Neural embedding of stress reactivity

Ryan Bogdan & Ahmad R Hariri

A report in this issue of *Nature Neuroscience* demonstrates that stress in infancy leading to altered cortisol levels in childhood culminates in vulnerability to dysregulated affect in adolescent girls by biasing the functional dynamics of core neural regions mediating the generation and regulation of emotional responsiveness.

One of the most intriguing and important questions across psychology, psychiatry and neuroscience is how experience 'gets under the skin' to shape individual differences in behavior and risk of mental illness. Early life adversity predicts nearly 45% of childhood-onset and 30% of adult-onset psychopathology[1]. Because the treatment of mental illness is expensive and often ineffective, a major goal of research is to prevent its development. Thus, it is important to understand the mechanisms through which early adversity forms a prelude to psychopathology so that therapeutic targets in etiologic chains and predictive biomarkers of risk may be identified.

Research across species has shown that early life stress alters the function of the hypothalamic-pituitary-adrenal (HPA) axis, the central regulator of stress responsiveness. Early life stress is also associated with alterations in the structure, function, and connectivity of a corticolimbic neural circuit, including the amygdala and ventromedial prefrontal cortex (vmPFC), which helps us recognize and react to challenges we encounter by orchestrating adaptive changes in both behavior and physiology. The effects of stress, particularly early in life, on this corticolimbic neural circuit are likely mediated by stress-related alterations in HPA axis function.

A wealth of research in humans and nonhuman models has demonstrated pleiotropic effects of HPA axis dysregulation on myriad mental and physical health outcomes[2]. Perhaps most relevant to understanding pathways to psychopathology, the hormonal end product of the HPA axis, cortisol, has been linked to variability in the structure and function of the corticolimbic circuit. Exposure to early life stress is associated with blunted diurnal cortisol variation (that is, relative to a normal profile, reduced cortisol on awakening but elevated levels later in the day and a less steep negative slope over the course of the day). Moreover, both early adversity and endogenous cortisol concentrations are positively correlated with the magnitude of threat-related amygdala reactivity and amygdala gray matter volume[3]. In contrast, emerging research in model organisms suggests that chronic adversity and cortisol release result in atrophy of the vmPFC[4]. Complementing these data is growing evidence that the vmPFC is critical in the integration and subsequent top-down regulation of amygdala reactivity and that this modulatory relationship predicts the severity of anxiety and depressive symptoms, as well as variability in diurnal cortisol[5–7].

In a study in this issue of *Nature Neuroscience*, Burghy *et al.*[8] now bridge these biological mechanisms by showing that early life stress increases adolescent symptoms of anxiety and depression and decreases amygdala-vmPFC functional connectivity through adversity-related elevations in childhood cortisol (**Fig. 1a**). Furthermore, these data emphasize the importance of developmental timing and gender differences in stress-related psychopathology by demonstrating that the changes in functional connectivity predict symptoms in adolescent girls only and are predicted by early, but not later, adversity.

In their study, 57 adolescents from the ongoing Wisconsin Study of Families and Work (http://www.wsfw.us/) were recruited into a functional magnetic resonance imaging study assessing the resting-state functional connectivity of the amygdala and vmPFC. Such resting-state measures, wherein subjects are not exposed to explicit triggers such as threatening facial expressions but rather allowed to rest quietly, have emerged as a useful index of general neural circuit architecture, which may represent more trait-like or persistent biases in neural function related to both normal and abnormal behavior[9]. In addition, participants completed self-report measures of current depression, anxiety and life stress, and they provided saliva samples from which basal afternoon cortisol was assayed. These newly collected data were then integrated with earlier measures of infant life stress (represented through a measure of maternal stress) and childhood basal afternoon cortisol. This integration allowed the current set of analyses, wherein Burghy *et al.*[8] found that in adolescent girls, but not boys, adversity during infancy is positively associated with later childhood cortisol levels, which predict decreased resting-state functional connectivity between the amygdala and vmPFC. Furthermore, this altered amygdala-vmPFC functional connectivity predicts increased symptoms of adolescent anxiety and depression. Interestingly, variability in resting-state functional connectivity and mediation models predicting adolescent anxiety and depression were not related to either recently experienced stressful life events or afternoon basal cortisol levels obtained during adolescence in either gender. The absence of significant associations between these more contemporaneous measures suggests the existence of a critical window during development wherein the neural embedding of stress reactivity affects later risk for psychopathology particularly in girls.

