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**ATTACHMENT PROCESSES AND GENE-ENVIRONMENT
INTERACTIONS: *Testing Two Initial Hypotheses Regarding the
Relationship between Attachment, and Methylation of the Glucocorticoid
Receptor Gene (NR3C1) and the Serotonin Transporter Gene (SLC6A4)***

By

Karen Jones-Mason

A dissertation submitted in partial satisfaction of the

Requirements for the degree of

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In

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In the

Graduate Division

Of the

University of California, Berkeley

Committee in charge:

Professor Jill Duerr Berrick, Co-Chair

Professor Steve Hamilton, Co-Chair

Professor William Runyan

Professor Daniela Kaufer

Professor Darlene Francis

Spring, 2011

Abstract

ATTACHMENT PROCESSES AND GENE-ENVIRONMENT INTERACTIONS: *Testing Two Initial Hypotheses Regarding the Relationship between Attachment, and Methylation of the Glucocorticoid Receptor Gene (NR3C1) and the Serotonin Transporter Gene (SLC6A4)*

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University of California, Berkeley

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This study seeks to determine whether methylation levels of a polymorphism in the serotonin gene-linked promoter region (5-HTTLPR) associated with the serotonin transporter gene (SLC6A4), and of the Glucocorticoid Receptor gene (NR3C1) (GR), determined from peripheral blood samples, differ among 93 participants, approximately 18-25 years of age, according to attachment “state of mind” and socio-economic status (SES). Attachment state of mind is determined by two measures: the Adult Attachment Interview (AAI) and a new measure called the Adult Attachment Projective (AAP). The extent of convergent agreement between the AAP and the AAI is reported. SES was determined by the Hollingshead Scale of SES (HSES) and the Family Affluence Scale (FAS), and the correlation between the HSES and the FAS is reported. Participants were also administered the MacArthur Measure of Subjective Social Status (MSSS) and the MacArthur Measure of Subjective SES (MSES) to determine if subjective measures of SES and social status differ based on attachment classification. It is hoped that determining whether the relationship between attachment state of mind and SES impacts genetic expression will help researchers understand the biological mechanisms underlying the dynamic between early life experience and development, and inform future treatment for those who develop pathology as a result of adverse life experiences. Findings: No convergence was detected between the AAI and the AAP; a significant correlation was found between the HSES and the FAS; the higher the participants objective and subjective perception of SES, the higher the MSSS score; in the lower HSES category, a trend toward higher MSSS scores among secure vs. insecure participants was detected; and ss genotype was associated with unresolved attachment classification (determined by AAI) when participants were divided into the U vs. non-U classifications, and into the ss vs. sl/ll genotypes only in participants self-identified as Hispanic. Using principal component analysis (PC), marginally significant differences in methylation levels were detected among all participants according to HSES among unresolved participants. Using PC analysis, lower HSES unresolved participants among the Asian-American and Hispanic participants had significantly higher levels of methylation than upper SES unresolved participants. Although more research with a larger sample size is necessary, results suggest that methylation levels may be set or moderated by attachment classification and SES.

Table of Contents

ATTACHMENT PROCESSES AND GENE-ENVIRONMENT INTERACTIONS: *Testing Two Initial Hypotheses Regarding the Relationship between Attachment, and Methylation of the Glucocorticoid Receptor Gene (NR3C1) and the Serotonin Transporter Gene (SLC6A4)*

Abstract.....	i
Table of Contents.....	ii
Dedication and Acknowledgements.....	iv
Introduction	1
Chapter 1: Literature Review.....	4
A. Attachment Theory.....	4
B. The Glucocorticoid Receptor Gene (NR3C1) and the Human Stress System.....	29
1. The Glucocorticoid Receptor (GR) Gene.....	29
2. The Human Stress Response: The Sympathetic Adreno- medullary System (SAM) and Limbic-Hypothalamic- PituitaryAdrenocortical (LHPA) System.....	31
C. Serotonin (“5-HT”), the Serotonin Transporter Gene (“SLC6A4”) and 5-HTTLPR.....	36
D. What is Epigenetics?.....	39.
Chapter 2: Research Design and Methods.....	46
Chapter 3: Results.....	51
Chapter 4: Discussion and Conclusion.....	62
REFERENCES.....	71
TABLES	
Table 1: Demographic Data.....	51
Table 2: Cross-tabulation of AAI and AAP.....	52

Table 3: Mean Scores of BDI.....	53
Table 4: Mean Scores of HSES, MSES & MSSS x Ethnicity.....	55
Table 5: MSSS x Low HSES Mean Scores.....	56
Table 6: Ethnicity X Genotype X Resolved vs. Unresolved (U/altF as U).....	57
Table 7: Genotype Distribution: Ethnicity X HSES.....	63
Table 8: Direction of Methylation Levels: Attachment Classification X HSES in Asian-American Participants.....	64

FIGURES

Figure 1: Targeted Regions for Methylation Data.....	58
Figure 2: Mean Methylation for SLC6A4 and NR3C1.....	58
Figure 3: SLC6A4 S1_4 Methylation.....	60
Figure 4: Methylation level in SLC6A4: Resolved vs. Unresolved Participants Across all Ethnicities and HSES.....	61
Figure 5: Methylation Level in SLC6A4 in Unresolved Asian-American and Hispanic Participants by HSES.....	61

APPENDICES

Appendix A Table of Selected Serotonin and Other Related Studies:.....	114
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ENDNOTES.....	115
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Dedication and Acknowledgements

There has never really been any question about who to dedicate this research to; the young people who donated their time, extraordinary effort, and hearts, far in excess of any compensation I could give them, have richly earned the honor. Despite the difficulty, and even tears that came forth in these interviews, with profound generosity and compassion for the other people who might benefit from this research, they pushed on to finish all measures fully and substantively. Someone once said that young people would not produce interesting transcripts. They could not have been more wrong. If these transcripts show anything, it is that by the time humans reach this age, compassion and empathy is in full swing. I have been honored and privileged beyond what I can adequately express to meet and work with each of the young people that participated in this study and I hope to make them all proud of the work that is ultimately produced.

In terms of acknowledgements, there are many people to thank. My first debt of gratitude goes to all my advisors. I am deeply grateful to my advisors from the social welfare department, Dr. Jill Duerr Berrick and Dr. William Runyon, who were able to think outside traditional notions of what constitutes “social welfare” and support this research. I know that our department has never produced research like this before and we all hope that perhaps this study can contribute to the creation of a new era in social welfare. You have reminded me, however, that, in a way, this study is not unique in it’s efforts to encourage social workers to learn the nomenclature of science in their efforts to advocate for justice, and that in fact, using scientific principles to create an evidenced based practice has been the legacy of over a century of social work. Perhaps this study can be viewed as just a continuation of that tradition. Moreover, if this research supports the notion that economic and social justice is directly involved in the health and quality of life of individuals, it will be harder and harder for legislators, policy makers and leaders to ignore social welfare’s plea for meaningful structural change. Dr. Berrick deserves special gratitude, for not only her unwavering support, but for other efforts too such as struggling to find rooms for the participants to be interviewed, and going above and beyond the call of duty by even sharing her own office! Of course, to my advisors from the biological and medical sciences, Dr. Steve Hamilton, Dr. Darlene Francis and Dr. Daniela Kaufer, I have a profoundly deep sense of gratitude for being willing to work with someone from a field so disparate from their own. With particular thanks to Dr. Hamilton, who did the lab work in this study and who patiently and kindly tutored me on the subtleties of genetic analysis. To Dr. Kaufer for her wise advise and counsel, for answering all questions thoroughly and with enthusiasm, and who even let me speak in front of her class to recruit participants! To Dr. Francis, who helped me understand that people from many diverse academic disciplines actually do, when all is said and done, care about the same things, and who is living proof that people from different specialties can successfully work together. To Dr. Mary Main and Dr. Erik Hesse, who kindly and quietly actually volunteered their time advising us on how to skillfully and accurately code the precious transcripts produced by this study. You are both the epitome of rigorous scientists and the lessons you taught me will be shared with what I hope will be multiple generations of students.

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“What is becoming clear...is that although most genes might be conserved, the regulatory connections that control their expression might not be. Closely related species can connect up their genes in very different regulatory networks, while keeping the end result deceptively unchanged. ‘Problems with many solutions are the rule rather than the exception in living systems’...there are many combinations of contributing factors that can reach the same outcome. ‘You can’t understand any of this if you think at the single-gene level’....The information that determines biological function lies at a higher, more abstract level, in the entire network of genes, proteins and other factors that each act on the others in a series of nonlinear feedback loops. The body plan or feature that results is what scientists who study complex systems call an ‘emergent property’ -one that is more than the sum of its parts.” (Chouard, 2008, p. 300)

INTRODUCTION

It is now well known that developmentally, very little about humans is genetically determined, and that most health issues are a result of interactions between genetic, environmental and/or social factors (Danese, Pariante, Caspi, Taylor & Poulton, 2007; McDade, Hawkey & Cacioppo, 2006; Hernandez & Blazer, 2006; Rutter, 2006). As the quote set forth above explains, genes alone do not determine phenotype; if they did humans could look like weeds. In fact, the largest genome reported to date is in a small plant called *P. japonica* estimated to have 152.13 +/- .39 billion base pairs followed closely by, “...a long-bodied muck dweller known as the marbled lungfish” (Satter, 2010, at para. 2) reported to have approximately 132 billion basepairs (Pellicer, Fay & Leitch, 2010). The human genome has a modest 3.2 billion base pairs in comparison. Instead, development involves a complex, interactive and non-linear set of events regulating many biological mechanisms, including genes.

Research has only just begun to uncover the connection between genes, environmental stress and health issues (Ellis, Boyce, Belsky, et. al., 2011; Danese, Pariante, Caspi, Taylor & Poulton, 2007; McDade, Hawkey & Cacioppo, 2006; Taylor, et. al., 2006; Caspi, et. al., 2006). For example, Caspi and colleagues found that children with the short version of the monoamine oxidase A gene (“MAOA”) (a gene responsible for degrading neurotransmitters like serotonin) had higher levels of conduct disordered behavior *only when they were maltreated* (Caspi, et. al., 2002). The next year Caspi and colleagues found that individuals with the short form of the serotonin transporter gene, an allele of the 5-HTT gene that makes serotonin less efficiently, *who experience stressful events after age 21*, report more depressive symptoms than those who do not possess the short allele (Caspi, et. al., 2003). Later, Taylor, Way, Welch, Hilmer, Lehman & Eisenberger (2006) found that those individuals with two short copies of the serotonin transporter gene *who came from a “supportive” family environment* had the lowest depressive symptomology in adulthood while those who experienced *a stressful early life* experience had the highest level of depressive symptoms. Accordingly, the research suggests that early life experience in combination with genetic vulnerability results in greater risk for psychopathology. It thus makes sense to ask whether early experiences with attachment figures in combination with genetic vulnerability also results in higher risk for negative health outcomes such as mood or behavioral disorders. At least one study has, in fact, found that children with a genetic

polymorphism (the 7 repeat DRD4 gene—a version of the dopamine gene) who have insensitive mothers display significantly greater externalizing behaviors (Bakermans-Kranenburg & van IJzendoorn, 2006). Another study found that the short version of the serotonin transporter gene actually acted as a protective factor against anxiety in insecurely attached adolescents (Olsson, et. al., 2005). Finally, a new study has found that participants with the 7 repeat DRD4 allele who reported “problems” with their adopted parents (i.e., marital conflict) were at risk for higher ratings of unresolved loss or trauma (Bakermans-Kranenburg, et. al., 2011).

One way the interaction between genes and environment takes place is through an epigenetic process called methylation. The term “epigenetic” literally means “above genetics” and refers to genetic change that does not involve the resequencing of nucleotides (Allis, Jenuwein, Reinberg & Caparros, 2007). Essentially, current research in epigenetics involves the study of how genes are regulated (e.g., what determines the level of gene expression). Methylation is one of the processes by which genetic expression is usually silenced.¹ The purpose of this study is to determine whether methylation levels of a polymorphism in the serotonin gene-linked promoter region (5-HTTLPR) associated with the serotonin transporter gene (SLC6A4; heretofore the “5-HTT gene”) and of the glucocorticoid receptor gene (NR3C1; heretofore the “GR gene”), determined from peripheral blood samples, differ among 93 participants, approximately 18-25 years of age, according to socio-economic status (SES) and “attachment state of mind” (i.e., how individuals conceive of and act upon early attachment experiences). Attachment state of mind is determined by the Adult Attachment Interview (AAI) and a newer measure, the Adult Attachment Projective (AAP). In other words, the study asks whether there is a relationship between attachment experiences and the level of gene expression. The serotonin transporter polymorphism was chosen because of its already established relationship with psychopathology such as depression (Caspi, et. al., 2003; Taylor, et. al., 2006). Because insecure attachment is a risk factor for mood disorders such as depression (Allen, Porter, McFarland, McElhaney, & Marsh, 2007; McMahon, Trapolini, & Barnett, 2008; Sund & Wichstrom, 2002; Easterbrooks & Biesecker, 1996) it seems logical to look for an epigenetic relationship between attachment classification and the serotonin transporter polymorphism. The GR gene was chosen because it is not only implicated in many psychopathologies, but is also central to the regulation of stress (McEwen, 2007). Since attachment theory hypothesizes that the ability to regulate stress is developed in early life through relationships with primary attachment figures, it also makes sense to look for an epigenetic relationship between attachment classification and methylation of the GR gene.

It is hoped that this study will mark the beginning of a larger study of perhaps hundreds of participants that seeks to determine whether early attachment experiences are correlated with differences in specific gene or genome wide expression. This is a new area of research and the author is aware of only a few studies published examining the relationship between social experience and gene or genome wide expression (Chen, Miller, Kobor & Cole, 2010; Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, and Philibert, 2010; Miller, Chen, Folk, et. al., 2009; Cole, Hawkey, Arevalo, Sung, Rose and Cacioppo, 2007). It is hoped that by furthering understanding of the impact of social experience on health outcomes new and effective interventions can be fashioned to treat diseases currently classified as “mental health disorders” such as depression or conduct disorder. Effective interventions could have a dramatic impact on children who are victims of trauma, maltreatment and neglect.

It is hypothesized that significant differences in methylation levels will emerge between resolved (secure and insecure classifications) and unresolved individuals. It is not clear whether significant differences will emerge between secure and insecure participants, but if such differences exist it is hypothesized that they will appear between secure participants and low socio-economic (SES) insecure participants. A finding that significant differences emerge in methylation levels between secure, insecure and unresolved participants may further knowledge about how self-regulation develops in humans as well as lead to new treatments for psychopathology in the future. In fact, one very recent study actually examined the methylation levels of the polymorphisms in the 5-HTTLPR and found a higher risk of unresolved attachment classification among carriers of the long version of the 5-HTTLPR polymorphism (ll) with high levels of methylation in a sample of adopted participants (Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, and Philibert, 2010.) This study hopes to investigate methylation levels of the polymorphisms in the 5-HTTLPR with a non-adopted population, as well as methylation levels of the GR gene. A finding that methylation profiles correlate with attachment state of mind among an adopted and a non-adopted population would strengthen the hypothesis that early attachment relationships do influence epigenetic mechanisms.

In addition, because the AAP is a relatively new measure, the results of a brief concurrent validity study comparing the AAI with the AAP is presented. If a correlation between the AAI and the AAP is found, the AAP could prove to be a reasonable alternative to the AAI for populations coping with trauma because it does not require the participant to directly discuss attachment related experiences, reducing the possibility of iatrogenic injury. The AAP is also faster to administer, easier to code and less expensive than the AAI and thus could act as an alternative attachment measure for those without the funds or time to use the AAI. The study also examines whether attachment state of mind influences perceptions of SES, and social status as measured by the MacArthur Scale of Subjective SES (MSES) and the MacArthur Scale of Subjective Social Status (MSSS). It is hypothesized that lower SES insecure or unresolved participants will rate themselves lower in subjective social status than lower SES secure participants. Finally, the study reports on the concurrent validity between the Hollingshead Scale of SES (HSES) and another measure of SES specifically designed for administration to adolescents called the Family Affluence Scale (FAS).

First, the paper presents a description of attachment theory examining classical attachment theory as well as the views of those who see attachment as modifying or mediating self-regulation. This discussion is included here because it is hoped that the results of this study will help inform the debate about what exactly the role of attachment relationships is in development. Second, since attachment classification, the 5-HTT gene and the GR gene are all highly implicated in stress regulation, the paper then presents a brief summary of how stress functions in humans, and explains what the GR gene actually is and why it is important to the stress system. Third, a similar and brief discussion of the 5-HTT and the polymorphisms in the 5-HTTLPR follows. Fourth, a description of epigenetics as well as a review of human studies conducted examining the relationship between social experience, including attachment, and epigenetic mechanisms is presented. Fifth, the methodology used in the present study is set forth. Sixth, the results of the study are reported followed by a discussion section. The paper concludes with suggestions for future research.

CHAPTER I

LITERATURE REVIEW

A. ATTACHMENT

Attachment Theory. First developed by Dr. John Bowlby, attachment theory is a biological and ethological theory that conceives of attachment as an instinct² in the child to maintain proximity to a source of safety, usually the biological parent. Bowlby explains attachment in the following manner:

To say of a child that he is attached to, or has an attachment to, someone means that he is strongly disposed to seek proximity to and contact with a specific figure and to do so in certain situations, notably when he is frightened, tired or ill. The disposition to behave in this way is an attribute of the child, an attribute which changes only slowly over time and which is unaffected by the situation of the moment. Attachment, by contrast, refers to any of the various forms of behaviour that a child commonly engages in to attain and/or maintain a desired proximity.... The theory of attachment advanced is an attempt to explain both attachment behaviour, with its episodic appearance and disappearance, and also the enduring attachments that children and older individuals make to particular figures. In this theory the key concept is that of a behavioural system... (Bowlby, 1969/1982, p. 372)

In other words, attachment is a “behavioral system” the purpose of which is to ensure that the child remains proximate to, and develops in, a safe environment. Bowlby laid out his theory in three volumes entitled *Attachment* (1969/1982), *Separation: Anxiety and Anger* (1973), and *Loss: Sadness and Depression* (1980).

In his first volume, *Attachment* (1969/1982), Bowlby establishes the ethological basis of attachment theory, describing such behaviors in primates and non-primates. Sucking, clinging, following, crying, and smiling are all examples of attachment behaviors used to maintain proximity to the attachment figure (Bowlby, 1969/1982). Bowlby rejects psychoanalytic “drive theory,” and asserts that the attachment relationship cannot be attributed to “secondary drives,” such as the need for food, liquid, warmth, and sex. In other words, the infant does not form an attachment relationship because the mother feeds him. This point is evidenced by the experiments of researchers such as Harry Harlow who demonstrated that baby rhesus monkeys preferred a cloth monkey mother to a wire monkey mother that provided food, and Konrad Lorenz who demonstrated that goslings and baby ducks follow their mothers without receiving food or other rewards, and other similar experiments. Notably, in the experiments Bowlby mentions even baby monkeys who were “gravely maltreated” attached to their mothers. This last point is important because it is one of the facts that demonstrates why attachment behavior cannot be explained by social learning theory—young attach even to those attachment figures who abuse them. Instead, attachment is a behavioral system³ that evolved over time to ensure the survival of infants who without the protection of attachment figures would fall victim to predators or other environmental dangers (e.g., falling or drowning).

Bowlby also distinguishes attachment theory from traditional psychoanalytic theory on several grounds. In essence, Bowlby asserts that psychoanalysis is unscientific. For example, psychoanalysis is based on retrospective study relying on memory, a source that is inherently unreliable, and may lead a mental health professional to consider only those facts consistent with a particular diagnosis. Psychoanalysis, in Bowlby's view, also can't be scientifically tested. Bowlby adopts an ethological approach because it is based on years of empirical research, which could be tested. Ethology suggests that what is important to the developing infant is real world experience, a point of view that is consistent with Bowlby's experience. Moreover, attachment is a theory in which a hypothesis can be stated and then tested through prospective naturalistic or experimental studies that follow children as they develop.

Bowlby identified four phases in which attachment behavior develops. Phase one is called "orientation and signals with only limited discrimination of figure." This is a period in which the child's sensory systems begin to orient to the world around them and although even newborns demonstrate a preference for their mother's voice, smell, and touch, the ability to discriminate among individuals is limited. The baby typically uses crying, grasping, and reaching, visual tracking of movement, smiling, and babbling to engage and maintain proximity. This phase lasts from birth to eight weeks of age.

Phase two is called "orientation and signals directed toward one or more discriminated figure." During this phase the infant behaviors described in phase one are more focused on the parent. This phase lasts from approximately 12 weeks to 6 months of age. Phase three is called "maintenance of proximity to a discriminated figure by means of locomotion as well as signals." During this time the infant is able to increasingly discriminate favored individuals, and behaviors expand to include following departing parents, greeting parents upon their return, and using parents as a "safe base" from which to explore. Stranger anxiety is growing and the child may have a hierarchy of attachment figures. Notably, Bowlby expressly states that the child's principal attachment figure does not have to be the natural mother. The child's behavior toward the parent may become "goal-corrected" or "homeostatic." Bowlby explains that the infant attempts to maintain "homeostasis" by taking continuous account of the location of the attachment figure, and adjusting his or her behavior to achieve the "goal" of maintaining proximity. This phase lasts from about six or seven months and continues into the third year. Phase four is called "formation of a goal directed partnership." This is the point at which the child starts to understand that the parent has his or her own feelings and motives. Bowlby states that this realization allows the parent and child to start to create a more complex relationship that he calls a "partnership." This phase starts at about the middle of the third year.

Bowlby (1969/1982) hypothesized that the child internalizes the relationship with the caregiver, creating what he called "an internal working model" in the child.⁴ The function of the internal working model is to allow the child to make accurate predictions about what will happen in his or her world, and give some understanding of self-worth (e.g., a secure child develops a sense of his or herself as important and efficacious. An insecure child may conclude that he or she is not worthy of attention, and cannot effectively exercise control over his or her life). An internal working model of attachment is believed to operate throughout life. Bowlby notes, however, that attachment behaviors and objects may change or expand to include others as humans grow older. For example, attachment in adolescence changes as "[o]ther adults may

come to assume an importance equal to or greater than that of the parents, and sexual attraction to age-mates begins to extend the picture.” (Bowlby, 1969/1982, p. 207). In addition, as a person grows older the nature of the relationship with the parent may change drastically (e.g., cutting off all contact with the parent). Alternatively, some individuals may be unable or unwilling to redirect attachment behaviors to others. As people enter old age, attachment behavior may even be directed toward younger individuals. According to Bowlby, most individuals keep the relationship with their parents intact throughout life, but expand ties to other people. Bowlby also argues that attachment behavior in adult life is a “continuation” of attachment in childhood, as evidenced by the fact that when ill an adult will frequently demand attention of others, and seek proximity to trusted individuals in times of disaster.

Bowlby adds that attachment behaviors may even be directed towards groups and institutions:

During adolescence and adult life a measure of attachment behaviour is commonly directed not only towards persons outside the family but also towards groups and institutions other than the family. A school or college, a work group, a religious group or a political group can come to constitute for many people a subordinate attachment- ‘figure’, and for some people a principal attachment- ‘figure.’ In such cases, it seems probable the development of attachment to a group is mediated, at least initially by attachment to a person holding a prominent position within that group. Thus, for many a citizen attachment to his state is a derivative of and initially dependent on attachment to its sovereign or president. (Bowlby, 1969/1982, p. 207)

For Bowlby, it appears that the phenomenon of attachment acts as a mechanism furthering a sense of cohesiveness and safety in cultural or political groups

In Volume 2, *Separation* (1973), Bowlby challenged the old psychoanalytic notion that the parent was simply a “need gratifying object,” and that as long as a child’s physical needs were taken care of, separation from the parent was unimportant. Instead, Bowlby found that deprivation of a parent can be profoundly traumatic for a child, and in this volume specifically describes the anxiety and fear children experience when deprived of their parent. Bowlby draws on his work with colleagues such as James Robertson and others to describe the three stages children who are separated from primary caregivers experience: “protest,” “despair,” and eventually “detachment” from the primary caregiver. Protest, according to Bowlby is motivated by stranger anxiety, and despair by grief and mourning over the loss of the attachment figure, while detachment serves as a defense measure. Here Bowlby describes how toddlers (between 15 to 30 months of age) with healthy family relationships and no significant previous separations, experienced severe emotional reactions upon the departure of their parents, which included heavy crying, breakdown of elimination control, clinging to objects brought from home, hostile behavior, and behavioral stereotypes such as rocking or remaining still. Upon reunion with their primary caregivers (i.e., their mothers) all the children showed detachment for as long as three days, commonly followed by a period of ambivalence toward parents—demanding their presence but ignoring them or acting hostile while they were present. For some of the children this attitude lasted for not less than twelve weeks. All the

children also showed fear of being taken away from family again. Bowlby reported that the presence of a familiar companion or possession (e.g., a teddy bear) or parenting from a substitute parent could mitigate the intensity of the response from the children (as opposed to placing children in a strange environment with strange people), but only within limits. The separation from the mother still produced the same behaviors, albeit in lower intensity. As long as the separation was not too prolonged, however, the children did eventually demonstrate attachment behaviors again.

Bowlby makes it clear that he is not presenting a general theory of anxiety. Instead, he explains:

What is attempted, instead, is more limited. Young children are upset by even brief separations. Older children are upset by longer ones. Adults are upset whenever a separation is prolonged or permanent, as in bereavement. A pile of clinical reports, moreover, starting with Freud's early studies of hysteria and swelling to increasing volume in recent years, shows that experiences of separation and loss, occurring recently or years before, play a weighty role in the origin of many clinical conditions....

Study of the problem suggests, indeed, that Freud was probably mistaken in claiming that missing someone who is loved and longed for is the key to an understanding of anxiety. As likely as not there is no single key: fear and anxiety are aroused in situations of many kinds. What seems certain, nevertheless, is that missing someone who is loved and longed for is one of the keys we need, and that the particular form of anxiety to which separation and loss give rise is not only common but leads to great and widespread suffering. That being so, let us grasp the key at hand and see what doors it opens. (Bowlby, 1973 p. 30)

Thus, Bowlby acknowledges that many situations may cause anxiety. Attachment seeks to understand only one source of anxiety; that of a possible defection of, or an actual loss or separation from, an attachment figure.

Also in contrast to Freud, Bowlby conceives of certain sources of fright in young children such as darkness, being alone, loud noises, height, animals, rapid approach, sudden movement, and strangeness, not as sources of neurotic anxiety, but rather as normal and evolutionarily adaptive fears that evolved in order to enhance survival of the vulnerable infant by motivating the infant to seek further proximity to an attachment figure. Bowlby is careful to add that there are other sources of "clues" to danger, such as cultural and learned cues. Bowlby also points out that fear can be learned from parents and cites the results of some of the few studies on the participant at the time (e.g., a child whose parent is afraid of dogs or insects will also be afraid of dogs or insects). This phenomenon may be of some interest as it appears to integrate certain principles of social learning theory with attachment as well as physiological self-regulation. Social learning theory, as conceived by Bandura (1977), predicts that a child can learn what is dangerous from a parent's behavior. Once fear is evoked, the child's physiological stress system is activated and, if the degree of fear is substantial enough, attachment behavior is evoked. Researchers know now that if a child is participated to constant stress, the physiological system regulating stress (the limbic-hypothalamic-pituitary-adrenal axis or LHPA axis) will eventually become hypersensitive. Thus, the child's ability to self-regulate is directly impacted by the attachment relationship, although in some instances mediated by

social learning. Bowlby makes the point that how children will react to a natural disaster may depend on how parents react (citing studies on child reactions to natural disasters such as tornados). Bowlby does appear to strongly criticize the position of psychoanalytic theory that the tendency of even adults to seek proximity to family members in times of disaster represents “regressive” behavior. In Bowlby’s view, behavior such as gathering family members together in time of crisis (e.g., war or natural disaster) is a healthy manifestation of the attachment system.

