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The Persistent Effects of Childhood Abuse through the Lens of Epigenetics

Childhood abuse has devastating effects on individuals that have long been studied in psychology for its lasting consequences on subjects into adulthood. This type of previous abuse has unusually robust and sustained effects, becoming associated with adult health problems (somatic, psychological, and substance abuse) whose associations are so strong that they are comparable to associations for patients currently experiencing abuse. It is conservatively estimated that more than 1,000,000 children are exposed to sexual abuse, physical abuse, or neglect each year. [1] The study of child abuse has more recently entered the realm of biology, and is now being studied through epigenetics. Epigenetics means "above the genome," and this active area of study within biology studies heritable changes in gene expression that are not caused by changes in the DNA sequence. Epigenetics most notably focuses on methyl groups and biochemical tags that can be added to or removed from chromatin and histone tails respectively to affect gene expression. Epigenetic markers, through experience, can be changed within a person's lifetime, and those changes can be inherited in the later generations, effectively passing on a change in gene expression due to environmental factors.

Childhood abuse is a form of traumatic stress that explicitly occurs early in life. There are many theories to address the potential mechanism of the adverse effects of childhood abuse on lifelong mental and physical health (especially depression), but the most promising and currently studied theory is the psychological theory of the stress diathesis model. This model hypothesizes that "excess reactivity of certain neural and endocrine systems [in response to traumatic stress] increases individual vulnerability to stress-related disease. Exposure to stress during developmentally critical periods results in persisting hyperreactivity of the physiological response to stress, increasing the risk of stress-related disease in genetically susceptible individuals."[1] The biological and neurological response to stress repeated over time, especially during the developmentally critical period at a young age, increases vulnerability to stress related disease because it results in relentless over reactivity of the stress response.

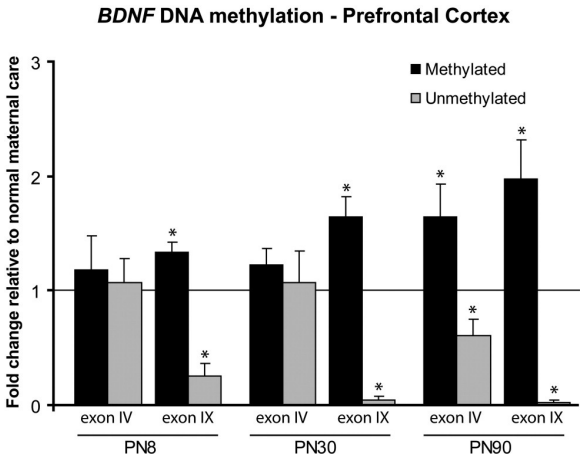
The biological response of the body to stress is mediated by the hypothalamic pituitary adrenal axis (HPA axis). In response to a stimulus, norepinephrine is released into circulation from the sympathetic nervous system and the same occurs with epinephrine from the adrenal medulla. Then, CRF (corticotrophin releasing factor) is released by the hypothalamic paraventricular nucleus to stimulate release of ACTH (adrenocorticotropic hormone) into general circulation from the anterior pituitary gland. ACTH is then transported to the adrenal cortex, stimulating the release of glucocorticoids (cortisol in humans). This activation of the HPA axis takes a couple of minutes to fully engage, and is later reduced by negative feedback of the glucocorticoids on glucocorticoid receptors in the hippocampus, hypothalamus, and the anterior pituitary. HPA axis activation allows for a focusing of biological resources to physiological functions that promote survival and escape.[1]

The psychological theory of the stress diathesis model, understood in the context of the biological view of the stress response, states that if the stress response becomes

chronic due to repeated stress, a sustained increase in the levels of stress hormones is developed. That sustained increase of glucocorticoids (that also mediate metabolism and immune function) will lead to biological changes (given genetic vulnerability) that result in stress related disease. A large study of adult female twins showed that childhood sexual abuse was associated with an increased risk of depression.[1] In a longitudinal study of major depression and co morbidity with 676 subjects who had experienced significant abuse or neglect before the age of 11 and 520 controls, children who were physically abused had an increased risk of lifetime major depression with an odds ratio of 1.59, and those who have experienced multiple types of abuse had an odds ratio of 1.75.[2] These ratios are disturbingly high, in one case, close to doubling an individual's chances of developing depression. These studies, with their alarming results, contribute support to the stress-diathesis model and leads to the questions of how these effects develop.

