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INTERACCIÓN ENTRE EL POLIMORFISMO DE LA REGION PROMOTORA DEL GEN DEL TRANSPORTADOR DE SEROTONINA Y FACTORES AMBIENTALES PARA PREDECIR SINTOMAS DEPRESIVOS: MÁS ALLA DEL MODELO DE VULNERABILIDAD

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INTERACTION BETWEEN A POLYMORPHISM OF THE GENE OF THE PROMOTER REGION LINKED TO SEROTONIN TRANSPORTER AND ENVIRONMENTAL FACTORS TO PREDICT DEPRESSIVE SYMPTOMS: BEYOND THE STRESS VULNERABILITY MODEL

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DEDICATED

With all my love to Pablo,

My profound loves, Diego and Pedro

All mothers, workers and researchers

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ABSTRACT

Introduction: Research on the potential role of gene-environment interactions (GxE) in explaining vulnerability to psychopathology in humans has witnessed a shift from a diathesis-stress approach to differential susceptibility approaches. This project critically reviews that body of research. Depression has been associated with alterations in the response systems to environmental stress. The serotonergic system is widely related to the stress response system, through the hypothalamic-pituitary-adrenal axis. Variations in this system—especially being a carrier of short allele (S) of the polymorphism of the serotonin transporter (5HTTLPR) —has been associated with an increased vulnerability to depression when exposed to adverse environments. However, few studies—and none in Chile—have analyzed the interaction between this polymorphism and the environmental factors from the differential susceptibility approaches.

Methodology: Databases were screened for studies of GxE in the prediction of personality traits, behavior, and mental health disorders in humans, published between January 2002 and January 2015. In total, 315 papers were included.

The project is a quasi-experimental study: mixed (through analysis between groups and within subjects), unifactorial, quantitative and transversal. The interaction between the 5HTTLPR polymorphism and the following variables in predicting depressive symptoms were evaluated: (1) childhood trauma; (2) recent life events (positive and negative); (3) social support; (4) attachment style, and; (5) personality style (anaclitic/introjective). Furthermore, an experimental task was performed, and salivary cortisol was measured to determine whether these interactions were related to changes in the neurobiological response to stress. The sample consisted of 151 adult subjects.

Results: Independent of the type of environment studied (early or recent life events, positive or negative environments), about 66.9-83.3% of the articles reported GxE interaction, which is consistent with the social susceptibility model. However, methodological considerations limit the ability to draw definite conclusions, especially since almost 90% (n=283/315) of the papers are based on samples from North America and Europe, and many studies (219/315) are based on overlapped samples. Methodological improvements in this area are shown by a significant increase in longitudinal and experimental studies as well as improved minimum genotyping.

In our study, S allele carriers showed fewer depressive symptoms when they presented high social support and low anxious attachment, compared to S allele carriers with low

social support and high anxious attachment. In turn, L allele carriers did not show these differences. Moreover, SS subjects with mixed personality configuration obtained significantly higher depressive scores. Furthermore, we found a GxE interaction between the 5HTTLPR polymorphism and social support and the depressive experience type for the average area under the curve (AUC) of cortisol during the experiment. **Conclusions:** The systematic review showed no differences on the GxE between different environment types to predict changes in human conduct, so it is possible that the model behind the interaction is one of differentiated sensitivity to the environment and not just vulnerability to psychopathology. Moreover, we observed that the short allele of the 5HTTLPR polymorphism confers a vulnerability to depressive symptoms in the presence of a mixed personality organization (high self-criticism and high dependence). But concerning attachment style and social support, it could provide a differentiated sensitivity to environmental stimuli, predicting lower depressive symptoms when interacting with positive environments and a worse outcome when interacting with more adverse environments. And, finally, the release of cortisol during the experiment depends on the interaction between the 5HTTLPR polymorphism and social support and the type of depressive experience**.**

INTRODUCTION

Depressive disorders are a public health problem worldwide because of their high prevalence and its serious consequences. Generating a severe impact on quality of life with high levels of disability (Ayuso-Mateos et al., 2001; Bijl, Ravelli, & van Zessen, 1998; KS Kendler et al., 1994; Kessler et al., 2003; New et al, 2010;.. Offord et al, 1996), becoming the second leading cause of years lost due to premature death or disability (DALYs) (WHO, 2001). These disorders are of concern in Chile, as we present figures above the expected for the region (Araya et al., 2007). According to the national health survey in Chile 2009-2010 the prevalence of depressive symptoms was 17.2% (women 25.7% and men 8.5%) (Chilean Ministry of Health). According to the Ministry of Health the prevalence of major depression is 6% of women and 3% in men, and if mild depressive episodes and dysthymia are added, the prevalence increases to 10.7% in women and 4.9% in men (Chilean Health Ministry, 2009).

From July 2006, in the context of health care reforms, treatment for depression is included in the Explicit Health Guarantee (GES) system, mechanism that grants specific services in prioritized areas for people and national health.

The efficacy of antidepressant treatment, whether pharmacological or psychotherapeutic, varies between 40 and 74% (Gaynes et al., 2008). The analysis of the STAR * D, North American study, which design approaches to what occurs in daily clinical practice of treatment of depressed patients, showed lower figures of remission to those observed in randomized clinical trials. Remission rate of depressive episode treated with any modality was 32%, and depression treated with individual psychotherapy was 27% (van der Lem, van der Wee, van Veen, & Zitman, 2012). These differences, besides from the obvious factors that keep away randomized clinical trials from reality (high patient selection), may due because particular aspects of depressed patients are not taken into account for the indication of treatment.

Currently depression is considered a systemic disease, recurrent, often chronic, that requires long-term treatment. Moreover, it is a complex disease involving etiologically multiple factors and multiple domains that act along the development. Its pathogenesis can be separated into different hierarchical levels of organization from genes to behavior and personality traits.

One of the difficulties presented by the clinical management of depression is that, so far, diagnostic and classificatory systems have proved insufficient when

addressing psychopathological complexities of mood disorders (Corveleyn & Blatt, 2005), which ultimately leads to pathogenesis, psychopathology, and therapy based on symptoms. Thus, in the current guidelines of the Ministry of Health (Chilean Health Ministry, 2009), psychotherapy is recommended for the treatment of depression depending on the severity of the clinical condition. The severity of depression according to ICD-10 is given by the number of symptoms presented by the person, regardless of the type or intensity of them (except for psychotic symptoms).

The potential interactions between genetic, neurochemical, and cognitive factors has only recently been demonstrated. The combination of findings from behavioral genetics and cognitive neuroscience opens new opportunities to integrate research results. It is suggested that a comprehensive study of the psychological and biological correlates of depression may grant a new way to understand this disabling disorder (Beck, 2008). Since last decade, investigators propose that the future of clinical research and therapeutic efforts should focus on the study of processes of vulnerability, which applies particularly to depression (Corveleyn & Blatt, 2005). It becomes especially urgent to accommodate these new proposals if we look at the results of meta-analytic review about the effectiveness of treatments empirical support (Westen, Novotny, & Thompson-Brenner, 2004). Because the low rates of response to treatment, researchers agree on the need to change research strategies to target from the beginning the question of which patients require what type of treatment (eg, pharmacotherapy or psychotherapy, brief or long term) being necessary to then identify dimensions related to patient treatment.

In the 90s, empirical studies on the interaction between genes and environment began in psychiatry. These investigations were designed to determine vulnerable to stress phenotypes. They conclude that some people carrying particular polymorphisms are more vulnerable to the effects of stressful environment. This is the case of the polymorphism of the promoter region of the serotonin transporter (5HTTLPR) gene, which has a variant (short allele) that would be more vulnerable to stressful environments. As we review in the development of the thesis, this model of vulnerable phenotype or diathesis to stress, has shifted in recent years, including positive aspects of the environment and considering these "vulnerable" alleles as "prosocial or plastic" alleles, that is, more sensitive to both negative and positive environment. The model changes from vulnerability to stress to different sensitivity to the environment. This research is designed under this new model. For this, environmental risk variables

(trauma, negative life events, insecure attachment), and protective variables (secure attachment, social support, positive life events) are included, to determine whether these environmental variables are related to levels of depressive symptoms and whether genetic variants have a role in this relationship. That is, if the relationship between genotype and environment show that carriers of the short allele of the serotonin transporter are more sensitive to environment. This is, the influence of the environment to predict depressive symptoms is stronger on plastic allele carriers.

