Toward a Mechanistic Understanding of How Variability in Neurobiology Shapes Individual Differences in Behavior

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Abstract Research has begun to identify how variability in brain function contributes to individual differences in complex behavioral traits. Examining variability in molecular signaling pathways with emerging and established methodologies such as pharmacologic fMRI, multimodal PET/fMRI, and hormonal assays are beginning to provide a mechanistic understanding of how individual differences in brain function arise. Against this background, functional genetic polymorphisms are being utilized to understand the origins of variability in signaling pathways as well as to efficiently model how such emergent variability impacts behaviorally relevant brain function and health outcomes. This chapter provides an overview of a research strategy that integrates these complimentary levels of analysis; existing empirical data is used to illustrate the effectiveness of this approach in illuminating the mechanistic neurobiology of individual differences in complex behavioral traits. This chapter also discusses how such efforts can contribute to the identification of predictive risk markers that interact with unique environmental factors to precipitate psychopathology.

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1 Introduction

Individual differences in trait affect, personality and temperament critically shape complex human behaviors, successfully navigating social interactions and overcoming challenges from our ever changing environments. Such individual differences may also serve as important predictors of vulnerability to psychopathology including depression, anxiety, addiction, and antisocial personality disorder, especially upon exposure to environmental adversity. Accordingly, identifying the biological mechanisms which give rise to trait individual differences affords unique opportunity to develop a deeper understanding of complex human behaviors, disease liability and treatment. Having established multiple modal neural processes supporting specific aspects of complex social behavior, research has now begun to reveal the neural substrates of inter-individual variability in these and related constructs. Moreover, recent studies have established that these neural substrates represent temporally stable and reliable indices of brain function. Thus, much like their behavioral counterparts, brain function represents an enduring, trait-like phenomenon, which in and of themselves may serve as important markers of individual differences as well as disease liability and pathophysiology.

As research continues to illustrate the predictive relationship between brain activation and trait-like behaviors (e.g., increased amygdala reactivity predicts trait anxiety), an important next step is to systematically identify the underlying mechanisms driving variability in brain circuit function. In this regard, neuroimaging studies employing pharmacologic challenge paradigms, principally targeting monoamine neurotransmission and hormonal systems, have revealed that even subtle alterations in dopaminergic, noradrenergic, serotonergic and neuroendocrine signaling can have profound impact on the functional response of brain circuitries supporting affect, personality and temperament. Similarly, multimodal neuroimaging approaches have provided evidence for directionally specific

relationships between key components of monoaminergic signaling cascades, assessed with radiotracer positron emission tomography (PET), and brain function, assessed with BOLD fMRI. Collectively, pharmacological challenge neuroimaging and multimodal PET/fMRI are revealing how variability in behaviorally relevant brain activation emerges as a function of underlying variability in key brain signaling pathways (e.g., increased serotonin signaling predicting increased amygdala reactivity). The next logical step is to identify the sources of inter-individual variability in these key neurochemical signaling mechanisms.

In the modern era of human molecular genetics, one step is firmly planted in the direction of identifying common variation in the genes that influence the functioning or availability of components in these pathways. As DNA sequence variation across individuals represents the ultimate wellspring of variability in emergent molecular, neurobiological and related behavioral processes, understanding the relationships between genes, brain and behavior is important for establishing a mechanistic foundation for individual differences in behavior and related psychiatric disease. Moreover, such genetic polymorphisms can be readily identified from DNA collected via cells from individual blood or even saliva samples using relatively well-tolerated, inexpensive and standardized laboratory protocols. Once collected and isolated, an individual's DNA can be amplified repeatedly providing an almost endless reservoir of material for genotyping of additional candidate polymorphisms as they are identified. When a precise cascade of related neurobiological and behavioral effects are clearly established, common polymorphisms can represent incredibly powerful predictive markers of such emergent properties that are more readily accessible (e.g., samples can be collected in doctor's offices), applicable (e.g., even newborns can be genotyped) and economical (e.g., costing only tens of dollars per sample in comparison to the hundreds and even thousands required for fMRI and PET) than their technological counterparts in neuroimaging and neuropharmacology. Of course, arriving at this ultimate reduction requires intensive and expansive efforts wherein all these technologies as well as epidemiological and clinical studies are first brought to bear on explicating the detailed biological mechanisms mediating individual differences in trait behaviors and related risk for neuropsychiatric disease.

In the last 5 years, significant progress has been made in describing the contributions of multiple common genetic polymorphisms to individual differences in complex behavioral phenotypes and disease liability—in particular, by identifying effects of functional genetic variation on the neural processes that mediate behavioral responses to environmental challenge (Caspi and Moffitt 2006; Hariri and Holmes 2006). The current chapter will review how the integration of psychology, neuroimaging, neuroendocrinology, neuropharmacology and molecular genetics can work toward the ultimate goal of understanding the detailed mechanisms mediating individual differences in human behavior and, in turn, establish predictive markers of disease vulnerability. The vast potential of such an integrated approach will be highlighted by reviewing recent studies whose collective results demonstrate that common sequence variation in human genes that bias key components of molecular signaling cascades results in altered brain

circuit function that mediates individual differences in complex behavioral traits such as temperamental anxiety, aggression, stress responsiveness and impulsivity. With their increased utilization and continued expansion each level of analysis in this integrative strategy—brain circuit function, neural signaling cascades and molecular genetics—also has the potential to uniquely illuminate clinically relevant information that can be used in efforts to devise individually tailored treatment regimes and establish predictive disease markers. In lieu of further describing a general framework, five specific examples will be used to illustrate the effectiveness of this integrated strategy to parse biological mechanisms mediating individual differences in complex behaviors.

Multiple mechanisms involving de novo biosynthesis, vesicular release, active reuptake, metabolic degradation as well as a myriad of both pre- and post-synaptic receptors contribute to the regulation of neurotransmission and its subsequent modulation of brain function. To illustrate the powerful capacity of functional genetic polymorphisms to model emergent variability in signaling pathways, the five exemplars below focus on different critical node in regulating the magnitude of neurotransmission, namely autoregulatory negative feedback, active synaptic reuptake, post-synaptic receptor binding, intracellular receptor binding, and enzymatic degradation. In the first example, individual differences in trait anxiety will be mapped onto threat-related amygdala reactivity. Variability in amygdala reactivity will, in turn, be mapped to serotonin signaling. Finally, variability in serotonin signaling will be mapped to a common functional polymorphism impacting the capacity for negative feedback inhibition of serotonergic neurons in the midbrain. In the second example, a similar relationship will be described between variability in aggression, amygdala reactivity, testosterone signaling and a variable number of tandem repeats in the androgen receptor. The third example describes variability in impulsivity, reward-related ventral striatum reactivity, dopamine signaling and a polymorphism impacting synaptic clearance of striatal dopamine. In the fourth example, a common polymorphism affecting the enzymatic degradation of endocannabinoids will be linked to divergent effects on threat-related amygdala reactivity and reward-related ventral striatum reactivity. In the fifth and last example, variability in stress-responsiveness and hypothalamic–pituitary–adrenal axis function will be linked to a missense polymorphism affecting the mechanistic action of the mineralocorticoid receptor.

2 Trait Anxiety, the Amygdala and Serotonin

The experience of anxiety is commonplace amongst both human and non-human primates as well as other highly social animals. In the context of social interactions, especially within delimited social hierarchies consisting of dominant and subordinate individuals, anxiety serves to shape appropriate and often opposing responses to precipitating events such as competition for limited resources (e.g., food, water, reproductive partners). Sensitivity to potentially threatening social

cues (e.g., affective facial expressions) varies considerably between individuals and represents a core component of commonly employed constructs representing trait anxiety. Individuals with high trait anxiety exhibit a propensity to more frequently appraise situations as more threatening than do others and are generally more sensitive to social cues including those representing both explicit and implicit threat (e.g., angry and fearful facial expressions). In turn, these individuals are at increased risk for developing psychopathology characterized by abnormal social and emotional behaviors such as depression and often precipitated by exposure to chronic or severe stressors. Examining the neural correlates of individual variability in dispositional temperament such as trait anxiety represents an important step in understanding key socioemotional behaviors as well as an effective means of elucidating pathophysiological processes contributing to related disordered states.

