Genetic Moderation of Stress Effects on Corticolimbic Circuitry

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Stress exposure is associated with individual differences in corticolimbic structure and function that often mirror patterns observed in psychopathology. Gene x environment interaction research suggests that genetic variation moderates the impact of stress on risk for psychopathology. On the basis of these findings, imaging genetics, which attempts to link variability in DNA sequence and structure to neural phenotypes, has begun to incorporate measures of the environment. This research paradigm, known as imaging gene x environment interaction (iGxE), is beginning to contribute to our understanding of the neural mechanisms through which genetic variation and stress increase psychopathology risk. Although awaiting replication, evidence suggests that genetic variation within the canonical neuroendocrine stress hormone system, the hypothalamic-pituitary-adrenal axis, contributes to variability in stress-related corticolimbic structure and function, which, in turn, confers risk for psychopathology. For iGxE research to reach its full potential it will have to address many challenges, of which we discuss: (i) small effects, (ii) measuring the environment and neural phenotypes, (iii) the absence of detailed mechanisms, and (iv) incorporating development. By actively addressing these challenges, iGxE research is poised to help identify the neural mechanisms underlying genetic and environmental associations with psychopathology.

INTRODUCTION

All organisms strive to maintain homeostasis by regulating physiology and behavior within a dynamic equilibrium. Stress, the perception of inadequate resources in the context of environmental pressures appraised as threatening, disrupts homeostasis by triggering physiologic and behavioral responses to meet the immediate demands on an individual (Ganzel *et al*, 2010; Selye, 1936). Although stress promotes adaptive responses to challenge when motivation is high and resources available, the relationship between stress, especially that which is chronic, unpredictable, and uncontrollable, and the experience of psychopathology is unequivocal (McEwen and Gianaros, 2010). Nearly 40% of individuals report experiencing adversity during childhood, which predicts 30% of adult-onset and 45% of childhood-onset psychiatric disorders (Green *et al*, 2010; Kessler *et al*, 2010). Similarly, evidence suggests that ubiquitous adult-onset stressful experiences, as well as perceived stress increase risk for the emergence and relapse of various forms of psychopathology (Dohrenwend, 2000; Monroe and Reid, 2009). These robust

transdiagnostic associations have propelled research across species to investigate the neurobiology of stress responsiveness and adaptation, as well as how individual differences in this neurobiology develop and confer relative vulnerability or resiliency to the pathogenic effects of stress (de Kloet *et al*, 2005; Hill *et al*, 2012; Lucassen *et al*, 2014; Lupien *et al*, 2009; McCrory *et al*, 2011; McEwen and Morrison, 2013; Teicher and Samson, 2013).

The neurobiology of stress responsiveness is well understood due in large part to its conservation across species. When a threat is detected coordinated autonomic, neuroendocrine, metabolic and immune system responses are initiated by governing interconnected corticolimbic circuitry, which also functions to return the body to homeostasis following stressor removal (de Kloet *et al*, 2005; Ulrich-Lai and Herman, 2009; Whalen and Phelps, 2009). The central structure, or hub, within this network is the amygdala, which has extensive afferent and efferent connections with other corticolimbic circuitry nodes including the thalamus, sensory cortex, autonomic and neuromodulatory brainstem nuclei, hypothalamus, insula, hippocampal formation, and prefrontal cortex (Figure 1; for more detailed reviews of this circuitry see (Duvarci and Pare, 2014; Hariri, 2015; Janak and Tye, 2015; Kim *et al*, 2011; Price and Drevets, 2012; Whalen and Phelps, 2009). Broadly, the amygdala and its connections to

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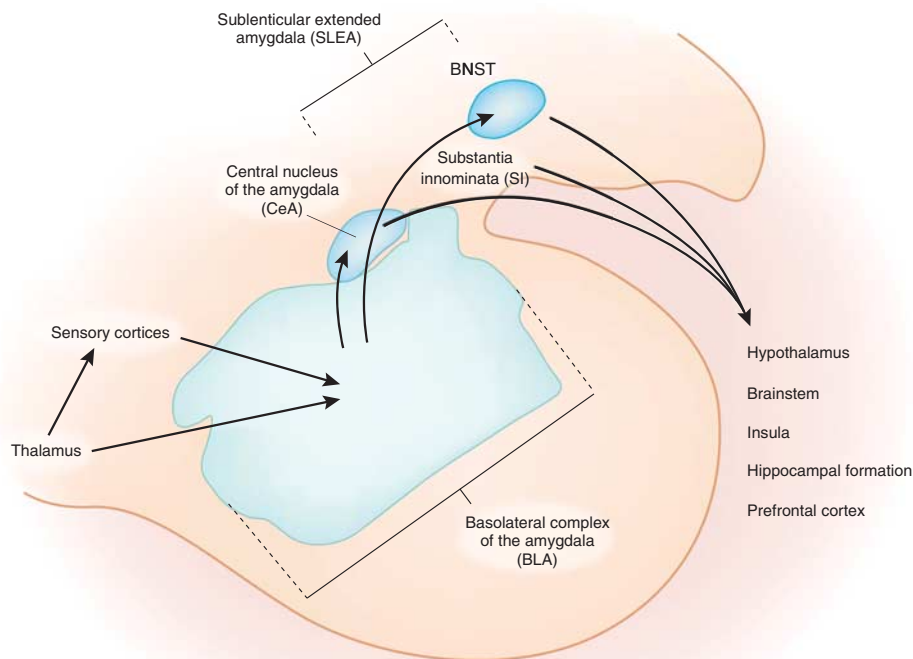
Figure 1. The amygdala functions as the hub of the corticolimbic circuit. The basolateral complex of the amygdala (BLA) receives low-resolution sensory information from the thalamus and olfactory tract, as well as high-resolution sensory information from unimodal sensory cortex and multimodal association areas. The BLA relays this information to the central nucleus of the amygdala (CeA), which further projects to the sublenticular extended amygdala (SLEA) composed of the substantia innominata (SI) and the bed nucleus of the stria terminalis (BNST). The CeA and SLEA drive physiobehavioral output through projections to corticolimbic nodes (brainstem, hypothalamus, insula, hippocampal formation, prefrontal cortex). CeA and SLEA projections to brainstem nuclei facilitate sympathetic arousal while relays to the hypothalamus excite the hypothalamic-pituitary-adrenal (HPA) axis producing a stress hormone response. Diffuse cholinergic projections from the nucleus basalis of Meynert within the SI to the cortex increase neuronal sensitivity to input, including sensory and interoceptive information, to facilitate acuity and awareness/alertness. BLA and SLEA projections to the insula facilitate interoceptive awareness in conjunction with convergent somatosensory and interoceptive projections from the body, allowing for a representation of one's bodily states (eg, heart rate). Amygdala and EA projections to the hippocampus potentiate encoding and recall of the context in which a stimulus triggered an amygdala response. A secondary role of the hippocampus within the corticolimbic system is to provide negative feedback inhibition of the HPA axis. Corticolimbic nodes further project back to the amygdala to further regulate its function. For instance, projections from the dorsomedial PFC can serve to inhibit amygdala response during emotion regulation and fear extinction. (For more detailed reviews of this circuitry see Duvarci and Pare, 2014; Hariri, 2015; Janak and Tye, 2015; Kim et al, 2011; Price and Drevets, 2012; Whalen and Phelps, 2009.)

these regions are necessary for first recognizing possible threat in the environment and then generating and regulating appropriate reactions in physiology and behavior.

Given the quintessential role of corticolimbic circuitry in stress responsiveness, it is not surprising to find consistent differences in corticolimbic function and structure across various forms of psychopathology and associated behavioral risk factors, such as negative emotionality or neuroticism (Hariri, 2015). In particular, relative hyperactivity of the amygdala to threatening contexts and stimuli has emerged as a core feature of stress-related disorders such as anxiety, PTSD, and depression (Bruhl et al, 2014; Groenewold et al, 2013; Hayes et al, 2012; Swartz and Monk, 2014a). Highly convergent findings from preclinical models of human psychopathology, especially anxiety disorders, further underscore the importance of corticolimbic circuitry broadly and the amygdala specifically in the emergence of stress-related disorders (Bukalo et al, 2014; Duvarci and Pare, 2014). Relative to the consistent and convergent observations of

amygdala hyperactivity in stress-related disorders, differences in amygdala structure have been mixed. For instance, first-episode depression is associated with larger amygdala volume, whereas smaller volumes or no differences have been observed in patients with recurrent depression (Frodl et al, 2002, 2003). Mixed volumetric findings have also been reported among individuals with PTSD and anxiety disorders as well as individuals stratified according to the experience of early life stress (Hanson et al, 2015; Hilbert et al, 2014; Luby et al, 2013; Sheridan et al, 2012; Tottenham et al, 2010). What is clear, however, is that there are vast individual differences in stress-related corticolimbic structure and function, with increasing evidence that these differences may mediate associations between stress and later psychopathology (Burghy et al, 2012; Gee et al, 2013; Gorka et al, 2014; Swartz et al, 2014b; 2015; Tottenham et al, 2011).

Imaging genetics is an emerging research strategy poised to identify mediating mechanisms through which variability in

the genome and epigenome shape individual differences in corticolimbic function and structure associated with risk for psychopathology (Bogdan *et al*, 2013a; Hariri, 2009). Inspired by: (i) non-human animal models and neuroimaging research documenting effects of the environment, and in particular stress, on brain function and structure (Gee *et al*, 2013; Lupien *et al*, 2009; Tottenham and Sheridan, 2009), and (ii) models of gene × environment interaction influencing psychopathology (Caspi *et al*, 2010; Karg *et al*, 2011; Zannas and Binder, 2014, but see also Duncan and Keller, 2011), imaging genetics studies have begun to examine how assessed and manipulated environments moderate associations between genetic variation and the brain.

Our review highlights emerging imaging gene × environment interaction (iGxE) research that is beginning to inform our understanding of individual differences in corticolimbic circuitry and how these differences may confer psychopathology risk (Caspi and Moffitt, 2006; Hyde *et al*, 2011a). We begin by providing a brief introduction of the basic structure and regulation of the hypothalamic-pituitary-adrenal (HPA) axis, as well as its associations with psychopathology and corticolimbic structure and function to help orient the reader to the processes subsequently considered within the iGxE framework. Next, we review iGxE research of the HPA axis and corticolimbic circuitry, as well as several iGxE studies that have been conducted with variants outside of the canonical HPA axis. Lastly, we address several key challenges that confront iGxE and are familiar to traditional genetic, neuroimaging, psychiatric, and environmental research, including: (i) small effects of common genetic variation further constrained by the frequency of environmental events (Duncan and Keller, 2011), (ii) how best to assay and/or manipulate the environment and neural phenotypes (Monroe, 2008), (iii) limited understanding of detailed mechanisms (Bogdan *et al*, 2013a), and (iv) the importance of considering developmental timing (Lupien *et al*, 2009). By actively confronting these challenges, iGxE, in concert with traditional neuroimaging, environmental, and molecular and behavioral genetic research can uniquely inform who is at risk for psychopathology and through what specific neurogenetic mechanisms this risk emerges. A deeper understanding of risk mechanisms can subsequently inform ongoing efforts to refine psychiatric nosology and identify novel therapeutic targets to combat the development of stress-related psychopathology.

