Eur Arch Psychiatry Clin Neurosci (2008) 258:97–106 DOI 10.1007/s00406-007-0788-1

SPECIAL ISSUE

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Elucidating risk mechanisms of gene–environment interactions on pediatric anxiety: integrating findings from neuroscience

Published online: 15 March 2008

Abstract Recent findings of gene–environment interaction on child and adolescent anxiety generate interest in mechanisms through which genetic risks are expressed. Current findings from neuroscience suggest avenues for exploring putative mechanisms. Specifically recent documentations of abnormality in brain function among anxious adolescents may reflect the end-result of gene expression. In turn these inherited predispositions may increase the likelihood of psychopathology in the presence of stress. The aim of the current article is to consider putative mechanisms reflecting genetic sensitivity to the environment (G × E). Thus we review data implicating biased processing of threat information and anomalies in brain circuitry in the expression of pediatric anxiety. These data suggest that links across development among genes, brain, psychological processes, and behavior are far from established. Accordingly, the article proposes strategies for examining these links. Exploring these relationships during development is crucial, given that these early life processes may potentially shape longer-term patterns of emotional behavior, and therefore life-long trajectories of anxiety.

Key words child and adolescent anxiety · gene–environment interaction · risk mechanisms · neural circuitry · psychological processes

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Introduction

Positive findings of gene–environment interaction (G × E) on child and adolescent anxiety have provoked interest in the mechanisms through which these effects arise. Both quantitative and molecular genetic studies have shown that the relationship between environmental risks for anxious behaviors is dependent on genetic characteristics [13, 22, 41]. At the conceptual level, such findings suggest that the expression of genetic risks for pediatric anxiety manifest through greater sensitivity to environmental stressors. This raises questions on the nature of intermediate processes through which these genetic risks emerge. Findings from neuroscience may provide clues on the nature of these intermediate processes by implicating specific neural systems in specific information-processing functions associated with anxiety. As the functioning of these systems impact emotional responses to the environment (in particular towards threat), they may reflect processes underlying G × E. Application of this "systems-neuroscience" approach within animal studies also demonstrates that risk processes operating early in development shape longer-term patterns of emotional behavior [16]. Thus in considering adult outcomes of anxiety, it becomes critical to examine links between genes, brain function and information-processing at earlier developmental stages, such as childhood and adolescence.

The current article reviews putative risk processes reflecting genetic sensitivity to the environment. We begin with a general framework on which core themes can be organized (Fig. 1), making three assumptions. First, consistent with findings of G × E, stress exposure increases risk for anxious behaviors more strongly among individuals with a high relative to low genetic predisposition for anxiety [41]. Second, the effects of genetic predisposition on behavior emerge through intermediate effects on functioning within a

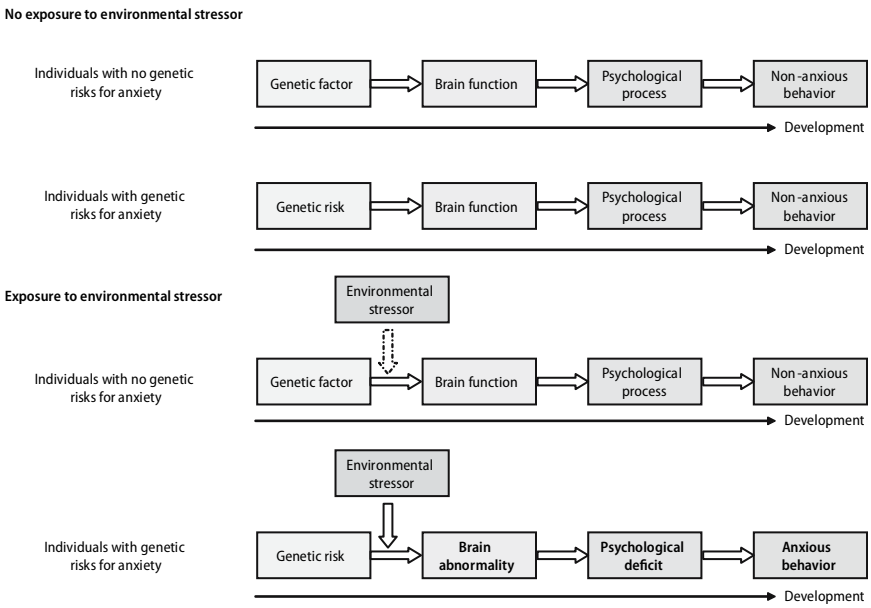

Fig. 1 A framework outlining how exposure to environmental stress can "trigger" genetic risks that become manifest through perturbed brain abnormalities and associated psychological processes to impact on anxiety outcomes

