Gene environment interaction in periphery and brain converge to modulate behavioral outcomes: Insights from the SP1 transient early in life interference rat model

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Abstract

It is generally assumed that behavior results from an interaction between susceptible genes and environmental stimuli during critical life stages. The present article reviews the main theoretical and practical concepts in the research of gene environment interaction, emphasizing the need for models simulating real life complexity. We review a novel approach to study gene environment interaction in which a brief post-natal interference with the expression of multiple genes, by inhibiting the activity of the ubiquitous transcription factor specificity protein 1 (Sp1) is followed by later-in-life exposure of rats to stress. Finally, this review discusses the role of peripheral processes in behavioral responses, demonstrating how specific behavioral patterns are linked to modulations in interwoven brain and body physiological processes due to gene and environmental changes.

Key words: Gene-environmental interaction; Specificity protein 1; Mithramycin; Stress; Animal-model; Essential amino acids; Tryptophan; Insulin

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Core tip: We review the main theoretical and practical concepts in the research of gene environment interaction. We present a novel approach to study gene environment interaction in which a brief post-natal interference with the expression of multiple genes by inhibiting the activity of the ubiquitous transcription factor specificity protein 1 is followed by later-in-life exposure of rats to stress. Finally, we discuss the role of peripheral processes in behavioral responses, demonstrating how specific behavioral patterns are linked to modulations in interwoven brain and body physiological processes due to gene and environmental changes.
INTRODUCTION

The role of nature vs nurture in shaping complex behavior and in mental disorders is a matter of long running dispute and creates a split between psychobiology, which emphasizes the dominancy of the being’s innate predispositions and psychotherapy supporters, which point out the surrounding influence. Evidence from decades of heredity research has suggested that complex behaviors and psychiatric disorders have a solid genetic basis\(^1\,2\). It has been reported by many studies that consistent differences in behavioral traits between subjects, such as stress responsivity and temperament, show a familial pattern\(^3\). On the other hand, the impact of environmental factors on physical illness is well established\(^4\,5\) and well recognized in behavioral disturbances\(^6\,7\). Numerous studies reported a correlation between candidate genes, and behavioral phenotype\(^8\,9\), yet with significantly lower rate of replication and a clear tendency toward positive results\(^10\,11\). Environmental aspects are formulated in the vast psychosocial literature and in models that use a scientific platform, such as the impact of different nursing abilities of female rats, on stress responsivity of their pups\(^12\,13\). Environmental physical factors such as intrauterine inflammatory reaction induced by Lipopolysaccharide, which simulates the impact of pre natal infection on behavior\(^12\,13\) were studied as well.

The stress diathesis theory, which suggests that disorders induced by the combination of varying degrees of predisposition with invers degrees of stressful stimuli, has become an accepted conceptual research framework for studying complex behaviors. Following this hypothesis numerous studies over the last decade assessed the relationship between candidate genes and behavior in the form of genome-wide association studies (GWAS)\(^14\), most of them focusing on the pathologic consequences of genetic alteration. Despite the remarkable advances in genetic tools and techniques, very few direct genetic effects on mental health have been identified and replicated\(^15\).

An alternative paradigm to the stress diathesis theory is the differential susceptibility theory, which assumes that individuals react differently to environmental stimuli depending on the plasticity of gene rather than their susceptibility. Thus showing higher responsiveness to both positive and negative external cues, frequently with opposing outcomes\(^15\,16\). For example, high frequency of antisocial behavior was correlated with childhood maltreatment the in low activity MAO-A allele carriers\(^17\). Interestingly, low activity MAO-A allele carriers, who were not exposed to childhood maltreatment showed the lowest anti-social behavior scores compared with normal activity MAO-A carriers\(^18\). An additional extensively studied genetic variant in psychiatry is the short allelic form of the serotonin transporter-linked polymorphic region (5-HTTLPR), which is associated with a reduction in the transporter activity\(^19\) and with high risk for major depression in individuals exposed to stressful life events\(^10\). Yet, 5HTT s-allele carriers were shown to be more responsive to the Attention Bias Modification training than long-allele carriers, supporting Belsky’s hypothesis that the s-allele may be considered as a plasticity gene rather than a susceptible gene\(^20\). Taken together, these examples support the differential responsiveness theory of sensitive genes in the etiology of complex behaviors\(^19\).

