Opinion

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Serotonin transporter gene, stress and raphe–raphe interactions: a molecular mechanism of depression

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Reports of gene–environment interactions (GxE) between the serotonin transporter gene and stress on risk of depression have generated both excitement and controversy. The controversy persists in part because a mechanistic account of this GxE on serotonergic neurotransmission and risk of depression has been lacking. In this Opinion, we draw on recent discoveries in the functional neuroanatomy of the serotonergic dorsal raphe nucleus (DR) to propose such a mechanistic account. We argue that genetically produced variability in serotonin reuptake during stressor-induced raphe–raphe interactions alters the balance in the amygdala-ventromedial prefrontal cortex (VMPFC)-DR circuitry underlying stressor reactivity and emotion regulation. In particular, the recently characterized stressor-responsive serotonergic interneurons originating from the dorsolateral DR may hold a key to unlocking the GxE mechanism of depression.

Introduction

Exposure to stressors is a primary risk factor for depression. However, whereas some individuals who experience severe life stress go on to develop the illness, others appear resilient to it. A mechanistic, neurobiological account of these individual differences in susceptibility to depression could not only inform our understanding of the illness, but also lead to more effective treatments in the clinic. One framework within which to tackle this problem is that of GxE (see Glossary), which holds that the impact of environmental stressors on risk of depression is modulated by genetic variation, particularly in genes related to brain function and stress response. In particular, increasing evidence from both animal studies [1] and human neuroimaging studies [2] suggests that genetic variation in the serotonin system affects the stress response and risk of depression through the critical brain circuitry underlying stressor reactivity and the regulation of emotion, which encompasses the amygdala, the VMPFC and the dorsal raphe nucleus (DR) [3].

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Serotonin (or 5-hydroxytryptamine, 5-HT) is a major modulatory neurotransmitter in the mammalian brain. It is known to regulate behavioral and physiological responses to environmental stressors [4], and has long been implicated in the pathophysiology of depression [5,6]. A prominent candidate in GxE research on depression has been the serotonin transporter. The serotonin transporter protein (5-HTT, SERT), encoded by the serotonin transporter gene (*SLC6A4*), is responsible for reuptake of serotonin from the extracellular space into presynaptic neurons, and serves as a key regulator of serotonergic signaling. Importantly, the promoter region of the gene harbors a 44 base pair insertion/deletion polymorphism, referred to as the 5-HTT-linked polymorphic region (5-HTTLPR), with the short allele (S) less efficiently transcribed than the long allele (L) in human

Glossary

5-HTTLPR: the 5-HTT-linked polymorphic region in the serotonin transporter gene (*SLC6A4*). The 5-HTTLPR comprises a 44 base pair insertion/deletion polymorphism in the promoter region of the gene. It is a functional polymorphism (i.e. it affects the function of the gene), with the short allele (S) less efficiently transcribed than the long allele (L) [7–10].
DRD and DRV: dorsal and ventral subdivisions of the dorsal raphe nucleus (DR). Both the DRD and the DRV contain cell bodies of serotonergic projection neurons to the forebrain, as well as axon terminals of serotonergic interneurons originating from the DRVL.
DRVL: ventrolateral subdivision of the DR. The DRVL contains cell bodies of serotonergic interneurons (also referred to as raphe–raphe interneurons). It is proposed that these serotonergic interneurons are activated by stressors and, in turn, inhibit the serotonergic projection neurons in the DRD and DRV.
GxE: gene–environment interactions. The view that the impact of environmental factors on risk of disease is modulated by genetic variation.
Serotonin (5-hydroxytryptamine, 5-HT): a major modulatory neurotransmitter in the mammalian brain, involved in regulating behavioral and physiological response to stressors [4] and implicated in the pathophysiology of depression [5,6].
Serotonin transporter protein (SERT, 5-HTT): a transmembrane transporter responsible for reuptake of serotonin from the extracellular space back into presynaptic neurons. It serves as a key regulator of serotonergic signaling.
Tryptophan hydroxylase (TPH): the enzyme that catalyzes the rate-limiting step in serotonin synthesis. Two isoforms of the TPH enzyme exist, encoded by *TPH1* and *TPH2* genes in humans and *tph1* and *tph2* genes in other mammals. TPH1 is expressed primarily in the periphery but is also present in the central nervous system (CNS), whereas TPH2 is expressed exclusively in the CNS [98].
VMPFC: the ventromedial prefrontal cortex. The VMPFC is thought to compute the degree of behavioral control over a stressor and to regulate the behavioral and physiological response to it via inhibition of subcortical and brainstem regions [30].

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cell lines *in vitro* [7–9] and in post mortem human brains [10] (conflicting findings [11] are possibly due to other, unmeasured functional variants in the same gene [12,13]). A seminal study by Caspi and colleagues [14] demonstrated that the 5-HTTLPR genotype modulates the impact of stress on the risk of depression. In the presence of childhood maltreatment or stressful life events, S/S homozygotes were at a higher risk of developing depressive symptoms compared to L/L homozygotes, with S/L heterozygotes at an intermediate level of risk. These findings have been replicated in a number of studies [15] and supported by a recent meta-analysis [16]. However, several troubling non-replications have also been reported [15], generating considerable controversy in the field [17].