specific neural circuit [18], which produces perturbations in information-processing functions. Third, this cascade of events emerges gradually across development, possessing the capacity to shape later-life processes, laying the foundation for later disordered outcomes of anxiety among vulnerable individuals. In summary, according to this framework clinical anxiety emerges from perturbations in brain function and associated information-processing functions occurring dynamically across development. These vulnerabilities may have a genetic basis, which are elicited in the presence of an environmental "trigger". Without this trigger, the effect of genetic factors on associated brain/information-processing dysfunction is reduced and in turn, the probability of developing the anxious phenotype. Of note, while this model speculates on how gene–environment interactions are expressed, it makes no assumptions on the presence or nature of alternate pathways by which anxiety manifests. For instance, anxiety may also result through environmental trauma or genetic risks alone.

While the present framework contains only the "bare bones" of a broader conceptual approach we supplement it in two ways, with findings on the relationships between anxiety and biased processing of threat, and anxiety and anomalies in brain function (see Fig. 2). First, we review the literature on biased processing of threats among anxious juveniles. Longstanding theories implicate clinical anxiety as a pathological response to a threatening event. In this context, threats represent objects or scenarios that organisms will extend efforts to avoid, since they are perceived as dangerous or capable of harming the organism. Such biases in the processing of threat may govern responses to the environment in general and as such represent promising candidates involved in G × E. The second portion of the review considers research approaches for examining the link between these various threat-processing anomalies and associated variations in brain function, focusing on two regions: the amygdala and prefrontal cortex (PFC). As these two regions also operate in a broader circuitry that is hypothesized to regulate responses to emotions, they too comprise promising candidate markers mediating genetic risk. The final section outlines a research agenda that attempts integration between a focus on the neural correlates of information processing biases in anxiety and findings of G × E.

Threats and information-processing biases

Considerable work in adults has found a repertoire of biases associated with the processing of threat-related information among anxious individuals. These biases are thought to play a critical role in both the development and maintenance of anxiety by moderating responses towards threat. As such, these biases may plausibly mark risks associated with genetic sensitivity to the environment (G × E). Work on anxiety-related processes has benefited from an integration of research in neuroscience that emphasizes a dissection of the relevant information-processing functions engaged as organisms confront threat. This approach provides a context for not only delineating distinct forms of bias as they relate to clinical conditions but also for relating each of these distinct biases to specific neural processes, as discussed in the next section. In the context of confronting a threat, all organisms, including humans, engage a series of information processing functions that interact with each other as they unfold. We briefly review findings for four "stages" of information-processing depicted in Fig. 2

