (*M* = -0.35, *SE* = 0.40, *p* = .019). Within cannabis users, there was no difference between masked (*M* = -0.54, *SE* = 0.37) and unmasked (*M* = -0.63, *SE* = 0.41, *p* = .568) conditions (Figure 9).

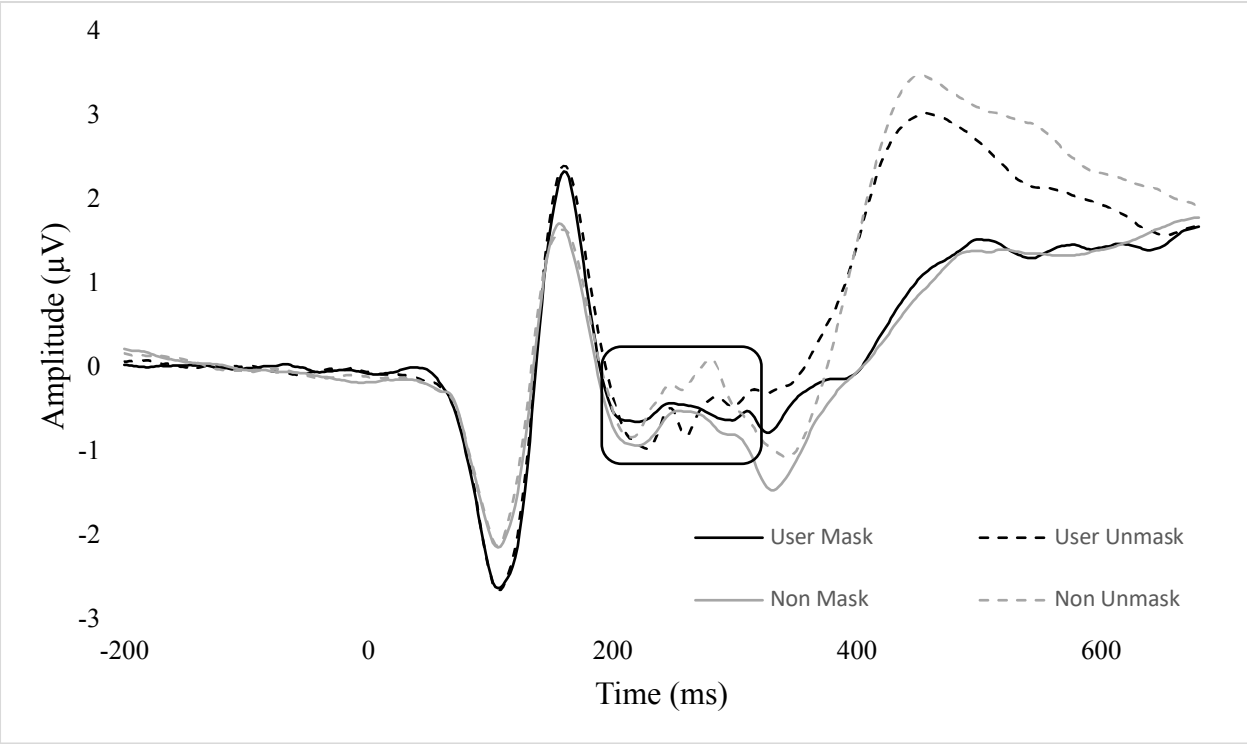

Figure 9: ERP wave from FZ, CZ, and PZ

P3

There was a significant main effect for emotion, $F(2, 68) = 11.01$, $p < .001$, $\eta_p^2 = .245$. Fear ($M = 3.15$, $SE = 0.32$) had significantly greater amplitude than neutral ($M = 2.56$, $SE = 0.30$, $p < .001$) and fear was approaching significance compared to happy ($M = 2.91$, $SE = 0.33$, $p = .081$). Happy was also approaching significance with neutral, $p = .053$. There was a significant main effect for duration, $F(1, 34) = 90.21$, $p < .001$, $\eta_p^2 = .726$. Amplitudes were significantly greater for unmasked faces ($M = 3.62$, $SE = 0.34$) compared to masked ($M = 2.13$, $SE = 0.30$, $p < .001$) There was also an interaction between emotion and duration, $F(2, 68) = 7.61$, $p = .001$, $\eta_p^2 = .183$. Within the masked condition there were no differences between fear ($M = 2.26$, $SE = 0.31$), happy ($M = 2.13$, $SE = 0.30$), and neutral ($M = 1.99$, $SE = 0.31$), ps $> .261$. In the unmasked condition, fear ($M = 4.04$ $SE = 0.37$, $p < .001$) and happy ($M = 3.70$, $SE = 0.38$, $p =$

.008) were greater than neutral ($M = 3.13$, $SE = 0.31$). Differences in fear and happy were approaching significance, $p = .056$. Additionally, each emotional expression had greater amplitude in unmasked compared to masked, ps $< .001$ (Figure 10).