Despite the considerable evidence regarding the importance gene environment interactions on the genesis of depressive disorders, there are at this moment few studies that address this interaction considering the different sensitivity model, and there are no investigations that analyse this relationship in our context.

Consequently, the research question will be: what is the effect of the interaction between the 5HTTLPR polymorphism, the personality and the environment over depressive symptomatology considering the model of "differential sensitivity", and does this interaction affect the neurobiologic reactivity to stress? To respond the question, a quasi-experimental mixed study was designed (analysis between groups and intrasubject), unifactorial, multivariate, quantitative and transversal. The dependent variable was the depressive symptomatology and the independent variables were the 5HTTLPR polymorphism. Moreover, the interaction between the polymorphism and the following variables in the prediction of the depressive symptomatology was evaluated: (1) child trauma history, (2) recent vital events, (3) social support, (4) attachment and, (5) personality style (anaclitic/introyective). The first four were considered "environmental variables" and the fourth "personality variable".

We consider that the relevance of this research is that it studies depressive disorders, a mental health highly relevant worldwide disorder but in spite that, the problem has been hardly analysed, and there are no studies that tackle this aspect in our context. Consequently, the contribution of this research can be defined on two levels. First, etiopathogenesis of depression, the findings of this work can expand the information regarding the way genes interact with the environment and the personality in the origin of depressive symptomatology, especially in an area where its relevance has been confirmed but has hardly been studied in our context. Second, treatment of depression, even though this project does not study directly the effect of GxE on response to treatment, it is a promising field, subjects more sensitive to environment may respond better to psychotherapy. Regarding psychotherapy, the identification of

“environmentally sensitive” genotypes can allow for the differentiation of clinical profiles that help to predict the response to the psychosocial interventions and, therefore, substantiate the basis for a differential indication in psychotherapy.

The manuscript of the thesis starts with the theoretical framework where the nature of the research problem is described, the available evidence is synthesized and the findings of a systematic review of the genetic polymorphisms that have been included on GxE studies are included. In the following chapter the hypotheses and the objectives are formulated. The working methodology is described and later the most relevant results are mentioned and are organized according to the studied variables, together with a list with the summary of the most significant findings. Finally, the conclusions are raised and contrasted with current scientific information, to end up with a series of recommendations for future investigations.

THEORETICAL FRAMEWORK

Psychopathology Models

To describe the theoretical model under which this research relies, we will review the etiopathologic models that attempt to understand and explain the development of depressive disorders. Lingering over the existing evidence and the implications of the research under different etiopathologic models.

1. Biopsychosocial model

Proposed by George Engel (Engel 1977), american psychiatrist frustrated with the classic biomedical model, which considered reductionist (a complex phenomenon could be explained by a single principle) and dualist (mind/body separated, and the only explanation of the disease were physical processes). The biopsychosocial model is quite suitable as a reference for social and biological sciences, because it is broad enough to incorporate genetic and environmental factors as potential contributors to health and disease. The proposed model considers that the factors that shape it are interdependent at all levels of the organization.

The problem with this model was the failure to create research designs (McCutcheon, 2006) by the slower development of science (McLaren, 1998). The model also describes that biological, psychological, and social components are interdependent but provides no hypothesis on how they interact.

The lack of communication between disciplines may have been another factor, the idea that life experiences and biology are factors that influence the development of disease and health is conceptually so broad, that no discipline can put together and test it with all the necessary data to support or refute its viability, so that multidisciplinary research is especially suitable to prove and evaluate this idea.

2. Stress diathesis model/Vulnerable phenotype model

The "stress diathesis" model of mental diseases proposes that stress activates a latent predisposition or diathesis, which then manifests itself as some form of psychopathology. This model assumes that a predisposition is necessary but not a sufficient condition for the development of a mental disorder and that the interaction

with stress activates the diathesis to increase the risk of developing a mental disorder (Zuckerman, 1999). Originally, the predisposition was presumed to be a genetic condition that was observable in certain biological traits; since then, the concept of diathesis has been expanded to include factors such as cognitive or social predispositions (Abela, 2001; Monroe & Simons, 1991). Under this broader concept, biological and psychological traits can be considered diathesis, i.e., the necessary precursors to develop the disorder. As such, in this theory, stress vulnerability is a predisposition or diathesis. This extension of the concept of vulnerability to stress has some conceptual problems, for example, a negative cognitive scheme that makes an individual more vulnerable to stress and anxiety can itself be influenced by genetic, social or both (Zuckerman, 1999). Under this concept, vulnerability to stress is a predisposition or diathesis (Zuckerman, 1999).

Stress can be defined as "a specific response of the body to a demand" (Lanfumey, Mongeau, Cohen-Salmon, & Hamon, 2008), but also can be described as "any environmental internal external change, or altering maintenance homeostasis" (Leonard, 2005). Its role as a risk factor for presenting psychopathology has been extensively studied. For this purpose, stress can be subdivided into 3 categories: acute stress, chronic stress, and stress in early life.

In the stress diathesis model, events that occur within the previous year of onset of the disorder are considered stressors or acute stress. Generally, life events that involve loss or humiliation have proved depressogenic (OR: 5.64) (K. S. Kendler, Karkowski, & Prescott 1999). Mild chronic stress studies have shown in animals and humans, that stress is related with neurobiological changes similar to those seen in depressed individuals (Grippo, Beltz, & Johnson, 2003; Tennant, 2002). Finally, stress in early life, such as childhood trauma (physical, sexual, or emotional abuse) and alterations in attachment, have shown to produce permanent biological changes that confer increased vulnerability to psychopathology (Gutman & Nemeroff, 2003, Christine Heim & Charles B. Nemeroff, 2001; Ladd et al., 2000; McCauley et al., 1997; Nemeroff et al., 2003; Plotsky, Owens & Nemeroff, 1998) and even different response to treatment, responding better to psychotherapy than drugs on chronic depressed women with a history of trauma (Nemeroff et al., 2003).

The distinction between early or remote and recent events is important for this model. This distinction is equally important for the psychoanalytic theory where it is considered that childhood events are predisposing factors for mental disorders in adults

(Marmor, 1968). Prior to the 90s, stress was considered as a non-specific and continuous concept, measured as high or low levels. The predisposition to stress was assumed as a threshold, below which the disorder is not expressed, no matter how severe was the stressor, and above which the disorder is expressed if you have sufficient levels of stress to activate the latent predisposition (Monroe & Simons, 1991). It incorporates the concept that early adverse experience can have lifelong effects on physical and psychological functioning, and become a vulnerability or diathesis for mental disorders.

The vulnerable phenotype model illustrates independent and interactive effects of genes and early environment in the development of the phenotype of the individual (Plomin, DeFries, McClearn, & Rutter, 1997). Adverse childhood experiences can exacerbate genetic vulnerability to stress. This can result in a phenotype that is hypersensitive to future exposures to stress and has an increased risk of developing psychopathology. Early social support and coping styles interact with the genetically determined temperament (Scarr & McCartney, 1983), and can act as buffers against the effect of early adversity in the development of the phenotype. Evidence from animal and human studies support the model of vulnerable phenotype, suggesting that early adversity induces neurobiological changes and that these changes inhibit the ability of the central nervous system (CNS) to regulate stress and emotions. This deregulation is accompanied by an increase in the rate of psychiatric disorders (S. J. Claes, 2004; Heim & Nemeroff, 2002; Shea, Walsh, Macmillan, & Steiner, 2005). Figure 1 summarizes the model of vulnerable phenotype and Figure 2 shows that individuals carrying the vulnerable genotype are more sensitive to adverse environments presenting a worse outcome than non-carriers of the vulnerable genotype. The latter are considered resistant to negative environments (resilient).