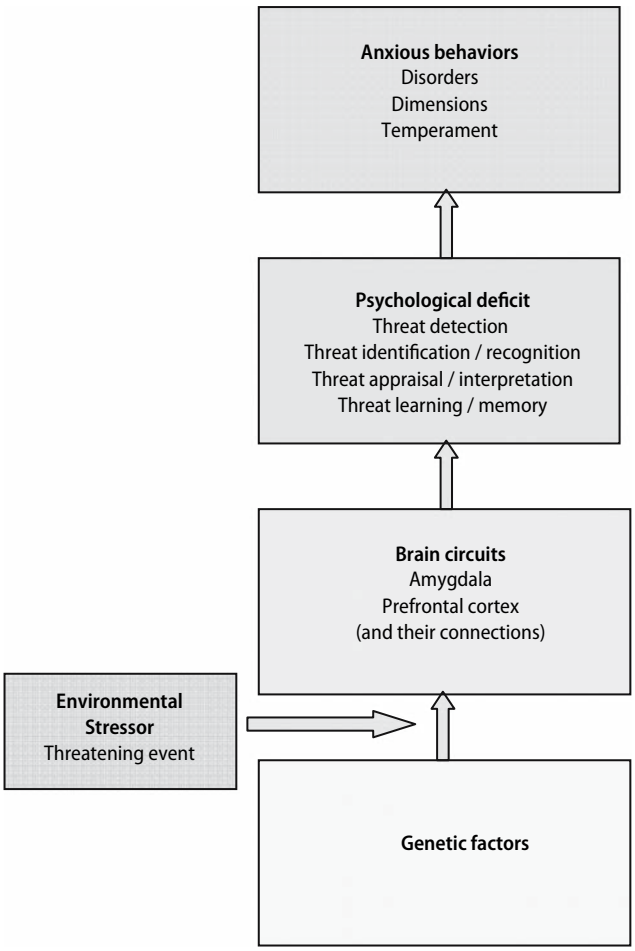

Fig. 2 A framework for representing specific biases in information-processing, brain circuitry and genetic variants that may account for greater reactivity towards threat among anxious individuals

(detection, recognition, interpretation/appraisal, learning/memory), focusing on associations with child and adolescent anxiety.

Biases in threat detection

A detection bias represents the first disorder-relevant process to manifest, appearing immediately following the presentation of a threat. When threats are extreme, they show an enhanced ability to co-opt attention resources in virtually all mammals, shortly after the threat is encountered. This effect manifests within tens of milliseconds. In comparison, attention biases occur when relatively mild threats show a greater capacity to influence attention in anxious patients or individuals at high risk for anxiety than in healthy subjects or individuals at low risk for anxiety.

Such attention biases can be replicated among humans in the laboratory, by assessing the presentation of threats on attention allocation within experimental paradigms. Two paradigms are typically used. In the emotional Stroop task, subjects are asked to name the colors of threatening relative to non-threatening words. Threat detection manifests through competition between attention resources directed to a task demand (color naming) and attention allocated to the threat. In other words, the degree to which reaction times for color naming are reduced as a function of the threat value of the word indexes vigilance towards threat. In this context, slower latencies in naming the color of threatening words among anxious individuals indicate greater interference associated with the threat content of the word stimulus among this group. In the dot-probe task, threat detection influences attention through the process of spatial orienting. Subjects are presented with a dot probe that either appears in the same spatial location as a previously presented threat cue (congruent condition) or in a different spatial location (incongruent condition). The underlying assumption is that if attentional resources were captured by the threat cue, this would result in quicker latencies to probes belonging to the congruent relative to incongruent conditions, signifying attention bias for threat. While the emotional Stroop task typically relies on words to convey threats, the dot-probe task uses either words or pictures providing greater flexibility in the type of threats that can be probed. Such manipulations allow inferences on whether attentional biases extend across verbal and non-verbal threat cues, including angry or fearful facial expressions. Studies applying these paradigms in child and adolescent samples have indeed found group differences between anxious and healthy subjects in attention biases. Although most studies concur with adult findings, with greater vigilance *for* threat among adolescents with clinical or high levels of anxiety [2, 9], occasional discrepancies of attention *away* from threat (i.e., avoidance) have also been reported [35, 44]. Regardless, robust support for perturbed attention deployment among anxious youth has emerged. The replicable nature of these findings facilitate efforts to relate this clinical variation first to variation in brain function, and then within genetically mediated pathways.

Biases in threat recognition

Following the detection of a threat, organisms engage more elaborative processes that categorize the degree of threat and other meaningful aspects of the stimulus. These processes can be placed later in the series of processes engaged by threats, based on the reaction times typically required to recognize relative to reaction times to detect a stimulus. Consistent with the temporal distinctions between detecting and recognizing threats, findings in clinical anxiety also appear distinct for these two processes.

