A Further Analysis and Commentary on: Profiling Changes in Cortical Astroglial Cells Following Chronic Stress

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ABSTRACT: The neuroplasticity hypothesis of depression proposes that major depressive disorders are related to decreased hippocampal and cortical neural plasticity, which is reversed by antidepressant treatment. Astroglial cells have emerged as key mediators of neural plasticity and are involved in the cause and treatment of depression and anxiety-like behaviors. One of the ways that astroglia modulate neuroplasticity is through the formation and maintenance of perineuronal nets (PNNs). Perineuronal nets are important extracellular matrix components that respond to stress and are implicated in anxiety-like behaviors. Normally, astroglial cells continuously turnover PNNs by degrading and donating PNN proteins; however, chronic stress slows PNN protein degradation and increases cortical PNN expression overall. In this report, we used weighted gene co-expression network analysis and eigeneuron analysis to further delineate the pathways and key regulators involved in the astroglial-PNN relationship following chronic stress. Our analyses indicate that chronic variable stress induces the expression of PNNs through inhibition of trophic pathways and key transcription factors in astroglial cells. These data further support the integral role of astroglial cells in the neuroplasticity hypothesis of depression through their modulation of anxiety-like behaviors and PNNs.

KEYWORDS: Stress, plasticity, mouse, translatome, glia, cortex

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The neuroplasticity hypothesis of depression described by Pittenger and Duman¹ postulates that the development of depression is characterized by decreases in neural plasticity, including neurogenesis, a reduction in dendritarborization, and synaptic plasticity. Decreases in neuroplasticity can be induced with chronic stress and have been described in the hippocampus and prefrontal cortex (PFC); moreover, antidepressant treatment increases the same neuroplastic events and is associated with a remediation of the associated behavioral phenotype. A growing body of work implicates astroglial cells in a number of neuroplastic events including neurogenesis and the development, maturation, and elimination of synapses.² Given this role, it is not surprising that recent work has suggested that astroglia are important mediators of both the cause¹ and the pharmacologic treatment⁴,⁵ of depression. In fact, ablation of PFC astroglia induces a depressive/anxiety-like phenotype, whereas PFC neuronal ablation does not.³ In addition to the critical role astroglial cells play in synapse integrity, they are involved in the cyclical production, degradation, and maintenance of perineuronal nets (PNNs)⁶ through the donation of component proteins and through enzymatic degradation. Perineuronal nets are proteoglycan-rich extracellular matrix (ECM) complexes that surround and stabilize, through inhibition of plasticity, inhibitory interneuron synapses in the cortex, hippocampus, and amygdala.⁷ In a recent study⁸ in which we sequenced the translatome of neocortical astroglial cells in response to chronic variable stress (CVS; a mouse model of depression),⁸ we found that several components of PNNs were increased including proteoglycans (BCAN), collagens (COL1A2, COL8A2, and COL9A3), laminins (LAMB2), and the link protein HAPLN1. To validate that this reflected a change in the PNNs themselves, we counted Wisteria Floribunda agglutinin (WFA, a PNN marker)-positive cells and found that the number of PNNs increased in response to CVS throughout the neocortex. Moreover, the PNN increase was related to increases in anxiety and depressive-like behavior, alluding to the importance of PNNs in the neuroplasticity hypothesis of depression. Finally, degradation of PNNs in the PFC alone was sufficient to reverse the effects of CVS on depressive and anxiety-like behavior.⁹ This suggests that although PNN upregulation was seen across the frontal and parietal cortices and is a global response to CVS or, perhaps, sustained glucocorticoid exposure, PNN integrity in the PFC is directly related to the effects of CVS on anxiety and depressive behaviors. This is not surprising given the central role of PFC plasticity in the control of these specific behaviors; whereas the parietal cortex is central to control of motor behaviors. Indeed, psychomotor retardation is a feature of major depressive disorder and has been associated with hypoconnectivity and hypoactivity of the parietal cortex, consistent with an increase in PNNs.
Previously, we generated a translatomic database of differentially expressed genes (DEGs) in cortical astroglial cells from mice exposed to CVS. The gene ontology of the top DEGs suggested that CVS impaired PNN degradation, contributing to a decreased neuroplastic environment.8 Using the expression levels of our entire data set from our original study,8 we performed weighted gene co-expression network analysis (WGCNA)9 to integrate the different altered gene sets into a coherent system-level context (Supplemental Data File 1). Weighted gene co-expression analysis was used to identify co-expression modules across the entire data set of gene expression levels as log-transformed RPKM (ie, log2(RPKM + 1)).9 The WGCNA function blockwiseModules was run with the following parameters:

