Journal of Neurological Sciences and Psychiatry

Research Article

Exposure to Stress and Separation-Induced BDNF Impairments in Adolescents

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Published: November 15, 2016

Abstract

Background: Although the deleterious effects of separation during early childhood have been extensively studied, little is known regarding other stress-sensitive periods in development, such as adolescence. Also unknown are the biological mechanisms explaining its deleterious effects. This study was carried out to determine how different types of separation can impact neurotrophic factors during adolescence.

Methods: A community sample of 450 Hispanic adolescents was queried in 3 separate visits about regarding four indicators of stress exposure: migration, low closeness to parents, divorce, and growing up with individuals other than their biological parents. Chronological age at the time of exposure to the stressor was documented. BDNF and Pro-BDNF levels were obtained at three time points during the length of the study.

Results: The expression of pro-BDNF and m-BDNF was altered by separation, both divorce and death. Of concern, near half of the sample reported their parents to be divorce as a result the majority had mothers that work full time. Exposure to recent life events such as a parent divorce resulted in a time-point dependent, differential down-regulation of m-BDNF levels. Parent-child conflict positively related to BDNF. Specifically, BDNF was affected only in those the father/male adolescent relationship.

Conclusion: Our data confirmed that separation triggers alterations in BDNF, even after the growth spurt of the brain during early childhood. The important implication of this study is the persistent abnormal levels of BDNF. The prolonged alteration is of concern when considering that BDNF plays a critical role in the pruning process occurring during adolescence. Additional studies are needed to assess whether these alterations can lead to neuropsychological disruptions.

Introduction: Decades ago, animal studies demonstrated that maternal separation is one of the strongest stressors that can lead to changes in the quality and quantity of maternal comportments, child development, and child behaviors. After separations, pups exhibited behavioral dysfunction, depression, heightened fear, and social withdrawal. As they grew they were more anxious, and had increased stress responsivity. Of concern, beyond mood disorders, there was evidence of structural changes, such as neuronal apoptosis and alterations in synaptic density [1]. In addition, scientists observed morphological modifications in limbic structures, the prefrontal cortex, and the hippocampus [2]. Since then, animals manipulated with maternal deprivation have been widely used as an experimental paradigm to examine the neuro-mechanisms of stress and adversity.

Given the well-known role of Brain derived neurotrophic factor (BDNF) in cognitive performance, mood control, and plasticity, BDNF was targeted by several researchers attempting to identify the mechanism mediating such observations. Those studies confirmed BDNF alterations were responsible, at least in part, for the observed developmental and behavioral problems [3]. Notably, these changes can remain pervasive across the lifespan. Such studies gave birth to epigenetics, when scientists discovered that changes in the BDNF protein were associated with chemical modifications

made to chromatin (DNA and the associated histone proteins) that help regulate transcription of the genome [4]. Observed changes include DNA methylation, post-translational histone modifications, and gene regulation by micro-RNA (miRNA) [4]. Among them, the Val66Met allele (methionine in place of valine at position 66) has been extensively studied as responsible for the decrease in intracellular trafficking, and activity-dependent secretion of BDNF [5]. Yet, the translation of these findings to humans has been rather slow.

Despite these significant advances, there are several gaps in knowledge that need to be addressed. For example, while it is evident that sustained stress, such as early separation, entails serious consequences, little is known about the results when separation occurs during adolescence. The roles of many other types of separation, such as migration, or divorce, as well as other forms of adversity, also remain unknown. Yet, there is growing interest in the effects of such events, given that Hispanics need to deal with bicultural management challenges. The migration process also can increase the frequency of single-parent families headed by mothers, fathers, or by grandparents. Despite differences in the national origin of Latinos, *familismo* (the salient role of family in the Hispanic culture) is a core characteristic of this group. According to the integrative model of ethnic minority child development, familismo is a protective factor that promotes positive outcomes for individuals [6-8]. For instance, statistics suggest that Hispanics have lower divorce rates than the general population. Researchers also recognize that familismo makes Hispanics more likely to "be more involved parents, and encourage higher levels of commitment to school and prosocial behavior in their children" [7,9-10]. Therefore, Hispanic adolescents and their families may represent a key sample to study the impact of family stressors.

Our goal is to provide a neurobiological model of adolescence that proposes an imbalance in BDNF and pro-BDNF as a potential mechanism for the deleterious effects of stressors. Such analyses are critical, given that Pro-BDNF received attention only a couple of years ago. It was demonstrated that Pro-BDNF was able to function as a ligand, and exerts the opposite effects of mature BDNF. These data can provide the basis for evidence-based intervention programming and decision-making, which may reduce stress and enhance parental and child health for millions of immigrants in the United States.