Model limitations

The stress-diathesis model is limited by its focus on stress which excludes other aspects of the environment that may interact with biological factors. As it was conceptualized to explain psychopathology, the focus is on environmental stressors that can contribute to the development of mental disorders. Leaving out environmental factors that can prevent, delay or treat mental disorders and promote resilience and health.

Figure 1 Integrated model of neurobiology of depression, based on the model of vulnerable phenotype

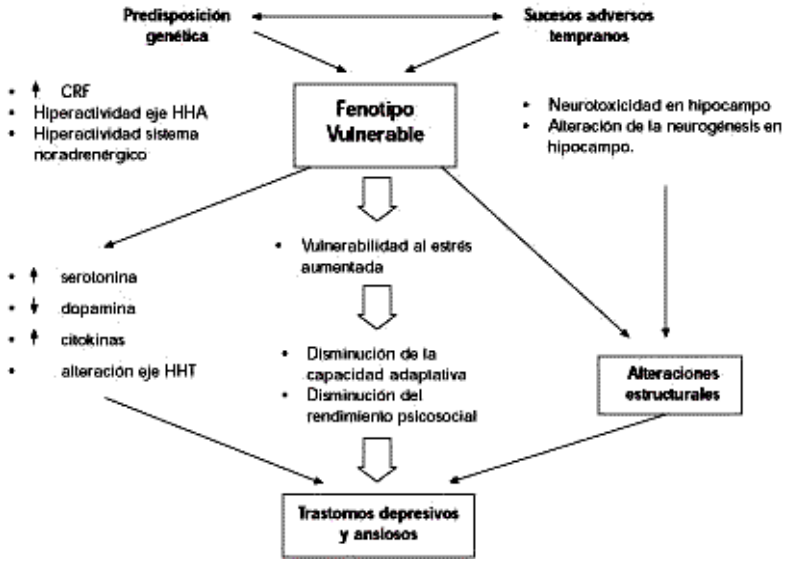


Figure 1 Obtained from: Silva, H. Nuevas perspectivas en la biología de la depresión. (2002). *Rev. chil. neuro-psiquiatr*, vol.40, pp. 9-20. CRF: Factor liberador de corticotropina, HHT: Hipotálamo Hipófisis Tiroídeo

Figure 2 Diathesis to stress model or vulnerable phenotype

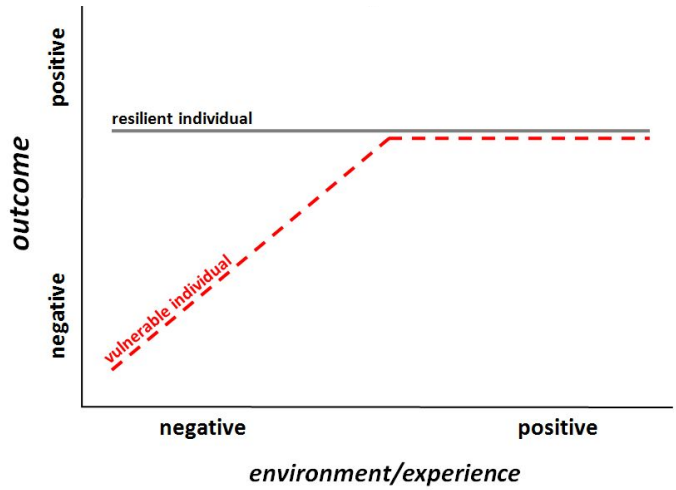


Figure 2 Obtained from Bakermans-Kranenburg & van Ijzendoorn MH. Genetic vulnerability or differential susceptibility in child development: the case of attachment. J Child Psychol and Psychiatry. 2007, 48(12);1160-73.

3. Environmental differential sensitivity model

Over recent years, investigators have reported about the relationship of certain genes, especially the serotonin transporter gene and increased sensitivity to environmental events. Their theories have minimal differences, so many of their publications have been made together. Taylor and Way (Way & Lieberman, 2010) have proposed the hypothesis that these polymorphisms predispose to greater social sensitivity, ie, they would be prosocial genes, while Pluess and Belsky (2009) proposed that these kinds of genes confer differential susceptibility to the environment, and would be plastic genes (malleable by the environment) (Fox, Zougkou, Ridgewell, & Garner, 2011). Previously, Ellis and Boyce (Ellis & Boyce, 2008), from an evolutionary perspective, proposed the model of biological sensitivity to context. Bringing together their theories, they proposed that these genes confer differential sensitivity to environment. Therefore, health and illness depend on the interaction between environmental and biological factors. That is, the genes (as biological factors) would give us more or less sensitivity to environmental factors, and the environment, as if it's positive or negative, would shape the individual, for worse or for better.

Unlike the vulnerable phenotype model, in which the presence of the short allele HTTLPR gene confers susceptibility to adverse environmental factors, in this model, the presence of this allele may provide greater sensitivity to the environment. This means that the S allele actually increases the sensitivity to the environment more generally, so exposition to adverse environments leads to worse outcomes, while supporters and positive environments lead to advantages (Bakermans-Kranenburg & van, 2015; Belsky et al, 2009;. & Pluess Belsky, 2009; Homberg & Lesch, 2011). This model includes the previous models of stress diathesis and vulnerable phenotype, but takes a more integrated vision of the environment (not only the negative aspects). "It seems that these models (diathesis stress and vulnerable phenotype) are only half the story" (Way, 2010). The serotonin transporter gene has been the most studied gene as plastic, known by its interaction with stress (environment) to develop psychopathology.

We will summarize the arguments supporting that the 5HTTLPR polymorphism behaves more like a prosocial/plastic allele than a polymorphism that only confers vulnerability: (a) sensitivity to positive environments and 5HTTLPR polymorphism, (b) differences in cognitive and brain function associated with the polymorphism, and (c) the relation between polymorphisms and culture. Figure 3 shows how in this case, the

individual carrying the most sensitive genotype has a negative result (worse) if exposed to negative environment and a positive result (better) if exposed to a positive environment. By contrast, the subjects not carrying the sensitive genotype are less sensitive to environmental events, ie, the result is independent of being exposed to positive or negative environments.

Figure 3 Model differentiated sensitivity to environment

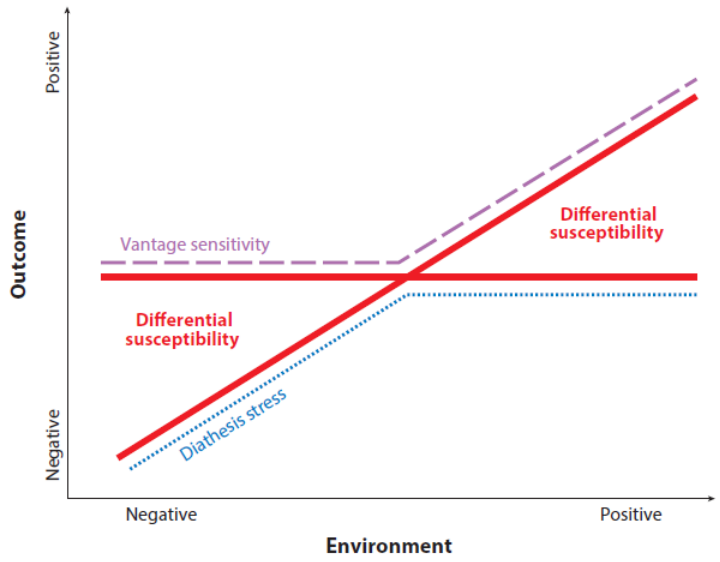


Figure 3 Obtained from Bakermans-Kranenburg, M. J., & van, I. M. H. (2015). The hidden efficacy of interventions: genexenvironment experiments from a differential susceptibility perspective. *Annu Rev Psychol*, *66*, 381-409. doi: 10.1146/annurev-psych-010814-015407

3.a. Exposure to positive environment and 5HTTLPR polymorphism

In general, as the model of vulnerable phenotype has prevailed, few studies have included positive environmental factors. And most works do not include actively a result of a better performance and greater well-being when exposed to favourable environments but seek for "no disease" or "no presence of symptoms". As described, the carriers of plastics genes, such as the short allele of 5HTTLPR, respond to the life experiences in a mode “for better or for worse way” depending on the nature of the experience in question (Belsky & Pluess, 2009).