HPA AXIS

Within corticolimbic circuitry, the neuroendocrine HPA axis is a central regulator of stress responsiveness and adaptation (for reviews see de Kloet *et al*, 2005; Lupien *et al*, 2009; Ulrich-Lai and Herman, 2009). HPA axis activity follows a daily oscillation governed by the circadian system and is provoked by stress. The HPA axis is controlled by the paraventricular nucleus (PVN) of the hypothalamus, which receives afferent innervation from the central amygdala

(CeA) and sublenticular extended amygdala (SLEA), as well as brainstem nuclei, other hypothalamic nuclei, the hippocampus, and prefrontal cortex (Ulrich-Lai and Herman, 2009). These projections convey a wide array of sensory, emotional, contextual, and perceptual information and serve to activate (and inhibit) a three-step hormonal cascade.

PVN activation stimulates the release of corticotropin-releasing hormone (CRH), which triggers adrenocorticotropic hormone (ACTH) secretion from the pituitary. ACTH stimulates release of cortisol (corticosterone in rodents), which operates on multiple targets through a binary corticosteroid receptor system consisting of mineralocorticoid (MR) and glucocorticoid (GR) receptors. Owing to their high affinity for cortisol, MRs are typically occupied throughout the circadian cycle allowing cortisol to provide a stable excitatory tone in the hippocampus that inhibits the HPA axis under basal and stressful circumstances. In contrast, GRs, which have a low affinity for cortisol, only become occupied following large spikes in cortisol, such as circadian rhythm peaks (eg, the awakening response) or following stress. Within the hippocampus, GRs inhibit continued HPA axis activity and facilitate a return to homeostasis after a stressor has passed (though cortisol-GR binding in other regions, such as the amygdala, can potentiate the HPA axis response; Kolber and Muglia, 2009). Generally, CRH and ACTH signaling stimulate HPA axis activity while cortisol-MR binding constrains the initial HPA axis response, and cortisol-GR binding returns the body to homeostasis after a stress-precipitated response (de Kloet *et al*, 2005; Lupien *et al*, 2009).

The influence of the HPA axis extends directly to other brain regions and the transcriptome. HPA axis hormone receptors are widespread throughout the brain with high concentrations in corticolimbic structures. For instance, CRH binding within the amygdala has been tightly linked to models of stress-related psychopathology in rodents, including anxiety and depression (Binder and Nemeroff, 2010; Hauger *et al*, 2009; Kehne and Cain, 2010). The HPA axis gains access to the genome through intracellular corticosteroid receptors. When bound with cortisol, these receptors can translocate to the nucleus where they bind to regulatory elements of DNA, known as GR response elements (GREs), to enhance or suppress the transcription of a wide range of genes (de Kloet *et al*, 2005, 2008; Menke *et al*, 2012). Access to the transcriptome allows the HPA axis to extend its reach to a diverse array of proteins and may contribute to the pleiotropic effects of stress, including subsequent vulnerability to psychopathology (Arloth *et al*, 2015). Interestingly, individual differences in cortisol-stimulated gene expression are more robust predictors of psychopathology than baseline differences (Menke *et al*, 2012), emphasizing the importance of considering the transcriptome in the context of stress and related psychopathology (Frodl *et al*, 2012, 2014a). Here, it is important to note that there are many additional factors that interact with the HPA axis (eg, urocortins, vasopressin, neuropeptide Y, inflammation) that are appealing candidates for further

study in the context of stress-related disorders; however, with few exceptions these additional factors have yet to be extensively investigated in the iGxE literature and hence are not further considered in our review (Hauger et al, 2006; Horowitz and Zunszain, 2015).

Genetic and Environmental Origins of HPA Axis Variability

There are vast individual differences in diurnal HPA axis rhythms and responsiveness to challenge that are generally stable over time and moderately to largely heritable (Federenko et al, 2004; Franz et al, 2010; Gustafsson et al, 2011; Van Hulle et al, 2012; Wust et al, 2000). Candidate gene and genome-wide association studies (GWAS) have begun to link common genetic variation within the HPA axis cascade to individual differences in diurnal and stress-evoked HPA axis function (Bolton et al, 2014; DeRijk, 2008, 2009). Furthermore, many of these variants have been shown to interact with stress exposure to convey vulnerability to psychopathology and other stress-related diseases (for reviews see: DeRijk et al, 2008; Zannas and Binder, 2014).

In addition to genetic factors, animal models have consistently documented that stress, particularly when chronic and early in life, results in long-lasting changes to the HPA axis (for review see: Lupien et al, 2009). For example, maternal separation in rodents increases CRH receptor expression in the pituitary and reduces hippocampal GR binding sites, resulting in heightened basal HPA axis output and an atypical diurnal pattern (Anisman et al, 1998; McEwen, 2000). Similar associations (ie, elevated ACTH and cortisol and reduced GR expression in the hippocampus) have been found in non-human primates and humans exposed to adversity in early life suggesting that these effects are conserved across species (Miller et al, 2007; Tyrka et al, 2013).

Rodent models suggest that early life stress-related HPA axis differences may emerge from epigenetic modifications. Most notably, Meaney and colleagues have shown that rat maternal care affects the later adult behavior of offspring through epigenetic regulation of the HPA axis (Turecki and Meaney, 2014; Weaver et al, 2004, 2005). Briefly, rats raised by a low-caring mother (ie, one providing little licking and grooming, as well as arched back nursing) show behavioral susceptibility to later stress (eg, anxiety-like behavior), which is mediated by a cascade of cellular changes that persist throughout the rat's lifespan. Offspring of low-caring mothers have reduced nerve growth factor-inducible protein A expression, which leads to increased methylation of the GR gene (NR3C2) in the hippocampus, and thus less GR expression. As GR-cortisol binding within the hippocampus provides negative feedback regulation of the HPA axis, this epigenetic change results in poor stress recovery, much like what is observed in humans with depression. Remarkably consistent findings in a human post mortem study suggest a striking conservation of this epigenetic pathway across species (McGowan et al, 2009). Perhaps most interestingly,

in cross-fostering experiments, this epigenetic change was transmitted inter-generationally whereby low-caring mothers raised offspring, who became low-caring mothers themselves transmitting this epigenetic signature across generations creating a heritable behavioral pattern uncoupled from DNA sequence.

HPA Axis Variability, Psychopathology, and Corticolimbic Structure and Function

Decades of research have shown that the HPA axis is disrupted across psychopathologies (de Kloet et al, 2005; Doom and Gunnar, 2013; Faravelli et al, 2012; Marques et al, 2009). The most consistent evidence is found regarding depression, which is often characterized by elevations in CRH, ACTH, and cortisol, as well as impaired negative feedback of the HPA axis (Pariante and Lightman, 2008). Alongside non-human animal models demonstrating that HPA axis manipulation (eg, knockout, pharmacologic challenge) produces anxiety- and depressive-like behaviors (Kolber and Muglia, 2009; Lupien et al, 2009), human studies indicate that chronic HPA axis stimulation (eg, for the treatment of medical conditions such as asthma or rheumatoid arthritis) can induce severe psychiatric symptoms that remit following treatment cessation (Fardet et al, 2012; Patten and Neutel, 2000; Wada et al, 2000). Moreover, successful treatment of stress-related disorders normalizes HPA axis function, prompting hope that novel treatments targeting this system may be more broadly therapeutically efficacious (de Kloet et al, 2008; Ising and Holsboer, 2007; Otte et al, 2010; Thomson and Craighead, 2008).

In contrast to many studies linking HPA axis function to psychopathology, far less research has examined HPA axis correlates of corticolimbic function and structure in humans. A few notable exceptions complement a rich foundation of non-human animal data (Dedovic et al, 2009; Lupien et al, 2009; McEwen and Gianaros, 2011). For instance, individuals with Cushing's syndrome (ie, a tumor leading to large amounts of ACTH and cortisol release) have increased threat-related activation of the amygdala and hippocampus, as well as diminished hippocampal gray matter volume (Andela et al, 2015; Maheu et al, 2007, 2008). Human pharmacologic challenge studies document that cortisol signaling increases amygdala activation and functional connectivity with other corticolimbic nodes in response to threat-related stimuli (Henckens et al, 2010, 2012; Vogel et al, 2015).

Similarly, circulating concentrations of endogenous cortisol, correlate with differential activity and connectivity within corticolimbic circuitry. Specifically, elevated cortisol is associated with increased amygdala and decreased ventromedial prefrontal cortex (vmPFC) activation during regulation of negative affect, providing an intriguing putative mechanism (ie, deficient emotion regulation) through which HPA axis dysfunction may contribute to psychopathology (Urry et al, 2006). Recent evidence further suggests that early life stress exposure leads to early 'maturation' of amygdala-

prefrontal connectivity during adolescence (ie, negative coupling comparable to that in adults) that is mediated by circulating concentrations of cortisol, and that individuals exposed to early life stress exhibiting relatively immature amygdala-prefrontal functional connectivity experience more anxiety (Gee et al, 2013). Lastly, in girls, greater early life adversity has been associated with heightened cortisol during childhood, which negatively predicts amygdala-vmPFC resting-state intrinsic coupling and greater subsequent risk for stress-related disorders in young adulthood (Bogdan and Hariri, 2012a; Burghy et al, 2012). Collectively, these studies suggest that dysfunction of the HPA axis may play a causal role in upregulating amygdala activity and altering its functional connectivity with the prefrontal cortex, which may weaken the capacity to effectively regulate stress responses and hence, contribute to the emergence of psychopathology.

iGxE OF CORTICOLIMBIC CIRCUITRY

Given clear associations between exposure to stress and expression of psychopathology, as well as transdiagnostic evidence for HPA axis dysregulation, understanding how genetic variation moderates the impact of stress on corticolimbic structure and function is critical for identifying mechanisms of risk and resilience. Although in its infancy, research adopting an iGxE framework is beginning to improve our etiologic understanding of psychopathology risk by identifying putative neural mechanisms. Current iGxE research has primarily focused on candidate polymorphisms of documented functional relevance in the HPA axis that are also predictive of psychopathology risk. More recently, and consistent with the polygenic architecture of HPA axis function, as well as the resolution at which neural and behavioral genetics research is conducted, additive genetic profiles have been constructed that seek to better capture the cumulative influence of multiple variants on HPA axis response and regulation, and subsequently, the impact of such variability on corticolimbic phenotypes, as well as associated risk for psychopathology. Below, we first review studies of candidate polymorphisms within the HPA axis, before turning to polygenic scores and investigations of iGxE outside of the canonical HPA axis (Table 1; Table 2).