Threat recognition processes can be engaged by asking subjects to label or identify stimuli encountered in the laboratory. Two categories of studies examine recognition of threat stimuli: those using

visual search paradigms to locate threatening faces among a set of distracter items (e.g., [17] and those explicitly instructing subjects to label facial emotions depicted in photographs (e.g., [8, 31]. The first set of studies find some evidence that high levels of anxiety predict faster search times to detect threatening but not neutral faces, but even here the findings are not well replicated [17]. For the second set of studies, the weight of the evidence suggests weak relationships with anxiety. Specifically, most studies find no differences between anxious and healthy subjects in their accuracy for labeling of angry or fearful faces [22, 31]. However, some small studies do find deficiencies in face-emotion recognition, both generally to all facial expressions [8, 29, 49], and to other emotions, such as sadness, disgust and happiness [42] among anxious subjects. Yet even for these positive findings, none of the studies corroborate adult findings of a superior recognition of negative facial emotions, including both angry [19] and fearful faces [45] among anxious subjects. While the reasons for these inconsistencies across studies remain unclear, these data suggest that attempts to extend G × E research through neuroscience approaches may benefit from focusing directly on threat detection than threat recognition paradigms.

Biases in threat interpretations and appraisals

Studies of threat recognition typically focus on the categorization of a stimulus by focusing explicitly on external features of the stimulus. While studies assessing this form of classification find minimal associations with anxiety, other forms of categorization have been linked more successfully to anxiety. Specifically, anxiety has been associated with biased categorizations when tasks require that subjects make a categorization by focusing explicitly on the subjects own responses to the stimuli. Thus, when subject's are asked to rate their internal reactions or to label stimuli as "safe" or "threatening", consistent associations with anxiety emerge. This form of categorization has been labeled as an "interpretive" or "appraisal", and biases in these stages of information-processing reflect reduced thresholds for making a threat categorization of a reaction to a situation.

Studies examining appraisal of ambiguous information among anxious youth have generated relatively consistent findings. Such studies have relied on both ratings of imagined scenarios or ratings of directly experienced threats, produced through experimental manipulations. In the former, subjects are presented with ambiguous words or stories that can give rise to both threatening and non-threatening interpretations. Subjects are instructed to produce a response that requires some appraisal of the content of the stimulus, such as constructing a sentence containing the word or to provide an explanation for the story. These responses are then coded according to their compatibility with the threatening or the non-threatening interpretation. Often, such studies will choose words or stories that involve social or physical elements in order to better discriminate between specific types of threat. Results indicate that anxious children and adolescents do produce significantly more sentences that coincide with threatening interpretations [46] and also select explanations for stories that subscribe to a threatening appraisal [4].

While these tests allow the assessment of interpretations to more global constructs of threat, broadly distinguishing between physical and social threats, the other approach has been to assess appraisals to more specific and directly experienced threats, by employing experimentally manipulated scenarios. Examples of these have included the monitoring of one's own heartbeat to assess sensitivity towards anxiety-related sensations [12], assessing changes in respiration during exposure to CO_2-enriched air [38], rating subjective levels of fear induced by viewing facial photographs [30], and rating negative affect in response to simulated peer rejection [23, 24]. Findings from these laboratory-induced scenarios are equally persuasive in suggesting that anxious children and adolescents are more likely to engage threat appraisals than their healthy peers. As with the findings on biases in the detection of threat, findings of interpretative and appraisal biases among anxious subjects are also worthy of further follow-up, both in terms identifying neural mechanisms and their participation in genetic pathways of risk.

Biases in threat learning and memory

Following the initial detection and classification of a threat, organisms will learn to alter their behavior based on cues associated with a threat. These processes emerge more slowly than detection and classification as they reflect processes that only emerge following multiple experiences with threats. Such learning-related processes form the basis for considerable work in neuroscience, particularly in animal models, stimulating efforts at translational work in humans, particularly in clinical populations. However, relative to work on threat detection or appraisal biases, associations in this area appear less consistent.

Perhaps the best forum for studying differences in threat-related learning between anxious and healthy subjects is the use of fear conditioning paradigms, developed initially from animal models. Due to difficulties implementing these paradigms in younger samples, this "stage" of information-processing has only recently been explored in this age group. Fear conditioning paradigms are based on the principle that repeated pairings of a neutral stimulus (e.g., tone) with an aversive unconditioned stimulus (e.g., shock) enables the neutral stimulus to become a conditioned stimulus (CS) invoking fear independently of the unconditioned stimulus (UCS). This

process, which occurs via associative learning mechanisms, is known as fear acquisition. Fear extinction, by comparison, is the process by which fear associated with the CS declines following repeated presentation of the CS in the absence of the UCS. Fear associated with the CS during acquisition may index emotion-related learning, whereas fear to the CS following extinction may probe emotion-related memory, which requires contextualization based on temporal cues.

