Journal Club

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DNA Methylation within the Amygdala Early in Life Increases Susceptibility for Depression and Anxiety Disorders

Ilan Vonderwalde
Faculty of Medicine, University of British Columbia, Vancouver, V6T 1Z3 British Columbia, Canada
Review of McCoy et al.

The presentation and development of psychiatric illnesses is affected by interindividual variability in brain morphology and genetics (Tost et al., 2012; Smoller, 2016). Epigenetic factors and early life experiences can also alter brain development and influence psychiatric illness susceptibility (LaSalle, 2011; Kundakovic and Champagne, 2015; Mitchell et al., 2016). For example, interactions between genetic and environmental factors may alter gene expression, resulting in neuroplastic and functional modifications that lead to anxiety and depression (Nieto et al., 2016; Uchida et al., 2018).

The hippocampus and amygdala, which are involved in emotion and memory processing, play important roles in the manifestation of depression and anxiety disorders. Many properties of these two brain regions likely contribute to psychiatric illnesses, including abnormal volume and density (Gatt et al., 2009; Walton et al., 2017), connectivity and signaling (Wheeler et al., 2018), cell function (Sild et al., 2017), genetics (Gatt et al., 2009; Lau et al., 2010; Wheeler et al., 2018), and/or epigenetic modifications (Simmons et al., 2012; McCoy et al., 2017).

One stable marker of epigenetic modifications is DNA methylation, a process which occurs when a methyl group is added to DNA at the C5 position in the DNA cytosine ring, most commonly at CpG dinucleotides. This process is catalyzed by DNA methyltransferases (DNMTs) and can result in modified gene expression, typically as a gene being "turned off" or repressed.

McCoy et al. (2019) hypothesized that DNA methylation patterns in the hippocampus and amygdala can drive behavioral phenotypes through modifiable mechanisms. To test this, they examined methylation patterns in low-responder (LR) rats, which were bred from LR lines shown to exhibit low responses to novel stimuli and increased behaviors associated with anxiety and depression (e.g., anxiety-like behavior, lower sex drive, passive coping, and being less social), and high-responder (HR) rats, from HR lines, which do not express such behaviors. Specifically, they examined levels of major protein regulators of methylation, such as DNMT1, DNMT3a, and DNMT3b, as well as global methylation levels in the amygdala and hippocampus of HR and LR rats within the first 21 postnatal days (P21). Although they observed no difference between the two populations within the hippocampus, significant differences were found in the amygdala; the LR rats generally showed greater levels of methylation, especially at early time points (1 week) after birth. Importantly, McCoy et al. (2019) also showed that modifying methylation during the late embryonic stages or early postnatal period can result in decreased anxiety and depressive-like demeanor on behavioral tests focused on emotional regulation in adulthood, such as the open-field test, the elevated plus maze, a social interaction test, a sucrose preference test, and the forced swim test.

Because the largest difference in methylation occurred early in development, McCoy et al. (2019) mapped and compared the methylome of HR and LR rat amygdalae 1 week after birth to identify specific DNA regions-of-interest that were differentially methylated between the two populations. There were 1881 differentially methylated regions, with >80% of them hypermethylated in the LR rats. These results differ from those in adult HR and LR rats, where HR rats tend to have increased methylation in the amygdala, albeit in different DNA regions and affecting different pathways (McCoy et al., 2017). Interestingly, rats that exhibited intermediate response (IR) between LR and HR rats in this study had a methylation profile more similar to HR than LR rats, suggest-

Received April 14, 2019; revised Aug. 27, 2019; accepted Sept. 11, 2019.
I thank Dr. Emily Gilbert and Carlos Vonderwalde for their input and comments.
The author declares no competing financial interests.
Correspondence should be addressed to Ilan Vonderwalde at Ilan.vonderwalde@alumni.ubc.ca.
https://doi.org/10.1523/JNEUROSCI.0845-19.2019

ing that the amygdala methylation pattern in LR rats is abnormal.

