Neuroimaging genetic approaches to Posttraumatic Stress Disorder

Lauren A. M. Lebois¹,², Jonathan D. Wolff¹,², and Kerry J. Ressler¹,²
¹Department of Depression and Anxiety, McLean Hospital, Belmont, MA
²Department of Psychiatry, Harvard Medical School, Boston, MA

Abstract

Neuroimaging genetic studies that associate genetic and epigenetic variation with neural activity or structure provide an opportunity to link genes to psychiatric disorders, often before psychopathology is discernable in behavior. Here we review neuroimaging genetics studies with participants who have Posttraumatic Stress Disorder (PTSD). Results show that genes related to the physiological stress response (e.g., glucocorticoid receptor and activity, neuroendocrine release), learning and memory (e.g., plasticity), mood, and pain perception are tied to neural intermediate phenotypes associated with PTSD. These genes are associated with and sometimes predict neural structure and function in areas involved in attention, executive function, memory, decision-making, emotion regulation, salience of potential threats, and pain perception. Evidence suggests these risk polymorphisms and neural intermediate phenotypes are vulnerabilities toward developing PTSD in the aftermath of trauma, or vulnerabilities toward particular symptoms once PTSD has developed. Work distinguishing between the re-experiencing and dissociative subtypes of PTSD, and examining other PTSD symptom clusters in addition to the re-experiencing and hyperarousal symptoms, will further clarify neurobiological mechanisms and inconsistent findings. Furthermore, an exciting possibility is that genetic associations with PTSD may eventually be understood through differential intermediate phenotypes of neural circuit structure and function, possibly underlying the different symptom clusters seen within PTSD.

Keywords

Intermediate Phenotype; MRI; Neural Circuit; Neurogenetics; Neuroimaging Genetics; PTSD; Risk Polymorphism; Trauma

Posttraumatic Stress Disorder (PTSD) is a debilitating disorder associated with increased suicide risk, educational dropout, unemployment, relationship instability, and the development of comorbid psychiatric disorders (Kessler, 2000). The majority of individuals in the US will experience a traumatic event in their lifetime, yet not all will develop PTSD.
There is evidence that genetic and epigenetic factors account for 30 – 70% of these individual differences (Afifi et al., 2010; Pitman et al., 2012), but the mechanisms by which they exert this influence are not well understood. In the long run, understanding these mechanisms will greatly inform both how to identify those at risk for certain clusters of PTSD symptoms, and how to tailor individual treatments for the best recovery outcomes. Brain structure and function, along with genetics, have emerged as important biological markers of PTSD, helping to identify risk for this disorder and further linking a complex cluster of behaviors to mechanisms of dysfunction and recovery (Greco & Liberzon, 2016; Michopoulos et al., 2015; Peterson et al., 2014; Stark et al., 2015). Combining the two methodologies of neuroimaging and genetics, however, offers an opportunity for an even more nuanced mechanistic understanding of PTSD (Bogdan et al., 2016). In addition to briefly outlining the current findings in the neuroimaging and genetics of PTSD, we focus on how the combination of these techniques has led to further insight into the vulnerabilities and patterns of PTSD dysfunction and recovery.

Classic Findings in the Neurobiology of PTSD

Brain Structure Overview

By examining the structural composition of brain regions we can identify potential dysfunctions. Reductions or increases in volume could point toward under or overuse, respectively, of that brain region or dysregulated communication between regions. PTSD research has consistently implicated abnormal structure in a number of brain regions involved in memory, emotion regulation and production (Figure 1). Several studies have found decreased hippocampal (HP) volume in participants with PTSD (Gurvitis et al., 1996; Stein et al., 1997; Kitayama et al., 2005; Wang et al., 2010; Smith, 2005; Karl, et al., 2006). Reduced volume has also been widely observed in the rostral ventromedial prefrontal cortex (vmPFC) and in the dorsal anterior cingulate cortex (dACC) (Kasai et al., 2008; Kityama, Quinn, & Bremner, 2006; Carrion et al., 2010; Schuff et al., 2008; Karl & Wrner, 2010; Sekiguchi et al., 2013). Work has yet to definitively determine whether these structural abnormalities are risk factors for developing PTSD or a consequence of the disorder or perhaps both. For example, there is evidence to support the hypothesis that lower HP volume is the result of exposure to trauma (Bremner, 2001), as well the hypothesis that it is a risk factor, environmental and/or genetic, for the development of PTSD (Gilbertson et al., 2002; Myslobodsky, 1995). We return to this point in the conclusions section.

Brain Function Overview

Using a non-contrast brain imaging technique, we can measure the concentration of paramagnetic deoxyhemoglobin in a particular area of the brain. Functional magnetic resonance imaging (fMRI) measures changes in the blood oxygen level-dependent (BOLD) signal, which is thought to reflect neural processing in a particular area of the brain (Ogawa et al., 1992). By measuring neural activity in this manner, we can identify potential dysfunctions in a psychological construct of interest as related to PTSD.
Several consistent findings have emerged in functional neuroimaging studies of PTSD. The amygdala, vmPFC, ACC, insula, and HP have been identified as key functional regions involved in PTSD (Figure 1). The amygdala appears to be hyper-responsive in PTSD. Exaggerated amygdala activity has been observed in response to trauma-related stimuli such as sounds (Liberzon et al., 1999; Pissiota et al., 2002), words (Protopopescu et al., 2005), and photographs (Hendler et al., 2003; Shin et al., 1997), trauma-unrelated affective stimuli such as fearful faces (Rauch et al., 2000; Shin et al., 2005; Williams et al., 2006), and during the acquisition of conditioned fear (Bremner et al., 2005). A positive correlation has been reported between amygdala activity and PTSD symptom severity (Rauch et al., 1996; Shin et al., 2004; Protopopescu et al., 2005; Armony et al., 2005). Amygdala hyperactivity may underlie increased salience and attention to threat in PTSD. In addition, PTSD participants often show increased activation in dACC during fear learning and extinction (Rougemont-Bücking et al., 2011; Milad et al., 2009; Bryant et al., 2005; Hayes et al., 2009), and increased insula activity when confronted with potentially aversive stimuli (Pitman et al., 2012; Simmons et al., 2008; Strigo et al., 2010; Aupperle et al., 2012).

