Published in final edited form as:

Physiol Behav. 2012 July 16; 106(5): 728–735. doi:10.1016/j.physbeh.2011.11.014.

Genetic sensitivity to the caregiving context: The influence of 5httlpr and BDNF val66met on indiscriminate social behavior

Stacy S Drury, M.D, Ph.D.^a, Mary Margaret Gleason, M.D.^a, Katherine Theall, Ph.D.^b, Anna T Smyke, Ph.D.^a, Charles A Nelson, Ph.D.^c, Nathan A Fox, Ph.D.^d, and Charles H Zeanah, M.D.^a

^aTulane University Health Sciences Center, 1440 Canal St TB 52, New Orleans La 70112, USA

^bTulane University School of Community Health. 1440 Canal St suite 2301, New Orleans La, 70112 USA

^cHarvard, Department of Pediatrics and Neuroscience. 1 Autumn St. Boston Ma, 02215. USA.

^dUniversity of Maryland, Department of Human Development. 3304 Benjamin Building, College Park Maryland, USA.

Abstract

Evidence that gene x environment interactions can reflect differential sensitivity to the environmental context, rather than risk or resilience, is increasing. To test this model, we examined the genetic contribution to indiscriminate social behavior, in the setting of a randomized controlled trial of foster care compared to institutional rearing. Children enrolled in the Bucharest Early Intervention Project (BEIP) were assessed comprehensively before the age of 30 months and subsequently randomized to either care as usual (CAUG) or high quality foster care (FCG). Indiscriminate social behavior was assessed at four time points, baseline, 30 months, 42 months and 54 months of age, using caregiver report with the Disturbances of Attachment Interview (DAI). General linear mixed-effects models were used to examine the effect of the interaction between group status and functional polymorphisms in Brain Derived Neurotrophic Factor (BDNF) and the Serotonin Transporter (5htt) on levels of indiscriminate behavior over time. Differential susceptibility, relative to levels of indiscriminate behavior, was demonstrated in children with either the s/s 5httlpr genotype or met 66 BDNF allele carriers. Specifically children with either the s/s 5httlpr genotype or met66 carriers in BDNF demonstrated the lowest levels of indiscriminate behavior in the FCG and the highest levels in the CAUG. Children with either the long allele of the 5httlpr or val/val genotype of BDNF demonstrated little difference in levels of indiscriminate behaviors over time and no group x genotype interaction. Children with both plasticity genotypes had the most signs of indiscriminate behavior at 54 months if they were randomized to the CAUG in the institution, while those with both plasticity genotypes randomized to the FCG intervention had the fewest signs at 54 months. Strikingly children with no plasticity alleles demonstrated no intervention effect on levels of indiscriminate behavior at 54 months.

Corresponding authors address: Stacy S Drury M.D., Ph.D. Department of Psychiatry and Neurology 1440 Tulane Ave, TB 52 New Orleans, La 70112 sdrury@tulane.edu 504-988-4794.

Dr. Gleason's mgleason@tulane.edu Dr. Smyke's asmyke@tulane.edu Dr. Zeanah's czeanah@tulane.edu Dr. Theall's ktheall@tulane.edu Dr. Nelson's Charles.nelson@childrens.harvard.edu Dr. Fox's fox@umd.edu

Disclosure: The authors report no conflicts of interest

These findings represent the first genetic associations reported with Indiscriminate social behavior, replicate previous gene x gene x environment findings with these polymorphisms, and add to the growing body of literature supporting a differential susceptibility model of gene x environment interactions in developmental psychopathology.

Keywords

BDNF; 5HTT; genetic plasticity; biological sensitivity; institutionalization; indiscriminate behavior

1. INTRODUCTION

Gene $\times$ environment studies have been a source of both tremendous enthusiasm as well as frustration. Although the initial demonstration of gene x environment interactions were promising[1], failed replications of both candidate gene and genome wide association studies (GWAS) have dampened the enthusiasm for genetic studies in psychiatry[2, 3]. The lack of consistent findings appears to challenge the utility of genetic studies for complex phenotypes, as well as the standard genetic risk and resilience model. An alternative conceptualization, differential susceptibility, may offer significant insight into these discrepant findings and enhance the understanding of the influence genetic variation has on complex psychological and behavioral phenotypes. In this model, specific polymorphisms confer a differential responsiveness to the environment, instead of risk or resilience *per se* [4]. These “plasticity alleles” are predicted to enhance outcomes in positive environments yet elevate vulnerability in adverse environments [5, 6]. Individuals with the non-plastic, “fixed” alleles, are expected to demonstrate few differences in behavior or outcomes between positive or negative environments. Randomized controlled trials provide a unique opportunity to directly test this model as there is a specific manipulation of the environmental context and established outcome measures by which responsiveness to the environmental change can be measured. [7] Randomized controlled trials of alterations of the environment, particularly the caregiving environment, though common in animal studies are rare in human research and thus represent an important source for hypothesis testing related to differential susceptibility.

Extremes of early caregiving adversity, including severe social deprivation as a result of institutional rearing, are associated with a range of clinical and behavioral problems that in normative social conditions often result in significant impairment. One behavioral construct which has been demonstrated across studies of children reared in institutions and proposed to be part of a deprivation specific pattern (as reviewed in[8]) is indiscriminate social behavior. Indiscriminate social behavior is also elevated in children exposed to early maltreatment. [9] The core features of indiscriminate behavior include lack of reticence with unfamiliar adults, inappropriate social boundaries and affection with strangers, willingness to accompany strangers, and failure to check back with a familiar caregiver when in an unfamiliar setting. While these behaviors may have an unidentified adaptive purpose in inconsistent or inadequate caregiving environments, these same behaviors in normative environments are impairing across multiple domains in part consistent with a mismatch

theory of early behavior. Recently, construct and criterion validity as well as stability of indiscriminate behaviors have been reported in a longitudinal study of institutionalized children [10-12]. Despite its etiologic association with adverse caregiving (13) indiscriminate behavior persists in a significant proportion of children years after restoration of adequate caregiving environment [11, 13-15] indicating that other factors contribute to both the development and the persistence of indiscriminate social behavior.