maxBlockSize=500000, corType="bicor", power=24, networkType="signed", deepSplit=2, minModuleSize=20, verbose=3, pamRespectsDendro=F).

The power parameter was estimated using the pickSoftThreshold function, setting networkType="signed".

Weighted gene co-expression network analysis module regulatory network analysis was done using the Expression2Kinases package, using the member of each module as the input list and default parameters.9 Analysis across the 6 samples grouped 14525 genes into 64 modules (plus the unassigned gray modules genes). Two of the modules were overrepresented with upregulated genes, one of which was enriched in pathways related to ECM proteoglycans as well as genes related to astroglial activation (Figure 1, magenta), with the other enriched in pathways related to lysosome and protein transport, and interestingly, some autism and Alzheimer disease–related genes (Figure 1, turquoise). Together, these may suggest increased production of ECM proteins, in particular proteoglycans, and an increase in protein degradation, consistent with astroglial activation. This pathway is particularly interesting because of the role astroglial cells play in the organization and maintenance of PNNs.6 Three of the modules were overrepresented with downregulated genes, one of which was enriched in MAP kinase genes associated with cytokine and growth factor signaling (nerve growth factor in particular) and Rho GTPases, which are important to changes in cytoskeleton and cell morphology (Figure 1, blue), with the others enriched in pathways related to signaling by growth factors and regulation of transcription (Figure 1, brown), whereas the smallest module shows weak enrichment of metabolic pathways (Figure 1, orange). Overall, these modules point to an impairment or downregulation of signal transduction and transcription, possibly associated with a decrease in astroglial metabolism. Downregulation of neurotrophin signaling is consistent with active inflammation,10 and the downregulation of MAPK pathway genes also supports decreased levels of growth factors such as fibroblast growth factor 2 and brain-derived growth factor, which is consistent with previous studies of depression and depressive behaviors and the role of fibroblast growth factor in the stress response.8,11,12

Eigengene analysis of the co-expression modules is a useful tool to understand module-to-module relationships and, in combination with upstream regulation analysis, can help elucidate the regulatory network driving the present transcriptional/translational alterations.13 The results from the eigengene analysis, after filtering for correlation coefficients (abs(cor) > 0.8), show a strong anticorrelation (1) between the turquoise and both the blue and brown modules and (2) between the brown
and magenta. Strong anticorrelations are, in part, expected as a result of the signed network definition, which splits positive and negative correlations. Nevertheless, it may suggest the presence of negative feedback loops or mutually exclusive programs of transcription. The brown module contains the largest number of strong (and significant) correlations, making the transcription factor activity and regulation of transcription a network hub.