In contrast, the vmPFC and rostral vACC are often hypoactive in PTSD. Decreased vmPFC and vACC activation has been reported in response to trauma-related stimuli such as narratives (Shin et al., 2004; Lanius et al., 2001; Shin et al., 1999; Bremner et al., 1999; Britton et al., 2005; Lindauer et al., 2004), photographs, and sounds (Bremner et al., 1999; Yang et al., 2004), as well as in trauma-unrelated stimuli such as fearful faces (Felmingham et al., 2010; Gold et al., 2011; Shin et al., 2005; Williams et al., 2006), and affective words (Bremner et al., 2003). PTSD symptom severity is often negatively correlated with mPFC activity (Shin et al., 2004; Shin et al., 2005; Williams et al., 2006; Britton et al., 2005). Hypoactivation in vmPFC and vACC may contribute to the maintenance of traumatic memories, and may underlie impaired emotion regulation.

Finally, the HP demonstrates mixed hyper- and hypo-activity across studies (Pitman et al., 2012). Inconsistent patterns of activity in HP and other regions (e.g., mPFC and amygdala) across studies could be caused by any number of design differences and symptom heterogeneity. One important potential cause of variation could also be the presence of dissociative symptoms. Often studies do not distinguish between the classic or re-experiencing vs. dissociative subtype of PTSD. The dissociative subtype of PTSD is a diagnosis that is characterized by significant symptoms of depersonalization and derealization, in addition to the classic PTSD symptoms of re-experiencing (e.g., flashbacks), avoidance, negative changes in beliefs and feelings, and hyper-arousal (DSM-5 American Psychiatric Association, 2013). Most generally, depersonalization and derealization are experiences of detachment from one’s self or surroundings, respectively. Recent research suggests that the re-experiencing subtype of PTSD shows classic amygdala hyper-activity and vmPFC hypo-activity, and in contrast, the dissociative subtype of PTSD shows the opposite pattern (Lanius et al., 2010). For detailed reviews of PTSD neuroimaging studies see Greco & Liberzon (2016), Liberzon and Martis (2006), Pitman et al. (2012), Peterson et al. (2014), and Shin, Rauch, and Pitman (2006).
Genetics Overview

Twin studies of PTSD demonstrate that heritability accounts for approximately 30% of the variance in risk for PTSD, and that genetic factors influence the risk of exposure to traumatic events (True et al., 1993; Xian et al., 2000; Stein et al., 2014; Koenen et al., 2003; Koenen et al., 2005). Both candidate gene and hypothesis-neutral genome-wide association studies (GWAS) have identified several genes that contribute to PTSD risk and symptomatology.

Although replication has been mixed, candidate gene studies have identified that the serotonin transporter gene, COMT, FKBP5, ADCYAP1R1, BDNF, GABARA2, and ApoE2 may play a role in PTSD. Genetic variation (e.g., polymorphisms) in these genes is often associated with PTSD. For example, the polymorphism 5-HTTLPR located in the promoter region of the serotonin transporter gene has been associated with PTSD. Specifically, the short (S) allele of 5-HTTLPR appears to confer risk for PTSD (Kilpatrick et al., 2007; Hans Jörgen Grabe et al., 2009; Koenen et al., 2009; Xie et al., 2009; Kolassa et al., 2010; Wang et al., 2011; Mercer et al., 2012). The MET158 functional polymorphism in the dopaminergic system is associated with a significant reduction in Catechol-O-methyltransferase (COMT) enzyme activity, and has been associated with PTSD (Lachman et al., 1996; Valente et al., 2011; Kolassa et al., 2010). The FKBP5 gene modulates glucocorticoid receptor sensitivity (Scammel et al., 2001) and its genetic variation has been associated with PTSD (Binder et al., 2008; Xie et al., 2010; Mehta et al., 2011; Sarapas et al., 2011; Klengel et al., 2013). Risk alleles of the PAC1 receptor gene (ADCYAP1R1) have been associated with PTSD primarily in African American Women (Ressler et al., 2011; Almli et al., 2013). Brain derived neurotrophic factor (BDNF) encoded by the BDNF gene is involved in fear extinction and recovery from stress, which are impaired in PTSD (Chhatwal et al., 2006; Heldt et al., 2007; Soliman et al., 2010). Finally, the GABA receptor gene (GABARA2) (Nelson et al., 2009) and ApoE2 (Freeman et al., 2014) have also been significantly associated with PTSD.

Genome-wide association studies have linked a number of additional genes and intergenic regions with PTSD: TLL1, RORA, COBL, PRTFDC1, linc01090, and BC036345 (Almli et al., 2015; Guffanti et al., 2013; Logue et al., 2013; Niervergelt et al., 2015; Xie et al., 2013). Several of the single nucleotide polymorphisms (SNPs) identified in these studies, however, did not reach genome-wide significance when replicated. Future studies with larger samples are needed to clarify the relationship between these genes and PTSD. For detailed reviews of PTSD genetics see Almli et al. (2014a), Skelton et al. (2012), and Koenen et al. (2007).

The What and Why of Neuroimaging Genetics

Given the genetic heritability of psychiatric disorders (Afifi et al., 2010; Pitman et al., 2012), understanding genetic vulnerabilities to and protective factors against these conditions is vital. Linking psychopathology and genetic underpinnings directly, however, has not produced stable, robust results. This may be, in part, because genes encode for biological activity within and between cells, and not for heterogeneous psychiatric symptoms (Rasetti & Weinberger, 2011). One way to address this issue is to identify an intermediate phenotype (see also, endophenotype) – a biological marker more directly linked to genetic code.
compared to the complex patterns of behavior and thought associated with psychiatric disorders (Admon, Milad, & Hendler, 2013; Meyer-Lindenberg & Weinberger, 2006). Brain structure and function are two examples of intermediate phenotypes that can bridge genetics and behavioral psychopathology.

The emerging field of neuroimaging genetics can provide one method for linking genes and psychopathology. Neuroimaging genetics is the examination of genetic variation and its influence on brain structure and function (Meyer-Lindenberg & Weinberger, 2006). Associations between genes and neuroanatomy or function can then be related to the behavioral and psychological symptoms of psychiatric disorders. As a result, there is a greater chance of detecting biological markers related to dysfunction or recovery from psychiatric disorders (Bigos & Weinberger, 2010; Meyer-Lindenberg & Weinberger, 2006; Morey et al., 2011). This technique may also reveal vulnerabilities and emerging physiological consequences of disorders much earlier, before they are perceivable in behavior (Pohlack et al., 2015). Another benefit of this method is that it can begin to distinguish between a vulnerability to a disorder and a consequence of the disorder (Admon, Milad, & Hendler, 2013).

