INFLUENCE OF THE BDNF VAL66MET POLYMORPHISM ON EMOTION FLEXIBILITY

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by

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TABLE OF CONTENTS........................................................................................................ iii
LIST OF TABLES................................................................................................................. iv
LIST OF FIGURES............................................................................................................... v
ACKNOWLEDGEMENTS.................................................................................................... vi

INTRODUCTION................................................................................................................. 1
METHODOLOGY................................................................................................................ 9
RESULTS............................................................................................................................ 19
DISCUSSION..................................................................................................................... 20
TABLES............................................................................................................................. 25
FIGURES............................................................................................................................ 27
REFERENCES.................................................................................................................... 30
LIST OF TABLES

Table 1. Sample Characteristics by Group………………………………………………………………………….25

Table 2. Paired Sample T-tests for Manipulation Check of Emotion Response to Emotion Response Assessment………………………………………………………………………….26
LIST OF FIGURES

Figure 1. Val66Met Genotype Differences in Mean Respiratory Sinus Arrhythmia (RSA) during Emotion Response Task ................................................................. 27

Figure 2. Val66Met Genotype Differences in Mean Positive Emotion during Emotion Response Task ................................................................. 28

Figure 3. Val66Met Genotype Differences in Mean Negative Emotion during Emotion Response Task ................................................................. 29
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Introduction

Flexible responding to environmental changes, a function of synaptic plasticity, is a highly adaptive process for which brain-derived neurotrophic factor (BDNF) is essential. In addition to the primary role of BDNF in neural plasticity across species, BDNF also influences differentiation, and growth (Allen & Dawbarn, 2006; Chao, 2003; Lu, 2003), as well as the development and maintenance of the human sensory and sympathetic nervous system (Huang & Reichardt, 2001). Synaptic plasticity, a critical function of BDNF, specifically refers to the strengthening and weakening of neural synapses in an experience-driven way, leading to changes in neural circuits and sometimes to changes in behavior (Holtmaat & Svoboda, 2009; Kessels & Malinow, 2009). For example, BDNF is central to learning and the ability to override previously learned information with newly acquired information (e.g. fear extinction) (Johnson & Casey, 2015). Importantly, decreased synaptic plasticity has also been identified as a risk factor for development of emotion-linked disorders, such as depression and anxiety (Colzato et al., 2011; Duman & Aghajanian, 2012; Duman, Aghajanian, Sanacora, & Krystal, 2016).

A single nucleotide polymorphism (SNP) (Val66Met, rs6265) in the proregion of the BDNF gene has been studied extensively in both human and rodent models due to its influence on levels of available BDNF (Notaras, Hill, & van den Buuse, 2015). The Val66Met polymorphism is located at position 66 of the prodomain region of the BDNF gene, and leads to a substitution of methionine for valine, causing reduction in available BDNF (Liebl, Tessarollo, Palko, & Parada, 1997) and impaired BDNF secretion in primary hippocampal neurons (Egan et al., 2003). Broadly, presence of the Met allele at the Val66Met polymorphism has been linked to maladaptive neurocognitive function (e.g. impaired episodic memory, reduced hippocampal and amygdala volumes) (for review, see Notaras et al., 2015) and impaired fear extinction in humans
(Asthana et al., 2015; Johnson & Casey, 2015), and emotion-related behaviors in rodents (e.g. continued freezing behavior following fear extinction training) (Da Silveira Da Luz & Dias, 2013; Soliman, et al., 2010). There has now been extensive research aimed at clarifying the complex relationships between variation in the Val66Met polymorphism and emotion-related psychopathology or symptoms (e.g. depression and anxiety). However, the current literature is mixed (Notaras et al., 2015), likely due to some methodological limitations and lack of objective measurement of emotion responses. Notably, there are currently no studies directly testing how Val66Met might influence specific emotion-related, clinically relevant behaviors (including emotion flexibility).

Across species, associations have emerged between reduced BDNF and behaviors that suggest decreased flexibility, greater rigidity, and risk for psychological illness (Da Silveira Da Luz & Dias, 2013; Soliman, et al., 2010). Much of the current understanding about the Val66Met polymorphism in humans has been derived through candidate gene studies, a scientific approach that has lately received criticism due to insufficient replication (Duncan & Keller, 2011; Koenen, Duncan, Liberson, Ressler, 2015; Tabor, Risch, Meyers, 2001). However, evidence from this research suggests consistent relationships between BDNF and psychiatric risk. For example, research conducted over the past decade has found that carriers of the Met allele at the BDNF Val66Met polymorphism (leading to reduced BDNF) generally report more symptoms of emotion-linked psychological disorders (e.g. depression) (Beevers, Wells, & McGeary, 2009; Lotrich, Albusaysi, & Ferrell, 2013). Moreover, Met carriers have been found to report greater symptoms of anxiety, nervousness, and insecurity, in response to lab-induced stress (the cold pressure test) (Colzato, et al., 2011). However, evidence of a significant association between the Met allele and psychiatric symptoms has also been debated (Notaras et al., 2015). In particular,
two meta-analyses found no association between BDNF Val66Met and major depressive disorder (MDD) (Chen et al., 2008; Lopez-Leon et al., 2008). Further, a more recent meta-analysis found a gender-dependent association, namely, a significant association between the Met allele and MDD diagnosis in male but not female participants (Verhagen et al., 2010). These mixed findings might stem from the emphasis in most candidate gene studies on measuring distal outcomes (e.g. retrospective self-report of symptoms), rather than specific and objective behavioral response patterns, more likely to be under influence of genetic variation (Hasler, Drevets, Manji, Charney, 2004; Insel & Cuthbert, 2009). Indeed, despite the recent criticism of candidate gene studies and insufficient replication, translational research suggests largely consistent findings across species (Caspi, Hariri, Holmes, Uher, Moffitt, 2010). Accordingly, the candidate gene approach might be better suited for examination of explicit behavioral phenotypes, as opposed to investigation of broad symptom patterns (Koenen et al., 2013).