There are, of course, several limitations that should be noted. First, the small sample size limits the utility of mediation analyses, which likely overestimate the variance in symptoms

Ryan Bogdan is in the Department of Psychology, Washington University in St. Louis, St. Louis, Missouri, USA, and Ahmad R. Hariri is in the Department of Psychology and Neuroscience, Institute for Genome Sciences & Policy, Duke University, Durham, North Carolina, USA.
e-mail: rbogdan@artsci.wustl.edu or ahmad.hariri@duke.edu

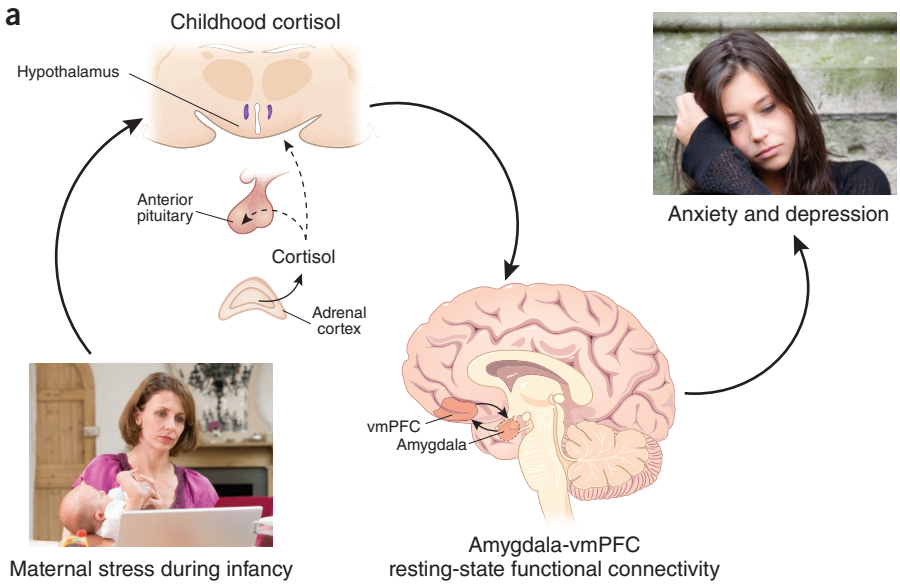

Figure 1 Biological pathways linking early life stress to later psychopathology. (**a**) In girls but not boys, a history of maternal stress during infancy predicts heightened basal afternoon cortisol during childhood, which predicts reduced resting-state amygdala-vmPFC functional connectivity during adolescence. Variability in this functional connectivity, in turn, mediates the association between childhood cortisol and adolescent symptoms of anxiety and depression. (**b**) Plausible epigenetic mechanisms contributing to links between early life stress, amygdala-vmPFC connectivity and symptoms of depression and anxiety. The glucocorticoid receptor (GR) is critical for negative feedback of the HPA axis, and increased promoter methylation (red ovals) of the GR gene (*NR3C1*), which results in decreased gene expression, is associated with childhood adversity. Hence epigenetic regulation of the GR may result in impaired negative feedback of the HPA axis that is gender independent. In contrast, childhood adversity may contribute to gender-dependent promoter demethylation (gray ovals) of the estrogen receptor-α (ERα) gene (*ESR1*), which results in increased gene expression and, possibly, anxiogenic changes in corticolimbic circuit function. It is further possible that epigenetic regulation of the GR can contribute to these gender-dependent effects through downstream regulation of *ESR1* transcription (dashed lines). In the absence of early adversity, stress-related GR activation may result in downregulation of ERα, which is associated with anxiogenic effects. In contrast, the methylation and subsequent downregulation of *GR* following early adversity leads to increased ERα expression and anxiogenic effects, possibly through alterations of functional connectivity between the amygdala and vmPFC.