A model of genetically driven differential susceptibility in this context would predict that indiscriminate behavior in some children, “sensitive” individuals, would be quite responsive to changes in the caregiving environment whereas others, “fixed” individuals, would demonstrate little change when moved to an improved environment. This model predicts that children who carry differentially susceptible genetic alleles would demonstrate both the greatest amount of symptoms in the adverse environment and *the least amount* of symptoms in the positive environment. To test this theory directly we explored the contribution of genotype to change in levels of indiscriminate behavior in the setting of a randomized controlled trial of foster care compared to institutional rearing, the Bucharest Early Intervention Project [16-19].

The biological substrate of indiscriminate behavior is unknown. However, given the association of indiscriminate behavior with a range of negative behavioral and psychological outcomes we selected functional polymorphisms in two genes, the 5httlpr in the serotonin transporter gene and the met66val polymorphism in BDNF. These genes were selected *a priori* because they have been associated with differential susceptibility in both preclinical animal studies and human research, have established roles in social behavior, and have been associated with a range of psychopathology. [3, 20-31] There exists significant evidence, particularly for the 5httlpr, that individuals with the short (“s”) allele, particularly those with the s/s genotype, are not only at increased risk for psychopathology with exposure to high levels of stress or adversity, but these same individuals also appear to benefit disproportionately from supportive environments. [32-34] BDNF is critically involved in neuroplasticity and neurodevelopment and BDNF levels have been found to moderate the association between early adversity and anxiety. [35] The functional val66met polymorphism has been studied across psychopathology. Although a number of studies have demonstrated that the val allele is the protective allele a recent meta-analysis revealed that the met allele was protective for neuroticism. [36] The differential impact of the met allele was also demonstrated in association with high levels of exercise and depression as well as protective relative to psychological disorders when associated with elevated fear processing. [37, 38] Because variation in gene expression levels have been demonstrated during early development for both genes we further evaluated whether these genotypes exhibited any differential timing effects.

The differential susceptibility model further predicts a multiplicative genetic effect where the responsiveness to the environment may be greater in individuals with more than one plasticity allele. Given that both gene-gene and epistatic interactions have been demonstrated repeatedly with these specific polymorphisms [20, 39-43] we examined both their independent and combined impact on indiscriminate social behavior. We hypothesized that cumulative genetic plasticity would be associated with the greatest sensitivity to the

caregiving environment. We predicted that children with both plasticity genotypes would exhibit the greatest number of symptoms in the negative caregiving environment (institutional) but the lowest amount of symptoms in the positive caregiving environment (foster care). We further predicted that children with no plasticity alleles would exhibit little difference in indiscriminate behavior between the extreme caregiving environments. [25, 44, 45].

2. METHODS

2.1 Participants

Participants were enrolled in the Bucharest Early Intervention Project (BEIP) [46], a randomized controlled trial of foster care as an alternative to institutional care in Romania. The study sample, with inclusion and exclusion criteria, has been described elsewhere [47, 48]. Briefly, participants included 136 abandoned children between 6 and 30 months of age who were living in one of six institutions in Bucharest, Romania. Following baseline assessments, 68 of the children (33 males and 35 females) were randomly assigned to care as usual (CAUG) and 68 (34 males and 34 females) were randomly assigned to foster care (FCG). Children were excluded for medical reasons including diagnosed genetic syndromes, significant evidence of fetal alcohol syndrome or microcephaly. The foster care network was created and supported by the project as an intentional alternative to institutional care [49].

Following randomization, all subsequent decisions regarding placement were made by the Romanian National Authority for Child Protection in accordance with Romanian law, with the expectation that no child removed from an institution and placed in project supported foster care would be returned to an institution. Over the four years of the project, there was considerable movement within the groups (Figure 1). Nevertheless, all analyses that include FCG or CAUG reported herein follow intent to treat, so that children are analyzed within their originally assigned group.

At 54 months of age, 112 children from the initial randomization continued to participate in the study (53 CAUG and 59 FCG). Complete psychopathology data at all four time points, BDNF and 5httlpr genotyping were obtained on 98 (CAUG 45, FCG 53) children.

2.2 Measures

The Disturbances of Attachment Interview (DAI)—[50] The DAI is a semi-structured interview of the caregiver about signs of disturbed or disordered attachment behavior in the child, including signs of indiscriminate social behavior. Ratings of three items, checking back with a caregiver when in unfamiliar settings, reticence with unfamiliar adults, and willingness to “go off” with a stranger, were coded on a 3-point Likert scale, where “0” was “rarely or minimally” demonstrated a behavior, “1” was “sometimes or somewhat” demonstrates a behavior, and “2” was “clearly” demonstrates a behavior. A composite score ranged from 0 to 6, with higher scores representing increasing signs of indiscriminate behavior. The DAI has strong internal validity, shows convergence with other caregiver report measures and has distinguished between institutionalized and never institutionalized children [51]. DAI scores were obtained at four time points: baseline

enrollment in the study and then at 30, 42, and 54 months of age. At each of these four time points, the interview was conducted with either the child's foster parent or the identified caregiver in the institution who knew the child best or was the child's identified preferred caregiver.

2.3 Genotyping

DNA was extracted from MasterAmp buccal swabs using Epicentre Biotechnologies MasterAmp DNA extraction solution following manufacturer's recommendations. 5httlpr allele status was determined using standard PCR methods and gel electrophoresis with careful attention to magnesium concentrations. Variation of Mg levels from 1mM to 2mM did not result in genotype differences as has been previously demonstrated in other studies[52]. Samples were all run in duplicate and with known controls[53]. The BDNF val 66 met (rs6265) polymorphism was genotyped using the TaqMan SNP assay, Applied Biosystems. All samples were run in triplicate, with known controls. Any discrepancies in genotype status were resolved by direct sequencing. Genotyping was done blind to other outcomes. Genotypes of both genes were tested to confirm they were in Hardy Weinberg Equilibrium 5httlpr, $p=0.545$ and BDNF rs6265 $p=0.52$, and consistent with the range of allele frequencies for both genotypes reported in the NCBI database and published studies. BDNF genotype did not differ from NCBI reported frequencies for European or Eastern European populations (chi square 2.272 df=2 $p=0.32$), (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6265).