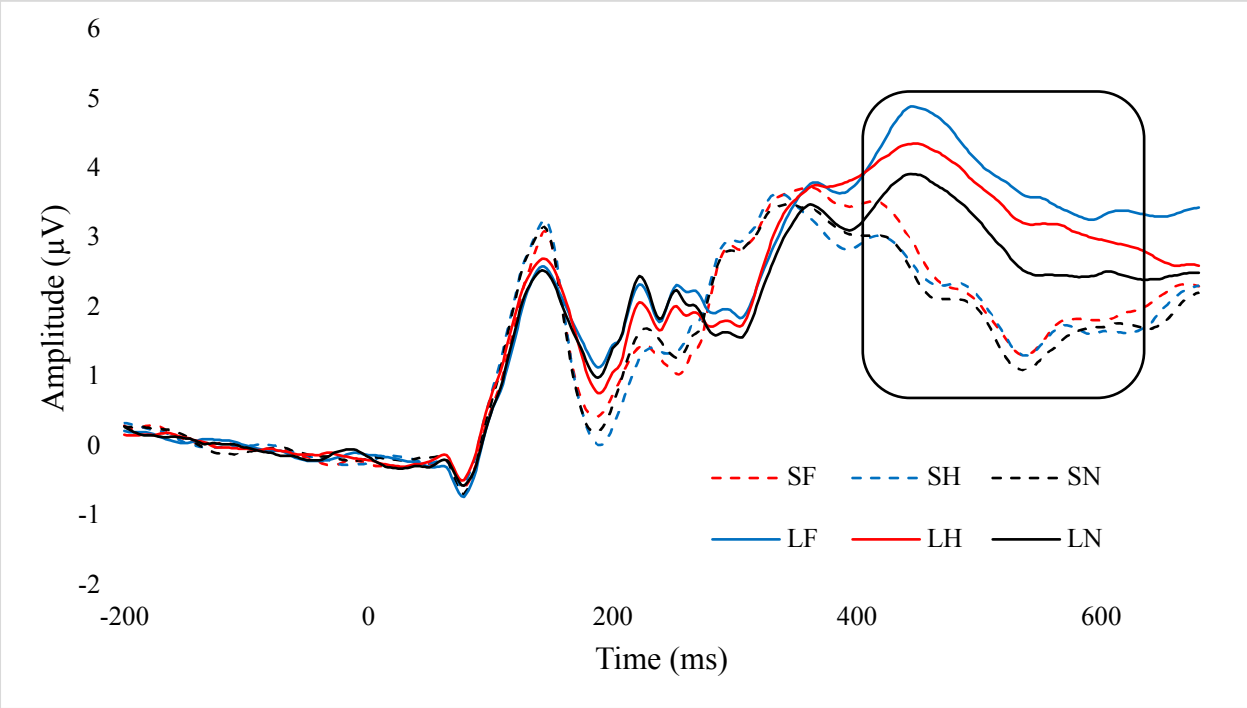

Figure 10: ERP wave from PZ

No group interactions were found for P3 amplitude in emotion $F(2, 68) = 0.07$, $p = .935$, $\eta_p^2 = .002$, duration $F(2, 68) = 0.94$, $p = .339$, $\eta_p^2 = .027$, and emotion by duration $F(2, 68) = 1.09$, $p = .304$, $\eta_p^2 = .030$.

Exploratory analysis

An exploratory analysis was conducted to examine potential differences between males and females. There were no ERP differences between males and females. There were also no correlations between questionnaire data and ERP components.

Discussion

The behavioral results suggested that masked facial expressions were not completely below the awareness threshold, each expression was greater than chance level (33.33%). Given that the actual refresh rate for the target face in the masked condition was 30 ms and not 16.66 ms, some of the participants might have been aware (Pessoa et al., 2005). However, on average, the participants were less accurate in correctly reporting the expression in the masked condition compared to unmasked. This suggests that awareness was restricted, but maybe not completely below the awareness threshold. There was no difference in accuracy between cannabis users and non-users suggesting cannabis use did not affect subjective perceptual awareness.

Although the interaction in P1 was approaching significance, cannabis users had increased amplitude towards happy facial expressions compared to fear and neutral expression. Previous research by Morel et al. (2014) indicated that individuals with high levels of trait anxiety showed a similar effect, in that there was enhanced P1 amplitude for happy faces but no difference in fear and neutral. In their experiment, the facial expressions (happy, fear, and neutral) were displayed for 500 ms and awareness was not restricted. The current results did not address whether or not cannabis users had greater anxiety, in fact, state anxiety, depression, and posttraumatic stress symptoms (PTSS) were statistically equal between users and non-users. However, our results suggest that cannabis users had increased attention towards a positive salient stimulus which is a similar finding to Morel et al. (2014) who also found an increased attentional bias towards positive stimuli in participants with high trait anxiety. Neither our results nor Morel et al. (2014) found differences between fear and neutral emotional expression which is dissimilar to other research (Harrewijn et al., 2017; Helfinstein et al., 2008: Holmes et al., 2008; Mueller et al., 2009; Torrence & Troup, 2017).

In previous backward masking studies, P1 differences were not modulated by emotional expression or masking conditions (Del Zotto & Pegna, 2015; Pegna et al., 2011, 2008). The results of the current study did suggest that across all participants P1 was greater in the masked condition compared to unmasked with happy having a greater P1 amplitude than fear and neutral. This is the first study, however, to suggest that masked faces elicited a greater P1 amplitude than unmasked faces. The P1 component is thought to reflect increased processing in the amygdala and visual cortex (Carlson et al., 2009; Pourtois et al., 2004; Vuilleumier & Pourtois, 2007). Etkin et al. (2004) found that masked faces increased basolateral amygdala activity and unmasked increased dorsal amygdala. They proposed that the visual, cingulate, and prefrontal connections of the basolateral amygdala represent the neural system related to the enhanced processing of masked faces. This could be a possible explanation of why we found enhanced P1 amplitudes in the masked condition.

The N170 results suggested that regardless of masking condition, emotional facial expressions had more enhanced N170 amplitudes. Specifically, happy was more enhanced than fear, and both were more enhanced than neutral. This is consistant with a recent review that suggested N170 is modulated by emotional expressions (Hinojosa et al., 2015). Additionally, we found that N170 for unmasked faces was more enhanced than masked faces. Del Zotto et al. (2015) found a similar result, that unmasked faces elicited a more negative N170 than masked. However, they also found an interaction between expression and duration and within the masked condition, fearful faces had more enhanced amplitudes than neutral, which we did not find in the current study. We did observe an interesting group interaction in N170. Within non-users, each emotion within each masking condition showed hemisphere lateralization, whereas cannabis users did not have this effect. A similar effect was found by Vukusic et al. (2017) in participants

with high autistic traits, which might indicate that faces are not as salient to cannabis users as they are to non-users. Alternatively, Maurer et al., (2008) examined N170 lateralization for faces and words and found reduced hemisphere lateralization for faces when the faces were presented one after another within the same block as compared to when faces and words were alternated. Their results suggested that a reduction in hemisphere lateralization indicated habituation in face processing. Taken together, cannabis users may have increased habituation to facial expressions compared to non-users.

This study only found N2 differences within the unmasked condition, similar to Pegna et al. (2008). However, Pegna et al. (2008) found differences in unmasked fear and neutral, we only found differences in happy compared to fear and neutral. Additionally, our results contradict Liddell et al. (2004) and Vukusic et al. (2017) in that we found no emotional expression differences within the masked condition. Our results also suggested that N2 was more prominent in frontal and central electrodes as opposed to posterior and may be related more with cognitive control (Folstein & Van Petten, 2008) whereas a more posterior N2 might be related to orientation of attention (Diao et al., 2017; Dowdall et al., 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015). Group comparisons revealed within non-users, N2 was enhanced for unmasked compared to masked facial expression. This difference, however, was not seen within cannabis users suggesting that non-users had better cognitive control over their response to unmasked facial expressions than cannabis users.