In other species, research has shown that BDNF deficient mice engage in more avoidance-related behaviors (e.g. less exploration of novel environments in open-field and elevated plus maze paradigms) (Bath et al., 2012; Chen et al., 2006; Chourbaji et al., 2008) and more helplessness-like behaviors (e.g. less mobility in a forced swim test) (Bath et al., 2012). Such behavioral patterns in rodents are widely accepted parallels to symptoms of depression (helplessness) and anxiety (avoidance) in humans. In addition, in fear conditioning paradigms, human Met carriers are more likely than Val homozygotes to generalize cued fear to novel contexts and show weaker fear extinction, consistent with inflexibility (Ashtana et al., 2016; Mühlberger et al., 2014; Soliman et al., 2010; Yu et al., 2009). Indeed, generalization of fear to novel cues demonstrate rigidity in fear response, and has been identified as a hallmark of anxiety disorders (e.g. panic disorder) (Lissek et al., 2010). Importantly, many of these findings have
been replicated in humans. For example, a translational study by Soliman and colleagues (2010), human Met carriers were found similar to Val homozygotes in their ability to learn fear cues, but showed impaired fear extinction. This study also demonstrated a parallel link between impaired fear extinction (but not fear conditioning) and the Met allele in a rodent model of the BDNF Val66Met (knock-in mice that have been genetically modified to express Val66Met). Such cross-species evidence suggests that reduced BDNF might in particular decrease the ability to override previously learned associations, resulting in rigid and potentially maladaptive behavioral responses. Although ample cross-species research has identified reduced BDNF as a risk factor for psychopathology, the Met allele might also afford some benefits. Indeed, research has found greater hypothalamic-pituitary-adrenal axis (HPA-axis) response, and higher levels of perceived stress, in human Val homozygotes in a laboratory stress test (public speaking) (Alexander et al., 2010). Human Val homozygotes have also demonstrated more symptoms of anxiety in response to early life stress (Gatt et al., 2009). Taken together, cross-species evidence suggests a link between reduced BDNF (presence of the Met allele) and greater behavioral rigidity, as well as a potential link between the ValVal genotype and greater reactivity to stress, both are risk factors for emotion-linked psychological illness.

Emotion inflexibility broadly serves as a risk factor for psychopathology, such that individuals with decreased capacity to both generate and modulate emotion responses, might be more likely to develop emotion-linked psychological illness (Coifman & Bonnano, 2010; Rottenberg, Gross, & Gotlib, 2005; Rottenberg, Kasch, Gross, & Gotlib, 2002). Emotions are biologically based, and have evolved across species for the purpose of survival and adaptation to the environment (Ekman, 1992; LeDoux, 1995). Specific emotions serve discrete functions, and are adaptive when flexibly limited or matched to the appropriate context, but can be maladaptive
when they occur outside of the evolutionary appropriate environment, or in conflict with individual needs. In humans, emotions are understood to manifest across several response dimensions including experiential, behavioral (e.g. emotional facial behaviors), and physiological (e.g. autonomic nervous system activity) (Ekman, 1992; Tooby & Cosmides, 1990). Emotion flexibility can be broadly defined as the ability to generate emotion in response to specific emotion contexts, and also to modulate emotional responses according to shifts in environmental demands. Indeed, inability to flexibly shift negative emotional responses is broadly associated with poor psychological and social functioning (Gotlib & Joorman, 2010; Kashdan & Rottenberg, 2010), and is present in both depression and anxiety. For example, depressed adolescents demonstrate more consistent (or inflexible) aggressive nonverbal actions and sad statements, as compared to non-depressed adolescents, in lab-induced discussions with family members about both positive and conflictual events (Kuppens, Allen, Sheeber, 2010). Furthermore, depressed adults show a diminished ability to reduce intensity of sadness by recalling positive memories, a strategy which often reduces sadness in healthy controls (Joorman, Siemer, & Gotlib, 2007). Moreover, an inflexible fear response (i.e. fear in non-threatening contexts) is a hallmark of most anxiety and stress-related disorders. For example, toddlers (two years of age) who demonstrate an increased fear behavior in low threat environments are more likely to develop symptoms of anxiety (i.e. maternal report of anxiety and social withdrawal) by age 5 years, as compared to toddlers observed to modulate their fear response according to environmental demands (i.e. fear in high threat but not in low threat environments) (Buss, 2011). Despite theorized, and empirically demonstrated, importance of emotion inflexibility in psychological illness, research on this specific construct is still sparse due in-part to methodological demands (Coifman & Almahmoud, 2015).
Although maladaptive negative emotion responses have received a great amount of research attention as a risk factor for psychiatric illness, inflexible or low positive emotion responsivity is also an important hallmark of psychopathology (Bylsma, Morris, & Rottenberg, 2008; Kashdan & Steger, 2006). Indeed, a central feature of depression is a decreased capacity to generate positive emotion (e.g. joy, contentment) in situations where such emotional responses are appropriate (American Psychiatric Association, 2013; Clark & Watson, 1991). For example, when engaged in pleasant activities (e.g. helping, playing, interacting) depressed individuals show less positive emotional responsivity as compared to non-depressed people (Catalino & Fredrickson, 2011). Notably, positive emotion responsivity may be a hallmark feature of emotion flexibility, as positive emotions are thought to broaden coping responses and cognitive resources, facilitating greater adaptation (Fredrickson, 1998). Indeed, the ability to generate positive emotions is linked to greater psychological health (Fredrickson, 1998; Fredrickson et al., 2003; Tugade & Fredrickson, 2007). In fact, positive emotion expression (smiling) has been found to help down-regulate negative emotion while discussing negative personal experiences (e.g. adjustment to college) (Papa & Bonanno, 2008) and down-regulate cardiovascular arousal (Fredrickson & Levenson, 1998). In addition, an association has emerged between greater capacity to generate positive emotion in daily life and increased autonomic flexibility, suggesting a link between positive emotion generation and increased capacity for the parasympathetic nervous system to modulate physiological arousal in response to contextual demands (Porges, 1995; Kok & Fredrickson, 2010).