FK506 Binding Protein 5 (FKBP5)

FK506 binding protein 5 (FKBP5) is a co-chaperone of the GR complex that diminishes GR sensitivity to cortisol, as well as its access to the transcriptome. Seminal studies by Binder, Ressler, and colleagues have identified a common functional polymorphism within FKBP5, rs1360780, associated with pleiotropic stress-related health effects (eg, depression, immune dysfunction (Zannas and Binder, 2014). The minor T allele of this C/T polymorphism is associated with greater FKBP5 expression following GR activation (Binder et al, 2004). The T allele further predicts a prolonged cortisol response to stress and impaired negative

feedback of the HPA axis, as well as elevated early life stress-related depression and PTSD (Binder et al, 2008; Zannas and Binder, 2014; Zimmermann et al, 2011).

Recently, the molecular mechanisms that likely drive the functional consequences of rs1360780 genotype have been detailed (Klengel et al, 2013). This polymorphism influences the 3D structure of FKBP5 by changing the physical proximity of a long-range enhancer region in intron 2 with the transcription start site. Specifically, the greater FKBP5 expression in T allele carriers appears to result from increased physical contact between a GRE within intron 2 and the transcription start site which is likely driven by T allele conferred increased affinity for TATA-box binding protein. As such, the T allele facilitates FKBP5 expression in the context of GR activation, and hence, reduced cortisol-GR binding and impaired HPA axis negative feedback. This effect can be further compounded by T allele-specific childhood stress-related demethylation of a functional GRE within intron 7 of FKBP5, which comes into physical contact with the transcription start site. Demethylation here enhances FKBP5 expression in the context of GR stimulation, resulting in a further reduction in HPA axis negative feedback among T allele carriers. This pattern of allele-specific methylation likely originates from prolonged HPA axis response following stress, owing to the 3D conformational changes linked to rs1360780 genotype. Interestingly, the epigenetic effects on FKBP5 may only emerge during early life, which may help explain the preponderance of research suggesting that adversity in early life, but not adulthood, robustly moderates the effects of this genotype on multiple stress-related phenotypes.

On the basis of clinical associations and the functional characterization of rs1360780 genotype, several recent iGxE studies have examined the impact of this polymorphism and stress exposure on corticolimbic structure and function. For example, we have shown that rs1360780 genotype interacts with childhood adversity to predict increased threat-related reactivity of the dorsal amygdala in European-American ($n = 139$) T allele carriers (White et al, 2012). This rs1360780 genotype x early life stress interaction has subsequently been replicated in young European ($n = 153$) and European-American ($n = 308$) adults in two independent studies (Di Iorio et al, submitted; Holz et al, 2014). That this finding has been most pronounced in the dorsal amygdala, which encompasses the CeA and thus the principal efferent innervation of the PVN (Figure 1), is consistent with rodent literature suggesting that stress upregulates FKBP5 in the CeA, but not the basolateral complex (Scharf et al, 2011). Thus, the interaction of FKBP5 genotype and early adversity on dorsal amygdala activity may reflect sensitized responses to threat. This interpretation is consistent with data suggesting that T allele carriers show an attention bias toward threatening stimuli, as well as increased function connectivity throughout corticolimbic circuitry (Fani et al, 2013; Holz et al, 2014).

In contrast to research on the interaction between early life stress and rs1360780 genotype, evidence for main effects with
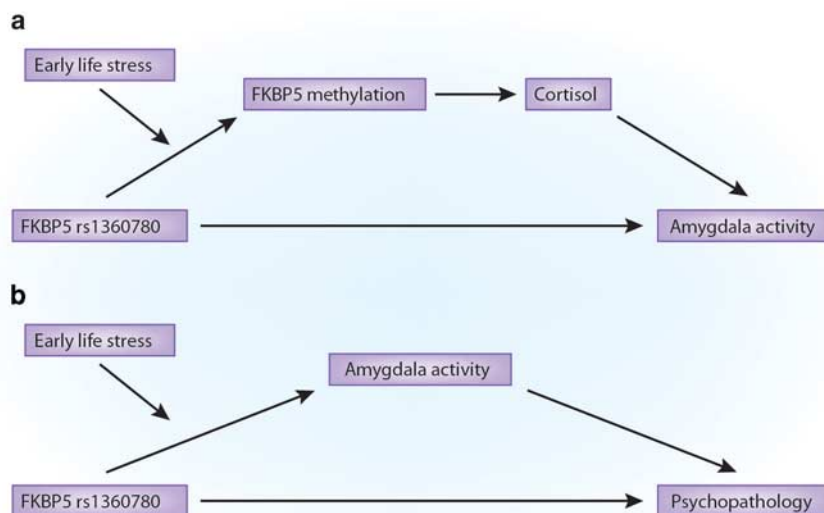
Figure 2. Understanding neural mechanisms underlying psychopathology in iGxE research: Moderated mediational models of *FKBP5*. Structural equation models allow researchers to examine indirect pathways to examine theoretical mechanisms. (a) *FKBP5* genotype is proposed to interact with early life stress to influence *FKBP5* methylation. This may, in turn produce differences in cortisol output (and its effect on the transcriptome), and in turn, amygdala activation to threat-related stimuli. (b) Similarly, amygdala activation (in conjunction with many other phenotypes) may partially mediate the association between the interaction of *FKBP5* genotype and early life stress on psychopathology. Although models a and b could be combined, it is unlikely that large enough data sets will exist in the near future that have adequately captured these phenotypes.

regard to corticolimbic structure and function has been less consistent. For instance, one study reported a main effect of rs1360780 genotype on threat-related amygdala reactivity, wherein the risk T allele was associated with greater reactivity (Holz *et al*, 2014). However, other studies have yielded null main effects on amygdala reactivity (Di Iorio *et al*, submitted; White *et al*, 2012). Hippocampal structure has been the most studied structural phenotype with null reports of rs1360780 main effects on hippocampal volume (Fani *et al*, 2013; Hernaus *et al*, 2014; Pagliaccio *et al*, 2014). Notably, *FKBP5* methylation in intron 7 has been negatively correlated with anterior hippocampal volume, further suggesting that neural effects may only be observable in the context of adversity (Klengel *et al*, 2013). Moreover, an effect of rs1360780 genotype on differences in hippocampal function and morphology has been observed in a trauma-exposed sample of African-Americans (Fani *et al*, 2013). Specifically, the risk T allele was associated with greater threat-related hippocampal activity, as well as greater spatial displacement in hippocampal morphology in the CA1 region. This finding is particularly intriguing in light of evidence that CA1 atrophy and impaired long-term potentiation are induced by stress and chronic corticosterone administration in rodents (Alfarez *et al*, 2003; Sousa *et al*, 2000), as well as abnormalities in CA1 shape in stress-related psychopathology (Kempton *et al*, 2011).

Lastly, some effects of rs1360780 genotype have been reported with the structure of the anterior cingulate cortex (ACC), which contributes to monitoring and regulating behavioral responses including those to threat. In a healthy

Japanese sample, the T allele has been linked to reduced dorsal ACC volume and reduced white matter integrity in the dorsal anterior cingulum as well as the posterior cingulum, two pathways linking the ACC with other brain regions. Impaired white matter integrity of the posterior cingulum has also been reported among African-American T homozygotes (Fani *et al*, 2014). These findings suggest that *FKBP5* rs1360780 genotype may disrupt white matter integrity within the cingulum and, possibly, white matter connectivity between the ACC and corticolimbic nodes including the amygdala and hippocampus, which may impact the capacity to effectively regulate stress and threat responses. However, notably, these white matter integrity results were not consistent with methodology (ie, mean diffusivity, fractional anisotropy) across these two studies suggesting that further validation is needed.

Collectively, iGxE studies of *FKBP5* rs1360780 genotype suggest that the risk T allele is associated with potentiated amygdala reactivity to threat in the context of early life adversity. Such potentiated reactivity may set the stage for the development of stress-related disorders later in life (Figure 2). That this association primarily arises in the context of early life adversity is concordant with evidence that allele-specific epigenetic signatures in intron 7 of *FKBP5* are developmentally constrained (Klengel *et al*, 2013). Importantly, however, this does not suggest that later life stress may not be important. Indeed, given evidence that *FKBP5* genotype moderates susceptibility to later PTSD (Binder *et al*, 2008), this interaction and the heightened amygdala reactivity it confers may predispose individuals to

greater sensitivity to future stressful experiences. Although requiring larger samples, it would be interesting to test whether the early life stress × rs1360780 genotype interaction better predicts future psychopathology and related neural phenotypes in the context of recent life stress (ie, early life stress × recent life stress × genotype). It will also be important to assess whether *FKBP5* methylation, HPA axis function, and related transcriptome effects mediate the relationship of the interaction between rs1360780 genotype and early life stress and corticolimbic function and structure and further, if these differences mediate the link to psychopathology (Figure 2).

Not surprisingly, this research has motivated attempts to develop FKBP5 treatments. Up until recently, this work has largely been unsuccessful due to the structural similarity between FKBP5 and FKBP2 (which has opposing functional effects). Notably, however, an FKBP5 specific inhibitor was recently developed that produced antidepressant like effects in mouse models of stress (Gaali *et al*, 2015). These results are particularly encouraging and are suggestive that FKBP5 inhibition may, eventually, become a useful tool in the psychiatric treatment arsenal.

MR Receptor (*NR3C2*)

A functional missense Iso/Val polymorphism (rs5522) located in exon 2 of the MR gene (*NR3C2*) influences its functional efficiency. The Val allele has been associated with reduced cortisol-related function, as well as blunted diurnal cortisol variation and heightened stress reactivity as indexed by neuroendocrine, autonomic, and self-report measures in some but not all studies (DeRijk *et al*, 2006, 2008; van Leeuwen *et al*, 2010). Interestingly, reduced MR expression is found in *post mortem* brains of depressed individuals consistent with observations that the Val allele predicts late life depression and depression-like HPA axis dysfunction (DeRijk *et al*, 2008; Klok *et al*, 2011a; van Leeuwen *et al*, 2010). Moreover, there is evidence from animal models that upregulated MR expression in the basolateral amygdala, wherein primary synaptic circuits for inhibition of amygdala output are located, can have anxiolytic and antidepressant effects (Mitra *et al*, 2009).

