Utilizing a unique animal model to better understand human temperament

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Abstract

Individual differences in temperament are associated with psychopathology in humans. Moreover, the relationship between temperament and anxiety-, depression-, PTSD- and addiction-related behaviors can be modeled in animals. This review will highlight these relationships with a focus on individual differences in the response to stressors, fear conditioning and drugs of abuse using animals that differ in their response to a novel environment. We will discuss behavioral and neurobiological commonalities amongst these behaviors with a focus on the hippocampus and, in particular, growth factors as promising novel targets for therapeutic intervention.

Introduction

Temperament represents stable features in an individual's reactivity to the environment and biases towards particular coping strategies following severe stress. Temperament is innate, highly heritable and emerges early in life. Moreover, temperamental tendencies predict the types of psychopathology that an individual might develop. Thus, some individuals gravitate towards novelty, risk and excitement, and they are more prone to "Externalizing Disorders," such as substance abuse and risk-taking behaviors. At the other extreme are individuals who are timid, avoid novelty and prefer predictable conditions, and they are more prone to "Internalizing Disorders," such as clinical depression and anxiety disorders. Indeed, recent evidence shows that nearly half of children with a highly inhibited temperament will develop an anxiety disorder, resulting in a heightened risk for depression [1]. Moreover, the quantity and quality of stress experienced may determine whether that individual will develop an "internalizing disorder", as well as the type of disorder (i.e. major depressive disorder (MDD) or post-traumatic stress disorder (PTSD)). Since temperament is the expression of the brain's response to the environment, we hypothesize that temperament is what determines the response to stressors and whether the individual will shift to psychopathology or not. Thus, understanding temperament can help understand the mechanisms that might lead to psychopathology.

Animal models are essential to disentangle brain responses related to temperament from those related to adaptation to stressors. Individual variation in stress responsiveness is evident in rodents, and we can exploit these differences to investigate the neural mechanisms underlying natural variation in emotionality and the associated psychopathology. For example, rats that exhibit high levels of locomotor activity in response to a novel environment (HRs) also exhibit low levels of depression- and anxiety-like behaviors; whereas rats with lower levels of locomotor activity in response to a novel environment (LRs) typically exhibit higher levels of anxiety-like behavior [2]. We are not suggesting that rodents exhibit psychopathology per se.

However, the locomotor response to a novel environment is an index of temperament, and this is associated with traits such as anxiety-like behavior. These traits can be selected for through genetic breeding, and in doing so, we have found that selectively bred high-responders (bHRs) provide an excellent model of vulnerability to a range of behavioral features of externalizing disorders; whereas the selectively bred low-responders (bLRs) provide a model of vulnerability to the behavioral features of internalizing disorders [3,4]. bHRs are representative of individuals prone to externalizing disorders or suffering from “behavioral disinhibition”. That is, relative to bLRs, bHRs are more impulsive, more aggressive and more likely to exhibit addiction-like behavior [5]. A common theme throughout this review will be how HRs/bHRs and LR/bLRs respond to stressors, fear conditioning and drugs of abuse. We will focus on the neurobiology of temperament in humans, and stress reactivity in animal models, drawing on parallels in the literature from the past three years.

Behavioral and neurobiological parallels between temperament in humans and stress responsiveness in rodents

The neural circuitry and some of the molecular players underlying features of personality and temperament in humans have emerged in recent years. This circuitry involves neural systems implicated in both reward and stress responsiveness, including the prefrontal cortex and its monoaminergic innervation, the nucleus accumbens, the amygdala and the hippocampus. Genetic studies of personality and temperament in humans have recently identified roles for the opioidergic system, the serotonergic system and the corticotropin-releasing hormone (CRH) system [1,6]. Interestingly, psychosocial stress can result in a blunted dopamine response in the nigrostriatal system [7,8]. A major controller of stress responsiveness is the hippocampus, which responds to threatening stimuli and senses circulating glucocorticoids from the hypothalamic-pituitary-adrenal (HPA) axis. For example, there are individual differences in hippocampal activation in response to threatening stimuli such that

individuals with a history of past deprivation or neglect, show increased in activation in response to emotional faces [9]. It is important to note that past neglect and abuse are associated with increased risk for psychopathology in youth [10]. When the emotional faces are threatening in nature, the increased response is an indicator of temperament and by extension associated with increased levels of anxiety and depression.