Although there were overall differences in P3 amplitudes between facial expression, differences were only observed within the unmasked condition. Specfically, fear elicited a greater P3 than happy, which was greater than neutral. Liddell et al. (2004) and Kiss and Eimer (2008) found similar effects and suggested that P3 amplitudes were related to higher emotional

processing which requires percpetual awareness. However, unlike Troup et al. (2016), these results suggested cannabis use was not related to differences in emotional processing as measured by P3. One explanation could be that Troup, Bastidas, et al. (2016) found differenes in implicit emotional expression processing and not in explicit. Given the nature of the current task, the participants were asked to pay attention to the target face expression. Therefore, when cannabis users are asked to pay attention to the emotion, there are no differences between them and non-users (Troup, Bastidas, et al., 2016).

There were limitations to this study. Firstly, the behavioral data suggests that there was variablitiy in awareness to facial expression (Figure 1). Although we found no differences between groups in STAI, CES-D, and PCL-5, we did not control for high and low levels of anxeity, depression, and PTSD. Given that some individuals self medicate with cannabis for these disorders (Crippa et al., 2009; Troup, Andrzejewski, et al., 2016) it would be interested to see the effects cannabis has on participants with high levels of anxiety. Additionally, we did not control for type of cannabis use. Since cannabis is recreationally legal in the State of Colorado, users have a wide variety of stains (e.g. indica and sativa) and method of use (e.g. flower, concentrates, edidbles, etc). Given that different ratios of cannabinoids effect the brain differently (Schubart et al., 2011) it would be worth exploring the effects on face processing. Early face processing requires the amygdala (Anderson & Phelps, 2001; Bach et al., 2015; Morris et al., 1996; Morris, Degelder, Weiskrantz, & Dolan, 2001; Suslow et al., 2006; Vuilleumier et al., 2004; Whalen et al., 1998) and cannabis effects amygdala activity (Phan et al., 2008; Rabinak et al., 2014; Rabinak & Phan, 2014). The ERP methods used in this current study cannot directly measure amygdala activity, even though the amygdala likelty influences the ERP

results. Future research could use fMRI to explore amygdala activity in a backward masking paradigm.

This study was the first to examine the effects of residual cannabis use on perceptual awaresness of emotional facial expressions as measured by ERPs. The results mostly support findings from previous research (Del Zotto & Pegna, 2015; Kiss & Eimer, 2008; Liddell et al., 2004; Vukusic et al., 2017) that discribed how the brain respondes to faces below and above the awareness threshold. Additionally, we observed differences in facial processing between cannabis users and non-users. Specifically, cannabis users displayed a hypervigilance towards happy faces, facial habituation, and reduced cognitive control to unmasked faces.

Chapter 4 – General Discussion

The results of these two studies suggested that there were some differences in attention and awareness of emotional faces between cannabis users and non-users. However, even with the differences between groups there were also similarities, especially in the second experiment. It is important to note that these were the first two experiments to study potential neurocognitive mechanisms of the anxiolytic effects of cannabis use. In the first experiment, a dot-probe task was used to test attentional bias towards fearful faces. The second experiment used a backward masking paradigm was used to measure perceptual awareness of fearful and happy facial expressions. Previous research has suggested that individuals with high anxiety typically have an over attentional bias towards threat-related stimuli (Bar-Haim et al., 2007) and increased emotional processing of emotional facial expressions (Harrewijn et al., 2017; Morel et al., 2014). Cannabis research has suggested that cannabinoids can have an anxiolytic effect (Viveros et al., 2005) and that residual effects of cannabis can affect processing of emotional expressions (Troup, Bastidas, et al., 2016; Troup et al., 2017). The general hypothesis that captures the work presented here as a whole was that cannabis users would have reduced ERPs in attending towards emotional facial expressions, similar to low anxious individuals in previous studies.

In the first study (attentional bias), cannabis users had lower P1 amplitudes when fearful faces were present compared to non-users. There were no differences in N170 amplitude. However, only cannabis users had differences in N2pc. The results of the second study (awareness), indicated that cannabis users actually had increased P1 amplitudes to happy facial expressions. It was observed that cannabis users did not have significant hemisphere lateralization in the N170, whereas non-users did have significant lateralization (Figure 8).

Cannabis users also exhibited no differences in N2 in masked and unmasked where N2 was difference for non-users. Although there were some group differences observed, there were some interesting non-group related findings.

General Face Processing

The aims of these present studies were to examine the residual effects of cannabis use on attentional processing of emotional facial expressions. However, these studies also addressed some general, non-group related, attentional processing. To date there is no published facial dot-probe ERP study that has examined both posterior-contralateral N170 and N2pc(Torrence & Troup, 2017). The main effects found for N170 and N2pc in experiment 1 were consistent with previous research. Carlson and Reinke (2010) and Rossignol et al. (2013) both found that the N170 was enhanced in the right lateral occipital electrode (near P8) for emotional expression displayed in the left visual field. The results here build on these studies suggesting that there is a posterior-contralateral N170 for fearful facial expressions, not just enhanced right N170 for left visual field faces. Additionally, Holmes et al. (2009, 2014) found enhanced N2pc to angry facial expressions. The current study found enhanced N2pc for fearful faces. In a visual search paradigm, Diao et al. (2017) suggested that greater N2pc towards angry facial expressions was correlated with increased theta oscillations. Similarly, the non-spatial N170 has been correlated with increased theta oscillations (Almeida et al., 2016; Zhang, Wang, Luo, & Luo, 2012). Theta oscillations could represent synchronization of the basal forebrain and the cortex (Lee et al., 2005). Visuospatial attention is modulated by cholinergic projections from the basal forebrain to the visual cortex (Chaves-Coira, et al., 2018; Pinto et al., 2013). Given the role of the amygdala in attentional processing (Anderson & Phelps, 2001; Bach et al., 2015) the N170 and N2pc could

represent amygdala mediated synchronization between the basal forebrain and the visual cortex in attending towards fearful facial expressions.