Finally, increasing evidence suggests a key role of the autonomic nervous system in emotion flexibility. Specifically, parasympathetic activity (often referred to as *vagal tone*: influence of the vagus nerve on the heart) has been theorized to function as a mediator for
individual differences in emotion responsivity (Porges, 1994). Vagal tone is often indexed by respiratory sinus arrhythmia (RSA: Porges, 2007), reflecting the respiratory rhythm in heart rate (greater RSA produces slower heart rate) (Porges, 2007). Notably, vagal tone might be directly linked to emotional facial expressions given the proximity of brain stem structures controlling facial expressions and those controlling autonomic responses (Porges, 1994). For example, newborns with lower resting vagal tone have been found to show less positive emotional facial expressions (e.g. joy and interest) (Stifter, Fox, & Porges, 1989). Moreover, adults with decreased RSA (low vagal tone) have been hypothesized to show a diminished capacity for emotion flexibility (Porges, 1994; Thayer et al., 2012; Thayer & Brosschot, 2005; Thayer, Hansen, Saus-Rose, & Johnsen, 2009), and decreased RSA has been associated with emotion linked disorders (e.g. panic disorder, posttraumatic stress disorder, depression) (Friedman, 2007; Friedman & Thayer, 1998). For example, a recent longitudinal investigation of young adults found that decreased RSA at rest was predictive of more reported depressive symptoms one year later (Yaptangco et al., 2015). Moreover, individuals that score high on measures of neuroticism show decreased RSA in response to lab-induced down-regulation of negative emotion (Di Simplicio et al., 2012). Accordingly, greater RSA serves as a broad indicator of health and regulatory resources (Friedman & Thayer, 1998; Porges, 2009; Thayer et al., 2012). In fact, higher RSA has been found in response to lab-induced joy (Kreibig, 2010) and has been associated with greater social connectedness and more daily experiences of positive emotions (Kok & Fredrickson, 2010). Lastly, individual differences in parasympathetic activation might be partly heritable (Singh et al., 1999). Indeed, Met carriers at the BDNF Val66Met polymorphism have been found to show decreased parasympathetic activity at rest, as compared to Val
homozygotes (Yang et al., 2010), suggesting a link between the Met allele at the Val66Met polymorphism and a less adaptive autonomic nervous system.

Flexible and adaptive responding to the environment may be a function of synaptic plasticity, a process for which BDNF is essential. Reduced plasticity has been identified as a risk factor for psychiatric illness (Ninan, 2014; Notaras, 2015), but the specific relationship between BDNF and emotion-linked psychological illness (e.g. depression) is still debated (Verhagen et al., 2010). An impressive body of cross-species behavioral research has presented associations that suggest a link between reduced BDNF (or presence of the Met allele at Val66Met) and inflexible behavioral response patterns (Bath et al., 2012; Chen et al., 2006; Chourbaji et al., 2008; Soliman et al., 2010). Behavioral inflexibility is indeed a risk factor for emotion-linked disorders (e.g. depression) (Gotlib & Joorman, 2010; Joorman, Siemer, & Gotlib, 2007). To the authors’ knowledge, inflexibility in an emotion context, specifically, has never been examined as a function of variation in the BDNF gene (or reduced BDNF). Therefore, the present study aims to explore the role of a single nucleotide polymorphism (rs6265, or Val66Met) in the BDNF gene in emotion flexibility. To examine emotion flexibility, an emotion response assessment was administered that consisted of two emotionally-evocative film clips of one of each, positive and negative, valence. Film clips were utilized to elicit emotion as previous research has suggested such stimuli provide a better proxy for emotion responses as they occur in daily life, as compared to static images or idiographic information (Kring & Gordon, 2008). Flexibility was operationalized as the ability to generate emotion in response to the eliciting context, and the ability to shift emotion as contextual demands changed, and emotion responses were monitored and recorded in real-time. Emotion responses were assessed across multiple dimensions, including self-reported emotion experience, coded facial behavior, and autonomic activity. In
particular, respiratory sinus arrhythmia (RSA: Porges, 2007), a representation of parasympathetic activity and a broad indicator of health and regulatory resources (Friedman & Thayer, 1998; Porges, 2009; Thayer et al., 2012) was indexed, as well as skin conductance, an established indicator of emotional engagement and sympathetic activity (Dawson et al., 2007).

Based on extensive cross-species literature where a relationship between behavioral rigidity and presence of the Met allele (BDNF reduction) has emerged, and the association between the Val allele and greater reactivity in response to stress, the following predictions were made: 1) Val homozygotes would generate greater emotion responses to negative emotion contexts (or greater reactivity), as compared to individuals with at least one copy of the Met allele, but 2) carriers of the Met allele would demonstrate a decreased ability to shift negative emotion response across contexts (i.e. down-regulate negative emotion when the context shifts from negative to positive), as compared to Val homozygotes. No a priori hypotheses were established regarding positive emotion responses due to limited evidence on potential effects of BDNF reduction and ability to generate or shift positive emotions (Van Winkel et al., 2014; Wichers et al., 2008). Similarly, no a priori hypotheses were established regarding RSA or skin conductance due to limited evidence on potential effects of BDNF reduction and autonomic nervous system activity (Yang et al., 2010). However, examination of genotype differences in generation of positive emotion response, shifts in positive emotion response, and autonomic nervous system activity, was conducted as well.