In this model, one would expect to find that S carriers were more sensitive to exposure to positive environments such as secure attachment, high social support, and

positive recent environmental events, that is, fewer subjects carrying the S allele would get sick comparing to the subjects carrying L allele. Taylor´s study (2006) on prediction of depressive symptoms, according to early family environment and recent life events, showed that the SS individuals, when they described a family atmosphere of low-risk and low number of recent stressors, presented the lower depressive symptoms rates of the sample, whereas if they described a high-risk family environment and many recent stressful events, they had the highest depressive symptoms rates of the sample. This indicates that individuals homozygous for the short allele are more sensitive to life events, both positive and negative ones, than the other genotypes, as shown in Figure 4.

Figure 4. Relationship between HTTLPR polymorphism and early family environment and depressive symptoms

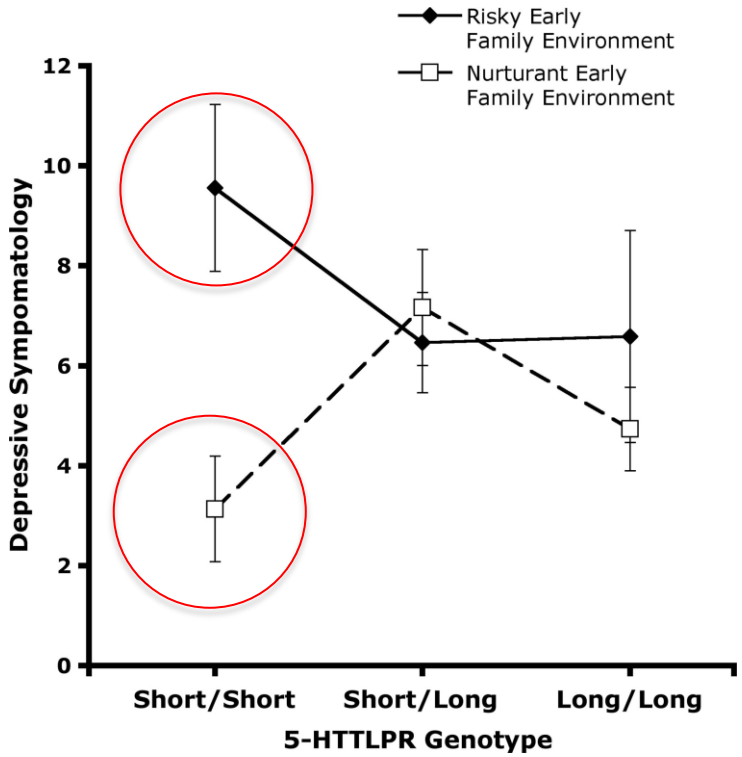


Figure 4 Obtained from Way, B. M., & Taylor, S. E. (2010). Social influences on health: Is serotonin a critical mediator? *Psychosomatic Medicine, 72*(2), 107-112.

Way, studied whether the nature of recent life events influences this interaction. He distinguished recent events between social events (e.g. end of romantic relationship, conflict with family or friends, death of a loved one) and non-social events (e.g. receiving a low grade, job loss, car accident). He noted that the relationship between genotype SS, life events, and depression, remains significant for recent social events

(Way and Taylor, 2010), but it´s lost for recent non social events, supporting the subtle difference between prosocial alleles instead of plastic alleles that he proposed.

For individuals carrying the S allele, social support appears to be an important factor in maintaining their well-being. Kilpatrick (2007) observed that subjects homozygous for the short allele that were exposed to a hurricane, had no greater risk for depression than those homozygous for the long allele, when they had a good perceived social support. However, if they perceived a bad social support they had 4.5 times greater risk of depression.

Kaufman (2004) found that social support moderated the risk for depression associated with the short allele and child abuse. Children with a history of abuse and SS genotype reported higher levels of depression. Maltreated children with the SS genotype and an absence of positive support had depression scores that were approximately twice as high as those of maltreated children with the SS genotype and positive social support. The authors conclude that the availability and frequency of social support may promote resilience even in children with high genetic vulnerability to depression and who have experienced adversity in childhood.

So, there is evidence to assume that positive environmental events mostly affect subjects carrying the short allele and especially when they have relational features. Summarized evidence, accounts for who are most affected by both positive and negative environmental events such as early and distant in time (trauma or dysfunctional/functional family) and recent events (social support or life events in the past year).

3.b. Cognitive and brain functioning according to 5HTTLPR polymorphism

Several studies on cognitive function, especially on voluntary attention and working memory in healthy individuals, have shown that carriers of the short allele perform better (Anderson, 2012; Enge, 2011a; Enge 2011b). Studies on emotional biases have shown that carriers of the S allele have a strong tendency towards negative material, especially related to threat (Beevers, 2009), and greater difficulty disengaging from emotional, positive, and negative stimuli (Beevers, Gibb, McGeary, & Miller, 2007; Beevers et al, 2011;. Beevers, Wells, Ellis, & McGeary, 2009; Fox, Ridgewell, & Ashwin, 2009; Kwang, Wells, McGeary, Swann, & Beevers, 2010; Osinsky et al, 2008;. Perez Edgar et al., 2010), and this was even observed in a meta-analysis (Pergamin-

Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012). While plasticity can operate towards negative and positive information, attention would respond more to negative bias (Fox et al., 2011).

Also, the S allele carriers are associated with a hyperactivity of the amygdala to threatening stimuli (Hariri, et al., 2002; Munafo, Brown, & Hariri, 2008). A study showed that carriers of the short allele, compared with homozygotes of the long allele, were faster in learning fear response in a paradigm of fear conditioning (Lonsdorf et al., 2009), which supports the idea that S allele carriers are more sensitive to environmental cues. This can be explained because learning to fear is a primary mechanism, which is done at a very prompt age because of its importance for survival. Cognitive malleability to increased environmental contingencies in the short allele carriers, explains why the S allele carriers learn faster to fear and develop neural circuits that are more sensitive to fear than subjects carrying the long allele.

Studies in healthy volunteers submitted to learning paradigms show greater and faster learning in short allele carriers. Fox (2011) underwent an attentional bias modification technique (AMB) (negative and positive bias) in healthy population. This technique has been tested in anxiety disorders and has been shown to decrease the biases associated with threat and is associated with improvement of clinical symptoms. In this experimental study, the results showed that the S allele carriers changed their attentional biases in a larger way than those homozygotes for the long allele. However, attention systems in S allele carriers respond more to positive and negative training (AMB) compared with the long allele homozygous, they responded more to AMB training, this supports the theory that the serotonin transporter gene expression is best conceived as a plastic gene rather than a vulnerability gene. "The form of low expression tunes people with the emotional significance of their environment, whether negative or positive. Looking beyond, the S allele is a genotype that confers vulnerability and the L allele is protective"(Fox, 2011, p. 1052). The author concludes that "one of the implications of this study is that carriers of the S allele could earn more of therapeutic interventions such as AMB" (Fox, 2011, p. 1053).