In addressing why individuals respond differently to fear, Bowlby acknowledges the role of genetics and biology, but he states that ultimately how an individual develops turns on a process of interaction between the person and his or her environment. Bowlby sets forth three major propositions relevant to self-regulation:

- 1) The individual who is confident that an attachment figure will be available when desired is “much less prone to either intense or chronic fear” than an individual who lacks such confidence.
- 2) A sensitive period exists from about six months to five years of age during which expectations about the availability of the attachment figure are formed. A less sensitive period continues for approximately ten years after that, although steadily diminishing as time passes. After this time expectations persist relatively unchanged throughout life.
- 3) Expectations regarding the availability of the attachment figure are “tolerably” accurate reflections of actual experience. The expectations an individual develops around the availability of the attachment figure create the internal working model discussed in Volume 1. (Bowlby, 1973, p.202)

Confidence in whether the attachment figure is accessible turns on two considerations: 1) “whether or not the attachment figure is judged to be the sort of person who in general responds to calls for support and protection”; and 2) whether or not the individual feels that they are “the sort of person towards whom anyone, and the attachment figure in particular, is likely to respond in a helpful way.” In practice, these factors are “confounded,” and thus, the model of the attachment figure and the image of the self are “complementary and mutually confirming.” Accordingly, a loved child is likely to feel not only loved by his parents, but lovable by others, as well. But an unwanted child is likely to feel unwanted by both his parents and others. A person who develops “anxious” or “insecure attachment” is one who has no confidence that his or her attachment figure will be available or responsive when needed, and will, therefore, adopt a strategy of remaining in close proximity to them in order to ensure their availability.

Citing a number of studies, Bowlby asserts that there are many factors that can cause anxious attachment, but the primary reasons center around experiences that shake a person’s confidence about the availability of the caregiver—including actual separations (perhaps due to hospitalization or illness of the parent) combined with unstable daily substitute care, rearing in an institution (even so called “good institutions”) threats to abandon the child as a disciplinary

measure, marital conflict, and attempted suicide. Bowlby adds one caveat: Moore's (1969) study of children who experienced substitute care before the age of five found that some of the children exposed to unstable care developed detachment rather than anxious attachment. These children, primarily boys, apparently did not trust or care for others and became aggressive, disobedient, and quick to retaliate. Bowlby categorically rejects the notion that anxious attachment is caused by "an excess of parental affection," noting that the evidence points in the opposite direction.

Anger is also closely connected to anxiety primarily because both emotions are elicited by the experience of separation, and once aroused each has the tendency to exacerbate the other. Consequently, following periods of separation a child is likely to develop anxious and possessive attachment behavior as well as anger toward the attachment figure for leaving the child in the first place. The purpose of the anger, however, is to provide an incentive to discourage future separation (i.e., avoiding angering the child). Anger also reflects the fact that the child cannot feel confident that the attachment figure will be available in the future should the need arise.

Bowlby continues to make the point that, contrary to traditional thought, loving, consistent, and timely parental attention not only does not spoil a child but is, instead, the key to the development of self-reliance. As Bowlby explains:

For not only young children, it is now clear, but human beings of all ages are found to be at their happiest and to be able to deploy their talents to best advantage when they are confident that, standing behind them, there are one or more trusted persons who will come to their aid should difficulties arise. The person trusted provides a secure base from which his (or her) companion can operate...Paradoxically, the truly self-reliant person when viewed in this light proves to be by no means as independent as cultural stereotypes suppose. An essential ingredient is a capacity to rely trustingly on others when occasion demands and to know on whom it is appropriate to rely. A healthily self-reliant person is thus capable of exchanging roles when the situation changes; at one time he is providing a secure base from which his companions can operate; at another he is glad to rely on one or another of his companions to provide him with just such a base in return. (Bowlby, 1973, pp.359-60)

To demonstrate this point Bowlby then draws on the results of a number of studies examining the family relationships of adults, adolescents, and younger children, including the work of his colleague, Mary Ainsworth. Bowlby explains that Ainsworth's findings show that parents who are sensitive, accessible, and responsive to a child, who accept his behavior and are cooperative, produce a child who, by his first birthday, is already developing self-reliance in addition to a trusting and enjoyable relationship with his parents.

Bowlby then presents a new model of personality development. Bowlby's model:

...conceives of personality as a structure that develops unceasingly along one or another of an array of possible and discrete developmental pathways. All pathways are thought to start close together so that, initially, an individual has access to a large range of pathways along any one of which he might travel. The one chosen, it is held, turns at each and every stage of the journey on an interaction between the organism as it has developed up to that

moment and the environment in which it then finds itself. Thus at conception development turns on interaction between the newly formed genome and the intra-uterine environment; at birth it turns on interaction between the physiological constitution, including germinal mental structure, of the neonate and the family, or non-family, into which he is born; and at each age successively it turns on the personality structure then present and the family, and, later, the wider social environments then current. At conception the total array of pathways potentially open to an individual is determined by the make-up of the genome. As development proceeds and structures progressively differentiate, the number of pathways that remain open diminishes. (Bowlby, 1973, p.364)

In illustrating the difference between psychoanalytic notions of personality development and attachment theory Bowlby employed the metaphor of two different railway systems:

The traditional model resembles a single main line on which are set a series of stations. At any one of them... a train can be halted, either temporarily or permanently; and the longer it halts the more prone it becomes to return to that station whenever it meets with difficulty...

The alternative model resembles a system that starts as a single main route which leaves a central metropolis in a certain direction but soon forks into a range of distinct routes. Although each of these routes diverges in some degree, initially most of them continue in a direction not very different from the original one. The further each route goes from the metropolis, however, the more branches it throws off and the greater the degree of divergence of direction that can occur. Nevertheless, although many of these sub-branches do diverge further, and yet further, from the original direction, others may take a course convergent with the original; so that ultimately they may even come to run in a direction close to, or even parallel with, routes that have maintained the original direction from the start. In terms of this model the critical points are the junctions at which the lines fork, for once a train is on any particular line, pressures are present that keep it on that line; although, provided divergence does not become too great, there remains a chance of a train taking a convergent track when the next junction is reached. (Bowlby, 1973, pp. 364–65)

In other words, when the individual encounters challenges or stresses, instead of regressing or “fixating” at a previous point in development, a new path of development is forged heading into a different direction than would have been the case had the stressor never occurred. Bowlby is said to have borrowed this concept from Winnicott, who characterized development as a tree whose branches grow into different directions depending on experience (Weinfeld, Whaley, & Egeland, 2004). Note that Bowlby is also saying that just because the train “diverges” from its original track, does not necessarily mean that it cannot return to that track at some point in the future.

In Volume 3, *Loss*, Bowlby asserts that children experience grief and mourning whenever attachment behaviors are activated but the attachment figure is unavailable (Bowlby, 1980). Earlier, psychoanalysts such as Anna Freud had argued that children could not mourn the loss

of a parent because of insufficient ego strength. In this volume Bowlby emphasizes that children grieve loss much as adults do. Bowlby identifies four main “phases” of “mourning.” The first phase is one of “numbing,” in which individuals are “stunned” or shocked by their loss. This phase can be interspersed with bursts of distress and anger. Anger can be directed to many possible targets including doctors, the lost loved one for “abandoning” the family, anyone that might have been involved in the death, and the bereaved himself. This is the phase Bowlby associates with the stage of “protest” a child encounters when separated from a parent. The second phase is “yearning and searching” for the lost loved one, a phase that can take months or years. At this point the reality of the loss begins to register. This phase is marked by “intense pining,” “spasms of distress,” restlessness, insomnia, preoccupation with thoughts of the lost loved one, and often a sense that the loved one is still present (e.g., a doorbell ringing at 5 pm is interpreted as the deceased husband returning from work). Anger may also be a part of this phase. Bowlby associated this phase with the searching behavior that a child engages in when seeking contact with a parent. The third phase is called “disorganization and despair.” Bowlby associates this phase with the “detachment” stage that infants who are separated for long periods from their caregiver experience. Bowlby feels strongly that individuals must go through a period of anger, despair, and pain in order to finally accept that the loss is permanent. Final acceptance is critical in order that the individual may go on to reorganize their life and form new attachment relationships ⁵

In *Loss* Bowlby hypothesized that internal working models (“IWMs”) may also bias the way individuals process separation and loss, and determine the “course” mourning will take. One way IWMs can “bias” the mourning process is through the operation of two major defensive strategies, the purpose of which is to protect the individual from anxiety, fear, and/or grief.⁶ The first, “defensive exclusion,” occurs when an individual has adopted two conflicting IWMs of the same relationship but only one is conscious and the other is suppressed, or parts of one may rise to the conscious level and then descend depending on circumstances. For example, one IWM could place the parents above criticism and view the child as “unjustifiably angry” and “ungrateful” and the other could view the parents as unavailable and unaffectionate, and the child as justifiably angry with the parents. Either model could result in pathological mourning resulting in either excessive self-blame or blame of others. Moreover, the two different IWMs can also fluctuate in terms of influencing awareness, possibly resulting in erratic behavior. The second defensive strategy, “segregation of principal systems,” appears to involve two completely different IWMs, each consistent in its own right, but neither communicating with the other. It has been suggested that Bowlby is probably talking about dissociation, possibly including dissociative identity disorder or fuges (Bretherton, 2005). A person whose attachment model is that of an available, responsive, and helpful caregiver, and who sees himself as a lovable and valuable person is probably not going to engage in a great deal of self-blame, or feel abandoned, and will be able to reorganize his life, “...fortified perhaps by an abiding sense of the lost person’s continuing and benevolent presence” (Bowlby, 1980, p. 243).

Bowlby sees detachment, as well as other insecure attachment behaviors, as a defense mounted against mourning by infants and children that is likely to stabilize and persist, resulting in pathology unless reversed. For example, prolonged absence of grieving is thought of as a deactivation of the attachment system by defensive exclusion with negative consequences such as suppression of care seeking or engaging in compulsive caregiving in an effort to compensate

for the inadequate care received earlier in life. Another example, alluded to above, occurs when children have information they know that their parents do not wish them to know (e.g., they witnessed the other parent abuse drugs, commit suicide, etc.); they may participate in this information to defensive exclusion for a variety of reasons including the possibility that a parent will withdraw love or abandon them if they do not. A problem arises when, upon reaching adulthood, these individuals who have essentially adopted a strategy of placating parents find that they are unable to create more appropriate strategies. In other words, insecure attachment behavior strategies found in the internal working model formed in childhood may originally have had adaptive value, but they lose their adaptive value when the environment changes and they cannot change their behavior accordingly.

As the individual ages, internal working models stabilize for multiple reasons. First, patterns of interactions between attachment figures and the child grow less accessible to awareness as they become habitual. Second, dyadic patterns of interactions are more difficult to change than individual patterns because of the pressures that attachment figures place upon the child, such as those suggested above. Third, Bowlby thought that IWMs may be difficult to bring to consciousness because they are developed before the child has verbal representation.

If Bowlby can be considered the “father” of attachment theory, then his friend and close colleague, Dr. Mary Ainsworth, can be considered the “mother” of attachment theory. The next section discusses Ainsworth’s contribution to the study of attachment in humans.

Contribution of Dr. Mary Ainsworth. Dr. Mary Ainsworth created the first empirical method of assessing attachment behaviors, called the “Strange Situation (SS).” Her work led her to conclude that the quality or security of the attachment relationship depended on the attachment figure’s sensitive and consistent response to the child’s signals—specifically, the caregiver’s ability to perceive the infant’s signals accurately, and to respond to those signals in a timely and appropriate manner (de Wolff & van IJzendoorn, 1997). Ainsworth laid out her thoughts around maternal sensitivity in her landmark study, *Infancy in Uganda* (1967). In this observational study of 28 infant-mother dyads among the Ganda of Uganda in the mid-1950s, Ainsworth explained:

The term “motherly care” is too unspecific. The connotation is one of routine care, of tending to the baby’s physical needs. Two more specific variables which have emerged as highly significant in my subsequent study of American babies during the first year of life, are (1) the sensitivity of the mother in responding to the baby’s signals and (2) the amount and nature of the interaction between mother and baby. Sensitivity of response to signals implies that signals are perceived and correctly interpreted and that the response is prompt and appropriate. The signals may be of need and distress or they may be social signals. Sensitivity to signals tends to ensure that the care the mother gives the baby, including her playful interaction with him, is attuned to the baby’s state and mood—at the baby’s own timing, not at the mother’s timing. Routine care may be undertaken with little interaction. It is interaction that seems to be most important, not mere care, and particularly conspicuous in mother-child pairs who have achieved good interaction is the quality of mutual delight which characterizes their exchanges. (Ainsworth, 1967, p. 397)

Ainsworth also added two other variables that she thought contributed to maternal sensitivity: “the extent to which she (the mother) is free from preoccupation with other activities, thoughts, anxieties, and grief so that she can attend to the baby and respond fully to him; and finally and obviously, the extent to which she can satisfy his needs, including his nutritional needs” (Ainsworth, 1967, p. 400). In other words, it is not mere interaction with the child that is critical, but consistent, meaningful interaction that communicates to the child that he or she has been noticed, heard, understood and is worth responding to on the child’s terms. Children with such a sensitive and predictable caregiver are then able to use that caregiver as a “secure base” from which to explore the environment with confidence that the caregiver will come should danger arise.⁷ Children with sensitive caregivers are usually classified as securely attached. Contemporary research has confirmed that sensitive and empathic parenting predicts security of attachment among infants (Posada, Jacobs, Richmond, Carbonell, Alzate, Bustamante, & Qiceno, 2002; Barnett, Ganiban, & Cicchetti, 1999; de Wolff & van IJzendoorn, 1997).

Although Ainsworth found a very strong correlation between maternal sensitivity and secure attachment (.78) some subsequent studies have found a clearly significant but more modest correlation. For example, van IJzendoorn’s meta analysis of 66 studies found a correlation of .24, a Columbian-American study found a correlation of .45 to .51 (Posada et al., 2002), and Canadian studies found correlations of .60 and .51 respectively (Pederson & Moran, 1996; Pederson, Gleason, Moran, & Bento, 1998). It is suggested by some that the problem is not that maternal sensitivity is not as important as once thought, but that researchers are having problems identifying sensitivity in parental-child interactions (Pederson et al., 1998). Studies that have more closely replicated Ainsworth’s original procedures have found the higher correlations (Posada et al., 2002; Pederson et al., 1998). Studies have also found a more modest role for maternal sensitivity as a mediator between attachment security in the parent and security in the child, however, leading to what is now commonly referred to as the “transmission gap” (Tarabulsy, Bernier, Provost, Maranda, Larose, Moss, Larose, & Tessier, 2005; van IJzendoorn, 1995). In other words, it appears that maternal sensitivity plays a role in attachment security in infancy, and that a robust relationship exists between parental attachment and infant attachment classification (van IJzendoorn, 1995). The issue is what is mediating the relationship between parental and infant security. Since maternal sensitivity seems to be a modest mediator between parent and infant security, other proposals have been forwarded such as parental cognitions regarding the desires and beliefs of the infant, and the role of attachment as a regulator of emotion (Pederson et al., 1998). Recently, a review of 13 studies of 962 dyads concluded that parental contingent responsiveness, a component of parental sensitivity, predicted infant attachment; the researchers found an effect size of 0.61 when contingent responsiveness was an independent variable and 0.58 when a dependent variable (Kassow & Dunst, 2004).

Ainsworth then developed a classification system for identifying attachment behaviors that she categorized as “avoidant” (or “A”), “secure” (or “B”), and “ambivalent/resistant” (or “C”). Solomon and George (1999, p. 291) succinctly describe the behaviors typically associated with each classification in the following manner.

Babies classified as “secure” use the attachment figure as a “safe base” from which to explore the environment. Secure babies will show some signs of distress or “missing” their parent when left alone but upon the parents return they will greet the parent with a “smile, vocalization or gesture” (Solomon & George, 1999, p. 291). If distressed, the baby will signal the parent (crying, reaching, making eye contact) and when they are sufficiently comforted they return to exploring their environment.

Babies classified as “avoidant” explore their environment but display little “safe base” behavior; they appear to avert their eyes from the attachment figure and focus on toys. These babies show little distress when left alone, and upon the parent’s return may actually seek distance from the parent, or if picked up may turn away.

Babies classified as “ambivalent” or “resistant” are palpably distressed when entering the playroom and do not engage in exploration. Upon separation they show a great deal of distress and cannot be easily comforted once the parent returns; they may even alternate between signaling that they want to be held but then rejecting the parent.

When attachment researchers started to work with high-risk families it became increasingly clear that some children did not fit any of the above categories. This realization led Dr. Mary Main and Dr. Judith Solomon to identify a fourth category, disorganized attachment (or “D”) (Main & Solomon, 1986, 1990). Infants showing disorganized attachment behaviors do not show a consistent pattern of attachment behaviors; the term disorganized actually refers to a breakdown of attachment strategies for organizing responses to the need for comfort and security when under stress. Disorganized behaviors are considered idiosyncratic to each child. Such behaviors include apprehensive, helpless, or depressed behaviors (e.g., crying and falling huddled to the floor or putting their hands to their mouths with hunched shoulders in response to their parent’s return following a brief separation), unexpected alterations of approach and avoidance toward the attachment figure, and other conflict behaviors such as prolonged “freezing” or “stilling”, or slowed “underwater” movements; elements of the three organized strategies may be present (Solomon & George, 1999, p. 291; Main & Solomon, 1986, 1990; Main & Hesse, 1990; Lyons-Ruth & Jacobvitz, 1999; Lyons-Ruth, 1996).

Disorganized strategies have been associated with maternal depression, maternal childhood histories of violence or abuse, maternal inpatient psychiatric histories, poverty, and documented child maltreatment, and with the parent’s unresolved loss or trauma (Barnett, Ganiban, & Cicchetti, 1999; Lyons-Ruth, 1996; van IJzendoorn, 1995; Lyons Ruth, Alpern, & Repacholi, 1993; Lyons-Ruth, Repacholi, McLeod, & Silva, 1991). In fact, Cicchetti and colleagues found, in one of the first series of studies to examine the correlation between maltreatment and disorganized attachment, that more than 80% of the maltreated children in their studies were disorganized (Barnett, Ganiban, & Cicchetti, 1999; Beeghly & Cicchetti, 1994, Cicchetti & Barnett, 1991; Carlson, Cicchetti, Barnett, & Braunwald, 1989). In 1999 van IJzendoorn and colleagues reported that only five studies had been published on maltreatment and disorganized attachment (Crittenden, 1988—reports on two studies; Lyons-Ruth, Connell, Grunebaum, & Botein, 1990; Barnett, Ganiban, & Cicchetti, 1999 Valenzuela, 1990) and found that across studies approximately 48% of maltreated children were disorganized, with effect sizes ranging from 0.03 to 0.60 (van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999).

Accordingly, the attachment system is active in adulthood, and there are individual differences in adult attachment behavior that develop as a result of early attachment experiences and are found in attachment representations. Those representations are measured by a structured narrative procedure called the “Adult Attachment Interview” (“AAI”), a semistructured interview for adults about childhood attachment experiences and the meaning the individual adult gives to those experiences (George, Kaplan, and Main, 1984/1985/1996). The AAI is based on a number of concepts:

- 1) That internal working models of attachment function at least partially outside consciousness;
- 2) That they are based on attachment-relevant experiences;
- 3) That infants begin to develop models that guide behavior in attachment relationships in the first year of life;
- 4) That representations provide guidelines for behavior and affective appraisal of experience;
- 5) That formal operational thought allows the individual to observe and assess a given relationship system, and hence that the model of the relationship can be altered without an actual change in experiences in the relationship; and
- 6) That the models are not templates, but are processes that serve to obtain or to limit access to information (Crowell, Fraley, & Shaver, 1999, p. 438).

During the AAI the adult is asked about his or her relationship with primary caregivers, ordinary events in which the attachment system is presumed to be activated (e.g., when the interviewee was ill or hurt), and experiences of loss, and the meaning that the adult attributes to all these experiences and how they apply to the interviewee in terms of his or her personality and own parenting. After being transcribed, the interview is then evaluated in terms of discourse, looking for unintended incoherencies and inconsistencies. The critical question is whether the individual is able to give an integrated, logical account of experiences and their meaning. Discourse style and language are said to reflect the individual’s “state of mind with respect to attachment” (Hesse, 1999, p. 421). The narrative is then assigned to one of four classifications: “autonomous” (a secure category); two insecure categories—“dismissing” (an avoidant category), and “preoccupied” (an ambivalent/resistant category); and for adults who report attachment-related traumas of loss and/or abuse, and who demonstrate confusion and disorganization during the interview, a fourth category called “unresolved.” A fifth classification is called “cannot classify” and involves individuals who show behaviors from more than one category. This category is associated with a high degree of incoherence and is considered highly insecure (Hesse, 1999; Crowell, Fraley, & Shaver, 1999).⁸

The correlation between parental state of mind with regard to attachment and infant classification based on the Strange Situation has consistently shown a robust correlation. Van IJzendoorn (1995) published a meta-analysis, looking at 18 samples in which infant SS response to a particular parent was compared to the classification assigned to the parent on the basis of the AAI (854 dyads). When the two insecure adult and infant attachment categories were collapsed to obtain a two-way classification, the correspondence between parental and infant attachment classifications was 75%. The combined effect size was 1.06. Correspondence for the three-way

cross tabulation was 70%. Studies in which the interview was administered prior to the birth of the first child (Fonagy, Steele, & Steele, 1991) showed 69% correspondence for the three-way classifications. Other studies have also reported that prenatal maternal characteristics such as maternal anxiety predict subsequent infant security as measured in the Strange Situation (Del Carmen, Pedersen, Huffman, & Bryan, 1993).

More current attachment research. Ainsworth's studies in Uganda and Baltimore represent the first two empirical studies of attachment relationships. Since then a number of longitudinal studies have been conducted. These include the Berkeley Longitudinal Study by Mary Main, Eric Hesse, and Nancy Kaplan, which led to the discovery of the disorganized classification in the Strange Situation and the development of the AAI (Main, Hesse & Kaplan, 2005); the Minnesota Longitudinal Study led by Alan Sroufe and colleagues, which followed a high-risk population for what is now over 30 years (Sroufe, Egeland, Carlson & Collins, 2005); the Pennsylvania Infant and Family Development Project and the National Institute of Child Health and Human Development ("NICHD") Study of Early Child Care conducted by Jay Belsky and colleagues, examining among other things the impact of child care on attachment classification (NICHD, 1997); the London Parent-Child Project conducted by Howard and Miriam Steele, Peter Fonagy, and colleagues who reported the links between pregnant women's responses to the AAI and later child ratings of security in the Strange Situation (they were also the first to report similar links between fathers and infants) (Steele, Steele & Fonagy, 1996); the Bielefeld and Regensburg longitudinal studies conducted by Karin and Klaus Grossmann in Germany—one of the first studies finding significant stability over time in attachment classification (Grossmann, Grossmann & Waters, 2005); the Haifa Longitudinal Study conducted by Ari Sagi-Schwartz and colleagues in Israel, examining the attachment status of children raised on a kibbutz (Sagi-Schwartz & Aviezer, 2005); Joan Stevenson-Hinde's work on attachment and temperament in Madingley, England (Stevenson-Hinde, 2005); Mary Dozier's studies of infants in foster care (Dozier, Stovall, Albus & Bates, 2001); and work by Michael Rutter and his colleagues (Rutter et al, 2004) following the development of Romanian adoptees in the United Kingdom. Although a comprehensive review of all these studies is beyond the scope of this paper, examples from all of them are used throughout this paper (for a review see Grossmann, Grossmann, & Waters, 2005).

Recall that Bowlby and Ainsworth asserted that the internal working models developed in childhood maintain themselves by biasing perceptions and cognitions (Bowlby, 1969/1982, 1973). Internal working models are thought to be the products of childhood attachment behaviors that extend into adulthood, eventually becoming part of the unconscious. If an individual's environment is stable, internal working models should become more stable as time passes. In other words, early attachment would be expected to predict subsequent parent-child relationships only if attachment is stable across this time span (Youngblade & Belsky, 1992). In fact, a number of studies have confirmed that attachment behavior can be stable throughout childhood, assuming intervening factors (e.g., highly stressful events such as divorce, death of a parent, illness or death of a child, etc.) do not occur (Weinfield, Whaley, & Egeland, 2004; Weinfield, Sroufe, & Egeland, 2000; Waters, Merrick, Treboux, Crowell, & Albersheim, 2000; Hamilton, 2000; Benoit & Parker, 1994; Wartner, Grossmann, Fremmer-Bombik, & Suess, 1994; Main & Cassidy, 1988). Stability rates have generally been reported between 73% and 96% (Benoit & Parker, 1994). The intergenerational transmission of attachment patterns is also notable—in their study of infants, mothers, and grandmothers, Benoit & Parker (1994) found that 65% of 77 grandmother-mother-infant triads had corresponding attachment classifications in all three

generations.

Secure attachments have been found to be correlated with positive developmental outcomes during childhood, including social competence with peers and less negative emotionality (Szewczyk-Sokolowski, Bost, & Wainwright, 2005; Schneider, Atkinson, & Tardif, 2001—a meta-analysis; Kochanska, 2001; Sroufe, Egeland, & Kreutzer, 1990; Erickson, Sroufe, & Egeland, 1985; Children with secure attachments in middle childhood and adolescence have been found to have enhanced attentional abilities, self-confidence, and cognitive performance (Jacobsen, Edelstein, & Hofman, 1994).

Later studies have also found that insecure attachment may be associated with childhood behavioral problems. For example, in Cohn (1990) researchers looked at 89 mother-child dyads and examined the association between attachment and child-peer social competence. Using peer nomination measures, peers identified insecure boys as significantly more aggressive than secure boys, and less liked. Teachers using the “Classroom Adjustment Rating Scales” and “Health Resources Inventory” also reported insecurely attached boys as having significantly more behavior problems than securely attached boys and being less competent than securely attached boys. In Park & Waters (1989) the security status of 33 four-year-olds and their best friends was ascertained, and their relationship was assessed using the Dyadic Relationship Q set (a measure designed to describe the behavior of a pair of children). The study found that statistically significant differences existed between secure-secure dyads (“SSDs”) and insecure-secure dyads (“ISDs”): SSDs more often negotiated a fair settlement, more often negotiated peacefully to settle issues, and more often easily reached agreement. In 1990 Speltz, Greenberg, and DeKlyen found in a study of 50 preschool-aged children with externalizing behaviors that 84% were classified as insecure as opposed to only 28% in the control group (these findings were successfully replicated in Greenberg, Speltz, DeKlyen, & Endriga, 1991). Speltz, DeKlyen, Greenberg, & Dryden (1995) found in a sample of 50 boys between the ages of 3.5 and 5.5 years, half of whom were diagnosed with Oppositional Defiant Disorder (the clinic group) and half of whom had no identified behavioral problems, that attachment assessment of the boys actually provided better discrimination of clinic and control groups than did behavioral measures long in use. Allen, Marsh, McFarland, McElhaney, Land, Jodl, & Peck (2002) found in a nonrandomized, observational, two-year longitudinal study of 117 at-risk adolescents that preoccupied adolescents were at heightened risk for delinquent behavior between age 16 and age 18.

Research has also found a statistically significant association between disorganized attachment and aggression even when family factors are controlled (Lyons-Ruth, Easterbrooks & Cibelli, 1997). For example, in 1997 Lyons-Ruth and her colleagues found that among a cohort of seven-year-olds whose attachment status was assessed, 83% of the children teachers identified as demonstrating high levels of externalizing behaviors were disorganized in their attachment behavior in infancy. It has been suggested that out of the insecure attachment classifications, disorganized attachment most closely approaches a clinical disorder, especially when considering that disorganized children are “drastically overrepresented” in the population of parents with “severe psychological abnormalities” (Zeanah, Keyes, & Settles, 2003, p. 25; van IJzendoorn & Bakermans-Kranenburg, 2002).

It should be emphasized that although most of the children demonstrating clinical levels of externalizing behaviors are insecurely attached, most insecurely attached children, at least in low-risk populations, do not show clinical levels of externalizing behaviors (Greenberg, 1999). Some studies have found that insecure attachment did not predict externalizing behaviors in low-

risk samples (Jacobsen & Hofmann, 1997). A meta-analysis of 12 studies involving 734 dyads by van IJzendoorn and colleagues (van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999) found an effect size of .29 between disorganized attachment and externalizing symptomology, not nearly as strong as other studies. It is of interest to note, however, that the overall average effect size was higher for those studies that measured attachment and behaviors at the same age (van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999).