Methods: Data were obtained from the ROBIM cohort (the Role of Brain Derived Neurotrophic Factor in Decision Making Participants), a longitudinal study of 450 Hispanic adolescents, which the American Academy of Pediatrics defines as minors 11 to 18-years-old. The study Ethnicity was determined using adolescents' self-identification, along with the country of origin, and ethnicity of the parents and the grandparents.

After obtaining IRB approval, adolescents were recruited between January 2012 and July 2016 from South Florida, US. The enrollment of the adolescents in this study entailed direct outreach in centers that provide recreational, social, and educational services for Hispanics, and from health care facilities. Adolescents were eligible if they did not have a history of major neurological/psychiatric disorders (i.e., autism, severe developmental problems, mental retardation, schizophrenia), or clinical diseases (i.e., cancer, renal or heart disease) that prevented their participation in the study. Adolescents receiving any neuro-pharmacological intervention, or taking bodybuilding substances (i.e. steroids, growth hormones) were ineligible.

After a complete description of the study to the adolescent and his/her legal guardian, written informed consent was obtained from all minors and guardians. If the adolescent was over 18 years of age, only their consent form was required.

Assessments: Participants were followed annually for two years. ROBIM's visits were conducted by trained bilingual interviewers, and consisted of a brief medical exam, structured survey questionnaires to obtain sociodemographic information, and the completion of several surveys to determine health behaviors. As part of the protocol approved by the Florida International and the University of Miami IRBs, parents were also invited to complete brief questionnaires regarding sociodemographics, and adolescent's health. Interviews were conducted in Spanish or English according to the preference of the subject.

Separation stress exposure: Both parents and adolescents were asked at baseline to identify adverse childhood experiences, and other plausible lifetime stressors (socioeconomic problems, neighborhood conditions, alcohol/drug use in the house, migration, who raised the adolescent, and disparities in acculturation with their parents).

However, subsequent follow-ups were focused on recent life events (deaths, divorce, and relationships in the past 12 months). Stressors were coded as 1, and absence of stressors as 0. If the adversity occurred, the participant was asked about the time and duration of the event.

Parent-child attachment: The adolescent-parent relationship was assessed using the Inventory of Adolescent Attachments. This tool assesses adolescents' perceptions of the positive and negative affective/cognitive dimension of relationships with parents [11]. The responses are on a 5-point scale, ranging from almost never/never true (1) to almost/always true (5)

Brain derived neurotrophic factor (BDNF): Circulating levels of BDNF were selected because prior studies had demonstrated that, although different from those in the cerebrospinal fluid (CSF), they are correlated with CSF measures in other CNS diseases. To obtain platelet-poor plasma, blood samples were collected in EDTA-coated tubes (plasma) (BD Diagnostic Systems, NJ, USA), and were stored on ice. Plasma was separated by centrifugation at 4ºC for 15 min at 1,500x g. This plasma was again re-centrifuged at 10,000xg, and aliquots of PPP were stored in polypropylene tubes at -80ºC until assayed.

BDNF concentrations were quantitatively determined using MILLIPLEX MAP Human Pituitary Magnetic Bead Panel from Millipore (EMD Millipore Corporation, Billerica, MA, USA).

The Standard Curve Range for the assay was from BDNF - 12 - 50,000 pg/mL, and the BDNF concentrations were expressed in pg/ml.

Control variables: Information was collected on potentially confounding variables, including sociodemographics (age, education, employment, and gender) and medical history. Data were collected with regards to household income (<$25,000, $25,000–$49,999 –$74,999, and $75,000 or higher), and immigrant status (born outside of U.S., U.S. citizen). We also gathered information on healthful diet, exercise (Stanford 7-day survey), body mass index, and drug abuse. These variables were used as both baseline and time-varying factors.

Statistical analyses: Descriptive statistics, such as minimum, maximum, median, and mean with SD were used to summarize the data. Group comparisons (i.e. by neighborhood, gender, BDNF levels) were assessed using the chi-square test for categorical variables, ANOVA for normally distributed variables, and the Wilcoxon rank sum test for non-parametrically distributed continuous variables. Alcohol use variables, BDNF, and neighborhood scores were assessed both as continuous and as categorical variables.