Converging evidence from animal and human studies clearly demonstrates that the amygdala is centrally involved in mediating both physiological (e.g., autonomic reactivity) and behavioral (e.g., reallocation of attentional resources) effects that allow an individual to respond adaptively to varied environmental and social challenges (LeDoux 2000). A large corpus of human neuroimaging research reveals that the amygdala is robustly engaged by varied biologically salient stimuli, most notably emotional facial expressions especially those representing threat. However, individuals differ appreciably in the magnitude of amygdala activation on exposure to emotionally expressive facial expressions, and these individual differences appear to be stable over time (Johnstone et al. 2005; Manuck et al. 2007). Thus, they may contribute to the emergence of stable differences in temperament such as trait anxiety.

Recent neuroimaging studies have reported positive relationships between the magnitude of amygdala reactivity to affective, especially threatening, stimuli and inter-individual variability in indices of trait (Dickie and Armony 2008; Etkin et al. 2004; Haas et al. 2007; Killgore and Yurgelun-Todd 2005; Most et al. 2006; Ray et al. 2005) and also state anxiety (Bishop et al. 2004; Somerville et al. 2004). In one study, Stein et al. (2007) report that high trait anxiety is associated with greater amygdala reactivity not only to angry and fearful but also happy facial expressions. Consistent with this pattern of normal variability, various mood and anxiety disorders (e.g., unipolar and bipolar depression, generalized anxiety disorder, social phobia) have been linked with greater amygdala responses to facial expressions depicting fear and anger, as well as sadness and disgust, and, more variably, to emotionally neutral facial expressions (Cooney et al. 2006; Evans et al. 2008; Phan et al. 2006; Phillips et al. 2003; Stein et al. 2002; Whalen et al. 2002). Such findings demonstrate that anxiety-related psychopathology is associated with a heightened amygdala response to diverse affective stimuli. More importantly, in the absence of such disorders, variability in the magnitude of threat-related amygdala reactivity is an important predictor of individual differences in trait anxiety.

Having first established a predictive link between amygdala reactivity and trait anxiety, factors that drive such behaviorally relevant variability in brain function can be now be identified in the broader context of detailing the biological

mechanisms mediating individual differences in temperamental anxiety. Converging preclinical and clinical evidence indicates that amygdala functioning is sensitive to the effects of central serotonin (Sadikot and Parent 1990), whose principle forebrain innervation is provided by the midbrain dorsal raphe nuclei (DRN). Available data from animal studies indicate that relative increases in local 5-HT result in potentiation of amygdala activation and associated behavioral phenomenon, such as fear conditioning (Amat et al. 1998, 2004; Burghardt et al. 2004, 2007; Forster et al. 2006; Maier and Watkins 2005). As advanced in the introduction of this chapter, recent neuroimaging studies using multimodal PET/fMRI or pharmacological challenge BOLD fMRI have provided direct evidence for parallel effects of 5-HT in humans. Specifically, in vivo PET has revealed that decreased endogenous capacity for local 5-HT reuptake (Rhodes et al. 2007) is associated with relatively increased amygdala reactivity. Acute IV administration of a selective serotonin reuptake inhibitor, which reduces capacity for 5-HT reuptake, during BOLD fMRI is likewise associated with not only increased amygdala reactivity but also decreased habituation of amygdala reactivity over time (Bigos et al. 2008). These data clearly indicate that variability in the regulation of 5-HT signaling is an important source of individual differences in amygdala reactivity.

Crucial among components regulating 5-HT neurotransmission and its subsequent modulation of brain function is activation of somatodendritic 5-HT_{1A} autoreceptors, which mediate negative feedback on DRN neurons resulting in decreased 5-HT release at postsynaptic targets in the forebrain (Sharp et al. 2007). Using multimodal PET/fMRI, we previously reported that the density of 5-HT_{1A} autoreceptors accounts for 30–44% of variability in amygdala reactivity in healthy adults (Fisher et al. 2006), confirming the important role of 5-HT_{1A} autoreceptors in modulating the activity of serotonergic target regions. Given the critical role of 5-HT_{1A} autoreceptors in regulating 5-HT signaling and its resulting influence on the functioning of major brain targets, such as the amygdala, as well as complex behavioral processes (Cowen et al. 1994; Hansenne et al. 2002; Lesch and Gutknecht 2004), it is important to identify sources of emergent variability in 5-HT_{1A} function.

Common sequence variation in the human 5-HT_{1A} gene (*HTR1A*) represents one potential source of such inter-individual variability. Recently, a relatively frequent single nucleotide polymorphism, C(-1019)G, in the promoter region of *HTR1A* was demonstrated to impact transcriptional regulation of the gene through altered binding of the transcription factors. Specifically, the -1019G allele abolishes or impairs transcriptional repression of the promoter and, as a consequence, is associated with increased 5-HT_{1A} expression (Lemonde et al. 2003), a phenomenon that appears to be specific to autoreceptors (Czesak et al. 2006). Consistent with this finding, in vivo human PET has revealed specifically increased 5-HT_{1A} autoreceptor density in both healthy adults and depressed patients carrying the -1019G allele (Parsey et al. 2006). However, a similar effect was not observed in an earlier PET study (David et al. 2005). Regardless, the in vitro effects of the *HTR1A* -1019G allele and the more general relationship

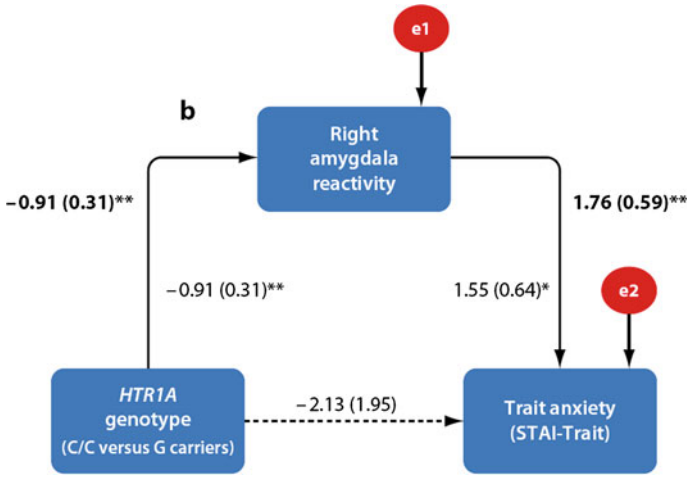

Fig. 1 Trait anxiety is indirectly predicted by *HTR1A* genotype (rs6295) through amygdala reactivity (adapted from Fakra et al 2009; Hariri 2009). Lines are labeled with unstandardized path coefficients and standard errors in parentheses. Bolded coefficients outside of the lines represent values from the trimmed model. Unbolded coefficients presented internally represent values from the full model with all paths included. Significant indirect effects of *HTR1A* genotype on trait anxiety were observed ($\alpha\beta = -1.60$, SE = 0.73, $P < 0.05$) while direct effects were nonsignificant and dropped from the model. E1 and e2 represent the residual variances not explained by variables included in the model. $^{*}P < 0.05$, $^{**}P < 0.01$

documented between increased 5-HT_{1A} autoreceptor density and decreased amygdala reactivity (Fisher et al. 2006) suggest that this common functional genetic variation may contribute significantly to the emergence of inter-individual variability in serotonin signaling which, in turn, biases amygdala reactivity.