As it is mentioned above, 5HTTLPR polymorphism affects the reactivity of the amygdala to process conscious and unconscious stimuli. Lonsdorf et al (2011) studied, in healthy population, the impact of genotype on the amygdala reactivity to facial expression of anger in a passive task. They found that S carriers showed greater amygdala reactivity to angry faces compared with individuals with genotype LL. The

amygdala is central for the fear system, is involved in the detection of environmental threat, fear learning and assessment of emotional meaning. Individuals carrying the S allele of the 5HTTLPR gene may be more sensitive in detecting biologically and socially relevant information, which is a critical function for social interaction and emotional functioning. The association of increased amygdala reactivity in short allele carriers has been demonstrated with scary faces and other negative emotions such as anger and grief, and with positive emotions such as joy.

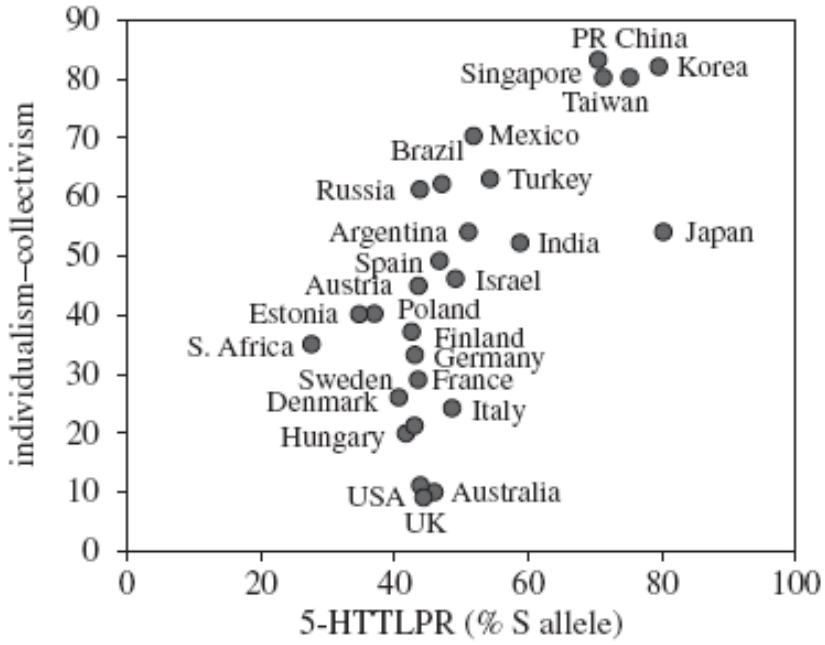
The individuals carrying the S allele of the 5HTTLPR gene may be more sensitive to the detection of biological and socially relevant information, which is a critical function for social interaction and emotional functioning. The association of increased amygdalian reactivity and short allele 5HTTLPR genotype has been demonstrated both with scary faces and with other negative emotions like anger and grief (Dannlowski et al., 2008), and with positive emotions such as joy (Domschke et al . 2006), both in healthy population, and patients with depression and panic disorder. This indicates, again, sensitivity to socially relevant information rather than only specific threat keys (Canli & Lesch, 2007).

Gyurak (2012) studied the effect of the polymorphism of the 5HTTPLR gene on emotional reactivity in two closer to social reality contexts, a) empathic response to others stress, b) self-awareness of the emotional response (to karaoke). Participants homozygous for the short allele reported higher levels of psychological and physiological stress in response to films showing other people suffer. SS participants reported higher levels of anger and amusement and a greater emotional expression in response to a social experiment that induced shame (seing a video with your karaoke singing). Moreover, another study (Schoebi, Way, Karney & Bradbury, 2012), held in marriages, observed that carriers of the short allele showed greater exchange of affection, both positive and negative, in social structured interactions in the laboratory, compared to individuals homozygous for the long allele. The affection of the carriers of the short allele moved more in line with their partners; the authors conclude that subjects carrying the SS genotype had responses to environmental stimuli that encouraged social interaction, compared with subjects with long allele, ie, favouring prosocial conducts.

3.c. Culture and 5HTTLPR polymorphism

Way (Way & Lieberman, 2010) and Chiao (Chiao & Blizinsky, 2010) conducted a review of the frequency of gene polymorphisms associated with individual social sensitivity of the serotonergic (5HTTLPR, MAOA-uVNTR) and opioid system (OPRM1 A118G), with the kind of culture of each population according to the degree of individualism and collectivism. The author hypothesized that collectivism may have developed and remained for a higher proportion of these alleles in the population. Consistent with this idea, they observed a correlation between the proportion of these prosocial alleles and the lifetime prevalence of major depression in the various nations. The relationship between frequency of these alleles and depression was partially mediated by the degree of collectivism/individualism, suggesting that reduced levels of major depression in populations with a high proportion of socially sensitive alleles is due to greater collectivism.

Figure 5 Correlation between collectivism/individualism and 5HTTLPR



polymorphism

Note Figure 5 Obtained from Chiao, J. Y., & Blizinsky, K. D. (2010). Culture-gene coevolution of individualism-collectivism and the serotonin transporter gene. *Proceedings.Biological Sciences / the Royal Society*, *277*(1681), 529-537. doi: 10.1098/rspb.2009.1650

Collectivist nations have a higher prevalence of S allele carriers (r (29) =. 7, p <.0001). If these polymorphisms were “vulnerable genes” for psychiatric disorders, one

would expect that populations with a higher frequency of the short allele, such as Asia, could present higher prevalence of depression or psychiatric disorders. It can be hypothesized, that because S carriers live in a more collectivist culture, i.e., giving more emphasis on interpersonal relationships than self-determination, these genes are influenced by this greater closeness in interpersonal relationships, which moderates the development of depression. Thus, one would expect subjects with these alleles, that live in individualistic nations, be at increased risk for depression, so that the prevalence of depression would be greater, for example, in Northern European countries. Emphasizing social norms that increase social harmony and foster social support, collectivism works as "anti-psychopathology" by creating an ecological niche that reduces the prevalence of chronic stress, and protects genetically susceptible individuals from environmental effects known to trigger negative emotions and psychopathology.

Genes and environment relations

Every human being is unique, despite sharing over 99% of genetic material with the rest of the human species ("The International HapMap Project," 2003; Rosenberg et al., 2002; Venter et al., 2001). Recent theoretical models stress the fact that a person's relationship with his environment from the moment of conception can be assumed to play a crucial role in this uniqueness (Christine Heim & Nemeroff, 2001; Nemeroff, 1998a, 1998b). The answer of what makes us distinctively different from other human beings may lie in the continuous reciprocal interaction between the environment and our biology. Such gene–environment relations are thought to result from both gene–environment correlations (rGE) and gene–environment interactions (GxE). Research on rGE explores the role of genes in the exposure to environmental factors (Kenneth S. Kendler & Eaves, 1986; Plomin, DeFries, McClearn, & Rutter, 1997). rGE refers to the tendency of individuals to select and generate their environment based on genetic features that influence behavior, thoughts, and feelings. Three types of rGE have been described in the literature: (a) passive, (b) reactive, provocative or evocative, and (c) active or selective (Jaffee & Price, 2008). (a) Passive rGE refers to the situation in which children inherit from their parents not only a genetic constitution, but also the environment in which they are raised (Plomin et al., 1997) (e.g., they inherit intellectual curiosity). The association between genetically related individuals is a requirement for passive rGE. (b) Evocative, provocative or reactive rGE refers to the tendency of certain genetically influenced behaviors or temperamental features to elicit certain types of

responses from people within their environment, (e.g., a child with a difficult temperament is more likely to elicit negative parenting behaviors). (c) Active or selective rGE refers to the active generation of certain environments based on genetic tendencies. This refers to the association between genetic features of the individual and the environmental niches that the individual selects or generates (e.g., a child with intellectual curiosity will tend to find intellectually rich environments while a child with behavioral disorder will seek peers with similar behaviors; that is, people who are more extroverted may seek very different social environments from those who are shy and withdrawn) (Plomin et al., 1997).

GxE, on the other hand, explain why people respond differently to environmental factors (e.g., why certain individuals are more prone to depression after being exposed to negative life events) (K. S. Kendler et al., 1995). Until relatively recently, GxE were thought to be rare in psychiatry, but research over the past decades has shifted toward a focus on GxE (Moffitt, Caspi, & Rutter, 2005; Rutter, 2010).