Neuroimaging genetics can be implemented in different populations, healthy controls and those with a psychopathology, to examine the functional relationship between genes and psychiatric disorders. Examining healthy controls allows identification of vulnerabilities before a disorder develops (Rasetti & Weinberger, 2011). Results are not confounded by medication, time course of the disorder, or other comorbid conditions (Rasetti & Weinberger, 2011). Healthy control studies, however, require extra steps to make a clear connection between their results and the disorder of interest before robust conclusions can be made (Rasetti & Weinberger, 2011). Studies conducted with the disordered population possess this direct link with the behavioral and psychological symptoms of the disorder. On the other hand, potential confounds abound with, for example, medication use that interferes with the interpretation of results. Studies with both populations are necessary to provide a complete picture of psychopathology biomarkers.

**Neuroimaging Genetics of PTSD**

The remainder of this article focuses on neuroimaging genetics studies of participants with PTSD as opposed to healthy control participants. The genes that we focus on are those that have emerged in the PTSD neuroimaging genetics literature as significantly linked to differences in brain structure and function. First, we review genetic polymorphisms, for example, single nucleotide polymorphisms (SNPs), paired with 1) structural and then 2) functional neuroimaging data. The genes in these studies were identified using either a candidate gene or genome-wide association approach. Finally, we end with studies examining epigenetic changes associated with neuroimaging data.

**Genetic variation associated with brain structure differences in PTSD as measured by MRI**

By examining the structural composition of brain regions and how they relate to genetic variation we can identify potential risk factors in developing PTSD or consequences of the disorder. However, as we will discuss in our conclusions section, it remains unclear in many
cases whether the origin of these structural differences is antecedent to trauma or is a change that occurs as a result of PTSD. Here we review findings from three genes, *FKBP5*, *COMT*, and *BDNF*, as they relate to differences in the brain structure of participants with PTSD.

**FKBP5**—FK506-binding protein 5 gene (*FKBP5*) modulates glucocorticoid receptor activity (Zannas et al., 2016). Increases in the protein produced by this gene inhibit glucocorticoid signaling either by decreasing the sensitivity or increasing the resistance of the receptor (Zannas et al., 2016). *FKBP5* is highly expressed in the hippocampus (Zannas et al., 2016), a structure involved in memory for consciously recalled events (declarative/explicit memory; Kim & Diamond, 2002; Squire, 1992). During memory formation the hippocampus (HP) helps bind together patterns of neural activity throughout the brain that comprise an episodic memory (Kim & Diamond, 2002; Squire, 1992). The SNP rs1360780 risk allele (T) is associated with increased *FKBP5* transcription, and therefore increased inhibition of glucocorticoid signaling (Zannas et al., 2016). Interestingly, low glucocorticoid levels and reduced glucocorticoid action have been implicated as vulnerabilities toward developing PTSD (Resnick et al., 1995; Yehuda et al., 1998).

In an adult sample of civilian African American women with a high trauma load, Fani et al. (2013) found that *FKBP5* SNP rs1360780 risk allele carriers (TT/TC) had altered left HP shape compared to nonrisk allele carriers (CC). This alteration in shape occurred especially in the CA1 region of the HP. Stress blocks neurogenesis in the HP, induces dendritic atrophy, and reduces long-term potentiation (Kim & Diamond, 2002; Sapolsky et al., 1990), which makes it a particularly vulnerable brain structure in PTSD. Approximately half of the sample had current PTSD as measured by the PTSD Symptom Scale, but this diagnosis and symptoms were not significantly different between the risk and nonrisk allele groups. Those with the risk allele (TT/TC), however, had increased attention toward threat cues on an attention task (described in detail in the fMRI section), a hallmark of PTSD symptomatology. One possible interpretation, given this cross-sectional sample, is that HP structural differences associated with increased threat bias in risk allele carriers represent a vulnerability toward developing PTSD.

Differential structure of the posterior cingulum has also been linked to *FKBP5* SNP rs1360780. The posterior cingulum is a bundle of white matter fibers that runs from the frontal lobe (anterior cingulate) to the temporal lobe (entorhinal cortex). It is a central line of communication between structures in the temporal and frontal lobes (Fani et al., 2014). Abnormalities in the posterior cingulum imply a disruption or dysregulation in communication between these areas. In an adult African American female sample, risk allele carriers (T) had lower fractional anisotropy in the left posterior cingulum, reflecting either lower fiber density, axonal diameter, myelination, or a combination thereof, in this region (Fani et al., 2014; Figure 2A). There was no interaction between genotype, posterior cingulum fractional anisotropy, and PTSD symptoms or diagnosis (approximately half of the sample had a current PTSD diagnosis as measured by the PTSD Symptom Scale). A tentative interpretation to be drawn from this cross-sectional sample is that decreased white matter integrity of the posterior cingulum is a vulnerability toward developing PTSD.
COMT—The COMT gene codes for Catechol-O-methyltransferase, which acts at both the level of synaptic terminals and the synaptic cleft to catalyze the breakdown of catecholamines such as dopamine (Tunbridge, Harrison, & Weinberger, 2006). COMT helps regulate levels of dopamine, especially in the prefrontal cortex where it is highly expressed (Tunbridge, Harrison, & Weinberger, 2006). Genetic variation in COMT is associated with executive function; for example, polymorphisms on this gene predict differential working memory performance (Tunbridge, Harrison, & Weinberger, 2006). The Val allele of COMT SNP rs4680 (Val158Met) is associated with the increased breakdown of dopamine, and consequently, decreased dopaminergic neurotransmission, decreased cognitive performance as seen behaviorally on neuropsychological tests (Dickinson & Elvevag, 2009), and decreased neural efficiency (i.e., greater activation in PFC during tasks; Mier, Kirsch, & Meyer-Lindenberg, 2010). In contrast, the Met allele is associated with the decreased breakdown of dopamine, and consequently, increased dopaminergic neurotransmission. Perhaps largely as a result of this, individuals demonstrate dysregulated emotional processing, for example, rigidity in emotional responding, and a propensity toward negative affect (Dickinson & Elvevag, 2009). These results highlight that it is important to remember that risk alleles for particular symptoms or psychopathologies may not be “risk” alleles in all contexts. Sometimes these alleles are protective, and the environmental circumstances determine their risk vs. resiliency.

