RESEARCH HIGHLIGHT

Epigenetic regulation of corticotropin-releasing hormone receptor 1: implication for anxiety-related disorders

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Recent literature corroborates that both, genes and environment, are crucial determinants contributing to psychiatric disorders. The selectively bred mouse models of anxiety-related behavior provide a great opportunity to investigate the interaction of a rigid genetic predisposition with environmental factors and are used to identify targets contributing to pathological anxiety. Here, we studied gene × environment (G×E) interactions using a mouse model of high (HAB) *vs.* low (LAB) anxiety-related behavior. By applying enriched environment (EE) and chronic mild stress (CMS), we succeeded in shifting the phenotypes of HAB and LAB mice towards "normal" anxiety. In this bidirectional shift, *Crhr1* was identified as a key player. Increased methylation of CpG1 within the *Crhr1* promoter region was shown to be critically involved in regulating the binding affinity of the transcription factor Ying-Yang 1 (YY1). The interplay between YY1 expression and DNA methylation might be the mechanism underlying the differences in *Crhr1* expression after EE and CMS. Other epigenetic mechanisms contributing to *Crhr1* expression are discussed here.

Keywords: G×E; anxiety; stress; enriched environment; epigenetics; Crhr1; YY1; methylation; microRNA; histone modification

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Successful attempts to constructively leverage advances of the recent years in understanding the nature of psychiatric disorders depend upon our ability to merge genetic and environmental factors in their etiology [1]. Indeed, the discovery of epigenetic mechanisms in the development of psychiatric diseases has changed the view of causality from genocentric towards gene × environment (G×E) interactions [2, 3]. Recent data indicate that epigenetics is the most important but largely uninvestigated field linking disease, environment and genetics [4].

Epigenetic modifications allow organisms to adapt to current environmental conditions through a dynamic regulation of their gene expression which is not mediated by changes in DNA sequence. Methylation, histone modification and non-coding RNA are the best understood examples of epigenetic instruments. Although the mode of interaction between these mechanisms is not completely known, it is likely that these processes are not independent

of each other [5]. Reasonably, rather than identifying merely genetic causes of psychiatric diseases, clinicians may broaden their "therapeutic toolbox" by shifting their focus on epigenetic mechanisms which predict or potentially prevent negative outcomes of GxE interactions. One should bear in mind that genetic manipulations of a patient's background is a priori difficult to perform or impossible due to ethical reasons and potentially delicate consequences.

The hyperactivity of the CRH system during stress exposure has been extensively studied in the etiology of anxiety and depression [9, 10]. Thus, it was shown that acute stress increases CRH in the central amygdala [11]. The later diffuse of CRH in the BlA [11, 12] might activate the CRH receptor 1 there and, thereby, cause alterations in anxiety-related behavior. A critical role of the amygdala in the observed phenotypic changes after EE and CMS was suggested in our recent studies [13, 14], which encouraged us