Results:

Sample characteristics: The demographic characteristics of the adolescents currently enrolled in ROBIM are shown in Table 1. The male to female ratio was nearly 1: 1. The age of the respondents ranged from 11 to 18 years, and the mean was 14.7 ± 2. Of the 450 youths, the majority of our participants were either middle or high school students (35% and 50% respectively), with the remaining participants being either in elementary (14%) or in the university (1%). The study successfully recruited a sample of both high (34% < 80,000) and middle/ low-income participants. Most adolescents were native-born Americans, and 27% were foreign-born.

Demographic Variable	Percent
Gender	
Male	47%
Female	53%
Age In Years	14.7 ± 2
Current Education	
Elementary	14%
Middle School	35%
High School	50%
University	1%
Income	
Low/Poverty	42%
Middle	26%
High Class	32%
Migration Status	
Immigrant	27%
Born in Florida	64%
Migrate From Another US State	9%

Table 1: Demographic Characteristics of the Adolescent Population (n = 450)

Stressors: Family life: Family separation can be another important source of stress among other plausible sources; the loss of a parent can be the most stressful condition, and was reported by three percent of our adolescents. A sizable proportion of the parents reported being divorced (48%) with the teens being raised by a single parent, mostly the mother. For the other 6% of the adolescents, the grandparent or another family member raised the teens.

Less than half of the adolescents (46%) grew-up in a two-parent household. As part of the familismo typical of Hispanics, 14% of the adolescents live in the same house with their grandparents. The vast majority of the Hispanic teens were raised in large families, as the average number of siblings reported was 3±2 siblings.

To our surprise, no significant difference in the type of family was observed between those with and without a history of migration. Overall, a smaller percentage (5 percent) of the adolescents reported that the mother or the father remarried following a divorce.

BDNF and Pro-BDNF: Among the 450 adolescents, there were a wide range of BDNF levels in circulation, from 290 to 23,000 (mean 2141 ± 149 pg/mL), with pro-BDNF being more abundant than mature BDNF (934-39,111 pg/mL, mean=5109 ± 243). While gender differences have been identified among adults, in this cohort males and females had similar BDNF levels, although females tended to have higher levels (1988.7 ± 197 vs. 1877 ± 182 pg/mL, $p = 0.6$).

BDNF and Divorce: For the purpose of these analyses, we only focused in incident cases. In other words, we analyzed first only those adolescents that reported a parental divorce after enrolling in the study. Analyses uncovered that a recent parental divorce resulted in a time-point dependent, differential down-regulation of m-BDNF levels (1047.3 ± 303 vs 2128.6 ± 357.8 ng/ml, *p=0.03*). Twelve months later, participants that experienced the stress of the divorce increased only their plasma m-BDNF levels by 548± 303, while those with a stable family raised both levels 1540± 350 ng/ml, (*p=0.001*). It needs to be noticed that those

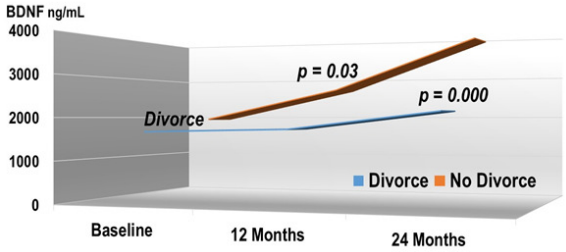

Figure 1: BDNF Trajectory after experienced a divorce

The burgeoning literature in this area has long questioned the importance of the timing of parental divorce, and it has thus remained unclear. Therefore, we teased apart whether a particular age range exists at which parental divorce would more greatly impact the individuals' health. The analyses regarding the total effect of a parental divorce during each interval on the child's m-BDNF and pro-BDNF levels did not identified significant difference. Neither the gender of the teen.

BDNF and loss of a parent: As depicted in Table 2, adolescents that have lost a parent had higher levels of m-BDNF, as compared to those with both parents still living (2117.9 ± 972 vs. 1962.8 ± 135 ng/ml, *p=0.09).* These differences pervaded 12 months later (5389.3 ± 2641 vs. 2561.6 ± 235 ng/ml, *p=0.09).* Levels of Pro-BDNF, which are deleterious, were also significantly higher (8514.3 ± 1646 vs. 4976.2 ± 243 ng/ml, p=0.05), and remained elevated 12 months later (7704.0 ± 2075 vs. 4265.2 ± 240 ng/ml, *p*= 0.05).