There is evidence that the COMT SNP rs4680 moderates the relationship between neural cortex structure and PTSD. Schulz-Heik et al. (2011) found that a military veteran sample with combat-related PTSD had decreased anterior cingulate cortex (ACC) gray matter volume compared to participants without a PTSD diagnosis (as measured by the Clinician-Administered PTSD Scale, CAPS Blake et al., 1998). This relationship was moderated by COMT SNP rs4680 genotype. Val allele carriers with PTSD had smaller right ACC volume compared to Val allele carriers without PTSD. In contrast, ACC volume was similar in Met allele carriers regardless of PTSD diagnosis. Overall the Val/Val genotype had the smallest right ACC volume compared to all other groups (PTSD and non-PTSD). The ACC is involved in the production and regulation of emotion (Kober et al., 2008). Functional divisions have been identified on the dorsal ventral plane. Dorsal ACC is often involved in the processing of pain, fear learning, response selection, and error detection, and ventral ACC is involved in visceral states, and conflict monitoring during emotional Stroop tasks (Kober et al., 2008; Pitman et al., 2012). As described earlier, PTSD participants often demonstrate hypoactivity in vACC during emotional tasks (e.g., Shin et al., 2001), and in contrast, hyperactivity in dACC during fear learning (e.g., Rougemont–Bücking et al., 2011). Schulz-Heik et al (2011) examined vACC and dACC together in their analyses, which speaks to a structural abnormality that could underlie either (or both) ventral and dorsal functional findings in the PTSD literature. Given that Val allele carriers without PTSD did not have reduced ACC volume, these results suggest the Val allele is a risk factor for developing structural abnormalities associated with PTSD. Reduced ACC volume linked to this genotype may not be a pre-existing vulnerability.

BDNF—The brain-derived neurotrophic factor gene (BDNF) encodes for the BDNF protein, a neurotrophin facilitating the development and survival of neurons. BDNF acts to facilitate
the activity-dependent strengthening (e.g., in response to thoughts, emotions, behavior) of connections between neurons (i.e., synaptic plasticity). Because of this, *BDNF* is heavily involved in learning and memory (Binder & Scharfman, 2004). It is expressed throughout the brain, but plays particularly crucial roles with regard to learning and memory in the HP. Notably, a functional coding SNP in the *BDNF* gene, SNP rs6265, is associated with increased plasticity (Lyoo et al., 2011). Furthermore, recent data suggests that rs6265 is also associated with altered extinction of fear in humans as well as in a humanized transgenic mouse that contains the same mutation in *BDNF* (Soliman et al., 2010).

*BDNF* SNP rs6265 may also play a role in PTSD recovery processes. Lyoo et al. (2011) longitudinally followed an Asian trauma-exposed civilian sample to examine *BDNF* polymorphisms and their relation to neural structure. Approximately 80% of the trauma-exposed sample was diagnosed with PTSD one-month post trauma (CAPS diagnosed). At one year follow-up assessments, they found that participants exposed to trauma had increased cortical thickness in dlPFC compared to non-trauma controls. This increase in dlPFC cortical thickness appeared most prominent for the Val/Val genotype vs. Met allele carriers at the rs6265 SNP (trend toward significance). The dlPFC thickness was associated with PTSD symptom improvement and recovery, and better performance on neuropsychological tests of executive function. Given the role of dlPFC in emotion regulation, reappraisal, and executive function (Miller & Cohen, 2001; Ochsner, Bunge, Gross, & Gabrieli, 2002; Spreng et al., 2013), this evidence suggests dlPFC may be involved in regulating trauma-related symptoms as individuals recover from PTSD. Furthermore, these data suggest that individuals with Val alleles at the rs6265 locus may have an advantage. Interestingly, BDNF has also been found to interact with glucocorticoid signaling. Higher levels of BDNF are associated with enhanced glucocorticoid receptor signaling, and glucocorticoids are important for neuronal plasticity (Arango-Lievano et al., 2015; Jeanneteau et al., 2012). Therefore, one way the *BDNF* SNP rs6265 may facilitate plasticity and be associated with improved PTSD-related recovery, is by enhancing glucocorticoid receptor signaling, thus normalizing recovery from stress-related emotional and memory ailments.

**GWAS evidence for the COBL gene**—Xie and colleagues recently reported a genome-wide significant single nucleotide polymorphism (SNP) \((p = 3.97 \times 10^{-8})\) rs406001, in a genome-wide association study (GWAS) of PTSD (Xie et al., 2013). The rs406001 SNP is intergenic with no known function; however, the closest gene is *COBL*, which may be related to actin polymerization, and neuronal development and function. Almli et al., (2014b) sought to replicate the top associations of Xie and colleagues, and to extend these findings with structural MRI to examine potential intermediate neural phenotypes. Although the main effects were not replicated in this secondary cohort, they found a significant genotype by environment interaction (GxE) with childhood trauma in the top SNP, rs406001 \((N = 3076, t = 14.98, p = 0.0006)\). In fact, all three SNPs close to the gene *COBL* gene reported in Xie et al., (2013), had significant GxE interactions with childhood trauma in the replication cohort. The brain imaging findings indicated that risk allele carriers of rs406001 demonstrate poorer white matter integrity in the uncinate fasciculus, connecting medial prefrontal with temporal lobe regions, including the amygdala (Almli et al., 2014b, Figure.
These regions thought to play a critical role in regulation and extinction of learned fear and trauma associations. These data serve as a partial replication and extension of the first large GWAS for PTSD, suggesting a potential white matter intermediate phenotype underlying PTSD risk.

**Genetic variation associated with differential brain activity in PTSD as measured by fMRI**

The majority of neuroimaging genetics papers with participants who have PTSD examine BOLD signal associated with attention to threatening stimuli. Given that a core symptom of PTSD is hypervigilance toward threat, this is a key paradigm of interest. Here we review genetic variance associated with four specific genes, *FKBP5*, *SLC6A4*, *ADCYAP1R1*, *OPRL1*, and the genes encapsulated at chromosome 4p15, in relation to neural activity associated with threat.