Unfortunately, the current gene environment interaction models have substantial limitations, ranging from lack of validation to poor predictive power and although genome studies have expanded our understanding of complex phenotypes and many human diseases, they hardly explain a small proportion of their heritability in the population\(^21\). Genetic variants are usually considered as having additive, suppressive or neutral effects on the phenotype, but the effect size for a single genetic variant is minor\(^22\). A more comprehensive model of real life interaction between multiple genes that influence the expression of each other and thereby the manifestation of a particular phenotype, is needed.

One possible gate for modelling real life gene environment interaction are through manipulation of key point genes, genes that are essential for the modulation of multiple genes’ activity, such as the ubiquitous transcription factor specificity protein 1 (Sp1). Sp1 is a member of the SP proteins family, which constitutes a group of highly conserved transcription factors present in a wide range of organisms. Their structure is defined by the presence of three highly conserved DNA-binding zinc finger domains which bind to similar, yet distinct, GC-rich target sequences. Members of the SP family function either as activators or repressors in cell via a promoter-dependent manner\(^23\). Sp1 is essential for the regulation of various physiological functions, maintains organ homeostasis, regulates tissue repair, and possibly serves as an anti-inflammatory mechanism that protects against organ inflammation and injury\(^24\). Sp1 regulates the expression of numerous genes in early developmental stages\(^25\) and the expression of most growth factors and their receptors depend on Sp1\(^26\,28\). Sp1 activity can be modulated by various environment-dependent factors including metabolic factors such as glucose and insulin, immunologic factors such as tumor necrosis factor-alpha (TNF-α), glucocorticoid receptors and several major kinases including CDK2 and ERK1/2\(^26\,32\). Sp1 also modulates the expression of many genes implicated in psychiatry research, including neuregulin-1\(^23\), reelin\(^34\), GAD67\(^35\), MAO A and B\(^36\,37\), NMDA receptor subunits (NR1 and NR2)\(^38\,39\), GABA A receptor\(^40\), DA receptors\(^41\) and genes of the oxidative phosphorylation

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system (OXPHOS)\cite{47}. In this article we will describe how simulating real life by minor manipulation of multiple gene expression, based on Sp1 unique characteristics, models more accurately gene environment interaction in behavioral sciences.