Whereas gene/environment correlation (rGE) refer to genetic exposure to the environment, gene/environment interaction (GxE) refer to the genetic sensitivity to the environment (Plomin et al., 1997). Once individuals are exposed to a environment, how sensitive are they to the potential environmental influences to develop psychiatric disorders? GxE interaction are implicit in the stress diathesis model and in differential sensitivity model.

rGE and GxE are not mutually exclusive. A polymorphism may correlate with some traits that generate changes in the environment. An example of such a mediational model is the finding that the short allele of the promoter region linked to the serotonin transporter gene (5HTTLPR) has been shown to correlate with neuroticism (Greenberg et al., 2000; Sen, Burmeister, & Ghosh, 2004), which in turn has been shown to be related to a tendency to have a negative interpretation bias related to life events (John & Gross, 2004). Moderator models in this context imply that there is an interaction with environmental factors. For example, studies suggest that 5HTTLPR may interact with negative life events in the prediction of depression (Avshalom Caspi et al., 2003), but also with social support, leading to lower levels of depression (Bakermans-Kranenburg & van Ijzendoorn, 2011; Kaufman et al., 2004; Kim et al., 2014). Figure 6 shows a diagram of the models of mediation and moderation.

Figure 6 Approaches to research in genetics of psychiatry

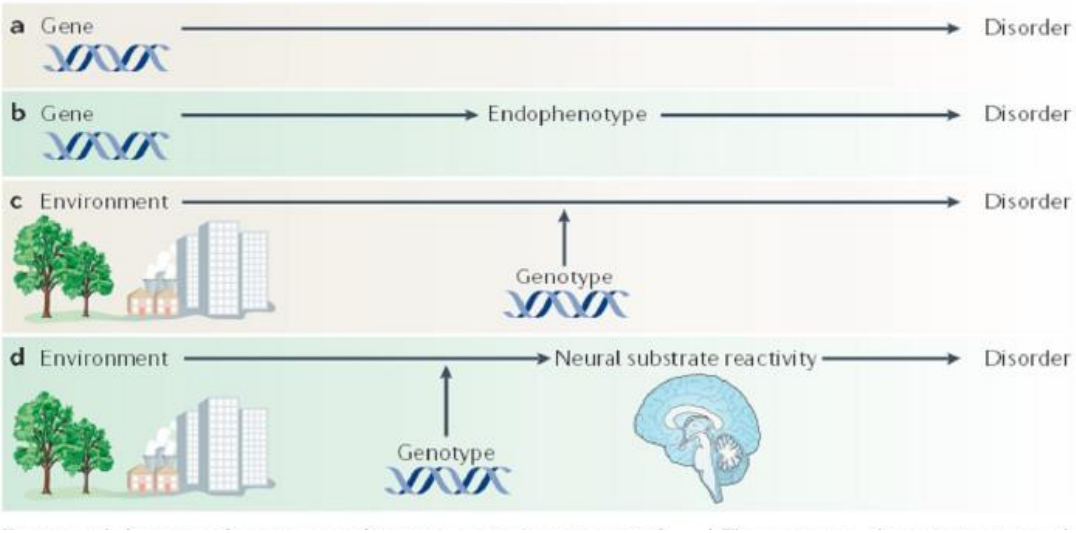


Figure 6 Note: a & b account for rGE models; c & d account for GxE models. Obtained from Caspi, A., & Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci, 7*(7), 583-590. doi: 10.1038/nrn1925

Evidence for Gene/Environment Interaction

There is now increasing consensus that most common psychiatric disorders, such as depression and anxiety, are best explained as complex disorders involving dysfunctions in several biological systems in interaction with environmental factors. One of the earliest studies of GxE was reported by Kendler and colleagues (K. S. Kendler et al., 1995). This study overthrew the concept of reactive or endogenous depression, because those individuals with a greater genetic risk for depression were shown to be also more reactive to negative environmental events.

Figure 7 Risk of major depression per person-month based on genetic risk and presence or absence of severe stressful events between 2060 female twins

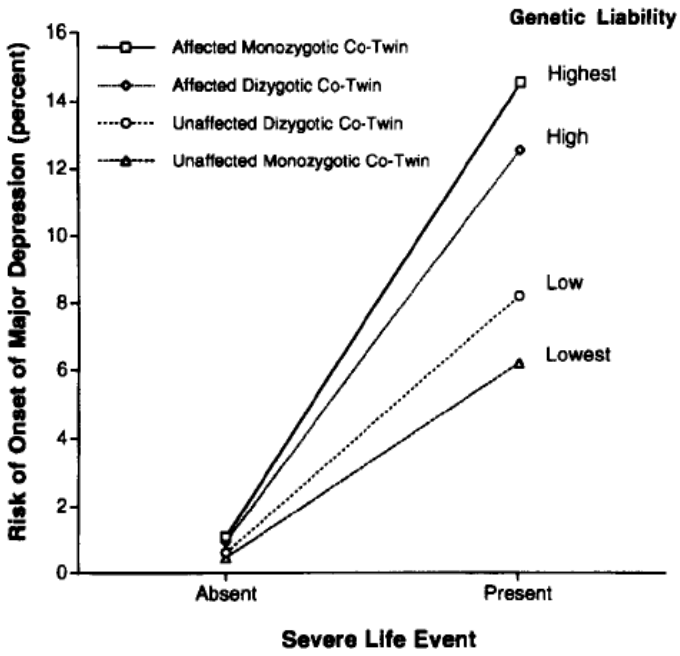


Figure 7 Obtained from Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., & Eaves, L. J. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *The American Journal of Psychiatry, 152*(6), 833-842.

In 2003, Caspi and colleagues (Avshalom Caspi et al., 2003) published a ground-breaking study which reported that carrying the short allele of the 5HTTLPR gene interacted with both early and recent negative events to predict depression. They began testing interactions between specific genes and life stress measurements. The focus on the serotonin transporter gene (5HTT) comes from animal and human studies that supported the hypothesis that this gene interacts with environment to model the stress response. The authors followed a representative sample of 953 individuals from Dunedin, New Zealand, since their birth in 1975, and evaluated them at 3, 5, 7, 9, 11, 13, 15, 18, 21, and for this study, at 26 years (Caspi, 2003). Data on child maltreatment was obtained by observation during childhood, parental report, and retrospective report as adults. Stressful life events were evaluated retrospectively from 21 to 26 years. These included labor, financial, health and relationship problems. Episodes of major depression, suicide ideation and attempt in the previous 12 months, were assessed at the age of 18, 21, and 26. A sample of DNA from saliva or blood was obtained from each participant. 3 groups of genotypes were formed according to 5HTTLPR polymorphism:

homozygous for the short allele (SS), heterozygous (SL) and homozygous for the long allele (LL). Using major depression as an outcome, the authors tested the effect of genotype, stressful life events, and their interaction for the risk of developing depression. The results revealed that individuals with at least one short allele were more strongly influenced by stressful life events to develop depression when compared with individuals homozygous for the long allele. They also were more likely to present suicidal ideation and suicide attempt. The authors conclude that child maltreatment predicts depression in individuals with a short allele, but not in individuals with two long alleles. 10% of the sample consisted of individuals with SS or SL genotype, with 4 or more recent stressful events, but were 23% of those diagnosed with depression. Based on these findings, Caspi speculates "that some multifactorial disorders, rather than result from small effects of many genes, can be produced by the variation of a few genes whose effects are conditioned by exposure to environmental risks." These findings are consistent with the biopsychosocial model, diathesis to stress, and vulnerable phenotype model.