Animal models can explore the neurobiological bases of individual differences in the response to stressors in greater detail. The recent human findings described above are consistent with findings in rodent models of stress where serotonin and CRH levels in the nucleus accumbens have been related to individual differences in depression-like behavior [11], and blockade of the kappa opioid receptor in the same brain region prevented depression-like behavior [12]. The prefrontal cortex also plays a role in individual differences in response to stressors [13]. Following acute stress, rats with a high GABA to glutamate ratio in the prefrontal cortex also had blunted activation and an exaggerated stress response relative to rats with a low GABA to glutamate ratio.

In contrast to the prefrontal cortex, the hippocampus may be the region that most determines the response to a novel environment, especially early in life. In rats that were selectively bred to differ in their response to a novel environment, the hippocampus showed the most divergence in gene expression between bHRs and bLRs during postnatal brain development [14]. Similarly, the hippocampus was the region with the most synchronized anatomical differences in mice susceptible to stress [15]. In this later study, the volume of the hippocampus was correlated with the volume of the hypothalamus, the cingulate cortex and the ventral tegmental area.

The hippocampus is enriched with glucocorticoid receptors (GR), which bind glucocorticoids (cortisol in humans, corticosterone in rodents) and mediate negative feedback to attenuate the stress response [16]. Thus, when a negative regulator of GR, FK506 binding protein 51 (FKBP51), was knocked out, mice had higher functional GR tone, reduced HPA axis

activity, and a blunted response to acute stress [17]. However, GR plays a dual and somewhat contradictory role, not only as a mediator of negative feedback but also as an initial detector of threat and mediator of anxiety [18]. Interestingly, the less anxious HRs also exhibit less GR expression in the hippocampus than LRs. Even though both bred and outbred HRs show elevated levels of circulating glucocorticoids, this does not deter them from exploring novel environments or seeking drugs of abuse. Whether these behaviors are mediated by GR in the hippocampus or other regions deserves further investigation. Perhaps HRs find glucocorticoids more rewarding than LRs, and this leads them to seek out rewarding stimuli [19]? This represents one example of the complex role of molecular players in mediating individual differences in various phases of the stress response-- initial reactivity, duration of the response, and its impact in shaping behavior.

Equally important is the long-term role of stress in shaping emotionality, and the interaction of this modulation with temperamental tendencies. Chronic stress, or chronically elevated levels of glucocorticoids, cause damage to the hippocampus [20]. Yet, chronic mild stress during adolescence in bLRs can promote resilience to depression-like behavior in adulthood [21]. This is in contrast to the high resiliency of bHRs to early life stress and perinatal stress [22,23]. Thus, sensitivity to stress can change over the lifespan and even subtle stressors can switch a phenotype from being prone to depression, to resilient [3]. In mice, genetic deletion of metabotropic glutamate receptor, mGluR2, leads to increased stress susceptibility [24]. This provides an avenue whereby glucocorticoids acting through the mineralocorticoid receptor in the hippocampus can decrease resilience to stress by downregulating mGluR2. This is achieved by epigenetic modifications of histones which regulate the expression of mGluR2. It is possible that early life stress epigenetically modifies the MR promoter, but this is still under investigation.

Growth factors also play a role in individual differences in stress responsiveness. Duclot & Kabbaj [25] have implicated hippocampal brain-derived neurotrophic factor (BDNF) expression in differential responsiveness to social stress in outbred animals. In our own work

with selectively bred animals, administration of fibroblast growth factor 2 (FGF2), which we have previously shown to decrease anxiety-like behavior only in LRs [26], increased social interaction following social defeat in both HRs and LRs (see Figure 1). This suggests that HRs can also benefit from early life FGF2 under conditions of severe stress. Thus, BDNF and FGF2 may each impart resilience to stress, but FGF2 can impart resilience to both bHRs and bLRs.

Relationship between stress responsiveness in rodents and anxiety disorders

As is the case with the response to stressors, individual differences in temperament can shape the neurobiological response to fear and fear memories. Therefore, personality traits such as anxiety can modulate the response to trauma [27]. This is likely because high anxiety can impair discriminatory learning and enable fear overgeneralization [28]. These individual differences are also gender-dependent. Females exhibit greater renewal of fear and PTSD symptoms [27]. Additionally, relative to males, females show greater variations in stress genes that predict brain activation in response to fearful faces [29].

Neuroimaging studies in humans suggest that individual differences in fear conditioning involve the amygdala and its connections with the prefrontal cortex [30]. There is evidence that hyperactivity in the amygdala is associated with the extinction process, particularly in the basolateral and central amygdala [31]. The basolateral amygdala (BLA) can integrate sensory and context information and lead to the expression of fear via central amygdala projections to the periaqueductal gray. Given the bi-directional connections of the basolateral amygdala to the prefrontal cortex and hippocampus, these regions can drive the expression of fear and fear extinction, respectively [32]. The amygdala and prefrontal cortex are also involved in the stress response via their connections with the hippocampus. Moreover, many of the stress-related alterations in the amygdala and prefrontal cortex recapitulate findings in extinction-impaired rodents. Thus, stress sensed by GR in the hippocampus has the ability to decrease fear

extinction. Understanding the extinction process following fear conditioning in animal models is necessary to understand PTSD and potential therapeutic interventions.