Consistent with the existing data (i.e., increased 5-HT_{1A} autoreceptors leading to increased negative feedback inhibition of DRN and decreased 5-HT release), we recently demonstrated that the *HTR1A* -1019G allele is associated with significantly decreased threat-related amygdala reactivity (Fakra et al. 2009). In addition, we found that *HTR1A* genotype effects on trait anxiety were mediated through its impact on threat-related amygdala reactivity, which presumably reflects the genotypes modulation of postsynaptic 5-HT release. Specifically, while path models revealed no significant direct genotype effect on trait anxiety they demonstrated that *HTR1A* C(-1019)G and amygdala reactivity indirectly predicted a significant proportion (9.2%) of individual differences in trait anxiety through their respective indirect and direct paths (Fig. 1). The data from this study is remarkably consistent with that reported for other common functional polymorphisms also associated with relatively increased 5-HT signaling, most notably the 5-HTTLPR short allele (Hariri et al. 2002b; Munafo et al. 2008) and *MAOA* low-activity alleles (Meyer-Lindenberg et al. 2006). More importantly, these findings represent an important step in this avenue of research by providing empirical documentation for the basic premise that genetic variation in neural signaling cascades indirectly

impact emergent behavioral processes by biasing the response of underlying neural circuitries (Hariri et al. 2006b; Hariri and Weinberger 2003).

3 Aggression, the Amygdala and Testosterone

Aggression, defined as any behavior directed toward the goal of harming or injuring another living being (Baron and Richardson 1994), has a major negative impact on society. For instance, the World Health Organization estimated that over 500 individuals between the ages of 10 and 29 die every day as a result of inter-personal conflict while countless more suffer psychologically and physically from aggression-related events. Nevertheless, despite these negative consequences, the use (or threat) of aggression can be beneficial under certain conditions (e.g., athletic competition, self-defense and establishment of status hierarchies).

Two factors contributing to the expression of aggressive behavior are the pursuit of a desired goal (e.g., money, territory, status, mates) and interpersonal provocation. Accordingly, researchers have typically classified aggressive behavior as either proactive or reactive. Proactive aggression, also referred to as instrumental aggression, occurs in the absence of direct provocation, does not involve physiological arousal, and is a goal-oriented behavior aimed at the acquisition of a valued resource (Dodge and Coi 1987). On the other hand, reactive aggression is a defensive response to perceived or actual provocation and is characterized by anger, impulsivity, affective instability and high levels of physiological arousal (Dodge and Coi 1987). Many cases of proactive aggression are highlighted in the media (e.g., assassinations, serial murders); however, reactive aggression likely accounts for most societal problems (Nelson and Trainor 2007). Thus, in the current section, we will focus primarily on putative neurobiological mechanisms underlying reactive aggression.

Non-human animal and human neuroscience research indicates that aggressive behavior is regulated by several inter-connected nodes of a 'social behavior network' including the hypothalamus, amygdala, bed nucleus of the stria terminalis, lateral septum, periaquaductal gray, and orbitofrontal cortex (see Nelson and Trainor 2007; Davidson et al. 2000 for reviews). In humans, a number of studies converge to implicate amygdala hyper-reactivity and/or reduced amygdala-orbitofrontal cortex (OFC) coupling in response to social threat among individuals prone to anger and reactive aggression (see Siever 2008 for review). For example, in a PET study with criminal offenders, Raine et al. (1997) reported that affective murderers (i.e., reactively aggressive inmates) demonstrated increased glucose metabolism in subcortical structures (including the amygdala) and decreased glucose metabolism in the prefrontal cortex. Subsequent imaging studies found differences in neural responses to threat among individuals characterized by reactive aggressive behavior. For instance, Coccaro et al. (2007) reported that adults diagnosed with intermittent explosive disorder displayed amygdala hyper-reactivity and decreased amygdala-OFC coupling to angry facial expressions.

Patients with borderline personality disorder, who are prone to engage in reactive aggression, demonstrate relatively increased amygdala reactivity to facial expressions depicting threat (Mauchnik and Schmahl 2010) and decreased amygdala-PFC coupling (New et al. 2007). Finally, spousal abusers characterized by reactive (but not proactive) aggression display heightened amygdala reactivity and also demonstrate attentional biases for aggressive words (Lee et al. 2008; Chan et al. 2010).

Studies in non-clinical samples indicate that even normal variation in constructs linked to aggressive behavior maps onto variability in threat-related neural responses. For instance, Beaver et al. (2008) reported that individual differences in approach motivation, a construct linked to reactive aggression (Harmon-Jones 2003), were positively correlated with amygdala reactivity to angry facial expressions. Other research has found that individual differences in approach motivation were associated with decreased ventral ACC-amygdala coupling during processing of angry facial expressions (Passamonti et al. 2008). Given the important role of highly interconnected prefrontal regions (e.g., ventral ACC, OFC) in mediating top-down regulation of amygdala driven emotional reactivity (see Davidson et al. 2000 for review), such decreased functional coupling may, in part, explain the positive link between approach motivation and aggressive behavior. Also, individual differences in trait anger are positively correlated with amygdala reactivity to angry faces, but only among men with relatively elevated trait anxiety scores (Carré et al. in press-a). Finally, in a more direct test of the hypothesis that amygdala reactivity to facial cues of threat represents a neurobiological marker for reactive aggression, we have found that variation in self-reported physical aggression is positively correlated with amygdala reactivity to neutral and angry facial expression in two independent samples of healthy men (Carré et al. in press-b). Together, these findings converge to suggest that amygdala hyper-reactivity and/or decreased amygdala-OFC coupling during processing of threat-related stimuli may represent a distinct neural signature for one's propensity to engage in reactive aggression (Siever 2008). It is important to note that individuals characterized by callous-unemotional traits and proactive aggression (e.g., conduct disorder, psychopathy) display amygdala and OFC *hypo*-reactivity to facial signals of threat (see Blair 2010 for review).

As described above, it is important to consider the underlying molecular substrates that give rise to individual variation in threat-related amygdala reactivity and amygdala-OFC coupling. Testosterone, the end-product of the hypothalamic pituitary gonadal axis, is one prime candidate. The physiological effects of testosterone occur mainly through binding to intra-cellular steroid hormone receptors (i.e., androgen and estrogen receptors) to ultimately influence gene transcription. Importantly, androgen (and estrogen) receptors are abundantly located in the amygdala and interconnected limbic structures involved in mediating aggressive behavior (see Newman 1999; Simon 2002 for reviews). Thus, through stimulation of steroid hormone receptors and subsequent modulation of cell function (see Adkins-Regan 2005 for review) testosterone can influence the functioning of neural circuits implicated in the expression of human aggression.

Recent functional neuroimaging studies have detailed some of the neural structures that are sensitive to individual differences in testosterone concentrations. In particular, individual differences in baseline testosterone concentrations are positively correlated with amygdala reactivity to facial expressions signaling threat (Derntl et al. 2009; Manuck et al. 2010) and negatively correlated with OFC reactivity to provocation (Mehta and Beer 2010). Consistent with these correlational studies, pharmacologic challenge experiments indicate that acutely raising testosterone concentrations causes an increase in amygdala reactivity and a decrease in amygdala-OFC connectivity in response to facial signals of threat (Hermans et al. 2008; van Wingen et al. 2008, 2010). These findings suggest that the association between acute fluctuations in testosterone and reactive aggression in men (Carré and McCormick 2008; Carré et al. 2009, 2010) may be due to the influence of testosterone on neural processing of threat (e.g., angry faces or provocation), which may ultimately bias aggressive behavior during social challenges.