We then used Expression2Kinases\textsuperscript{14} to infer key upstream regulators that cause the observed transcription/translation patterns by testing each of the 5 main network modules as the input. We assessed each group of regulators for differential expression, co-expression, and module of origin, to identify the potential upstream regulators responsible for the observed translational patterns. Strikingly, 50% of all the differentially expressed upstream regulators belong to the blue module (Supplemental Data Files 2 and 3), placing it potentially upstream of the other modules. In particular, gene members of the brown, magenta, and turquoise modules are under the control of differentially expressed regulators in the blue module, and furthermore, most of the differentially expressed regulators of the blue module itself are also a part of the blue module (Supplemental Data Files 2 and 3). This is particularly interesting because the blue module represents decreases in growth factor signaling and Rho GTPases, which potentially control (ie, promoting or no longer inhibiting) the upregulation of ECM proteoglycans, astroglial activation (magenta), and protein degradation components (turquoise) and the downregulation of transcription factor activity (brown). Furthermore, the upstream regulators of the magenta module are mostly in the blue and turquoise modules, suggesting that the upregulation of ECM proteoglycans (ie, PNNs) is potentially driven by the downregulation of the growth factor signaling and upregulation of the protein degradation components of the system.

The module-to-module relationships implied by the distribution of differentially expressed upstream regulators (based on transcription factors (TFs), protein-protein interactions (PPIs), and kinase databases) are in agreement with the (data driven) eigengene analysis, which suggests internal consistency across our analyses (Supplemental Data Files 2 and 3).

Altogether, these analyses suggest that chronic stress impairs trophic activity (Figure 1, blue) in astroglial cells. These lead to inefficiencies in degradation of proteins associated with PNN maintenance, therefore increasing PNN expression and subsequently decreasing plasticity. Perineuronal nets have been associated with fear conditioning in the amygdala, such that increases in PNNs are observed following fear conditioning.\textsuperscript{15} Perineuronal nets appear to preserve memories and prevent extinction of the fear conditioning cues, presumably as an adaptive response to protect the organism and allow it to recognize future threats. It is possible, therefore, that a similar process occurs in the PFC; however, in response to chronic rather than acute stress, this becomes maladaptive and leads to decreased plasticity and cognitive flexibility. Indeed, cognitive functions are also impaired when PNNs are not present\textsuperscript{26} and of course are a core feature of mood disorders in humans.\textsuperscript{7,27}

Perineuronal nets surround only a subset of the cortical parvalbumin, somatostatin, and reelin interneuron populations, and functional changes in each of these cell populations have been implicated in major depressive disorder.\textsuperscript{18,19} Importantly, it is not clear what is the functional difference between a PNN- or non-PNN-surrounded interneuron, although it is believed that these cells show different plasticity potential.\textsuperscript{20} The putative link between PNNs, GABAergic function, and depressive and anxiety-like behaviors remains to be elucidated.

The question as to how stress induces the astroglial changes noted in our previous\textsuperscript{8} and this study remains. One obvious factor may be that these changes reflect direct responses to prolonged glucocorticoid exposure. Indeed, astrogial express glucocorticoid receptors and we demonstrate that our CVS model induces sustained glucocorticoid increases.\textsuperscript{8} Moreover, several studies have shown direct functional changes in astroglia in response to glucocorticoids.\textsuperscript{21,22} Both in vivo and in vitro models show that glucocorticoid receptors act as transcriptional regulators in astroglia to change levels of adenosine triphosphate, glutamate transporters, and connexin component proteins,\textsuperscript{23-25} inducing functional changes in neocortical astroglial cells.

Another possibility (ie, not mutually exclusive) is that astroglial changes occur in response to sustained decreases in PFC activity previously reported to be induced by CVS and in major depressive disorder.\textsuperscript{26,27} Further studies are required to test these hypotheses and understand the upstream factors involved in stress-induced changes in astroglia. Altogether, these data consider the effects of stress in the PFC from a multicellular, systems, perspective. It is important to note, however, that these are predicted models and future in vivo work will be needed to confirm the translational validity of these findings. Nevertheless, taken together with previous work,\textsuperscript{5,8} these findings reinforce an important role for astroglia in the interplay between stress, neurons, and anxiety and depressive-like behaviors.

Author Contributions
GC, SS, and NS contributed to the experimental design, implementation, analysis of the results and writing of this manuscript. GMR contributed to the experimental analysis, data interpretation and writing of the manuscript.

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