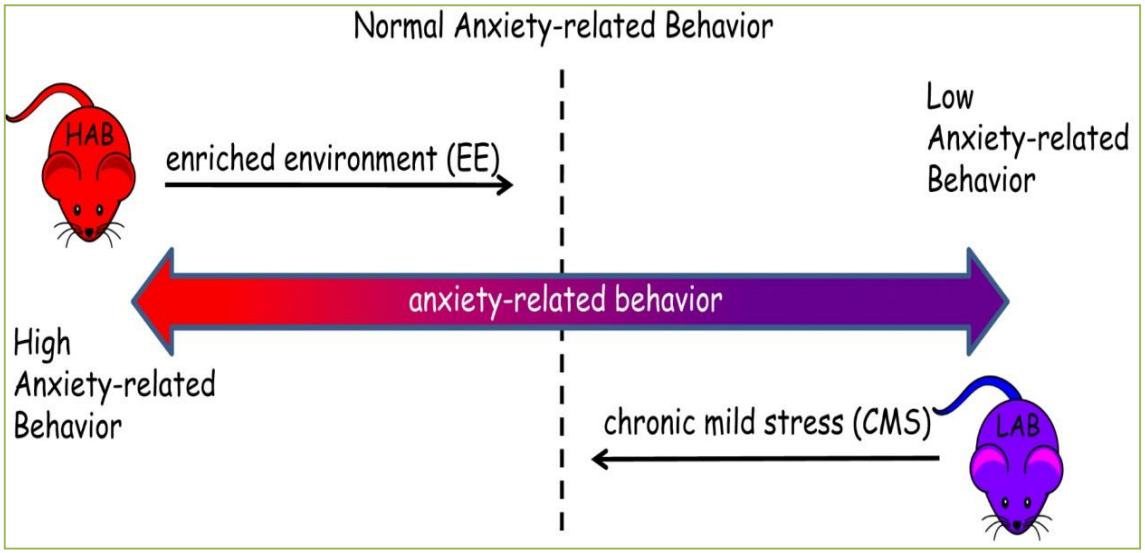

Figure 1. Conceptual framework and behavioral effects of environmental modifications. Chronic mild stress (CMS) induced an anxiogenic effect in low anxiety-related behavior (LAB) mice shifting their phenotype toward "normality". Similarly, enriched environment (EE) rescued the inborn phenotype of high anxiety-related behavior (HAB) mice.

Recent literature supports the important role of animal models to unravel G×E interactions. However, many studies consider only detrimental environmental effects, whereas the absence of adversity is considered as the "good" end of the environmental continuum [6]. Subsequently, such studies ignore the positive effects of environmental factors, and, therefore, fail to measure the multi-faceted range of psychological and behavioral reactions. By using a bidirectional approach to study the role of G×E interactions in the development of extremely high (HAB) or low (LAB) anxiety-related behavior of mice we have tried to avoid this bias [7]. In order to shift these extreme anxiety-related behaviors towards the "normal" range of the anxiety continuum [8], LAB mice were exposed to chronic mild stress (CMS) (adverse environment), whereas enriched environment (EE) provided improved housing conditions for HAB mice (beneficial environment) (Fig 1). We were able to show that even genetically determined anxiety-related behavior can be shifted from the extremes of the anxiety continuum via exposure to CMS or EE, respectively, thereby rescuing "normal" behavior.

to further investigate the involvement of distinct CRH system components in our mouse model. HABs display high anxiety-related behavior in a variety of tests [15]. One underlying rationale might be a higher expression of *Crhr1* in the BlA compared to LABs. EE was indeed capable of decreasing this difference in *Crhr1* receptor expression, whereas the opposite effect on gene expression was observed in LABs exposed to CMS.

Earlier studies reported that the effects of early life stress on *Crh* expression can be mediated via changes in DNA methylation of its promoter [16, 17]. Similarly, we observed changes in *Crhr1* promoter methylation;

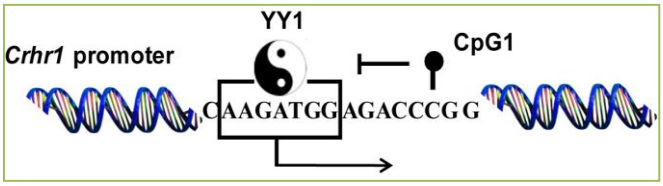

Figure 2. Binding of transcription factor Ying-Yang 1 (YY1) to the Crhr1 promoter enhances its activity. Methylation of CpG1 significantly reduced binding affinity of YY1 and, thereby, decreased YY1-induced promoter activity.