The second experiment examined the ERP correlates of awareness to emotional facial expressions. The results indicated that emotional expressions and awareness modulated ERPs. The P1 component was greater for masked faces than unmasked and there was also an effect for emotion, in that happy and fearful were greater than neutral. Previous backward masking studies suggested that the P1 component was not modulated by expression or awareness (Del Zotto & Pegna, 2015; Pegna et al., 2011, 2008). Consistent with a recent meta-analysis (Hinojsa et al., 2015), emotional expression was related to more enhanced N170. That is, happy was greater than fear, and both were greater than neutral. Similar to Del Zotto et al. (2017), this study also found that the N170 was modulated by awareness, unmasked faces had enhanced amplitude compared to masked. Vuilleumier et al. (2002) indicated that activation of the amygdala to emotional faces was not modulated by awareness, that is the amygdala was active in both aware and unaware conditions. Addiontally, faces processed below the level of awareness activated the extrastriate cortex and the orbital frontal cortex. The P1 component has been suggested to indicate increased processing in the extrastriate cortex, mediated by the amygdala (Carlson et al., 2009; Pourtois et al., 2004; Vuilleumier & Pourtois, 2007). The results here would imply that masked faces can modulate increased visual attention (i.e. P1) but the N170 represent further processing that requires awareness.

Unlike experiment 1 that examined N2pc (posterior N2) which represents orientation of visuospatial attention (Diao et al., 2017; Dowdall et al., 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015), experiment 2 examined the anterior N2 which represents cognitive control (Folstein & Van Petten, 2008). Similar to Pegna et al. (2008), we found that emotional

expression only modulated the N2 in the unmasked condition suggesting the anterior N2 component reflects awareness of emotional expressions. The P3 is another component linked to awareness and higher order emotional processing (Johnston et al., 1986; Polich, 2007). Consistent with previous research (Kiss & Eimer, 2008; Liddell et al., 2004), P3 amplitudes were modulated by emotional expression only in the unmasked condition.

Overall, the results of the present two experiments have been consistent with previous research examining attentional bias and awareness of emotional facial expression in the non-clinical samples. The posterior-contralateral N170 and N2pc seem to measure initial orientation of spatial attention in the dot-probe task (Torrence & Troup, 2017) and early components (i.e. P1) reflect attention whether aware or unaware of emotional face, whereas later ERP components (i.e. N170, anterior N2, and P3) represent awareness and emotional processing. Therefore, examination of ERP components seems to be a reliable indicator of attention and awareness of facial expressions and can represent neural networks of attention.

Effects of Cannabis

Given that cannabis users have neuroanatomical difference (Lorenzetti et al., 20016) and there is high CB1 receptor density in areas of the brain associated with attentional bias and awareness (Burns et al., 2007), it is plausible that cannabis use could interfere with attentional processing. In addition, cannabis has been suggested to have anxiolytic effects when used in small doses (Viveros et al., 2005) and hypervigilance towards threat-related stimuli has been associated with high levels of anxiety (Bar-Haim et al., 2007). These two experiments examined the residual effects of cannabis use on attentional bias and awareness of facial expressions.

In experiment 1, cannabis use was associated with reduced attention when a fearful face was present. That is, comparing non-users with users, users had reduced P1 amplitudes to fearful

faces. Previous research has suggested that individuals with high levels of anxiety had increased P1 amplitudes in response to negative facial expressions (Harrewijn et al., 2017). Phan et al. (2008) examined the acute effects of THC on the amygdala reactivity to emotional faces (i.e. angry and fearful) in cannabis users. They found that THC was associated with reduced amygdala activity as compared to the placebo. However, THC did not affect the visual or motor cortex. The P1 component is thought to reflect early visual cortex activity (Pourtois et al., 2004) and this increased visual cortex activity is mediated by the amygdala (Carlson et al., 2009; Pourtois et al., 2004; Vuilleumier & Pourtois, 2007). The dot-probe results would suggest that the residual of cannabis use was correlated with reduced activity in the early attention network and additional use of THC could further reduce amygdala activity (Phan et al., 2008).

Wilcockson and Sanal (2016) used eye-tracking to examine attentional bias towards anxiety related stimuli. The researchers' results suggested that cannabis users had attentional avoidance of anxiety related stimuli (e.g. snakes). That is, daily cannabis users looked away from the anxiety related stimulus and towards the neutral stimulus (e.g. book). However, eye-tracking measures overt attention and this later attentional avoidance of threat-related stimuli is actually related to increased anxiety, whereas early attention towards a threat-related stimulus is related to anxiety (Bar-Haim et al., 2007). Therefore, the current dot-probe results suggest that there is a decrease in early attentional bias in cannabis users which has been correlated with reduced anxiety (Bar-Haim et al., 2007), conversely Wilcockson and Sanal (2016) found cannabis users resembled anxiety type attentional bias in later attentional processing. Another difference between their study and the present one is that the present study included moderate cannabis users and Wilcockson and Sanal (2016) only included daily users. According to a review by Viveros et al. (2005), heavy, long-term cannabis use can have anxiogenic effects whereas

moderate use can have anxiolytic effects. Future research could include two cannabis groups, a moderate user group (less than weekly) and a heavy user group (daily).

The P1 results from experiment 2 were opposite of what was found in experiment 1. That is, in experiment 2, cannabis users had increased P1 amplitudes to happy facial expressions which is similar to what Morel et al. (2014) found in participants with high anxiety. However, monthly use was, approaching, significantly lower in experiment 1 (M = 12.79, SD = 17.11) than in experiment 2 (M = 27.33, SD = 31.08), $t(35) = -1.78$, $p = .084$. There were no differences between experiment 1 users and experiment 2 users in STAI ($t(32) = 1.08$, $p = .289$), CES-D ($t(35) = 0.70$, $p = .487$), and PCL-5 ($t(34) = 0.72$, $p = .479$). As previously discussed, heavy cannabis use was associated with anxiogenic effects (Viveros et al., 2005), which might explain the differences between the two experiments. Although the experiments were different in the P1 results, the N170 was consistent.