2.4 Human subjects

The study was approved by Institutional Review Boards at Children's Hospital Boston/Harvard Medical School, Tulane University, University of Maryland, and by the local commissions on child protection in each sector of Bucharest. Ethical issues in this study are numerous and complex because of the extremely vulnerable population, have been discussed by us [54] and by others [55, 56] in detail elsewhere. Briefly, we highlight that the study was conducted in Romania because the investigators were invited by a government official to contribute meaningful data to an on-going policy debate about how to best care for abandoned children. Prior to this research project there existed little extant data demonstrating which type of care was in the best interest of abandoned children and the vast majority of abandoned children across the globe were in institutional care. The study involved minimal risks to participants, and no child had their custody or placement affected by study participation. That is, if the child protection authorities ordered a child to be returned to parents, or adopted or placed in government sponsored foster care, we did not interfere. Therefore, no child was made worse by participating. The proposed study was reviewed by IRBs in the U.S. institutions of the 3 principal investigators, as well as in 2002 by an ad hoc ethics commission appointed by the Government of Romania[47, 54-57].

2.5 Data Analysis

Bivariate relationships were examined through Likelihood Ratio Chi-square tests of independence or Fisher's exact, where appropriate. General linear mixed effects models were used to compare DAI scores over time and explore the impact of group and genotype centered at 54 months. Repeated measures within children were modeled with time coded as

a categorical variable and not centered to facilitate comparison over the four time points. A three way interaction of genotype, group and time was added to the model to assess for the differential impact of genotype between the two treatment groups and potential timing effects. Genotype was examined separately as: 1) BDNF, which compared met carriers to val/val homozygotes, 2) 5htt, which compared s/s homozygotes to s/l and l/l genotypes, and 3) cumulative plasticity genotype. In this cumulative model children with neither the s/s or met/* genotype were classified as 0, children with either the s/s or the met/* genotype were considered 1, and children with both the s/s and the met/* genotype were categorized as 2 consistent with proposed models of cumulative plasticity.[42] Post-hoc linear regressions were run to examine the relations between genotype and change in indiscriminate behavior between all time points after accounting for gender and the clustering of individual responses over time to determine if there were differences in timing. Bonferroni corrections were employed for the linear regression analyses due to the comparison of genotype over 3 different independent variables. All analyses were performed using SAS 9.2.

3. RESULTS

98 individuals had complete data sets that included DAI scores at all time points and valid genotype for both the BDNF and 5htt polymorphisms. No differences in DAI scores were found in those children from whom genotype was available and those who did not have genotype data and DAI scores were not associated with gender or ethnicity. Genotype was not associated with gender, randomization group, or ethnic background (Table 1). Frequency of non-collapsed genotypes were s/s=22, s/l=54, and l/l=24 for the 5httlpr and met/met=1, met/val=24 and val/val=73. Genotype categorized in this manner was not significantly associated with gender, group or ethnic background.

3.1 Genetic plasticity in mixed effects model

The interaction of genotype and group in the mixed-effects models was statistically significant for 5httlpr ($F=7.65$, $df=320$, $p=.006$), BDNF ($F=10.41$, $df=332$, $p=.0014$) and the cumulative plasticity genotype ($F=16.78$, $df=320$, $p<.001$) over time. Full estimates and p values for all levels of interactions are reported in Table 2. Graphical representation of DAI scores stratified by genotype and group are displayed in Figure 2 (BDNF), Figure 3 (5httlpr). The effect of the cumulative plasticity using baseline and 54 months is displayed graphically in Figure 4a and b. Genotype did not have a significant direct association with indiscriminate behavior at baseline or at 54 months consistent with a differential susceptibility model as opposed to diathesis stress model.[58]

As the mixed effects model with repeated measures demonstrated significant interaction over time, post hoc analyses were performed to determine if differential timing effects related to genotype existed. Change in levels of indiscriminate behavior between all assessment points was examined. BDNF genotype significantly interacted with group ($p=.009$, $\beta=2.90$, $r^2=.06$) between 30 and 54 months. 5httlpr genotype interacted with group earlier in the course, between baseline and 30 months ($p=.03$, $\beta=1.2$, $r^2=.04$), baseline and 42 months ($p=.02$, $\beta=1.1$, $r^2=.05$) and baseline to 54 months ($p=.042$, $\beta=1.24$, $r^2=.05$)

The cumulative plasticity genotype interacted significantly with group between 30 and 54 months ($p=.036$, $\beta=1.4$, $r^2=.03$) and baseline to 54 months ($p=.0048$, $\beta=2.4$, $r^2=.08$).

To provide additional support for the model of differential susceptibility compared to stress diathesis or cumulative risk post-hoc analysis was performed using baseline and 54 month time points. T-Tests were performed with plasticity categorized as 0 or 1 (both plasticity alleles present) within the FCG and the CAUG. At baseline no significant difference between levels of indiscriminate behavior were found between the FCG and the CAUG or by plasticity genotype. However at 54 months while no significant association was found with in the CAUG, in the FCG there was a trend significant finding $t(36)=1.92$ $p=.06$.

Analysis 54 months including both groups but accounting for group interaction resulted in a significant full model $F(2,105)=2.31$, $p=.04$ and significant group by genotype interaction $F(2, 105) 4.5$, $p=.013$.

4 DISCUSSION

These results support our hypothesis that differential susceptibility to the caregiving context, determined by the s/s genotype of the 5httlpr and the met allele of BDNF, contributes significantly to indiscriminate behavior in children exposed to early severe social deprivation and adds to the growing body of literature supporting a differential susceptibility model in developmental psychopathology. [7] Our findings demonstrate, in the setting of a longitudinal randomized controlled trial, that individual differences in responsiveness to changes in the caregiving environment are influenced by genetic variation. Children with “sensitive” or plastic genotypes (s/s 5httlpr or met allele carriers of BDNF) had the most signs of indiscriminate behavior when randomized to CAUG. However children with this same “sensitive” genotype, when placed into the enhanced caregiving environment of high quality foster care, had the least signs of indiscriminate behavior at 54 months of age and the greatest decrease in indiscriminate behavior. As expected children with the alternative “fixed” genotypes (5httlpr l/s and l/l or BDNF val/val) demonstrated little change in indiscriminate behavior with alterations in the caregiving environment at any time point.

Interestingly, we also noted that the influence of genotype appears to follow a different time course for each genotype in this study. The 5httlpr genotype appears to have an earlier impact, before the 42 month assessment, while the impact of the BDNF genotype happened slightly later in development, between 30 months and 54 months. This may reflect developmental differences in the impact of these genes or alternatively differences in the underlying mechanism through which they influence changes in indiscriminate behavior in response to caregiving changes. Our findings also indicate that genetic sensitivity to change in the caregiving context did not occur immediately. Rather the decline in indiscriminate behavior occurred over time as their experiences in a different caregiving environment accumulated. The fact that a period of time in the new caregiving context is required for the expression of this differential sensitivity is consistent with a model that includes neuronal plasticity or alteration in neurodevelopmental trajectories occurring prior to detectable changes in a behavioral phenotype. This hypothesis should be tested in existing animal models of early adversity and maternal separation paradigms.