In addition, some of the studies cited by van IJzendoorn use parental self-report measures (e.g., CBCL) rather than teacher or third-party observers. It is possible that parents may not be accurate reporters of their children's behaviors. Also note that some of the studies examine attachment classification concurrently with externalizing behavior, and some assess security at an earlier or later age. It appears that the highest correlation between attachment and externalizing behavior occurs when attachment status is assessed concurrently with behavior. It is also possible that insecure children from low-risk populations have access to other resources that compensate for insecure parental attachment relationships (e.g., a secure relationship with another family member or a teacher, etc.). In addition, it is possible that disorganization itself is not enough to produce externalizing behaviors and that other factors such as family adversity or maltreatment are necessary before externalizing behaviors reach a clinical level (recall that disorganization can result from maltreatment or from the effects of parental trauma on the child). The evidence does appear strong, however, that disorganization and insecure attachment can be considered a risk factor for behavioral problems.⁹

Perhaps the most compelling evidence supporting Bowlby's emphasis on the importance of experience are the results of studies examining attachment relationships between adopted and foster youth and their parents. Many of these studies are led by Dr. Mary Dozier of the University of Delaware. In 2001, Dozier had found in an observational prospective study of 50 foster-parent–foster-child dyads (part of a larger longitudinal study) placed with their foster parents between birth and 20 months of age, that a two-way correspondence between maternal state of mind (as measured by the AAI) and infant attachment (as measured by the Strange Situation) was 72%, similar to the level seen among biologically intact mother-infant dyads (Dozier, Stovall, Albus, & Bates, 2001). Dozier also published later studies making similar findings among younger children (Stovall-McClough & Dozier, 2004). Other research involving children adopted as infants (van Londen, Juffer & van IJzendoorn, 2007; Stams, Juffer, & van IJzendoorn, 2002) and children adopted during latency (Hodges, Steele, Hillman, Henderson, & Kaniuk, 2005) showed that children could develop secure attachments to their adopted parents and experience improvements in socio-emotional and cognitive development.

Finally, to date research has generally supported Bowlby's assertion that attachment is a universal phenomenon, (Behrens, Hesse & Main, 2007; Posada, Jacobs, Richmond, Carbonell, Alzate, Bustamante, & Qiceno, 2002; van IJzendoorn & Sagi, 1999; Vereijken, Riksen-Walraven, & Kondo-Ikemura, 1997; Posada, Gao, Wu, Posada, Tascon, Schoelmerich, Sagi, Kondo-Ikemura, Haaland, & Synnevaag, 1995; Grossmann, Grossmann, Huber, & Wartner, 1981; Ainsworth, Blehar, Waters, & Wall, 1978; Ainsworth, 1967), although some suggest that culture can impact the way security is expressed (Posada & Jacobs, 2001) and some assert that much more research is needed in cultural differences over the meaning of sensitivity and competence (Rothbaum, Weisz, Pott, Miyake, & Morelli, 2001).¹⁰

It should also be noted that current attachment theorists have repeatedly pointed out that

although attachment may be one of the most profoundly important human developmental influences, it is not the only influence on development, and not the only influence on even social development (Sroufe, Egeland, Carlson, & Collins, 2005). Sroufe has repeatedly argued that theorists need to observe the parameters of attachment theory in making predictions about later life. He suggested limiting those predictions to constructs that have some relationship to attachments—i.e., inner confidence and self-worth, and intimacy in relationships (Sroufe, 1985). Small effect sizes (.20) found in a meta-analysis of 63 studies examining the link between secure attachment and peer relationships in general, but higher for close friendships than for unfamiliar peers (.24 vs. .14), appear to support his point (note that higher effect sizes were reported for other countries such as Germany (.43 and .47), the United Kingdom (.57), and Israel (.28) (Schneider, Atkinson, & Tardif, 2001). Moreover, when attachment is related to life “outcome,...attachment variations are not properly viewed as linear, inevitable causes.” From the outset, Bowlby (e.g., 1973) argued for a pathway/process model in which both early and later experiences of various kinds worked together to shape developmental outcomes and in which cause was viewed in probabilistic terms” (Sroufe, Egeland, Carlson, & Collins, 2005, p. 51). For example, parents may provide ethical guidance and academic support, which may not necessarily be linked to attachment per se but can affect life outcomes. There can also be other influences—for example, peer relationships. Moreover, although the evidence supports the proposition that early attachment experiences are represented and carried forward in life, “setting conditions for seeking, interpreting, and reacting to later experiences,” later experience can also alter representations (Sroufe, Egeland, Carlson, & Collins, 2005, p. 67). In other words, “the development of attachment is not considered to be fixed during the first year of life...but should be regarded as ‘environmentally labile,’ particularly in the early years of life” (van IJzendoorn, 1995, p. 412). In fact, the longitudinal studies of high-risk populations conducted by Sroufe and colleagues (Weinfeld, Sroufe, & Egeland, 2000) has demonstrated that security in childhood does not ensure security later in life. Negative life experiences such as maltreatment, poverty, being raised by a single parent, and death or serious illness of a primary caregiver are all associated with an altering of attachment classification from secure to insecure or disorganized. A secure attachment classification in infancy might, however, provide a child with some resiliency to stress in later years; Sroufe, Egeland, and Kreutzer (1990) found that children with poor adaptation in various social, cognitive, and emotional competencies in preschool but who had secure attachments in infancy showed the “greatest capacity for rebound” in terms of emotional health/peer competency in early elementary school than children who showed similar problems in preschool, but were insecure in infancy. Sroufe himself stated as he and his colleagues were about to start his longitudinal studies:

We would not expect a child to be permanently scarred by early experiences or permanently protected from environmental assaults. Early experience cannot be more important than later experience, and life in a changing environment should alter the qualities of a child’s adaptation. (Sroufe, 1978, p. 50, as quoted in van IJzendoorn, 1995, p. 412)

In other words, it is all of life’s experiences that matters: “...Established patterns of adaptation may be transformed by new experiences while, at the same time, new experiences are framed by, interpreted within, and even in part created by prior history of adaptation. Bowlby’s was a dynamic view of development” (Sroufe, 2005, p. 350).

In sum, according to Bowlby, attachment refers to the instinct in the child to seek proximity to a source of safety, the parent figure. Attachments evolved because they increased the infant's chance of surviving in the early evolutionary environment. Attachment behaviors take any form that results in maintaining proximity to a parent and are mediated by a behavioral system that becomes "goal corrected" or "homeostatic"—the infant attempts to maintain homeostasis by taking continuous account of his or her attachment figure's location. The infant uses perception of himself or herself and the environment when engaging in goal-corrected behavior. Intense emotions (i.e., love) exist during the creation of these attachments, and the threat of loss of an attachment figure causes anxiety, and actual loss causes grief and mourning. Anxiety and loss is likely to create anger. Because emotions are connected to attachment relationships, emotional psychopathology is frequently found in pathological affectional bonds. Psychopathology does not occur because an individual is "fixated" to some earlier developmental period, but because development has taken a "deviant pathway" (Bowlby, 1980). Overreactivity or complete deactivation of the attachment system is pathological and can occur at any time during life. Affectional bonds in adulthood are built upon attachment patterns initially created in childhood. Attachment behavior leads to the development of attachments at first between parent and child, and later between adults. Attachment relationships continue throughout life, and impact personality and psychosocial development through their influence on expectations individuals possess about themselves, and about their relationships with others.

Attachment as a mediator between biology/genetics and behavior. Those who conceive of attachment as a mediator argue that self-regulatory mechanisms create the behaviors associated with the attachment relationship. Frequently, this discussion occurs in the context of what is called "temperament." In other words, attachment acts as a mediator between "temperament" and behavior. "Temperament" is a construct that has been defined in various ways but is generally viewed as the manner in which self-regulation and motor/emotional reactivity is manifested in the individual (Posner & Rothbart, 2000), and is more a function of the biological or genetic make-up of the child and less, if any, a product of the child's relationship with caregivers (Zeanah & Fox, 2004). A significant literature has also developed examining the relationship between attachment and temperament (for a literature review see Vaughn & Bost, 1999). What is of interest here is whether attachment impacts self-regulation conceived of as "temperament," or whether current measurements such as the Strange Situation really assess temperament, which creates the character of the attachment relationship.

Although others have argued that what is considered a variation in attachment security is really temperament (Chess & Thomas, 1982),¹¹ the researcher who may have adopted the most extreme version of this view is Jerome Kagan, a professor emeritus of psychology from Harvard. As noted above, Kagan does not dispute that attachment exists or that it is critical for early development. But Kagan asserts that insecure attachment does not put children at "high risk for later psychological problems," (Kagan, 1996) and what is being measured in attachment studies is really temperament, which is biologically and genetically determined (Shea, 2004; Kagan, 1996). In other words, Kagan feels that what is currently being studied is not behavior reflecting a transactional history between parent and child, but rather a genetic and biologically determined set of behaviors he calls "temperament."

Kagan defines temperament as "...the stable moods and behavior profiles observed in infancy and early childhood" (Schwartz, Wright, Shin, Kagan, Whalen, McMullen, & Rauch, 2003, p. 1952). During the 1980s Kagan and his colleagues followed a cohort of children through their first eight years of life, and some until adulthood (Kagan, Reznick, & Snidman, 1988; Kagan, 1989; Kagan, Reznick, & Gibbons, 1989; Kagan & Snidman, 1991; Kagan, Snidman, & Arcus, 1998; Schwartz et al., 2003). They found that approximately 15% of the children in their sample demonstrated "inhibited" behavior and 15% demonstrated uninhibited behavior. Children with inhibited temperament tended "to be timid with people, objects and situations that are novel or unfamiliar" and uninhibited children spontaneously approached "...novel persons, objects and situations" (Schwartz et al., 2003, p. 1952). Kagan and his colleagues discovered that uninhibited and inhibited children also had physiological differences in terms of heart rate, papillary dilation during cognitive tasks, vocal cord tension when speaking under moderate stress ("perturbation"), and salivary cortisol levels (Kagan, Reznick, & Snidman, 1988; Kagan, Reznick, & Gibbons, 1989). Kagan refers to uninhibited infants as "high reactive infants," who have a low threshold of activation in the amygdala to sensory stimulation and as a result react more physically and emotionally—e.g., move their limbs and "fret or cry" when their low threshold has been passed (Kagan, Snidman, & Arcus, 1998). Similarly, uninhibited infants are "low reactive infants" and show minimal motor activity and distress in response to the same stimulation. In a later study, Kagan estimated that 20% of the healthy European-American infants he studied were inhibited and 40% were uninhibited (Kagan, Snidman, & Arcus, 1998). Kagan asserts that an uninhibited temperament in early childhood, along with a specific rearing environment, is associated with externalizing behaviors and antisocial behavior in adolescence, and an inhibited temperament is associated with anxiety disorders in children and adults (Schwartz et al., 2003). Relatively recently Kagan and colleagues did a follow-up study of 22 adults who had been categorized in the second year of life as inhibited or uninhibited and found that inhibited adults did show a significantly greater response in both the right and left amygdalae to novel faces compared with those who had been categorized as uninhibited (Schwartz et al., 2003). Kagan and his colleagues suggested that the differential amygdala findings may be influenced by or caused by temperamental factors persisting from early childhood, supporting his earlier hypothesis that inhibition is caused by a low threshold for activation in the amygdalae. Thus, avoidant infants are not invoking a strategy that minimizes attachment behavior, but are simply less inhibited or fearful than secure and ambivalent babies. Ambivalent babies are more inhibited and irritable, and secure babies fall in between.

Although Kagan has repeatedly argued that the behaviors captured by the Strange Situation reflect temperament, he has never conducted a study testing this hypothesis. Studies that examine the fear level of avoidant babies have found that avoidant infants were as distressed or significantly more distressed than some secure infants (B1 and B2 infants) during separation (Ainsworth et al., 1978; Braungart & Stifter, 1991; Frodi & Thompson, 1985). As noted above, other studies have found that avoidant infants are less behaviorally distressed on separation but have the same or higher levels of physiological distress (e.g., heart rate and cortisol levels) than secure infants (Spangler & Grossmann, 1993; Thompson & Lamb, 1984). Two studies also found that ambivalent babies are no more distressed on separation than some secure infants (B3 and B4) (Braungart & Stifter, 1991; Frodi & Thompson, 1985). Moreover, there is no evidence that irritable babies are more likely to be

classified as ambivalent (van den Boom, 1994; Crockenberg, 1981 as cited in Cassidy, 1994). There is some evidence that ambivalent babies may be prone to irritability, however. One 1985 Japanese study found a disproportionate number of C babies showed irritability and proneness to distress; this finding was interpreted as supporting Kagan's theory, but researchers in that study failed to follow the specific Strange Situation instructions (Miyake, Chen, & Campos, 1985). Traditionally, Japanese infants were never left alone during their first year of life, and this sample was drawn from a population practicing traditional parenting. When these babies were left alone in the Strange Situation they demonstrated significant distress—not surprising since they were never without their mothers. Strange Situation instructions require that the mother re-enter the playroom when babies experience such distress, but here the babies were allowed to cry for the entire three-minute period. Researchers have suggested that any baby that is distressed for that long is bound to be hard to calm (Takahashi, 1986; Sroufe, 1985).

Generally, most of the studies examining attachment and its relationship to temperament find no correlation between temperament and behavior in the Strange Situation. Of four studies that did find a relationship between attachment and temperament, all used self-report measures of the mother to ascertain infant security, such as the Attachment Q-Sort, which was found to be an unreliable measure of attachment security by van IJzendoorn in a meta-analysis examining the reliability of the version using parent-reports and observer ratings,¹² and/or temperament was assessed after attachment patterns had a chance to develop (Szewczyk-Sokolowski, Bost, & Wainwright, 2005; Kochanska, 1995; Vaughn, Stevenson-Hinde, Waters, Kotsaftis, Lefever, Shouldice, Trudel, & Belsky, 1992; Seifer, Schiller, Sameroff, Resnick, & Riordan, 1996). Even when the mother-reports of attachment were used, the correlation between attachment categories and temperament constructs was weak (i.e., less than 25% of the variance in the attachment score found in common with temperament dimensions; Vaughn et al., 1992). Generally, in those studies that do not use self-report measures of attachment, infant security is unrelated to measures of temperament (Shamir-Essakow, et. al., 2005¹³; Vorria, Papaligoura, Dunn, van IJzendoorn, Steele, Kontopoulou, & Sarafidou, 2003; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Del Carmen, Pederson, Huffman, & Bryan, 1993; Belsky & Rovine, 1987; Mangelsdorf, Gunnar, Kestenbaum, Lang, & Andreas, 1990; Weber, Levitt, & Clark, 1986; Egeland & Farber, 1984). But it should be noted that some of these studies, although they did not find overall statistical significance between measures of temperament and attachment security, did find infant negativity to be significantly related to security of attachment. For example, in Del Carmen et al., (1993) although the overall statistic for discriminating attachment classification by temperament (defined as “positivity, negativity, sociability and soothability”) was not significant, one temperamental variable, “infant negativity,” was related to attachment security. Also, the study found that, using discriminant analysis, the strongest predictor of infant security was distress management interaction (i.e., how mothers respond to infants' signals of distress).

Relatively recently (2003), Nathan Fox published a longitudinal observational study of 172 children followed from 14 months to 4 years of age that appears to be somewhat at odds with Kagan (Burgess, Marshall, Rubin, & Fox, 2003). The purpose of the study was to determine the extent to which both child temperament and attachment relationships

independently or interactively predicted child behavioral outcomes. Child attachment classifications were assessed in the Strange Situation at 14 months, behavioral inhibition at 24 months, and social behavior and cardiac variables at age 4. The study found that children who were classified as avoidant in the Strange Situation and uninhibited at 24 months showed the highest levels of externalizing behaviors, while secure children who were also classified as uninhibited showed the lowest level of externalizing behaviors (although the difference between secure uninhibited children and extremely inhibited ambivalent children did not appear significant, the difference was in the same direction). The study also found that although heart rate did not differ between avoidant and secure or ambivalent children at age 14 or 24 months, by age 4 avoidant children had a significantly lower heart rate than either secure or ambivalent children. The authors opined that the “most notable” finding was that attachment relationship quality in infancy appeared to influence physiological functioning in early childhood, rather than the reverse. The authors stated, “Indeed, there were no concurrent associations between cardiac functioning and inhibition or attachment, and the predictive relation was one of early attachment quality predicting later physiology” (p. 829). This study not only fails to support Kagan’s assertions, but also appears to lend support to classical attachment’s view of the development of self-regulation.

There are a number of other arguments advanced against interpreting attachment behaviors as reflecting mere temperament rather than a transactional history of the relationship between attachment figure and child. Sroufe and others have pointed out that if the behaviors captured by the Strange Situation were really just manifestations of temperament, why do children show different behaviors with different caregivers? Research has shown that children can have different attachment classification with mother vs. father vs. other caregivers (Steele, Steele, & Fonagy, 1996; Goossens & van IJzendoorn, 1990; Belsky, Garduque, & Hrnacir, 1984; Grossman, Grossman, Huber, & Wartner, 1981; Main & Weston, 1981), although there is some evidence that the attachment relationship with the primary caregiver (usually the mother) can influence the attachment relationships with others—but not vice versa. In other words, in Steele, Steele, & Fonagy (1996) researchers found that the infant-mother Strange Situation classification was significantly related to the father-infant classification (e.g., if the relationship with the mother was secure, the relationship with the father was more likely to be secure), but the infant-father relationship was not significantly related to the infant-mother Strange Situation classification. This finding is also consistent with Bowlby’s contention that the relationship with the primary caregiver influences other attachment relationships. Moreover, attachment classifications have repeatedly been associated with sensitivity of caregiving (Ainsworth et al., 1978; De Wolff & Van IJzendoorn, 1997), and the association between maltreatment and disorganized attachment is particularly compelling. In addition, attachment classification is participant to change when primary caregivers are participant to life stress, a phenomenon that supports the notion that attachment patterns are a function of relationships.

Today, there appears to be growing support for the notion that “temperament” and attachment behavior are two different phenomena; temperament may influence the way attachment behaviors are expressed, but not their organization (Sroufe, 1985). As Sroufe has explained:

Whether an infant cries none, a little, some or a lot is of little relevance

to the determination of the security of attachment (i.e., B or non-B). Rather, it is how the infant responds to the caregiver when distressed (contact seeking, absence of anger, returning to play when settled) or not distressed (greeting, seeking interaction upon reunion) that allows the classification of secure attachment. (Sroufe, 1985, p. 3)

There is evidence to support this position. Thompson and Lamb (1984) and Frodi and Thompson (1985) discovered that avoidant infants and those classified as B1 and B2 (the B classification has four subgroups: B1, B2, B3, and B4) showed similar arousal patterns in the Strange Situation (such as low separation distress, long onset latency and rise time, and a brief recovery) as did resistant infants and those classified as B3 and B4 (strong and quick reaction to mothers' departure, brief onset latency and rise time, and an extended recovery time). In 1987 Belsky and Rovine asked whether the emotional expression differences documented by Frodi and Thompson (1985) and by Thompson and Lamb (1984) between the A/B1–2 and C/B3–4 groups were temperamental in origin. After replicating the results found in the Thompson studies, Belsky and Rovine surmised that newborns with less central nervous system (CNS) integrity are more likely to express insecurity in a resistant as opposed to avoidant manner, and the reverse is true for infants with more CNS integrity. Infants with difficult temperaments would express their security by crying, approaching, and clinging to their mothers (C/B3–4) and those who are temperamentally less vulnerable to stress would express their security by greeting mother across a distance and engaging in other forms of distal interaction (A/B1 and B2). They concluded that the SS security-based classification system does assess the quality of the infant-parent relationship rather than temperament, whereas the A1–B2 vs. B3–C2 classification system assesses temperament. In other words, infant temperament affects the manner in which security or insecurity is expressed, rather than whether or not the infant develops a secure or insecure attachment.¹⁴

Accordingly, the great weight of research does not support the notion that attachment behaviors as measured in the Strange Situation are simply measures of temperament. In fact, it appears that current measures of temperament are measuring something different from attachment strategies, although it is not clear that temperament and attachment are not related in some fashion. As Belsky and Rovine have postulated, it is possible that temperament influences how attachment behaviors are expressed.

Attachment as a moderator of development. Of particular relevance to the present study are the views of those who view attachment relationships as a moderator of development. These theorists see the self-regulatory process as a separate entity from the attachment relationship, but they see the attachment relationship as modifying this process in some fashion. In other words, attachments impact the relationship between self-regulation as an original variable and behavior as an outcome variable. Attachment is, in essence, a coping mechanism.

One of the major researchers representing this point of view is Megan Gunnar. Gunnar is well known for her studies regarding psychophysiology and childhood development. In Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss (1996), Gunnar and colleagues explain that several studies have used factor analysis to demonstrate that physiological responses to stressful situations involve two factors: an “effort” factor that is significantly associated

with vigilance, attention, and involvement, and a “distress factor” that involves feelings of helplessness, loss of control, fear, and anxiety. High cortisol levels are associated with the distress factor. Relevant to this discussion, research shows that “the ability to predict and control stimulation”, and “the presence of supportive others,” reduces the responsiveness of the limbic-hypothalamic-pituitary-adrenocortical system (“LHPA”)—part of the neuroendocrine system which secretes cortisol from the adrenal glands (Nachmias, et. al., 1996, p. 509). Gunnar does not appear to see the operation of the LHPA axis as created by an environmental stimulus, but as influenced by an environmental stimulus, which in turn effects how people influence their own environment. Gunnar hypothesizes that the availability and behavior of the parent should act to help the child cope with stress and thus keep cortisol levels low. In fact, in Nachmias et al. (1996), Gunnar and her colleagues examined the role of attachment security in moderating the level of cortisol production in 77 “inhibited” toddlers who were exposed to novel events. The study found that cortisol levels rose only in toddlers with insecure attachments. It was also observed that the mothers of the insecure toddlers consistently interfered with their child’s own coping efforts.

In a somewhat similar study, Gunnar and her colleagues assessed 83 babies at 2, 4, 6, and 15 months of age during a well-baby exam (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996). Cortisol readings were taken before the exam. During the exam two inoculations were given, and cortisol readings were taken again 20 minutes after the exam. Maternal responsivity was assessed using Ainsworth’s sensitivity criteria (Ainsworth et al., 1978), and mothers were coded as “responsive,” “ignoring,” or “intrusive.” Infant behavior and distress was also coded. *The Toddler Behavior Assessment Scale* was filled out by the mothers when the babies were 15 months old, the fearful scale being of most interest. At 18 months the toddlers were assessed in Strange Situation. The study found that in the high fearful group, insecurely attached toddlers (5 As and 2 Cs) had higher cortisol levels than secure toddlers. But in the average to low fearful group cortisol score did not differ by attachment security. Follow-up univariate tests revealed significant differences in maternal responsiveness; infants classified as secure at 18 months had more sensitive and responsive mothers in the well-baby visits at 2 to 6 months than infants who would be classified as insecure. Pretest cortisol levels were significantly lower at 2 to 6 months for securely attached infants compared to insecure infants. The authors concluded that the results confirmed that secure attachment essentially “buffers” infant brains from elevations in cortisol and that these protective effects may be particularly apparent for inhibited children. The authors opined that one function of the attachment relationship may be to protect an infant's brain from damage due to increased cortisol levels. The fact that insecurely attached children do not receive this protection may be what puts them at risk of anxiety disorders.

Another recent Gunnar study, conducted in Germany, found that after entering child care secure toddlers had lower cortisol levels than insecure toddlers (Ahnert, Gunnar, Lamb, & Barthel, 2004).

Van Bakel & Riksen-Walraven (2004) sought to determine whether attachment security moderated the relationship between cognitive level and cortisol reactivity. Their study states that infants with higher levels of cognitive competence demonstrate higher levels of cortisol. The assumption is that higher levels of cognitive competence help the infant recognize the stress involved in important situations. The study used a modified version of

the Strange Situation to assess attachment security, and evaluated infants in a task designed to elicit temperamental dimensions of social fearfulness and anger. Cognitive capacity was assessed with the Bayley Mental Scale of Infant Development. The study found that infant cortisol reactivity was significantly and positively related to infant anger and cognitive competence. The study also found that attachment security did moderate the relation between cognitive level and cortisol reactivity; insecure infants with high cognitive levels did have the highest cortisol levels while secure infants of any cognitive level and insecure infants with low cognitive level had lower cortisol reactivity. This study is at odds with the view that cognitive competence usually implies better emotional and behavioral control. The authors acknowledge this fact, but small increases in cortisol may increase competence and essentially, that may be the price that insecurely attached children pay for higher cognitive competence without the buffering effect of a secure attachment. This does appear to be an interesting point; higher levels of cognitive competence mean that individuals are more aware of the stresses involved with important events. Attachment security may influence the individual by providing enough confidence and self-esteem to be able to weather the stress that such knowledge brings.

One point that should be noted about all these studies linking secure attachment with reduced levels of cortisol is that the effect was observed in the presence of the attachment figure, usually the mother. Recently, Gunnar stated that she is unaware of any research demonstrating that a history of secure attachment acts as a buffer to damaging levels of cortisol outside the presence of the attachment figure (Gunnar & Vasquez, 2006). Nevertheless, she does point out that her own research shows that nine-month-old infants, separated from their mothers for 30 minutes, showed no elevations in cortisol when they were randomly assigned to a sensitive and supportive caregiver (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992). Thus, it appears that the factor “moderating” cortisol is sensitive and supportive behavior.

Other studies have reported that securely attached children either do not experience increases in cortisol during the Strange Situation or experience lower increases; arguably, these other studies also support the conception of secure attachment as a moderator of behavior (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Spangler, Schieche, Ilg, Maier, & Ackermann, 1994; Spangler & Grossman, 1993). It must be emphasized, however, that later studies have not been able to replicate the finding that avoidant children demonstrate just as high or higher cortisol levels in the Strange Situation (Spangler & Schieche, 1998)—although Spangler and Schieche did find that children classified as ambivalent in the Strange Situation did experience adrenocortical activation—and it is now apparent that children who are exposed to chronic stress have what is described as “hypocortisolism” or low levels of cortisol (Gunnar & Vasquez, 2001). Current studies have found that children who have been sexually or physically abused have higher morning cortisol than other children (Cicchetti & Rogosch, 2001), while children who suffer from depression have higher nighttime levels of cortisol (Forbes, Williamson, Ryan, Birmaher, Axelson, & Dahl, 2005); and children in foster care have atypical patterns of cortisol production compared to children who were never in foster care (Dozier, Manni, Gordon, Peloso, Gunnar, Stovall-McClough, Eldreth, & Levine, 2006). Accordingly, it may be that insecurity of attachment is linked to both high levels of cortisol and lower basal cortisol in the morning hours, and possibly the development of what Gunnar calls a “more blunted

cortisol response to stressors post infancy” (Gunnar & Vazquez, 2001) (it is thought that children with a history of exposure to chronic violence “upregulate” glucocorticoid receptors as an adaptation to protect the brain from the damaging effects of high cortisol levels (Heim & Nemeroff, 2001)). Thus, studies linking attachment insecurity with cortisol levels must now be approached with considerable caution.

One point made by Marshall and Fox (2005) in their study of temperament and Strange Situation subgroup classifications was that infants classified as B3 or B4 tended to have high levels of negative affect during separation, much like C babies, but the difference was that their negative affect decreased rapidly upon reunion with their mother. Marshall and Fox also cited Braungart and Stifter’s (1991) findings that B3 and B4 babies also showed a high level of regulatory behaviors such as orientation to people and objects during separation by looking at the stranger or the door. C1/C2 babies displayed lower levels of such regulatory behaviors. The “ontogenesis” of these regulatory abilities would be the history of the infant’s caregiving and ecology. Thus, maternal sensitivity, in addition to creating attachment security, could also have a moderating effect on temperament.