	Baseline Lost a Parent	Parents Alive	P value	Raised by others	Raised by the Parents	P value
BDNF ng/ml	2117.9 ± 972	1962.8 ± 135	0.09	1047.3 ± 303	2129 ± 358	0.03
Pro-BDNF ng/ml	8514.3 ± 1646	4976.2 ± 243	0.03	6985 ± 1451	4406 ± 431	0.03

Table 2

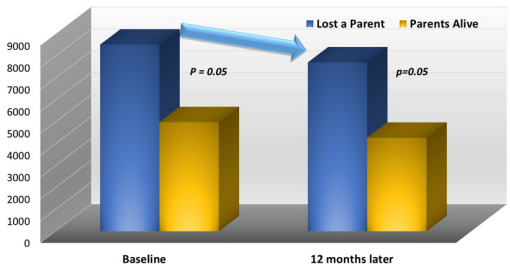

Figure 2: Longitudinal effect of parent lost on pro-BDNF

BDNF and family integrity: Growing in a single parent household did not seem to exert any differences over neurotrophic factors. However, youths raised by other family members (i.e., grandparents) exhibited lower m-BDNF levels than those cared for by both parents (1047.3 ± 303 vs. 2129 ± 358 ng/ml, *p=0.03*). They also had higher pro-BDNF (6985 ± 1451 vs 4406 ± 431 ng/ml, *p=0.03*). Since only one adolescent had lost both parents and were raised by others we do not have enough power for additional analyses.

BDNF and parental attachment: Most adolescents felt closest to their mothers. As depicted in Table 3, Hispanic mothers were seen as more caring. Then we examined the attachment of adolescents to their father and the role that gender differences. As illustrated in Table 4 male and females rated differently their relationship with their father. As compared to females, male adolescents' perceived in most cases quality of father/adolescent less than optimal.

Variables	Mother	Father
Do a good Job	76%	58%
I wish I have a different	10%	33%
Talking with….about my problems make me feel foolish	28%	70%
I get upset easily around my	20%	33%
My mother/father has her/his own problems, so I don't bother her/him with mine	31%	33%
I don't get much attention from my	15%	30%

Table 3: Adolescent Parent Attachment

	Gender	Mean	Std. Deviation	P Value
I wish I had a different father.	1	1.33	0.817	0.022
	2	1.56	1.11	
I like to get my father's point of view on things I'm concerned about.	1	3.95	1.278	0
	2	3.15	1.487	
My father can tell when I'm upset about something.	1	3.74	1.285	0
	2	3.05	1.436	
I get upset easily around my father.	1	1.85	1.17	0
	2	2.34	1.424	
When we discuss things, my father cares about my point of view.	1	4.02	1.17	0.02
	2	3.74	1.295	
My father trusts my judgment.	1	4.12	1.084	0.006
	2	3.8	1.306	
My father helps me to understand myself better.	1	3.82	1.319	0
	2	3.16	1.337	
I tell my father about my problems and troubles.	1	3.44	1.392	0
	2	2.72	1.47	
I feel angry with my father.	1	1.62	0.995	0.007
	2	1.93	1.254	
I don't get much attention from my father.	1	1.61	0.988	0
	2	2.25	1.428	
My father understands me.	1	4.18	1.093	0
	2	3.44	1.428	
When I am angry about something, my father tries to be understanding.	1	3.98	1.163	0.001
	2	3.55	1.383	
I trust my father.	1	4.51	0.985	0
	2	3.89	1.422	
My father doesn't understand what I'm going through these days.	1	2.22	1.385	0.01
	2	2.59	1.433	
I can count on my father when I need to get something off my chest.	1	3.93	1.244	0
	2	3.22	1.437	

(1) Almost never or never true; (2) Not very often true (3) Sometimes true (4) Often true (5) Almost always or always true

Table 4: Father Attachment by Gender

Since family disruption can impact adolescent parenthood attachment, we explored this relationship and found that adolescents raised by a single parent and those raised by another family member were more likely to report poor attachment, as reflected by high scores on questions such as 'I wish I had a different mother or father' (mother: 1.33±0.8 vs. 1.1±0.4 *p=0.01* father: 3.33±1.4 vs. 2.87±1.4, *p=0.002*).

Further analyses confirmed that the parent-adolescent relationship discord can impact neurotrophic factors. Boys who expressed that "they wish they have a different father" exhibited significantly higher pro-BDNF levels (6574.3 ± 1860 vs. 3610.1 ± 664.6, p=0.04). In contrast, females wishing to have a different father or a different mother had similar m-BDNF and pro-BDNF levels.