Two designs have been used to instantiate fear acquisition and follow its extinction. Simple conditioning paradigms involve pairing one neutral cue with an anxiety-provoking UCS. Emotional learning and memory are then inferred from fear associated with this neutral cue following acquisition and extinction, respectively. Discrimination conditioning paradigms in contrast present two neutral stimuli, one of which is paired (CS+) with the UCS while the other remains unpaired (CS−). In this design, emotional responses to the CS+ following either acquisition or extinction are adjusted relative to fear of the CS−. A recent meta-analysis of adult data documents abnormalities in fear conditioning, but these are relatively subtle [28]. Compared to healthy controls, anxious patients show greater fear to the CS when this stimulus is presented alone (i.e., in simple conditioning paradigms). However, patients and controls manifest comparable levels of differential fear conditioning, that is, both groups show greater fear of the CS+ relative to the CS−. These results are suggestive that patients in general exhibit greater fear to both conditioned stimuli (CS+ and CS−) than controls, reflecting a tendency to generalize fear across stimuli. The same pattern emerges for extinction data: patient-control differences in the resistance to extinction are greater following simple conditioning than in discrimination conditioning paradigms.

Only a handful of three studies have examined fear acquisition and extinction in pediatric anxiety samples, yielding mixed results. While one study replicated adult findings of greater overall levels of fear among anxious adolescents following acquisition [22], the other two failed to document these group differences [27, 39]. For extinction, only one study reported greater fear responses to the CS+ relative to the CS− among anxious relative to healthy subjects [27]. While some of these discrepancies can be attributed to methodological variation, more work is needed to establish the presence of anxiety-linked biases in these indices of threat-related learning and memory.

The amygdala and PFC

Research on information processing provides one avenue for examining the psychological substrate of G × E interactions. However, individual differences in information processing are likely to reflect individual differences in brain function, and measures of brain function provide an index of between-subject variability that lies closer to the causal chain through which genes and the environment influence behavior. Thus, research that combines measures of information processing and direct measures of brain function provides a sound means for exploring mechanisms through which G × E shape behavior. Moreover, available research in animal models provides clues concerning the manner in which genetic influences shape fear-related information processing and behaviors, through effects on specific neural circuitry encompassing the amygdala and PFC. Such genetic mechanisms may well account for greater sensitivity to environmental stressors, thus underlying G × E. Such work in basic neuroscience provides a backdrop for a focused approach in children.

Studies in affective neuroscience have begun to map brain regions engaged when children confront mildly threatening stimuli [34]. Parallel to work in animal models, two anatomical regions (and their connections) are implicated: the amygdala, located bilaterally in the temporal lobe, and the PFC, a neocortical region of the frontal lobe, which can be divided broadly into dorsolateral, medial, and ventral regions, and in which further sub-divisions are possible [14, 33]. We briefly review functions associated with each of these regions, as well as any studies that have charted group differences between anxious and healthy youth in the structure or activity of these regions, in particular during the processing of threat.

Anxiety-linked amygdala function

The amygdala is thought to play a pivotal role in the formation of stimulus-reinforcement learning, whereby associations are formed between neutral events and punishments or rewards [26]. Within the context of such learning, the amygdala has been implicated specifically in the modulation of attention deployment, which facilitates learning of these associations.

Studies examining anxiety-linked perturbations in the amygdala have explored structural as well as functional differences. Two studies assessing the anatomical integrity of this region have reported conflicting results: while both found structural group differences, one reported enlarged total volume of the right amygdala [7], whereas the other noted reduced gray matter volume in the left amygdala [32]. Although these results are undoubtedly intriguing, such structural data can provide only limited insights on the mechanisms that might disrupt function, perturb information-processing and precipitate anxiety conditions.