Consistent with other studies our results demonstrate a cumulative effect of plasticity genotype. Children who were carriers of both the BDNF met allele and the s/s 5httlpr genotype, two plastic genotypes, had the greatest decline in indiscriminate behavior by 54 months- if they were placed in foster care. However if they remained in the institutional care they had the highest level of indiscriminate symptoms at 54 months. Children who had neither sensitive genotype had little change in symptoms.

The 5httlpr and BDNF val66met polymorphisms have been studied across a range of disorders. Although some have suggested that this raises the question of false positive findings, others propose this reflects a more widespread involvement in neurodevelopment consistent with their broad expression in the central nervous system. The BDNF val66met polymorphism has been associated with anxiety and mood disorders, neuroticism and HPA axis reactivity [26, 59, 60]. Additionally BDNF is known to modulate neuronal plasticity, increase hippocampal dependent memory and learning, is up-regulated in response to stress exposure, and implicated in serotonergic neuronal development and function [61, 62]. Consistent with our results, previous studies have demonstrated that the s/s genotype of the 5httlpr is protective within supportive early or current environments but risk amplifying with exposure to adversity, as noted in one previous study of adolescents with a history of institutionalization[24, 63, 64]. An additional study demonstrated the lowest level of plasticity in response to early childhood adversity in children with the val/val BDNF genotype and long allele of the 5httlpr in effortful control [25] similar to our results which demonstrated little change in levels of indiscriminate behavior with this genotype combination.

The finding of an additive impact of these two polymorphisms is consistent with the evidence of their interaction at the biological and cellular level [43, 65, 66]. Additionally, a significant number of studies have demonstrated both epistatic and gene x gene interactions with 5httlpr and BDNF, particularly in the presence of environmental adversity [20, 25, 39, 67-71]. In a study of HPA axis reactivity in preschool children, Dougherty and colleagues demonstrated an interaction between the s/s genotype and BDNF met carriers on cortisol levels and stress reactivity in preschool children consistent with their hypothesis that children with this genetic combination maybe less reactive under low stress conditions and more reactive under high stress conditions. These findings of genetic plasticity with the s/s and met allele are consistent with our own findings in which children with this combination of polymorphisms demonstrated significant improvement in indiscriminate social behavior when placed in foster care but elevated indiscriminate behavior with prolonged institutional rearing[39].

There are several limitations to this study. First, we did not correct for genetic admixture and possible population stratification. However, for population stratification to present a statistical problem, two conditions need to be met [72]. First, there must be significant differences in allele frequencies by ethnicity. Second, baseline differences in indiscriminate behavior between ethnic groups must be present. As neither of these conditions was present, we did not correct for genetic admixture. Second, we examined only the s and l alleles of the 5httlpr. Other studies have considered an additional polymorphism in the 5httlpr (rs 25531), but we did not further subdivide the genotypes due to concerns about sample size and

uncertain functional significance of additional SNPs located within this polymorphism. Although there is some evidence that this additional SNP has functional significance more detailed studies have revealed up to 10 allelic versions in this same polymorphism thus questioning whether other variants could be functionally significant [73]. Additionally we characterized the plasticity genotype for the 5httlpr alleles as s/s compared to s/l and l/l genotypes together. This was done apriori as in previous studies the s/s genotype has demonstrated the highest level of plasticity compared to s/l or l/l genotypes. [27, 74, 75] A third limitation is sample size and the resulting limited statistical power, particularly when considering stratification by group and combined genotype, increasing the risk of a Type II error. The number of children with both plasticity alleles was particularly small and while this is the largest randomized controlled trial of foster care compared to institutional care the issue of sample size needs to be considered. Lastly, we performed analysis with two genes thereby increasing the potential for type I errors. To address this limitation we only presented findings that remained significant after Bonferroni correction had been applied. Our analyses were driven by clearly delineated *a priori* hypotheses based on previous studies that have demonstrated plasticity with these genotypes, the known interactions between these genes at the molecular and cellular level, and previous evidence of gene x gene x environment interactions. [40, 68, 76, 77].

5. CONCLUSIONS

These results replicate and extend previous findings related to differential susceptibility in general and these polymorphisms in particular. The demonstration of this effect in a randomized controlled trial utilizing the same outcome measure over time represents one of the first studies to demonstrate differential susceptibility in the same individuals over time and offers significant support for the model. [78] The development of disinhibited social behavior in children exposed to extremes of early adverse caregiving which, in the majority of individuals appears to be resistant to later alterations of the caregiving environment, is also consistent with the mismatch hypothesis in that this type of behavior, in the context of unpredictable or limited social stimulation may be adaptive and developmentally set a behavioral repertoire of seeking social interaction from any individual [79]. For the majority of children who experience adverse early caregiving resistance to relying on a specific caregiver, if there is little future expectation that a reliable caregiver would be identified, may be an appropriate evolutionarily adaptation. However by having a limited number of individuals within a population able benefit from novel positive environmental exposures, such as supportive caregivers, at the risk of increased vulnerability to negative environments, could offer evolutionary advantage to the group as a whole by permitting a subset to have significant benefit should rare changes in circumstances occur.

Conceptualizing gene x environment interactions in terms of differential susceptibility represents a potential important paradigm shift for genetic studies of developmental psychology and psychopathology that often treat genetic influences as fixed effects rather than fluid interactions. The redefining of genetic variants, particularly ones with a high prevalence in a population, as neither “good” nor “bad” but rather in terms of responsiveness to environmental changes is more consistent with the complexities of biological systems. Thoughtful testing of hypotheses derived from previous research, combined with evidence

found in preclinical and translational studies, may decrease the number of failed replications and enhance our understanding of the genetic influence on developmental psychopathology.

Acknowledgments

Special thanks to Dr. Michael Scheeringa for statistical assistance, Dr. Elizabeth Shirtclif for comments, and Mr. Arden Nelson for assistance with figures.

Affiliations and funding: This research was supported by the John D. and Catherine T. MacArthur Foundation Research Network on "Early Experience and Brain Development" (Nelson), a grant from the NIMH (1R01MH091363) (Nelson), a NARSAD Young Investigator Award (Drury) and the Tulane University CTREC (Drury).