Methodology

Participants
One hundred and nineteen adult participants (79% Caucasian, 62.2% female, mean age = 20.87 years) were recruited from the subject pool within the Psychology department at a large public university in the Midwest in the United States. Participants needed to be 18 years of age or older to be eligible for the study. No other exclusion criteria were established for this study. Recruitment took place over the course of two academic years, and study sessions were 90 minutes long.

All participants completed questionnaires that included factors known to influence emotion responses including age, gender, race, ethnicity, and depressive symptoms (Bradley et al., 2001; Gotlib & Joorman, 2010; Kring & Gordon, 1998; Vrana & Rollock, 2002; Zimmerman & Iwanski, 2014), in order to isolate the effect of genotype as much as possible. To index depressive symptoms, participants completed the Center for Epidemiologic Studies Depression Scale (CES-D). A summary of demographic characteristics of this sample can be found in Table 1.

The frequency of the BDNF Val66Met alleles in this sample (ValVal =68 (61.26%); ValMet =40 (36.04%); MetMet =3 (2.7%)) did not differ from the Hardy-Weinberg equilibrium (Gaunt, Rodriquez, & Day, 2007). $\chi^2 = 1.04$, $p = 0.30$ thus, the genotype distribution of this sample is likely representative of the general population. Based on prior research on the BDNF Val66Met polymorphism (e.g. Beevers, Wells, & McGeary, 2009; Egan et al., 2003; Lotrich, Albusaysi, & Ferrell, 2013; Soliman, et al., 2010), participants in this sample were grouped into two groups, one group consisting only of individuals with the ValVal genotype, and one group consisting of individuals carrying the Met allele. Demographics variables (age, gender, ethnicity, race) and symptoms of depression (CES-D) were examined for differences between the genotype groups. Specifically, Pearson’s chi-square tests were utilized to compare carriers of the ValVal
genotype versus the ValMet or the MetMet genotype. A group difference in age emerged with a trend toward significance, such that Met carriers were found to be older than Val homozygotes $\chi^2=22.02$, $p=.08$. No other significant group differences across the genotype groups were found on any key study variables (see Table 1).

**Procedures**

The present study took place in the laboratory of the Principal Investigator, and study sessions were conducted by trained research assistants. Approval for the study was obtained by the university’s institutional review board governing research involving human subject, and all participants gave informed consent before study participation. Participants received a copy of the informed consent to read and discuss with the research assistant, prior to engaging in any study activities.

After informed consent was obtained, participants provided demographic information, and then completed the CES-D to index depressive symptoms. This scale has 20 items, responses are indicated on a three-point Likert scale, and scores above 16 reflect clinically significant symptoms of depression (Radloff, 1977). A CES-D mean of $M=11.1$ $SD=7.2$ ($\alpha = .85$) was found in this sample. Based on the mean, this sample demonstrates comparable levels of depressive symptoms to other healthy, college-age, samples (Shean & Baldwin, 2008).

Following completion of questionnaires, saliva (2 ml) was collected using Oragene-DNA Self-Collection Kits. Samples were then stored at -20°C. Saliva was collected following established procedures (Gilman et al., 2015). Once saliva collection was complete, the participant was seated in a private 4’x4’ study room.
DNA extraction was completed using prepIT-L2P. DNA extraction was conducted by following the manufacturers recommendations (DNA Genotek Inc., Ottawa, Canada). DNA was then purified using Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA) and quantified using SYBR Green I dye (Lonza, Walkersville, MD). DNA was resuspended to 5 ng/µL prior to polymerase chain reaction (PCR). The Val66Met polymorphism was then amplified using primers adopted from Hünnerkopf et al. (2007) (Forward: 5’-AAA GAAGCA AAC ATC CGA GGA CAA G; Reverse: 5’- ATT CCT CCA GCA GAA AGA GAG G). In a final volume of 20 µL, 1 µL DNA was amplified, and each reaction consisted of 0.5 µM forward primer, 0.5 µM reverse primer, 0.5 µL Taq polymerase solution, 5 mM MgCl2, 2 mM dNTPs, and 10% glycerol. PCRs were run on an Eppendorf PCR Mastercycler pro (model no. 6321, Hamburg, Germany) using a touchdown PCR cycle protocol adopted from Anchordoquy et al., (2003) and modified to have a 65°C annealing temperature for 10 cycles, followed by a 55°C annealing temperature for 35 cycles. Following amplification, a restriction digest was performed to discern the presence or absence of the G to A substitution. 10 µL of the PCR product was used in the total reaction with 1X Cutsmart buffer and 4 U/reaction NlaIII enzyme (New England BioLabs, Ipswich, MA) in a total volume of 20 µL and was incubated for 1 hour at 37°C. Restriction fragments were visualized on a 3% agarose gel. In the presence of methionine substitution (A allele) yielded product lengths of 140, 77, and 57 bp. In the absence of the substitution, (G allele) the PCR product was undigested and a 274 bp product was visualized. 12% of sample genotypes were separately reconfirmed with 100% concordance.