Jacobsen, Huss, Fendrich, Kruesi, & Ziegenhain (1997) is arguably another study of attachment as a moderator. The purpose of the study was to determine whether children’s ability to delay gratification was related to attachment classification. The ability to delay gratification is considered a critical component of the development of self-regulation because it is linked with the ability to inhibit impulses, deal better with frustration, and achieve higher levels of social and cognitive competence (Sethi, Mischel, Aber, Shoda, & Rodriguez, 2000). The authors expected that secure attachment would “facilitate” children’s delay behavior, explaining that because secure children develop “ego resilience,” a result of having experienced an available and responsive caregiver, they have developed enough “inner strength and persistence” to tolerate a delay in a frustrating task (Jacobsen et al., p. 412). They found that children’s ability to delay gratification at age 6 was predicted by both concurrent measures of attachment as well as infant attachment measured in the Strange Situation at age 12; secure children were able to wait the longest periods of time while insecure children had the most difficulty waiting, with disorganized children demonstrating the shortest waiting tolerance. After further analysis, the study concluded that overall attachment quality, but not attachment quality at age 6, was significantly related to children’s ability to wait. The authors interpreted this result as lending support to Bowlby’s contention that self-regulatory abilities are best predicted by children’s overall attachment experience during the years of early childhood. It was also found that avoidant children who exhibited high cognitive functioning could wait as long as securely attached children, but not avoidant children with average or low cognitive functioning, suggesting that high cognitive ability may serve as a protective factor with respect to delay capabilities. High cognitive ability did not, however, appear to play this role for secure or disorganized children and the authors do not explain why not. It might be that even high cognitive ability cannot compensate for the regulatory problems experienced by disorganized children, or it could be that the measure of cognitive ability used in the study was not adequate (the Bayley scales were administered at 19 months of age). In addition, secure children scored higher on the Bayley scales than the insecure groups—thus, it is possible that the secure children also had a cognitive advantage. Recall that one of the advantages of security is supposed to be the development of flexible thinking—primarily because the child does not

have to adopt defense mechanisms for self-protection. A child who could think more flexibly would probably be better able to reason that delay was in their self-interest than a child whose thinking was more rigid. Thus, security of attachment might impact delay abilities indirectly. In other words, security in effect acts as a moderator on cognitive development.

Jay Belsky and his colleagues have also proposed a moderating effect for attachment. That is, secure attachments may be linked to certain outcomes depending on the environmental variations existing in the population studied. Belsky and Fearon have suggested that secure attachment may act as a sort of protective factor and insecurity functions as a risk factor. Thus, secure children would be less affected by high-risk environments than insecure children (Belsky & Fearon, 2002). In 2004 Fearon and Belsky examined data from 918 children from the National Institute of Child Health and Human Development Study of Early Child Care Attachment security was assessed in the Strange Situation when the children were 15 months old, and attentional skills were evaluated at 4.5 years of age using maternal reports and a computerized laboratory attentional task. The study found that secure attachment was associated with better attentional skills as tested by the computer tasks, and moderated the effects of gender or risk or both. The study supported the notion that attachment security moderates a child's cognitive and attentional capacities, particularly when the child lives in a high-risk environment.

As the discussion above suggests, there are few studies directly examining the role between self-regulation and attachment. Researchers argue that attachment plays at least one of three roles. Researchers like Kagan suggest that although early attachments are clearly important to the development of an infant, the infant is nevertheless born with a certain temperament, which stays relatively stable throughout life and is essentially determined by genetics and biological factors, and influenced very little by environmental factors. According to Kagan, "attachment behaviors" are really manifestations of temperament. Classical attachment theorists argue that the attachment relationship actually creates self-regulation. The third school of thought argues that the attachment relationship actually moderates the relationship between self-regulation and behavior. There is very little evidence to support Kagan's argument that attachment behaviors are merely a function of temperament. There are, however, a number of studies finding that attachment does moderate the reactivity of the LHPA axis, and cardiac, cognitive, and social variables. Those studies finding that attachment moderates cortisol levels are problematic, because it is now understood that glucocorticoids may be dysregulated in many different ways (e.g., in some children cortisol levels will rise in response to stress but in children exposed to chronic stress cortisol production may be "blunted"). Moreover, the fact that attachment may act as a moderating factor in some instances does not preclude self-regulatory capacities such as attention or the ability to delay gratification from mediating the relationship between attachment and behavior. In fact, in the studies that do attempt to perform a moderating and a mediating analysis, the model that uses self-regulatory capacities as a mediator fits the data better (Eisenberg, Gershoff, Fabes, Shepard, Cumberland, Losoya, Guthrie, & Murphy, 2001). It is also possible that all three models are right under some circumstances. Temperament may play a role in so far as how secure behaviors are expressed, as suggested by Belsky & Rovine (1987). Attachment may act as a moderator for children born with significant temperamental differences. The study by Burgess, Marshall, Rubin, & Fox (2003) is

particularly instructive in this regard: Recall that the researchers found that avoidant uninhibited children had the highest level of externalizing behaviors of any of the other attachment classifications. But who had the lowest level of externalizing behaviors? Children with the same uninhibited temperament, but who were securely attached. This study suggests that Kagan may be quite right that children are born with some temperamental characteristics, but how these characteristics manifest themselves is determined by environmental influence. In other words, attachment matters very much—so much so that one can tell very little about an individual's behavioral outcomes from their “temperament.” Moreover, other factors besides attachment may impact self-regulation, such as parental warmth and gentle discipline (Davidov & Grusec, 2006; Kochanska, Aksan, Knaack, & Rhines, 2004).

All these studies leave at least one overarching question: How is it that attachment relationships impact self-regulation? This is actually a very complicated question, the answer to which requires knowledge of regulatory physiology, brain development, and genetics. The connection between attachment, regulatory development, and genetics is an area scientists are just beginning to investigate. This study attempts to contribute to this investigation by examining two genes closely related to stress regulation—the GR gene and polymorphisms associated with the 5-HTT gene. Accordingly, the next section will present a brief description of the GR gene, the human stress response and the 5-HTTLPR polymorphism.

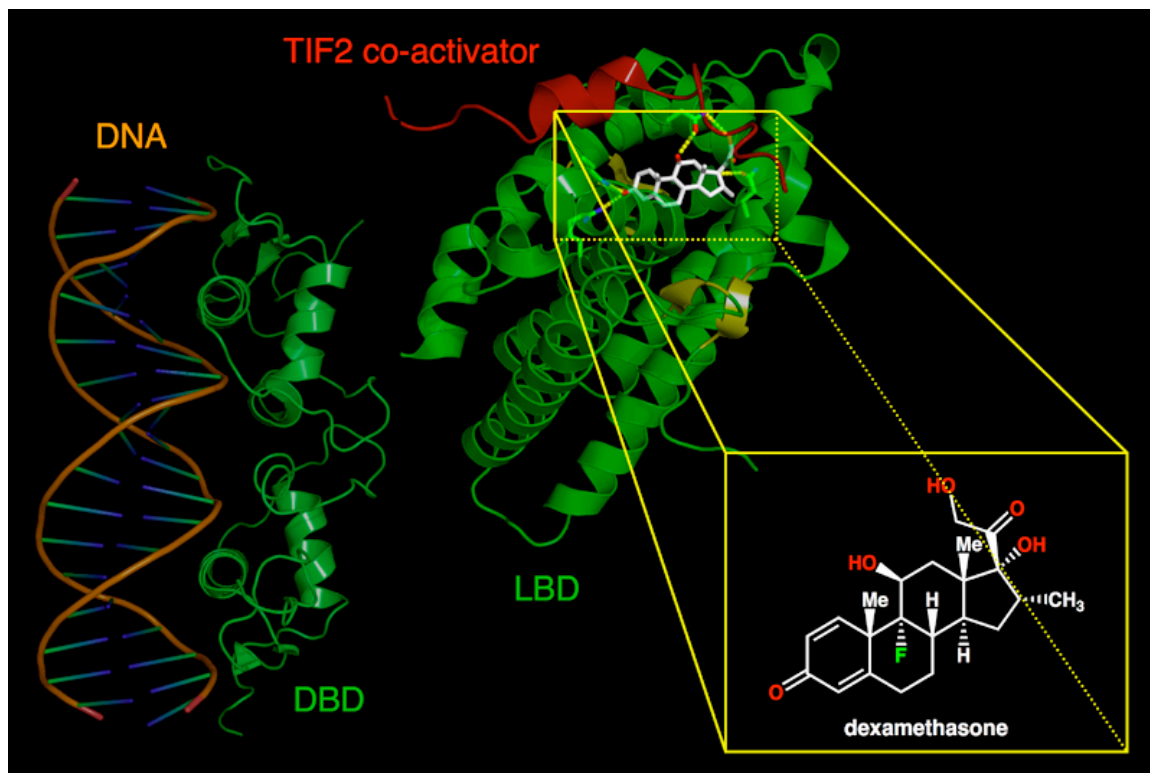
B.

THE GLUCOCORTICOID RECEPTOR GENE AND THE HUMAN STRESS SYSTEM

The Glucocorticoid Receptor (GR) Gene. The GR is encoded by the NR3C1 (nuclear receptor subfamily 3, group C, member 1) gene and is found on chromosome 5 (5q31—the letter “q” refers to the long arm of the chromosome, region 31). The GR gene was chosen for this study because it mediates the actions of one of the most important steroids in the human body—cortisol. Cortisol is critical to adults because it regulates glucose metabolism and blood pressure, impacts the immune system, and controls lipid metabolism and maintenance of energy homeostasis (Van Craenenbroeck, De Bosscher, Vanden Berghe, Vanhoenacker, Haegeman, 2005). Cortisol is also vital to embryonic development, particularly lung development and tissue maturation (Van Craenenbroeck, De Bosscher, Vanden Berghe, Vanhoenacker, Haegeman, 2005). When cortisol binds to the GR its primary function is gene transcription. Although, as noted above, cortisol has many functions, one of the most important is the role it plays in the stress system. Accordingly, cortisol and the GR are discussed in the context of the stress system. But first it may be useful to review briefly what the GR is and how it impacts genetic expression.

Cortisol functions by binding to two receptors in the human body; the GR and the mineralcorticoid receptor (MR). There are GR's and MRs in every cell. The MR's are thought to have a higher affinity to cortisol; in other words, because they bind to cortisol more easily, they are occupied at lower cortisol levels and may inhibit the HPA axis during periods of low activity. GR's, however, are occupied when levels of circulating cortisol are higher.

The actions of cortisol and its GR receptor are represented below. In this illustration, cortisol is surrounded by the ligand binding domain ((LBD)--the green ribbons--a ligand is simply a molecule that binds with another chemical to form a more complex entity—so here cortisol would be the ligand). The molecular structure of cortisol is displayed in white. The LBD is responsible for binding the cortisol to the receptor. The DNA binding domain simply binds the GR and cortisol with relevant short sequences of DNA located in the promoter region of the gene called glucocorticoid response elements (GRE).



*Image Retrieved from 5/1/10 from http://en.wikipedia.org/wiki/File:Glucocorticoid_receptor.png.

How does cortisol impact gene expression? Cortisol easily crosses cell membranes and binds to GR located in the cytoplasm of the cell (Barnes, 2006). GRs in the cytoplasm are usually bound to proteins called “molecular chaperones” such as “heat shock protein 90” which help protect the receptor. Once cortisol binds to the GR receptor, it enters into the nucleus and then the DNA where it binds at GRE’s, and leads to changes in gene expression (Barnes, 2006). When GR interacts with GRE’s it typically leads to an increase in gene transcription but can lead to a decrease, one of the most established examples of which is decreasing CRF (corticotrophin releasing factor). It is believed that cortisol directly regulates an estimated 10-100 genes per cell, but may also influence additional genes indirectly (Barnes, 2006).

The occupied GR can impact gene expression in number of ways. GR can (a) turn on inflammatory genes; (b) enhance expression of genes already expressed; (c) silence certain genes such as the CRH gene by dislocating activators and binding to DNA; (d) dislocating activators but not binding to DNA; (e) and silencing activators.

As already noted, there are GR receptors in every cell. Accordingly, GRs must have a wide influence on genetic expression. The following chart shows the genes that are currently known to be regulated by GRs. The green reflects genes that are turned on by GRs and the red are genes that are turned off by GR. It is fairly clear that the GR has broad influence over genetic expression in the human body.

The above discussion offered a brief summary of what the GR is, how it affects gene expression, and underscores its influence on many human genes. The following section will review the GRs central role in the regulation of the human stress system.

The Human Stress Response: The Sympathetic Adrenomedullary System (SAM) and Limbic-Hypothalamic-Pituitary Adrenocortical (LHPA) System

Before describing the human stress response it may be useful to ask what exactly is “stress?” There is no definitive definition, but stress is commonly thought of as both a consequence of uncontrollable threatening events, and an actual cause of disease. Dr. Bruce McEwen, considered one of the founding fathers of modern stress research, has pointed out that stress can be many things:

For many of us, it [stress] refers to a state of complete overload. External events unite with the discomfort of our own response to overwhelm us and exhaust our ability to cope. As a result we begin to feel tired, edgy and run down; eventually, this state tips toward illness. In trying to keep up with the ever-increasing pace of the world around us, we start catching colds that we can't seem to shake off. Or in struggling to meet the needs of innumerable friends, co-workers, mates and children we develop a sense of inadequacy that spirals into helplessness and depression. When things get to this point we are no longer simply under stress – we're 'stressed-out.'

(McEwen & Lasley, 2002, p. 2). Some researchers also distinguish between what has been called “good stress” and “bad stress.” Good stress might be characterized as being a Giants fan at a Giants vs. Braves baseball game, and watching as Buster Posey (the Giants' catcher), in the bottom of the 9th inning when the score is tied and there are 2 outs and 2 strikes, brings home the winning run. Bad stress is being a Braves fan. Good stress, in other words, “...leave[s] a sense of exhilaration and accomplishment, whereas ‘bad stress’...refers to experiences where a sense of control and mastery is lacking and which are often prolonged or recurrent, irritating, emotionally draining and physically exhausting...” (McEwen 2007 at p. 874). Although it is important to remember that there are many definitions of stress possible, in the context of the present research it may be useful to think of “bad” stress as the inability to feel safe in the face of a perceived threat. The concept of “bad stress” raises the question of whether insecurity or disorganization may be viewed as a form of “bad stress.”

The nervous system is composed of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain and the spinal cord. The PNS, part of the nervous system outside the CNS, consists of the autonomic nervous system (ANS) and the somatic nervous system (SNS). The SNS is associated with voluntary control of body movements. The ANS consists of the sympathetic and the parasympathetic system. The sympathetic system acts to, “mediate vigilance, arousal, activation, [and] mobilization”(e.g., “flight or fight” response that stimulates the heart to beat faster and the digestive system to

slow down) (Sapolsky, 2004, p. 22). The parasympathetic system acts to slow down body functions such as heart rate and generally stimulates functions that occur when the body is resting such as digestion, elimination and salivation, and promotes growth; for that reason it is known as, “the rest and digest system “ (Kolb & Whishaw, 2008; Sapolsky, 2004). A third system, the enteric system, essentially focuses on digestive regulation.

When the human brain perceives danger many regulatory systems respond including the metabolic, the ANS, the neuroendocrine, and the immune system. Here the focus is primarily on the sympathetic adrenomedullary (SAM) system and the LHPA system (sometimes referred to as the HPA axis but now contains the “L” to emphasize the importance of limbic system participation (Gunnar & Vazquez, 2006). The LHPA system is thought to be the “slow acting” pathway, and the SAM is thought to be the “fast acting” pathway (Kolb & Whishaw, 2001, p. 229).

Both the fast and the slow pathway are activated when the brain senses a threat. The fast response, (SAM), part of the sympathetic nervous system response, results in an increase in epinephrine and norepinephrine, preparing the body for a “fight” or “flight “ (Gunnar & Vazquez, 2006; Lavallo, 2005; Sapolsky, 2004). Assume for the moment that someone is taking a walk and stumbles upon a rattlesnake. Rattlesnake bites can be fatal so it is highly likely that the SAM system would be triggered—possibly before the individual was even aware of it. Essentially, what happens is that light (photons) hits light receptors (i.e., cells in the retina called cones and rods) which react to the light by sending an electric impulse (called an “action potential”) through the optic tract to the thalamus (Kandel, Schwartz, & Jessell, 2000). The thalamus can be thought of as the initial reception desk for all sensory information coming into the brain (except for olfactory information which goes directly to the hippocampus). The thalamus then sends out efferent signals all over the cortex, the hypothalamus, the amygdala and the hippocampus. When detecting a rattlesnake or something else of an equally threatening nature a direct signal is sent from the thalamus to the amygdala, a part of the brain directly related to fear processing¹⁵ (Rodrigues, LeDoux & Sapolsky, 2009; Panzer, Viljoen & Roos, 2007). An action potential is then sent from the hypothalamus through the spinal cord via the thoracic splanchnic nerve fibers which synapse on cells in the adrenal medulla, releases acetylcholine and triggers the production of norepinephrine and epinephrine; the norepinephrine and epinephrine then bind to adrenoreceptors in a number of organs sending humans into “fight or flight” mode (Gunnar & Quevedo, 2007).

In the “slow acting” pathway (LHPA Axis) the hypothalamus also releases corticotrophin-releasing hormone (CRH) and vasopressin (VP) from the paraventricular nuclei (PVN) of the hypothalamus (Gunnar & Vazquez, 2006; Kolb & Whishaw, 2001). Central to the stress response, the PVN has been described as, “the main integration site for control of the HPA” (Marques de Souza & Franci, 2008, p. 686). CRH and VP travel to the anterior pituitary where they stimulate the production of ACTH which then interacts with receptors on the cortex of the adrenal glands to stimulate the production of the glucocorticoid, cortisol.

Once cortisol is released from the adrenal gland what is commonly referred to as a “negative feedback loop” ensues. In other words, cortisol acts as a signal to the brain to turn off the stress response. Once again, the PVN plays a critical role in this process. There are many pathways leading into the PVN from other areas of the brain.

Although, many parts of the brain interact with the PVN, the hippocampus has been long noted to be a major influence on CRH inhibition (Sapolsky, 2004). It appears that once cortisol is released from the adrenal cortex during a stress episode and reaches the hippocampus, GABA (gamma aminobutyric acid—the main inhibitory transmitter in the human brain that influences cell communication by inhibiting cells from firing by manipulating levels of calcium, potassium or chloride (Kandel, Schwartz & Jessell, 2000)) is expressed via a long neuronal¹⁶ pathway through the fornix to a GABA receptor enriched area that is near the PVN (Marques de Souza & Franci, 2008; de Kloet, Joels & Holsboer, 2005; Miklos & Kovacs, 2002)). CRH neurons have GABA receptors that bind with released GABA, and thus disrupt the production of CRH. Other areas of the brain influence the PVN such as the medial pre-frontal cortex, also acting as an inhibitor of CRH production through GABA expression.

CRH production is also inhibited when cortisol reaches the PVN, binds to GRs and acts as a transcription factor to halt the genetic expression of CRH. As discussed in the previous section, increases in cortisol act as transcription factors silencing the expression of genes in the PVN that produce CRH and VP. The inhibition is performed by cortical responsive cells in the hypothalamus, the hippocampus and the medial regions of the prefrontal cortex—these are the GRs (Gunnar & Vazquez, 2006; Kolb & Whishaw, 2001). Cortisol attaches to the GR and then moves into the nucleus where it binds to DNA and silences CRH and VP genes.

The more GRs present, the better able the brain is to detect cortisol and move quickly to inhibit the stress reaction. As noted above, the hippocampus plays a critical role in silencing the stress response through this process. Up until this point the hippocampus had been signaling through neuronal fibers traveling indirectly to the PVN of the hypothalamus to produce CRH. (Lovallo, 2005). When cortisol binds to the GR receptors (in fact, the hippocampus has the greatest number of GR receptors in the brain) signaling between the hippocampus and the PVN is stopped resulting in a cessation of CRH production. Thus, the inhibition of the stress response is a complicated process involving many areas of the brain.

It is extremely important to emphasize that various structures in the limbic system also influence the HPA axis. These structures are significant because they influence the stress appraisal process. The hippocampus' role in *inhibiting* the stress reaction has already been discussed but because the hippocampus has pathways to the PVN and the amygdala, and plays a role in storing declarative memories (memories for facts and events) the hippocampus may also help to *trigger* the HPA axis (McEwen & Gianaros, 2010); Lovallo, 2005). An already noted, when the SAM system is activated, the central nucleus of the amygdala produces CRH which travels through the lateral hypothalamus, and activates epinephrine and norepinephrine production in the adrenal medulla (Jankord & Herman, 2008). In addition, the locus coeruleus, produces norepinephrine, called the brains' "global arousal system," aminergic cells such as the raphe nucleus produces serotonin (discussed in detail below), and the ventral tegmental area produces dopamine (influences mood and is associated with the nucleus accumbens, called the "reward center" of the brain) (Lovallo, 2005, p. 108) all of which could influence HPA activity. The central nucleus of the amygdala could also activate the HPA in response to psychological stressors but would take an indirect path through the bed nucleus of the stria terminalis to the PVN (it may of interest to note that the amygdala may act indirectly by eliminating inhibitory influences on the PVN)(Jankord & Herman, 2008). In fact, CRH receptors appear in parts of the brain used to appraise psychological stressors such as the








anterior cingulate cortex (ACC), the orbital frontal cortex (OFC) and the mPFC, as well as subcortical regions such as the bed nucleus of the stria terminalis (BNST), the hippocampus, the hypothalamus and the brain stem (Gunnar & Quevedo, 2007). Accordingly, some commentators have stated that multiple pathways exist to HPA activation to allow “fine tuning” of the stress response (Gunnar & Quevedo, 2006). In fact, Gunnar and Quevedo conceive of the human response to psychological stressors as consisting of three levels of neurobiological organization; the “cortico-limbic level,” the “hypothalamic-brain stem level,” and the “neural to adrenal level of organization.” The point is to underscore the fact that the stress response system is ultimately much more complicated than a simple description of the HPA axis would suggest.

What happens to humans physiologically when the SAM/HPA system is triggered? When the stress response is triggered the body immediately starts to conserve resources in some areas of the body and direct energy to other areas that are needed to “fight” or flee. For example, the immune and reproductive system is suppressed (who needs to fight disease when fleeing a rattlesnake? The individual will also need all his energy or he won’t live long enough to reproduce); the digestive tract is shut down or compromised (he is not eating while he is running); heart rate and respiration increases; blood pressure increases; pupils dilate; blood vessels to the muscles dilate and constrict to other parts of the body; hearing loss and tunnel vision may develop; and the body may begin to sweat to cool down and counteract the heat he is generating as he prepares to run as fast as he can (Lovallo, 2008; Sapolsky, 2004). Critically, epinephrine stimulates “glycogenolysis” –a process that occurs in the liver to increase levels of glucose and give the organism enough energy to respond to the threat. Neither norepinephrine nor epinephrine cross the blood brain barrier so the brain produces norepinephrine on its own (in a region called the locus coeruleus) intensifying, “vigilance, arousal and narrowing of attention, along with participating in the processes that activate the other arm of the mammalian stress system, the HPA axis.” (Gunnar & Quevedo, 2007, p. 147).

The “...process of maintaining stability (homeostasis) by active means, namely, by putting out stress hormones and other mediators” is sometimes referred to as “allostasis” (McEwen, 2007, p. 874). HPA hormones, catecholamines (e.g., epinephrine) and cytokines may be thought of as some of the tools of allostasis. In other words, allostasis refers to actions the body takes to maintain healthy levels of important biological functions (e.g., blood glucose levels). This response is sometimes called, the “allostatic load” a term coined by Dr. Bruce McEwen, meaning, “...the wear and tear on the body and brain caused by use of allostasis, particularly when the mediators are dysregulated, e/e., not turned off when stress is over or not turned on adequately when they are needed” (McEwen, 2007, p. 874).


As noted earlier, the importance of glucocorticoids, can hardly be overstated; cortisol itself interacts with every cell in the body and influences development and functioning in a myriad of ways including regulating metabolism, signaling the liver to raise blood sugar to enable the brain and muscles to have sufficient energy to respond to stress (called “gluconeogenesis”), regulating blood pressure and controlling inflammation. The stress reaction is designed to help individuals escape predators but the reaction is meant to last only for a short period of time. As Dr. Robert Sapolsky has pointed out, zebras don’t get ulcers because they don’t worry—they either elude the lion chasing them or they are lunch, but the stress reaction does not continue day after day (Sapolsky, 2004). As also noted above, not all stress is bad; moderate short term stress can even improve cognition (Sapolsky, 2004).

But when stress is overwhelming (high allostatic load), and/or chronic, however, stress can be lethal. Consequences include;

-  **immune suppression**
-  **reproductive distress**
-  **digestive tract: ulcers,
decreased nutrient
absorption, stunted growth**
-  **cardiovascular:
arteriolosclerosis**
-  **bone: stunted growth,
osteoporosis**
-  **metabolism: late-onset
diabetes**
-  **accelerated aging**

***Image from Kaufer (2010)**

Cortisol levels can inhibit the production of, shrink or actually destroy cells in the hippocampus resulting in what is called hippocampal atrophy, causing memory impairment (Sapolsky, 2004; Merke, et. al., 2003). Moreover, chronic exposure to stress can dysregulate the entire stress response system either resulting in either a hypersensitive response or a “blunted” response (Gunnar & Vasquez, 2006). Chronic stress can also suppress growth hormones leading to arrested growth (Charmandari, Tsigos & Chrousos, 2005). In contrast to the effects on the hippocampus, chronic stress can cause “hypertrophy” of the nerve cells of the amygdala, resulting in a hyperactive response to stress thereafter. The hypertrophy of the amygdala that comes with chronic exposure to stress appears to be due to long-term-potential (LTP)¹⁷ in the amygdala (Rogan, Staubli & LeDoux, 1997). In other words, because of the plastic nature of the brain, exposure to chronic stress leads to heightened LTP which leads to amygdala hypertrophy which in turn leads to hyperreactivity to stress (Sapolsky, 2004; Sah, Faber, Lopez de Armentia & Power, 2003). A hyperactive amygdala is associated with conditions such as anxiety and depressive disorders, eating disorders, substance abuse disorders, personality disorders including conduct disorders as well as hypertension, osteoporosis and heart disease (Charmandari, Tsigos & Chrousos, 2005). Other disorders associated with dysregulation of cortisol include;



Disorders Linked to Over and Underproduction of Cortisol	
<u>Overproduction</u>	<u>Underproduction</u>
<ul style="list-style-type: none"> ■ Cushings ■ Melancholic depression ■ Diabetes ■ Sleep deprivation ■ Anorexia nervosa ■ Excessive exercise ■ Malnutrition ■ OCD ■ Panic disorder ■ Chronic alcoholism ■ Childhood abuse, physical sexual ■ GI disorders 	<ul style="list-style-type: none"> ■ Atypical/seasonal depression ■ Chronic fatigue ■ Fibromyalgia ■ Hypothyroidism ■ Nicotine withdrawal ■ Rheumatoid arthritis ■ Allergies ■ Asthma

***Image from Francis (2008).**

Accordingly, proper regulation of cortisol is critical not only to optimal health but to survival. The GR gene is critical to human functioning because it mediates the effect of cortisol.

The next section briefly describes the polymorphism associated with the 5-HTT gene and a sampling of some of the relevant research .

C. Serotonin (“5-HT”), the Serotonin Transporter Gene (“5-HTT” or “SLC6A4”) and the Polymorphism of the Promoter Region of the 5-HTT Gene (5-HTTLPR)

Why care about serotonin? Serotonin (typically represented as “5-HT”) is, “a phylogenetically ancient signaling molecule; It is the most widely distributed neurotransmitter in the brain and is implicated in the regulation of emotions” (Lesch, 2007, p. S25). Serotonin pathway reaches almost every major area of the brain including the median pre-frontal cortex, amygdala and hippocampus (Holmes, 2008).

In fact, serotonin influences many important functions such as sleep, neurogenesis in the hippocampus (Djavadian, 2004), appetite, cognition, and various psychopathologies such as depression, conduct disorder, alcoholism and anxiety (Holmes, 2008; Wilhelm, et. al., 2006; Caspi, et. al., 2002; Taylor, et. al., 2006; Olsson, et. al., 2005; Foley, et. al., 2004)(a brief review of selected studies is attached as Appendix A). Serotonin is also implicated in adult and early brain development impacting the migration, proliferation and differentiation of cells as well as synaptogenesis (Lesch, 2007).

Serotonin influences the HPA axis through a number of different mechanisms including neural

connections between the dorsal raphe and the PVN, and the median raphe and the hippocampus (recall the dorsal raphe and the median raphe both produce serotonin) (Holmes, 2008; Lopez, 2002). Serotonin may help trigger the HPA axis through stimulation of CRH release from the PVN, ACTH and glucocorticoids (Hanley & Van de Kar, 2003).