References

1. Caspi A, Sugden K, Moffitt T, Taylor A, Craig I, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003; 301:386–9. [PubMed: 12869766]
2. Risch N, Herrell R, Lehner T, Liang K, Eaves L, Hoh J, et al. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events and risk of depression: a Meta-analysis. *JAMA*. 2009; 301:2462–71. [PubMed: 19531786]
3. Caspi A, Hariri A, Holmes A, Uher R, Moffitt T. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *American Journal of Psychiatry*. 2010; 1–19. Aia. [PubMed: 20068118]
4. Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? *Molecular Psychiatry*. 2009; 14:746–54. [PubMed: 19455150]
5. Belsky J, Bakermans-Kranenburg MJ, Van Ijzendoorn MH. For better and for worse: differential susceptibility to environmental influences. *Curr. Dir. Psychol. Sci*. 2007; 16:300–4.
6. Belsky J, Pluess M. The nature (and nurture?) of plasticity in early human development. *Perspectives on Psychological Science*. 2009; 4
7. Bakermans-Kranenburg MJ, Van Ijzendoorn M, Pijlman F, Mesman J, Juffer F. Experimental evidence for differential susceptibility: dopamine D4 receptor polymorphism (DRD4 VNTR) moderates intervention effects on toddler' externalizing behavior in a randomized controlled trial. *Development Psychology*. 2008; 44:293–300.
8. Rutter M, Sonuga-Barke EJ, Beckett C, Castle J, Kreppner J, Kumsta R, et al. Deprivation-Specific Psychological Patterns: Effects of Institutional Deprivation Monographs of the Society for Research in Child Development. 2010; 75:1–252.
9. Pears K, Bruce J, Fisher P, Kim H. Indiscriminate Friendliness in Maltreated Foster Children. *Child Maltreatment*. 2009; 1–12.
10. Zeanah, C.; Smyke, A. Disorders of attachment.. In: Zeanah, C., editor. *Handbook of Infant Mental Health*. 3rd ed.. Guilford Press; New York: 2009. p. 421–34.
11. Gleason M, Fox N, Drury S, Smyke AT, Egger HL, Nelson C, et al. Validity of evidenced-derived criteria for Reactive Attachment Disorder: Indiscriminately Social/Disinhibited and Emotionally Withdrawn/Inhibited Types. *The Journal of the American Academy of Child and Adolescent Psychiatry*. 2011; 50:216–31.
12. Glowinski A. Reactive Attachment Disorder: An Evolving Entity. *The Journal of the American Academy of Child and Adolescent Psychiatry*. 2011; 50:210–1.
13. Rutter M, Colvert E, Kreppner J, Beckett C, Castle J, Groothues C, et al. Early adolescent outcomes for institutionally-deprived and non-deprived adoptees. I: Disinhibited attachment. *J Child Psychol Psychiatry*. 2007; 48:17–30. [PubMed: 17244267]
14. Chisholm K. A three year follow-up of attachment and indiscriminate friendliness in children adopted from Romanian orphanages. *Child Dev*. 1998; 69:1092–6. [PubMed: 9768488]
15. Hodges J, Tizard B. Social and family relationships of ex-institutional adolescents. *Journal of Child Psychology and Psychiatry*. 1989; 30:77–97.

16. Boris NW, Hinshaw-Fuselier SS, Smyke AT, Scheeringa MS, Heller SS, Zeanah CH. Comparing criteria for attachment disorders: Establishing reliability and validity in high-risk samples. *J Amer Acad Child Adolescent Psychiatry*. 2004; 43:568–77.
17. Zeanah CH, Scheeringa M, Boris NW, Heller SS, Smyke AT, Trapani J. Reactive attachment disorder in maltreated toddlers. *Child Abuse & Neglect*. 2004; 28:877–88. [PubMed: 15350771]
18. O'Connor T, Rutter M. Attachment disorder behavior following early severe deprivation: Extension and longitudinal follow-up. *Journal of the American Academy of Child & Adolescent Psychiatry*. 2000; 39:703. [PubMed: 10846304]
19. O'Connor, T. Attachment Disorders in infancy and childhood.. In: Rutter, M.; Taylor, E., editors. *Child and Adolescent psychiatry: Modern Approaches*. 4th ed.. Blackwell; Oxford, England: 2002. p. 776-92.
20. Kaufman J, Yang B, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, et al. Brain-Derived Neurotrophic Factor–5-HTTLPR Gene Interactions and Environmental Modifiers of Depression in Children. *Biological Psychiatry*. 2006; 59:673–80. [PubMed: 16458264]
21. Kaufman J, Yang B, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal J, et al. Social supports and serotonin transporter gene moderate depression in maltreated children. *PNAS*. 2004; 101:17316–1721. [PubMed: 15563601]
22. Barr C, Newman T, Lindell S, Shannon C, Champoux M, Lesch K, et al. Interaction Between Serotonin Transporter Gene Variation and Rearing Condition in Alcohol Preference and Consumption in Female Primates. *Archives of General Psychiatry*. 2004; 61:1146–52. [PubMed: 15520362]
23. Bennett A, Lesch K, Heils A, Long J, Lorenz J, Shoaf S, et al. Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry*. 2002; 7:118–22. [PubMed: 11803458]
24. Champoux M, Bennett A, Shannon C, Higley J, Lesch K, Suomi S. Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Molecular Psychiatry*. 2002; 7:1058–63. [PubMed: 12476320]
25. Nederhof E, Bouma EMC, Riese H, Laceulle OM, Ormel J, Oldehinkel AJ. Evidence for plasticity genotypes in a gene–gene–environment interaction: the TRAILS study. *Genes, Brain and Behavior*. 2010; 9:968–73.
26. Vinberg M, Trajkovska V, Bennike D, Knorr U, Knudsen G, Kessing L. The BDNF val66met polymorphism:relation to familiar risk of affective disorder, BDNF levels and salivary cortisol. *Psychoneuroendocrinology*. 2009; 34:1380–9. [PubMed: 19473771]
27. Alexander N, Kuepper Y, Schmitz A, Osinsky R, Kozyra E, Hennig J. Gene-environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression. *Psychoneuroendocrinology*. 2009; 34:1294–303. [PubMed: 19410377]
28. Krishnan V, Han M, Graham D, Berton O, Renthall W, Russo S, et al. Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*. 2009; 131:391–404. [PubMed: 17956738]
29. Whittle S, Yap M, Sheeber L, Dudgeon P, Yucel M, Pantelis C, et al. Hippocampal volume and sensitivity to maternal aggressive behavior: A prospective study of adolescent depressive symptoms. *Development and Psychopathology*. 2011; 23:115–29. [PubMed: 21262043]
30. Wilhelm K, Mitchell P, Nive H, Finch A, Wedgewood L, Scimone A, et al. Life events, first depression onset and the serotonin transporter gene. *British Journal of Psychiatry*. 2006; 188:210–5. [PubMed: 16507960]
31. Zalsman G, Huang Y, Oquendo M, Burke A, Hu X, Brent D, et al. Association of the triallelic serotonin transporter gene promoter region (5HTTLPR) polymorphism with stressful life events and severity of depression. *American Journal of Psychiatry*. 2006; 163:1588–93. [PubMed: 16946185]
32. Belsky J, Pluess M. Beyond Diathesis Stress: Differential Susceptibility to Environmental Influences. *Psychological Bulletin*. 2009; 135:885–908. [PubMed: 19883141]
33. Brody G, Beach SRH, Philibert R, Chen Y, Murry V. Prevention effects moderate the association of 5-HTTLPR and youth risk behavior initiation: Gene x Environment hypotheses tested via randomized prevention and design. *Child Development*. 2009; 80:645–61. [PubMed: 19489894]