We have found evidence that the val allele interacts with early life stress to influence threat-related amygdala reactivity in children ($n = 279$) (Bogdan *et al*, 2012b). Specifically, there was a positive association between emotional neglect and threat-related amygdala reactivity in Iso allele homozygotes. In contrast, in the context of low childhood adversity, val-allele carriers had increased amygdala reactivity relative to iso allele homozygotes. There were no genotype differences in reactivity at high levels of maltreatment. Thus, even in the context of low prior adversity, Val allele carriers display amygdala activation similar to individuals with stress-related psychopathology. As such, Val allele carriers may be more vulnerable to the development of stress-related illness, even in the absence of significant environmental stress, perhaps due to the relative reduction in HPA axis inhibition. This

interpretation is consistent with evidence that MR blockade raises basal cortisol levels but does not affect stress-induced HPA axis response (Vogel *et al*, 2015). Notably, this relationship has only been observed in children just entering adolescence and was not replicated in young adults ($n = 308$; Di Iorio *et al*, submitted). Given evidence of continued amygdala development into adulthood, it is possible that stress-related increases may be observed in Val allele carriers later in life; however, it is also possible that this is a false positive. Clearly, additional replication attempts and extensions to different developmental periods are needed. Lastly, emerging research has shown that along with rs2070950, rs5522 forms a haplotype that is further predictive of differential MR function and risk for psychopathology (Klok *et al*, 2011b). This haplotype may help explain conflicting reports of the relationship between the rs5522 variant in single SNP analyses of HPA axis function and psychopathology (Klok *et al*, 2011a; van Leeuwen *et al*, 2011) and allow future iGxE research to more accurately characterize functional consequences of variation in the MR gene on stress-related risk phenotypes (Di Iorio *et al*, submitted).

Polygenic Profiles

The vast majority of iGxE research has examined the association between single-polymorphic loci and individual differences in neural phenotypes. In light of small effects typically conferred by single polymorphisms and the utility of multilocus approaches in traditional psychiatric research (Purcell *et al*, 2009), imaging genetics broadly has begun to incorporate multilocus genetic profiles to quantify aggregate genetic influence (Bogdan *et al*, 2013a; Holmes *et al*, 2012; Nikolova *et al*, 2011). This approach has two primary scoring schemes: (i) the summation of risk alleles or weighted effects providing a 'risk' score for a given condition across the genome or within a specified set of genes, or (ii) a biologically informed multilocus profile score (BIMPS) based upon previously reported associations with gene function or downstream consequences representing the function of a biological system (eg, the HPA axis).

Most polygenic research in imaging genetics has used BIMPS. In this approach, genetic polymorphisms are selected based upon known functional associations (eg, receptor expression). In the case of iGxE, this approach has been most often used with regard to measures of HPA axis function (eg, cortisol levels). Strengths of this approach include the use of *a priori* knowledge, which enhances the interpretation of findings and better integrates this research within the general field of neuroscience and psychiatry. A related limitation, of course is the necessity for *a priori* functional knowledge. With few exceptions (eg, *FKBP5* rs1360780), common polymorphisms have generally been poorly characterized with little to no replication of functional associations. Moreover, additive polygenic profiles typically assume additive effects, despite the strong likelihood of epistatic interactions. Notably, when polygenic approaches are constrained to neural systems and pathways they likely

TABLE 1 iGxE of Polymorphisms within the HPA Axis

| Gene/ profile	Variant (s)	Environment	Outcome measure	Finding	Sample size	Study
FKBP5	rs1360780 (C/T)	Early life stress	Amygdala activation to threatening faces and amygdala volume	T carriers: increased amygdala activation and reduced amygdala volume in the context of early life stress	308	Di Iorio et al, submitted
		Trauma-exposure	Hippocampal activation to threatening faces and volume	T carriers: increased activation of bilateral hippocampus Greater spatial displacement in hippocampal morphology in the CA1 region	36 women	Fani et al, 2013
		Early life stress	Amygdala activation to threatening faces, amygdala volume, and amygdala-OFC-hippocampal connectivity	T carriers: increased activation of right amygdala in the context of early life stress	153	Holz et al, 2014
		Childhood emotional neglect/ Early life stress	Amygdala activation to threatening faces	T carriers: increased activation of bilateral dorsal amygdala in the context of early life stress	139 children	White et al, 2012
NRC32	rs5522 (iso/val)	Childhood emotional neglect	Amygdala activation to threatening faces	Iso/Iso: increased activation in the context of elevated emotional neglect Decreased activation in the context of low adversity	279 children	Bogdan et al, 2012b
		Early life stress	Amygdala activation to threatening faces and amygdala volume	No effect	308	Di Iorio et al, submitted
HPA BIMPS	FKBP5 (rs1360780), CRHBP (rs10473984), CRHR1 (rs110402), and NR3C2 (rs5522, rs4635799)	Early life stress	Amygdala activation to threatening faces and amygdala volume	Higher BIMPS was associated with elevated amygdala activation and reduced amygdala volume in the context of elevated early life stress	308	Di Iorio et al, submitted
	CRHR1 (rs4792887, rs110402, es242941, rs242939, rs1876828), NR3C2 (rs5522), NR3C1 (rs41423247, rs10482605, rs10052957), and FKBP5 (rs1360780)	Cortisol response to an acute stressor and early life stress	Amygdala and hippocampal volume	Higher BIMPS was associated with increased volume of the left hippocampus and left amygdala in the context of elevated early life stress	120 children	Pagliaccio et al, 2014

bring genetic data to a resolution that is more consistent with neural and behavioral phenotypes. That is, as neuroimaging assesses higher-order circuit function reflecting the overall activity of thousands of neurons and glia, a polygenic profile assesses higher-order function of a signaling cascade in comparison with a single polymorphism with a likely small effect on only one component of a signaling cascade.

In a study of young adult college students ($n = 308$) we have found that a BIMPS reflective of HPA axis function comprised of FKBP5 rs1360780, CRHBP rs10473984, CRHR1 rs110402; NR3C2 rs5522/rs4635799, predicts threat-related amygdala reactivity and amygdala gray matter volume in the context of early life stress (Di Iorio et al, submitted). Specifically, individuals with elevated BIMPS, reflective of relatively increased HPA axis activity and/or impaired negative feedback, also reporting greater exposure to early life stress had increased threat-related amygdala activity and reduced amygdala gray matter volume, whereas an opposite pattern of findings was observed in those with low BIMPS. In addition, we found that HPA BIMPS predicted elevated anxious arousal, though there was no evidence that the effects on the amygdala mediated this relationship. These functional findings are consistent with a wealth of literature

suggesting that HPA axis hyperactivity predicts relatively exaggerated corticolimbic responses and, importantly, identifies individual polygenic liability to these effects.

That elevated exposure to early life stress was associated with smaller amygdala gray matter volume in those with high HPA axis BIMPS (ie, associated with heightened HPA axis activity), but reduced amygdala gray matter volume in those with low BIMPS provides insight into inconsistent (ie, larger, smaller, equivalent) amygdala volume reports in those exposed to early life stress (Hanson et al, 2015; Sheridan et al, 2012; Tottenham et al, 2010). Specifically, these data suggest that individual differences in HPA axis function may meaningfully contribute to variability in early stress-related brain structure. Some prior evidence is consistent with this speculation as temporally-limited ELS predicts increased amygdala volume, whereas a longer duration of exposure, which is associated with impaired HPA axis negative feedback, has been linked to reduced amygdala volume (Mehta et al, 2009). This interpretation is consistent with the hypothesis that initial stress-related hypertrophy within the amygdala may eventually lead to amygdala atrophy in the context of continued adversity and/or HPA axis activity (McEwen, 2003).
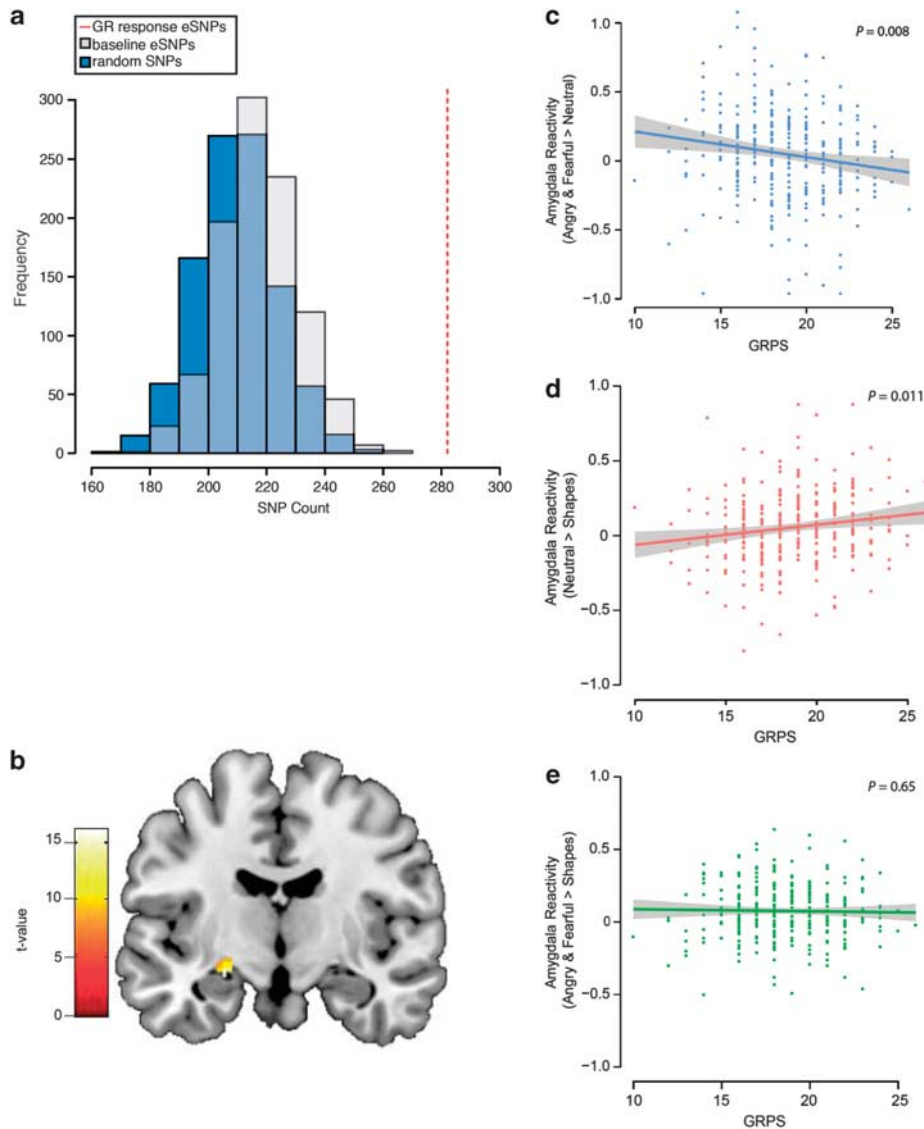
Figure 3. Genetic determinants of the transcriptome response predict depression and generalized amygdala function. In a multisite study, the GR agonist dexamethasone was administered to healthy and depressed participants who were genotyped. RNA expression was measured before and after (+3 h) dexamethasone administration. First, we identified SNPs that were associated with dexamethasone-related changes in gene expression. Next, we examined whether these SNPs were significantly enriched in psychiatric disorders. (a) Evidence of significant enrichment in patients with depression from the PGC depression mega analysis. The red line indicates the number of SNPs that were predictive of GR-related gene expression and depression; the blue and gray bars represent enrichment with regard to random SNPs and SNPs associated with baseline gene expression, respectively. As can be seen, permutation analyses indicated that GR-expression related SNPs are overrepresented in their association with depression. (b–e) the results of these genetic polymorphisms on threat-related amygdala function. We first created a multilocus profile score representing SNPs associated with both GR-related gene expression and depression before regressing this score on threat-related amygdala activation. Curiously, the genetic profile was predictive of relatively reduced amygdala reactivity to threatening stimuli ((c) angry and fearful faces > neutral faces). However, post hoc tests revealed that this profile was predictive of elevated amygdala response to neutral faces ((d); ie, neutral faces > shape matching control task) while being unrelated to threatening faces ((e) angry and fearful faces > shape matching control task). These findings suggest that SNPs associated with both GR-related gene expression and depression predict an overgeneralized amygdala response, wherein neutral faces elicit relatively increased activation. Figure adapted from Arloth et al, 2015.