**FKBP5**—As described earlier, *FKBP5* modulates glucocorticoid receptor activity. *FKBP5* SNP rs1360780 risk allele (T) is associated with increased *FKBP5* transcription, and increased inhibition of glucocorticoid signaling (Zannas et al., 2016). In an adult sample of civilian African American women with a high trauma load, Fani et al. (2013) found that *FKBP5* SNP rs1360780 risk allele carriers (TT/TC) had increased attention toward mild threat cues. In this dot probe paradigm, participants were presented with a pair of faces. The pair was comprised of either two neutral faces or one neutral face and one valenced expression (happy or threatening). At a certain point, an asterisk replaced the faces, and the task was simply to indicate whether the asterisk appeared on the left or right side of the screen. In general, participants with a threat bias attend to the threatening face. With these participants, if the threatening face’s location matches the location of the asterisk (threat congruent), their left/right responses will be faster vs. if the locations are a mismatch (threat incongruent). Fani et al. (2013) found the risk allele carriers demonstrated this threat bias not only in their behavioral responses on the task, but also in their neural activity. On threat incongruent trials compared to threat congruent trials, participants with the risk allele (TC/TT) had more activity in their HP and parahippocampal gyrus vs. the CC genotype. Given the role of the HP and parahippocampal gyrus in memory encoding and retrieval, these results suggest an increased sensitivity to perceived threat in the environment. Findings were independent of PTSD symptoms or diagnosis. A tentative interpretation of this cross-sectional data suggests this *FKBP5* risk allele is associated with increased threat bias (behaviorally and neurally), which in turn may represent a vulnerability toward developing PTSD.

**SLC64A**—The serotonin transporter gene (*SLC6A4*) encodes a protein that transports serotonin back into presynaptic terminals (i.e., reuptake). Serotonin is linked to mood with decreased serotonin levels associated with lower mood (Young, 2007). Classically studied polymorphisms in this gene include SNPs (e.g., rs16965628, GG genotype and CG genotype) and deletions (e.g., 5-HTTLPR short allele, “S” and long allele, “L”). The rs16965628 G allele and 5-HTTLPR S allele are both associated with decreased expression of *SLC64A* (Martin et al., 2007). Both polymorphism types can also be examined in tandem. For example, in one triallelic variation of 5-HTTLPR, the SLₐ, LGₐ genotypes offer a

*Exp Neurol*. Author manuscript; available in PMC 2017 October 01.
baseline for the expression of SLC64A, the LG, SLG, and SS genotypes are low expressing, and the LAL genotype is high expressing.

In a sample of combat-trauma exposed veterans with and without PTSD (as measured by Davidson Trauma Scale, Davidson, 1996), Morey et al. (2011) found the rs16965628 and 5-HTTLPR polymorphisms predicted differential neural activity on a working memory task. Participants were shown a series of faces, a short delay occurred, and then they were shown a subsequent face. Their task was to indicate whether this subsequent face was previously displayed (“old”) or new. During the delay period between initial face exposures and the memory rating (old vs. new), different kinds of distracters were displayed. The distracters were combat-related, non-combat related, or scrambled images, and all were irrelevant to the task at hand. All groups, regardless of PTSD symptom severity or genotype had a similar behavioral performance on this task, but distinct differences emerged between genotype, PTSD diagnosis, and neural activity.

When comparing BOLD signal during the delay period for combat-related distracters vs. non-combat related distracters, rs16965628 genotype affected ventrolateral PFC (vlPFC) activity and 5-HTTLPR genotype affected amygdala activity. In participants with PTSD, those with the rs16965628 GG genotype had more activity in vlPFC to combat distracters compared to trauma-exposed control participants with the GG genotype. CG genotype was not associated with differential vlPFC activity in PTSD and non-PTSD participants. Because all groups performed similarly on the working memory task, this evidence suggests PTSD-positive participants with the GG genotype required more activity in vlPFC to maintain performance when confronted with trauma-related distracters. At the same time, the 5-HTTLPR polymorphism trended toward modulating amygdala activity in response to combat distracters. That is, participants with PTSD and an S allele trended toward greater left amygdala activity compared to S allele carriers without PTSD. There was no difference in amygdala activity between PTSD diagnostic groups in the 5-HTTLPR LL genotype. Although this trend level significance should be interpreted with caution, this result suggests that PTSD-positive participants with an S allele found the threat cues more salient. Taken together, this evidence tentatively suggests that these two polymorphisms are associated with biased neural activity to threat once PTSD has developed.

**ADCYAP1R1**—Pituitary Adenylate Cyclase-Activating Polypeptide Type I Receptor (PAC1) is encoded by the ADCYAP1R1 gene, and it was previously associated with increased PTSD risk, particularly in women (Ressler et al., 2011; Almli et al., 2013). PAC1 is selective for Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP). The PAC1 receptor is highly expressed in the amygdala, hypothalamus, and HP, among other regions (Kormos & Gaszner, 2013; Shen, Gehlert, & Collier, 2013). PACAP has a diverse set of functions, including both the facilitation and inhibition of neuroendocrine release in response to stressors (Vaudry et al., 2009). The ADCYAP1R1 SNP rs2267735 risk allele (C) is associated with decreased expression of ADCYAP1R1, and less ADCYAP1R1 mRNA (Ressler et al., 2011).

The ADCYAP1R1 SNP rs2267735 may also predict neural activity related to threat processing (Stevens et al., 2014). Urban civilian women with a heavy trauma load passively
viewed fearful and neutral faces during fMRI. Participants were divided into two groups based on rs2267735 genotype, risk (CC) and nonrisk (GC/GG). These groups were matched for trauma load and PTSD symptom severity as measured by the modified PSS (Falsetti, 1993). When BOLD signal to fearful faces was compared to neutral faces, the rs2267735 genotype modulated activity in the amygdala and HP. The risk genotype (CC) had greater activation in amygdala and HP to fearful faces compared to the nonrisk group (G allele carriers) (Figure 2C). The number of risk alleles was also positively correlated with activity in these regions. Furthermore, the risk genotype had significantly decreased amygdala hippocampal connectivity compared to the nonrisk allele carriers. Together these results suggest the risk group found the fearful faces more salient, and had more fear reactivity (Stevens et al., 2014). Because this was a cross-sectional sample, and groups were matched on PTSD symptom severity, these results may represent a vulnerability toward developing PTSD.