Figure 8 Regression analysis between the history of early trauma and likelihood of adult depression according 5HTTLP

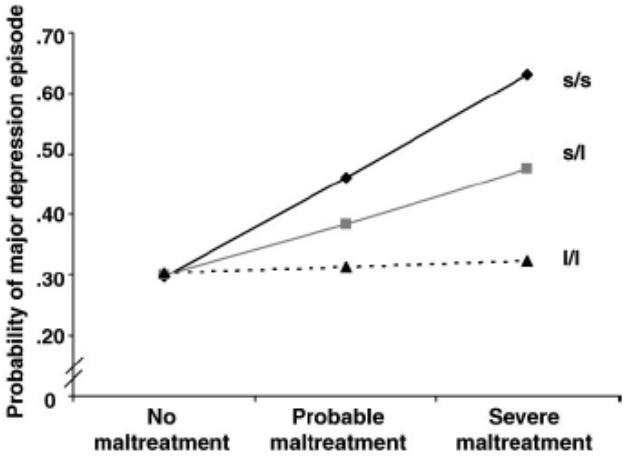


Figure 8 Obtained from Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., Poulton, R. (2003). Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. *Science, 301*(5631), 386.

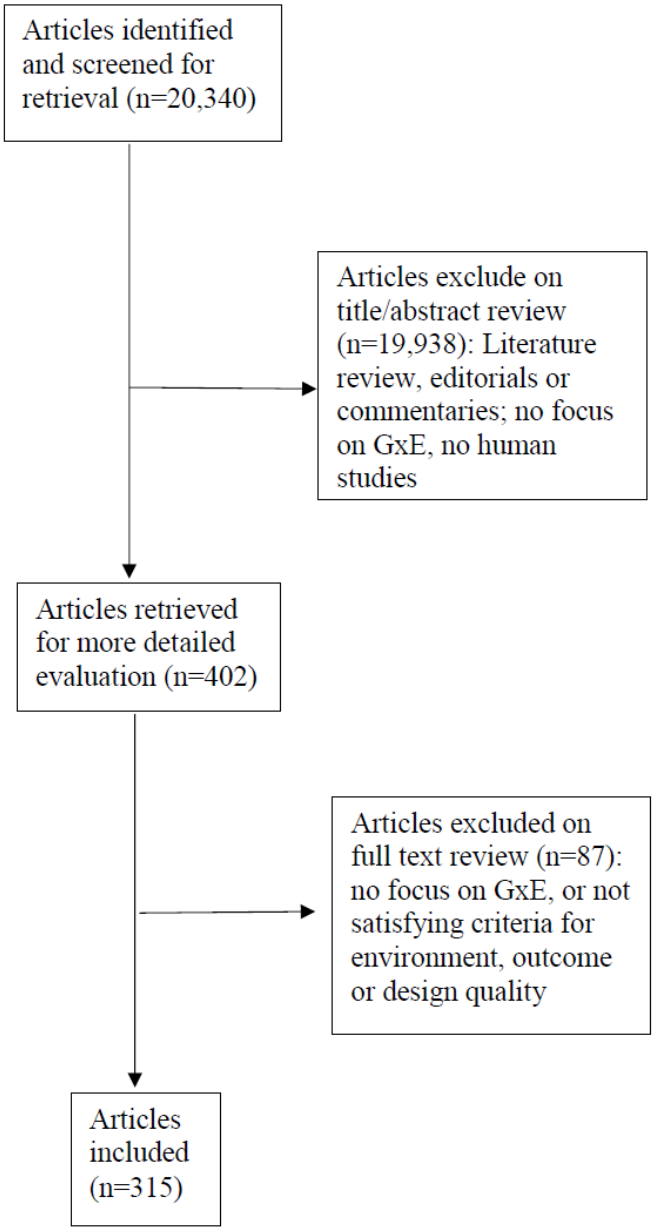
Caspi study findings have been replicated in multiple studies; (Bozina, Mihaljevic-Peles, Sagud, Jakovljevic, & Sertic, 2006; Eley et al., 2004; Frodl et al.,

2004; Gonda, Juhasz, Laszik, Rihmer, & Bagdy, 2005; Gonda et al., 2006; Hariri et al., 2005; Hoefgen et al., 2005; K. S. Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Lenze et al., 2005; Lotrich & Pollock, 2004; Mandelli et al., 2007; Munafo, Clark, Roberts, & Johnstone, 2006; Wilhelm et al., 2006; Willeit et al., 2003). Yet, findings have not always been consistent. Two meta-analyses, for instance, failed to corroborate an interaction between the 5HTTLPR gene and stressful life events in predicting depression (Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009). By contrast, a meta-analysis by Uher and McGuffin (Uher & McGuffin, 2010) did find evidence for an interaction between the 5HTTLPR gene and adversity in predicting depression. Differences between these studies' conclusions may be due to differences in their methodology and inclusion criteria. But it is clear that there still is controversy regarding the role of GxE and rGE in psychiatric disorders (Bakermans-Kranenburg & van Ijzendoorn, 2011, 2014; Dick et al., 2015; Fraley, Roisman, Booth-LaForce, Owen, & Holland, 2013; Kim-Cohen et al., 2006).

Systematic review of GxE

We conducted a systematic review with the aim to critically review the research on GxE with the aim of fostering research in this area. Specifically, we provide a systematic qualitative review of research on all genes that have been investigated in GxE research, focusing on five areas: (a) the candidate genes studied; (b) the phenotype or effect studied for each gene; (c) the type of environment investigated; (d) the samples investigated in terms of age group and geographical regions where the studies took place; and (e) methodological considerations. Based on this review, we also formulate a number of recommendations for future research (see conclusions). We present a summary of the most interesting findings of the review that relate to the thesis project. Figure 9 shows the flowchart of search and selection of articles included in the review.

Figure 9 Flowchart of search and selection of articles



Candidate genes studied in gene-environment interaction, since the seminal publication of Caspi in 2002 (A. Caspi et al., 2002), are summarized in Table 1. In total, we identified polymorphisms of 34 different genes that have been studied in GxE research (see Table 1) in 315 papers using 160 original samples (see below).

The most investigated gene is 5HTTLPR, with about half (51.4%, 162 articles) of the total number of studies on GxE focusing on this polymorphism.

Table 1

Type and number of genes included on GxE studies

Gene	Name	N° of Articles
SLC6A4 (5HTTLPR)	Serotonin transporter	162
BDNF	Brain-derived neurotrophic factor	44
DRD4	Dopamine receptor	36
MAOA	Monoamine oxidase A	36
OXTR	Oxytocin receptor	19
COMT	catechol-O-methyltransferase	17
5HTR (1A/1B/2A/2C/3A)	Serotonin receptors	15
DRD2	Dopamine receptor	13
FKBP5	FK506 binding protein 5	10
CRHR1	Corticotropin-releasing hormone receptor 1	9
SLC6A3(DAT1)	Dopamine transporter	6
TPH1/TPH2	Tryptophan hydroxylase	5
NR3C1 (GR)	Glucocorticoid receptor	4
NR3C2 (MR)	Mineralocorticoid receptor	4
OPRM1	μ1 Opioid receptor	3
GABRA2/ GABRG1	γ1 and α2 subunits of GABA-A receptor	3
RGS2	Regulator of G-protein signaling 2	3
CHRM2	Cholinergic muscarine 2 receptor	2
ANKK1	Ankyrin repeat and kinase domain containing 1	2
PER1/ PER2	Period circadian clock 1 and 2	2
OXT	Oxytocin	2
NPY	Neuropeptide Y	1
ACE	Angiotensin 1 converting enzyme	1
GRIN2B	Glutamate receptor, ionotropic, NMDA 2B	1
NPSR1	Neuropeptide S receptor	1
CACNA1C	Calcium channel, voltage-dependent, L type, α 1C subunit	1
CREB1	cAMP responsive element binding protein 1	1
FOXP2	Forkhead box protein 2	1
GALR1/ GALR2/ GALR3	Galanin receptors	1
MAOB	Monoamine oxidase B	1
SLC6A2 (NET)	Norepinephrine transporter	1
NOS1	Nitric oxide synthase 1 (neuronal)	1
ODC1	Ornithine decarboxylase 1	1
DRD1/DRD3/DRD5	Dopamine receptor	1

Over the phenotype or the results included on GxE studies, almost half of the studies focused on different types of psychopathology (n=150/315 studies, 46.8% of the total number of papers). Depression has been by far the most studied pathology (n=102/315 papers, 32.3%), with studies focusing mainly on 5HTTLPR (n=79/102, 77.5%), BDNF (n=20/102, 19.6%), and the remainder investigating 5HTR (1A/1B/2A/2C/3A) (n=7/102, 6.9%), CRHR1 (n=7/102, 6.9%), MAOA (n=6/102, 5.9%) and OXTR (n=6/102 papers, 5.9%). These genes have been mostly studied in interaction with early stressful events or chronic stress to predict depression and (less frequently) anxiety.