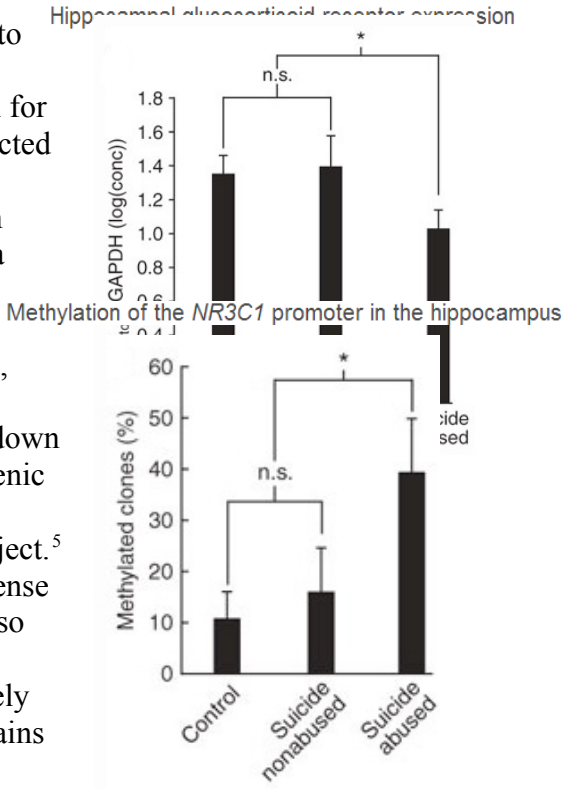
The study of epigenetics is being used to study the biological changes that cause and perpetuate these effects of childhood abuse. Animal studies have given us the most insight for the foundation into this. One example of such are rat studies comparing normally reared pups and pups that suffered repeated or long periods of maternal separation. The deprived rat pups showed an exaggerated HPA response, increased glucocorticoid response along with lower expression of the glucocorticoid receptors in the hippocampus, increased levels of ACTH in the blood, and changes in the mRNA expression of CRF. [3] Experiments with rhesus monkeys similarly showed that the HPA axis was altered in infants raised in isolation from their mothers with an exaggerated ACTH response to CRF and altered basal cortisol levels.[1] These studies illustrate that sustained, increased levels of stress hormones lead to a deregulation of the HPA axis. This deregulation of the HPA axis has been observed in regards to traumatic stress early in life and has been proposed as a potential mediator of the long-term effects of abuse.

Not many specific epigenetic markers have been found, but a couple have been discovered within the past decade that are starting to shed some light. A further study on rats by Roth et al. showed that early abuse produced persistent changes in DNA methylation of the BDNF (brain-derived neurotrophic factor) gene, altering its expression in the adult prefrontal cortex and that the altered methylation persisted in the offspring. Subjects tested for two of the nine exons that make up the BDNF gene, exon IV that contains the transcription start site, and exon IX, that lies downstream of the transcription start site and contains a large number of CpG sites, all show a remarkable increase in methylation over chronology, as the results chart shows (PN stands for postnatal day, so PN8 are infants, PN30 are adolescents, and PN90 are adults). Even though the maltreatment ended at PN7, the effects on methylation persisted and increased over time. BDNF is a key mediator of neural plasticity in the prefrontal cortex and hippocampus, and suppressed BDNF expression (verified by reduced BDNF mRNA levels) is implicated in the onset of several mental illnesses associated with early-life adversity.[4]



There have been few epigenetic studies done with humans and maltreatment. One of the most prominent ones focused on glucocorticoid receptor expression (to address HPA axis responses to stress) and promoter methylation of the NRC31 gene (the glucocorticoid receptor

gene) examined in hippocampal samples from suicide victims and controls (individuals who died suddenly of unrelated causes and had no history of childhood abuse). The glucocorticoid receptor expression was examined using reverse transcription PCR of glucocorticoid receptor mRNA, and it was found that the mRNA for suicide victims that suffered child abuse was significantly lower than the mRNA quantity of both the non-abused suicide victims and controls who were found to be virtually the same.[5]



Twenty clones were sequenced for each individual for methylation mapping of the promoter region and the expected results of methylation levels were found. There were no differences in the percentage of methylated clones for non abused suicide victims and control. There was, however, a significant increase in methylation, specifically cytosine methylation. The methylation occurred on certain sites along the promoter instead of throughout. One of the sites, NGFI-A, a site that has not yet been studied in the human brain, does have some evidence in rat studies that shows down regulated expression in the prefrontal cortex of schizophrenic rats. It should also be noted that there were no significant correlations between levels of methylation and age of subject.[5] With the reduction in glucocorticoid receptors, it makes sense that a higher basal glucocorticoid hormone level would also exist, and thus heighten the HPA axis's stress response. However, there is not enough information yet to definitively conclude why the increased basal levels occur, and it remains unclear whether a reduction in receptors facilitated an increased basal glucocorticoid hormone response or vice versa.

Another study comparing brain samples from suicide victims with a history of childhood abuse or severe neglect and controls who died suddenly from unrelated causes without histories of abuse examined the methylation of rRNA genes that encode ribosomal RNA. There were no significant differences in post-mortem interval, age, brain pH, and ancestry between the suicide victims and the controls (all subjects were of French-Canadian descent). Twenty clones were sequenced, and methylation mapping was performed for the rRNA. Results show that there was very heavy methylation throughout the promoter region and 5' regulatory region of the suicide victims.[6, A1] With high methylation of the rRNA, there are few ribosomes to produce proteins in the brain. This finding goes hand in hand with the other results listed in this paper and with Professor Brutlag's opinion in lecture that many of the genes are methylated because they are not being transcribed. If there are not enough ribosomes available to transcribe the genes in the first place, then they may become methylated because there is no reason for those genes to be loosely packed and take up more space when they could become heterochromatin. If this were to be true, then this then asks the question of how and why the rRNA became methylated in the first place.