Participants were comfortably seated in a private study room, in front of a computer, and were instructed to engage with the content of a sequence of two well-validated (Gilman et al., 2016), emotionally evocative video clips known to elicit negative and positive emotions. Each
clip was five-minutes long and followed by a two-minute long break before the next video started. The video sequence started with a neutral content clip ("Big Cat Diaries," BBC Earth) in order for the participant to acclimate to the study conditions. For the purpose of explicitly examining shifts in emotion responses between a high arousal negative context and a low arousal positive context, participants were first shown a high arousal negative emotion (e.g. disgust, anger) video (Road to Guantanamo Bay, Revolution Films, 2006) followed by a low arousal positive emotion video (Alive, Paramount Pictures, 1993). Specifically, this video sequence (Road to Guantanamo Bay to Alive) allowed for examination of spontaneous down-regulation of high arousal negative emotion in response to a low arousal positive emotion context, in addition to examination of contextually appropriate negative and positive emotion generation, abilities that are central to emotion flexibility. Thereby, the assessment increased the potential for capturing genetic variability in emotion responses, and to specifically explore emotion inflexibility in Met carriers and emotion reactivity in Val homozygotes. The two video clips were part of a larger investigation which contained two additional, well-validated films (Gilman et al., 2016), known to elicit low arousal negative (The Champ, Metro-Goldwyn Mayer, 1979) and high arousal positive emotion (Between two Ferns, www.comedyordie.com, 2010). This assessment was administered using EPrime 2.0 (Psychology Software Tools, Sharpsburg, PA). During the entire assessment, emotional responses were measured in real-time on multiple dimensions including: coded facial expressions and sympathetic and parasympathetic arousal (autonomic activity). Participants were also asked to provide self-report of their emotional experience following each film clip, during the two-minute break.

*In-vivo Assessment of Emotion Responses during Emotion Flexibility Assessment*
Electrocardiogram (ECG) was recorded in real time, in order to calculate a value to represent RSA (Porges, 2007) for each participant. Electrodermal activity (EDA) was also recorded in real time, in order to calculate a value to represent Skin Conductance (SC) for each participant. This procedure was conducted as follows. The study room was kept at a steady temperature of 74.3 degrees Fahrenheit and participants were encouraged to drink one 8-ounce bottle of water before the assessment began. These procedures were followed in order to minimize interference with EDA recording. To record the ECG signal, three disposable Ag/AGCL ECG spot electrodes were placed on the participant. One electrode was placed underneath the right collarbone, and two electrodes were placed on either side of the ribcage. To record the EDA signal, an electrolyte gel of sodium chloride was applied to two Beckman electrodes that were attached to the palmar surface of the participant’s middle phalanges of the first and second fingers of the non-dominant hand. The ECG signal was acquired using a 2-slot BioNex bioamplifier (Mindware Technologies, Gahanna, OH) and sampled at a rate of 1000 Hz. ECG data was amplified with a gain of 500 uS/Volt. To filter out noise, a high pass filter was set at .5 Hz and a low pass filter was set at 45 Hz. A bandpass filter (.25 to .40 Hz) and notch filter (60 Hz) were used to filter out frequencies in the electrocardiogram not relevant to RSA. The EDA signal was acquired through a 31-channel A/D converter operating at a resolution of 12 bits and with an input range of 2.5V to +2.5V. Amplification rates, high-pass filter (HPF), and low-pass filter (LPF) settings were as follows: ECG (gain=500, HPF=0.1Hz, LPF=1000Hz) and EDA (gain=0.1V/S, HPF=none/DC, LPF=10Hz, 6dB/octave, single pole RC).

Physiological data were cleaned and artifacts removed using customized Bio-Lab™ Software (MindWare Technologies, Gahanna, OH) and visual inspection of artifacts. Missed or mislabeled parts of the ECG wave were deleted and problematic beats were interpolated, and
Problematic segments of the EDA wave were excluded following visual inspection. RSA was derived by spectral analysis of the interbeat interval (RR) series derived from the ECG. High frequency spectral power was then integrated over the respiratory frequency band (0.12-0.4 Hz) and averaged for each video resulting in one average value of RSA for each video. Mean skin conductance data was averaged across each film and a skin conductance response (SCR) value (in uSiemens) was calculated for each film, for each individual.

To measure behavioral expression of emotion during the emotion response assessment, participant’s emotional facial behavior was recorded with a high-resolution camera. Emotional facial behaviors were later coded by four independent coders, naïve to any study details. Coders viewed participant videos individually, on a 23” computer monitor without sound. After viewing each video, coders rated degree of positive and degree of negative emotional facial behaviors to each video on a 7-point Likert scale (Coifman & Bonanno, 2010), resulting in a positive and a negative emotion expression score for each participant. Coders were sufficiently reliable (average ICC = .80, range .74-.90), and ratings were averaged across coders to increase reliability.

To measure emotional experience, participants were instructed to self-report their emotional response following each film by rating emotion-words on a 7-point Likert scale. Emotion-words were of both negative (fear, sadness, disgust, guilt, distress, anger) and positive (happiness, enjoyment, amusement, affection, relief) valence. Self-reported ratings of negatively valenced words were utilized to generate a mean negative affect score in response to both negative (The Road to Guantanamo) and positive (Alive) emotion contexts, for each participant. Similarly, self-reported ratings of positively valenced words were used to generate a mean

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1 Evidence suggests that facial coding by relatively naïve coders may be as valid as highly trained coders (e.g., Dondi et al., 2007).
positive affect score in response to both negative (*The Road to Guantanamo*) and positive (*Alive*) emotion contexts, for each participant. Emotion-words were chosen from contemporary circumplex models of affect (e.g. Rafaeli, Rogers & Revelle, 2007; Russell, 1980). These emotion-words have been used reliably in prior studies (e.g. Coifman & Bonanno, 2010), and demonstrated sufficient internal consistency in this sample (negative emotion experience in response to negative emotion context; $\alpha = .88$, and positive emotion experience in response to positive emotion context; $\alpha = .85$). Ratings were completed immediately after each video, during the two-minute break between the videos.

For the final analyses, emotion expression and emotional facial behavior scores were standardized to $z$-scores, and then averaged to create a mean negative emotion score, and a mean positive emotion score, in response to negative (*The Road to Guantanamo*) and positive (*Alive*) contexts, for each participant.