explained by the biologic measures. Second, the expression of symptoms related to anxiety and depression was not above clinical thresholds for disease diagnosis, and thus the direct relevance of these patterns for treatment and prevention of psychopathology remains to be determined. It is likely that further alterations within the corticolimbic circuit (for example, hippocampal function and connectivity with both the amygdala and vmPFC) would emerge in individuals with clinical diagnoses of mood and anxiety disorders. Third, diurnal variability in cortisol, which represents a better marker of disordered HPA axis function than a single time-point measure, was not assayed, and thus the extent of dysfunction in this system is unclear. Finally, the relationship between resting-state measures of functional connectivity and the responsiveness of neural circuits to actual provocation, such as that elicited by life stress commonly leading to psychopathology, is uncertain and an area of active inquiry. Presumably, resting-state neural circuit function predicts how the circuit responds to challenge.

Despite these limitations, the work of Burghy *et al.*[8] provides fertile ground for further speculation regarding the molecular and cellular mechanisms mediating the gender- and developmentally specific neural embedding of stress. Nonhuman primate research has shown that the most rapid rate of amygdala development occurs during infancy, which may represent a critical period during which the amygdala is particularly sensitive to environmental input[3]. Moreover, females may be more sensitive to early life stress because their amygdala reaches adult-like volume in childhood, whereas in males it grows in volume at least throughout adolescence[10]. Estrogen signaling in the amygdala, which can produce anxiogenic and anxiolytic effects mediated though estrogen receptor-α and estrogen receptor-β, respectively, could further contribute to these patterns[11,12].

Interestingly, gender-specific evidence of epigenetic regulation related to early adversity has been documented for estrogen receptor-α. Female rats experiencing less maternal care have reduced methylation of the estrogen receptor-α gene and corresponding increased estrogen receptor-α expression in the amygdala[13]. Given that estrogen receptor-α function has been associated with anxiogenic effects and with impaired negative feedback of the HPA axis, adversity during infancy may specifically leave females vulnerable to HPA axis dysregulation, culminating in altered resting-state amygdala-vmPFC functional connectivity and increased symptoms of anxiety and depression. This speculation is particularly intriguing in light of recent evidence that estradiol modulates both vmPFC and amygdala reactivity in women[14]. Moreover, gender-specific epigenetic regulation of estrogen receptor-α may magnify more general epigenetic effects occurring in both genders, most notably in the glucocorticoid receptor gene[15], which collectively may leave females particularly vulnerable to HPA axis dysregulation, corticolimbic circuit dysfunction and related risk for psychopathology (**Fig. 1b**).

Regardless of the molecular mechanisms underlying the findings reported by Burghy *et al.*[8], their report offers important insight into the neural embedding of stress reactivity and the mechanisms through which early life adversity may precipitate risk for later psychopathology. Perhaps the most important question arising from the present report is whether we can use these findings and those from similar longitudinal research to inform

the development of new therapeutic targets (for example, estrogen receptor-α expression in the amygdala) and, more importantly, new markers of risk (for example, genetic polymorphisms predicting greater HPA axis responsiveness) that may ultimately allow the prevention of mood and anxiety disorders and mitigation of their long-term negative impact on individuals, families and societies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Breathless without Hox

Valérie Castellani & Artur Kania

Sustained activity of Hox5 transcription factors is needed for the development and maintenance of motor neurons that innervate the diaphragm, reports a study in *Nature Neuroscience*.