In an independent longitudinal study of children ($n = 120$), we have found that an HPA axis genetic risk profile predicts cortisol response to an acute stressor, which mediates the relationship between the genetic profile and later childhood amygdala volume (Pagliaccio et al, 2014). Here, the risk profile was comprised of ten SNPs (CRHR1 (rs4792887, rs110402, es242941, rs242939, rs1876828), NR3C2 (rs5522), NR3C1 (rs41423247, rs10482605, rs10052957), and FKBP5 (rs1360780)) associated with HPA axis function and/or depressive phenotypes. Consistent with literature from which the profile was derived, the profile was associated with elevated cortisol response to acute stress. Most interestingly, the profile interacted with stressful life events during early childhood to predict increased amygdala and hippocampal gray matter volume measured between ages 7–12, which is consistent with a cortisol-mediated

mechanism predicting later structural differences. Notably, this finding is opposite the finding we have observed in young adults mentioned above (Di Iorio et al, submitted). This lack of consistency may be the result of developmental timing wherein, in young childhood, early life stress correlates with larger amygdala volume (ie, hypertrophy) in the context of elevated HPA axis activity; however, later, in the context of continued HPA axis activation, this association may transition to atrophy as has been speculated (McEwen, 2003). It will be critically important for further longitudinal research to examine transitions between developmental windows (eg, childhood to adolescence) to directly test this speculation.

Lastly, the pleiotropic effects of stress likely emerge, at least in part, due to the influence of HPA axis function on the transcriptome. In a multisite collaborative study led by Binder's group (Figure 3), we recently identified genetic polymorphisms that predict transcript-specific mRNA expression differences following GR agonism (Arloth et al, 2015). Interestingly, we found that polymorphisms predicting GR-driven differences in gene expression were also overrepresented in the major depression mega analysis conducted by the Psychiatric Genomics Consortium (PGC; http://www.med.unc.edu/pgc/downloads). Further, we found that an additive profile across these SNPs predicted depression in a community sample, as well as abnormal amygdala activity (specifically, increased reactivity to neutral relative to threatening facial expression) in a sample of young adult college students. Thus, these results suggest that SNPs predicting individual differences in GR-related gene transcription and depression may potentiate a non-specific amygdala response that speculatively may lead to fear overgeneralization that alters perception of otherwise neutral stimuli as threatening. We are currently collecting concurrent imaging and gene expression data in the context of a dexamethasone challenge to ascertain whether GR-related gene expression mediates associations between dexamethasone challenge and corticolimbic function.

iGxE Outside of the HPA Axis

Several candidate polymorphisms residing outside the HPA axis have been examined in an iGxE framework with regard to corticolimbic structure and function in the context of stress (Table 2). Because the vast majority of this work has investigated the 5-HTTLPR variant of the serotonin transporter gene (SLC6A4) and the Vall66Met (rs6265) polymorphism of the brain-derived neurotrophic factor gene (BDNF) we focus predominantly on these lines of research.

The serotonin transporter-linked polymorphic region (5-HTTLPR) is an imperfect repeat polymorphism within the 5′ regulatory region of the serotonin transporter gene (SLC6A4) that, in conjunction with 5′ SNPs rs25531 and rs25532, may alter serotonin transporter expression, confer vulnerability to the depressogenic effects of stress, and be associated amygdala function. Relative to the long allele, the short allele at this locus has been linked in some, but not all,

studies to reduced 5-HTT expression, increased vulnerability to the depressogenic effects of stress, and heightened amygdala reactivity to threatening stimuli (Bastiaansen et al, 2014; Duncan and Keller, 2011; Hariri et al, 2002; Karg et al, 2011; Kaufman, 2015; Lesch et al, 1996; Murphy et al, 2013; Sharpley et al, 2014). A handful of iGxE studies have examined whether this polymorphism interacts with the environment to predict variability in corticolimbic circuitry with evidence that the short allele is associated with elevated amygdala activity at rest and in response to threatening stimuli among those exposed to relatively elevated life stress (Table 1; Alexander et al, 2012; Canli et al, 2006; Williams et al, 2009). Moreover, two 5-HTTLPR iGxE studies suggest that 5-HTTLPR short allele carriers have smaller hippocampal volume in the context of early life stress (Frodl et al, 2010) and stressful life events (Rabl et al, 2014); however, these findings were not replicated with regard to ELS (Gatt et al, 2009) and were in an opposing direction when considering perceived stress in another study (Zannas et al, 2013). Lastly, one study provides evidence that, in women, 5-HTTLPR genotype moderates the association between stressful life events and resting-state functional connectivity of the parahippocampus and posterior cingulate cortex, as well as structural connectivity of the hippocampus and both the amygdala and putamen (Favaro et al, 2014). Notably, iGxE research on the 5-HTTLPR to date has been conducted in relatively small samples with no direct replications (ie, same neural phenotype and environmental assessment/manipulation). As such, although this preliminary research suggests that stress and 5-HTTLPR genotype may interact to shape corticolimbic structure and function, which may contribute to increased stress-related psychopathology in short allele carriers, validation of these results are needed, particularly in light of mixed effects reported in the literature (Bastiaansen et al, 2014; Duncan and Keller, 2011; Karg et al, 2011; Kaufman, 2015; Murphy et al, 2013). Given evidence that 5-HTTLPR × stress effects are most pronounced when considering early life adversity, as opposed to lifetime measures of stress, iGxE studies focusing on early life stress exposure may prove most fruitful (Bogdan et al, 2014; Karg et al, 2011).

The Met allele of the BDNF Vall66Met (rs6265) polymorphism has been linked to reduced BDNF function, as well as depression and reduced hippocampal volume in some, but not all studies (Egan et al, 2003; Elzinga et al, 2011; Verhagen et al, 2010). Perhaps most interestingly from a GxE perspective, there is evidence that Met allele carriers who have been exposed to childhood abuse had the lowest levels of serum BDNF (Elzinga et al, 2011). Given the key role of BDNF supporting the survival and growth of neurons, several iGxE studies have examined associations between rs6265 genotype and gray matter volume. Across these studies, reduced hippocampal volume has been reported among Met allele carriers who have been exposed to elevated levels of stress (Table 2; Carballedo et al, 2013; Frodl et al, 2014b; Gatt et al, 2009; Rabl et al, 2014; but see also Gerritsen et al, 2012). These results suggest that