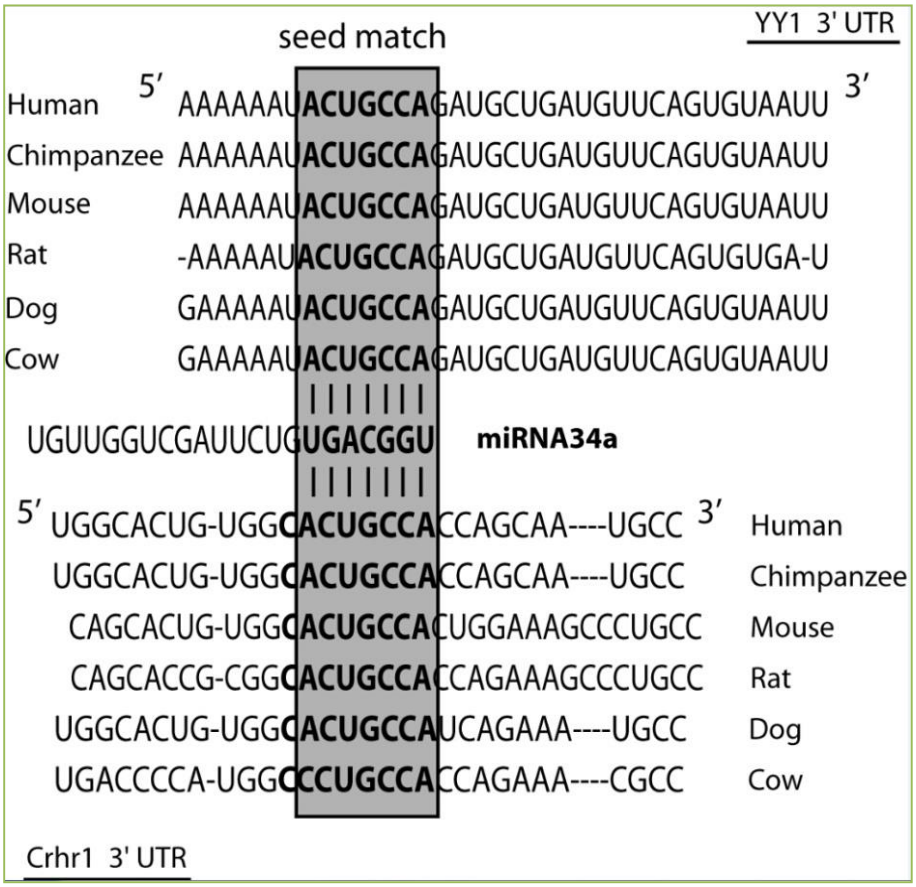

Figure 3. An evolutionary conserved binding site for the miRNA34 family. Using TargetScan (http://targetscan. org) a recognition sequence for miRNA34 family was found on the *Crhr1*-3'UTR and *YY1*-3'UTR.

surprisingly, both EE and CMS increased the methylation of the first CpG dinucleotide in the promoter region (CpG1). This CpG site is located close to a binding site of the transcription factor Ying-Yang 1 (YY1) shown to regulate promoter activity of several genes in a methylation-dependent [18,19] and -independent [20, 21] manner. Here, YY1 enhanced *Crhr1* promoter activity and mRNA expression, whereas CpG1 methylation significantly reduced the binding affinity of YY1, thus causing decreased *Crhr1* promoter activity (Fig. 2). We hypothesized that increased CpG1 methylation and decreased *YY1* expression, as observed after EE, could mediate lower expression of *Crhr1*, whereas increased CpG1 methylation might prevent the CRH system from YY1-induced *Crhr1* over-expression and, thereby, might play a stress-protective role in LABs.

Our research group is working to uncover other epigenetic mechanisms contributing to *Crhr1* gene expression. Recently, it was found that chronic and acute stress induce expression of several microRNAs (miRNA) in the CeA, among others miRNA-34c [22]. The miRNAs of this family could down-regulate *Crhr1* expression via binding to the 3'UTR and, consequently, effect anxiety-related behavior. Our data support these results, since expression of miR-34a in the BlA was found to be higher in LAB compared to HAB mice [23]. Interestingly, systematic proteome analysis, performed by Chen et al. [24], demonstrated that miR-34a can down-regulate *YY1* through binding to a specific recognition sequence in the 3'UTR region. These data indicate that both *Crhr1* and *YY1* seem to be regulated by miR-34a (Fig. 3), which predisposes this miRNA as a promising candidate for drug discovery.

As mentioned earlier, there is a close interaction between different epigenetic mechanisms. Thus, binding of YY1 attracts other co-factors which control accessibility of DNA for the transcription machinery. The HDAC2/1 complex was found to be one of them [25, 26] and suggests a possible involvement of histone modifications in the regulation of *Crhr1* expression. The available ChIP-seq data (Ensemble Genome Browser) on embryonic stem cells suggest that the *Crhr1* promoter might be a critical site for histone modifications (Fig. 4A). Indeed, treatment of neuro-2a neuroblastoma cells with valproic acid, a well-