To this end, functional Magnetic Resonance Imaging (fMRI) studies comparing group differences in the activity of a particular region during the performance of a certain task may be of greater utility. A

growing body of literature implicates abnormal amygdala function among anxious children and adolescents. Mirroring adult findings, abnormally high amygdala activity in response to emotional facial expressions, in particular fear, has been reported in groups of children and adolescents manifesting behaviors on the anxiety spectrum. These include anxiety disorders (McClure et al. 2006) [47], high levels of anxiety symptoms [21], and behavioral inhibition, which often precedes anxiety disorders [36]. Detailed examination of these abnormalities reveals unique modulation of amygdala activity by attentional state. For example, anxiety-linked perturbations in amygdala activation to fearful faces emerged only under conditions where attention was directed towards an internal evaluation of fear ("how afraid are you?") and not under conditions where attention was directed towards external features of the face stimuli ("how wide is the nose" or "how hostile is this face") (McClure et al. 2006) [36]. Significant amygdala *de*activation was, however, noted among anxious and inhibited pediatric subjects under passive viewing conditions, where attention was unconstrained. That is, under conditions when there were no explicit instructions to attend to a threat cue, anxious and at-risk adolescents were more likely to show reduced activation in the amygdala relative to a neutral baseline condition. While the majority of studies have examined perturbed amygdala responses selectively towards fearful faces [21, 47] (McClure et al. 2006), these responses have on occasion generalized to happy faces too [36].

In summary, these studies converge on the finding anomalies associated with amygdala function may characterize anxious children and adolescents. However, beyond viewing negative emotional faces, no published studies have yet assessed amygdala function during more specific information-processing functions engaged when encountering explicit threats. In such work, various threats should be employed to map the neural processes governing the detection, recognition, interpretation, learning and memory of distinct threat stimuli. Specific anxiety disorders may arise through distinct G × E effects on some subset of processes for isolated classes of threat. In order to reach this goal, future studies will need to develop more specialized paradigms that tap into these specific processes for diverse threat stimuli. For instance, imaging studies of brain function have begun to assess neural responses to experimentally manipulated stressors that tap into anxiety-relevant cognitions, such as appraisals during social rejection or learning associations between fear and neutral cues.

Anxiety-linked PFC function

The PFC is thought to influence brain responses to emotional stimuli by regulating primary affective responses, such as those instantiated in the amygdala. However, two considerations complicate the elucidation of more specific brain functions within this general region. First, the PFC is anatomically and functionally heterogeneous. As alluded to previously, three basic PFC components can be delineated: dorsolateral, medial, and ventral regions. The medial region also can be further divided to include a dorsal and ventral area, as well as a posterior expanse that encompasses the cingulate gyrus, and an anterior expanse that encompasses Brodmann's areas nine and ten. Within the ventral PFC, orbital-medial and ventrolateral components can also be distinguished, though these regions overlap with regions considered components of the inferior medial regions. Another anatomical distinction that has received some attention pertaining to functional differences in approach and avoidance behaviors is that between right and left cerebral hemispheres [6]. While approach-related behaviors and the expression of positive emotions has been attributed to activity in left frontal regions, avoidance and withdrawal behaviors and the expression of negative emotions have been localized to activity in the right frontal regions. What is thought to be key to psychopathology, however, is the asymmetry between these regions, that is, the balance in activity between right and left-brain areas. A second complication in studying PFC function is that each of these regions operates within a larger circuit involving reciprocal connections with each other. Moreover, through ventral regions and their dorsal interconnections, the PFC shows strong associations with the amygdala. Importantly, it is through interactions among these regions that the brain implements processes associated with emotional responses, making it difficult to localize specific brain function.

Despite the inherent difficulty of this work, several promising findings have emerged. First, orbital-frontal regions have been linked to the updating of associations formed between neutral (sensory) stimuli and unconditioned (aversive) stimuli during fear acquisition, with changes in punishment and reward contingencies in the environment [40]. In comparison, ventral regions may serve to extinguish these associations following changes in punishment/reward contingencies. Second, there is some work suggesting that the medial PFC plays a role in representing and monitoring subjectively experienced emotional states [1], a key aspect of appraisal. Third, preliminary findings indicate involvement of the ventrolateral PFC in selective attention processes during the presentation of threat [35]. Finally, the anterior cingulate cortex (ACC) has also been implicated in emotion regulation during challenging situations by responding to conflict and facilitating subsequent response selection (see Lewis and Steiben 25 for details).