34. Manuck S, Flory J, Ferrell R, Muldoon M. Socio-economic status covaries with central nervous system serotonergic responsivity as a function of allelic variation in the serotonin transporter gene-linked polymorphic region. *Psychoneuroendocrinology*. 2004; 29:651–68.
35. Carolla V, Gross C. BDNF moderates early environmental risk factors for anxiety in mouse. *Genes, Brain and Behavior*. 2010; 9:379–89.
36. Frustaci A, Pozzi G, Gianfagna F, Manzoli L, Boccia S. Meta-Analysis of the Brain-Derived Neurotrophic Factor Gene (BDNF) Val66Met Polymorphism in Anxiety Disorders and Anxiety-Related Personality Traits. *Neuropsychobiology*. 2008; 58:163–70. [PubMed: 19088493]
37. Mata J, Thompson R, Gotlig I. BDNF Genotype Moderates the Relation Between Physical Activity and Depressive Symptoms. *Health Psychol*. 2010; 29:130–3. [PubMed: 20230085]
38. Lonsdorf T, Weike A, Golkar A, Schalling M, Hamm A, Ohman A. Amygdala-dependent fear conditioning in humans is modulated by the BDNFval66met polymorphism. *Behavior Neuroscience*. 2010; 124:9–15.
39. Dougherty L, Klein D, Congdon E, Canli T, Hayden E. Interaction between 5httlpr and BDNF val66met polymorphisms on HPA reactivity in preschoolers. *Biological Psychiatry*. 2010; 83:93–100.
40. Goodyer I, Croudace T, Dudbridge F, Ban M, Herbert J. Polymorphisms in BDNF (val66met) and 5httlpr, morning cortisol and subsequent depression in at-risk adolescents. *The British Journal of Psychiatry*. 2010; 197:365–71. [PubMed: 21037213]
41. Terracciano A, Tanaka T, Sutin A, Deiana B, Balaci L, Sanna S, et al. BDNF val66met is associated with introversion and interacts with 5httlpr to influence neuroticism. *Neuropsychopharmacology*. 2010; 35:1083–9. [PubMed: 20042999]
42. Belsky J, Beaver KM. Cumulative-genetic plasticity, parenting and adolescent self-regulation. *Journal of Child Psychology and Psychiatry*. 2011; 52:619–26. [PubMed: 21039487]
43. Mossner R, Daniel S, Albert D, Heils A, Okladnova O, Schmitt A, et al. Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). *Neurochem Int*. 2000; 36:197–202. [PubMed: 10676853]
44. Aguilera M, Arias B, Wichers M, Barrantes-Vidal N, Moya J, Villa H, et al. Early adversity and 5-HTT/BDNF genes: new evidence of genexenvironment interactions on depressive symptoms in a general population. *Psychological Medicine*. 2009; 39:1425–32. [PubMed: 19215635]
45. Wichers M, Kenis G, Jacobs N, Mengelers R, Derom C, Vietnick R, et al. The BDNF val(66)met × 5HTTLPR X child adversity interaction on depressive symptoms: an attempt at replication. *American Journal of Medical Genetics B. Neuropsychiatric Genetics*. 2008; 147:120–3.
46. Zeanah C, Nelson C, Fox N, Smyke A, Marshall P, Parker S, et al. Designing research to study the effects of institutionalization on brain and behavioral development: The Bucharest Early Intervention Project. *Development and Psychopathology*. 2003; 15:885–907. [PubMed: 14984131]
47. Nelson C, Zeanah C, Fox N, Marshall P, Smyke A, Guthrie D. Cognitive recovery in socially deprived young children. *The Bucharest Early Intervention Project Science*. 2007; 318:1937–40.
48. Zeanah C, Egger H, Smyke A, Nelson C, Fox N, Marshall P, et al. Institutional rearing and psychiatric disorders in Romanian preschool children. *American Journal of Psychiatry*. 2009; 166:777–85. [PubMed: 19487394]
49. Smyke A, Koga S, Johnson D, Fox N, Marshall P, Nelson C, et al. The caregiving context in institution reared and family reared infants and toddlers in Romania. *J Child Psychol Psychiatry*. 2007; 48:210–18. [PubMed: 17300560]
50. Smyke AT, Dumitrescu A, Zeanah CH. Attachment disturbances in young children I: The caretaking casualty continuum. *J Amer Acad Child Adolescent Psychiatry*. 2002; 41:972–82.
51. Zeanah CH, Smyke AT, Koga SF, Carlson E. Attachment in Institutionalized and Community Children in Romania. *Child Dev*. 2005; 76:1015–28. [PubMed: 16149999]
52. Yonan A, Palmer A, Gillian T. Hardy-Weinberg disequilibrium identified genotyping error of the serotonin transporter (SLC6a4) promoter polymorphism. *Psychiatric Genetics*. 2006; 16:31–4. [PubMed: 16395127]
53. Heils A, Treufel A, Petri S, Stober G, Riederer P. Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*. 1996; 66:2621–4. [PubMed: 8632190]