TABLE 2 iGxE of Polymorphisms Outside of the HPA Axis

Gene	Variant	Environment	Outcome measure	Finding	Sample size	Study
ADRA2B	−4825 indel	Induced stress (movie clips)	Amygdala BOLD activation to emotional morphing faces	Non-deletion homozygotes: blunted phasic amygdala activation under stress relative to control manipulation and deletion carriers under stress	41 Men	Cousijn et al, 2010
		Induced psychological stress after encoding, before recognition	Amygdala and hippocampal BOLD activation during fearful and neutral face recognition	Deletion carriers: blunted amygdala response to neutral faces under stress, increased amygdala response to neutral faces under control manipulation Non-deletion homozygotes: increased right hippocampal activation to fearful faces after stress	22	Li et al, 2015
BDNF	rs6265 (Val66Met)	Early life stress	Hippocampal volume	Met carriers: smaller hippocampal volume in the context of higher lifetime stress. Val/Val no differences.	133 (62 MDD patients)	Carballedo et al, 2013
		Early life stress	Hippocampal subfield volume	Met carriers: smaller hippocampal subfields in the context of higher lifetime stress Val/Val: no differences	82 (38 MDD patients)	Frodl et al, 2014b
		Early life stress	Hippocampal, amygdala, and prefrontal volume	Met carriers: smaller hippocampal and lateral PFC volumes in the context of early life stress Val/Val: larger amygdala and prefrontal volume in those exposed to early life stress	89	Gatt et al, 2009
		Early life stress	Cingulate, hippocampal, amygdala, and prefrontal volume	Met carriers: smaller subgenual ACC volume in the context of early life stress with no differences in any other region	568	Gerritsen et al, 2012
		Pain acute stress	Ventral striatal D2/3 receptor availability	Met carriers showed increased dopamine release during pain and reduced release during placebo	49	Peciña et al, 2014
		Lifetime stress	Hippocampal volume	Met carriers: smaller hippocampal volume in the context of elevated lifetime stress Val/Val: no association between volume and life stress	153	Rabl et al, 2014
COMT	rs4680 (Val158Met)	Lifetime stress	Hippocampal volume	Met/Met: smaller hippocampal volume in the context of elevated lifetime stress Val/Val: larger hippocampal volume in the context of elevated life stress	153	Rabl et al, 2014
		Lifetime stress	rs4680 methylation and PFC activation during N-back	Greater stress predicts reduced methylation among Val homozygotes, which in turn predicts elevated PFC activation	84	Ursini et al, 2011
		Moderate acute stress	PFC activation during N-back	Val carriers show blunted activation during control and elevated activation following stress	41 (within subject design)	Qin et al, 2012
NPSR1	rs324981	Acute psychosocial stress and urban upbringing	Amygdala and ACC activation during stress	T/T: positive association between urbanicity and amygdala activation during acute stress A/A: negative association between urbanicity and amygdala activation during acute stress	42	Streit et al, 2014
OPRK1	rs6989250	Acute psychosocial stress	BOLD activation during stress	C/G: increased activation of the amygdala and midbrain during stress	13 (5 CG) abstinent cocaine-dependent	Xu et al, 2013
SLC6A4	5-HTTLPR	Lifetime stress	Amygdala BOLD activation to faces; Amygdala-hypothalamic functional connectivity	Short/Short: positive association between life stress and amygdala reactivity to fearful faces, as well as functional coupling of the amygdala and hypothalamus	48	Alexander et al, 2012
		Lifetime stress	Amygdala BOLD resting activity and response to neutral faces	Short carriers: positive correlation between life stress and amygdala activity at rest; negative correlation with response to neutral faces Long/Long: negative correlation between life stress and amygdala activity at rest; positive correlation with response to neutral faces	48	Canli et al, 2006
		Negative life events	Hippocampal resting-state fMRI and DTI	Short carriers: increased connectivity between the parahippocampal gyrus and posterior cingulate in the context of elevated life stress Long/Long: altered DTI tractography between the hippocampus and amygdala and putamen in the context of elevated life stress	34 women	Favaro et al, 2014
		Early life stress	Hippocampal volume	Short carriers with a history of emotional neglect have reduced hippocampal volume	24 MDD patients	Frodl et al, 2010
		Early life stress	Amygdala and medial prefrontal cortex volume	No effect	89	Gatt et al, 2009
		Lifetime stress	Hippocampal volume	Short carriers: negative association between hippocampal volume and stress Long/Long: positive association between hippocampal volume and life stress	153	Rabl et al, 2014
		Early life stress	BOLD conscious and activation to faces	Short carriers: early life stress potentiated conscious face processing-related activation in the medial prefrontal cortex and brainstem Early life stress potentiated unconscious face processing-related activation in the amygdala, medial prefrontal cortex, and anterior cingulate cortex	39	Williams et al, 2009

BDNF reductions in Met allele carriers exposed to early life stress, may potentiate stress- and cortisol-related effects on hippocampal volume, perhaps by not buffering the survival of these cells (Frodl and O'Keane, 2013). Such stress-related moderation may explain inconsistent main effect associations between rs6265 genotype and hippocampal volume (Molendijk et al, 2012).

In addition to the *SLC6A4* 5-HTTLPR and *BDNF* Val66Met polymorphisms, a handful of other iGxE studies have explored polymorphisms within other genes (*ADRA2B*, *NPSR1*, *COMT*, *OPRK1*; Table 1). Because these findings have largely emerged in single studies, we only briefly review polymorphisms for which two or more iGxE reports exist (additional single studies are noted in Table 2). First, a common deletion (−4825 indel) in the gene coding for the presynaptic noradrenergic α2B receptor (*ADRA2B*) is associated with blunted agonist-promoted receptor desensitization, elevated re-experiencing of traumatic memory and elevated amygdala reactivity to negative pictures, may moderate the effects of acute stress on corticolimbic circuitry (de Quervain et al, 2007; Rasch et al, 2009; Small et al, 2001). However, the two reports iGxE reports on this findings have used vastly different paradigms. In one study, acute stress blunted amygdala reactivity to emotionally morphing faces among non-deletion carriers (Cousijn et al, 2010); however, in the other, it was deletion carriers who had blunted amygdala reactivity when recognizing pictures depicting neutral facial expressions, whereas fearful faces were not associated with −4825 indel genotype (Li et al, 2015). Whether these different findings are the result of task differences or may be indicative of false positives is presently unclear.

Three studies have examined whether a common functional polymorphism (rs4680; Val158Met) within the catechol-O-methyltransferase gene (*COMT*), which codes for a catecholamine catabolic enzyme, moderates associations between stress exposure and corticolimbic neural phenotypes. The Val158Met polymorphism has been primarily associated with differential prefrontal dopamine function, schizophrenia, and working memory, though meta-analyses suggest these main effects may not be stable (Bilder et al, 2004; Egan et al, 2001; Munafo et al, 2005; Rabl et al, 2014; Wardle et al, 2013). Met allele homozygosity, which is associated with reduced *COMT* expression and hence elevated catecholamine levels in the prefrontal cortex, is predictive of a negative relationship between stressful life events and hippocampal volume; an opposing, positive pattern was observed in Val homozygotes (Rabl et al, 2014).

An independent line of research has examined associations between COMT rs4680 genotype and stress with working memory and related neural function. One such study found that among Met allele homozygotes, acute stress induced poorer working memory performance, and blunted dorsolateral prefrontal cortex (DLPFC) activation; whereas performance benefits and increased DLPFC activation was observed in Val allele carriers (Qin et al, 2012). Lastly, in one of the first neuroimaging studies incorporating measures of

methylation, Ursini et al (2011) noted that the Val allele at rs4680 creates a CpG site wherein methylation can occur that is absent in the Met allele. Here, in Val homozygotes, a composite measure of lifetime stress was associated with reduced methylation in *COMT*, which in turn was associated with increased peripheral measures of *COMT* expression. Perhaps most interestingly, elevated methylation, which was associated with reduced stress exposure, was predictive of greater working memory performance and reduced prefrontal cortex activity (interpreted as greater efficiency) among Val homozygotes. Thus these results, suggest that in the absence of stress exposure, the increased methylation present among Val homozygotes may produce *COMT* expression more similar to the Met allele and improvements in working memory performance; whether this would then leave individuals homozygous for the Val allele with no lifetime stress exposure susceptible to acute stress-induced deficits in memory performance and blunted DLPFC activation is unclear. However, it is possible that such GxE interactions may at least partially contribute to mixed main effects observed in the literature of this candidate polymorphism (Munafo et al, 2005; Wardle et al, 2013). Collectively, these results point to the importance of considering GxE in the context of neuroimaging, particularly when there are probable molecular mechanisms that may promote such effects (eg, the creation of a methylation cite by the Val polymorphism of the Val158Met polymorphism).

CONCLUSIONS AND FUTURE DIRECTIONS

Genetic variation, particularly within the HPA axis, predicts stress-related differences in coticolimbic structure and function. The most replicated association has been an interaction between *FKBP5* rs1360780 genotype and early life adversity predicting threat-related amygdala function. This finding complements psychiatric associations by providing evidence that potentiated stress-related enhancement of corticolimbic function is a plausible neurobiological mechanism underlying clinical association. Greater faith in this finding comes from convergent research in non-human animals and the molecular characterization of this polymorphism (Klengel et al, 2013; Scharf et al, 2011; Zannas and Binder, 2014). With the exception of the *BDNF* rs6265 Met allele conferring reduced hippocampal volume in the context of elevated stress, other investigations of single-polymorphic loci have not yet been replicated (Frodl et al, 2014b). Recently, polygenic approaches have been used which aggregate genetic variation across the HPA axis. This approach is intuitively appealing as it is likely more consistent with the resolution at which neural and behavioral genetics research is conducted and polygenic HPA axis research is already beginning to shed light on individual difference factors that may contribute to mixed findings within the psychiatric/early life stress literature (eg, amygdala structure enlargement and reductions (Di Iorio et al, submitted; Pagliaccio et al, 2014)). However, notably, such

polygenic analyses have not been regularly conducted and are in need of replication and developmental extension. Like all research approaches, iGxE research is confronted by many challenges, four of which are among the most daunting: (i) small effects, (ii) measuring the environment and neural phenotypes, (iii) understanding detailed mechanisms, and (iv) incorporating development into research design. Here we review these challenges and discuss strategies to address them.

Small Effects—Increasing Sample Size and Polygenic Analyses

Much like the effects of common genetic variation on psychiatric vulnerability and behavior, evidence suggests that common polymorphisms will have, at most, only a small effect on neural measures (Hibar et al, 2015; Iacono, 2014). Such weak penetrance is difficult to detect and may account for inconsistent findings (ie, false positives and negatives) within imaging genetics, particularly in studies using small samples, such as those within the iGxE field reviewed here. Traditionally, the prohibitive monetary and resource cost to both neuroimaging and genetic research has constrained sample sizes. However, improvements in availability, affordability, and data management and analyses in both neuroimaging and genetics have allowed for the development of large-scale studies (Bogdan et al, 2012b; Holmes et al, 2015; Nikolova et al, 2012; Schumann et al, 2010), multisite data pooling protocols (Barch et al, 2013; Holmes et al, 2012), and data sharing networks (Thompson et al, 2014) in an effort to increase power to detect small effects.

Neuroimaging measures in genetics research have been lauded for their value as biological processes intermediate to genes and the expression of clinical syndromes (ie, as intermediate phenotypes; Bogdan et al, 2013b; Cannon and Keller, 2006; Gottesman and Gould, 2003; Hariri and Weinberger, 2003; Hasler et al, 2004; Meyer-Lindenberg and Weinberger, 2006). Consequently, we and others have suggested that these phenotypes should produce relatively larger effects than more distal behavioral and psychopathology phenotypes (Bogdan et al, 2013b; Hariri and Weinberger, 2003; Hasler et al, 2004; Meyer-Lindenberg and Weinberger, 2006). Although there is some evidence to support the assumption of larger effects sizes in neuroimaging relative to clinical and behavioral phenotypes, this evidence is also confounded by studies characterized by smaller samples, which generally report larger effects (Rose and Donohoe, 2013). A series of reports originating from the Minnesota Twin Family Study, the largest genetic study of EEG-related phenotypes ($n = 4905$), suggests that neural phenotypes may be linked to common genetic variation with approximately the same strength of traditional psychiatric and behavioral phenotypes (Iacono, 2014). The largest investigation of intermediate phenotypes (discovery $n = 13$-171; replication $n = 17\,546$), the ENIGMA consortium, recently identified genome-wide significant signals for measures of brain volume (Hibar et al, 2015). The strongest

effect emerged within the putamen for an intergenic SNP, rs945270, which is associated with KTN1 expression; critically, the effect of this SNP is sobering as it explained only 0.52% of variance in putamen volume. This relatively small effect, is consistent with effects of single variants on other complex traits (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Although intermediate phenotypes have been assumed to provide better traction into genetic architecture contributing to psychopathology, this evidence challenges this assumption. Future research would ideally compare effect sizes to neural intermediate phenotypes and psychiatric and behavioral phenotypes within the same sample. If indeed, neural intermediate phenotypes are not associated with larger genetic effects, intermediate phenotype research may prove most useful for understanding the neurobiological mechanisms through which genetic variation influences behavior.