Few published studies explicitly link structure and function of the PFC to pediatric anxiety disorders. Of the available literature, there is some suggestion of greater ventral and medial PFC in anxious adolescents

when appraising internal fear levels [30]. Such findings are generally agreement with adult data, which also support links between anxiety and hyperactivation in ventro-medial and ventro-lateral PFC regions. With regard to asymmetry in frontal engagement, preliminary findings indicate that anxious children show a distinct pattern of activity compared to their healthy counterparts [3]. However, these anomalies are not entirely consistent, as they appear to vary subtly with age and gender: prepubescent anxious girls exhibited greater right to left activity, corresponding to a behavioral pattern of avoidant over approach tendencies. This profile was not observed among healthy female controls or anxious boys, but emerged unexpectedly in healthy boys. Moreover, other work implicates posterior asymmetry in pediatric or adolescent anxiety more consistently than frontal asymmetry [20]. Such findings await further replication before strong conclusions can be drawn. Data also implicate perturbed PFC-amygdala connectivity in pediatric anxiety, findings resonating with data among adults [30]. Specifically, perturbations in the balance of activity between the amygdala and regions of the PFC may characterize subjects at high risk for anxiety during the engagement of specific emotional stimuli [5]. Such hypotheses await further verification both in adults and in children.

Future directions

Endophenotypes

While interesting preliminary findings are accumulating, considerable work is needed before firm conclusions can be drawn on the nature of psychological and neural deficits among anxious children and adolescents. Nevertheless, as work continues, the stage has been set for relating measures of information processing and brain function to markers of genetic risk, in particular those that are implicated in genetically-mediated sensitivity to environmental stressors (G × E). From this perspective, measures of information processing and brain function can be considered "risk markers".

Several different sets of criteria have been proposed to help locate these putative risk markers of genetic influence, termed "endophenotypes" [15, 43, 48]. Although variations between these suggestions exist, several key elements are apparent. First, the marker should co-occur with the disorder and its symptoms in the general population as well as in clinical groups, as has begun to occur for measures reviewed in this summary. It is particularly important to verify that such risk markers are measured with tools possessing good reliability and validity. Although disease-specificity and universality (i.e., the risk marker applies to all such conditions only) are ideal, these are not requirements given a common gene variant may affect multiple phenotypes (pleiotropic) and that there may be etiologically heterogeneous sub-groups within the disorder. The second criterion is that the marker should be relatively state-independent rather than an epiphenomenon of the illness. Ideally it should also possess temporal stability and developmentally precede the condition. The third and fourth key criteria are that endophenotypes should show evidence of heritability and familial (genetic) overlap with the disorder of interest, respectively.

Fulfillment of these criteria can be adopted as a research strategy to stimulate future studies. The most progress has been made in relation to criteria one: establishing associations between a putative marker and a condition, both as a disorder and as symptoms. Consistent findings to arise from our brief review of possible candidates were a greater detection for threat cues and an increased tendency to draw threatening interpretations from ambiguous scenarios, in addition to perturbed amygdala function during the presentation of negative emotions (fear) among anxious children and adolescents. While one priority is clearly to establish relationships between these psychological deficits (biases in detection and interpretation of threat) and perturbed neural (amygdala) function, parallel steps can be taken to examine criteria two, that is, to verify whether such anomalies precede or arise as a result of the condition. This objective is probably most effective when carried out using longitudinal studies, preferably by employing high-risk designs. In these, the development of psychopathology is compared between non-symptomatic individuals who manifest a particular risk marker (e.g., increased attention for threat) and non-symptomatic individuals who do not manifest this vulnerability. Given the interest in delineating intermediate pathways that are involved in sensitivity to the environment, it would be of additional interest to further divide these groups of non-symptomatic individuals into those who have experienced recent threatening events relative to those without this exposure. This additional manipulation in the study design can allow tests of interaction between the pre-existing risk marker and the occurrence of the environmental stressor on symptoms.