As discussed above, many of the physiological effects associated with testosterone are mediated by activation of intra-cellular androgen receptors. Specifically, when activated by testosterone, androgen receptors (AR) migrate to the cell nucleus where they regulate gene transcription by activating hormone response elements (HRE) located within gene regulatory sequences. Importantly, the transcription potential of the androgen receptor varies with the expansion of a polyglutamine stretch in the N-terminal domain of the AR protein, as encoded by a trinucleotide (CAG) repeat polymorphism in exon 1 of the X chromosome-linked *AR* gene (Zitzmann and Nieschlag 2003). Specifically, in vitro work indicates that the transactivation potential of the androgen receptor declines in relation to an increase in the number of CAG repeats (Chamberlain et al. 1994), and that androgen receptor concentrations decline with an increasing number of CAG repeats (Choong et al. 1998).

Recent evidence indicates that the number of CAG repeats correlate negatively with testosterone responses to social interactions with attractive women (Roney et al. 2009). In other words, men with fewer *AR* CAG repeats demonstrate a more robust neuroendocrine response to potential mates, suggesting that this androgen receptor polymorphism may influence the efficiency with which an individual may mount an endocrine response to social interactions. Furthermore, genetic studies have found that high testosterone men with fewer CAG repeats are more aggressive (Rajender et al. 2008; Vermeesch et al. 2010). Finally, Manuck et al. (2010) found that individual differences in CAG repeat length were negatively correlated with ventral amygdala reactivity to facial expressions depicting threat, particularly among men with relatively high baseline testosterone concentrations. Thus, variation in the number of *AR* CAG repeats modulates testosterone responses to social interactions and amygdala reactivity to facial signals of threat.

Collectively, these findings provide support for the idea that heightened amygdala reactivity to social threat (e.g., provocation and/or facial signals of threat) may represent a neurobiological mechanism through which androgens modulate human aggressive behavior. Pharmacologic challenge experiments in

which aggressive behavior is measured directly during fMRI are needed to confirm the causal role of testosterone in modulating human aggressive and threat-related neural responses.

4 Impulsivity, the Ventral Striatum and Dopamine

Discounting future outcomes underlies much of human decision making and figures prominently in several overlapping psychological constructs such as self-regulation, impulse-control, delay of gratification and intertemporal choice (Manuck et al. 2003). Moreover, individuals who strongly prefer immediate over deferred rewards of larger nominal value are often generally impulsive or lacking in self-control and at risk for addictive disorders such as pathological gambling, cigarette smoking and drug and alcohol abuse (Alessi and Petry 2003; Bickel et al. 1999; Kirby et al. 1999; Madden et al. 1997). In experimental research on intertemporal choice, discounting of future rewards or delay discounting (DD) is a well-characterized behavioral measure of preference for immediate over delayed rewards and provides an index of impulsive tendencies in humans (Green and Myerson 2004). Behavioral tests used to derive estimates of DD commonly ask participants to choose between multiple immediate rewards that vary in value and a constant, larger reward available after varying intervals of delay. In such tasks, rates of discounting often differ appreciably and consistently among individuals (Simpson and Vuchinich 2000). Thus, DD represents a potentially important psychometric index of individual differences in present versus future-oriented tendencies.

Similar to the research on trait anxiety and amygdala reactivity explication of the underlying neural processes that give rise to such inter-individual variability has the potential to allow for a more comprehensive understanding of the mechanisms leading not only to normal variability in such behaviors but also the pathophysiology of addiction and related disorders. Through reciprocal cortical and subcortical connections, the nucleus accumbens (NAcc) and, more broadly, the ventral striatum (VS), contribute to the motivational salience of stimuli and abet appetitive or reward-dependent behaviors (Berridge and Robinson 2003). Activity of the VS increases in response to both the anticipation and receipt of rewarding stimuli including primary (e.g., food) and secondary (e.g., money) reinforcers (O'Doherty 2004). Moreover, in addiction, craving and compulsive drug seeking as well as sensitivity to drug cues are associated with dysregulated increases in VS activity (Kalivas and Volkow 2005). Because the response of the VS involves an immediate response to rewards, the magnitude of VS activity may contribute to individual differences in a relative preference for immediate, compared to delayed, rewards.

Using BOLD fMRI, we have demonstrated that the magnitude of VS reactivity predicts individual differences in a simple laboratory measure of DD (Hariri et al. 2006a). Specifically, analyses revealed that individual differences in DD correlate

positively with magnitude of VS activation in response to both positive and negative feedback as well as with differential reward-related VS activation in response to positive compared with negative feedback. Consistent with the strong general correlation between DD and traditional self-report measures of impulsivity (De Wit et al. 2004, 2007), we have also found that reward-related VS reactivity is positively correlated with scores from the Barratt Impulsiveness Scale (Forbes et al. 2009). Collectively, our results suggest that increased self-reported impulsivity as well as the preference for smaller immediate over larger delayed rewards reflect both a relatively indiscriminate and hyper-reactive VS circuitry. Similar variability in VS function has also been associated with more complex measures of incentive-based decision making (Knutson et al. 2007). Moreover, dysregulation of the VS contributes to addiction, perhaps by affecting impulsive decision making (Kalivas and Volkow 2005). As such, inter-individual variability in VS reactivity to reward-related stimuli likely contributes to the emergence of differences in the intermediate behavioral risk factors for, as well as the clinical expression of, addiction. Identifying variability in neural signaling pathways that contributes to individual differences in VS function offers additional traction in the search for underlying biological mechanisms.

Dopamine modulation of neuronal activity, especially in the VS (i.e., mesolimbic system), serves as a nexus for the expression of DA signaling at the level of reward-related behaviors (Cardinal et al. 2004; Kelley 2004). Functioning of the DA system has been linked to normal individual differences in reward-related traits (Depue et al. 1994), and disorders involving enhanced reward-seeking, such as addiction, have been hypothesized to reflect maladaptive alterations of this mesolimbic reward system (Hyman et al. 2006; Volkow et al. 1999). Multimodal and pharmacological neuroimaging studies of DA effects on brain function again offer a unique opportunity to more directly evaluate underlying molecular mechanisms regulating this circuitry. A recent in vivo human study reported a direct relationship between striatal DA synthesis, assessed with PET and brain activity, assessed with BOLD fMRI (Siessmeier et al. 2006). Acute increase of DA release via oral amphetamine has also been linked with relatively increased extent of BOLD fMRI assessed VS activity (Menon et al. 2007). More generally, acute pharmacologic increase of DA in both healthy volunteers (Hariri et al. 2002a) and patients with Parkinson's disease (Tessitore et al. 2002) results in relatively increased BOLD fMRI assessed activity in closely related limbic brain regions, namely the amygdala. Given the importance of DA in modulating this behaviorally relevant neural circuitry, identifying factors that determine inter-individual variability in DA signaling and its related impact on the reactivity of the VS will facilitate our understanding of the neurobiological mechanisms governing reward-related behaviors and augment efforts to improve the treatment and even prevention of pathological behaviors such as drug abuse and addiction.

We have explored the role of altered DA signaling, resulting from a common functional polymorphism impacting active synaptic reuptake in the striatum, in determining inter-individual variability in reward-related VS reactivity and correlated variability in behavioral impulsivity. Consistent with the research on serotonin

signaling, amygdala reactivity and trait anxiety, the selection of our candidate polymorphism was driven by available in vitro and/or in vivo assays demonstrating significant impact of the variant on aspects of biological function related to DA neurotransmission and not on available data from association studies with behavioral (e.g., impulsivity) or clinical (e.g., alcoholism) phenotypes. While association studies are necessary for understanding the ultimate contribution of genetic polymorphisms to variability in behavioral and clinical phenomena, they do not readily allow for inferences regarding polymorphic effects on gene or protein function. Such inferences are instrumental for the development of biologically plausible and tractable hypotheses regarding the impact of genetic variation on inter-individual variability in brain function and associated behaviors such as those pursued in our current work (Hariri et al. 2006b; Hariri and Weinberger 2003).