Although GxE research is intuitively appealing on a theoretical basis, it is met with unique challenges from a statistical power perspective (Dick et al, 2015; Duncan and Keller, 2011). In GxE research, both genotype frequency and variability in environmental experience constrain the sample; this is potentiated in SNPs with relatively rare minor alleles and in environments that are extreme or specific. For example, a recent report suggests that opioid-related genetic variation moderates the association between stressful life events and depression in a modestly sized sample ($n = 420$). However, the only group that differed had a cell sample size of 6 (ie, carriers of the rare G allele at rs1799971 who were also exposed to targeted rejection events (Slavich et al, 2014)); effects driven by such small cells, in the absence of rare highly penetrant mutations, are unlikely to be stable. If iGxE research is associated with similar effects of traditional psychiatric GxE research (eg, Duncan and Keller, 2011), all iGxE studies to date have been vastly underpowered. Lastly, the statistical power of GxE is further reduced, particularly in small studies, due to the need to account for genotype × covariate and environment × covariate interactions in analyses, which limits degrees of freedom more than main effect analyses (Keller, 2014). Going forward it will be important for iGxE research to consider such interactions between predictor variables and covariates of no interest (ie, G × covariates and E × covariates), which is becoming increasingly common (Carey et al, in press; Di Iorio et al, submitted; Pagliaccio et al, 2014, 2015; Rabl et al, 2014).

In addition to increasing sample size, iGxE research may be able to increase power by using polygenic analytic techniques. Following increasing accessibility of genome-wide data, novel methods have been developed to leverage its high dimensionality. In particular, in addition to the additive profile scores discussed above, three additional approaches may prove fruitful: (i) polygenic risk scores, (ii) gene/systems-level testing, and (iii) regression trees.

The polygenic risk scoring approach generally relies upon well-powered GWAS results (though investigations have been conducted in an unweighted fashion using candidate genetic loci (Pearson et al, 2014) that are relevant to the

neural phenotypes under study such as those provided by mega analyses of categorical disorder risk conducted by the PGC (http://www.med.unc.edu/pgc/downloads; Smoller et al, 2013). A benefit of this approach is that it likely better captures the polygenic nature of psychopathology. A limitation is that these genetic risk scores are based on statistical association with a phenotype and in isolation provide limited insight into potential biological mechanisms underlying observed neural associations. Moreover, like the vast majority of widely employed polygenic methods, this approach assumes an additive model, that neglects potential epistatic effects. Notably, although epistatic effects are suggested from molecular interactions in non-human animal models (eg, Gray et al, 2015), reported epistatic interactions in the imaging genetics literature have not been replicated to our knowledge (eg, Andreasen et al, 2012; Tan et al, 2007).

Perhaps the most important limitations of disorder-based polygenic risk scores when used in iGxE research, is that they were generated without consideration of the environment and are inherently constrained by the phenotype from which they were generated. Psychiatric disorders are characterized by heterogeneity in presentation, stability, and symptoms (Flint and Kendler, 2014; Hasler et al, 2004). For instance, according to DSM-5, five of a possible nine symptoms are required to be diagnosed with depression (with at least one of these symptoms being either depressed mood or anhedonia; American Psychiatric Association, 2013). This results in hundreds of possible different symptom combinations that qualify for the same diagnosis but presumably reflect distinct underlying pathophysiologies. This heterogeneity within disorders produces polygenic risk scores that may not clearly map onto discrete behaviors associated with a particular neural phenotype. For example, although anhedonia, which reflects an inability to anticipate and/or experience pleasure, is a cardinal symptom of depression, according to some studies it is only the fifth most common symptom endorsed by depressed patients with a varied expression ranging from 37–77% (Lewinsohn et al, 1998; Pelizza and Ferrari, 2009). As such, polygenic risk scores generated from a GWAS of depression diagnosis alone likely include estimates unrelated to anhedonia that may not be appropriate predictors for a neuroimaging study of reward processing.

Put more generally, it is possible that distinct underlying pathophysiologies may have a more specific polygenic basis than what is captured in polygenic disorder risk scores. If this in indeed the case, a research domain criteria approach to generating polygenic risk scores that is free from categorical diagnostic status may help catalyze research that will be more readily translated to iGxE research (Insel et al, 2010). Notably, however, given the small effects of common genetic variants, this approach is constrained by access to large data sets with measured phenotypes; as such, GWAS of individual symptoms may be a powerful starting point. In addition to their potential utility for iGxE research, such analyses could potentially inform psychiatric nosology by showing genetic consistency or divergence across specific symptoms and syndromes (Bulik-Sullivan et al, 2015).

This limitation notwithstanding, polygenic risk scores hold great promise for iGxE research. In addition to their use across the genome, polygenic scores could be calculated for sets of SNPs within distinct neural systems. Such an approach may be particularly important for providing SNP level priors, when there is evidence to support a systems-level involvement in a given neural phenotype, but limited evidence to prioritize particular SNPs (eg, the circadian system). Integrating prior information from psychiatric disorder risk (ie, prioritizing particular SNPs) and neuroscience (ie, collating SNPs involved in a specific neural system/s) may be informative for iGxE research of neural risk mechanisms.

From an atheoretical perspective, various ways to mine GWAS data have been developed. For example, software packages have been developed (eg, GATES (Li et al, 2011); VEGAS (Liu et al, 2010)) that combine single SNP p-values generated from GWAS and assigns each SNP to gene to create a summary statistic representing gene-based associations (Liu et al, 2010). This approach has identified significant gene-based contributions to phenotypes when single SNP GWAS have failed (eg, Agrawal et al, 2014). A similar approach, gene-set enrichment, which can be conducted with GWAS-based single SNP p-values using various software packages (eg, Aligator (Holmans et al, 2009); INRICH (Lee et al, 2012); MAGENTA (Segre et al, 2010), evaluates whether sets of genes in predefined biological processes or functionally-related genes (based upon gene sets in online databases or user specified gene sets) show patterns of enriched associations with a given phenotype. For example, gene-set enrichment analyses have linked voltage-gated cation channel activity-related genes to working memory performance, related brain function, and schizophrenia (Heck et al, 2014).

Lastly, in an attempt to characterize epistatic relationships, machine learning approaches have been applied to data (Andreasen et al, 2012; Arnedo et al, 2015). Although there are promising associations reported, this approach is highly susceptible to false positives and what constitutes replication is unclear as the primarily predictive variables are generated internally from the data set. It will be critical for future regression tree-based exploratory research to ascertain precisely what interactions are driving effects so that these specific interactive pathways can be evaluated within independent data set; this has been done in some (Andreasen et al, 2012), but not other (Arnedo et al, 2015) machine learning approaches. It will be important for future iGxE studies, and imaging genetics research more generally to complement traditional candidate gene approaches with big data approaches to synthesizing complex data sets (eg, gene-set enrichment, machine learning) that are becoming more normative in psychiatric genetics. Moreover, in the context of iGxE, it will be important for iGxE research to use new developments in knowledge to target particular systems, such as transcripts influenced by cortisol signaling (Arloth et al, 2015).

Measuring the Environment and Neural Phenotypes

The ability to conduct research on a given construct is constrained by how precisely it can be measured. With sufficient quality control, genotyping can be measured very reliably. However, measuring stressful experiences and neural phenotypes of interest is fraught with difficulty (for review see Glover, 2011; Monroe, 2008). Measuring life stress in iGxE studies is particularly difficult, because the studies that are powered to detect moderations are typically designed to recruit a large number of participants to complete an imaging protocol. The ability to assess other factors is often limited by resources and the vast majority of these large studies assess the environment with retrospectively reported measures of early life stress and recent life stress checklists. Although there are psychometric data supporting these measures, there is also some evidence that more thorough assessments may better capture stress (Bernstein et al, 2003; Dohrenwend, 2006; Monroe, 2008). Notably, however, such interview based methods such as the Life Events and Difficulties Schedule (LEDS) or Stressful Life Events Schedule (SLES) can often take hours to administer and further hours from multiple trained raters to score (Brown and Harris, 1978; Williamson et al, 2003). This additional burden in already complex studies has largely excluded their use in large-scale imaging genetics studies, despite their potential utility. Notably, new computer-based stress assessments have recently been developed that are less time intensive to administer and score that may prove beneficial for large-scale studies of stress exposure (Slavich and Epel, 2010). A further difficulty confronting large imaging genetics studies incorporating the environment is that, with few exceptions, these studies are generally cross-sectional which prohibits the examination of change in neural phenotypes in the context of a changing environment (Swartz et al, 2014b, 2015). Moreover, within some large cross-sectional studies assaying stress, it is impossible to ascertain the temporality of stress exposure in relation to concurrently measured symptoms; indeed this temporality problem has resulted in many studies evaluating only early life stress, as opposed to recent life stress, when attempting to link environmental experience, brain function, and psychiatric symptoms or related behavior (Moffitt and Caspi, 2014).

A complementary approach to measuring the environment is to acutely manipulate stress to examine its influence on neural function in the context of genetic variation. Although such studies are rare in a neuroscience context, some studies have used stress manipulations while measuring neural phenotypes and assaying genetic variation (Bogdan et al, 2011; Cousijn et al, 2010; Streit et al, 2014; Xu et al, 2013). These within-participant studies are especially powerful because they afford researchers a unique opportunity to experimentally manipulate stress and ensure that each participant is exposed to the same stressor, providing greater reliability and validity of what stress is across participants that is free from reporting biases. However, such studies are

intensive to run and exceptionally difficult to have large numbers of participants complete and their ecological validity is questionable. A related powerful and perhaps more difficult methodology to implement is pharmacologically challenging circuits involved in stress response (eg, HPA axis, norepinephrine), ideally in a placebo-controlled crossover design (Bigos et al, 2008; Henckens et al, 2010, 2011). Pharmacologic challenge has identified genetic differences in stress-related gene transcription that are further associated with psychopathology and related brain function (Arloth et al, 2015). However, it has yet to be commonly employed in human genetic research. Moreover, the ecological validity of such challenge studies is reduced due to their specificity as these studies traditionally influence a single receptor that in typical life experience is activated alongside a symphony of other neural cascades.