The final two key criteria involve testing for the heritability and genetic associations between putative risk markers and the phenotype. There are several different designs available for examining these relationships. Perhaps the simplest are studies comparing risk markers among offspring of anxious parents relative to the offspring of healthy parents (e.g., [38]. While group differences in the degree to which a risk factor is present are suggestive of links with genetic risk, the results do not negate the possibility that at-risk offspring develop these risk markers through social mechanisms, such as modeling or learning from their parents. Other family based designs such

as twin studies are better equipped at disentangling the role of genetics on a risk marker from the role of the shared family environment. To this end, large studies of anxious twins have already begun to collect measures of information-processing biases in order to quantify genetic effects (criteria 3) as well as the degree to which shared genes account for relationships between the risk marker and symptoms (criteria 4) [11, 22]. A final approach, which is attracting much excitement is to examine associations between candidate gene polymorphisms and these intermediate phenotypes. Made possible largely by technological advances in the procurement and analysis of DNA, this approach has been exemplified in adults, by significant associations between serotonin transporter gene variants and amygdala function during the viewing of negative facial expressions [18]. Intriguingly, such predictive associations have also been demonstrated in relation to connectivity between the amygdala and regions of the PFC [37]. Such "imaging genomics" studies that examine gene–brain links using a paradigm that exploits findings from cognitive psychology await exploration in child and adolescent clinical samples. The incorporation of data on the presence of environmental threatening events will further enable inferences on the degree to which these links only manifest in response to stress.

Remaining issues; in summary, we have reviewed various aspects of psychological and neural functioning that may pertain to markers of genetic sensitivity to the environment (G × E). We have also elaborated on new research strategies that can be adopted for extending these speculations and thus testing the links illustrated in Fig. 2. However, there remain two additional issues that may serve to complicate these links requiring consideration.

The first issue concerns the degree of disorder-specificity in these links. Anxiety is not a unitary phenotype but instead manifests as various different subtypes including generalized anxiety, panic anxiety, social anxiety, separation anxiety, specific phobias and obsessive-compulsive disorder. Findings from behavioral genetic studies are suggestive that while genetic and environmental factors on anxiety symptom types strongly correlate, they do not necessarily reflect a common set of shared factors [10]. Furthermore, distinct patterns of gene–environment interaction have been found across symptom-types [22], such that only genetic risks for early separation and later panic anxiety varied significantly across life events. Given these findings of differences in etiology, it is not surprising to expect variations in underlying pathways of genetic risk. Yet with exception to data suggesting distinct threat-related biases in the appraisal and interpretation of threat across anxious subtypes, it is unclear how biases associated with the other stages of threat-processing and anomalies in amygdala-PFC function map onto nosological categories.

The second issue pertains to how relationships among genes, brain function, psychological processes and behavior may change across developmental stages. In particular, changes associated with development, such as time-dependent gene expression, maturation of certain brain regions and cognitive capacities means that links between variables are unlikely to be static but instead may vary dynamically with time. The main implication associated with this possibility is that it cannot be assumed that risk processes operating at one stage of development mirror those at later or earlier periods. Furthermore, it may be more important in this respect to consider "development" in terms of pubertal status rather than chronological age. Given that biological, cognitive and social changes are likely to correspond with stage of puberty, it would be interesting for future studies to examine the links between genes, neural function, information-processing biases and anxiety, across pubertal transitions rather than age.

Conclusions

Until recently the disciplines of behavioral genetics, affective neuroscience and cognitive psychology have pursued disparate but parallel trajectories in attempts to identify risk *markers* of anxiety. Yet as methodological advances have allowed increasingly sophisticated information to be gathered on the role of genes, brain circuitry and information-processing factors, the time could not be more optimal for combining these efforts to understanding their relationships that could ultimately provide insights into risk *mechanisms*. Furthermore, studying these relationships during development is of particular importance. The plasticity of neural circuitry and associated psychological functions means that any risk processes occurring in this age range may shape longer-lasting diatheses that lay the foundation for later stress-related illnesses. Given these implications, such initiatives may not only enhance theoretical models of anxiety, but also powerfully inform treatment protocols too.

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