The dopamine transporter is responsible for the active clearance of synaptic DA and, thus, plays a critical role in regulating the duration of postsynaptic DA signaling, especially in the striatum (Sesack et al. 1998). Accumulating evidence indicates that a 40-base pair variable number of tandem repeats (VNTR) polymorphism in the 3′ untranslated region of the DAT gene (*SLC6A3*) impacts the expression and availability of DAT (Bannon et al. 2001). Although a genotype effect has not been consistently observed across all studies (Martinez et al. 2001; Michelhaugh et al. 2001; Mill et al. 2005; van Dyck et al. 2005), several suggest that in comparison to the 9-repeat allele, the 10-repeat is associated with relatively increased levels of DAT both in vivo (Cheon et al. 2005; Heinz et al. 2000) and in vitro (Mill et al. 2002; Van Ness et al. 2005). We hypothesized that there would be relatively greater VS reactivity associated with the 9-repeat allele, which is linked with reduced DAT expression and presumably greater striatal synaptic DA, in comparison with the 10-repeat allele. Consistent with our hypothesis, the DAT1 9-repeat allele was associated with relatively greater VS reactivity and accounted for nearly 12% of the inter-individual variability. In contrast, genetic variation directly affecting DA signaling only in the prefrontal cortex (i.e., COMT Val158Met) was not associated with variability in VS reactivity. These results highlight an important role for a genetic polymorphism affecting striatal DA neurotransmission in mediating inter-individual differences in reward-related VS reactivity. They further suggest that altered VS reactivity may represent a key neurobiological pathway through which these polymorphisms contribute to variability in behavioral impulsivity and related risk for substance use disorders.

5 Endocannabinoids, Threat- and Reward-Related Brain Functions

Modern neuroscience methodologies have greatly advanced our understanding of the intrinsic mechanisms mediating and regulating endogenous cannabinoid or endocannabinoid (eCB) signaling in the CNS (Piomelli 2003). Such eCB signaling has emerged as a potent modulator of neural circuitries mediating both basic

physiological (Calignano et al. 1998; Meng et al. 1998) and advanced behavioral responses (Maldonado et al. 2006; Scherma et al. 2008; Viveros et al. 2005). Experimental manipulation of these mechanisms has revealed significant behavioral effects, especially in threat- and reward-related domains, which are generally consistent with the effects of *Cannabis* intoxication, which are largely driven by the constituent chemical Δ^9-tetrahydrocannabinol (Robson 2005). The elucidation of molecular mechanisms regulating eCB signaling, akin to that for serotonin and dopamine, has motivated attempts to understand its possible contribution to the emergence of variability in brain circuit function and related individual differences in behavioral attributes (e.g., anxious or impulsive temperament) associated with increased risk for psychiatric disorders.

After their biosynthesis from arachidonic acid, eCBs such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) typically modulate synaptic neurotransmission through stimulation of CB1, the principal CNS cannabinoid receptor widely expressed on multiple neuronal subtypes and their distributed circuitries. In turn, the duration and intensity of eCB signaling, especially for AEA, is regulated by two complementary mechanisms: enzymatic degradation via fatty acid amide hydrolase (Cravatt et al. 1996) and active synaptic clearance via the AEA transporter (Piomelli et al. 1999). The psychotropic and THC-like effects of AEA, however, appear to be coupled with fatty acid amide hydrolase (FAAH), but not AEA transporter function (Solinas et al. 2007). Thus, FAAH, an integral membrane enzyme, may uniquely regulate behaviorally relevant eCB signaling by mediating the hydrolytic breakdown of AEA into arachidonic acid and ethanolamine.

Again, common genetic variation (i.e., polymorphisms) affecting the functioning of components involved in eCB neurotransmission (e.g., AEA, CB1, FAAH) may represent a significant potential source of inter-individual variability in eCB signaling that mediates emergent differences in emotion- and reward-related behaviors (Onaivi et al. 2002). Because of its critical role in regulating the signaling duration and intensity of AEA (Cravatt et al. 1996), and its selective contribution to the psychotropic effects of AEA (Solinas et al. 2007), we have recently examined the neurobiological and behavioral effects of a common functional nonsynonymous SNP resulting in the conversion of a conserved proline residue to threonine (P129T) in the amino acid sequence of FAAH (Hariri et al. 2009). In vitro, *FAAH* 385A is associated with normal catalytic properties, but reduced cellular expression of FAAH, possibly through enhanced sensitivity to proteolytic degradation (Chiang et al. 2004; Sipe et al. 2002). Moreover, the C385A is the only common mutation in *FAAH* (Flanagan et al. 2006) and the 385A, which putatively augments AEA signaling via decreased enzymatic degradation, has been associated with reward-related pathologies including street drug use and problem drug/alcohol abuse, as well as being overweight and obese (Flanagan et al. 2006; Sipe et al. 2002).

In animal models, both pharmacologic and genetic disruption of FAAH function result in *decreased* anxiety-like behaviors, as well as *increased* consumption and preference for ethanol (Basavarajappa et al. 2006; Blednov et al. 2007; Kathuria et al. 2003; Moreira et al. 2008; Solinas et al. 2007). Moreover, a recent pharmacologic fMRI study in human subjects has reported that acute oral administration of

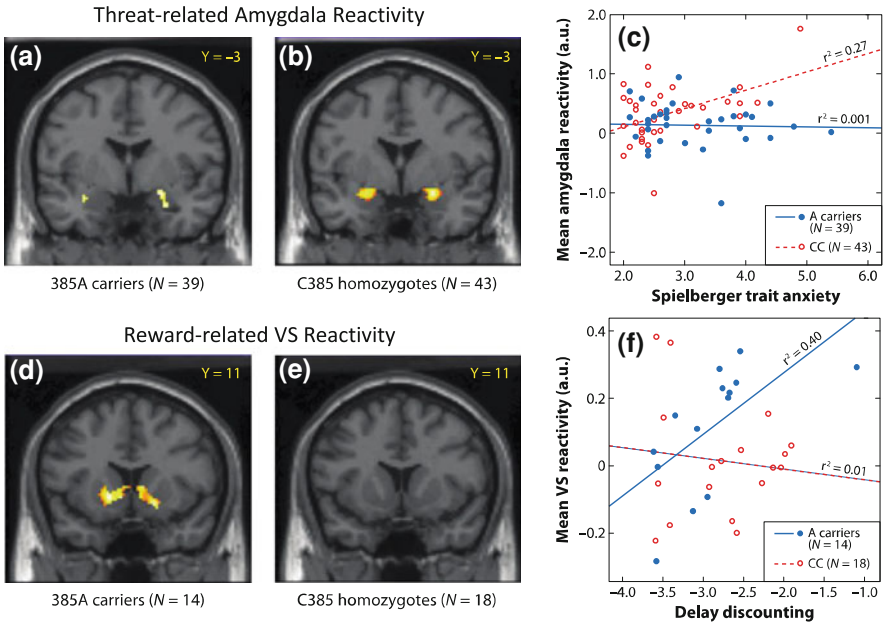

Fig. 2 Effects of *FAAH* genotype (rs324420) on threat- and reward-related brain activation (adapted from Hariri et al. 2009; Hariri 2009). Statistical parametric maps displaying the correlation between threat-related amygdala reactivity and trait anxiety in **a** *FAAH* 385A carriers and **b** C385 homozygotes. **c** Plots of the correlation between threat-related amygdala reactivity and trait anxiety according to *FAAH* C385 genotype. Statistical parametric maps displaying the correlation between reward-related ventral striatal (VS) reactivity and delay discounting in **d** *FAAH* 385A carriers and **e** C385 homozygotes (no significant correlation observed). **f** Plots of the correlation between reward-related VS reactivity and delay discounting according to *FAAH* C385A genotype. FAAH: fatty acid amide hydrolase

THC is associated with reduced amygdala reactivity to threat-related facial expressions of emotion (Phan et al. 2008). Consistent with these effects, we hypothesized that the *FAAH* 385A would be associated with relatively *decreased* threat-related amygdala reactivity, but *increased* reward-related reactivity in the VS. Analyses revealed that carriers of the *FAAH* 385A, associated with reduced enzyme expression and, presumably, increased AEA signaling, have decreased threat-related amygdala reactivity. In contrast, carriers of the FAAH 385A exhibited increased reward-related VS reactivity in comparison to C385 homozygotes. Moreover, divergent effects of *FAAH* C385A genotype on brain function were manifest in a consistent manner at the level of brain-behavior relationships (Fig. 2). Relative to C385 homozygotes, *FAAH* 385A carriers showed a diminished relationship between amygdala reactivity and trait anxiety. In contrast, 385A carriers exhibited a markedly increased relationship between VS reactivity and delay discounting, a behavioral index of impulsivity and reward-sensitivity.