**Data Cleaning and Processing**

Data from $n=27^2$ participants were dropped from some of the final analyses, but not from all final analyses. Thus, the degrees of freedom vary slightly across the final analyses. Data were dropped for the following reasons. ECG signal data was lost due to equipment malfunction (e.g. detachment of electrodes, large muscle movements disrupting signal recording) that resulted in lack of a detectable signal. EDA signal data was lost due to equipment malfunction resulting in

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$^2$ Note regarding missing data for this study: pairwise deletion was utilized for missing data and resulted in deletion of data from a total of $n=27$ participants according to the following. ECG data was lost for $n=21$ participants due to equipment malfunction, $n=4$ of these participants were also missing EDA data. EDA data was lost for an additional $n=3$ participants due to equipment malfunction, and EDA data for $n=2$ participants was lost due to naturally occurring skin conductance (see below footnote). Facial behavior data was lost for $n=5$ research participants. For $n=4$ participants, video recording was unsuccessful due to camera software malfunction, and $n=1$ participant requested deletion of their video data. Two of the participants with missing facial behavior data were also dropped from ECG or EDA analyses.
lack of a detectable signal and naturally occurring irregular or low skin conductance\(^3\). Physiological data (ECG and EDA signals) was lost for one participant due to administration error (electrodes not placed properly). Facial behavior data was lost due to unsuccessful video recording following camera software malfunction, and facial data was dropped for one participant upon their request. No significant differences on key study variables emerged between participants who were included in final analyses for the present study and participants with missing data points.

Efficacy of the emotion response assessment was confirmed in this sample by conducting paired samples \(t\)-tests on negative and positive self-reported affect in response to each emotional context. Participants were found to report significantly more negative than positive affect during the negative emotion video (*The Road to Guantanamo*) and significantly more positive than negative affect during the positive emotion video (*Alive*) (see Table 2). Moreover, a significant change in negative affect emerged across the task, such that negative affect increased from baseline to the negative video \((t(118)= -16.327, p=.000)\) and decreased from the negative video to the positive video \((t(118)= 16.217, p=.000)\). To further confirm the strength of the emotion response assessment, Pearson correlations between affect and facial behavior in response to positive and negative stimuli were examined. A significant positive correlation emerged between negative affect and negative facial behavior in response to the negative video, suggesting intense negative emotion (*The Road to Guantanamo*) \((r=.30, n=115, p<.01)\). Further, a significant positive correlation was found between positive affect and positive facial behavior in response to the positive film, suggesting intense positive emotion (*Alive*) \((r=.25, n=114, p<.01)\). These

\(^{3}\) Notably, due to individual skin conductance variation within the general population, some people naturally show irregular or low skin conductance (termed “nonresponders”) (Dawson et al., 2007). When such irregularities were discovered during data collection for the present study, the research investigator attempted to adjust the electrodes and massage fingers of the participant in order to better collect the EDA data. Adjustments were unsuccessful for \(n=2\) participants, resulting in lack of a detectable signal.
findings are in line with past research suggesting that emotions manifest as a more organized response at high intensity, as compared to emotions at low intensity (Mauss et al., 2005).

Analyses for the present study were conducted to examine the influence of genetic variation in the BDNF Val66Met polymorphism on emotion flexibility (operationalized as; emotion generation and modulation). First, three repeated measures analysis of covariance (ANCOVA) tests were utilized to analyze group differences (BDNF ValVal versus ValMet/MetMet) in patterns of emotion generation measured during the emotion response assessment (physiological signals, positive emotion experience/expression, and negative emotion experience/expression). Specifically, these omnibus ANCOVA tests followed a 3 (video type: baseline video, negative emotion video (The Road to Guantanamo), positive emotion video (Alive)) x 2 (valence of emotion: negative and positive) x 2 (genotype: ValVal versus ValMet/MetMet) design. Demographic variables (age, sex, race, and ethnicity) and depressive symptoms (CESD) were entered into all analyses as covariates. These variables are well-known to influence response to emotional contexts, and were included in order to assist in isolating the effect of genotype on emotional responses. Next, follow-up univariate tests were examined for group differences in shifts in emotion responses (emotion modulation) according to changes in contextual demands (when the emotion response task moved from a negative emotion to a positive emotion context: The Road to Guantanamo to Alive). Specifically, follow-up univariate tests were examined for group differences on negative emotion experience/expression in a positive emotion context (Alive), while controlling for negative emotion experience/expression in a negative emotion context (The Road to Guantanamo). Likewise, follow-up univariate tests were examined for genotype differences on positive emotion experience/expression in a positive emotion context (Alive), while controlling for positive emotion experience/expression in a
negative emotion context (*The Road to Guantanamo*). The alpha level was set at 0.05 for all analyses, and effect sizes are reported as *partial eta*². Assumptions of ANCOVA were met, including sphericity and equality of error variances. All statistical analyses were conducted with PASW Statistics 21 (SPSS Inc., Chicago, IL, USA).

**Results**

Examination of group differences (BDNF ValVal versus ValMet/MetMet) in emotion generation during the emotion response assessment, utilizing omnibus ANCOVA tests, resulted in significant genotype differences on RSA, positive emotion experience/expression, and negative emotion experience/expression. First, using Pillai’s trace, a significant multivariate effect of genotype emerged on RSA across the emotion response assessment $F(2,92) = 3.731$, $p=0.028$, *partial eta*² = .075. The results show a within-subjects effect of genotype and RSA $F(2,92) = 3.682$, $p=.027$, *partial eta*² = .038, such that Val homozygotes had greater RSA across videos (Figure 1). Moreover, follow-up contrasts revealed a significant difference between Val homozygotes and Met carriers in RSA during the negative video (*The Road to Guantanamo*), $(F(1,93)=2.15$, $p=.03$, *partial eta*² = .05, 95%CI: .04-1.05). Specifically, Val homozygotes displayed significantly higher RSA in response to a negative context (M=6.62, SD=1.12) as compared to Met carriers (M=6.01, SD=1.19) (see Figure 1). Second, a significant between-subjects effect emerged on positive emotion experience/expression across the emotion response assessment $F(1,104)=5.711$, $p=.019$, *partial eta*² = .052 (see Figure 2). Third, using Pillai’s trace, a significant multivariate effect of genotype also emerged on negative emotion experience/expression $F(2,103)=3.095$, $p=.049$, *partial eta*² = .057 (Figure 3). The results specifically show a within-subjects effect of genotype and negative emotion...
experience/expression, $F(2,103)=3.091$, $p=.048$, partial $\eta^2 = .029$, and follow-up contrasts revealed that Met carriers show greater negative emotion experience/expression at baseline (M=.364, SD=1.878), as compared to Val homozygotes (M=-.30, SD=1.077). No significant genotype differences in skin conductance emerged in this sample.