In both experiments, there were no group interactions in N170. The only differences observed between non-users and users was in experiment 2, in which cannabis users had no significant hemisphere lateralization where non-users did have this effect. Hemisphere lateralization was not examined in the dot-probe task since the faces were displayed in different visual fields. In fact, the dot-probe study found that the contralateral electrodes had more enhanced N170 amplitudes than ipsilateral, regardless of hemisphere. However, there was a correlation between monthly use and difference between contralateral and ipsilateral N170 amplitudes. That is, the more a participant used in month, the less of a difference there was between contralateral and ipsilateral amplitudes. Given that cannabis users in experiment 2 used more frequently than users in experiment 1, the correlation between monthly use and N170 might be related to the lack of hemisphere lateralization found in experiment 2. There has been

other research that has examined lateralization of the N170. One study found reduced hemisphere lateralization in participants with high autistic traits (Vukusic et al., 2017) and another suggested the reduced lateralization was related to habituation (Maurer et al., 2008). Together, these results might suggest that emotional facial expressions are not as salient for cannabis users, much like in autism, as compared to non-users. If cannabis use is associated with anxiolytic, or anxiogenic, attentional processing then the lack of difference between groups in N170 is consistent with a recent review (Harrewijn et al., 2017).

In a recent review, Harrewijn et al. (2017) discussed that the N170 was not modulated by anxiety in explicit tasks. Similarly, the review also discussed that in explicit tasks anxiety was not related to differences in P3. In experiment 2, there were no group differences in P3 amplitude. Troup, Bastidas, et al. (2016) and Troup et al. (2017) had three different emotional expression processing task. They had implicit (identify male or female face), explicit (identify the emotional expression), and empathic conditions (indicate whether one can empathize with that expression). The researchers found that during implicit and empathic processing, cannabis use was associated with reduced P3 amplitides. However, the researchs indicated that when explicitly processing emotional expressions, cannabis use had no effect. The reduction in P3 amplitude in implicit and empathic might indicate that cannabis use is related to reduced higher order processing of expressions when not expliticly told to attend to the emotion.

Cannabis use was also related to alterations in the different N2 components. The results of experiment 1 indicated that cannabis use was associated with more enhanced N2pc amplitudes, suggesting greater orientation of visuospatial attention (Diao et al., 2017; Dowdall et al., 2012; Luck & Hillyard, 1994b, 1994a; Tan & Wyble, 2015). However, there was a trend in non-users and the difference between the N2pc difference score (contralateral – ipsilateral) was

not significant. This would indicate that there might be a third variable contributing to the slight differences in N2pc. Experiment 2 examined the anterior N2 component which is connected to cognitive control (Folstein & Van Petten, 2008). This study found that in non-users, unmasked facial expressions had enhanced N2 amplitudes, whereas within cannabis users, there was no difference in N2 amplitudes between masked and unmasked faces.

Limitations

Although both experiments found interactions between cannabis use and face processing, there were some limitations. One limitation is the differences in monthly cannabis use between the two experiments. Participants in experiment 1 used less frequently than users in experiment 2. These differences could account for some of the inconsistencies between the two. Previous research has indicated that frequent cannabis use had anxiogenic effects, whereas moderate use had anxiolytic effects (Viveros et al., 2005). As previously mentioned, future research would benefit from including at least two different cannabis groups (e.g. moderate users and heavy users). Defining cannabis use in the literature has been inconsistent and difficult. Cousijn, Núñez, and Filbey (2017) discussed how most studies rely on self-repost measures, as the current two experiments did, and this self-report data might not be reliable. Given cannabis's history of illegality, some participants may be less likely to report they use and might report they use less often than reality. Another difference between the two experiments was age. The participants in the first experiment (M = 19.67, SD = 2.18) were significantly younger than the participants in experiment 2 (M = 23.75, SD = 3.94), $t(73) = -5.61$, $p = .000$. This difference in interesting in that the first experiment had mostly participants under the age of 21, which is the legal age for recreational cannabis use in Colorado and had lower monthly use than the participants in experiment 2, most of which were the legal age.

Monitor refresh rate was a limitation, especially in the second experiment (awareness). The refresh rate was not examined in the first experiment (dot-probe), although the dot-probe task has been effective in eliciting attentional bias related ERPs from 33 ms to 150 ms (Torrence & Troup, 2017). However, in experiment 2, the refresh rate of 16 ms was critical for subliminal processing (Pessoa et al., 2005). The results of our test revealed that the actual refresh rate for 16 ms was actually about 30 ms. The longer refresh rate would explain the behavioral data, that the accuracy was significantly lower than the unmasked condition, but still above chance level.

Both experiments had more females than males. Previous research has indicated that in males and females have differences in emotional processing and the related ERP components (Campbell & Muncer, 2017; Filkowski, Olsen, Duda, Wanger, & Sabatinelli, 2017; Pfabigan, Lamplmayr-Kragl, Pintzinger, Sailer, & Tran, 2014; Torrence et al., 2017; Victor, Drevets, Misaki, Bodurka, & Savitz, 2017). In addition, the questionnaire used did not ask about sex or gender, it simply had a check box for male, female, and other (see Appendix D). Therefore, in neither experiment can we determine whether a participant was male or female in terms of sex or gender. Another limitation with the personal inventory (Appendix D) was assessing neurological and psychiatric disorders. The questionnaire asked about them but there it does rely on self-report which knowledge of a disorder is required. That is, a self-report questionnaire was not effective in assessing preclinical or undiagnosed disorders.

Neither experiments had differences between non-users and users in anxiety, depression, and PTSD. Additionally, there were no differences in these measures between the two experiments. Now that these two experiments suggest that cannabis use is associated with anxiety related attentional processing, future research could include additional anxiety groups. These two studies were also purely correlational and does not offer insight as to how cannabis

use changes attentional processing. These experiments did not control for type of cannabis use. Since cannabis has been legal in Colorado, participants have a wide range of use methods available to them, flower, concentrates, edibles, etc. Even within flower, ratios of cannabinoids vary, some have high THC and low CBD, where other strains may have more even ratios. Schubart et al. (2011) suggested that participants who use cannabis strains with higher CBD percentages had less psychotic symptoms, as compared to participants who use strains with low CBD. Green et al. (2003) discussed how method of use and the environment mediate cannabis related anxiety. Specifically, after oral THC use, participants reported higher anxiety symptoms and after smoking in a small group, participants reported feeling less anxious. Future research could select participants that have different methods of use and use different ratios of cannabinoids to determine if these factors affect attentional processing.