It is important to note that there were no direct associations between *FAAH* genotype and behavioral phenotypes (i.e., anxiety or impulsivity) in this study, a common occurrence when working with relatively small samples, possibly reflecting the minimal effect the proximal biological impact associated with any genotype has on any distal behavioral phenotype (Hariri et al. 2006b; Hariri and Weinberger 2003), as well as the importance of environmental stressors in unmasking genetically driven effects on behavior (Caspi and Moffitt 2006). However, there were robust differences in the relationships between regional brain function and complex behaviors as a function of *FAAH* C385A genotype. These observed brain-behavior patterns may reflect the influence *FAAH* C385A associated differences in endogenous eCB tone on stimulus-driven neural circuit function mediating complex behavioral processes. Relatively higher levels of AEA in the amygdala of *FAAH* 385A carriers may reduce the responsivity of this structure to salient input (possibly through CB1-mediated potentiation of local GABAergic interneurons) and, as a consequence, lead to reduced anxiety-like behaviors predicted by amygdala function. In contrast, higher levels of AEA may increase the responsivity of the VS in *FAAH* 385A carriers (possibly through CB1-mediated increased dopamine release and potentiation of VS neuron activity) leading to increased reward-sensitivity predicted by VS function. Support for this speculation exists in studies reporting a failure of restraint stress to effect changes in amygdala activation in knockouts lacking FAAH or animals treated with FAAH inhibitors (Patel et al. 2005), and increased food-intake as a result of local FAAH inhibition in the nucleus accumbens (Sorice-Gomez et al. 2007). Thus, the endogenous state of eCB signaling associated with either constitutive genetic variation such as the *FAAH* C385A or acute pharmacologic manipulation likely biases the responsivity of neural circuits to behaviorally relevant information and their subsequent regulation of complex behaviors.

Decreased threat-related amygdala reactivity and associated trait anxiety may contribute to the emergence of pathologies such as addiction and obesity, previously associated with the *FAAH* 385A (Flanagan et al. 2006; Sipe et al. 2002; Tyndale et al. 2007), by reducing the sensitivity of these individuals to potential environmental threat or harm. In fact, blunted amygdala reactivity has been reported in individuals at high familial risk for alcoholism and this has been interpreted as possibly contributing to decreased threat-sensitivity and subsequently increased risk-taking behaviors in these genetically predisposed individuals (Glahn et al. 2007). An increase in reward-related VS reactivity and associated impulsivity (e.g., steeper discounting of future, relative to immediate rewards) may likewise contribute to disinhibitory psychopathologies through heightened reward-sensitivity and impulsive decision making. Studies in addicted patients have generally reported a sensitization of the neural circuitry for reward, including the VS (Kalivas and Volkow 2005). And, increased behavioral impulsivity and reward-sensitivity are significant risk factors for addiction (de Wit and Richards 2004). Thus, through divergent effects on both threat- and reward-related brain functions, the influence of *FAAH* C385A on eCB signaling may have a compound and accelerated effect on risk for related pathologies.

6 Stress, the HPA Axis and the Mineralocorticoid Receptor

All organisms strive to maintain homeostasis by regulating physiological states within a dynamic equilibrium. Stress, the perception of inadequate coping resources in the context of environmental demands appraised as threatening, is a common experience that disrupts homeostasis, and triggers a biological-behavioral stress response to promote adaptation/survival. While stress can promote adaptive coping to environmental challenges by recruiting necessary resources, it is also associated with many adverse physical and mental health conditions such as cardiovascular disease, depression, post-traumatic stress disorder (PTSD) and immune system dysfunction (Cohen et al. 2007; McEwen and Gianaros 2010). Importantly, there is tremendous variability in which environmental demands individuals perceive as stressful as well the extent of physiological and psychological response to these demands (Dickerson and Kemeny 2004; Kudielka et al 2009). Because this variability is associated with stress-related physical and mental health outcomes and differences in related neural activation (Marques et al. 2009; Yehuda 2002), identifying the factors that contribute to variability in stress reactivity is an important step for understanding the etiology of stress-related disorders as well as limiting the sequelae of stressful experiences.

A wealth of research has established that stress reactivity is centrally regulated by the hypothalamic–pituitary–adrenal (HPA) axis (for reviews see de Kloet et al. 2005; Ulrich-Lai and Herman 2009). Briefly, pathways from the medial prefrontal cortex, hippocampus, amygdala and brainstem involved in the behavioral, neuroendocrine, autonomic and immune responses to stress converge within the paraventricular nucleus of the hypothalamus to regulate the release of corticotropin releasing hormone (CRH) in response to perceived stress. CRH triggers the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which upon binding to receptors in the adrenal gland, stimulates cortisol release. As part of a negative feedback loop, increases in cortisol inhibit CRH and ACTH release from the hypothalamus and anterior pituitary gland, respectively. At all levels in the cascade, HPA axis function varies widely across individuals and is relatively stable over time (Fox et al. 2006; Kudielka et al. 2009; Márquez et al. 2005) suggesting that trait-like variation in HPA axis function may contribute to stable differences in responses to stressors.

Rodent knockout and non-human and human pharmacologic challenge studies have demonstrated that disruption at all nodes of the HPA axis (e.g., hypothalamus, pituitary, adrenal gland) can induce anxiety and depressive-like behavior (e.g., Kolber and Muglia 2009; Marques et al. 2009; Müller et al. 2002). Moreover, nearly 50 years of research has strongly linked abnormal function at all levels of the HPA axis to stress-related psychopathology (Gibbons and McHugh 1962; Marquis et al. 2009; Yehuda 2002). For instance, depression is characterized by elevated CRH, ACTH and cortisol as well as a disrupted negative feedback loop whereby cortisol does not effectively inhibit CRH and ACTH (van Praag et al. 2004). PTSD is associated with elevated CRH but diminished ACTH and cortisol

as well as an enhanced cortisol-induced inhibition of CRH and ACTH (Yehuda 2002). Furthermore, HPA dysregulation is associated with a number of risk factors for psychopathology such as childhood maltreatment (Tarullo and Gunnar 2006) and low social support (Abercrombie et al. 2004).

More recently, and consistent with earlier non-human animal work (Rodrigues et al. 2009; Ulrich-Lai and Herman 2009), translational neuroimaging research suggests that HPA axis function is associated with structural and functional differences in brain regions, such as the hippocampus, amygdala, basal ganglia and prefrontal cortex, that are relevant to stress-related psychopathology (for review see Pruessner et al. 2010; McEwen and Gianaros 2010). For example, Urry et al. (2006) show that heightened amygdala and reduced ventromedial prefrontal cortex activation during the regulation of negative affect is associated with dysregulated HPA axis function. Moreover, prefrontal glucose metabolism, an index of neuronal activity, predicts variability in HPA axis function (Jahn et al. 2010; Kern et al. 2008), and lesions to these brain regions result in HPA axis dysregulation (Buchanon et al. 2010).