54. Zeanah C, Koga S, Simion B, Stanescu A, Tabacaru C, Fox N, et al. Ethical dimensions of the BEIP: Response to commentary. *Infant Mental Health Journal*. 2006; 27:581–3.
55. Millum J, Emanuel E. The ethics of international research with abandoned children. *Science*. 2007; 318:1874–5. [PubMed: 18096792]
56. Miller F. The randomized controlled trial as a demonstration project: An ethical perspective. *American Journal of Psychiatry*. 2009; 166:743–5. [PubMed: 19570933]
57. Zeanah C, Fox N, Nelson C. Case study in ethics: The Bucharest early intervention project. *Journal of Nervous and Mental Diseases*. in press.
58. Kochanska G, Kim S, Barry R, Philibert R. Children's genotypes interact with maternal responsive care in predicting children's competence: diathesis-stress or differential susceptibility. *Development and Psychopathology*. 2011; 23:605–16. [PubMed: 23786699]
59. Montag C, Schoene-Bake JC, Faber J, Reuter M, Weber B. Genetic variation on the BDNF gene is not associated with differences in white matter tracts in healthy humans measured by tract-based spatial statistics. *Genes, Brain and Behavior*. 2010; 9:886–91.
60. Alexander N, Osinsky R, Schmitz A, Mueller A, Kuepper Y, Hennig J. The BDNF val66met polymorphism affects HPA axis reactivity to acute stress. *Psychoneuroendocrinology*. 2010; 35:949–53.
61. Djalai S, Holtje M, Grobe G, Rothe T, Stroh T, Grobe J, et al. Effects of brain derived neurotrophic factor (BDNF) on glial cells and serotonergic neurons during development. *Journal of Neurochemistry*. 2005
62. Dagnino-Subiabre A, Zepeda-Carreno R, Diaz-Veliz G, Mora S, Aboitz F. Chronic stress induces upregulation of brain-derived neurotrophic factor (BDNF) mRNA and integrin alpha 5 expression in the rat pineal gland. *Brain Research*. 2006; 1086:27–34. [PubMed: 16626638]
63. Kumsta R, Stevens S, Brookes K, Schlotz W, Castle J, Beckett C, et al. 5HTT genotype moderates the influence of early institutional deprivation on emotional problems in adolescence: evidence from an English and Romanian Adoptee (ERA) study. *The Journal of Child Psychology and Psychiatry*. 2010
64. Taylor S, Way B, Welch W, Hilmert C, Lehman B, Eisenberger N. Early family environment, current adversity, the serotonin transporter promoter polymorphism, and depressive symptomatology. *Biological Psychiatry*. 2006; 60:671–6. [PubMed: 16934775]
65. Duman R, Heninger G, Nestler E. A molecular and cellular theory of depression. *Archives of General Psychiatry*. 1997; 54:597–606. [PubMed: 9236543]
66. Djalali S, Hölte G, Große G, Rothe T, Stroh T, Große J, et al. Effects of brain-derived neurotrophic factor (BDNF) on glial cells and serotonergic neurons during development. *Journal of Neurochemistry*. 2005
67. Kim J-M, Stewart R, Kim S-W, Yang S-J, Shin I-S, Kim Y-H, et al. Interactions Between Life Stressors and Susceptibility Genes (5-HTTLPR and BDNF) on Depression in Korean Elders. *Biological Psychiatry*. 2007; 62:423–8. [PubMed: 17482146]
68. Pezawas L, Meyer-Lindenberg A, Goldman AL, Verchinski BA, Chen G, Kolachana BS, et al. Evidence of biologic epistasis between BDNF and SLC6A4 and implications for depression. *Mol Psychiatry*. 2008; 13:709–16. [PubMed: 18347599]
69. Terracciano A, Tanaka T, Sutin AR, Deiana B, Balaci L, Sanna S, et al. BDNF Val66Met is Associated with Introversion and Interacts with 5-HTTLPR to Influence Neuroticism. *Neuropsychopharmacology*. 2010; 35:1083–9. [PubMed: 20042999]
70. Hiio K, Merenäkk L, Nordquist N, Parik J, Oreland L, Veidebaum T, et al. Effects of serotonin transporter promoter and BDNF Val66Met genotype on personality traits in a population representative sample of adolescents. *Psychiatric Genetics*. 9000, Publish Ahead of Print:10.1097/YPG.0b013e32834371e8.
71. Mattson M, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *TRENDS in neuroscience*. 2004; 27:589–94.
72. Hutchison K, Stallings M, McGeary J, Bryan A. Population stratification in the candidate gene study: fatal threat or red herring? *Psychological Bulletin*. 2005; 130:66–79. [PubMed: 14717650]

73. Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry*. 2000; 5:32–8. [PubMed: 10673766]
74. Gotlib I, Joormann J, Minor K, Hallmayer J. HPA axis reactivity: a mechanism underlying the association among 5-HTTLPR, stress and depression. *Biological Psychiatry*. 2008; 63:847–51.
75. Mueller A, Broke B, Fries A, Lesch K, Kirschbaum C. The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology*. 2010; 35:289–96.
76. Carver CS, Johnson SL, Joormann J, LeMoult J, Cuccaro ML. Childhood adversity interacts separately with 5-HTTLPR and BDNF to predict lifetime depression diagnosis. *Journal of Affective Disorders*. In Press, Corrected Proof.
77. Bhang S, Ahn J-H, Choi S-W. Brain-derived neurotrophic factor and serotonin transporter gene-linked promoter region genes alter serum levels of brain-derived neurotrophic factor in humans. *Journal of Affective Disorders*. 2011; 128:299–304. [PubMed: 20674984]
78. Kegel C, Bus A, Van Ijzendoorn M. Differential susceptibility in early literacy instruction through computer games: the role of the dopamine D4 receptor gene (DRD4). *Mind, brain and education*. 2011; 5:71–8.
79. Schmidt M. Animal models for depression and the mismatch hypothesis of disease. *Psychoneuroendocrinology*. 2011; 36:330–8. [PubMed: 20674180]

Figure 1.

Consort diagram representing the flow of participants in the study and current placements. BDNF genotype was available on 102 subjects and 5httlpr genotype was available on 98 participants. Complete data sets with DAI scores at all time points and both genotypes were available on 98 total subjects. No demographic differences were found between those with incomplete data and complete data sets.

Figure 2.