There is much more research that needs to be performed within this area of study, and within the field of epigenetics, to answer the questions raised in this paper. Multiple studies have implicated the stress diathesis model, viewed through the context of biology and epigenetics, to be a prominent theory of how the effects of child abuse can persist into adulthood, both physically and mentally, especially noted with the known methylation of the BDNF, NRC31, and

rRNA genes. However, much more needs to be known before a definitive hypothesis, and thus, a definitive solution, can be posited. I imagine that this solution would attempt to result in demethylation of these genes that have been affected by childhood abuse.

One of the most important things I have learned by writing this paper is that the genes that have been found to be associated with the lasting effects of childhood abuse into adulthood are all methylated. The information known of what allows these disabling effects to persist all stem from a lack of necessary proteins. This lack of necessary proteins works in concert with a hyperactive stress reactivity system that increases basal hormone levels as a coping mechanism to the low number of receptors. As well, the epigenetic features can be and have been shown to be inherited in the next generation. These acquired genetic features can become genetic predispositions for the next generation. The studies reported in this paper show that the lasting effects of childhood abuse into adulthood are, in effect, genetics exacerbated by stress in a critical period, and this leads to a cycle of mental illness or depression from one generation to the next that is enforced by epigenetics.

Resources

1. The Neurobiological Toll of Child Abuse and Neglect
   http://tva.sagepub.com/content/10/4/389.full.pdf+html

2. Neurobiological and Psychiatric Consequences of Child Abuse and Neglect
   http://onlinelibrary.wiley.com/doi/10.1002/dev.20494/full

3. Research Review: The Neurobiology and Genetics of Maltreatment and Adversity
   http://onlinelibrary.wiley.com/doi/10.1111/j.1469-7610.2010.02271.x/full

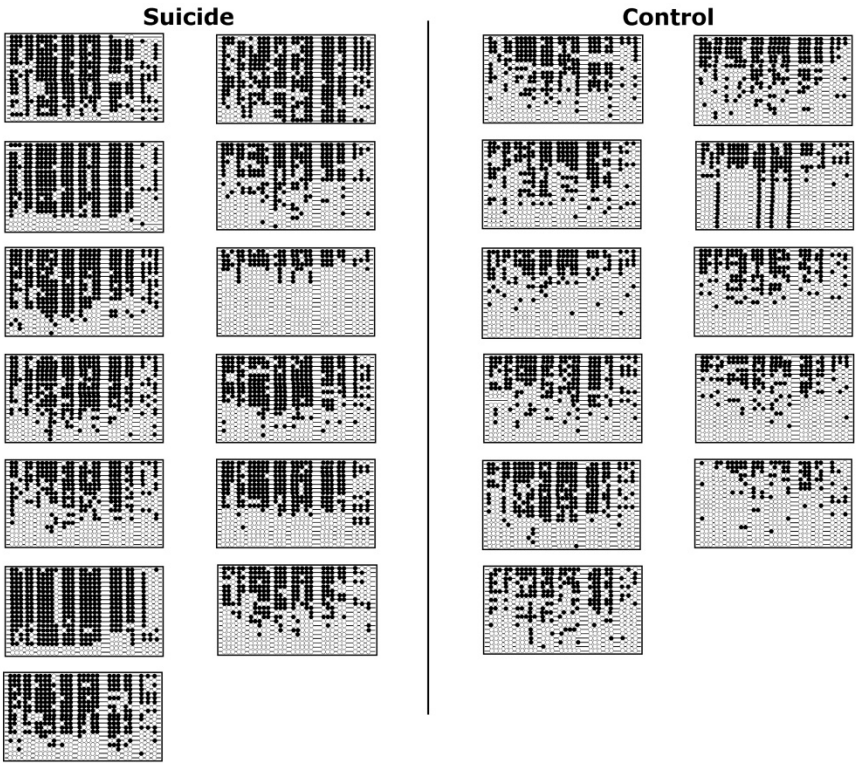
4. Lasting Epigenetic Influence on Early-Life Adversity on the BDNF Gene
   http://www.sciencedirect.com/science/article/pii/S0006322308015308

5. Epigenetic Regulation of the Glucocorticoid Receptor in Human Brain Associated with Childhood Abuse
   http://www.nature.com/neuro/journal/v12/n3/full/nn.2270.html

6. Promoter-Wide Hypermethylation of the Ribosomal RNA Gene Promoter in the Suicide Brain
   http://www.plosone.org/article/info:doi/10.1371/journal.pone.0002085?imageURI=info:doi/10.1371/journal.pone.0002085.g002#pone-0002085-g002

Appendix

A1.



This is the methylation mapping of the rRNA promoter sequence. Twenty clones (each line representing one clone) were sequenced for each subject from multiple independent PCR reactions. Each circle represents CpG dinucleotides, in the 5' to 3' order, and the filled (darkened) circles represent methylation while the open circles do not.