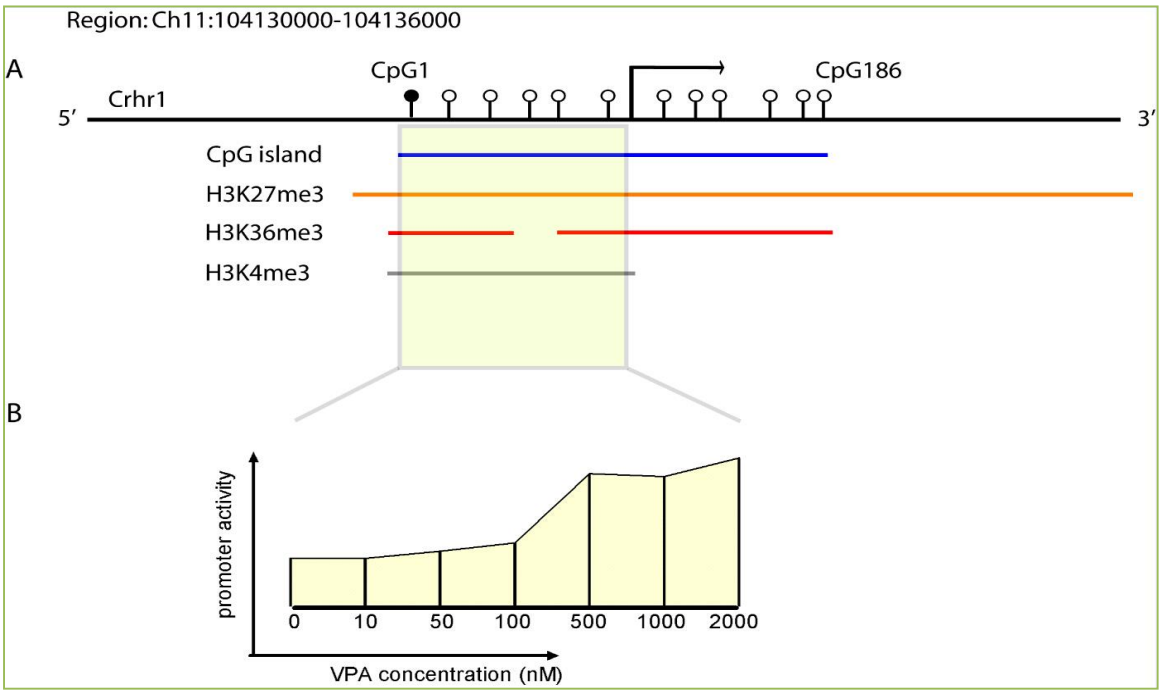

Figure 4. *Crhr1* promoter is suggested to be a vulnerable place for regulation via histone modifications. A - Lines represent enriched domains of histone modifications within promoter and exon 1 of the *Crhr1* using ChIP-seq data on embryonic stem cells (Ensemble Genome Browser). B - Treatment of neuronal-2a cells with increasing concentration of the histone deacetylase inhibitor valproic acid (VPA) significantly enhanced promoter activity. Promoter strength was evaluated by measuring luciferase activity of the construct containing a 1.2kb promoter fragment of the *Crhr1* gene (yellow box) cloned upstream of a firefly *luciferase gene* (relative luciferase activity). Cell culture, transfection and reporter gene assay were performed as described earlier in Sotnikov et al.[7].

known histone deacetylase inhibitor (HDACi), anticonvulsant and mood-stabilizer, induced a significant increase of both *Crhr1* promoter activity (Fig. 4B) and mRNA expression [23]. Recent experiments in our group [27] suggest a mild anxiolytic effect of valproic acid in HAB mice when applied chronically, highlighting its therapeutic potential to treat anxiety disorders.

Altogether, using a mouse model of pathological anxiety, we succeeded in showing that epigenetic processes triggered by detrimental or beneficial environmental stimuli are able to rescue genetically determined extreme anxiety-related behavior. In particular, we were able to demonstrate an involvement of CpG1 methylation in the regulation of *Crhr1*. However, an additional involvement of histone modification and miRNA is likely as well, thus creating the probability for an intricate interplay to fine-tune gene expression. These data provide novel opportunities for treatment of anxiety-related disorders which can be utilized complementary or as an alternative to already existing ones.

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