**SP1 IN SCHIZOPHRENIA AND OTHER NEUROPSYCHIATRIC DISORDERS**

The alterations in different genetic trajectories in schizophrenia reported by numerous studies\cite{43}, can result from transcriptional dysregulation in the disorder\cite{44}. Our group showed that the expression of Sp1 is disrupted in brain samples and peripheral blood lymphocytes of schizophrenia patients as compared with those of healthy subjects. Specifically, downregulation in Sp1 mRNA expression was prominent in the prefrontal cortex and the striatum, while upregulation was observed in the parieto-occipital cortex and in blood lymphocytes of schizophrenic patients. Sp1 levels were highly and significantly correlated with two subunits (NDUFV2 and NDUFV1) of the first complex of the OXPHOS in lymphocytes and brain specimens of normal subjects, while abolished in schizophrenic patients\cite{45}. We have shown that Sp1 is a transcription factor of both subunits, which have been repeatedly implicated in schizophrenia\cite{46,47}. A defect in Sp1 transcriptional activity, which leads to abnormal expression of complex I subunits, can be one of the causes for reduced complex I activity associated with mitochondrial dysfunction and reduced energy metabolism observed in schizophrenia brains by numerous imaging studies\cite{48}. Such distortion in brain energy production can affect synaptic plasticity and connectivity of neuronal networks and thereby cognitive and emotional behaviors\cite{49}. Additional studies by other groups substantiated the role of Sp1 in mental and neurological disorders by showing a reduction of Sp1 protein and mRNA in the postmortem prefrontal cortex brain of chronic schizophrenia patients\cite{50}, increased Sp1 mRNA levels in the hippocampus\cite{51} and a reduction in Sp1 and Sp4 protein levels in lymphocytes of first-episode psychosis patient\cite{52}. These reports are in line with other studies demonstrating an altered expression of Sp1 in the regulation of many genes associated with neuropsychiatric psychiatric disorders. Thus, elevation in Sp1 protein levels was observed in autistic brains, which was associated with altered expression of autism candidate genes such as OXTR and PTEN\cite{53}. Sp1 mRNA and protein was also found to be up-regulated in Alzheimer’s disease (AD) brains and in a transgenic mice model of the disease\cite{54}. In Huntington disease, Sp1-regulated huntingtin transcription is dysregulated. In adrenal medulla-derived PC12 cell cultures it was shown that Sp1 is involved in the regulation of epinephrine biosynthesis in response to acute and chronic stress\cite{55}. The dual characteristic of Sp1, having specific environmental and internal signal regulated transcriptional activities, together with its role in the regulation of multiple genes, coincide with the multi-gene alteration and the heterogeneous symptomatology of mental disorders.

**SP1 MANIPULATION MODELS**

Complete inhibition of Sp1 is incompatible with life and Sp1 knockout mice die *in utero* with multiple phenotypic aberrations\cite{56}. However, Sp1 transcriptional activity can be inhibited by Mithramycin (MTR)\cite{57}. MTR is an antineoplastic antibiotic, which binds to GC-rich regions on the DNA displacing the Sp family transcription proteins from their binding sites\cite{58}. MTR is a clinically approved antibiotic that is effective for the treatment many cancers such as testicular cancer\cite{59} and also for cancer induced hypercalcemia\cite{60}. Our group has reported that MTR induced a time dependent decrease in the expression levels of complex I subunits NDUFV1, NDUFV2 and NDUFS1, as well as of reelin, all regulated by Sp1 and implicated in schizophrenia\cite{61}. The availability of a simple pharmacologic agent that modulates the transcription of different genes, turns it into an attractive tool for modelling multiple gene dysregulation. Indeed, neonatal rats treated for a few days (7-10 postnatal days) with MTR, showed three months later cognitive and behavioral deficits such as spatial working memory impairment and anxious behavior, without any impact on their bodily wellbeing\cite{62}. The effect of MTR treatment was also studied in AD experimental models. Thus, MTR injections to AD transgenic adult mice for several months, resulted in greater memory impairment in these mice and increased amyloid β peptide levels\cite{63}, with no additional behavioral differences. These data suggest that manipulation of Sp1 transcriptional activity at adulthood has long lasting effects on behavior depending on predisposing genetic aberration earlier in life. In contrast to these results chronic MTR administration to AD transgenic mice by another group resulted in cognitive improvement\cite{63}, emphasizing the need for better understanding the role of Sp1 transcriptional activity in the pathophysiology of AD. In a mouse model for Huntington disease chronic MTR treatment from PND 20 throughout life extended survival, enhanced motor performance, and improved brain histopathology\cite{64}. The neuroprotective effect of MTR was also demonstrated in adult rats exposed to repeated administration of methamphetamine\cite{65}, an accepted model for schizophrenia\cite{66}.