OPRL1—Opiate Receptor-Like 1 gene (OPRL1) encodes the nociceptin receptor (Andero, 2015). Among many biological roles, this receptor can modulate the perception of pain (Andero et al., 2013). OPRL1 plays a particularly functional role in the amygdala. Evidence suggests polymorphisms of this gene may be related to risk for PTSD. In an urban civilian sample of women with a high trauma load, Andero et al. (2013) found that OPRL1 SNP rs6010719 predicted PTSD symptoms, and behavioral and neural responses to two different fear-related tasks. As described in the previous section, participants passively viewed fearful and neutral faces while they were in the magnetic resonance imaging scanner. Outside of the scanner, participants also completed a fear potentiated startle task. Andero et al (2013) found that as participants’ reported trauma load increased, risk allele carriers (G) had increased PTSD symptoms (as measured by the PSS and CAPS) compared to the CC genotype. Risk allele carriers also had poorer discrimination between safety and danger signals in the fear potentiated startle paradigm compared to the CC genotype. In addition, on the fMRI task, risk allele carriers had increased functional connectivity between amygdala and posterior insula while viewing fearful faces compared to the CC genotype (Figure 2E). The insula is involved in pain perception and the posterior insula especially is involved in visceral state awareness (Craig, 2003). Together these results suggest risk allele carriers have increased PTSD symptom severity, and dysregulated fear responding. The OPRL1 SNP rs6010719 G allele carriers may represent a genotype at potentially higher risk for developing PTSD.

SNP rs717947 at chromosome 4p15—The only GWAS with PTSD participants to use the functional neuroimaging genetics methodology to date identified a SNP (rs717947) at chromosome 4p15 that correlates with neural activity related to threat processing (Almli et al., 2015). The functional role of this SNP is unclear, but it is a methylation quantitative trait locus, suggesting that is may have important roles in influencing the epigenetic regulation of adjacent genetic loci or regulatory regions (Banovich et al., 2014). Participants who completed the neuroimaging genetics portion of this study were urban civilian women with a high trauma load. Participants passively viewed fearful and neutral faces while in the scanner. Risk allele (T) carriers had higher PTSD symptoms compared to the CC genotype. The number of risk alleles negatively correlated with dlPFC and dmPFC activity to fearful faces compared to neutral faces. That is, risk allele carriers had decreased activity in dlPFC.
and dmPFC to fearful faces (Figure 2D). Given these regions are associated with emotion regulation (Ochsner et al., 2002; Ochsner & Gross, 2005), these results imply that risk allele carriers had increased emotional dysregulation to fearful faces perhaps an inability to regulate fearful emotion or extinguish fear (Almli et al., 2015). Together these results suggest the SNP rs717947 at chromosome 4p15 T allele carriers may represent a genotype at higher risk for developing PTSD.

**Epigenetic variation associated with brain structure and function differences in PTSD as measured by MRI and fMRI**

Not only can we look at genetic variation as it relates to risk for PTSD, but we can also assess the dynamic regulation of gene expression. One way to measure gene expression is through the methylation status of a gene. For the most part, increased methylation implies decreased gene expression (Jaenisch & Bird, 2003). Here we examine methylation status as it relates to structural and functional differences in the brains of participants with PTSD symptoms. We begin with findings from two genes, *SKA2* and *FKBP5*, as their epigenetic differences relate to brain structure, and then we end with epigenetic findings related to *NR3C1* and brain function.

**SKA2**—Spindle and kinetochore-associated complex subunit 2 (*SKA2*) encodes for a protein that enhances the activation of glucocorticoid receptors (Rice et al., 2008). In this way it facilitates negative feedback inhibition of the HPA axis, and is protective against the toxic effects of chronic HPA axis activity (Rice et al., 2008). The *SKA2* SNP rs7208505 is associated with suicidal behavior (Guintivano et al., 2014), and recent evidence suggests it is also associated with brain structure differences in PTSD (Sadeh et al., 2015). The rs7208505 risk allele (C) can have varied methylation status, but the nonrisk allele (T) cannot be methylated (Guintivano et al., 2014). Because of this, Sadeh et al. (2015) controlled for *SKA2* SNP rs7208505 genotype so they could look at the relationship between this gene’s methylation status and PTSD. Participants were white, predominately male military veterans with Criterion A trauma. Over half of the participants had a current PTSD diagnosis as measured by the CAPS. *SKA2* methylation (adjusted for genotype) was associated with decreased cortical thickness in frontopolar cortex, superior frontal gyrus (SFG), and orbital frontal cortex (OFC). In turn, reduced cortical thickness in these areas was associated with increased PTSD symptom severity. Frontopolar cortex, SFG, and OFC are involved in diverse cognitive functions, for example, complex decision-making, attention, prospective thinking, introspection, and reward/punishment-related processing (e.g., Christoff & Gabrieli, 2000; Kringelbach & Rolls, 2004; Okuda et al., 2003). Differences in frontopolar cortex, SFG, and OFC structure could point toward dysfunction in their processing, for example, emotional dysregulation, impulsivity, and difficulty picturing the future (Sadeh et al., 2015). A path analysis suggested that *SKA2* methylation status mediated the relationship between PTSD and cortical thickness in frontopolar cortex, SFG, and OFC. This indicates that *SKA2* methylation (decreased expression of *SKA2*) may represent a risk for developing PTSD.

**FKBP5**—As already discussed, *FKBP5* modulates glucocorticoid receptor activity. *FKBP5* SNP rs1360780 risk allele (T) is associated with increased *FKBP5* transcription, and
increased inhibition of glucocorticoid signaling (Zannas et al., 2016). Methylation of this gene inhibits its expression, which in turn can cause increased glucocorticoid signaling (Klengel et al., 2012). Klengel et al. (2012) replicated the finding that an interaction between childhood trauma and FKBP5 SNP rs1360780 genotype predicted lifetime PTSD as measured by the CAPS (Binder et al., 2008). Risk allele carriers (T) with childhood trauma were more likely to have had a PTSD diagnosis over their lifetime. Building on these findings, Klengel et al. (2012) found an interaction between FKBP5 SNP rs1360780 genotype and childhood trauma that predicted methylation status of this gene. Participants who experienced childhood abuse and had the risk allele (T) had decreased methylation of this gene (associated with more FKBP5 expression and altered GR sensitivity) compared to participants with the protective genotype (CC). In the risk allele carriers (T), methylation was negatively correlated with overall childhood abuse, especially physical and emotional abuse subscales as measured by the Childhood Trauma Questionnaire (Pennebaker & Susman, 1988). That is, risk allele carriers (T) with greater levels of childhood abuse had demethylation of this gene, and presumably more expression of FKBP5, inhibiting glucocorticoid signaling. FKBP5 SNP rs1360780 methylation also positively correlated with right HP volume. That is, demethylated FKBP5 seen in the risk group was associated with decreased right HP volume, a region especially vulnerable to the effects of chronic stress. Together this evidence suggests that risk allele specific FKBP5 demethylation may mediate the interaction between this genotype and childhood trauma as it relates to PTSD.