"What is the mode of growth of the natural breath and its mode of maintenance?" asked Aristotle in his treatise "On Breath." Much headway into this question has been made at the anatomical and physiological level, complemented by electrophysiological recordings. Such studies have located the vertebrate breathing circuits in the cervical spinal cord and the brainstem. The breathing rhythm has its origin mainly in the pre-Bötzinger complex, and the rhythms generated by this circuit are coordinated with behaviors such as swallowing, locomotion and speech. The main outputs are motor neurons that control the contraction of the diaphragm muscle. Despite the importance of breathing for an organism's survival, however, we know very little about how such motor neurons are specified and how they innervate their targets. A report from Philippidou *et al.*[1] in this issue of *Nature Neuroscience* now shows that neuronal identity and connectivity patterns of the phrenic motor neurons, which innervate the diaphragm, are specified by the transcription factor Hox5, thus providing mechanistic insight into how this important breathing circuit is constructed in mammals.

All motor neurons are born in the ventral neural tube as naive precursors that, over the course of their development, acquire distinct identities through the expression of motor neuron subtype–specific molecules. To better understand how the diaphragm-innervating motor neurons of the phrenic motor column (PMC) are specified, Philippidou *et al.*[1] first characterized PMC markers such as Scip and ALCAM through retrograde PMC cell body labeling from the diaphragm. Next, they used these markers to define PMC molecular identity in terms of Hox protein expression: PMC neurons express Hoxc5 and Hoxa5 but not other Hox proteins, such as Hoxc6 (**Fig. 1**). Hox proteins are specialized transcription factors that define the anteroposterior identity of many cell types, including neurons[2]. This regionalization is in part accomplished through mutual cross-repression that helps to refine anteroposterior boundaries between neuronal and non-neuronal cells alike. Indeed, Philippidou *et al.*[1] show that removal of more posteriorly expressed *Hox* genes that are normally not expressed in the PMC leads to a posterior expansion of the phrenic nucleus. Furthermore, removal of *Foxp1*, which encodes a transcription factor expressed in limb-innervating motor neurons lying close to the PMC but not in PMC neurons themselves, leads to an increase in PMC neuron numbers, and, conversely, Foxp1 overexpression suppresses generation of PMC neurons.

The authors did several experiments that point to Hoxa5 and Hoxc5 (collectively known as Hox5) as key determinants of PMC cell fate, in a presumably cell-autonomous manner. Mice in which *Hox5* is selectively deleted from motor neuron precursors appear grossly normal at birth and yet have one important defect: they do not breathe. Using their PMC molecular markers in such mutants, the authors found a steady decrease in PMC motor neuron numbers starting at the early embryonic stages when these neurons are first generated. Strikingly, the phrenic nerve that carries the diaphragm-innervating axons of the PMC is thinner in *Hox5* mutants, and, concomitantly, few PMC axons reach their target, and those that do disappear over time. The authors also showed that another important phase of PMC–diaphragm connectivity is compromised by *Hox5* loss: the PMC axons that reach the diaphragm fail to branch and form the synapses that normally elicit the contractions of this muscle that lead to lung expansion and inhalation. Using genetic approaches to prevent motor neuron apoptosis in *Hox5* mutants did rescue the decline in PMC neuron numbers but did not ameliorate the innervation and branching defects, suggesting that *Hox5* genes control these processes. Finally, the authors showed that if Hox5 is selectively deleted from mature mouse motor neurons, PMC neurons are generated in normal numbers, cluster into a nucleus and send sufficient numbers of their axons to the diaphragm to initiate breathing. However, in such mice, intramuscular branching is stunted, arguing that *Hox5* gene function is required both for early PMC development and for PMC maintenance.

The results from Philippidou *et al.*[1] point to Hoxa5 and Hoxc proteins as regulators of PMC neuron cell fate specification, maintenance, migration and clustering, axon guidance and target innervation, an impressive list that places these proteins at the top of a hierarchy of regulators of PMC neuron development. The authors' manipulations also suggest that if Hox5 proteins were to be expressed in the context of a naive motor

Valérie Castellani is at the CNRS, Université de Lyon, Lyon, France, and Artur Kania is at the Institut de recherches cliniques de Montréal, Montréal, Quebec, Canada.
e-mail: valerie.castellani@univ-lyon1.fr or artur.kania@ircm.qc.ca