**STRESS EXPOSURE MODEL**

Studying the additive effect of environmental variables on top of the predisposing susceptibility is complex and may have many bias pitfalls. Stress is commonly used to mimic environmental insults in models of mental disorders and complex behaviors. We have used peripubertal mild unpredictable stress protocol. One major parameter in modeling environmental effects is the timing of exposure to insult. However, timing and duration of exposure to stress differ between studies. There are early in-life stress models, mainly maternal separation, which increase...
stress reactivity in the offspring\cite{67}, while there are adult stress models, including the unpredictable chronic mild stress model, which differ in chronicity, protocol elements and actual age of stress exposure, adolescence or adulthood\cite{68-70}. Both prenatal period and postnatal mid to late adolescence were shown to be particularly vulnerable to stress in rats\cite{61}. Chronic adolescence stress was repeatedly shown to be associated with HPA dysfunction\cite{71}, hippocampal volume reduction and impairments in spatial learning\cite{72} later in life. To elaborate our view on the impact of environment we compared two stress regimens differing only in duration, chronic and sub-chronic regimens, in adolescence. Interestingly, high serum corticosterone levels and higher anxiety index were related to the sub-chronic stress regimen, while rats exposed to chronic stress did not differ significantly from the controls, which implies adaptation to stress\cite{61}. Although chronic mild stress is an accepted paradigm for induction of depressive-like symptoms in rats\cite{73}, several studies show resilience effects of long-term stress\cite{74,75} which is in line with the adaptation to the chronic stress regimen.

**MANIPULATION OF GENE EXPRESSION AND THE ENVIRONMENT**

Studies modeling genetic predisposition for behavioral alterations, induce predisposition in one or more of the four following paradigms: Manipulation of a candidate gene, interference with a candidate system/pathway, intrauterine insults or exposure to early post-natal stressors that induce epigenetic changes.

Numerous studies using candidate gene knockout mice and chronic stress were published. Candidate system interference studies mostly involve HPA axis manipulation either pharmacologically by glucocorticoids administration\cite{76} or induced by early life stress\cite{77}. Examples for intrauterine insult models include the prenatal protein malnutrition, which affects development of the brain in utero and induces cognitive impairment and severe widespread morphological abnormalities similar to schizophrenia\cite{78,79}. Other models are based on the intrauterine infection theory for schizophrenia\cite{80}. These models include prenatal exposure of mice to viruses, such as the influenza virus\cite{81} which cause brain developmental damages similar to those observed in schizophrenia brain, or maternal immune activation by lipopolysaccharide or polyinosinic:polycytidylic acid (Poly I:C) during pregnancy, which model schizophrenia and autism in the offspring\cite{82,83}. The best studied model for epigenetic changes induced by early life stressors is the maternal separation model, which enhances behavioral changes\cite{84,85}, and causes epigenetic modifications that can be transmitted through generations\cite{11}. We hypothesize that a transient interference with the expression of many various genes, by MTR for example, at a critical developmental stage of the brain together with an exposure of the animal to stressful environment later in life, will provide an animal model to study the role of gene environment interaction in long lasting complex behavior relevant to mental disorders. Although it may be argued that modification of the expression of numerous genes is inaccurate and difficult to monitor, we believe that it is a closer model to real life complexity. Indeed, we found that MTR treated rats exposed to sub-chronic stress demonstrated higher anxiety index, anhedonia and indifference to novel objects. However, MTR treated rats exposed to the chronic stress paradigm demonstrated normal sucrose preference, low anxiety index and high novelty seeking behavior. These findings support the differential sensitivity theory, claiming increased reactivity to environmental stimuli in genetically sensitive individuals, with differential responses to various stimuli\cite{60}.