NR3C1—The nuclear receptor subfamily 3, group C, member 1 (NR3C1) gene is the glucocorticoid receptor gene. The glucocorticoid receptor is where glucocorticoids, for example, cortisol, bind. Because of this, the glucocorticoid receptor is essential for HPA axis regulation. Increased methylation of NR3C1 is associated with decreased expression of this gene (Yehuda et al., 2013).

Recently methylation status of NR3C1 has been associated with neural activity related to distressing interpersonal interactions and PTSD. Schechter et al (2015) examined NR3C1 methylation in a sample of mothers who had experienced interpersonal violence. One group of mothers had PTSD as measured by the CAPS and PCL-S (Weathers et al., 1993), and the other group of mothers did not meet PTSD diagnosis criteria, although all participants had experienced interpersonal trauma. Both groups watched video clips of mother-child interactions while they were in the magnetic resonance imaging scanner. The interactions included a child playing with their mother and a child being separated from their mother. Some videos were of the participant and their own child; others were of an unfamiliar mother and child. In this sample, decreased methylation of the NR3C1 promoter region was associated with increased PTSD symptom severity. When BOLD signal for child separation videos was compared to the signal for child playing videos, it was also associated with NR3C1 promoter region methylation status. Less methylation of NR3C1 promoter region was associated with decreased activity in vmPFC, dmPFC, dlPFC, precuneus, and thalamus to viewing child separation, and greater PTSD symptom severity. NR3C1 promoter region methylation status was also a significant predictor of activity in dlPFC and vmPFC. These results suggest that increased expression of NR3C1 (demethylation of the promoter region) is associated with increased PTSD symptom severity and decreased activity in areas of the
brain related to emotion regulation (e.g., dIPFC, vmPFC) when viewing a distressing interpersonal interaction.

Interestingly, increased expression of glucocorticoid receptors is also associated with enhanced negative feedback inhibition of the HPA axis (Herman et al., 2012). The PFC is heavily populated with glucocorticoid receptors, and actively plays a role in HPA axis negative feedback inhibition (Diorio, Viau, & Meaney, 1993; Herman et al., 2012). Lower levels of glucocorticoids and enhanced HPA axis negative feedback are often understood to be features of PTSD (Yehuda et al., 1990; Yehuda et al., 1993; see Yehuda, 2006 for a discussion of mixed findings). Demethylation of the NR3C1 promoter region, which is expected to increase receptor expression and thus enhance negative feedback of HPA, may therefore represent a risk factor for developing PTSD.

Conclusions and Future Directions

The growing body of research on the neurobiology of PTSD suggests the amygdala, vmPFC, ACC, insula, and HP are consistently implicated, and SLC6A4, COMT, FKBP5, ADCYAPI1, BDNF, GABARA2, ApoE2, TLL1, RORA, COBL, PRTFDC1, linc01090, and BC036345 are often predictors of dysfunction in the disorder. Neuroimaging genetic studies that associate genetic and epigenetic variation with neural activity or structure related to PTSD provide an even more nuanced layer of mechanistic information on top of each field studied separately. The neuroimaging genetics results reviewed here reveal that genes related to the physiological stress response (e.g., glucocorticoid receptor and activity, neuroendocrine release), learning and memory (e.g., plasticity), mood, and pain perception are tied to neural intermediate phenotypes associated with PTSD. These genes are associated with and sometimes predict neural structure and function in areas involved in diverse functions, for example, attention, executive function, memory, decision making, emotion regulation, salience (e.g., of potential threats), and pain perception. These relationships appear to be vulnerabilities toward developing PTSD, or vulnerabilities toward particular symptoms once PTSD has developed.

However, the specific relationship between neuroimaging genetic results and PTSD is still tentative in most cases. The extent to which identified biological markers represent vulnerability factors that increase the likelihood of developing PTSD following a significant trauma, or are due to the neurotoxic effects of traumatic stress associated with PTSD has yet to be definitely determined. Likely some differences pre-date trauma and PTSD, and others are corollaries of the disorder. For example, there is evidence to suggest that the reduced hippocampal volume often associated with PTSD is a vulnerability toward developing PTSD (Gilberson et al., 2002; Kasai et al., 2008; Pitman et al., 2006). This has been established through studies of monozygotic twins, one of the twins having experienced military combat exposure and one having not. Of the twin pairs, some of the combat exposed individuals developed PTSD and some did not, affording a comparison between PTSD vs. non-PTSD twin pairs. For those who did develop PTSD, both the trauma-exposed twin with PTSD and the unexposed twin without PTSD and smaller hippocampi compared to the twin pairs in which the trauma-exposed twin did not develop PTSD (Gilberson et al., 2002). The reduced HP volume for the unexposed monozygotic twin in the PTSD-twin pair suggests this
difference predates combat trauma exposure, and may have been a vulnerability toward developing PTSD.

However, there is strong rodent and human evidence to suggest that the brain, and the HP especially, is subject to structural remodeling due to the effects of stress and glucocorticoids. Thus many structural differences in PTSD may reflect consequences of the disorder. For example, there is evidence to suggest that chronic stress exposure and glucocorticoids can affect the hippocampus by reducing neurogenesis and synaptic plasticity, hindering long-term potentiation and lowering dendritic spine density, among other alterations (see Conrad, 2008 for a review). If not directly causing hippocampal cell death (see Glucocorticoid Cascade Hypothesis, Sapolsky, Krey, & McEwen, 1986), chronic stress exposure and glucocorticoids at the very least make the HP vulnerable to such damage for extended periods of time (see the Glucocorticoid Vulnerability Hypothesis; Conrad, 2008). This evidence for the detrimental effects of stress and glucocorticoid exposure on the HP points towards PTSD potentially causing reductions in HP. Similarly, Kasai et al (2008) found that reductions in pregenual ACC gray matter were a consequence of PTSD in a study of monozygotic twins discordant for combat exposure. The combat exposed twins had smaller pgACC compared to their twins who did not experience combat and did not have PTSD. More longitudinal research that assesses individuals before and after PTSD develops is necessary to definitely delineate pre-existing vulnerabilities and consequences of PTSD.