This controversy persists in part because a simple, mechanistic account of exactly how environmental stressors interact with the serotonin transporter genotype to affect serotonergic neurotransmission and risk of depression has been lacking. In this Opinion, we draw on recent advances in understanding of the functional neuroanatomy of the central serotonin system to propose such a mechanistic account. More specifically, we propose a model for how genetically produced variability in serotonin reuptake during stressor-induced raphe–raphe interactions may alter the balance in the amygdala-VMPFC-DR circuitry. Admittedly, much more complex neurobiological models – reflecting the mechanistic interactions of multiple environmental factors (both positive and negative), multiple genes, multiple neurotransmitter systems and multiple brain circuits – will be required to explain all of the nuances and controversies in GxE research on depression. Nevertheless, the proposed model represents an advance in that direction. We conclude with a discussion of the implications and future research directions, including how the proposed model fits into a broader theoretical framework of biological susceptibility to the environment [18–20].

A molecular mechanism of depression

Serotonergic circuitry

The largest group of serotonergic neurons in the brain originates from the DR in the midbrain and pons. From the DR, the serotonergic neurons project to nearly every area of the forebrain. The DR also receives inputs from some of its projection regions, including the amygdala and the VMPFC, forming feedback loops that are thought to be critical for reactivity to stressors and regulation of emotion [21]. However, not all serotonergic neurons in the DR are projection neurons. To the contrary, the DR is also known to contain serotonergic axon terminals [22], and increasing evidence suggests that these serotonergic neurons serve as raphe–raphe interneurons, mediating crucial interactions between different subdivisions of the DR [23]. In particular, serotonergic interneurons originating from the ventrolateral part of the dorsal raphe nucleus (DRVL) appear to exert inhibitory control over the serotonergic projection neurons in the dorsal (DRD) and ventral (DRV) parts of the DR (Box 1).

Serotonergic activity in the absence of severe stressors

During active wakefulness, in the absence of stressors, the DRD and DRV serotonergic projection neurons are tonically active [24] (Figure 1a). This tonic activity of the serotonergic projection neurons yields a steady synaptic concentration of serotonin and a sustained activation of the post-synaptic serotonin receptors in the target regions, enabling active, goal-directed motor and cognitive function. Likewise, for as long as no severe stressors occur, the DRVL serotonergic interneurons should have a sustained low level of activity. This is because, in the absence of severe stressors, glutamatergic projections from the VMPFC maintain tonic inhibition of the DRVL via a large population of GABAergic interneurons in the DRVL [25,26]. Similarly, compelling evidence suggests that behavioral control over a stressor – as mediated by the VMPFC [27–29] – blocks the impact of that stressor both on serotonergic transmission and on behavior [30].

Severe stressors induce raphe–raphe interactions

By contrast, severe and uncontrollable stressors elicit a large release of serotonin within the DR [31], alter serotonin release in the projection regions of the DR [32,33], and produce lasting behavioral changes described as learned helplessness [4] or behavioral depression [34], when contrasted with equal but controllable stressors (Figure 1b). Such a stressor activates the DRVL serotonergic neurons

Box 1. Topography of the DR: focus on serotonergic raphe–raphe interneurons

The dorsal raphe nucleus (DR) is topographically organized and contains several anatomically and functionally distinct groups of serotonergic neurons [23,64,65]. These subdivisions of the DR include the dorsal raphe nucleus, dorsal part (DRD), which is bordered ventrally by the dorsal raphe nucleus, ventral part (DRV) and laterally by the dorsal raphe nucleus, ventrolateral part (DRVL), also referred to as the lateral wing (LW) [35] (Figure I).

The DRD and DRV receive inputs from a similar set of brain regions, including the VMPFC, amygdala and hypothalamus [66–68]. The DRD sends collateral serotonergic projections to some of the same regions from which it receives inputs, including the VMPFC, amygdala and hypothalamus, as well as the hippocampus and nucleus accumbens, suggesting a role in the regulation of emotional behavior [69,70], whereas the serotonergic projections from the DRV target primarily the motor, sensorimotor and frontal cortices, suggesting a role in motor and cognitive function [71–76].

The adjacent DRVL has several features that set it apart from both the DRD and the DRV. In addition to inputs from the VMPFC, amygdala, and hypothalamus [67,68,77], the DRVL also receives direct visual inputs from the retina [78–80] as well as viscerosensory inputs from the glossopharyngeal and vagal nerves [81]. The serotonergic neurons from the DRVL innervate the hypothalamus, medulla, periaqueductal gray (PAG) and subcortical somatosensory regions, and are thought to regulate the presympathomotor neurons in the spinal cord [74,82–88]. These anatomical features of the DRVL, together with functional neuroanatomical studies [37–40], suggest a role in physiological and behavioral responses to severe stressors, including freezing and fight-or-flight responses.

Critically, the DRVL serotonergic neurons innervate and appear to provide tonic inhibitory input to the DRD and DRV [23,82], acting as serotonergic raphe–raphe interneurons. Furthermore, preliminary evidence suggests that the DRVL serotonergic interneurons and the DRD/DRV serotonergic projection neurons may have opposing patterns of activity. When the DRVL serotonergic neurons are active, the serotonergic neurons in the DRD and DRV are not [38,89]; conversely, when the DRD serotonergic neurons are active, the DRVL serotonergic neurons are not [90–96]. This pattern of dissociation is most consistent with the DRVL neurons exerting inhibitory control over the DRD/DRV projection neurons – an important revision to the current models of serotonergic brain circuitry.