Given the firm association between HPA axis function and stress-related psychopathology and related neural circuitry, factors that shape the responsiveness of this system can be studied with the ultimate goal of identifying mechanisms underlying individual differences in response to stress. As mentioned above, in addition to numerous regulatory mechanisms inside and outside of the HPA axis, cortisol is a major regulator of HPA axis function and related neural systems. In support of a causal relationship between cortisol and stress-related psychopathology, 20% of patients prescribed chronic high doses of hydrocortisone (a synthetic form of cortisol), develop psychopathology including depression, mania and psychosis. In addition, while 75% report some psychiatric symptoms these disappear following treatment cessation (see Marques et al. 2009).

Cortisol operates through a binary corticosteroid receptor system, binding to both the high affinity mineralocorticoid receptor (MR) and low affinity glucocorticoid receptor (GR) which are widely co-expressed in limbic neurons including those in the amygdala (de Kloet et al. 2005; Joëls et al. 2008). These intracellular receptors function as transcriptional regulators whereby binding can alter the expression of 70–100 genes (de Kloet et al. 2005), which likely has widespread consequences for neural activation and stress responsiveness. Recent research provides support for the existence of long-hypothesized membrane-bound MR and GR, although the mechanism(s) through which these effects occur remain unclear (Bartholome et al. 2004; Karst et al. 2006; Joëls et al. 2008).

Because of its low affinity, the GR is only extensively occupied in the wake of large spikes in cortisol such as those associated with stressors or circadian rhythms. One of the primary functions of the GR is to normalize brain activity following the extinction of a stressor and to inhibit continued HPA axis response. On the other hand, because of its high affinity, the MR is almost always occupied and non-human animal research suggests that it is necessary for a stable excitatory tone in the hippocampus which inhibits the HPA axis under basal and stressful circumstances (Reul et al. 2000). In addition to tonically inhibiting HPA axis

response, MR binding is relevant to behavioral stress response functions including the appraisal of novel situations as well as the selection of appropriate responses to deal with challenge (de Kloet et al. 2005; Joëls et al. 2008). Thus, it is believed that the MR is prominently involved in basal stress system regulation and the onset of a stress response while the GR primarily functions to terminate an initial stress reaction. As such, the MR is an ideal candidate to contribute to differences in stress reactivity.

In mice, MR knockout or antagonism increases basal and stress-evoked HPA axis activity (Gass et al. 2001) and worsens response to antidepressant medication (DeRijk et al. 2008). Conversely, enhanced MR expression is associated with reduced depressive- and anxiety-like behaviors and corticosterone (the rodent homolog of cortisol) secretion under stressful and basal conditions (Mitra et al. 2009; Rozeboom et al. 2007). Consistent with these mechanisms, antidepressant medications increase MR expression (Brady et al. 1991). Collectively, these findings suggest that reduced MR function may contribute to elevated HPA axis activity at baseline and in dysregulated responses to stressors. Thus, given the prominent role of the MR in stress-responsiveness, stress-related behavior and psychopathology, it is important to identify sources of variability in MR function.

A missense isoleucine(Iso)/valine(Val) polymorphism (rs5522) located in exon 2 of the MR gene (*NR3C2*) occurs in approximately 10% of the Caucasian population. Importantly, in vitro studies show that the Val allele is associated with reduced cortisol, but not aldosterone, binding and reduced cortisol-induced transactivation (De Rijk et al. 2006; Arai et al. 2003). Thus, the MR Val allele may promote elevated HPA axis activity under basal conditions and in response to small stressors due to reduced cortisol-MR binding and subsequently decreased inhibition of the HPA axis. The Val allele may promote the development of HPA axis dysregulation, not only through reduced inhibition of HPA axis responsiveness but also because of reduced MR expression typically elicited by cortisol.

Consistent with these functional associations and speculations, the MR Val allele has been linked to heightened endocrine and autonomic responses to acute stress and stress perception. Specifically, the Val allele is associated with elevated cortisol and heart rate responses to acute social stress (DeRijk et al. 2006), skin conductance responses evoked by acute physical stress (threat-of-shock, Bogdan et al. 2010), perceptions of the aversiveness of shock levels (Bogdan et al. 2010) and waking cortisol levels (van Leeuwen et al. 2010). Furthermore, relative to Iso homozygotes, youth Val carriers have heightened threat-related amygdala reactivity, particularly in the context of low emotional neglect (Bogdan et al. in press). Collectively these finding suggest that the functional effect of the Val allele does result in enhanced stress responsiveness and perception. Furthermore, the Val allele has been associated with enhanced reward learning under basal conditions, but a vulnerability to stress-induced reward learning deficits (Bogdan et al. 2010) as well as depressive symptoms (Kuningas et al. 2007). Thus, it appears that the heightened stress responsiveness characteristic of the Val allele may translate to important behaviorally relevant differences related to psychopathology.

Taken together, individual differences in HPA axis function are predictive of stress-related physical and mental health disturbance in what appears to be a causal relationship. In addition to work showing that environmental experience (e.g., childhood maltreatment) can have long-lasting and even epigenetic effects (McGowan et al. 2009) on HPA axis system function, emerging research has shown that common polymorphisms including that in the MR gene detailed here as well as in the genes for GR (DeRIjk et al. 2008), CRHR1 (Binder 2009; Binder and Nemeroff 2010; Thode et al. unpublished observation) and FKBP5 (REF) influence variability in HPA axis function, stress sensitivity and psychopathology. Given the association of both environmental and genetic factors with individual differences in HPA axis function, this system may be a particularly fruitful area to further pursue gene-by-environment interactions research.

7 Summary and Future Directions

As detailed above, multimodal techniques assessing brain function, have begun to identify how variability in neural substrates associated with processing specific forms of information contribute to emergent individual differences in stable and enduring aspects of human behaviors such as personality and temperament. In parallel, the application of pharmacologic fMRI, multimodal PET/fMRI, and neuroendocrinology is allowing for an understanding of how variability in specific molecular signaling pathways influences individual differences in behaviorally relevant brain function. Moreover, information on DNA sequence variation in humans and related identification of functional genetic polymorphisms is now being utilized to understand the biological origins of variability in component processes of molecular signaling pathways as well as to efficiently model how such emergent variability impacts behaviorally relevant brain function. Such ongoing efforts to understand the detailed mechanisms that mediate individual differences in complex behavioral traits and related psychopathology at the level of brain circuit function, molecular signaling pathways and functional genetic polymorphisms have the potential to inform clinically relevant issues and provide guiding principles for the development of more effective and individually tailored treatment regimes. In addition, the elucidation of such mechanisms, especially those mapped to functional genetic polymorphisms, can lead to identification of predictive risk markers that interact with unique environmental factors to precipitate psychopathology.

While the five examples highlighted in this chapter are evidence for the potential of an informed and integrated research strategy to identify the neurobiology of individual differences in complex behavioral traits and their related clinical endpoints, much work is left to be done. First, to allow for tractable experimental designs and testable hypotheses in existing samples, the studies highlighted above have focused on the effects of a single signaling pathway on behaviorally relevant brain circuitry. Of course, it is very clear that there are

numerous complex interactions between signaling pathways and that more than one pathway contributes to the regulation of any brain circuitry. For example, we know that DA plays an important role in modulating amygdala function and anxiety (Hariri et al. 2002a; Tessitore et al. 2002), and that 5-HT can influence reward-related brain circuitry and impulsivity (Manuck et al. 1998). However, existing studies lack the power and sophistication to model such complex interactions while effectively controlling for other important modulatory factors (e.g., age, gender, stress exposure) in the context of BOLD fMRI, pharmacologic fMRI or multimodal PET/fMRI protocols. To do so, we must aggressively expand the scale and scope of our studies to include hundreds and, preferably, thousands of subjects. This will afford opportunities to effectively examine interactions between signaling pathways (e.g., 5-HT and DA) on brain function and behavior through modeling of multiple functional polymorphisms (e.g., *HTR1A* -1019 and DAT1), and examine the effects of genetically driven variation in signaling pathways on multiple behaviorally relevant brain circuitries.