Change over time in indiscriminate behavior by genotype and group for the BDNF genotype. The figure on the left is for participants with the met/* genotype, while the figure at the right shows the level of indiscriminate behavior for participants with the val/val genotype at each time point. BL=baseline, 30 mo= 30 months, 42 mo=42 months, 54 mo=54 months. CAUG= care as usual group, children who were randomized to continued institutional care. FCG= foster care group, children who were randomized to the foster care intervention.

Change in Indiscriminate behavior by 5httlpr genotype.

Figure 3.

Change over time in indiscriminate behavior by genotype and group for the 5httlpr. The figure on the left is for participants with the s/s genotype, while the figure at the right shows the level of indiscriminate behavior for participants with the l/* genotype at each time point. BL=baseline, 30 mo=30 months, 42 mo=42 months, 54 mo=54 months. CAUG= care as usual group, children who were randomized to continued institutional care. FCG= foster care group, children who were randomized to the foster care intervention.

Figure 4.

Change over time in indiscriminate behavior by genotype and group using a cumulative plasticity model. To simplify the appearance of the graph only baseline and 54 month time points were included. Figure a represents all three classifications of plasticity alleles- 0, 1 and 2. Figure b demonstrates the change in indiscriminate behavior from baseline to 54 months in individuals with 0 plasticity alleles compared to 2 plasticity alleles. CAUG= care as usual group, children who were randomized to continued institutional care. FCG= foster care group, children who were randomized to the foster care intervention.

Table 1

Demographics by genotype (N, %)

Genotype	5ht1pr N=98			BDNF N=102			Plasticity Category** N=98		
	s/s	s/l + l/l	p-value	Met/*	Val/val	p-value	0	1	2
Total			P=0.30			P=0.39			p=0.85
CAUG (n=52)	8 (8%)	37 (38%)		14 (14%)	33 (33%)		28 (28%)	13 (13%)	4 (4%)
FCG (n=57)	14 (14%)	39 (40%)		12 (12%)	43 (41%)		32 (33%)	16 (17%)	5 (5%)
Ethnic			P=0.33			P=0.40			p=0.63
Romanian	10 (11%)	41 (42%)		12 (13%)	42 (40%)		32 (33%)	16 (16%)	3 (3%)
Roma	7 (7%)	26 (27%)		11 (11%)	22 (20%)		20 (21%)	8 (8%)	5 (5%)
Unknown/other	5 (5%)	9 (10%)		3 (2%)	12 (13%)		8 (8%)	5 (5%)	1 (1%)
Sex			P=0.75			P=.09			p=0.21
Female	9 (10%)	34 (34%)		7 (6%)	38 (38%)		30 (31%)	11 (11%)	2 (2%)
Male	13 (14%)	42 (42%)		19 (18%)	38 (38%)		30 (31%)	18 (18%)	7 (7%)

* met/* represents both met/met and met/val genotypes

** plasticity definition is 0 for neither s/s or met/* status; 1 is defined as presence of either the s/s genotype or met/* genotype and 2 is for individuals with both the s/s and the met/* genotypes. Note. P-values based on Chi-square analyses unless cell sizes < 5, where p-value based on Fisher exact estimates.

Table 2

	BDNF	5htt Estimate (df) p value	Plasticity
Group	.25 (110) p=NS	-.04 (106) p=NS	.46 (106) p=NS
Genotype	.99 (110) p=NS	1.6 (106) p= 0.01	1.1 (106) p=NS
Group x genotype	- 2.3 (110) p=0.01	-1.3 (106) p=NS	- 1.7 (106) p=.009
Genotype x time x group	.98 (322) p=.0014	.9 (320) p=.006	.85 (320) p=<.0001
Intercept	1.36 p=.03	1.71 p=.013	1.68 p=.014

Mixed effects results. Time treated as a categorical variable due to nonlinearity of time between assessment points and baseline age. BDNF compares met carriers to val/val homozygotes. 5htt compares s/s homozygotes to s/l and l/l genotypes. Plasticity genotype is 0,1, or 2. NS= not significant $p>.05$. Intercept is provided for the complete model with the outcome centered at 54 months.

Table 3

Genotype and change in Indiscriminate Behavior over time

Time	BDNF !	5htt	Plasticity
Bl to 30	BDNF=NS	5htt=NS	genotype=NS
	Group=NS	Group=NS	Group=NS
	Interaction=NS	Interaction $p=0.03$, $\beta=1.2$ $r^2=.04$	Interaction=NS
30 to 42	BDNF=NS	5htt=NS	genotype=NS
	Group=NS	Group= NS	Group=NS
	Interaction=NS	Interaction=NS	Interaction=NS
Bl to 42	BDNF=NS	5htt=NS	genotype=NS
	Group $p=0.09$, $\beta=.9$	Group $p=.002$, $\beta=1.0$	Group=NS
	Interaction=NS	Interaction $p=0.02$, $\beta=1.1$ $r^2=.05$	Interaction=NS
30 to 54	BDNF=NS	5htt=NS	genotype $p=.036$, $\beta=1.4$
	Group $p=0.003$, $\beta=.9$	Group=NS	Group= NS
	Interaction $p=0.009$, $\beta=2.9$ $r^2=.06$	Interaction=NS	Interaction $p=.06$, $\beta=1.4$ $r^2=.03$
42 to 54	BDNF $p=0.049$ $\beta=1.2$	5htt=NS	genotype=NS
	Group=NS	Group=NS	Group $p=.036$, $\beta=.35$
	Interaction $p=0.045$, $\beta=1.4$ $r^2=.04$	Interaction NS	Interaction= NS
Bl to 54	BDNF=NS	5htt=NS	genotype=NS
	Group $p=.006$, $\beta=1.0$	Group $p=.011$, $\beta=.97$	Group=NS
	Interaction $p=0.03$, $\beta=1.2$ $r^2=.06$	Interaction $p=0.042$, $\beta=1.24$ $r^2=.05$	Interaction $p=0.0048$, $\beta=2.4$ $r^2=.08$

*Interaction indicates a significant group by genotype interaction on DAI score between each specified time period. Time is treated as a categorical. BDNF compares met carriers to val/val homozygotes. 5htt compares s/s homozygotes to s/l and l/l genotypes. Plasticity genotype is 0, 1, or 2. NS= not significant $p>.05$; Bl= baseline, 30= 30 month assessment point; 42= 42 month assessment point; 54= 54 month assessment point. Analysis was done on change in DAI score for all time points and controlled for gender and ethnicity. p values represent Bonferoni corrected values. All r^2 values represent the r^2 of the interaction effect alone.