Examination of univariate follow-up tests revealed a group difference in shifts of positive emotion experience/expression with a considerable trend toward significance, such that Val homozygotes demonstrate a greater increase in positive emotion experience/expression when the context shifts from a negative emotion context (The Road to Guantanamo) to a positive emotion context (Alive), $F(1,104)= 3.31$, $p=.07$, partial $\eta^2 = .03$, as compared to Met carriers. No significant genotype difference in shifts of negative emotion experience/expression emerged in this sample.

Discussion

The impact of genetic variation on psychological illness has received much research attention over the past decade, and there is evidence to suggest that variation in the BDNF gene might play a role in emotion-linked psychological disorders (Colzato et al., 2011; Lotrich et al., 2013; Notaras et al., 2015; Soliman, et al., 2010). However, there is little consistency and methodological specificity in the literature to clarify the link between BDNF and psychiatric risk.

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4 Regarding the video stimuli that were not included in the present analyses (The Champ and Between two Ferns), non-significant trends of a similar pattern as reported results, emerged across the emotion response assessment. Specifically, individuals with the ValVal genotype showed higher RSA in response both excluded videos, as compared to Met carriers. Val homozygotes also showed an increase in reported negative emotion experience in response to the negative emotion context (The Champ) which then decreased as the context shifted to positive emotion (Between two Ferns). Met carriers were observed to increase reported negative emotion experience in response to positive emotion (Between two Ferns) context. Lastly, a non-significant trend emerged such that Val homozygotes showed a decrease in reported positive emotion experience in response to the negative film (The Champ) and then an increase in reported positive emotion experience as the emotion context shifted to positive (Between two Ferns), whereas Met carriers showed the opposite pattern of responding, consistent with reported results.
The current investigation expands this work by providing the first evidence of an association between the Val66Met polymorphism in the BDNF gene and emotion flexibility in humans. In particular, this study presents evidence to suggest that individuals who carry at least one copy of the Met allele at BDNF Val66Met show inflexible emotion response patterns (emotional facial behaviors, self-reported emotion experience, and autonomic activity) in response to changing environmental demands. Specifically, Met carriers in this sample demonstrate less parasympathetic nervous system activation), as compared to Val homozygotes. Met carriers also show poor ability to generate positive emotion to an explicit positive emotion context. Instead, these individuals decrease their positive emotion responses as the emotion context shifted from negative to explicitly positive. In contrast, Val homozygotes in this sample were able to adaptively generate positive emotion in response to positive emotion context, and also demonstrate overall patterns of greater parasympathetic activation. However, unlike some previous research there was no evidence of greater reactivity in Val homozygotes, in this sample.

Inconsistent with the present study hypothesis, Met carriers in this sample were not found to demonstrate more persistent and rigid negative emotion responses (emotion expression/experience). Non-significant findings might reflect a similarity between the genotype groups on negative emotion responses, or lack of sensitivity in measurement of this construct. This investigation also did not find any significant group differences in skin conductance, an indicator of sympathetic activation, across the emotion response assessment. Measurement of skin conductance (an index of reactivity) was included in the present study based on previous evidence that suggest a link between the Val allele and greater stress reactivity. Non-significant findings might reflect a similarity between the genotype groups on sympathetic activation in an
emotion context, but call for further research on specific patterns of autonomic activity and reduced BDNF.

These findings compliment the current body of cross-species literature that has suggested a link between reduced BDNF and behavioral rigidity (Soliman et al., 2010), and expand on the current understanding of psychiatric risk afforded by the Met allele at Val66Met in the BDNF gene in humans. The current investigation also provides novel evidence of emotion inflexibility through the use of a naturalistic emotion response assessment where objective behavioral responses (emotional facial expressions; autonomic activity) were measured across contexts. Such methodology provides greater specificity of measurement, and allows for capturing enduring emotional responses that more closely approximate those occurring in daily life where emotions often occur in response to dynamic contexts (Gross & Levenson, 1995). Due to extensive past research focus on symptoms and more distal processes of psychopathology, there has been a need for behavioral evidence to demonstrate how BDNF levels could influence risk. Thus, these results suggest the importance of further investigation of BDNF Val66Met by naturalistic measurement of emotion flexibility.

The present study contributes to the continued understanding of BDNF by showing that individual differences might contribute to greater tendency for healthy individuals to display patterns of emotion inflexibility, thereby increasing risk for emotion-linked psychological illness (e.g. depression and anxiety). Clinically, the present findings suggest that individuals with the Met allele may be more vulnerable to decreased psychological well-being, potentially due to increased emotion inflexibility. Inflexible emotion responses have indeed been both theorized and demonstrated to contribute to higher risk for psychological illness (Buss et al., 2004; Graham & Milad, 2011; Joorman & Gotlib, 2010). More broadly, these findings contribute to the
understanding of dominant models of psychopathology, which have demonstrated that psychological disorders likely develop from complex interactions between genetically predisposed vulnerability and environmental factors (Cuthbert & Insel, 2013; Insel et al., 2010).