Serotonin is regulated by a number of enzymes, but especially by the serotonin transporter gene. Technically, the correct designation for the serotonin transporter gene is “SLC6A4” (“solute carrier family 6 neurotransmitter transporter, serotonin, member 4”) although many articles simply designate the gene “5-HTT.” Here, the serotonin transporter gene will be referred to as “5-HTT” for simplicity.

5-HTT has been described as “central to the regulation of brain and peripheral serotonergic neurotransmission” (Wilhelm, et. al., 2007). 5-HTT is a protein that acts by regulating the concentration of serotonin in the synaptic cleft, and thus controls serotonin’s effect on the receiving neuron.

What is called a “functional polymorphism,” exists in the promoter region of the 5-HTT gene (“promoter region” refers to DNA that allows or “promotes” the expression of a gene; usually the promoter for a particular gene is located near or “upstream” from the gene regulated) and is referred to as “5-HTTLPR (the serotonin-transporter-gene-linked polymorphic region). In other words, various versions of 5-HTTLPR exist. One version is called the “short variant” and is designated by “ss,” another version is called the “long variant” and is designated by “ll.” A third version is a heterozygous form made of one allele of the short version (s) and one allele of the long version (l) and designated as “ls.” The short version or “ss” is usually associated with reduced transcriptional efficiency (Wilhelm, 2006). The short and long version of the 5-HTTLPR has different levels of repetitive sequencing. The short, long and heterozygous versions of the 5-HTTLPR appear to differentially impact human development and psychopathology. For example, researchers have detected structural differences in the brain anatomy of individuals with different versions of the 5-HTTLPR polymorphism. As noted earlier, both the amygdala and serotonin play an important role in the regulation of stress. Weinberger and colleagues discovered that participants with at least one short s allele have smaller amygdalas as well as a smaller cingulate cortex than those participants with the ll allele (Pezawas L, Meyer-Lindenberg A, Drabant EM, et al. (2005). The researchers further discovered that the connectivity between the amygdala and the cingulated cortex differed; in participants with at least one small s allele, the signaling between the amygdala to the cingulated cortex is not as strong. Consequently, it is theorized that ss/sl participants have more difficulty calming an activated amygdala; in other words, it is thought that ss individuals take longer to recover from a stressful experience than ll individuals.

The short (ss) version or imbalanced levels of serotonin has been linked to increased vulnerability to depression and anxiety (Uher & McGuffin, 2007; Caspi & Moffit, 2006; Hariri & Holmes, 2006; Brummett, Boyle, Siegler, et. al., 2008) (although two recent meta-analyses have questioned those findings (Munafo, Durrant, Lewis & Flint, 2009; Risch, Herrell, Lehner, et. al., 2009)); poor responsiveness to CBT in participants with post traumatic stress disorder (Bryant, Felmingham, Falconer, et. al., 2010); childhood aggression (Beitchman, Baldassarra, Mik, et. al., 2006; Sakai, Young, Stallings, et. al., 2006; Cadoret, Langbehn, Caspers, et. al., 2003) drug use (Gerra, Garofano, Castaidini, et al., 2005) and mental illness (Lyons-Ruth, Holmes, Sasvari-Szekely, et.al., 2007) and higher risk of post traumatic stress disorder when

exposed to natural disasters (Kilpatrick, Koenen, Ruggiero, et. al. 2007). Although it should be noted that in Cadoret, Langbehn, Caspers, et. al., (2003) the ss/sl alleles were associated with higher levels of aggression in males, they were associated with *lower* levels in females. In addition, in the two meta-analyses that failed to find an interaction effect between the 5-HTTLPR, stressful life events and depression, not one attachment study was included in the meta-analyses (Munafo, Durrant, Lewis & Flint, 2009; Risch, Herrell, Lehner, et. al., 2009). Moreover, meta-analyses that relied on studies using objective measures of depression rather than retrospective self-report measures have confirmed the relationship between 5-HTTLPR, adversity and depression (Uher & McGuffin, 2010).

A number of studies underscore the importance of taking into account genetic and environmental interactions. Although Kaufman, et. al., (2006) did not deal with attachment classification directly, the study did find that the interaction between maltreatment by parents, the ss allele, and BDNF variants (brain derived neurotrophic factor) predicted the highest depression scores. Note that in Kaufman, the ss allele interaction with maltreatment alone resulted in a “trend” toward significance. In fact, the highest significance levels were realized when maltreatment X ss allele X BDNF variant X social support were taken into account. In other words, social support had a significant impact on maltreated children with the ss allele but not on the maltreatment group as a whole. Kaufman, et. al., (2006) is significant because it suggests that some of the diversity of results in this area of research may be a result of failing to take into account interactions such as maltreatment, SES, social support, etc.. Similarly, Kilpatrick, Koenen, Ruggiero, et.al. (2007) found that ss participants exposed to a 2004 Florida hurricane were at greater risk of developing post traumatic stress disorder (OR=4.5) than ll participants but only under conditions of high exposure to the hurricane in combination with a low level of social support. In Manuck, Flory, Ferrell & Muldoon (2004) researchers found that low SES participants with less education showed an attenuated serotonin response to a drug-challenge test compared to participants with higher education/income but only in participants with the ss/sl alleles. A French study, Ritchie, Jaussent, Stewart et. al. (2009), found no significant relationship between depression and the 5-HTTLPR alleles, but did find participants with the ll/sl alleles who experienced poverty in childhood were at higher risk for depression later in life (Ritchie is an interesting study because author’s propose that some of the different 5-HTTLPR allelic findings might be due to the fact that different alleles have different risks at different stages of life—Ritchie looked at the impact of early life poverty on late life depression suggesting that the l allele may be more of a risk factor for older individuals).

The importance of SES has been underscored in a number of other studies. Williams, Marchuk, Siegler, et. al. (2008) found at baseline that participants raised in a low SES environment had blood pressure that was 25% higher than participants raised in a high SES environment. But when genotype was taken into consideration, those low SES in childhood participants with the ll allele had diastolic blood pressure that was 3.7 times greater than high SES in childhood participants with the ss allele. Nobile, Giorda, Marino, et. al., (2007) found that an interaction between low SES, and the long version of the dopamine gene (DRD4) and the ll allele was associated with higher aggressive behavior scores than other genotype-SES combinations. Finally, Chen, Miller, Korb & Cole (2010) found that participants who were raised in low SES homes but who reported high levels of “maternal warmth” had statistically significantly lower levels of pro-inflammatory signaling than low SES participants who reported low levels of “maternal warmth.”

Only very recently have a number of studies examined the relationship between the serotonin genotypes and attachment classification. For example, Caspers, Paradiso, Yucuis, et. al. (2009) found in a sample of adopted older adults, an correlation between the ss genotype and unresolved attachment classification, although in a later German study this finding was not replicated (Reiner & Spangler, 2010). Spangler, Johann, Ronai & Zimmerman (2009) found an interaction between the ss allele and maternal insensitivity was associated with an increased risk of disorganized classification (but no significant effect found for an interaction between genotype and maternal behavior on attachment *security*). Kochanska, Philibert & Berry (2009) found that insecure children with the ss/sl genotype developed poor self-regulatory skills on a host of tests (i.e., effortful attention). Barry, Kochanska and Philibert (2008) found that mother responsiveness predicted infant security only in children carrying the ss/sl genotype, not the children carrying the ll allele (although this study included about 57% of children from families with incomes over \$57,000 and 43% with incomes below, it is not clear if any of the children came from high risk families or families with very low incomes. It could be that the genotypes would not be significant in a purely high risk population. In other words, if the stress is high enough, it could be that the ll genotype would not act as a protective factor). Bakermans-Kranenberg and van IJzendoorn (2008) found that mothers with the ss allele were less sensitive than mothers with the sl/ll allele, and mothers with a polymorphism of the oxytocin gene “AA or AG” were less sensitive than mothers with the “GG” genotype. It must be noted, however, that only 3% of the variance in was explained by genotype while 15% of the variance was explained by education (the authors also point out that the sample was well educated with no deprivation). Gilissen, Bakermans-Kranenberg, van IJzendoorn & Linting (2008) found that secure children with the ll allele showed the lowest levels of stress reactivity during a trier of social stress test. Finally, Zimmerman, Mohr & Spangler (2009) found that insecure adolescents with the ss allele were more aggressive with their mothers during a joint social task designed to elicit emotion than secure ss participants. (A brief summary of many of these studies appears in Appendix A).

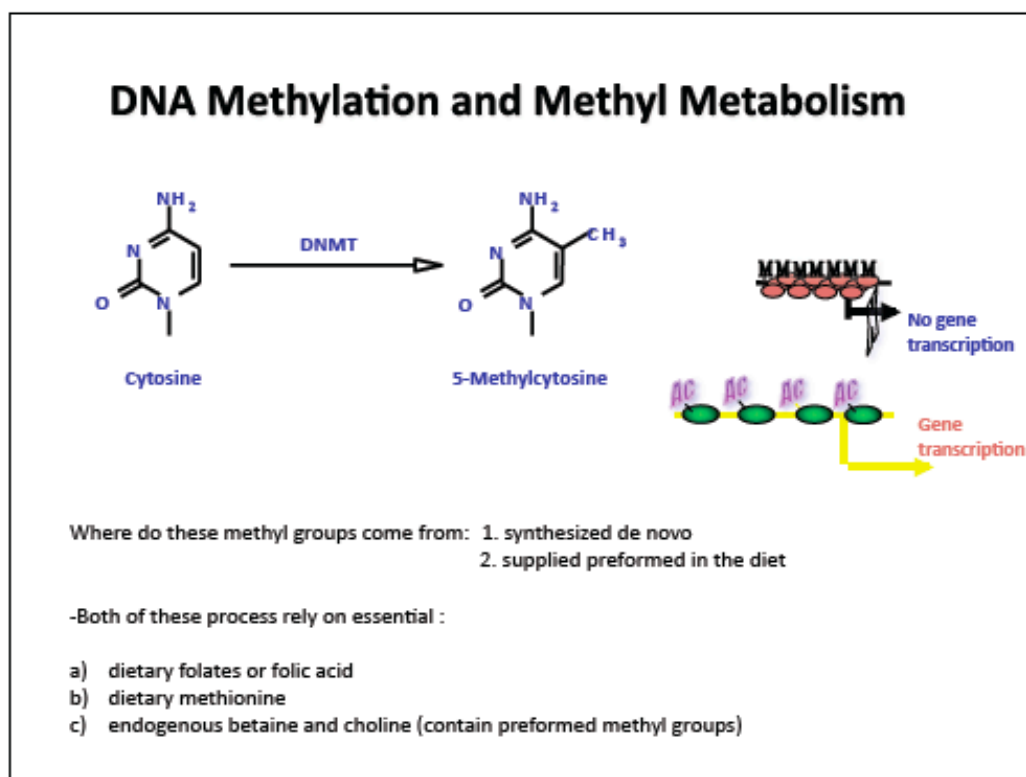
The discussions above strongly suggests that stress regulation is probably influenced in some manner by attachment relationships. But what does this relationship look like and does any research confirm that such a relationship exists? It is possible that a new area of science called “epigenetics” may help answer such questions. There are a few studies now that suggests the 5-HTT gene and the GR gene is involved in the development of early regulation through epigenetics. But before discussing those studies it might be helpful to define epigenetics, briefly explain the process and then present a sampling of what research currently exists implicating attachment. It should be noted that most of the research is in the area of animal studies but some early studies have emerged suggesting epigenetic processes are at work in humans that involve early relationships.

WHAT IS EPIGENETICS?

“Epigenetics” literally means “above genetics” (Waterland, 2006, p. 1706S) and may be defined as, “All meiotically and mitotically heritable changes in gene expression [the process by which DNA sequencing is transformed into the structure and functioning of a cell] that are not coded in the DNA sequence itself” (Egger, Liang, Aparicio & Jones, 2004, p. 457). Epigenetic effects may have evolved to permit humans to adapt to changing environmental

conditions (Cooney, 2006). It is believed that epigenesis happens in a number of ways but the two most studied methods to date are methylation and histone modification. Methylation is a method that allows cells, which all carry the identical DNA code, to specialize. Methylation prevents expression of a gene through the addition of a methyl group to a cytosine (“C”) nucleotide within the gene. This addition prevents transcription factors from accessing the gene, thereby rendering it unexpressed. Methylation is said to act as a sort of “gum” that prevents the expression of unwanted genes (Venkat, 2004, para. 11). Abnormalities in methylation can lead to death of a fetus or other devastating conditions such as Rhett syndrome (Egger, Liang, Aparicio & Jones, 2004). In histone modification, DNA, which is negatively charged, is tightly wrapped around what is called a histone core, which is positively charged, making the genes inside that DNA unavailable for expression through transcription (Venkat, 2004). Negatively charged functional groups called “acetyl groups” loosen up the DNA, allowing those genes to be expressed. Removing too many acetyl groups increases the positive charge of the histone core, and thus has the effect of making the DNA wrap around the core ever more tightly, making critical genes such as tumor suppression genes unable to express themselves – a condition associated with diseases such as cancer (Venkat, 2004). In sum, cytosine methylation is associated with primarily the silencing of genes, and histone acetylation is associated with primarily activation of genes.

The illustration below contains the molecular model for methylation and how DNA behaves during methylation and acetylation;



***Image from Francis (2010)**

Notice in the illustration above how close DNA nucleosomes are bunched together when methylated effectively preventing transcription. During acetylation, however, the nucleosomes are spread apart allowing transcription factors to access promoter regions.

There are now a number of articles which discuss the epigenetic effects of parenting on development in offspring (Meaney & Szyf, 2005; Branchi, Francia & Alieva, 2004; Fish, Shahrokh, Bagot, Cald'r, Bredy, Szyf & Meaney, 2004).

How might maternal care impact methylation and histone modification? Meaney and his colleagues (Meaney & Szyf, 2005, p. 460) constructed a model demonstrating how maternal care in rats (in this case licking) could affect histone modification and methylation. Essentially, Meaney and Szyf explain that in rats, maternal licking increases levels of serotonin (5-HT) which triggers the release of enzymes that activate nerve growth factor transcription which, in turn, enhances histone modification which then facilitates the process of demethylation resulting in expression of the GR receptor gene, which has the effect of lowering stress reactivity.

Significantly, Weaver and colleagues have also shown that maternal methylation is, in theory, reversible, at least in rats using a chemical called “trichostatin A.

At the present time, it is not known for certain whether methylation can be reversed in humans, although experiments are ongoing. Exciting new research involving the brains of deceased individuals who used antidepressants compared to those who did not do suggest that antidepressants are associated with a decrease in methylation (Chen, Ernst & Turecki, 2010).

Epigenetic changes are thought to be heritable (Gluckman, Hanson & Beedle, 2007). Thus, what an individual's grandparent experienced might increase that person's vulnerability to disease. There are a number of examples of this phenomenon. Many of these studies include work performed by Meaney and his colleagues, and Suomi. Studies have started relatively recently in humans, but the numbers are increasing. The classic example is that of the arrowleaf plant—if it is grown in a dry environment it grows an “elephant-ear leaf,” but if it is grown in a wet environment it grows lily pad-like leaves, and if it is grown in deep water it grows skinny leaves (Coniff, 2006, p. 61). Examples involving animals and humans include:

*Babies of low LG-ABN mothers have higher levels of cortisol than babies of high LG-ABN mothers primarily because babies of high LG-ABN mothers have higher levels of GR gene demethylation, which leads to higher GR gene expression, so they have more functional GR genes operating in the brain and thus are better able to regulate stress (Francis, Szegda, Campbell, Martin, & Insel, 2003).

*Baby rats that are genetically from a low LG-ABN strain but are raised by high LG-ABN mothers will show “**indistinguishable**” methylation patterns from naturally high LG-ABN rat babies. These effects are present in adulthood (Weaver, Cervoni, Champagne, D'Alessio, Sharma, Seckl, Dymov, Szyf, & Meaney, 2004).

*Epigenetic effects can be reversed in adulthood by pharmacological interventions (Weaver, Cervoni, Champagne, D'Alessio, Sharma, Seckl, Dymov, Szyf, & Meaney, 2004).

*Maternal separation decreases hippocampal glucocortical receptor expression in the PVN of the hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the locus coeruleus/parabrachial nucleus. But postnatal handling of baby rats increases receptor expression in the PVN of the hypothalamus and the central nucleus of the amygdala. It is argued that handling even adult rats decreases the stress response by decreasing CRF mRNA expression in the hypothalamus and amygdala (Plotsky, Thrivikraman, Nemeroff, Caldji, Sharma, & Meaney, 2005; Meaney, 2001; Maccari, Piazza, Kabbaj, Barbazanges, Simon, & Le Moal, 1995).

*Over **900 genes** have been found to be “stably regulated by maternal care” (Weaver, Meaney, & Szyf, 2006).

*Hippocampal neurons in the offspring of low LG-ABN mothers are “more vulnerable to loss” through pruning (Weaver, Meaney, & Szyf, 2006).

*Mother’s diet (e.g., high in folic acid) before and after pregnancy results in changing a pup’s hair color from yellow to brown (Wolff, Kodell, Moore, & Cooney, 1998).

*Female rat babies of high LG-ABN mothers show higher levels of oxytocin receptors in the amygdala and bed nucleus of the stria terminalis, and male babies show higher levels of vasopressin (Francis, Young, Meaney, & Insel, 2002).

*Babies of high LG-ABN mothers but reared by low LG-ABN mothers show more fear and have higher basal cortisol levels than high LG-ABN babies raised by high LG-ABN mothers (Priebe, Brake, Romeo, Sisti, Mueller, McEwen, & Francis, 2005).

*When baby rats were removed from their mothers for eight hours every other day during infancy, 15 genes were up-regulated and 9 genes were down-regulated. (Kohda, Jinde, Iwamoto, Bundo, Kato, & Kato, 2005).

*Baby rats born to low LG-ABN mothers but who were raised by high LG-ABN mothers had cognitive levels that were indistinguishable from babies born to and raised by high LG-ABN mothers; baby rats born to high LG-ABN mothers but who were raised by low LG-ABN mothers had cognitive levels that were also indistinguishable from babies born to and raised by high LG-ABN mothers—suggesting genes may provide some protection against deprivation. Maternal behavior may be related to different expression of gene coding for NMDA receptors, which enhances “hippocampal sensitivity to glutamate” and increases brain-derived nerve growth factor, leading to increased synaptic development (Liu, et al., 2000, p. 802).

*Some of the damaging effects of maternal deprivation in rats (e.g., higher anxiety, stress, lower learning function) are reversible through environmental enrichment (Bredy, Humpartzoomian, Cain & Meaney, 2003) but it does not reverse the effects on CRF gene expression (Francis, Diorio, Plotsky, & Meaney, 2002), although later studies do show differences in genetic expression when peripubertal rats with low licking mothers are raised in an enriched environment on expression of genes encoding for the glutamate receptor (Bredy, Zhang, Grant, Diorio & Meaney, 2004).

*Of those monkeys who had a short version of a gene that improves the transmission of serotonin, only those who were peer raised had significantly lower serotonin metabolite levels (Soumi, 1999).

*HR (high reactive) monkey babies placed with highly nurturing monkey mothers displayed precocious behavior, leaving their mothers early, exploring more than either the HR monkeys

raised by normal mothers or the normal infants raised by either mother; they later became leaders in their troupe; when HR babies became mothers themselves they adopted the parenting style of their foster mothers (Suomi, 1999).

*When a short version of the serotonin transporter gene (5-HTT) is present in baby monkeys, only those who are maternally deprived (peer raised) show altered serotonin functioning in the CNS (Bennet, Lesch, Long, Lorenz, Shoaf, Champoux, Suomi, Linnoila, & Higley, 2002), increased levels of ACTH when separated from peers, and, during chronic separation, evidence of blunted cortisol levels (Barr, Newman, Shannon, Parker, Dvoskin, Becker, Schwandt, Champoux, Lesch, Goldman, Suomi, & Higley, 2004).

*PET scan studies of peer- and mother-raised monkeys show that regional brain serotonin transporter binding is lower in PR monkeys than in MR monkeys in a variety of brain areas, including the thalamus, hypothalamus, caudate and putamen, globes palladium, anterior cingulate gyros, and medial temporal region, which includes the hippocampus and amygdala (Ichise, Vines, Gura, Anderson, Suomi, Higley, & Innes, 2006).

*Monkey babies born to non-abusing monkey mothers who were cross-fostered by abusive monkey mothers grew up and eventually abused their own babies; in fact, those babies who were born to non-abusive mothers but raised by abusive mothers were more likely to abuse their children (Maestripieri, 2005).

*Baby rodents who are separated from their mothers experience an upregulation of dopaminergic and serotonergic receptor density in the hippocampus, but when the babies hear their mother's cries, upregulation is suppressed (Ziabreva, Poeggel, Schnabel, & Braun, 2003).

As the above points demonstrate, there is a rich and developing literature in animal studies illustrating that development does take place primarily through a gene and environment interaction, but research on humans is just beginning. The research on humans still primarily focuses on genetic X environmental interaction and rarely examines epigenetic influences. The following is a brief summary of some of this literature:

*In an epigenetic study comparing the methylation and histone modification levels of MZ twins, it was found that the youngest twins (approximately three years of age) had almost identical levels, while the oldest twins whose lives had diverged the most had the greatest differences (Fraga, Ballestar, Paz, et al., 2005). The study demonstrates that humans experience epigenetic change.

*The first human methylation profiles have been reported; researchers look specifically at chromosomes 6, 20, and 22 (Eckardt, Lewin, Cortese, et al., 2006). The authors report that 17 percent of the genes examined were differentially methylated in at least one of the tissues.

*Phenylketonuria ("PKU") involves a single gene mutation that results in high levels of phenylalanine, causing mental retardation. Yet, simply removing phenylalanine from what is referred to as the "dietary environment" prevents the expression of that gene (Todd & Constantino, 2003).

*Maternal dietary intake of folic acid regulates methylation in animals and humans. Adding folic acid to a pregnant mother's diet prevents neural tube defects and helps maintain imprinting genes (genes that are expressed from only one parent and need to be methylated in order to function) that control cognitive function (Duttaroy, 2006).

*Humans with one or more copies of the short allele version of the 5-HTT gene who also experienced stressful events after age 21 report more depressive symptoms than those who have the long allele (Caspi, Sugden, Moffit, Taylor, Craig, Harrington, McClay, Mill, Martin, Braithwaite, & Poulton, 2003; Taylor, Way, Welch, Hilmert, Lehman, & Eisenberger, 2006).

*Children who had lived in Romanian orphanages for an average of approximately 17 months after birth had lower overall levels of vasopressin than children raised in birth homes, and did not experience a rise in oxytocin levels after interacting with their adopted mothers, unlike children raised by birth mothers (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005).

*Children with the T.7 haplotype (a group of genes or alleles inherited as a group) of the DRD4 gene (a gene regulating dopamine) are more likely to develop secure attachments, as they presumably possess more efficient dopamine systems and can perceive environmental stimuli such as parenting differently; the authors suggest a possible environmental-genetic interaction (Gervai, Nemoda, Lakatos, Ronai, Toth, Ney, & Sasvari-Szekely, 2005).

*In the Netherlands twin study, children with insensitive primary caregivers and the 7 repeat DRD4 gene display significantly more externalizing behaviors (in fact, a sixfold increase) than children with the gene but with sensitive mothers, and more than children without the gene regardless of parental sensitivity (Bakermans-Kranenburg & van IJzendoorn, 2006).

*Humans with two copies of the short 5-HTTLPR alleles who came from a “supportive” family environment had the lowest depressive symptomology in adulthood and those with the two short alleles who came from an early stressful family environment had the highest level of depressive symptomology; family environment did not moderate depressive symptomology for those with one short and one long allele, or two long alleles (Taylor, Way, Welch, Hilmert, Lehman, & Eisenberger, 2006). Thus, the short alleles are “protective” in a supportive environment but a risk factor in a stressful family environment. A similar relationship existed for current stress. The study is also noteworthy because the degrees of adversity in the families were “relatively mild.” In essence, the short alleles may represent a “sensitivity to environmental influence” (p. 674).

There is an advantage to animal studies in that researchers can control for environment in a way unethical and illegal to do in humans. Unfortunately, it is difficult to draw any conclusions from such studies, because what may be true for other species may not be true for humans. For example, hormonal triggers are necessary to elicit maternal behavior in some species, but not in humans, although hormones can impact the intensity of human parental behavior (Nunan & Innes, 2003). Moreover, although we share as much as approximately 99% of our DNA sequencing with animals such as chimpanzees, we may differ significantly in the way our genes are actually expressed (Gilad, Oshlack, Smyth, Speed, & White, 2006), making the study of gene expression in humans more compelling. Nevertheless, the above information does confirm that many phenomena appear to be conserved across species. For example, dysregulation occurs in the LHPA axis when children experience parental deprivation and abuse; the mother’s diet during pregnancy has some impact on children; prenatal stress affects the regulation of the LHPA in offspring; methylation is a form of gene expression that occurs in many species, including plants; oxytocin and vasopressin are implicated in social relationships in humans, primates, and rodents; and most of all, one *cannot predict the characteristics of an individual from genotype alone*. Recall Suomi’s study of highly fearful monkey babies raised by highly nurturing monkey mothers that produced nurturing mother monkeys who eventually

attained leadership positions in their troupe (Soumi, 1999); Maestripieri's troublesome study of abused infant monkeys, which showed that those babies of nonabusive mothers were more likely to abuse their children when raised by abusive mothers than the babies who were raised by their own abusive mothers (Maestripieri, 2005); Caspi's studies of genetic alleles linked to increased vulnerability to depression or conduct disorders but only in the face of increasing life stress or maltreatment; the study by Burgess, Marshall, Rubin, and Fox (2003) finding that insecure children who are uninhibited have the highest rates of externalizing behaviors but secure uninhibited kids have the lowest; and the Bakermans-Kranenburg & Van IJzendoorn (2006) study that found externalizing behaviors increased sixfold among children with the DRF4 gene but only when they were insecurely attached.

New Studies Examining Epigenetic Mechanisms: SL64C polymorphisms and Experience

Just in the last few years a number of new studies have emerged looking at the relationship between the 5-HTTLPR polymorphisms and behavior and/or methylation. Most of the most relevant studies have been done by Philibert and colleagues using the Iowa Adoption Study. For example, Philibert and colleagues have found that abuse experienced in childhood, including physical and sexual abuse, is correlated with hypermethylated 5HTT CpG islands in females (Beach, Brody, Todorov, Gunter & Philibert, 2009) (a short summary of these studies is included in the table of studies attached as Appendix A). In a very recent study Beach, Brody, Todorov, Gunter & Philibert (2011) found methylation levels correlated with symptoms of Anti Social Personality Disorder in female participants but only in participants with the ss and sl alleles and not the ll allele.

Only two studies, one just published, attempt to examine the relationship between methylation of SLC6A4 and attachment. Caspers, Paradiso, Yucuis, Troutman, Arndt, & Philibert, (2009) found that the s allele predicts increased risk of U classification. In Bakermans-Kraneberg & van IJzendoorn (2010) researchers found that lower levels of methylation in the ss participants predicted increased risk of U classification. Higher methylation levels in ss participants actually associated with lower risk of U. This is a surprising finding but the authors opine that the vulnerability to pathology associated with the ss allele may be reduced by high methylation levels. One possible problem with the study may be that transformed lymphoblast cell lines were used which can produce different methylation levels than the original samples although the authors site some support for the proposition that the levels are actually comparable. This study is different from the present study in that it involved only adopted participants with a median age of 39. The present study involves children raised by birth parents and are all late-adolescents. Moreover, this study used blood taken directly from the participants, and controls for SES. Accordingly, it will be of some interest to see if the results differ. Nevertheless, the studies all indicate that methylation levels do differ between unresolved and secure individuals.

The above discussion makes clear that development is not controlled by genes, or environment or any one particular factor but rather is a result of a complex interplay between many different factors, some of which we undoubtedly are not even aware. The present research seeks to contribute just a small part to the human storage of knowledge by looking at the GR gene and the 5-HTTLPR polymorphism. Each is intimately involved in the process of development and in the protection and regulation of the organism during

times of stress. It is hoped that this research can some day inform treatment and interventions to help those suffering from diseases and disorders linked to stress and trauma. The following section presents a review of the methodology used in this study.