Similarly on the functional imaging side, for example, it is often unclear whether the neural activity associated with potential threat is a predisposing risk for PTSD or a PTSD-acquired dysfunction. It is perhaps likely that the biomarkers discussed here represent both vulnerability factors and the results of traumatic exposure. A recent review, however, proposes a potential causal model of PTSD risk and corollaries (Admon, Milad, & Hendler, 2013). Based on growing evidence including monozygotic twin studies, Admon et al (2013) suggest increased amygdala activity to emotionally salient stimuli and hyperactivity in the dACC during fear learning appear to be predisposing vulnerabilities toward developing PTSD (e.g., Shin et al., 2011). On the other hand, reduced connectivity between mPFC and HP resulting in reduced capacity to extinguish fear may be an acquired PTSD dysfunction. Combining results from healthy controls, healthy controls whose immediate family members have PTSD, and patients with PTSD themselves will continue to refine this relationship. Admon et al (2013) also suggest a renewed focus on neuroimaging genetics in twin studies and prospective studies that catch participants before trauma occurs.

Neuroimaging genetic approaches may also be valuable in the study of sex differences in PTSD. Males are more likely to experience potentially traumatic events, however, females are more likely to meet criteria for PTSD (Kessler et al., 1995). These differences may in part be accounted for by higher incidences of highly pathogenic types of trauma for females compared to males (e.g., childhood sexual abuse, sexual assault; Tolin & Foa, 2006). However, females are more likely to meet criteria for PTSD even in categories more frequently experienced by males (e.g., non-sexual assault, accidents; Tolin & Foa, 2006), suggesting the relevance of biological mechanisms for PTSD related sex differences. Recent findings on the dynamic regulation of PACAP and the PAC1 receptor gene by estrogen point to genetic variation contributing to these sex differences (Jovanovic et al., 2012; Ressler et
The enhanced ability of neuroimaging genetics to identify biological markers related to psychiatric disorders may be especially relevant to the understanding of sex differences in PTSD.

Future research should also aim to distinguish between the re-experiencing and the dissociative subtype of PTSD to help clarify neurobiological mechanisms and inconsistent findings. Notably, the majority of work has focused on the re-experiencing and hyperarousal symptoms associated with PTSD. It may be beneficial to concentrate on other symptom clusters, for example, negative alterations in cognitions and mood, to identify novel neural and genetic mechanisms of PTSD related to self-referential processing. Understanding the neurobiological mechanisms of these symptoms, and how genetics underlies differential neural phenotypes, could prove very fruitful in breaking down barriers to PTSD treatment and recovery.

Acknowledgments

Many thanks to Guia Guffanti and Evan Lebois for their genetic discussions and comments. This work was primarily supported by the National Institutes of Mental Health (MH096764 and MH071537 to KJR).

References


Almli LM, Stevens JS, Smith AK, Kilaru V, Meng Q, Flory J, Ressler KJ. A genome-wide identified risk variant for PTSD is a methylation quantitative trait locus and confers decreased cortical activation to fearful faces. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2015

Andero R. Nociceptin and the nociceptin receptor in learning and memory. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2015


Banovich NE, Lan X, McVicker G, Van de Geijn B, Degner JD, … Gilad Y. Methylation QTLs are associated with coordinated changes in transcription factor binding, histone modifications, and gene expression levels. 2014


Blake, DD.; Weathers, FW.; Nagy, LM.; Kaloupek, DG.; Charney, DS.; Keane, TM. Clinician-Administered PTSD Scale for DSM-IV, Revised (CAPS), National Center for Posttraumatic Stress Disorder, Medical Center & Neuroscience Division, West Heaven VA, Medical Center; Boston, VA, USA: 1998.


Davidson, J. Davidson trauma scale. Multi-Health Systems Inc; Toronto, ON: 1996.


Sadeh N, Spielberg JM, Logue MW, Wolf EJ, Smith AK, Lusk J, Miller MW. SKA2 methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed veterans. Molecular psychiatry. 2015


Exp Neurol. Author manuscript; available in PMC 2017 October 01.


Yehuda R, Daskalakis NP, Desarnaud F, Makotkine I, Lehrner AL, Koch E, Bierer LM. Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. Frontiers in psychiatry. 2013; 4


Figure 1. Brain Regions most frequently associated with Posttraumatic Stress Disorder

This diagram of the human brain illustrates some of the most frequent brain regions associated with PTSD in two decades of work related using fMRI approaches to understand brain activation in PTSD. The prefrontal cortex (PFC) and the hippocampus have strong connections to the amygdala, which is important for conditioned fear and associative emotional learning. The PFC is involved in emotion regulation and is hypoactive in PTSD with some studies showing decreased gray matter density. The hippocampus is thought to play a role in explicit and contextual memories of traumatic events and in mediating extinction of conditioned fear. In PTSD, the hippocampus is decreased in volume. The amygdala is the most well-known area in regulating fear responses, involved in conditioned fear and recovery from fear. Hyperactivation of the amygdala to fearful cues is a robust intermediate phenotype in patients with PTSD. The end result of these neuroanatomical alterations is increased stress sensitivity, generalized fear responses and impaired extinction. Other regions including the anterior cingulate cortex, the orbitofrontal cortex, the parahippocampal gyrus, the thalamus and the sensorimotor cortex also play a secondary role in the regulation of fear and PTSD. (Figure Adapted from Mahan & Ressler, 2012).
Figure 2. Genetic Associations with Structural and Functional Neural Pathways in PTSD

Shown are examples of neuroimaging genetic approaches to identifying associations of PTSD-associated SNPs with intermediate phenotypes of neural structure and activation using structural and functional MRI. A) Differential structure of the posterior cingulum connecting hippocampus to cingulate cortex has been linked to *FKBP5* SNP rs1360780. Risk allele carriers had lower fractional anisotropy in the left posterior cingulum (from Fani et al., 2014). B) The risk allele carriers of rs406001 (identified in a PTSD GWAS, Xie et al., 2013) demonstrate poorer white matter integrity in the uncinated fasciculus, connecting medial prefrontal with temporal lobe regions, including the amygdala (from Almli et al., 2014b). C) Carriers of the *ADCYAP1R1* SNP rs2267735 risk allele show greater activation in amygdala to fearful faces compared to the nonrisk group (from Stevens et al., 2014). D) The number of risk alleles of SNP rs717947 (identified in a GWAS for PTSD) negatively correlated with dmPFC activity to fearful faces compared to neutral faces (from Almli et al., 2015). E) The *OPRL1* gene SNP rs6010719 was found to be associated with PTSD and intermediate phenotypes of PTSD. With fMRI, enhanced bilateral amygdala activation in response to fearful versus neutral face stimuli was observed in all participants, irrespective of genotype. However, when viewing fearful faces, risk allele carriers had increased functional connectivity between amygdala and right posterior insula (from Andero et al., 2013).