This study had several limitations. Primarily, the sample size of this study was relatively small. The costly nature of in-vivo measurement of emotion makes the sample size necessarily limited. Indeed, such ecologically valid measurement of emotion is crucial in order to better understand the complex patterns of emotion linked to risk for psychopathology, but it is time intensive and laborious. Another limitation of the present study was the sole focus on Val66Met, without consideration of potential interactions of this polymorphism with other SNPs within the BDNF gene, or other related genes. Currently, there is a small body of research that has examined interactions between BDNF Val66Met and other genes (Bredemeier et al., 2014; Dougherty et al., 2010; Nederhof, 2010). Although results are generally mixed, there is a clear need for more methodologically complex studies on the BDNF gene and emotion responses, and it is essential that future research take epistatic interactions into account in such investigations.

The present investigation aimed to explore the role of the BDNF Val66Met polymorphism in emotion flexibility by utilizing a naturalistic emotion response assessment. Emotion responses were captured on multiple dimensions (emotional facial expressions, self-reported emotion experience, autonomic activity) while participants viewed two emotionally evocative film clips, known to elicit both negative and positive emotions. Results from the present study suggest an association between presence of the Met allele at the Val66Met polymorphism and greater emotional inflexibility. These findings echo patterns found in emotion-linked disorders, where greater rigidity in emotion responses often are found in individuals who suffer from both depression and anxiety-related illness (APA, 2013; Kuppens,
Allen, & Sheeber, 2010; Rottenberg, Gross, & Gotlib, 2005), as well as research demonstrating the role of emotion inflexibility in prospective prediction of emotion-linked disorders (Bonanno et al., 2004; Coifman & Bonanno, 2010). Undeniably, BDNF plays an essential role in plasticity, learning, and thereby psychiatric risk thus, it is essential to gain further understanding about how reduced BDNF might influence clinically relevant emotion responses. Greater specificity in the present study was achieved by exploring these associations in healthy individuals, where the effect of genotype could be further isolated. Given the complexity of BDNF and the call for replication within candidate gene research, further exploration of genetic variation in the BDNF gene and clinically relevant emotion responses is necessary.
## Table 1. Sample Characteristics by Group

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>Full Sample (n=119)</th>
<th>Val’Val</th>
<th>Val’Met/ Met’Met</th>
<th>Chi-square B v. C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hardy-Weinberg:</strong></td>
<td></td>
<td>68 (61.26%)</td>
<td>43 (38.74%)</td>
<td></td>
</tr>
<tr>
<td>$\chi^2=1.04, p=0.30$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Age</strong></td>
<td></td>
<td>M=20.87, SD=6.43</td>
<td>M=20.7, SD=6.4</td>
<td>$\chi^2=22.02, p=.08$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M=21.1, SD=6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td>74 (62.2%)</td>
<td>50 (66.7%)</td>
<td>$\chi^2=1.73, p=.13$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 (54.5%)</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td>94 (79%)</td>
<td>57 (76%)</td>
<td>$\chi^2=3.31, p=.35$</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td>37 (84.1%)</td>
<td>37 (84.1%)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>14 (11.8%)</td>
<td>11 (14.7%)</td>
<td>3 (6.8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.5%)</td>
<td>1 (1.3%)</td>
<td>2 (4.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 (6.7%)</td>
<td>6 (8%)</td>
<td>2 (4.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td>$\chi^2=.13, p=.50$</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>108 (90.8%)</td>
<td>69 (92%)</td>
<td>39 (88.6%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>7 (5.9%)</td>
<td>4 (5.3%)</td>
<td>3 (6.8%)</td>
<td></td>
</tr>
<tr>
<td>Depression Symptoms</td>
<td>M=11.1, SD=7.2</td>
<td>M=11.2, SD=7.6</td>
<td>M=10.9, SD=6.4</td>
<td>$\chi^2=22.21, p=.77$</td>
</tr>
</tbody>
</table>
Table 2. Paired Sample T-tests for Manipulation Check of Emotion Response to Emotion Response Assessment

<table>
<thead>
<tr>
<th>Condition</th>
<th>M (SD)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative affect during baseline video</td>
<td>1.07 (0.17)</td>
<td></td>
</tr>
<tr>
<td>Positive affect during baseline video</td>
<td>3.05 (1.22)</td>
<td>(t(118) = -17.51, p=.00)</td>
</tr>
<tr>
<td>Negative affect during negative emotion video</td>
<td>2.91 (1.23)</td>
<td>(t(118) = 10.28, p=.00)</td>
</tr>
<tr>
<td>((The Road to Guantanamo))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive affect in during negative emotion video</td>
<td>1.47 (0.69)</td>
<td></td>
</tr>
<tr>
<td>((The Road to Guantanamo))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative affect during positive emotion video</td>
<td>1.18 (0.32)</td>
<td>(t(118) = -13.58, p=.00)</td>
</tr>
<tr>
<td>((Alive))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive affect during positive emotion video</td>
<td>2.6 (1.25)</td>
<td></td>
</tr>
<tr>
<td>((Alive))</td>
<td></td>
<td></td>
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</tbody>
</table>
**Figure 1.** Val66Met Genotype Differences in Mean Respiratory Sinus Arrhythmia (RSA) during Emotion Response Task
Figure 2. Val66Met Genotype Differences in Mean Positive Emotion during Emotion Response Task.

![Graph showing differences in mean positive emotion across baseline, negative video, and positive video conditions for ValVal and ValMet/MetMet genotypes. The graph includes a significant difference (*p<.05*) for ValVal genotype.](image-url)
**Figure 3.** Val66Met Genotype Differences in Mean Negative Emotion during Emotion Response Task.
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