CHAPTER II

RESEARCH DESIGN AND METHOD

- 1. Study Design.** This study follows an exploratory observational (non-randomized) design. The target population consists of older adolescent youth, 18-25 years of age. Our study population, however, consists of undergraduates at a public university in the western United States. This study ultimately involved 101 participants, 93 of which participated in the analysis using the Adult Attachment Interview (AAI). Eight participants were dropped because interview recordings were inaudible or because the participants were of mixed ethnic background. All 101 participants were used in the analysis using the Adult Attachment Projective (AAP).

The participants live in a large urban area, the ethnic composition is highly diverse, and they attend one of the top universities in the country. Accordingly, great care should be taken before generalizing the results of this research to beyond the study population here (external validity).

This study determined self-identified race/ethnicity, sex and socioeconomic status (“SES”) primarily because each one of these factors could impact life experience in a significant manner. A short measure called the Family Affluence Scale (“FAS”) (Currie et al., 1997) and a longer measure called the Hollingshead Socio-economic Scale (“HSES”) (1957/75) was used to determine SES. The FAS was used because it is specifically designed to be more user friendly to adolescents.

Initially, students were screened using the FAS, HSES, the Beck Depression Inventory (“BDI”) and the Beck’s Anxiety Inventory (“BAI”), and a short questionnaire to determine age, self-identified ethnicity and sex. The AAI and AAP were administered thereafter. The AAI was always administered first as required by AAI protocol. In approximately half the cases a blood draw was made before the measures were administered and in half the cases after the measures were administered. An amount of \$50 was given to all volunteers after completion of the measures.

- 2. Sample.** As noted above, we successfully interviewed 101 students, and could have interviewed more, if necessary.
- 3. Study site.** The data was collected from a large western U.S. university from almost exclusively undergraduate students.
- 4. Sampling plan:** Participants were solicited initially from the Department of Social Welfare web site. Participants were also recruited via fliers on women’s and men’s restroom walls, on the notice boards outside the Department of Psychology’s office, and then generally on

bulletin boards around the campus. In addition, the author was allowed to address one undergraduate neuroscience class to introduce the study. Most students were either social welfare or psychology students, although students from other diverse disciplines such as physics, political science and business also participated.

5. Operationalization of concepts:

Attachment state of mind is operationalized through the use of two measures, the AAI and the AAP. As noted above, the AAI is a semistructured interview for adolescents/adults about childhood attachment experiences and the meaning the individual gives to those experiences (George, Kaplan, and Main, 1984/1985/1996). As explained above, during the AAI the participant is asked about his or her relationship with primary caregivers, ordinary events in which the attachment system is presumed to be activated (e.g., when the interviewee was ill or hurt), experiences of loss, the meaning that the adult attributes to all these experiences and how they apply to the interviewee in terms of his or her personality and own parenting. If the participant does not have children, they are asked to imagine that they are the parent of a child. After being transcribed, the interview is then evaluated in terms of discourse, assessing for unintended incoherencies and inconsistencies. The critical question is whether the individual is able to give an integrated, logical account of experiences and their meaning. The narrative is then assigned to one of four classifications: “autonomous” (a secure category—designated the “F” category); two insecure categories—“dismissing” (an avoidant category—designated the “D” category), and “preoccupied” (an ambivalent/resistant category—designated the “E” category); and for adults who report attachment-related traumas of loss and/or abuse, and who demonstrate confusion and disorganization during the interview, a fourth category called “unresolved” (designated “U”). Participants who received a U classification also receive a secondary F, D or E organized classification. A fifth classification is called “cannot classify” and involves individuals who show behaviors from more than one category (designated “CC”). This category is associated with a high degree of incoherence, is considered highly insecure (Hesse, 1999; Crowell, Fraley, & Shaver, 1999) and, consistent with other attachment research, is grouped with participants falling in the U classification (Ward, Lee & Polan, 2006).

The AAI was coded by two raters certified reliable by Drs. Mary Main and Erik Hesse of the University of California, Berkeley. In addition, all AAI's were coded by the author, also a certified reliable rater (Sroufe, 2001; Gojman, 2009)

For purposes of this study, a new measurement of attachment state of mind called the AAP will be used to test the construct validity of the AAP. George & West (2001) describe the measure as follows:

The AAP is comprised of a set of eight drawings, one neutral scene and seven scenes of attachment situations. These drawings were carefully selected from a large pool of pictures drawn from such diverse sources as children's literature, psychology text books, and photography anthologies. (See West & Sheldon-Keller (1994) for a complete discussion of the picture selection process.) The AAP drawings depict events that, according to theory,

activate attachment, for example, illness, solitude, separation, and abuse. The drawings contain only sufficient detail to identify an event; strong facial expressions and other potentially biasing details are absent. The characters depicted in the drawings are culturally and gender representative (George & West, 2001, p. 32).

In other words, the AAP uses drawings to elicit information about attachment relevant experiences. Each interview is then transcribed verbatim. The AAP codes for three major categories; discourse, content and defensive processing. There are eight subscales scales organized under each of the three major categories. The table below summarizes the AAPs coding scheme:

<i>Dimensions</i>	<i>Pictures coded</i>	<i>Definition</i>	
<i>Discourse dimensions</i>			
Personal experience	All	Story includes own life experience in response.	Present; Absent
Coherency	All	Degree of organization and integration in the story as a whole	3-point rating scale combining quality, quantity, relation, manner
<i>Content variables</i>			
Agency of Self	Alone	Designates degree to which story character is portrayed as integrated and capable of action.	Internalized Secure Base, Haven of Safety; Capacity to Act; No agency
Connectedness	Alone	Expression of desire to interact with others	Clear signs of a relationship in the story. Relationship not possible (e.g. someone walks away, someone is dead); engaged in own activity
Synchrony	Dyadic	Characters' interactions are reciprocal and mutually engaging.	Mutual, reciprocal engagement; Failed reciprocity; No relationship is acknowledged in the story.
<i>Defense variables</i>			
Deactivation	All	Evidence of deactivation and demobilization	Negative evaluation, Rejection, Social roles, Power, Stereotyped scripts, Achievement, Minimization, Nullification, Shutting down narrative, Distancing, Neutralizing, Demotion, Deactivating language
Cognitive disconnection	All	Evidence of uncertainty, ambivalence, and preoccupation	Uncertainty, Withdrawal or Withhold, Anger, Busy or /Distracted, Feisty, Entangled, Disconnection, Glossing over, Unfinished thoughts, Stumbling, Literal descriptions, Passive language
Segregated systems	All	Evidence of being overwhelmed by attachment trauma	Danger or Failed protection, Helplessness or Out of control, Emptiness or Isolation, Odd or Disturbing themes, Dissociation, Intrusion, Constriction

George & West (2001, pp. 41-42).

The AAP uses the same main classifications used by the AAI (e.g., secure, dismissing, preoccupied, and unresolved). George and West report “empirical validation of the AAP” in three studies with a combined sample size of 75 (George & West, 2001, p. 32). Reported results indicate, “strong interjudge reliability and convergent agreement” with AAI classifications (George & West 2001, p. 33).¹ The author of the AAP, Dr. Carol George, coded all AAPs used in the study.

Dr. Steve Hamilton, a geneticist from the University of California, San Francisco, who is familiar and experienced in the technology necessary to conduct methylation profiles, particularly of the 5-HTTLPR gene, performed the methylation analysis. Generally, the process of defining methylation profiles followed a protocol used by UCSF in genetic research. More specifically, the analysis used a process called sodium bisulfate methylation mapping.

In the present case DNA was extracted from peripheral blood samples. An assay is designed to amplify specific regions of the genome. A polymerase chain reaction (PCR) process is then used to create multiple copies of a specific DNA base sequence. The amplified DNA is then treated with sodium bisulfite in order to help identify quantitative methylation patterns. Analysis of the PCR product can take place by using mass spectrometry to measure the proportion of methylation at each cytosine (Zilberman & Henikoff, 2007). The process is reported in greater detail in Zilberman & Henikoff (2007). Blood was taken at the university student health center by licensed phlebotomists.

This study will inform us about what, if any, relationship exists between attachment classification, and 5-HTT gene or GR gene methyl profiles. The study will also give us practical information about the feasibility of the study.

All coding of the AAI was blind to coding of the AAP, and all coding of the AAP was blind to coding of the AAI. All AAI and AAP coders were blind to methylation levels and Dr. Hamilton's lab was blind to any and all coding for the AAI's or AAPs.

The Beck Depression Inventory –II (BDI) and the Beck Anxiety Inventory (BAI) was used to assess current levels of depression or anxiety (BDI-II; A. T. Beck, Steer, & Brown, 1996). The BDI is a validated and commonly used self report measurement for determining various levels of depression in adult participants. Participants rate 21 depressive symptoms on a scale ranging from 0 (not present) to 3 (severe) that may have occurred during the prior 2 weeks. Scores range from 0-63. Strong internal consistency and convergent validity has been reported (Beck, et. al., 1996).

Similarly, the BAI (Beck et al., 1988;1961) requires participants to answer 21 questions about various symptoms of anxiety that they may have experienced in the last week (e.g., sweating, numbness, trembling, etc.). There are four possible answers (1. Not at all; 2. Mildly: it did not bother me much; 3. Moderately; it was very unpleasant, but I could stand it; 4. Severely: I could barely stand it). The BAI has four possible scores (0-7; minimal anxiety: 8-16; mild anxiety: 16-25 moderate anxiety and 26-63; severe anxiety). The BAI has been found to be internally consistent and reliable (Cronbach's alpha of .94 and test-retest reliability coefficient of .67) (Fydrich, Dowdall & Chambless, 1992).

The Hollingshead index (Hollingshead, 1975), commonly represented as a "gold standard" measure, was used for assessing SES. Alternative scales, the Family Affluence Scale (FAS) and the MacArthur Scale of Subjective SES, were used to examine SES. The MacArthur Scale of Subjective Social Status-Youth Version (MSSS) was developed to ascertain an adolescent's sense of his or her own social and economic status. The measure presents two simple pictures of "social ladders," each of which has 10 rungs, and the participant is asked to place an "X" on the rung which they feel best represents their economic status and their social standing in school. SES has long been recognized as relevant to health outcomes in children but research with the MSSS has demonstrated that subjective social status is also associated with health and obesity (Goodman et al., 2001;2003). The FAS was used because it was specifically designed to be easy and more accurate for adolescents to report SES. The FAS has been used and validated in a number of studies in Europe in the *World Health Organization-Health Behavior*

in *School-aged Children survey*, and recently in Taiwan (Boyce, Torsheim, Currie & Zambon, 2006; Holstein, Currie, Boyce, et. al., 2009; Lin, 2010). The FAS asks four questions concerning how many cars the family owns (0, 1, 2 or more), how many computers (0, 1, 2, 3 or more), number of family vacations (0, 1, 2, 3 or more), and whether the participant has their own bedroom (no = 0, yes = 1). Scores range from 0 to 9 (0-3=low SES, 4-5=middle SES and 6-7 high SES). A correlation analysis between the Hollingshead and the FAS was performed to test construct validity.

6. Data Collection Methods: As noted earlier, volunteers were solicited to participate in the study through the Department of Social Welfare's website, posting notices on the notice board outside the psychology student affairs office, in numerous restrooms and on bulletin boards around campus, and through one classroom presentation. Students were invited to either call the author or send an email to participate in the study. As explained above, the AAI and the AAP are structured interviews. All measures were administered in a private office to ensure complete confidentiality. Participants were compensated \$50.00 after completion of all measures. The author has been trained to administer both the AAI and the AAP. Because participants were required to be over the age of 18 to participate in the study, only the informed consent of the student was necessary. Interviews were audiotaped and transcribed by an individual experienced in transcribing AAIs. As also noted above, all blood was obtained from the student health center by licensed phlebotomists and frozen until they could be delivered to Dr. Hamilton's lab for storage and extraction. Blood draws were performed for approximately half the students before the administration of the measures, and half after the administration of the measures. Exclusionary criteria included any participant that had used psychotropic medications or glucocorticoids within the last month. Only one participant reported ever using psychotropic drugs and no participants were found to have ever used glucocorticoids.

7. Preliminary data analysis plan. The independent variables in this study are attachment classification, determined, as stated above, by the administration of the AAI and the AAP, and SES determined by the FAS and the HSES. The dependent variable is methylation level. The MSES and MSSS may also be considered dependent variables.

Professor Hamilton's lab prepared the assays that were delivered to the UCSF Helen Diller Family Comprehensive Cancer Center Genome Analysis Core for methylation analysis. Each sample was separately genotyped to determine if the participant carries a long or short allele of the 5-HTTLPR and then methyl profile measured with sodium bisulfate methylation mapping, as described above, for both the 5-HTTLPR and the GR gene. As noted above, Professor Hamilton's lab was blind to all attachment classifications, and all AAI and AAP coders were blind to methyl profiles.

First, inter-rater reliability for the AAI was determined using Cohen's Kappa, and an ANOVA was performed to determine if BDI and/or BAI is associated with SES, attachment classification alone and attachment classification x SES.

Second, a Spearman correlational analysis was performed to determine if the AAI correlated with the AAP. Since the AAP is a new instrument such a correlation could contribute meaningfully to the field by revealing a realistic alternative measure to the AAI.

Third, a Spearman correlational analysis was performed to determine if the HSES correlated with the FAS.

Fourth, an analysis of variance (ANOVA) was performed to determine if attachment state of mind predicted how participants would subjectively rate themselves on the MSES and the MSSS. The hypothesis is that lower SES unresolved participants would rate themselves lower in MSSS.

Fifth, because earlier Philibert had found an association between the ss genotype for the 5-HTTLPR polymorphism of the serotonin transporter and unresolved attachment classification (Caspers, et. al., 2009), a chi square analysis was conducted to determine if participants with the ss genotype were over-represented in the unresolved attachment classification.

Finally, statistical testing using both standard regression and principal component analysis was conducted to determine if any meaningful differences in methylation of the 5-HTT and GR genes exist according to attachment classification, and according to attachment classification across the two HSES categories. We hypothesize that there will probably be no statistically significant differences between participants in methylation levels based on attachment classification alone. We hypothesize that upper/mid HSES resolved (secure and insecure) participants will have significantly higher levels of cytosine methylation than lower HSES participants in both genes. It is unknown if any significant differences in methylation levels between secure and insecure participants will be detected, but if any significant differences exist, they will only exist between low HSES insecure participants and high HSES secure participants.

Human Participants. Human participants approval was obtained from the University of California's Committee for the Protection of Human Participants ("CPHS) as well as from the Committee on human Research at the University of California, San Francisco.

Chapter III

RESULTS

The demographic, AAP, BAI, BDI, HSES, FAS and 5-HTTLPR genotype results for 101 participants, and the AAI results for 97 participants are presented in Table 1

Table 1	
Characteristic	Value
Age (years, mean/SD)	19.8 (0.4)
Female (n,%)	82 (81.2%)
European-American(n,%)	34(33.7%)
Asian-American (n,%)	49 (48.5%)
Hispanic (n, %)	12 (11.9%)
African-American (n,%)	2 (2%)
Other	4 (4%)
BDI (mean/SD)	6.9 (6.5)
Severe	1 (.9%)
Moderate	5 (4.95%)
Mild	10 (9.9%)
Minimal	85 (84.1%)
BAI (mean/SD)	6.1 (5.7)
Severe	2 (1.9%)
Moderate	4 (3.9%)
None or mild	95 (94.0%)
Hollingshead Index (mean/SD)	47.9 (15.1)
Hollingshead Index groups (n,%)	
≥54	44 (43.6%)
40-54	32 (31.7%)
30-39	9 (8.9%)
20-29	9 (8.9%)
≤20	7 (6.9%)
≥30	85 (84.2%)
<30	16 (15.8%)
Family Affluence Scale (mean/SD)	5.66 (1.1)
Range	2.0 (min) 7.0 (max)
Family Affluence Scale index groups (n,%)	
Low (0-3)	5 (4.95%)
Middle (4)	10 (9.9%)
(5)	19 (18.8%)
High (6-7)	66 (65.3%)
AAI (n,%)	
Secure (“F”)	46(45.5%)
Insecure(“D/E”)	31 (30.6%)
Unresolved (“U”)	20 (19.8%)
Ambiguous or missing data	4 (3.9%)
AAP (n, %)	
Secure (“F)	8 (7.9%)
Insecure (“D/E”)	57 (56.4%)
Unresolved (“U)	36 (35.6%)
5-HTTLPR genotype (n,%)	
S/S	33 (32.7%)
S/L	49 (48.5%)
L/L	19 (18.8%)

The distribution of AAI classifications (secure-autonomous [F], insecure-preoccupied [E], insecure-dismissing [D], and unresolved [U]) is not different from reported rates in large surveys (Bakermans-Kranenburg and Van Ijzendoorn, 2009; Van Ijzendoorn, 1995). The sample was enriched with females. The majority of participants self-identified as Asian-American. Recent data indicates that 45.7% of the student body at the study site is Asian-American.¹⁸ Accordingly, there was a slight enrichment of Asian-American students in this

study (48.5%). Similarly, European-American students were also slightly over-represented (31.7% on campus vs. 33.7% in the study). African-American students were under-represented (3.4% on the campus vs. 2% in the study). The category classified as “other” which generally held participants of mixed ethnic background, was overrepresented at 4% vs. 0% on campus.

Inter-rater Reliability. Inter-rater reliability was high for general classifications. All the raters were blind to each other, and to any statistical data generated in the study including methylation levels, BDI, BAI, MSES, HSES, and MSS scores. Rater 1 scored all the transcripts (n=97), rater 2 scored 81 of the transcripts, and rater 3 scored 31 of the transcripts. Accordingly, inter-rater reliability was based on 81 transcripts between rater 1 and rater 2, and 31 transcripts between rater 1 and rater 3. Inter-rater reliability between rater 2 and rater 3 was made on the basis of 15 transcripts. Inter-rater agreement scores across all four classifications were as follows:

Rater 3 vs. Rater 1: Kappa = 0.89 (93.6% agreement)
 Rater 3 vs. Rater 2: Kappa=0.77 (86.7% agreement)
 Rater 1 vs. Rater 2: Kappa = 0.96 (97.5% agreement)

Correlation between AAI and AAP. The coding for the AAI and the AAP is given in Table 2. Below is the cross tabulation for AAI and AAP scores.

Table 2

*AAI Final Ratings * Adult Attachment Projective Crosstabulation*

		Adult Attachment Projective			Total
		Secure	Insecure	Unresolved	
AAI Final Ratings	Secure	5	22	19	46
	Insecure	2	20	9	31
	Unresolved	1	13	6	20
Total		8	55	34	97

Correlation between the AAI and the AAP used a non-parametric Spearman correlation since the data are not normally distributed. No correlation was found between the AAI and the AAP across all found classifications ($r = -0.07$, $p\text{-value} = 0.52$).

Does BDI/BAI vary across levels of attachment classification? The vast majority of participants scored in the minimal or mild range on the BDI. Only one participant scored in the severe depression range and five participants scored in the moderate depression range. An analysis of variance (ANOVA) was performed to compare mean scores across three groups, secure, insecure and unresolved (dismissing and preoccupied participants were combined) No difference was detected in means across categories of AAI alone ($p=0.50$). An ANOVA was done using the AAP and no difference in means across attachment classifications were detected on the BDI ($p=.39$). Similarly, only two participants scored in the severe anxiety range on the BAI, and only four participants scored in the moderate anxiety range. Another ANOVA was performed comparing means across the three attachment classifications and no differences in

means across categories of AAI were detected ($p=.71$). An ANOVA was also done across attachment classifications determined by the AAP, and no meaningful differences were detected in BAI scores ($p=.40$).

Does BDI/BAI vary across attachment classification when SES is taken into account? An ANOVA was conducted to determine whether statistically significant differences in BDI would emerge across AAI classification when HSES was taken into account. No significant differences emerged for participants in the high or low HSES categories ($p=0.30$ for high HSES and $p=0.68$ for low HSES.) Similarly, no significant differences in BAI scores emerged between attachment classifications when HSES was taken into account ($p=0.24$ for high SES and $p=0.24$ for low SES).

Does BAI/BDI vary across levels of Hollingshead SES (HSES) score alone? An ANOVA was performed to compare mean scores on the BAI across the five categories of the HSES. Statistically significant differences in BAI scores were not detected ($p=0.20$). Statistically significant differences in scores on the BDI, however, were detected across all SES categories ($p=.03$). Using post-hoc pairwise testing, significant differences were detected between groups 1 and 5 (the highest and lowest SES groups); 2 and 5, 1 and 3; 2 and 3; 1 and 4 and 2 and 4. The greatest differences existed between categories 1 and 5. Means and SD are given below in Table 3:

Table 3
Mean BDI scores X Hollingshead category

Holl_cat	N	Mean (sd)
1	44	4.95 (4.55)
2	32	5.94 (4.77)
3	9	11.0 (5.92)
4	9	10.6 (10.7)
5	7	14.0 (10.2)

Again, these results must be treated with caution because of the low number of depressed individuals in this sample.

Does BDI/BAI differ based on sex or ethnicity? A nonparametric test (Wilcoxon rank sum test) was used since these scores are not normally distributed; no statistically significant differences in BDI scores emerged between the sexes ($p=0.16$.) Similarly, using the Wilcoxon rank sum test, no significant differences in BAI scores emerged between the sexes ($p=0.64$). The Kruskal-Wallis test (a non-parametric version of the ANOVA) detected no significant differences between ethnicities in either the BDI ($p=0.46$) or BAI ($p=0.95$).

In sum, neither attachment nor ethnicity was related to BDI or BAI scores. BAI scores were not related to HSES. BDI scores were significantly related to HSES although extreme caution must be exercised given the low number of depressed participants in this sample.

HSES and self-reported ethnicity. Using logistic regression, as well as the Kruskal-Wallis (a non-parametric version of the ANOVA) the only association detected between perceived

ethnicity and HSES when Hollingshead categories are divided into low HSES (Hollingshead categories 4,5) and high HSES (Hollingshead categories 1,2, and 3) was between low SES and the Hispanic ethnicity ($p=0.003$; $p=0.0005$). This is almost certainly because there were only 12 participants who self-identified as Hispanic and half of those individuals were in the lower HSES categories.

HSES and attachment classification. When all 5 Hollingshead SES categories are used, using a Fisher's Exact test, HSES is not significantly associated with attachment class ($p=0.42$).

Attachment and sex? No significant differences emerged in attachment classification based on sex: Although there were a higher number of U's among women (5 males vs. 15 females), these differences were not significant after a chi-square analysis was performed ($p=0.611$).

Attachment and ethnicity; A Fisher Exact test revealed no association between ethnicity and attachment ($p=0.51$).

Sex and HSES. Chi square analysis revealed no significant differences in HSES between participants based on sex ($p=0.386$).

Sex and ethnicity. Chi square analysis revealed no significant differences between the sexes based on ethnicity ($p=0.822$).

Correlation between Hollingshead SES (HSES) and Family Affluence Scale (FAS). The study also tested another measure of SES called the FAS. The distribution of FAS and HSES scores is presented in Table 1 above. A non-parametric Spearman correlation was used because the data here are not normally distributed. A significant correlation between the FAS and the HSES was detected ($r=.26$, $p\text{-value}=0.008$).

The MacArthur Subjective Social Status Scale (MSSS) and the MacArthur Subjective Scale of SES (SES). Mean scores on the HSES, MSES and the MSSS stratified by ethnicity are set forth below in Table 4. There was a strong significant correlation between *objective* SES as measured by the HSES and *subjective* SES as measured by the MSES ($r=.72$; $p<0.0001$). No significant differences between the sexes emerged on either the MSES ($p=0.77$) or the MSSS ($p=0.83$).

Table 4
HSES, MSES and MSSS Mean Scores X Ethnicity

Ethnicity	HSES	MSES	MSSS
Euro-Am	1.5	7.47	7.8
Asian-Am	2.1	5.98	6.9
African-Am	2.0	7.0	7.0
Hispanic	3.4	4.17	6.4

Objective SES (HSES) was significantly positively correlated with subjective perception of social status (MSSS) ($r=0.36$, $p=0.0002$). Subjective SES (MSES) was also significantly positively correlated with MSSS ($r=0.53$, $p=0.0001$).

Significant differences in objective SES (HSES) were observed across ethnicities when participants were divided into the five individual Hollingshead categories; European-American participants reported the highest SES on the HSES (1.5), followed by African-American participants (2.0), Asian-American participants (2.1) and Hispanic participants (3.4). Similarly, European-American participants reported the highest subjective MSES scores (7.47), followed by African-American participants (7.0, $n=2$), Asian-American participants (5.98) and Hispanic participants (4.17).

Significant differences by ethnicity also emerged on the MSSS: European-American participants classified themselves as higher in subjective social status on the MSSS than either Asian-American or Hispanic participants ($p=0.02$); Asian-American and Hispanic participants did not differ significantly from each other on the MSSS.

Do secure participants report higher subjective social status than insecure or unresolved participants on the MacArthur Subjective Social Status Scale (MSSS). When attachment classifications were compared as a whole, without taking SES into account, no significant differences emerged in social status ratings across the attachment classifications ($p=.93$). When stratified by SES, high SES participants similarly did not appear to differ in subjective status score across attachment classification. However, among low SES participants, a significant difference was observed in MSSS score across attachment groups ($p=0.05$). In post-hoc pairwise testing, significant differences at the $p<0.05$ level were detected between unresolved participants and both secure and insecure participants. Unresolved participants rated themselves *higher* in social status in both cases (difference in mean scores between unresolved and secure participants, 1.68; difference in mean scores between unresolved and insecure participants, 2.80); No significant difference was observed between secure and insecure low SES participants, but the difference in mean scores between secure and insecure lower HSES participants were in the predicted direction (6.1 for secure vs. 5.0 for insecure). Due to a limited number of lower HSES insecure participants ($n=2$), we cannot preclude the possibility that we had insufficient power to detect a significant effect between the two groups. It should be noted that three out of five unresolved participants in the lower HSES category were unresolved with an organized attachment classification of secure (U/F). Because a participant rated a U/F is sometimes treated as simply secure in the literature, the U/F's were moved to the secure category. When the U/F's were moved to the lower HSES secure column, the mean scores for the lower HSES secures rose to 6.54 (confidence intervals were 3.52 – 9.56 for secure and 2.18 – 7.82 for insecure) but the differences between secure and insecure participants still failed to reach statistical significance ($p=0.21$) again, possibly due to the low number of insecure lower HSES participants. Nevertheless, there still appeared to be a trend toward higher MSSS scores in the lower HSES secure category. Table 5 reports the means for the lower SES participants.

Table 5
Mean Score MSSS by attachment classification
for low HSES

Attachment Classification (AAI)	MSSS (sd) Mean Score
Secure	6.54 (1.5)
Insecure	5.0 (1.3)
Unresolved	7.8 (1.1)

Genotype and Attachment

Distribution of 5-HTTLPR genotype, SES and attachment classification: Did any of the attachment classifications appear to be overrepresented in any of the 5-HTTLPR genotypes? . Previously, Philibert and colleagues found an association between the short variant of the 5-HTTLPR and unresolved participants (Caspers, Paradiso, Yucus, et.al., 2009) (although note, as stated above, that finding was not replicated in Reiner & Spangler, 2010). In the present study, although participants with a homozygous ss genotype represented only approximately 32.9% of the sample, approximately 50 % of the U's fell into the ss category. Considering the three genotype categories separately, and classifying the participants as secure, insecure and unresolved, (3 class vs. 3 class comparison) produced a non-significant p -value of 0.4225.

Genotype categorized as ss/sl vs. ll. Following Philibert's (Caspers, Paradiso, Yucuis, et. al., 2009) lead, participants were divided into U vs. non-U categories; genotype was divided into ss/sl vs. ll categories (suggesting that the short allele is dominant). ¹⁹One participant was coded secure but alternatively unresolved (S/altU). It was decided to run the remaining statistical analyses including this participant as secure and then again as unresolved. No statistically significant association between U vs. non-U and genotype was found when including the secure/altU participant as secure ($p=0.225$), or when including the S/altU participant as unresolved ($p=0.189$).