**INTERTWINED PERIPHERAL AND BRAIN INTERACTION**

The molecular and biochemical pathways that contribute to behavioral phenotypes are still a mystery and it is almost impossible to differentiate between genetic and environmental impacts. The currently common dominate hypothesis is that changes in brain cellular pathways are responsible for alterations in behavioral responses. We and others suggest that peripheral factors are essential for formulating behavioral responses. In our rat model for example, we showed that exposing MTR treated rats to chronic stress (MTR + stress) caused a significant reduction in tryptophan brain levels, which in part stems from peripheral changes. Alteration in peripheral tryptophan levels was found to be associated with behavioral and cognitive phenotypes. For example, aggression tendencies associated with a low serum tryptophan levels\cite{86} and impulsivity\cite{87} was observed in the course of manic episodes\cite{88}, while increased serum tryptophan levels were observed during the recovery periods in bipolar manic patients\cite{89}. Tryptophan depletion studies have reported association with worsening of depressive symptoms in human, yet the data are inconclusive\cite{89,90}. In addition, it was reported that a reduction in tryptophan levels interrupts memory consolidation yet improves attention\cite{92}. Dietary tryptophan depletion is also used in modeling major depression in rats\cite{93} and dietary prenatal protein deprivation is used to model cognitive impairment observed in schizophrenia\cite{78}. In our model, the reduction in brain tryptophan in the MTR + stress rats was probably not due to its extensive metabolism in brain, as no change was observed in its two major metabolic pathways the serotonin and kynurenine pathways\cite{94}. However, being an essential amino acid tryptophan level in brain depends also on its availability, which can be modulated by several variables including its serum level and its BBB transporter (LAT1) activity\cite{95}. Serum level of amino acids, which compete with tryptophan on its transporter, the branched chain amino acids (BCAA) for example\cite{96,97}, can affect tryptophan availability to the brain. Indeed, serum tryptophan/BCAA ratio is an established measure to
estimate brain tryptophan levels. In the MTR + Stress rats, reduced tryptophan brain levels were associated with reduced LAT1 protein levels and its light chain SLC3A2 transcript levels. In addition, we observed a reduction in serum tryptophan/BCCA ratio, implying a peripheral contribution to reduced brain tryptophan levels. We further suggest that tryptophan/BCCA reduction is due to a failure of these MTR treated rats to respond to stress by increasing serum glucose and insulin, a known regulator of serum BCAA, as did rats exposed to chronic stress only. Taken together, these data suggest that interference with brain tryptophan homeostasis is due to joint brain and peripheral physiological processes. In line with the latter is the finding that brain tryptophan levels were only affected in rats receiving the combined treatment of MTR + stress, while serum tryptophan/BCCA ratio or brain LAT1 were affected by either Stress or MTR, respectively. Our data suggest that a mild modulation of both peripheral and central processes, which converge and mutually interact, can influence behavioral phenotype. A similar interaction can be seen in circuits of energy balance regulation in the body. Thus, adipose tissues secrete leptin as an afferent signal, which influences the activity of the hypothalamus. The hypothalamus signals decrease food intake by inhibiting anabolic circuits, and enhance energy expenditure through the activation of catabolic circuits. It is quite intuitive, but sometimes neglected, that the brain collects both central and peripheral internal inputs, as well as external inputs and executes reaction based on the sum of predisposition and experience. The recent increasing interest in the link between microbiome and brain function and its role in mental disorders further substantiates a role for peripheral inputs in behavior.

CONCLUSION
The ubiquitous transcription factor Sp1 plays a role in the regulation of many genes in response to internal and environmental signals and is suggested to have implication in neuropsychiatric disorders and complex behaviors. Using simple manipulation of Sp1 we showed that a wide and transient interference with gene expression in inbred rats at a critical developmental stage, can induce a long lasting impact on metabolic and behavioral response to environmental stimuli, with different and even opposite outcomes, depending on the characteristics of the environmental stimuli/insult. Already at 1963, Bleuler wrote that “unfavourable nature and environment develop together and influence each other. They are interwoven from babyhood. The environment influencing the manifestation of the hereditary disposition is already a reflected image of this disposition”. Peripheral and central physiological processes, which are both subjected to genetic and environmental changes, interact reciprocally to induce specific behavioral patterns (Figure 1). Further studies could shed light on the importance of these brain-periphery reciprocal interactions for whole body homeostasis and its influence on behavior. In addition, new targets may emerge from such a perspective of behavioral modulators for future clinical intervention.

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