A second important consideration is that existing studies have been largely conducted in ethnically and racially homogenous populations. Thus, the observed effects may not generalize to other populations. This is especially true of studies utilizing functional genetic polymorphisms because the potential effect of any single genetic variant on a complex biological and behavioral phenotype is likely to be small against the background of the approximately 20,000–25,000 human genes and the multitude of other neurobiologically relevant functional variants they likely harbor. In fact, we have already seen that the well-replicated effects of a common functional polymorphism affecting 5-HT signaling on amygdala reactivity in Caucasian subjects may be reversed in those of Asian ancestry (Lee and Ham 2008; Munafo et al. 2008). Importantly, our most recent studies have experimentally controlled for occult genetic stratification independent of self-reported race or ethnicity as well as the independence of the target genotype from other functional polymorphisms impacting the brain functions under study. While such efforts allow for the attribution of emergent variability in brain and behavior to the candidate variant of interest and not to other possible polymorphisms or more general differences between genotype groups in genetic background, it is important to explicitly test the independence of functional polymorphisms through rigorous statistical modeling in larger samples and also to test the validity of any associations derived in one sample population (e.g., Caucasian) to populations with different genetic backgrounds (e.g., Asian or African).

A third important consideration for the future of this research is the need to conduct large-scale prospective studies beginning in childhood to determine any developmental shifts in neurogenetic pathways mediating individual differences in behavior as well as their predictive utility in identifying risk for psychopathology as a function of environmental or other stressors. All of the studies described above and most of the studies available in the literature as a whole have been conducted in adults carefully screened for the absence of psychopathology. Because of this, these findings identify mechanisms contributing to variability in the normative range of behavior only. The utility of these markers of individual

differences in behavior be they neural, molecular or genetic in predicting vulnerability to psychopathology is unclear. Such predictive utility is ideally tested through prospective studies beginning with premorbid populations that account for the moderating effects of environmental stress in the emergence of clinical disorder over time (Caspi and Moffitt 2006; Viding et al. 2006).

A fourth issue is the need to further integrate pharmacologic challenge protocols with multimodal PET/fMRI to determine if variability in molecular components of signaling pathways mediate effects of specific neurotransmitters or neuromodulators on individual differences in behaviorally relevant brain circuit function. For example, despite the remarkable convergence of findings implicating variability in eCB signaling in threat- and reward-related brain functions, the exact nature of the downstream signaling pathways through which *FAAH* C385A may modulate neuronal and neural circuit function cannot be determined from the available results. FAAH catalyses the hydrolysis of other biologically active endogenous fatty acid amides (e.g., oleamide and oleoylethanolamide), which impact threat- and reward-related behaviors independently of AEA (Wei et al. 2007; LoVerme et al. 2005). Although, FAAH has high selectivity for AEA (Desarnaud et al. 1995) the effects of *FAAH* C385A cannot be specifically linked to AEA neurotransmission without additional data. If the neural and behavioral effects of *FAAH* C385A are mediated by genotype-driven differential availability of AEA, then these effects should be sensitive to manipulation of CB1 receptors. An interesting test of this putative mechanism would be to examine the impact of CB1 antagonists, such as rimonabant, on neural phenotypes associated with *FAAH* C385A genotype using pharmacologic fMRI. The availability of a PET radiotracer for CB1 (Burns et al. 2007) also allows for the determination of any *FAAH* C385A effects on endogenous receptor concentrations. If this polymorphism biases brain function through AEA stimulation of CB1, then antagonism of the receptor should eliminate the divergent effects on amygdala and VS reactivity documented here. Any genotype related alterations in AEA concentrations may also be reflected in relative up- or down-regulation of CB1 receptors assayed via PET. If CB1 antagonism fails to abolish the differential effects of *FAAH* C385A on brain function or if there are no differences in CB1 concentrations based on the genotype, then the existing effects are likely mediated by non-eCB fatty acid amides. In addition to testing this mechanistic hypothesis with pharmacologic fMRI and multimodal PET/fMRI, future studies with substantially increased sample sizes can model allele load effects of *FAAH* 385A, as well as potential *FAAH* interactions with functional genetic polymorphisms affecting other components of eCB neurotransmission (Chakrabarti et al. 2006).

Fifth, in light of evidence for environmental modulation (Tarullo and Gunnar 2006), gene-by-environment interactions (Caspi and Moffitt 2006) and epigenetic regulation (MacGowan et al. 2009), it is important for future research to not only assess genetic variation, but also to optimize assessments available to assess the objective and subjective impact of positive and negative environmental variables. Examples of this include interview-based methods to assess the objective and subjective impact of stressful life events and prospective study designs to examine the influence of stress over the course of study assessments (e.g., the Life Events and

Difficulties Schedule; Brown and Harris 1978; Monroe 2008; Williamson et al. 2003). Furthermore, in light of theoretical arguments that genotypes associated with adversity may reflect plasticity to the environment and not just vulnerability (e.g., Belsky et al. 2009; Manuck 2010), it is critical for these assessments to also assess positive effects of the environment such as social support (e.g., Hyde et al. 2011).

Finally, there is tremendous potential in developing large databases (again preferably thousands of subjects) with detailed measures of behavioral traits, neuroimaging-based measures of multiple brain circuitries and extensive genotyping. One of the most exciting applications of molecular genetics is in identifying novel biological pathways contributing to the emergence of complex traits (Gibson and Goldstein 2007; McCarthy et al. 2008). The continued refinement of a detailed map of sequence variation across the entire human genome (i.e., SNPs that "tag" every gene) and production of technologies supporting efficient high-throughput identification of such variation in individuals have dramatically accelerated the discovery of genes involved in the emergence of complex disease processes (Fellay et al. 2007; Link et al. 2008) as well as normal variability in continuous traits (Lettre et al. 2008). Many of the genes identified in such studies have illuminated novel pathways not previously implicated in these processes or traits, spurring intensive efforts to understand the potential biological effects of the proteins produced by these genes. As such, these "genome-wide" screens represent an opportunity to leap forward beyond the available pool of candidate molecules and pathways in parsing the mechanisms of complex biological processes. Because neuroimaging-based measures of brain function reveal key mechanisms involved in the emergence of individual differences in behavioral traits and are closer to the biological effects of functional genetic polymorphisms, they are ideal substrates for genome-wide screens. For example, BOLD fMRI estimates of amygdala reactivity predicting variability in trait anxiety can be used as the continuous trait in a genome-wide screen. Any significant associations that emerge between genetic variation and amygdala reactivity may confirm existing relationships (e.g., the importance of genes biasing 5-HT signaling) or, more importantly, reveal unexpected candidate molecules or pathways (e.g., a gene producing a molecule that is expressed in the brain and may function in second-messenger signaling cascades). Once identified and, ideally, replicated in large-scale databases that effectively address confounds common to genome-wide screens (e.g., controlling for multiple comparisons resulting from testing the association of a phenotype with hundreds of thousands or millions of SNPs), the impact of variation in novel genes associated with amygdala reactivity can be explored at each level of the biological cascade leading to trait anxiety (i.e., be fed back into the discovery loop outlined in the introduction). In addition to exponentially improving our understanding of neurobiological pathways leading to individual differences in complex behavioral traits these efforts may lead to the discovery of novel therapeutic strategies targeting related disease processes.

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