Next, McCoy et al. (2019) asked whether reducing methylation in LR rats would influence behavior. To decrease methylation in developing rats during the late embryonic phase when limbic system development and refinement predominates (Moore et al., 2006; Soma et al., 2009), they fed LR dams a diet lacking in methyl donors starting at gestational day 17 and lasting until P21. Although no global changes in methylation were identified in offspring from LR dams that were restricted in methyl-donor intake, they still showed improved behavioral results, including increased exploratory behavior, less anxiety, more social exploration, and less depression-like behavior in adulthood (P70+); overall, their behaviors were similar to those exhibited by IR rats. As expected, there was no change in offspring functioning when HR dams were fed the same diet. Future work could explore the inverse relationship by feeding HR dams excess methyl donors to evaluate whether inducing hypermethylation during the perinatal period is also sufficient to change their offspring behavior.

To determine whether changes in methylation exerted direct or indirect effects on behavior, McCoy et al. (2019) directly suppressed amygdala methylation in IR rats by infusing a specific siRNA to target $DNMT_{3B}$ into the amygdala of P10 IR pups. This treatment led to a transient 17% reduction of $DNMT_{3B}$ levels 4 d after siRNA infusion, but did not alter expression in adulthood. Nonetheless, transient suppression of $DNMT_{3B}$ in developing amygdala of IR rats resulted in less anxious behavior in males, but not in females, suggesting that temporary changes in methylation patterns during development can have long-term effects. These experiments also highlight potential differences in male and female neurodevelopmental pathways and epigenetic susceptibility.

An important question to consider is how changes in DNA methylation within the amygdala during key developmental periods can affect function later in life. The most commonly hypermethylated pathways in LR rats were those involved in long-term depression, glutamatergic transmission, and signaling cascades involving Ras-related protein 1 (Rap1), oxytocin, and phosphoinositide 3-kinase-protein kinase B (PI3K-Akt), which have various roles in brain function including cell-cycle regulation, proliferation, survival, and migration. Interestingly, previous work found that decreased activity of these pathways in the amygdala, as well as downregulation of brain-derived neurotrophic factor signaling, was associated with depression and anxiety in mammals, including humans (Pape and Pare, 2010; Duman and Voleti, 2012). It is possible that early changes in these pathways can influence cell connectivity and function during sensitive periods of brain development (Moryś et al., 1998; Bouwmeester et al., 2002; Stead et al., 2006). For example, impaired synaptic plasticity and the inability to prune connections between neurons or achieve long-term depression can lead to hyperactivity in the basolateral amygdala (BLA) and/or hyperconnectivity and increased functional coupling between amygdala regions (BLA, centromedial amygdala) and various other brain regions (hippocampus, anterior cingulate cortex, and medial prefrontal cortex); in turn, this can lead to an inability to properly regulate emotions and increased susceptibility for developing anxiety or depressive disorders (Qin et al., 2014; Ehrlich and Josselyn, 2016; Johnson et al., 2018). Some regulatory changes that could be responsible for these alterations in neurodevelopment include dysregulated PI3K-Akt-mTOR pathway signaling (Huang et al., 2016), diminished Rap1 expression and pathway downregulation (Pan et al., 2008; Ye and Carew, 2010), or decreased signaling within the oxytocin pathway (Fan et al., 2015; Sobota et al., 2015; Koch et al., 2016). Ultimately, the precise mechanism by which the methylation and interaction of these pathways produces long-term effects should be an important avenue to pursue in the future studies.

Regardless of the mechanistic details, the findings that early methylation changes can have measurable effects on behavior later in life has important implications for clinical translation. McCoy et al. (2019) suggest that potential future strategies for stress-related psychiatric illnesses may include early intervention in at risk populations, such as children that display atypical behaviors (Frenkel et al., 2015). However, important considerations include defining who is at risk, whether methylation changes can help change brain function, and how to target the therapy, as drastic global dietary changes involving methyl donor restriction would likely have adverse side effects.

Ultimately, McCoy et al. (2019) show that DNA methylation within the amygdala during early developmental periods plays a fundamental role in the development of psychiatric illnesses later in life. Further experiments will be necessary to gain a better understanding of the implications of epigenetic manipulation on development and function before translation is possible, but the knowledge acquired from this study has potential to help in the creation of future mitigation strategies to manage, predict, and prevent anxiety and depression.

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