Conclusion

These two experiments were the first to examine the residual effects of cannabis use on attentional bias towards and awareness of emotional expression using ERPs. The main effects and interactions, non-group interactions, suggested that both experiments found similar ERP results as previous research. Specifically, in the dot-probe task the N170 and N2pc results were consistent with previous research (Torrence & Troup, 2017) showing that early spatial attention can be measured with ERP components. In the awareness task the ERP results were consistent with previous research (Kiss & Eimer, 2008; Liddell et al., 2004; Pegna et al., 2008). That is, faces have an effect on early ERPs, even when awareness was restricted, and ERPs can be used to measure perceptual awareness. The consistency suggests that the experiments were testing the correct attentional effects. Therefore, the differences found between cannabis users and non-users should be accurate. Experiment 1 suggested that cannabis use was associated with a

decrease in attentional bias, similar to low anxiety (Harrewijn et al., 2017), whereas experiment 2 suggested cannabis use was related to increased attention towards happy faces, similar to high anxiety (Morel et al., 2014). The mixed findings could be related to differences in average monthly use between groups. Additionally, experiment 1 suggested heavy cannabis use was correlated with less N170 differences between contralateral and ipsilateral electors. Similarly, experiment 2 indicated less hemisphere lateralization of N170 in cannabis users (mostly heavy users in experiment 2). Anxiety related attentional processing could be a mechanism of the anxiolytic and anxiogenic effects of cannabis use. Future research might use the implications of the current findings to examine the effects of cannabis on attentional bias modification training. Previous research has suggested that acute use of cannabis facilitates fear extinction learning and could be helpful in patients with PTSD (Rabinak et al., 2014). Similarly, acute use of cannabis could potentially facilitate learning to orient attention away from threat related stimuli using attentional bias modification, which has been suggested to reduce anxiety (Mogg, Waters, & Bradley, 2017).

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Appendix A

Instruction Script

Protocol for "Attentional bias"

Greet & Welcome the Participant –

1 Seat and Give the participant the consent form and allow them time to read it over.

   a. While they read it over, enter their data into the computer program and start up the NIRS program.

   b. Once they are finished with the consent form, ask them if they have any questions and would like a copy of the consent form.

   c. Sign their consent form and keep the signed copy, File it away.

   d. Remind the participant that they are volunteering to participate in the study and they can leave any time without penalty.

   e. ASK them to **TURN OFF** or **AIRPLANE MODE** their CELL PHONES and leave it in the experimenter's room.

2 Seat the participant 59cm from the screen. Apply the EEG equipment to the participant – Tell them to limit movements.

   a. Ask if it is comfortable, and Give them the following instructions:

Dot-Probe Task: *Each trial of the experiment will start with a small '+' (plus sign) in the center of the screen. At all times keep your eyes fixated on the plus sign. After an initial period of fixation two stimuli will be briefly presented: one on each side of the screen. After these stimuli disappear, a small dot will appear either on the left or on the right side of the screen.*

Your task is to locate this dot: left or right. To do this, use your right hand. Use your right index finger on the "1" button on the key board to indicate left-sided target dots. Use your right middle finger on the "2" button on the key board to indicate right-sided target dots. IT IS IMPORTANT THAT YOU RESPOND AS QUICKLY AS POSSIBLE. AS SOON AS YOU LOCATE THE DOT MAKE A RESPONSE. The experiment will be divided into several blocks. Between block you can take a small break, if you like. When you are ready to begin the next block press the "1" button. DO YOU HAVE ANY QUESTIONS?

3 After the experiment administer the STAI-S, CES-D, PCL-5, RCUE Questionnaires

4 Ask if they have any questions about the study

Appendix B

Instruction Script

Protocol for "Awareness"

Greet & Welcome the Participant –

1 Seat and Give the participant the consent form and allow them time to read it over.

a. While they read it over, enter their data into the computer program and start up the NIRS program.

b. Once they are finished with the consent form, ask them if they have any questions and would like a copy of the consent form.

c. Sign their consent form and keep the signed copy, File it away.

d. Remind the participant that they are volunteering to participate in the study and they can leave any time without penalty.

e. ASK them to **TURN OFF** or **AIRPLANE MODE** their CELL PHONES and leave it in the experimenter's room.

2 Seat the participant 59cm from the screen. Apply the EEG equipment to the participant – Tell them to limit movements.

f. Ask if it is comfortable, and Give them the following instructions:

Awareness Task: *Each trial of the experiment will start with a small '+' (plus sign) in the center of the screen. At all times keep your eyes fixated on the plus sign. After an initial period of fixation a face will appear and will be immediately replaced by another face. The first face will either be fear, happy, or neutral, the second face will always be neutral. Your task is to*

indicate the facial expression of the first face. To do this, use your right hand. Use your right index finger on the "1" button on the key board to indicate fear. Use your right middle finger on the "2" button on the key board to indicate happy. Use your right ring finger on the "3" button on the key board to indicate neutral. IT IS IMPORTANT THAT YOU RESPOND AS QUICKLY AS POSSIBLE. AS SOON AS YOU SEE THE QUESTION MAKE A RESPONSE. If you are unsure of what the facial expression was, indicate neutral The experiment will be divided into several blocks. Between block you can take a small break, if you like. When you are ready to begin the next block press the "1" button. DO YOU HAVE ANY QUESTIONS?