Differential responsiveness to fear learning and extinction, and its linkage to differences in trait anxiety, can readily be modeled in animals. For example, outbred HR rats extinguish their conditioned fear response faster than LR rats [33]. However, our unpublished studies (Prater *et al.*, unpublished) suggest more dramatic differences between the selectively bred rats that differ in anxiety, with bLRs having reduced extinction learning and retention when compared to outbred animals. In various animal models of anxiety, differences in neurotransmitter systems have been identified following fear conditioning. These studies focused on the serotonin 1a receptor (5HT1A), the GABA-A receptor and the dopamine D1 receptor (DRD1) [34-36]. For example, animals with high anxiety had increased D1 receptor responsivity following fear conditioning in the prefrontal cortex [34]. Differences in GABA-A receptors have also been found between high-anxiety and low-anxiety rats in the prefrontal cortex [37]. Here, high-anxiety rats had reduced GABA-A receptor density. This is consistent with compensation in the GABA system in response to stressors. The HPA axis also plays a significant role in determining susceptibility to trauma. In a rodent model of individual differences in trauma exposure using a rodent-model of PTSD, a recent comprehensive gene expression study found that GR signaling was the most significant pathway identified using upstream transcription factors to predict behavior postexposure, across the hippocampus and amygdala and between sexes [38]. Moreover, corticosterone treatment following the predator-stress-scent paradigm prevented the characteristic behavioral responses.

The role of growth factors in the amygdala is opposite to that seen in the hippocampus. In the hippocampus, growth factors tend to be protective against fear learning, and rats with high context- or cue-induced conditioned fear responses exhibit lower FGF2 levels [39]. Moreover, the difference in FGF2 levels is sustained long after fear conditioning, suggesting differences in hippocampal plasticity and/or function [40]. By contrast, higher levels of growth

factors in the basolateral amygdala are associated with reactivity to fear conditioning, as stress susceptible animals had increased BDNF levels [41]. Thus, individual differences in fear processing are not simply due to a common dysregulation in underlying plasticity, as BDNF is upregulated in the amygdala and FGF2 is downregulated in the hippocampus in rats with increased fear conditioning. BDNF is located on neurons, whereas FGF2 is located primarily on astrocytes in the hippocampus. This suggests that the neurotrophic tone in the hippocampus, and hence fear processing, may be driven by neuron-glia interactions. It is possible that growth factor signaling can control both fear learning and fear extinction through different cell types, and this balance can be shifted following stress. Moreover, the neurotrophic tone in the hippocampus may influence the activity of the amygdala through direct connections.

Relationship between stress responsiveness in rodents and risk for addiction

A strong literature has linked traits such as impulsivity and sensation-seeking to drug taking behavior in the clinical population. Studies have related behavioral differences in impulsivity and anxiety to differences in serotonin receptor genotype (5HT_{2C}) and reduced volume or connectivity in the mesolimbic system [42-46]. As with many psychiatric disorders, individual differences in addiction liability are evident in the brain prior to the first experience with a drug of abuse. This predisposition to drug self-administration can be modeled in animals.

The bHRs, which are selected based on their propensity for "sensation-seeking" acquire cocaine self-administration more rapidly than bLRs [47], and the same is true in outbred HR/LR rats [48]. However, when exposed to psychosocial stress, drug-taking behavior is enhanced in the more anxious LR rats [48]. Thus, this model of temperament instantiates two different paths to drug seeking--- sensation-seeking and stress-induced drug-taking behavior. However, it should be mentioned that our data demonstrating that bHRs are more prone to addiction-like behavior relative to bLRs is discordant with a series of papers suggesting that the locomotor response to novelty trait is not relevant to the transition to addiction or compulsive drug use, but associated

only with the initial propensity to take drugs [49,50]. However, it should also be noted that the bHRs are different from outbred rats characterized based on locomotor response to novelty in that the bHRs have been co-selected for a number of traits that are not apparent in outbred HRs. For example, in bHRs, locomotor response to novelty is correlated with measures of impulsive action and the propensity to sign-track, two traits that are not related to “sensation-seeking” in outbred rats. Thus, we have created a line of rats that exhibit a constellation of traits relevant to addiction, rather than a single trait, and this constellation of traits is what likely renders them more susceptible to compulsive drug use.