Final Model: Table 5 illustrated the final predictors of BDNF at the last visit. We have also examined the interaction effect of divorce and low closeness with mom and the interaction effect of divorce and immigration. Neither one indicated statistical significance.

Model	Un Standardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta		
(Constant)	-79.821	1673.688		-0.05	0.96
Single household score	757.125	580.922	0.128	1.303	0.2
I / wish I had a different mother.	642.45	327.521	0.182	1.962	0.05
I / wish I had a different father	-214.588	306.982	-0.066	-0.7	0.49
Gender	-459.976	544.328	-0.078	-0.85	0.4
Divorce	-2649.586	5095.627	-0.181	-0.52	0.6
Age divorce groups	316.47	372.784	0.084	0.849	0.4
What / country are you currently a citizen of?	1865.25	758.136	0.233	2.46	0.02
Household / Income	-255.784	170.387	-0.139	-1.5	0.14
Interaction migration divorce	1245.325	1180.384	0.371	1.055	0.29
Interaction hate mother divorce	-1760.044	1773.416	-0.216	-0.99	0.32

Table 5: Coefficients[a]; a. Dependent Variable: v12bdnf

Although not statistically significant, the output shows that immigrant adolescents will reduce the effect of parent divorce on BDNF and adolescents who hate mom will increase the effect of parent divorce on BDNF.

Discussion: The present study makes a few important contributions to this area of research. Firstly, because the detrimental influence of inter-parental conflict on adolescent's development in minority groups remains scarce [12]. Secondly, the hypothesis that Hispanic familismo would be reflected in lower rates of single household families and divorce was incorrect. Our data indicates that this is an issue far worse in severity than anticipated. Today in the U.S., single parents head an estimated 30% of all families with children [13]. In reality, almost half of our adolescents were raised in a single parent household, a much higher rate than in the general population.

In addition, while the literature in large focus on early childhood, we focused on another stress-sensitive period during development: adolescence. Stressors experienced during puberty may be particularly influential as the brain continues to develop, relative to similar exposures at early ages, if they lower the rate of developmental growth. The gap between these two trajectories, one growing at a slower rate due to the developmental insult, will increase with age, and the difference will be larger if the exposure occurs earlier in life [14]. To the extent that developmental outcomes affect adult health, the timing of influential events will help to determine the size of health disparities later in life. Furthermore, developmental outcomes are more vulnerable to change at some ages than others [14-16]. It follows that the pathways connecting parental divorce to adult health may vary depending on when the event occurs, and which developmental outcomes are the most vulnerable.

Third, we examined how family stressors affect the development of Hispanic youth. Specifically, we examined whether family disruption is associated with significant biological changes in neurotrophic factors. After looking at the divorce literature, the focus was largely on children's internalizing and externalizing problems. In addition, available studies were cross-sectional and none focused on biological parameters. Thus this study represents a departure from prior literature concentrating on toxic stressors, as we described the deleterious effects of divorce on their levels of BDNF.

In terms of the effects of these stressors, while one can imagine that these alterations reflect temporary adjustments, we were surprised to discover that changes remained stable a year later. Neurobiological consequences of family stressors are especially important because BDNF plays a critical role in brain development, mood, and cognition. As a result, these stressed youths may experience a higher risk/higher incidence of mood disorders, problems adapting, and academic difficulties [17-18].

When the family is sensitive to, and supportive of the developmental needs of offspring, it may then serve as a buffer to handle stressors. Unfortunately, a sizable proportion of adolescents showed deficits in their father/child attachment. Previous models of attachment explained poor father-child relationships through the societal expectation of the father being the economical provider only, and the mother being the nurturer of the children and home. According to our findings the poor attachment, particularly with fathers and their children, seems to be attributable to the increasing rates of divorce. On the other hand, the mother's childcare role is still as prevalent as it was before, only that now they also have to be the breadwinners.

Further examinations of attachment of adolescents to their father and the role that gender differences play in this development indicate clear difference between male and female adolescents. Male adolescents reported more conflicts with the fathers, sufficient to alter BDNF. These findings are of concern in light of studies showing that to form close and secure relationships, father's parenting, but not mothers, is a decisive factor [19]

Nonetheless this study has some limitations. First of all, the sample is limited to Hispanics and thus findings should be replicated in other groups. On the other hand, the sample size is a fairly large with a similar distribution of gender to derive conclusions.

However, a possible solution in animal models suggest that physical exercise can reverse Brain derived neurotrophic factor (BDNF) signaling alterations, and will be the focus of our next project.

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