3 After the experiment administer the STAI-S, CES-D, PCL-5, RCUE Questionnaires

4 Ask if they have any questions about the study

Appendix C

Preprocessing Script

```
% Convert to EEGLAB
%Put the subject numbers here
subject_list = {'400', '401', '402', '403', '404', '405', '406', '407',
'408', '409', '410', '411', '412', '413', '414', '415', '416', '417', '418',
'419', '420','421', '422', '423', '424', '425', '426', '427', '428', '429',
'430', '431', '432', '433', '434', '435', '436', '437', '438', '439'};
numsubjects = length(subject_list); % number of subjects
%Put the file path of the location of raw EEG data
parentfolder =
'/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Curry_Raw/';

for s=1:numsubjects

    subject = subject_list{s};
    % replace dap with .raw or .mat
    subjectfolder = [parentfolder subject '.dap'];

    fprintf('\n\n\n*** Processing subject %d (%s) ***\n\n\n', s, subject);
    %replace loadcurry with the ERPLAB function for netstation
    EEG = loadcurry(subjectfolder, 'CurryLocations', 'False');
    EEG.etc.eeglabvers = '14.1.1';
    %Put path to EEGLAB files
    EEG = pop_saveset( EEG, 'filename',[subject
'_.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Aware
_EEGLAB/');
end



%Preprocess EEG/ERP data
% Note trials are only marked for rejection, to completely reject the
% trials you will need to do in the GUI
% tools/reject data epochs/reject marked epochs

%Clear workspace
clear

%Put the subject numbers here
subject_list = {'400', '401', '402', '403', '404', '405', '406', '407',
'408', '409', '410', '411', '412', '413', '414', '415', '416', '417', '418',
'419', '420','421', '422', '423', '424', '425', '426', '427', '428', '429',
'430', '431', '432', '433', '434', '435', '436', '437', '438', '439'};

numsubjects = length(subject_list); % number of subjects
%Put the file path of the location of EEGLAB data
parentfolder =
'/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Aware_EEGLAB/';
%Put the file path of the location of to put ERP data
```

```
erpfolder =
'/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Aware_ERP_excel/';

for s=1:numsubjects

    subject = subject_list{s};

    subjectfolder = [parentfolder subject '.set'];
    suberpfolder = [erpfolder subject '.txt'];

    %Show what subject it is running
    fprintf('\n\n\n*** Processing subject %d (%s) ***\n\n\n', s, subject);
    %Load EEG data
    EEG = pop_loadset('filename',[subject '_.set'],'filepath',parentfolder);
    %Rereference to the average reference then save
    EEG = pop_reref( EEG, []);
        EEG = pop_saveset( EEG, 'filename',[subject
'_ref.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Aw
are_EEGLAB/');
    %Bandpass filter 0.1 to 40 Hz then save
    EEG  = pop_basicfilter( EEG,  1:33 , 'Boundary', 'boundary', 'Cutoff', [
0.1 40], 'Design', 'butter', 'Filter', 'bandpass', 'Order',  2 );
        EEG = pop_saveset( EEG, 'filename',[subject
'_ref_filt.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Da
ta/Aware_EEGLAB/');
    %Create event list Add evenlist file path after 'List'
    EEG  = pop_editeventlist( EEG , 'AlphanumericCleaning', 'on',
'BoundaryNumeric', { -99}, 'BoundaryString', { 'boundary' }, 'List',
'/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/AwareBinList2.txt',
'SendEL2', 'Workspace&EEG', 'UpdateEEG', 'binlabel', 'Warning', 'off' );
        EEG = pop_saveset( EEG, 'filename',[subject
'_ref_filt_event.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Data/Aware_
S18_Data/Aware_EEGLAB/');
    %Epoch the data from -200 to 1000 with baseline correction
    EEG = pop_epochbin( EEG , [-200.0  1000.0],  'pre');
        EEG = pop_saveset( EEG, 'filename',[subject
'_ref_filt_event_epo.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Data/Aw
are_S18_Data/Aware_EEGLAB/');
    %Mark threshold trials of -100 to 100 micro V
    EEG  = pop_artextval( EEG , 'Channel',  1:32, 'Flag',  1, 'Threshold', [
-100 100], 'Twindow', [ -200 998] );
        EEG = pop_saveset( EEG, 'filename',[subject
'_ref_filt_event_epo_art.set'],'filepath','/Users/bobtorrence/Desktop/Dis_Dat
a/Aware_S18_Data/Aware_EEGLAB/');
    %average events/bins
    ERP = pop_averager( EEG , 'Criterion', 'good', 'ExcludeBoundary', 'on',
'SEM', 'on' );
    %save ERP file Add the file path to where you want ERP data to be
    ERP = pop_savemyerp(ERP, 'erpname', subject, 'filename', [subject
'.erp'], 'filepath',
'/Users/bobtorrence/Desktop/Dis_Data/Aware_S18_Data/Aware_EEGLAB/',
'Warning', 'on');
end
```

Appendix D

Personality Inventory

Age: ____ Check one: ☐ Male / ☐ Female / ☐ Other (Specify: _____)
Handedness: ☐ Right / ☐ Left / ☐ Ambidextrous
Native English speaker? ☐ Yes / ☐ No • Other languages: _____.
Check all that apply:

Have you had any of the following…	In the past 8 hours? How much?		In the past 24 hours? How much?	
Caffeine	☐	_____	☐	_____
Alcohol	☐	_____	☐	_____
Tobacco	☐	_____	☐	_____
Cannabis	☐	_____	☐	_____
Other substance/prescription medicine. Please list: _____	☐	_____	☐	_____

Have you ever been diagnosed with…?	Past diagnosis	Current diagnosis	Please list:
Vision problem	☐	☐	_____
Neurological problem	☐	☐	_____
Psychological disorder Please specify:	☐	☐	_____
Mood Disorder	☐	☐	_____
Anxiety Disorder	☐	☐	_____
Substance-Related	☐	☐	_____
Other	☐	☐	_____

Has anyone in your immediate family been diagnosed with…?		Please list:
Psychological disorder Please specify:	☐	_____
Mood Disorder	☐	_____
Anxiety Disorder	☐	_____
Substance-Related	☐	_____
Other	☐	_____

Appendix E

Recreational Cannabis Use Questionnaire. (R-CUE) Troup, Andrzejewski & Bastidas 2015

Complete the following and check the most appropriate response. Please answer each question honestly and to the best of your ability. You may add any extra information next to the question or on the back of each page.

1. **Age___**

2. **Are you part of Colorado's Medical Marijuana Registry (do you own a red card)?** ☐ Yes ☐ No

3. If you answered **YES** to #2, **for how long have you been a member of the registry?**

☐ N/A (Never a member)	☐ 2-4 years
☐ Less than one year	☐ 4-7 years
(This is my first red card)	☐ 7-10 years
☐ 1-2 years	☐ 10+ years

Specify exactly how many years you have been in the registry:_____
Specify the STATE-APPROVED medical ailment that is being addressed with your Colorado MMJ card:_____

4. If you answered **NO** to #2, **do you use cannabis solely recreationally, or for any medical purpose not specified by Colorado's Medical Marijuana Registry?**

☐ Use recreationally	☐ Use for medical benefits

5. **If you use cannabis for a medical purpose other than what is designated by the Colorado, what ailment do you use it for?**