Beyond the initial likelihood to take drugs, temperamental differences play a role in the conversion to addiction. We have recently shown that following a prolonged cocaine self-administration paradigm, bHRs, but not bLRs, develop addiction-like behaviors, including drug-seeking behavior when the drug is no longer available and a greater propensity for relapse following abstinence [5]. Thus, the *a priori* knowledge that bHRs are more susceptible to ‘addiction’ than bLRs, allows us to investigate any pre-existing neurobiological differences that are associated with addiction liability. Indeed, we have shown that under “baseline” conditions bHRs have lower levels of dopamine receptor D2 mRNA in the core of the nucleus accumbens, but more D2^{high} receptors, the functionally active state of the receptor. In addition, there are differences in the epigenetic regulation of the D2 receptor under basal conditions, with a greater association of the repressive mark on histones (H3K9me3) at D2 in bHRs relative to bLRs [5]. Interestingly, these phenotypic differences in D2 expression and its epigenetic regulation are no longer apparent following prolonged cocaine self-administration and abstinence. However, the epigenetic regulation of D2 does seem to play a role in susceptibility to reinstatement, or relapse [5].

CRH may be another mediator of individual difference in the response to drugs of abuse. In LR mice, CRH overexpression in the brain was associated with stronger rewarding

effects of cocaine [51]. Thus, CRH and D2 receptors may be key players in mediating individual differences in the response to drugs of abuse.

Due to the role of context in drug-seeking behaviors and relapse, the hippocampus has gained increasing attention in addiction research [52,53]. Drug-taking and drug sensitization can be modulated by FGF2 with corresponding changes in hippocampal gene expression [54]. In response to cocaine abstinence, FGF2 expression decreased in the dentate gyrus of bLRs and increased in the nucleus accumbens of bHRs, suggesting that different circuits may be mediating the individual differences [55]. In agreement, neonatal FGF2 in bLRs increased cocaine sensitization and FGF2 expression in the nucleus accumbens compared to bHRs [56]. Given the diverse and widespread effects of FGF2, this growth factor may mediate susceptibility to substance abuse via different pathways in different individuals.

FGF2 is also a target and a trigger of epigenetic mechanisms [57]. Relative to bLRs, bHRs have lower FGF2 expression and increased association of the repressive mark on histones (H3K9me3) at FGF2 in the nucleus accumbens core [5]. Remarkably, and unlike D2, these differences persist long after cocaine self-administration and prolonged abstinence. Thus, low levels of FGF2 may serve as a protective factor against addiction liability. Future studies should assess how epigenetic regulation of these molecules influences the development of temperament.

Conclusions

Temperament is a highly stable trait that influences emotional responsiveness including the type of psychopathology that an individual might express. Individual differences in temperament regulate the response to stressors, fear conditioning and drugs of abuse in animals. GR, the serotonergic and the dopaminergic systems are involved in the response to stressors, fear conditioning and drugs of abuse; however, different players are implicated in different regions. The hippocampus is involved in the development of temperament and is

known to mediate the response to context, which can drive anxiety, depression, PTSD and addiction. As illustrated in Figure 2, stress can impact different brain regions. These brain regions are interconnected and exhibit differences in gene expression in HR-like and LR-like brains, paving the way for novel therapeutics.

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Figure Legends

Figure 1. Early life FGF2 is protective of social stress in both bHRs and bLRs. FGF2 was administered (20ng/g, s.c. in 50 μ l 0.1M PBS with 0.1% BSA) the day after birth. The animals were tested for social interaction in adulthood following 10 days of repeated social defeat stress. FGF2 increased time in the interaction zone in both bHRs and bLRs compared to vehicle-injected controls. $*p < 0.05$. This study followed the National Institutes of Health guide for the care and use of laboratory animals.

Figure 2. Individual differences in the response to stressors, fear conditioning and drugs of abuse. The effects of stress on the brains of animals that differ in their response to stressors are illustrated by lightning bolts. The regions are interconnected and can influence the response to fearful stimuli and drugs of abuse. Gene expression differences in the brains of HR-like and LR-like animals are listed below each region.

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bHR/bLR animal model. Perhaps most importantly, the epigenetic regulation of DRD2 may underlie relapse, whereas low FGF2 expression and its epigenetic regulation may prevent addiction-related behavior. Interestingly, the epigenetic findings with FGF2 persisted following prolonged cocaine self-administration and abstinence; whereas the DRD2 findings were no longer evident at this time point.

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