Genotype categorized as ss vs. sl/ll. Because some studies suggest a recessive effect for the ss genotype, we then divided genotype categories into ss vs. sl/ll. Performing a chi-square analysis, and including the secure/altU participant as secure, produced a p -value of 0.069. When the secure/altU participant was included as unresolved, a significant p value emerged (Pearson chi-square p -value: 0.033). A p value of 0.057 was also detected when the secure/altU participant was eliminated altogether. When ethnicity was controlled, however, using the Fisher Exact test, only the participants self-identified as Hispanic had a significantly greater number of U classifications in the ss genotype (p -value; 0.018). These results are set forth in Table 6 below. When the secure/altU participant was designated as secure, the p -value for the Hispanic group was insignificant (Fisher Exact Test; $p=0.127$).

Table 6***Ethnicity X Genotype X Resolved vs. Unresolved (includes U/altF participant as U)***

Ethnicity	Genotype	Resolved		Unresolved		p-value*
		n	(%)	n	(%)	
Asian-American	ss	17	(0.71)	7	(0.29)	0.50
	sl/II	18	(0.82)	4	(0.18)	
European-American	ss	4	(0.80)	1	(0.20)	1.00
	sl/II	25	(0.86)	4	(0.14)	
Hispanic	ss	0	(0.0)	3	(1.00)	0.018
	sl/II	8	(0.89)	1	(0.11)	
African-American	ss	0		0		**
	sl/II	1	(0.50)	1	(0.50)	
Other	ss	0		0		**
	sl/II	3	(1.00)	0		

* calculated using two-sided Fisher's Exact test

** could not be calculated

Methylation of the 5-HTT/SLC6A4 gene, the GR/NR3C1 gene and Attachment Classification

This study targeted regions of the GR/NR3C1 gene and the 5-HTT/SLC6A4 gene for methylation analysis that are commonly used in the research literature (McGowan et al., 2009; van IJzendoorn et al., 2010) (Figure 1) (for purposes of the remainder of the analysis, the 5-HTT gene will be referred to as the SLC6A4 gene, and the GR gene will be referred to as the NR3C1 gene). We identified data for 45 CpG sites. Ten CpG sites for NR3C1 and 30 CpG sites for SLC6A4 met the requirement of >5% difference in methylation between minimum and maximum methylation fraction among the participants; the five sites that did not meet this requirement were not included in Figure 1 below, but were included in the final analysis in order to adjust the findings. Figure 1 below sets forth the targeted regions for the methylation analysis, and Figure 2 the mean levels of methylation for 101 participants.



Figure 1: Targeted regions for methylation data. Upper panel, NR3C1, with block of sequence in CpG island (green) upstream of 1F exon. Lower panel, two amplicons used to cover SLC6A4, in CpG island upstream of exon 1.

Figure 2

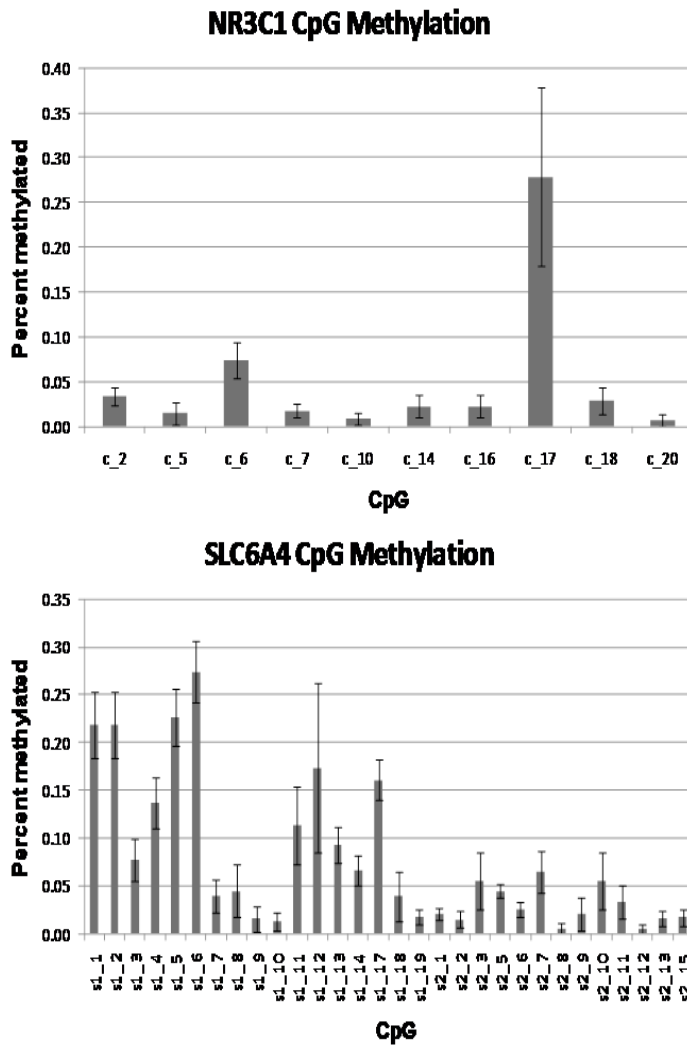


Figure 2: Mean methylation, with standard deviation, for NR3C1 amplicon (upper panel) and two SLC6A4 amplicons (lower panel) for 101 participants. (Hamilton, 2011).

Methylation levels.

After conducting an ANOVA we found some evidence of a difference in mean methylation levels between ethnic groups (adjusted ²⁰p-value=0.04). Recall, no significant differences between the sexes were detected on the basis on attachment classification or HSES. No significant difference was detected between the sexes based on genotype. We found that only one CpG site did differ based on sex in the SLC6A4 gene with very slightly higher levels in females (adjusted p=0.04).

Initial investigation: Our initial investigation found that four CpG sites in the two gene (1 in NR3C1, 3 in SLC6A4) were nominally associated with low HSES at the $p \leq 0.05$ level. Five non-overlapping CpGs (all in SLC6A4) were associated with the U vs. non-U classifications. To test the hypothesis that secure attachment may be protective against the effects of SES as

measured by Holl-30, we found that three of the four Holl-30 CpGs were no longer associated with CpG methylation when participants were divided into secure and unresolved classifications. For example, the association between SES and one CpG in SLC6A4 (s1_4) appears to be dependent on unresolved vs. secure classification, where CpG methylation is not correlated with SES in individuals with secure (F) attachment ($p=0.46$), while those with unresolved attachments (U) showed CpG methylation differences based on SES ($p=0.03$), as shown in **Figure 3**. In other words, no significant difference in methylation levels appeared between secure participants across HSES, but significant differences in methylation did appear between unresolved participants across HSES.

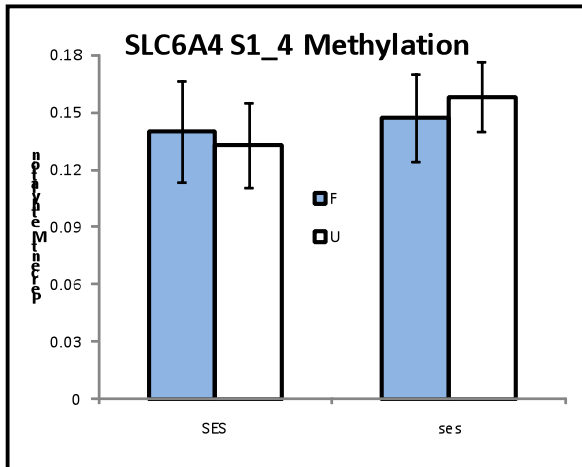


Figure 3: Percentage CpG methylation at SLC6A4 site S1_4, stratified by SES (SES, ≥ 30 , high; ses, < 30 , low) and then by AAI U vs F attachment classification.

After adjusting for multiple testing (i.e., 45 CpG testing sites as well as SES, sex, ethnicity, and attachment classification), however, all statistical significance was lost. Using regression analysis, no significant differences in methylation levels were found between participants based on SES alone (adjusted p values; SLC6A44=0.17, and 0.35; NR3C1: $p=0.45$) or based on attachment classification (unresolved vs. resolved) alone (adjusted p values; NR3C1: $p=0.34$; SLC6A44: $p=0.34$). No significant differences in methylation levels between lower SES secure participants and upper SES secure participants emerged, in either the SLC6A44 or NR3C1 gene. We also found no significant differences in methylation levels between upper SES participants who were secure and lower SES participants who were insecure or unresolved. We concluded that at least two

explanations could explain these findings; there were no significant differences to detect, or the findings were a consequence of lack of power and the impact of multiple testing corrections.

Aggregation of signal across sites improves signal and reduces the impact of multiple testing corrections. Accordingly, principal component analysis (PC), a technique that creates a weighted average of methylation levels for each participant, was used which reduced multiplicity from 45 CpG's to 3 sets (one for the NR3C1 gene and two for the SLC6A4 gene). Marginal differences were detected between participants based on sex with females showing very slight increases in methylation compared to males (adjusted $p=0.019$). Surprisingly, a marginally significant difference in methylation levels was detected between participants based on attachment classification (resolved vs. unresolved) alone in the SLC6A44 gene across all HSES and ethnic categories; unresolved participants had lower methylation levels than resolved participants (adjusted p-value: 0.055) as shown in Figure 4²¹. Lower SES unresolved participants had marginally higher levels of methylation than upper SES unresolved participants (adjusted p value=0.058) in the SLC6A4 gene.

The low HSES-high HSES marginal result was complicated by the fact that ethnicity composition differed substantially between the two groups; one of the groups was about half European-American while the other had no European-American participants. Accordingly, we

stratified to (1) ensure that we were not confounding ethnicity with SES effect, and (2) possibly improve power by reducing heterogeneity. This stratification clarified the picture: controlling for ethnicity and SES, a stronger relationship between attachment and methylation emerged; lower SES Asian-American unresolved participants had higher levels of methylation than higher/mid SES Asian-American unresolved participants (adjusted p-value= 0.0016). No significant differences in methylation levels were detected between upper/mid HSES resolved participants and lower HSES resolved participants. No significant differences in methylation levels were found between unresolved and resolved Asian-American participants independent of HSES. Collapsing Hispanic identified participants with Asian identified participants produced similar results, although not quite as strong (adjusted p-value; .0066) suggesting that there are differences between these ethnic groups. Figure 5 presents methylation levels for unresolved upper HSES participants vs. unresolved lower HSES participants among the Asian-American and Hispanic sample.

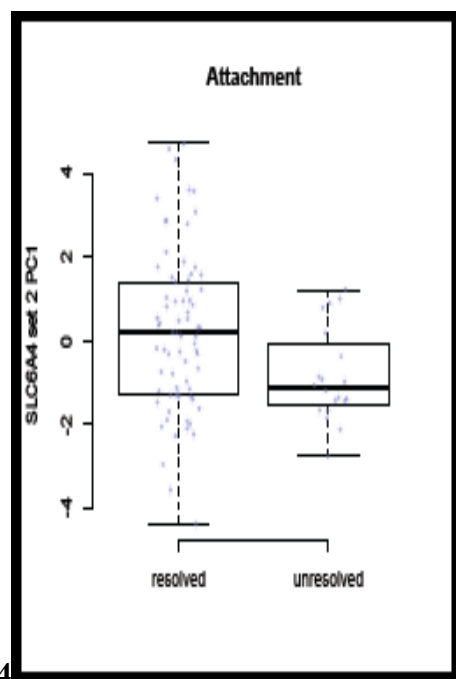


Fig. 4

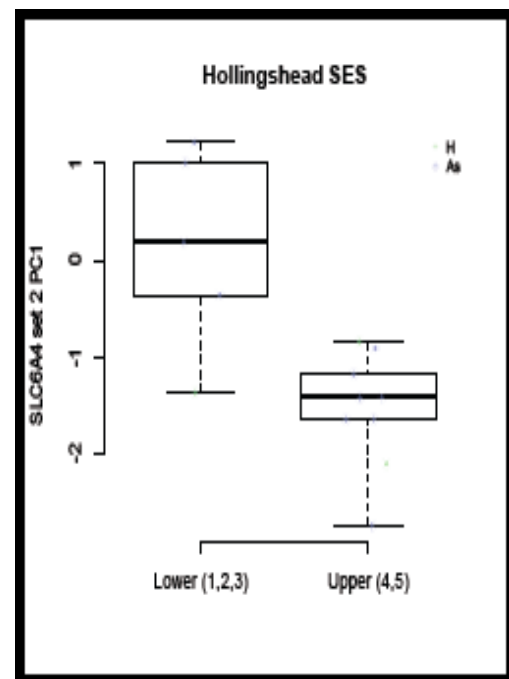


Fig.5

Figure 4: Methylation level in SLC6A4 Compared in Resolved vs. Unresolved Participants across Ethnicity and HSES. Figure 5: Methylation level in SLC6A4 in Unresolved Asian-American and Hispanic Participants by HSES.

No significant differences in methylation levels were detected between participants in the NR3C1 gene using PC analysis.

Additional testing revealed no significant differences in methylation levels within the Asian-American sample between the following participants;

1. Upper HSES secure (ss) vs. Low HSES unresolved (ss);
2. Upper HSES secure (ss/sl) vs. Low HSES unresolved (ss/sl);
3. Upper HSES secure (ss) vs. Lower HSES unresolved (ss/sl);

4. All secure (ss/sl) vs. all secure (ll);
5. All with ss/sl genotypes vs. all with ll genotypes;
6. All with ss genotypes vs. sl/ll genotypes.

Finally, using the *AAP* to identify unresolved participants, among Asian-American participants, no statistically significant differences in 5-HTTLPR methylation levels between lower HSES unresolved participants and upper HSES unresolved participants were detected.

In sum, the hypothesis that lower HSES unresolved participants will have higher methylation levels than upper HSES resolved participants was not supported. Marginally significant differences in methylation levels were detected between all resolved and unresolved participants using PC: unresolved participants actually had lower methylation levels than resolved participants. This relationship changed, however, once HSES was taken into account. Across all ethnicities and HSES, lower HSES unresolved participants had marginally higher levels of methylation than upper HSES unresolved participants. Controlling for ethnicity clarified this relationship: in participants self-identifying as Asian-American, lower HSES unresolved participants had statistically significant higher levels of methylation than higher HSES unresolved participants. Other findings in this study include Hispanic participants appeared to demonstrate a disproportionate number of ss participants in the U classification. In addition, the higher participants were in HSES status, the higher they rated themselves in subjective social status (MSSS). Finally, there were no differences in subjective social status (MSSS) detected among upper HSES participants across attachment classifications. Although differences in subjective social status between secure and insecure lower HSES participants were in the predicted direction, these differences did not reach the level of significances; significant differences in MSSS scores were found between lower HSES unresolved participants and secure and insecure participants although not in the predicted direction.

CHAPTER IV DISCUSSION

This study attempted to answer a number of questions designed to further knowledge about the manner in which environment impacts development focusing on early life experiences, SES and genetic expression.

The study hypothesis that lower HSES unresolved participants would have higher levels of methylation than upper HSES secure participants was *not* supported, possibly due to power issues and the impact of multiple testing requirements. In other words, the p-value here had to be adjusted for testing at no less than 45 different CpG sites. To increase power a statistical analysis called principal component analysis (PC) was used which essentially created a weighted average for 3 sets of 13 different CpG sites and reduced the p-value adjustment from 45 to three. PC analysis produced some unpredicted results. For example, although the results were marginal (p-value=0.055), it was a surprise that any differences in methylation levels were detected between participants on the basis of attachment classification alone (unresolved vs. resolved). Moreover, the direction of these differences were unexpected; unresolved participants had lower methylation levels than resolved participants. This finding might

actually be consistent with a new study from Philibert and colleagues that found that higher levels of methylation in participants with the ss genotype is associated with lower scores on unresolved loss or trauma (Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach & Philibert (2010)). In other words, less U is associated with higher methylation levels among those participants with the ss genotype. Philibert and colleagues opined that since the ss genotype is associated with environmental sensitivity, higher methylation levels may act to protect individuals from the impact of trauma or loss. It must be remembered that Philibert's participants came from the middle or upper SES category.

The present study, however, included participants from the middle/upper HSES and lower HSES categories. Controlling for SES turned out to be important in the present study because the relationship between higher methylation and lower levels of unresolved attachment that Philibert and colleagues detected reversed itself when HSES was taken into account. Across all ethnicities lower HSES unresolved participants had marginally higher levels of methylation than unresolved HSES participants. Only after controlling for ethnicity and HSES, and using PC, and only in Asian-American and Hispanic participants, was it found that lower SES Asian-American and Hispanic unresolved participants had significantly higher levels of methylation than higher/mid SES Asian-American and Hispanic unresolved participants (adjusted p-value=0.0066). Moreover, the fact that Asian-American participants had a lower p-value than prior to the addition of Hispanic participants (p-value=0.0016) suggests that there are differences between these two groups that may have not been fully registered because of the small number of Hispanic participants. Accordingly, the results could be viewed as positive primarily in Asian-American participants. No differences in methylation levels were detected between Asian-American resolved participants across HSES, which was consistent with the hypothesis in this study.

The question remains why would these differences appear in a statistically significant form only among Asian-American participants? The obvious answer would be genotype and HSES distribution. As the present study demonstrates, genotype and ethnicity is confounded. Table 7 sets forth self-reported ethnicity x genotype (by count and percentage with significant data highlighted in red). Examining the genotype distribution in this sample reveals that 96% of all Asian-American participants carry at least one "s" allele; 76% of all ss participants and 45% of all sl participants were Asian-American. Among European-American participants, however, only 15.2% carried the ss genotype and 28% carried the sl genotype; however, 79% carried the ll genotype. Among Hispanic participants 83% carried at least one s allele. Moreover, the only ethnic groups represented in the lower HSES group as defined in this study were Asian-American and Hispanic participants. Accordingly, if Philibert and colleagues are correct that attachment impacts methylation to a detectable and statistically significant extent in those participants with the ss genotype, the findings in the present study could be explained by the fact that the ss genotype was present to the highest numbers in Asian-American participants.

Table 7
Genotype Distribution: Ethnicity X HSES

HSES123vs45			Genotyp5HTTLPR			Total
			ss	sl	ll	
123	Self-reported Ethnicity (n,%)	Asian American	20(51%)	17(44%)	2(5%)	39 (100%)
		% within Geno5HTT	77%	42.5%	10.5%	46%
		European-American	5(15%)	14(41%)	15(44%)	34 (100%)
		% within Geno5HTT	19.2%	35.0%	79%	40.0%
		Hispanic	1 (17%)	3 (50%)	2 (33%)	6 (100%)
		% within Geno5HTT	3.8%	7.5%	10.5%	7.1%
		African-American	0 (0%)	2 (100%)	0 (0%)	2 (100%)
		% within Geno5HTT	0%	5%	0%	2.4%
		Other	0 (0%)	4 (100%)	0 (0%)	4 (100%)
	% within Geno5HTT	0%	10%	0%	4.7%	
Total		26(30.6%)	40(47%)	19(22%)	85 (100%)	
% within Geno5HTT		100%	100%	100^	100%	
45	Perceived Ethnicity(n,%)	Asian American	5 (50%)	5(50%)		10 (100%)
		% within Geno5HTT	71.4%	55.6%		62.5%
		Hispanic	2(33%)	4(67%)		6(100%)
		% within Geno5HTT	28.6%	44.4%		37.5%
	Total		7(44%)	9(66%)		16(100%)

To make the picture even murkier, however, there was some evidence that the opposite pattern in methylation levels exists among secure participants (secure upper/mid HSES participants had higher levels of methylation than secure lower HSES participants) although the sample size was too small and the variance too great to produce a significant p-value. Thus the following table might be what ultimately emerges when larger sample sizes are present;

Table 8

Methylation Levels; Attachment Classification X HSES in Asian American participants.

Attachment Classification	Methylation Levels in Upper/Mid HSES	Methylation Levels in Lower HSES	Methylation levels in Combined HSES
Resolved (secure and insecure participants collapsed)	High/Mid HSES resolved participants = Lower HSES resolved participants	High/Mid HSES resolved participants = Lower HSES resolved participants	RESOLVED = UNRESOLVED
Secure	\wedge ?	\vee ?	
Unresolved	Unresolved Up/mHSES \vee Unresolved Lower HSES participants	Unresolved Lower HSES \wedge Unresolved Upper/mid HSES	

What would explain such a result? As stated above, the ss genotype is hypothesized to be associated with environmental sensitivity (Caspi, et. al., 2003). If it is true that the presence of the ss genotype increases environmental sensitivity, then the combination of the unresolved attachment classification and the stress of low SES could explain higher methylation levels in lower HSES participants vs. high/mid HSES participants. In other words, higher methylation levels could have a protective effect on unresolved participants because they mute sensitivity to environment explaining why Philibert found higher levels of methylation associated with lower U scores in middle/upper class participants (so more U associated with low levels of methylation). Unresolved upper/mid HSES participants may possess low methylation levels increasing environmental sensitivity enough to increase the risk of an unresolved classification in an otherwise low risk environment. But why would this relationship be reversed in secure participants? One possibility is that low levels of methylation in the ss genotype is adaptive among low SES children because it increases the child's ability to experience the benefits of sensitive parenting helping to counteract the negative impact of poverty. In a way, if it does turn out that secure lower SES children have lower levels of methylation, it might help explain Suomi's (1999) finding that highly reactive baby monkeys raised by highly nurturing mother monkeys eventually display precocious behaviors, becoming leaders in their troupe. These anxious baby monkeys could have had low methylation levels, enhancing their ability to take advantage of maternal sensitivity.

In summary, the evidence here suggests that in a population in which the ss genotype is present to a sufficient extent, a SES effect appears among at least unresolved participants; lower HSES participants have higher levels of methylation than upper/mid HSES participants, presumably because higher levels of methylation may act to decrease sensitivity to a low SES and possibly abusive environment.

What remains to be seen is whether these results can be replicated in a study with a larger sample size, and whether a larger sample size could produce significant results among secure participants.

This study also found no significant differences in methylation levels between participants based on attachment classification or HSES in the GR gene. The GR methylation results did not come as a surprise since the recent publication of Miller, Chen, Fok, et. al. (2009) which found no differences in genetic expression between participants based on SES in the GR gene. We hypothesized that adding attachment status might produce different results, but no significant differences between participants were detected even considering attachment classification. Once again, Miller and colleagues had a comparable sample size as this study (n=103) so it is entirely possible that both studies just have sample sizes that are too small to detect significant differences in methylation levels.

How does this study inform us with respect to whether attachment relationships act as the cocoons for self-regulation, simply moderate self-regulation or just mediate the relationship between biological processes and self-regulatory behavior? It is very difficult to draw any conclusions from this study given the fact that standard regression could not produce significant results. But if a larger sample size does eventually confirm the results of the analysis performed with PC, then it is possible that the attachment relationship plays both an influential role when it comes to forming epigenetic profiles, as well as a modifying role. Recall that humans are born with certain epigenetic profiles in place already. The fact that we are seeing methylation levels differ in adolescence after taking into account SES and attachment status suggests that both environmental factors are modifying methylation levels interacting in a more general sense; so, for children in the low SES with a sensitive parent, methylation levels might decrease. For children born to a low SES environment with insensitive or possibly abusive parents, methylation levels could increase. What is still difficult to explain is why children in the upper/mod HSES that are unresolved would have lower methylation levels to begin with (remember, no significant differences in methylation levels were found between resolved and unresolved Asian-American participants not controlling for HSES—therefore, methylation levels among unresolved upper/mid HSES participants would have to be lower than resolved participants). Could they just have had the unfortunate luck to have inherited a low methylation profile from their parent? If so, this raises two points. One, methylation profiles probably involve an interaction between what an individual is born with, and what s/he experiences during life. Two, could the heritability of methylation profiles help explain why illnesses such as depression and anxiety run in families?

But here another question presents itself; could the high methylation levels that are thought to be protective against an insensitive and possibly abusive environment also help explain why children with behavioral disorders (e.g., conduct disorder) are so resistant to therapeutic intervention²²? If a child becomes less sensitive to environment in order to survive in the birth home, that very insensitivity could make an environmentally based intervention less successful. It is possible that this research will eventually inform treatment for children with so-called “behavioral disorders” by suggesting that environmental intervention must be combined with some type of intervention directed at bringing methylation levels down enhancing the potential success of treatment. In fact, recently Chen, Ernst & Turecki (2010) found that antidepressants reduced methylation levels (and increased gene expression) among individuals with MDD. If the findings of this study are replicated, then mental health professionals will have to be concerned about the possibility that treating youth with conditions such as conduct disorder with psychotropic drugs (and thereby increasing environmental sensitivity) without providing a concurrent change in environment could end up making these youth, in fact, worse.

The MacArthur Subjective Social Status Scale (MSSS) and the MacArthur Subjective Scale of SES (SES). The higher the participant was in objective *SES* (HSES) the higher they rated themselves in terms of subjective social status (MSSS) ($r=.36$; $p=0.0002$). In addition, the higher the participant rated themselves in subjective *SES* (MSES) the higher they rated themselves in terms of subjective social status (MSSS) ($r=0.53$; $p\text{-value}=0.0001$). In other words, both the objective fact of, and the perception of a higher SES lead to a higher subjective perception of social status. Arguably, such a finding suggests that these participants live in an environment in which high SES is associated with high social status, hardly a shocking finding (although admittedly disturbing to find among adolescents). This is not, however, an unimportant finding. These results suggest that not only does a child growing up in a low SES environment have to deal with the concrete hardships of poverty, but that poverty itself might exact a psychic cost in terms of the perception of lower social status, and consequently, the perception of less control over environment. Recall, that low MSSS has been found to be associated with poor self-rated health outcomes (Goodman, Huang, et. al., 2007).

What might also be of some interest is that despite the fact that significant differences in objective HSES emerged between Asian-American and Hispanic participants, they did not differ in terms of MSSS, and both scored significantly lower in MSSS than European-Americans. It may be of some interest to note that although Hispanic participants were overrepresented in the lower HSES category, all were securely attached raising the question of whether this could have contributed to the trend toward higher MSSS scores in the secure lower HSES participants.

No differences in MSSS emerged across attachment classifications or across attachment classification in the upper HSES. Significant differences in MSSS ratings did emerge among lower HSES participants but in the opposite direction of the hypothesis of the study; unresolved participants had higher MSSS ratings than either secure or insecure participants. This result is almost certainly explainable by the fact that three out of five of the low HSES unresolved participants were assigned a secure organized classification (recall that all unresolved participants receive two ratings; a U for lack of resolution and a second organized rating of D, E or F). Typically, these were participants that had experienced a normative loss such as the death of an aged grandparent in the last year or so. Arguably, these participants should be included among the secure participants in future studies given the low likelihood that the loss of say, an aged grandparent, is going to negatively impact subjective social status. In fact, when those three were added to the secure category in this study, the trend toward a higher MSSS score for secure participants in the lower HSES became clearer. Only two unresolved –insecure lower HSES participants remained and they did rate themselves highly on the MSSS. It is difficult to draw any conclusions from such a small sample size, but it is at least possible that being a student at a major university in the United States is a source of status that compensates for loss of social status that lack of resolution may influence. Future studies should include participants who are unresolved because of abuse and neglect, rather than normative loss.