☐ **Alleviate Anxiety/Anxiety-related symptoms (including panic attacks)**	☐ **To improve Sleep**
☐ **Alleviate Depression/Depression-related symptoms**	☐ **To improve appetite**
☐ **Gastrointestinal ailments (Crohn's, Acid Reflux, Diarrhea, etc.)**	☐ **To reduce side-effects of other medications**
☐ **Seizure Prevention**	☐ **To reduce other prescription use**
☐ **Other:_____**	

6. **How long ago did you first try cannabis?**

☐ N/A (Never tried)	☐ 2-4 years
☐ Less than one year	☐ 4-7 years
☐ 1-2 years	☐ 7-10 years

	☐ 10+ years

Specify how old you were when you first tried cannabis:_____

5. How often do you use Cannabis (in any form)?

☐ N/A (Don't use)	☐ A couple (1-2) times a week
☐ N/A (No longer use)	☐ A few (3-6) times a week
☐ Less than monthly (1-11 times/year)	☐ Daily
☐ Less than weekly (1-3 times/month)	☐ 2-4 times a day
☐ Once a week	☐ More than 4 times a day

Specify how many times you have used this this month (if use is less frequent, how many times this year): _____

6. Have you ever used Cannabis more heavily than you do now?

☐ No, have never used.
☐ No, same amount as now.
☐ Yes, I used to use but no longer do (*how much did you use on average?)*_____
☐ Yes. *How often did you use at that time?* _____

7. Which of the following types of Cannabis and methods of intake do you prefer? Check all bolded items that apply (*and check subcategories to the best of your knowledge/ability*)**.**

a. ☐ N/A (Don't use/No longer use)

b. ☐ Smoking Cannabis flower (Bud, Nugget, etc.)

i.*Strain (type) of cannabis:*

☐ Indicas ("Body high")	
☐ Sativas ("Mind high")	
☐ Hybrids:	☐ Sativa-dominant
	☐ Indica-dominant
	☐ True hybrid (50/50 Sativa/Indica)

c. ☐ Smoking Cannabis Concentrates (Hashish/"Dabs")

i.*Strain (type) of cannabis:*

☐ Indicas ("Body high")	
☐ Sativas ("Mind high")	
☐ Hybrids:	☐ Sativa-dominant
	☐ Indica-dominant

	☐ True hybrid (50/50 Sativa/Indica)	

ii. ☐ Strain-specific hash. If so, list strains that you have used:

_____

_____

_____

_____

_____

iii. Method of THC extraction (type of concentrate):

1. ☐ *Solvent based extraction:*

☐ Butane Honey Oil (BHO)
☐ Carbon Dioxide (CO_2)
☐ Quick Wash Isopropyl Alcohol (QWISO)
☐ Hexane solvent concentrates
☐ Propane solvent concentrates
☐ Ethanol solvent concentrates
☐ "Shatter" hash (High purity butane/ethanol extraction)

2. ☐ *Solvent-less concentrates:*

☐ Cold Water Extraction (CWE)/Icewax/Solvent-less wax/"grease"/"jewce"
☐ Bubble hash
☐ Screen filtered hash (Finger hash/Keif)

d. ☐ Cannabis Edibles

i. Specify:

☐ Pre-packaged baked edibles
☐ Hard candy/gummy edibles
☐ Chocolate edibles
☐ Drink-based edibles (THC-infused sodas, teas, etc.)
☐ Tinctures: ☐ Glycerin based
☐ Ethanol based
☐ Cannabis butter (Cannabutter)
☐ Other. Please list:

_____

ii. Cannabinoids contained:

☐ CBD ☐ THC ☐ CBD/THC

e. ☐ Dermal Cannabis Application: ☐ Skin patches

☐ Lotions/balms/oils

8. In order of preference (1 being most preferred, 4 being least preferred), what is your preferred form of consuming Cannabis?

▪ Cannabis flower/nugget ▪ Concentrates/hash
▪ Edibles ▪ Topical/Dermal Absorption

☐ N/A (Don't use)

1. _____ (Most Preferred)
2. _____ (Second Most)
3. _____ (Second Least)
4. _____ (Least Preferred)

9. If applicable, **what smoking devices do you use? (Select all that apply) If you NO LONGER USE, which devices did you use with cannabis?**

a. ☐ **None**

b. ☐ **Water-filtration devices:**

☐ Bong (upright/waterpipe)
☐ Bong (gravity)
☐ Bubbler

c. ☐ **Dry smoking devices:**

☐ Pipe (glass/metal)
☐ Steamroller
☐ Joint
☐ Blunt

d. ☐ **Vaporizers:**

☐ Bag vaporizers
☐ Whip vaporizers
☐ Portable/Pen vaporizers

e. ☐ **Dabs:**

☐ Spoon dabs
☐ Nail dabs
☐ Noodle dabs
☐ Health stone dabs
☐ Skillet dabs

10. If applicable, **in an average month, how much in Cannabis flower/nugget do you smoke?**

☐ **None** (0 grams)	☐ **An ounce or less** (14.1-28 grams)
☐ **A gram or less** (0-1 grams)	☐ **More than an ounce** (1-2 ounces)
☐ **An eighth of an ounce or less** (1.1-3.5 grams)	☐ **More than two ounces** (2-4 ounces)
☐ **A half of an ounce or less** (3.6-14 grams)	☐ **More than a quarter pound** (4+ ounces)

In an average month, roughly how many grams of flower/nugget do you smoke (please round to nearest half gram if possible):_____

11. If applicable, **in an average month, how much in Cannabis concentrates do you smoke?**

☐ **None** (0 grams)	☐ **An ounce or less** (14.1-28 grams)
☐ **A gram or less** (0-1 grams)	☐ **More than an ounce** (1-2 ounces)
☐ **An eighth of an ounce or less** (1.1-3.5 grams)	☐ **More than two ounces** (2-4 ounces)
☐ **A half of an ounce or less** (3.6-14 grams)	☐ **More than a quarter pound** (4+ ounces)

In an average month, roughly how many grams of cannabis concentrate have you smoked (please round to the nearest half gram if possible):_____

12. If applicable, **in an average month, how many Cannabis edibles do you consume? (One edible is equal to what is considered one dose by the manufacturer).**

☐ **None**	☐ **4-8 edibles**
☐ **One edible**	☐ **8-20 edibles**
☐ **2-4 edibles**	☐ **20+ edibles**

In an average month, roughly how many micrograms of THC/CBD have you consumed through (round to the nearest hundred if possible):_____