Lastly, with regard to the environment, theoretical work suggests that some genetic variants that have typically been conceptualized as conferring risk to psychopathology in the context of negative environments may be more accurately conceptualized as conferring sensitivity to the environment, for better or worse (Belsky et al, 2009). This theory has begun to receive empirical support in candidate gene studies showing that some genotypes previously conceptualized as conferring risk to negative environments also confer benefits in the context of positive environments (Hankin et al, 2011). As such, it will be important for iGxE research, to not only measure adverse environmental conditions, which is most common in psychopathology-related research, but to also measure enriching experiences (eg, social support, positive parenting; Hyde et al, 2011b). It is highly probable that such data would also further help untangle the complexity of factors that influence neural phenotypes (eg, buffering effect of supportive social relationships on negative life experiences). There are cautions to be aware of with this research approach, however. Most notably, data suggest that such crossover interactions are more likely to emerge by chance relative to other shapes of interaction (Dick et al, 2015); given that the vast majority of results supporting the plasticity model have been in small samples, it will be important for these effects to be observed in more well-powered studies.

Another outstanding issue in imaging genetics research, and the field of neuroimaging more generally, is the largely unexplored test–retest reliability of neural phenotype measures. Although structural measures such as gray matter volume appear to be highly reliable (Bartzokis et al, 1993), the reliability of functional measures is mixed. With regard to tasks designed to elicit corticolimbic activation, some studies suggest moderate to good reliability whereas other studies suggest relatively poor reliability (Manuck et al, 2007; Plichta et al, 2012, 2014; Sauder et al, 2013). Critically, the vast majority of test–retest reliability research has used exceptionally small samples (ns < 30) and further attention to this issue is clearly needed, which may be achieved through collaborative releases of large neuroimaging data sets (eg, Zuo et al, 2014). Moreover, it will be critical for future

research to not only assess test–retest reliability but to also understand factors that may reduce reliability (eg, time of day, hydration, fatigue, mood, *etc*) so that future studies may be optimized (Nakamura *et al*, 2015). In the context of iGxE research reliable imaging measures are needed to produce replicable associations with genetic variation, environmental experience, and psychopathology symptomatology.

Mechanistic Understanding

One of the primary goals of neuroscience, psychology, and psychiatry is to understand the mechanisms that drive behavioral variability. iGxE complements psychological and psychiatric genetic research by adding a plausible biological mechanism—the brain—through which genetic variation and environmental experience give rise to the vast array human behavior. iGxE is uniquely positioned to explicitly test whether genetic variation and the environment contribute to individual differences in neural phenotypes and whether these neural differences indirectly link GxE to behavior and psychopathology (Hyde *et al*, 2011a). To do this, iGxE can use structural equation modeling tools such as path analysis, which were developed by geneticist Sewall Wright and are now most commonly used in the social sciences (Figure 2; Duncan, 1966; Hyde *et al*, 2011a; Wright, 1920). Although testing these models allows one to evaluate theoretical neural mechanisms underlying behavior, there are important considerations when conducting these analyses. First, this analytic approach further emphasizes the need for large samples which is potentiated when considering less common alleles and environmental experiences. Second, the ideal manner in which to conduct mediational analyses is between temporally distinct assessments. However, with few exceptions, the vast majority of iGxE research assesses psychiatric symptoms and behaviors within days of imaging data collection; as such, the strength of interpretation that neural differences or environmental experiences preceded the development of psychiatric symptoms or other behavior differences is an unsupported assumption that needs to be considered in interpretation. Ideally, prospective studies would be conducted (Burghy *et al*, 2012; Swartz *et al*, 2015). In addition to examining the brain as a mediator between genetic variation and environmental experience and behavior, it will be further critical to examine whether biomarkers (eg, cortisol, gene expression) mediate links between genetic variation and environmental experience and brain phenotypes (Figure 2; Pagliaccio *et al*, 2014). Ideally, such measures could be taken in the context of longitudinal assessment and be measured under different conditions (eg, cortisol: diurnal variation, stress-evoked activation).

Transdisciplinary research can be further used to detail mechanisms underlying effects of genetic, environmental, and neural mechanisms on behavior. Natural orthologous genetic variants and the ability to manipulate genetic code in non-human animals (eg, transgenic animals) and cell-based assays are useful for interpreting iGxE research (Caspi *et al*, 2010). Transgenic mouse models have provided insight into

mechanisms through which differences in genetic sequence impact neural function and behavior; these models are particularly powerful in the context of iGxE as the environment can be explicitly controlled and manipulated. For example, a knock-in mouse model that parallels a common human polymorphism, rs324420, in *FAAH* within the eCB system was recently developed (Dincheva *et al*, 2015). The rs324240 polymorphism has been linked to functional differences in *FAAH* and related correlations to behavioral and neural phenotypes (eg, amygdala habituation, stress sensitivity; Gunduz-Cinar *et al*, 2013a, b; Hariri *et al*, 2009). This genetic knock-in model shows remarkable parallels in not only biochemistry, but also neurocircuitry and behavior that are consistent with the naturally occurring human polymorphism. Given reciprocal relationships between AEA and the HPA axis, this provides a novel model organism to better understand how this particular variant might influence behavioral susceptibility to stress. Recent developments in genetic engineering using the CRISPR/Cas9 system to disrupt genes across species provides more precise targeted genetic manipulation that may ultimately prove useful for not only clinical treatment but also modeling the effects of polymorphisms (Jinek *et al*, 2012; Sander and Joung, 2014); however, the recent use of this technology in human embryos has generated significant ethical controversy (Cyranoski and Reardon, 2015; Lanphier *et al*, 2015).

Lastly, an important potential mechanism underlying iGxE results is epigenetic regulation. For example, genetically-conferred differences in epigenetic regulation may underlie *FKBP5*-related observations in psychopathology and brain function (Klengel *et al*, 2013). Moreover, there is evidence that a widely studied variant in *COMT*, rs4680, confers the presence or absence of a methylation site that may underlie at least some of this polymorphisms effects (Ursini *et al*, 2011). Indeed, the potential impact of methylation on human brain function has been recently demonstrated (Booij *et al*, 2015; Dannlowski *et al*, 2014; Frodl *et al*, 2015; Klengel *et al*, 2013; Nikolova and Hariri, 2015; Nikolova *et al*, 2014; Ziegler *et al*, 2015). For instance, methylation within the serotonin transporter gene predicts increased threat-related amygdala reactivity, as well as decreased *SLC6A4* expression in *post mortem* brain tissue (Nikolova *et al*, 2014). These results suggest that considering methylation is critical to understand the impact of the genome on psychiatrically-relevant brain function and suggests that peripheral measures of methylation may, at least in some cases, be of utility in imaging genetics research, provided that such measures find convergence with *post mortem* tissue. In particular, it will be important to examine epigenetic regulation as a potential mechanism underlying GxE effects (eg, Ursini *et al*, 2011; Figure 2). In addition to assaying methylation in ongoing studies, novel bioinformatic tools, such as the Wash U Epigenome Browser (http://epigenomegateway.wustl.edu/) may be of utility for understanding the epigenetic landscape within which a genetic polymorphisms reside (Carey *et al*, in press; Zhou *et al*, 2011, 2013). This may be particularly useful for understanding the potential impact of polymorphisms

that have not been characterized functionally and may provide very preliminary evidence of potential functional consequences of a SNP, even if curated data sets (eg, Brain Cloud: http://braincloud.jhmi.edu/, Colantuoni et al, 2011) suggest the variant is unrelated to expression. Such knowledge could prioritize genetic variants to examine in the context of methylation-related research.

Developmental Timing

Despite evidence that there are vast differences within corticolimbic circuitry according to development, very few studies have examined neural phenotypes across developmental windows (Giedd et al, 2015). Indeed rapid neural growth and pruning in childhood might lead to a developmentally sensitive period wherein the corticolimbic circuitry may be most sensitive to negative and positive experiences (Andersen et al, 2008; Teicher et al, 2003). For example, a host of studies suggest that adolescence is characterized by heightened amygdala responsivity coupled with a low capacity for frontal regulation that may impair emotion regulation and provide a developmental window that is ripe for the development of stress-related psychopathology (Johnson and Casey, 2015). Similarly, there is evidence for differential HPA axis function across development, as well as its association between early life stress and indices of HPA axis function in childhood and adulthood (Lupien et al, 2009; Shirtcliff et al, 2012; Tarullo and Gunnar, 2006). Although rare in neuroimaging studies, prospective studies that allow for dynamic changes across development are clearly needed in which both neural, environmental, and genetic (eg, epigenetic, gene expression) factors are assayed longitudinally; such designs, in large samples, would be ideally positioned to assay the mechanisms through which psychopathology develops (Luby et al, 2014; Pagliaccio et al, 2014; Swartz et al, 2014b).

CONCLUSIONS

The integration of the environment into imaging genetic research has the potential to inform our understanding of the origins of neural mechanisms underlying stress-related psychopathology. Indeed, this research approach has yielded novel insights into the neural mechanisms that may underlie associations between both *FKBP5* rs1360780 genotype and *BDNF* rs6265 genotype with stress-related psychopathology (Gatt et al, 2009; White et al, 2012). Research on additional single variants and polygenic profiles have begun to report significant effects that, if replicated, would help inform the individual difference factors that give rise to variability in stress-related neural phenotypes (Tables 1 and 2). It is critical for iGxE research to address the challenges of: (i) small effects, (ii) measuring the environment and neural phenotypes, (iii) the lack of detailed mechanisms, and (iv) integrating research into a developmental framework. To address the problem of small effects, imaging genetics has begun to rely on larger more well-powered studies and use

polygenic approaches such as enrichment to compliment more traditional candidate polymorphism research (Hibar et al, 2015). Imaging genetics has just begun to consider the environment; going forward it will be important for this research to measure environmental experience as accurately as possible and to conduct challenge studies to explicitly target stress-related neural circuitry (Dick et al, 2015). Moreover, it will be important for the test–retest reliability of imaging designs to be evaluated in large studies that attempt to account for confounding factors. To understand mechanisms, it will be important for well-powered iGxE to assess moderated mediation models and for collaboration across iGxE researchers and non-human animal models, while also assessing signatures of epigenetic regulation (Bogdan et al, 2013a; Hyde et al, 2011a). Lastly, given evidence of differences across development in corticolimbic circuitry and HPA axis function (Johnson and Casey, 2015; Lupien et al, 2009), future iGxE studies using longitudinal prospective designs will be particularly useful. Although these challenges are undoubtedly daunting, addressing them is paramount to the potential insight offered by iGxE research. In doing so, iGxE will be uniquely able to identify neural mechanisms through which genetic variation and environmental experience confer vulnerability to psychopathology.

FUNDING AND DISCLOSURE

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