The AAI and the AAP. Early life experiences were operationalized by two measures, the AAI and a new measure, the AAP. The AAP was tested for concurrent validity. The AAP is shorter, cheaper and easier to administer and code than the AAI, and because it does not ask about

early life directly, arguably avoids possible iatrogenic consequences. If a correlation between the AAP and the AAI, the “gold standard” of attachment measures (Bakermans-Kranenburg, et. al., 2011), emerged, it is possible that the AAP could have been used with high-risk populations such as foster youth. No correlation was found, however, between the AAI and the AAP across all four categories. This was a surprising finding given that George & West (2001, 2003) reported a high convergence (e.g., 0.94 for all AAI classifications) in three smaller studies between the AAI and the AAP: Study one involved 25 mothers from a Canadian infant risk study (mean age 26); study two involved 23 female participants in a depression study; and study three used 48 participants (42 women and 6 males) in a sample of community and university participants. Recently, George communicated a yet unpublished study in which a concordance rate of 84% for the four AAI classification groups, 91% for secure-insecure, and 88% for unresolved-resolved (George, C., personal communication. January, 19, 2011). One possibility is that the population here consisted of almost entirely non-clinical participants and the population sample studied by George & West did include some clinical participants. The George & West study also included some older (average 26 years and 42 years) participants—it is possible that adolescents were just too tired after spending as long as two hours taking measures to really focus on the AAP. Adolescents may also have a hard time taking the pictures seriously as some of the participants were giggling or laughing through the measure. The most obvious explanation for the lack of correlation between the two measures is the fact that the AAI asks questions about early life directly, while the AAP simply requires the participant to make up stories, albeit stories that are theoretically supposed to tap into attachment constructs. It is possible that a projective measure just can’t access conscious or unconscious attachment constructs as well as direct questioning. Finally, it is possible that the AAP is measuring something different than the AAI, although it is unclear what that might be. Recall that, unlike the AAI, methylation levels did not correlate with attachment classification as determined by the AAP, even though PC analysis was used. Since the AAP is a new measure further studies will be needed to determine what, if any, correlation exists between the AAI and AAP. At the moment, the results of this study do not suggest that the AAP can be used as a substitute for the AAI, but efforts should continue to refine and possibly alter the AAP. Until a reasonable alternative is found, however, any research requiring assessment of unconscious attachment constructs would be well advised to continue using the AAI.

Depression and SES. Low SES has been associated with increased risk of depression (Gilman, Kawachi, Fitzmaurice & Buka, 2003). Accordingly, the finding that low HSES was associated with depression measured by the BDI was expected. Extreme caution should be exercised before drawing any conclusions from this result, however, simply because only one severely depressed participant existed in this sample, and only five moderately depressed participants.

HSES and FAS. The study also found a correlation between two measures of SES, the Hollingshead Measure of SES (HSES) and the Family Affluence Scale (FAS). As noted, the FAS is much shorter and specifically designed for use with adolescents, thus positive results might be helpful in future studies given the length and complexity of the HSES. But extreme caution should be exercised. The FAS is vulnerable to criticism on a few grounds. For example, the questions asked could be viewed as culturally biased; in some cultures it is common to share a room with other family members and this fact does not necessarily reflect SES. In addition, it became clear that ownership of a computer could be misleading when some

participants pointed out that yes they had computers, but they had purchased them from their own earnings. The most useful measure of SES probably turned out to be a combination of the HSES and the AAI. Recall that the AAI does ask questions about what families did for a living, giving researchers a better idea of the participant's actual SES. The HSES was very difficult for adolescents to fill out, however. Accordingly, it is not recommended that the HSES be used alone to assess SES among adolescents, but a combination of measures is optimal. Here, the combination of information gleaned from the AAI and the HSES gave researchers more specific information about actual SES than the FAS could do alone.

Strengths and Limitations. The primary strength of the study was the use of what are considered “gold standard” measures; the AAI, the HSES, the BDI and the BAI. It is probably because these instruments were used that the study was able to detect any significant differences. In addition, this study used blood instead of buccal swabs to obtain DNA, a more difficult process but one which is thought to involve less risk of contamination. The main limitation of this study is clear; we needed a bigger sample size, particularly of lower HSES participants. The sample size was considered large for an attachment study, but it became clear that if we were to detect what turned out to be very subtle differences, we should have had a bigger sample size. In addition, it must be remembered that this is a study of college students in late adolescence. Accordingly, it can be generalized only with great caution. The study was also enriched with females, for reasons that are unclear since males were solicited in the same manner. A more focused effort should be made in the future to attract male participants. In addition, most of the study participants were social welfare or psychology students, although students from around the campus were eventually recruited. Future studies may wish to create a more diverse balance of students from other disciplines, although it should be remembered that the distribution of attachment classifications did not appreciably differ from other studies involving adolescents. Finally, the study was not balanced ethnically and, as it turned out, ethnicity turned out to be a crucial factor here because it was confounded with genotype. But in another sense, the multi-ethnic composition of the sample could be viewed as a strength because it allowed us to determine what patterns of genotypes would emerge within different populations, and will help guide us in future research. How these limitations will guide future research are discussed in more detail in the conclusion set forth below.

CONCLUSION

There are a number of conclusions that could be drawn from this study. Remember that no relationship at all was found between attachment and methylation of the GR gene, and the relationship between attachment and methylation of the 5-HTTLPR was detected only with the use of PC analysis, a way of reducing the complexity of the data. Accordingly, it would not be unreasonable to conclude that there are no significant differences in methylation levels to detect between participants using a peripheral blood sample, and that we should be looking at different tissue, particularly neurons. It is almost certainly too early to definitively conclude that studies with DNA drawn from peripheral blood should stop. The fact that results obtained with PC analysis pointed in the direction of significant and/or marginally significant differences in methylation levels between participants based on HSES and attachment classification, and even on attachment classification alone, do not support this conclusion, but it is still entirely feasible. A larger sample size should inform this question. Second, this study

only examined two genes in isolation. Genes generally do not operate in isolation. It could be more fruitful to examine groups of genes linked by “themes” or pathways such as those genes involved in immunity or stress function. For example, recall that Miller, Chen, Fok, et. al. (2009) found that participants raised in a low SES environment did not differ from participants raised in a high SES in terms of expression of the GR gene, but did differ in the expression of genes with response elements for the GR gene—in other words those genes that are controlled by the GR gene while regulating the stress response. Uddin, Aiello, Wildman, et. al. (2010) found that genes related to immune function were differentially methylated in participants with PTSD compared to participants without PTSD. Third, again, this study only looked at two genes, there are approximately 19, 998 other genes still that have not been examined. We examined 45 individual sites in the genome, while current technologies now make possible investigation of ~450,000, while novel high-throughput DNA sequencing technologies make it feasible to catalogue the entire methylome of an individual. Fourth, the difficulties of using brain tissue are fairly obvious; there would have to be available a large cohort of participants who have taken the AAI, left their brain to a brain bank and died. The chances of such tissue being available, although not impossible, is highly improbable. Moreover, it is still possible that genetic information drawn from peripheral blood sources may come close to representing events in the brain. In short, this research literature is relatively recent, and much more research is necessary before any definitive conclusions can be made. Indeed, the results of this study are at least encouraging but they do offer valuable lessons for future studies.

First, strict controls should be exercised on ethnicity for many reasons but particularly because it is clear that genotype distribution can differ between ethnic groups, and methylation levels could differ by genotype. Second although the differences were marginal, all future studies should control for sex. Third, it is absolutely critical to control for SES. The results in this study suggest that SES can actually reverse the direction of methylation, at least in unresolved participants. Fourth, participants that are unresolved because of normative loss should be analyzed separately from those who are unresolved because of trauma and/or abuse. This study did not contain enough traumatized or abused participants to analyze separately to any significant extent. Therefore, a stronger effort needs to be made to increase participants from an at risk population, and many more participants are needed from the lowest SES levels. Fifth, a much larger sample size than the one used in this study is necessary to detect what at this point appear to be subtle differences in methylation levels. Sixth, future research should consider conducting an agnostic genome-wide data collection, instead of examining just a few hypothesis-driven genes. Finally, it cannot be emphasized enough that what may appear to be subtle differences to researchers may not be subtle to nature. The differences detected in this study could very well be important biologically, and may someday make an important contribution to our understanding of human development, and even inform future treatment options.

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APPENDIX A

ENDNOTES

¹ It might be helpful to offer a brief explanation of the concept of genetic expression. It might also be useful to note that genes do not directly cause phenotype (actual outward manifestation of genotype), but are rather part of a complex chemical process that is not fully understood (Rutter, 2006). The current understanding is that DNA (deoxyribonucleic acid) contains four “bases” made up of hydrogen, carbon, and nitrogen, and oxygen (one of the bases does not contain oxygen). These bases are called “nucleotides” and consist of adenine (A) (the only base without oxygen), cytosine (C), guanine (G), and thymine (T). A sequence of three nucleotides is called a “codon” which directs how to construct an amino acid, the building block of proteins. Essentially, DNA exists in the form of a double helix. The double helix is wrapped around a core of eight histone molecules. The DNA and histone create what is referred to as a nucleosome a string of which creates “beads on a string.” The nucleosomes are then wrapped tighter and tighter (think of a rubber band being wound over and over again) and packed into chromatin which is then packed into chromosomes—in fact, it is said that approximately six feet of DNA exists in every human nucleus (Schwartz & Ahmad, 2006). Humans have 20 amino acids. Amino acids make up proteins. DNS stores the instructions to make a protein but these instructions must be “transcribed” onto mRNA (messenger RNA-ribonucleic acid-a single strand of DNA). This process is called “transcription” and is assisted by what are called “transcription” factors (proteins that participate in the syntheses of mRNA). The process is very much like DNA replication but only the instructions of genes are transcribed into mRNA. When mRNA is complete it is transported to ribosomes outside the cell nucleus. Inside the ribosome the process of “translation” occurs; amino acids are constructed from nucleotides, polypeptides are constructed from amino acids and finally proteins are constructed from polypeptides.

² Bowlby defines “instinct” as comprising four main characteristics:

- a. it follows a recognizably similar and predictable pattern in almost all members of species (or all members of one sex);
- b. it is not a simple response to a single stimulus but a sequence of behavior that usually runs a predictable course;
- c. certain of its usual consequences are of obvious value in contributing to the preservation of an individual or of the continuity of a species; and
- d. many examples of it develop even when all the ordinary opportunities for learning it are exiguous or absent (Bowlby, 1969/1982, p. 38).

2 The term “behavioral system was used instead of “instinct.”

⁴ Bowlby indicates that he based his idea of internal working models on the concept created by Craik (1943) who proposed that individuals construct mental models for different kinds of social or physical events as heuristic devices that help them understand the functioning of those events. It may be worthy of note that Mary Ainsworth, a colleague of Bowlby’s and discussed later in this paper, felt that internal working models could be seen as analogous to Piaget’s concept of a “schema” (Ainsworth, 1967). She stated that the “prototypical patterns of attachment behavior’ to a “large extent” overlap with Piaget’s sensorimotor schemata. Like Piaget’s theory of cognitive development, attachment theory asserts that development takes place through transactions with the environment but the transactions are with people rather than merely physical objects. Attachment theory, like Piaget’s cognitive theory, also emphasizes the importance of the infant’s own actions and his or her experiences of the consequences of those actions.

⁵ Although children do grieve in much the same way as adults, Bowlby points out that some differences exist. First, it is much more important that children have an alternative attachment figure to give comfort or support because they don’t know they can survive without such a figure. Second, children do not have the same access to information as an adult—they cannot readily understand the circumstances under which they are experiencing grief or loss. Third, children cannot necessarily seek comfort from others should those close to them fail to give comfort. Fourth, children are less able to understand what has happened, particularly if the adults around them use figures of speech. Finally, children’s moods are more participant to change so those caring for them may be “fooled” into thinking that they are better off than they are. Bowlby advises that the best approach to helping a

child cope with grief and loss is to be honest and open, and explain the facts in terms the child can understand. In this way the child is able to integrate the loss in a healthy manner and proceed with normative development.

⁶ Bowlby points to dichotic listening studies (i.e., individuals given different pieces of information in each ear via headphones are able to selectively attend to one of them but do remember the discarded information when repeated) showing that humans selectively retain or reject information for processing, even after information has gone through fairly significant levels of encoding.

⁷ Ainsworth notes, in another landmark study conducted among 26 mother-infant dyads in Baltimore, Maryland, that the concept of a “secure base” and parent-child security is not new. Ainsworth credits Dr. William Blatz, a psychology professor at the University of Toronto. Blatz constructed a personality theory around the basis of security. Ainsworth states that she first heard Blatz’s hypothesis that children use their parents as a secure base from which to explore their environment in a course Ainsworth took at the University of Toronto in 1934–35 (Ainsworth, Blehar, Waters, and Wall, 1978) although he did not publish his theory until 1966 in a book called *Human Security*.

⁸ George, Kaplan, and Main (1984/1985/1996) report that the adult attachment categories were first generated through study of a development sample of 44 interview transcripts taken from Bay Area parents observed with their infants in Ainsworth’s Strange Situation. The development sample produced the three “organized” adult attachment categories, each corresponding to one of the original organized forms of infant Strange Situation response. Specifically, the mothers and fathers of the six year olds who had been secure with them in the Strange Situation five years earlier were identified as valuing attachment, yet being seemingly objective in describing any particular relationship or experience. The parents of children who had been insecure avoidant were identified as being dismissive of attachment, and the mothers and fathers of children who had been insecure-ambivalent/resistant were described as being preoccupied by past attachment relationships and experiences. The system was then successfully applied to a remaining 66 parent transcripts by a coder blind to the infant Strange Situation behavior. Main and Hesse later reported that the parents of disorganized/disoriented infants in the Bay Area sample exhibited brief lapses in the monitoring of reasoning or discourse during the attempted discussion of traumatic events, and these individuals were assigned to a fourth category—unresolved or disorganized adult attachment. The authors then discussed a meta-analysis authored by Van IJzendoorn (1995) which looked at 18 samples in which an infant’s Strange Situation response to a particular parent was compared to the classification assigned to the parent on the basis of the AAI (854 dyads). When the two insecure adult and infant attachment categories were collapsed to obtain a two-way classification, the correspondence between parental and infant attachment classifications was 75% ($k=.49$). The combined effect size was 1.06 (equal to a Fisher’s Z of .51), $r=.47$ (biserial $r=.59$, Cohen, 1988). Correspondence for the three-way cross tabulation was 70% ($k=.46$), and studies in which the interview was administered prior to the birth of the first child (Fonagy, Steele, & Steele, 1991) showed 69% correspondence for the three-way classifications ($k=.44$, $N=389$).

⁹ Finally, it should be noted that there are other measures that claim to measure attachment constructs, but these are primarily self-report measures. Why are self-report measures sometimes problematic in attachment research? The AAI purports to measure “state of mind with respect to early attachment relations and experiences” (Hesse, 1999—time and space restrictions preclude a thorough discussion of the differences between the AAI and self-report measures; for a more thorough discussion of this topic see Hesse, 1999). As stated above, according to attachment theory, attachment constructs are, at least partially, unconscious. For example, strong defense mechanisms may be employed to protect the individual from consciously acknowledging painful realities (e.g., that a parent was neglectful or abusive). Self-report measures of attachment are thought to reflect conscious beliefs. A number of studies have attempted to illustrate some of the differences between the AAI and self-report measures. In 1992 Dozier and Kobak reported that dismissing individuals show high levels of physiological arousal on skin conductance tests when questioned about attachment experiences on the AAI, despite the their insistence that attachment experiences and emotions were not a source of concern. In 1999 Hesse reported on research he conducted on a sample of 50 college students in which he found no relation between the AAI status of the participants and their self-reported attachment classification. The attachment behaviors of children in the Strange Situation can also be predicted from the parent’s AAI, but not from self-report measures (Hesse, 1999). A recent study compared the scores of the AAI and a self-report measure (the Inventory of Parent and Peer Attachment) to response latencies in an attachment priming task (Maier, Bernier, Pekrun, Zimmermann, & Grossmann, 2004). The study found that attachment organization as assessed by the AAI correlated with the priming effects, whereas the self-report measure scores were actually inversely related or not related at all to

priming. In other words, the AAI was found to assess, to a certain degree, unconscious working models of attachment, while the self-report measure did not.

¹⁰ Some researchers have suggested that specific attachment behaviors may be expressed differently across cultures (Grossmann, Grossmann, & Kepler, 2005; Posada & Jacobs, 2001; Rothbaum, Weisz, Pott, Miyake, & Morelli, 2000), and that methods used to assess attachment in Western cultures may not necessarily be valid when applied to other cultures (Rothbaum et al., 2000; Miyake, Chen, & Campos, 1985). Particular care needs to be exercised when assessing securely attached children to ascertain which outcomes are truly attachment related and which are merely cultural artifacts (Rothbaum et al., 2000). On the other hand, it might also be said that attachment theory should not be confused with traditional western values. For example, Rothbaum and colleagues criticize attachment theory for emphasizing autonomy, stating, “The path of relying on others, so often devalued in the West, is often favored, even prescribed in Japan.” In fact, Bowlby and Ainsworth both said that the key to self-reliance was the development of the capacity to trust and rely on others. Attachment theory does not value separation, but values autonomy in the sense that it allows the individual to recognize that others have different feelings or interpretations of events than they. Autonomy strengthens the ability to think flexibly about the world and nurtures the development of empathy and sensitivity toward others—it is not a term intended to mean independence from others. A clear understanding of both attachment theory and culture will be necessary before researchers will be able to gain a better understanding of the interplay between culture and attachment.

¹¹ A complete review of the temperament literature in general is beyond the scope of this paper. But it might be helpful to note that although the concept of temperament has been around at least since the time of the ancient Greeks, Chess and Thomas are frequently credited with starting with what has been called the “renaissance” of research in this area (Zeanah & Fox, 2004). Chess and Thomas identified nine dimensions of temperament from their studies of infants and parents in a variety of settings. Those dimensions included activity level, rhythmicity or regularity of functioning, approach-withdrawal in new situations, intensity of emotional expression, overall valence of mood, adaptability to changes in routine, persistence, distractibility or soothability, and threshold of sensory responsiveness. These dimensions were then distilled into three famous categories: “difficult,” “easy,” and “slow-to warm up.” It is worthy of note that a substantial minority of these children did not fall into any of these categories. Chess and Thomas argued that healthy infant development occurs when there is a “goodness of fit” between parent and child, but developmental problems occur when parental and infant temperaments differ. Problems occurred with Chess and Thomas’ temperamental dimensions essentially because of problems with vagueness in the terms. As a result, other researchers developed alternative means of measuring temperament. For example, Mary Rothbart proposed that temperament has two components: reactivity and regulation (Rothbart & Derryberry, 1981). Reactivity is essentially biological in origin and present at birth, whereas regulation is something that develops over the lifespan and involves the interaction of reactivity and environmental experiences.

¹² The observer version of the Attachment Q-sort was found to a reliable attachment measure, however.

¹³ In Shamir-Essakow’s study there was no significant difference between the proportion of children with secure or insecure attachments in the “behaviorally inhibited” or “uninhibited” groups. But when the individual subgroups (A, C, and D) were included in the statistical analysis it became clear that a significantly greater number of inhibited children were classified as insecure ambivalent (20.8%) than uninhibited (3.1%).

¹⁴ Later studies have supported the finding that B3/B4 or C1/C2 infants are “temperamentally more negatively reactive” than A1/A2 or B1/B2 infants (Burgess, Marshall, Rubin, & Fox, 2003; Marshall & Fox, ND, p.1; Fox, Kimmerly, & Schafer, 1991). Marshall and Fox concluded that their findings were consistent with evidence from behavioral genetics studies indicating that temperament and attachment security have “diverse origins”—attachment security is determined by unique and shared environmental factors while temperament has genetic and environmental origins (citing Bokhorst et al., 2004; Calkins & Fox, 2002). The research is not unanimous in this result, however. In Mangelsdorf, Gunnar, Kestenbaum, Lang, and Andreas (1990) not only were the traditional attachment classifications as assessed in the Strange Situation (A,B,C,D) found to be unrelated to temperamental dimensions, but even the A1-B2 vs. B3-C2 classifications were unrelated to temperament. The researchers did find a significant proneness-to-distress x security interaction for what was called maternal “constraint” behavior—which indicated more rigid or traditional forms of responsiveness to the infant. In other words, infants who were highly prone to distress were more likely to be classified as insecure when mothers displayed more “rigid” responsiveness. In this sense, the authors argued that the infant’s temperament does contribute to attachment

security. The problem with this study is that measures of infant temperament were taken at nine months of age—long after internal working models of attachment are fully established, and “rigid” responsiveness is arguably an insensitive parental characteristic fully consistent with infant insecurity. Moreover, other studies of infants described as temperamentally “irritable” do not find higher levels of infant resistant insecurity as would be predicted if temperament was responsible for the attachment relationship (van den Boom, 1994). In fact, in van den Boom’s study of 100 temperamentally “irritable” infants in a low SES group, the majority of control infants were classified as insecure avoidant (52%).

¹⁵ The amygdala is frequently referred to as an “almond shaped” structure in the temporal lobe of the cerebrum, is connected with the hypothalamus and the hippocampus (and the dentate gyrus, the part of the hippocampus that is said to generate new neurons throughout life) and plays a crucial role in the regulation of emotion, particularly fear (Sah, Faber, Lopez de Armentia & Power, 2003). In fact, animal studies have shown that when the amygdala is removed the animal no longer experiences fear (Bertolino, Arciero, Rubino, Latorre, De Candia, Mazzola, Blasi, Caforio, Hariri, Kolachana, Bardini, Weinberger & Scarabino, 2005; Sapolsky, 2004), and studies on humans with amygdala damage indicate that the same is true for humans (LaBar, LeDoux, Spencer & Phelps, 1995).

¹⁶ It may be useful to describe what a neuron is. Neurons are brain or “nerve” cells that consist of a soma (the cell body containing the cell nucleus, chromosomes, genes and DNA), branching dendrites that receive signals coming from other neurons, and axons, projections that conduct the nerve signal (an electric signal). Axon terminals transmit an electrochemical signal using neurotransmitters which are located in synaptic vesicles in the axon terminals. Neurotransmitters are sent across the synaptic cleft, the space between the axon terminal of one neuron and the dendrites of another neuron. Neurons tremendously vary in size ranging from a fraction of an inch to several feet in length and from .004 mm to .1 mm in diameter. Within the CNS are groups of axons called nerve pathways. Myelin, a combination of fat (70-80%) and protein (20-30%) otherwise known as white matter, is produced by Schwann’s cells and coats and insulates the axon except at periodic breaks along the axons called nodes of Ranvier. Myelin develops throughout adolescence and increases the transmission of electric currents (or nerve impulses) along the axon. Neurons can have as few as one axon, but typically have between 1,000 to 10,000 synapses through which they communicate to the same number of other neurons.

¹⁷ Long term potentiation or LTP refers to the neural process that is thought to underlie learning. Neurons communicate with each other through what are called “action potentials.” An action potential occurs when a neuron sends information via an electric current down its axon to another neuron. In order for an action potential to occur the electric charge inside the postsynaptic neuron (which is normally differs in its charge from the outside of the neuron by about -70 millivolts --it’s “resting potential”) must increase toward 0, and thus increase the possibility that an action potential will occur (an action potential will occur when the electric charge on the inside of the cell differs from the outside by approximately -55 millivolts). A cell that is experiencing a drop in amplitude is called an “excitatory postsynaptic potential” because the decrease in amplitude increases the possibility that an action potential will occur. This change in amplitude results in a strengthening of the connection between pre and post synaptic connections creating a memory storage mechanism (Weiss, 1999). It should be noted that it usually takes EPSPs coming from hundreds or thousands of neurons to create one action potential. This is the process is called LTP.

Although LTP takes place all over the cortex, the strongest correlation between declarative memory and LTP occurs in a hippocampal pathway called the Schaffer collateral pathway, where LTP does not occur unless the NMDA (N-methyl-D-aspartate) glutamate receptor is “activated in the postsynaptic cell” (glutamate is an amino acid neurotransmitter that excites neurons) (Weiss, 1999, p. 113). Thus, there needs to be simultaneous activity both pre and postsynaptically. Normally the NMDA receptor’s mouth is plugged by magnesium ions that can be unplugged only when a particularly strong signal is generated in the postsynaptic cell, a signal that reduces or depolarizes the resting potential of the postsynaptic membrane significantly. In experiments, such a strong signal can be produced artificially by firing the presynaptic cells at a high frequency; it is thought that during learning a similar strong firing occurs. The strong firing is said to reduce the membrane potential of the postsynaptic cell sufficiently to expel the Mg plug from the mouth of the NMDA receptor, thereby allowing Ca to flow into the postsynaptic cell through the NMDA receptor channel. The entry of Ca through the NMDA receptor channel initiates the sequence of steps that leads to the persistent enhancement of synaptic transmission.

Evidence supporting the notion that LTP is a storage mechanism for memory includes rat studies which show impaired memory when NMDA receptors are blocked by either antagonists or by genetic “knockout” techniques, as well as

those interesting studies which show that when NMDA receptors are increased rats show increased cognitive capacities (Tang, Shimizu, Dube, Rampon, Kirchner, Zhou, Liu & Tsein, 1999).

How is memory then stored and maintained? Long term memories are said to be stored in the synaptic connections that LTP creates. Specifically, interfering with NMDA receptors seems to interfere with the stability of “place fields” which are cognitive maps generated in the hippocampus (experiments have found that mice create cognitive pictures of where they think food is – not where they actually see it, smell it, etc. (Weiss, 1999). These place fields are formed in minutes and once formed the cognitive maps they help create can remain stable for weeks. Interfering with NMDA receptors appear to undermine place fields by either preventing neurons from firing together or creating instability in the place fields. How LTP is maintained is still being studied but researchers are exploring the idea that although LTP is initiated in the postsynaptic cell, the maintenance of LTP requires not only enhanced activity of non-NMDA receptors in the postsynaptic cell, but also enhanced activity in the presynaptic neuron. There is evidence that humans also generate cognitive maps in the hippocampus (Maguire, Gadian, Johnsrude, Good, Ashburner, Frackowiak, & Frith, 2000).

LTP is the mechanism by which cortical plasticity takes place. In other words, experience and learning impacts the shape of the brain and its cortical mapping. For example, a common feature of sensory cortical areas primarily devoted to touch, vision and learning is that they all represent their respective sensory epithelial surfaces in a topographic manner. These maps undergo plastic changes in response to both peripheral manipulations and behaviorally important experience throughout life. Buonoman and Merzenich (1998) explain that animal studies support the proposition that changes in cortical map organization occur as a result of training animals on tasks that produce specific differential patterns of activity in identified cortical sectors (e.g., monkeys who learned to put one or two fingers on a revolving, grooved disk developed a several fold increase in the cortical representation of the specific surfaces of the digit tips that were in contact with the disk during training; the results are similar for humans-Pascual-Leone and Torres (1993) used MEG (magnetoencephalography) to examine the differences in the hand representations of adult Braille readers and showed that the scalp area over which potentials were recorded was significantly larger for the finger used to read with (i.e., the right index finger) as compared with the left finger or the right finger of a participant that could not read Braille. Moreover, representations of parts of the hand that were not used to read Braille were smaller than the hands of control participants. In a later study, Pascual-Leone and colleagues found that merely *mentally* practicing piano finger movements also resulted in cortical changes (Pascual-Leone, Dang, Choen, Brasil-Neto, Cammarota & Hallett, 1995). Importantly, Buonoman and Merzenich (1998) point out that blocking NMDA receptors or CaAM kinase activity does interfere with cortical map development and plasticity (referring to mice studies). So, within limits the cortex can allocate cortical area in a use dependent manner-in other words, use part of your brain or lose it! This also explains why NMDA is so important during critical periods - During development expression of NMDA changes – higher expression could result in greater learning and preservation of that part of the brain.

¹⁸ Office of Student Research, Office of Planning and Analysis (2010). New freshman enrollment by ethnicity: Fall 2006 through Fall 2010. Retrieved on 1/1/11 from <http://opa.berkeley.edu/institutionaldata/campusenroll.htm>.

¹⁹ No significant differences between the sexes emerged in genotype distribution or attachment classification. Accordingly, sex was collapsed. No significant differences in ethnicity emerged when participants were classified according to attachment classification. No significant HSES (≥ 30 , < 30) differences emerged according to attachment classification or genotype distribution (ss vs. sl/ll). But significant ethnic differences in genotype distribution did emerge (see Table 1). The target population was late adolescence and all participants were in late adolescence.

²⁰ The adjustment used is called the Benjamini and Hochberg Adjustment to Control the False Discovery Rate (Benjamini and Hochberg, (1995).

²¹ Recall that the percentage of methylation at each CpG site was originally identified. PC analysis centered and scaled each one of these data points. The vertical axis of Figure 4 and 5 represents a relative conception of the direction of methylation levels using these scaled data points; “0” is the average, “negative” refers to all data below the average and “positive” refers to any data above the average.

²² Moffitt reports that only approximately 12% of children with CD respond to treatment (Moffitt, 2006). Moreover, there are very limited treatments available for youth who are diagnosed with CD. In fact, the AACAP

(American Academy of Child and Adolescent Psychiatry) practice parameters for CD state that research does not support treatment with psychodynamic psychotherapy or short-term interventions (Steiner & Dunne, 1997).