Other phenotypes or results that have been of interest in studies of GxE are: social behavior (n=86/315, 21.3% of total papers) has been primarily studied in interaction with genes related to the dopaminergic system (DRD4, DRD2, MAOA, DAT1). These genes have been mostly studied in interaction with parenting to predict behaviors such as criminal activity, alcohol use and behavioral problems in adolescents. Studies on the neurobiological mechanisms (studies that include as an outcome intermediate pathways that could be involved in the GxE mechanism i.e. changes in cortisol levels or changes on methylation rates) involved in GxE have been relatively scarce, at least in humans (n=39/315, 12.2% of the total number of papers). Genes related to the glucocorticoid system have focused the most on neurobiological outcomes, (e.g. FKBP5, GR and CRHR1) and only a small proportion of articles on 5HTTLPR (n=18/162) have focused on the neurobiological outcomes of GxE.

Among the kinds of environmental factors that have been studied, early and negative environments such as poor parenting and childhood trauma have been the most frequent focus of research. In total, 70.8% (n=223/315) of the articles included early life events (ELE). Recent life events (RLE) such as psychosocial interventions, experimentally induced stress or recent important experiences have been studied less often (n=113/315, 35.9% of articles). Over the type of environment, negative or positive, negative environments were the most studied, according to vulnerability to stress model. 95.9% (n=302 articles) of the 315 articles included a negative environment, only 22.2% (n=70/315 articles) focused on interactions with positive events. Interestingly studies with early environments found evidence for GxE in 77% (172/223) of the studies; for recent environments, 73.5% (83/113) of the studies; for negative environments in 78.1% (n = 236/302) of the studies; and for studies of

interaction with positive environments found evidence for GxE in 81% (n = 57/70) of the studies. In general, independent of the type of environment studied (early or recent life events, positive or negative environment) the proportion of papers that showed evidence for GxE was the same, χ^2 (3, n= 708) = 1.76, ns.

Focusing on 5HTTLPR 62.3% (n=101/162 studies) of the studies included ELE while 45.7% (n=74 studies) included RLE. Negative events were focused upon in 96.9% (n=157/162) of the studies, and positive events were the focus in 17.9% (n=29/162) studies. There were no significant differences in type of environment (positive, negative, ELE or RLE), and evidence of GxE in 5HTTLPR studies, χ^2(3, n= 362) = 0.09 ns.

Examining the type of samples included in the studies the vast majority of studies (almost 90 %, n=283/315) were conducted in North America or Europe. There were no differences between the country, continent or ethnicity from which the sample came and evidence of GxE (χ2(9, n=322) = 15.89, ns; χ2(5, n=304) = 10.00, ns, and χ2(3, n=237) = 3.94, ns, respectively.).

The overlap of samples used in different research papers was very high. Of the 315 articles included in this review, only 96 used samples that did not overlap with samples reported on in other papers. Hence, 69.5% (n=219) of the papers used samples that were also used in other GxE studies. From the 219 overlapping papers, original samples reduced to 64. So there were only 160 original samples studied for GxE, mainly from North America and Europe. When taking into account overlap of samples in papers from different countries, the original proportion of 90% of the samples (articles) coming from the US or Europe diminished to 84.3%. Figure x (map) shows the world distribution of the samples and original samples (not overlapped) used to study GxE.

Figure 10 shows the world distribution of the samples and original samples (not overlapped) used to study GxE. Studies including 5HTTPLR polymorphism show the greatest overlap of samples (162 papers, using 102 samples).

Figure 10 World distribution of GxE studies

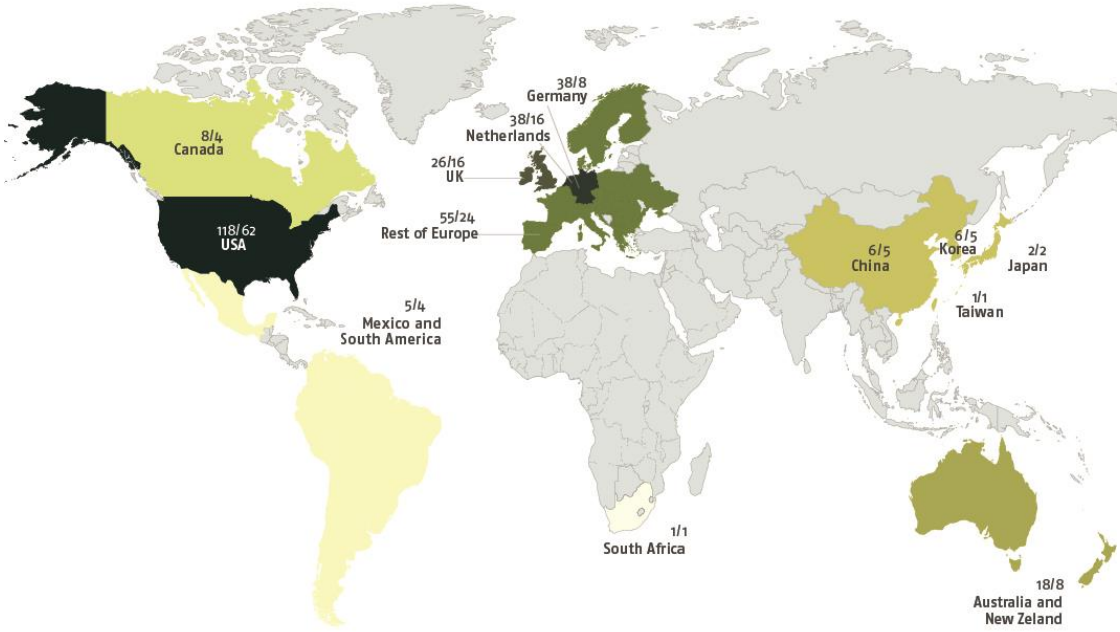


Figure 10 Note: The first number refers to the number of articles per country and the second is the number of original samples (non-overlapping). The frequency of articles is shown in gradient from darker (higher frequency) to lighter (less frequent).

When considering the age of the samples included on GxE studies, regarding the 5HTTLPR gene, 61.5% (n=112/182) of articles included adult samples, while 35.7% (n=65/182) included children and adolescents. In total, 81.1% (n=116/143) of GxE papers using child and adolescent samples found positive results; in young adults the proportion was 77.2% (n=159/206), while 62.5% (n=5/8) of GxE studies using samples of adults or older adults reported positive findings, $\chi^2(2, n=357) = 2.00$, ns.

Most of the articles showed positive results for GxE, with positive findings ranging from 63.8% for MAOA studies to 83.3% of studies including DAT1 and CRHR1, (mean 72.9% of all articles included). The quality of the studies is an important consideration in trying to eliminate false positives in GxE studies. One

indication of the quality of studies is the nature of the design. Only 11.4% (n=36/315) of the articles included in this review were experimental in nature; 39.4% (n=124/315) were cross-sectional studies, rendering interpretation of causality difficult. Somewhat more encouraging is that 48.9% (n=154/315) of the identified papers were longitudinal in nature. Furthermore, it is also encouraging that there are a growing number of longitudinal prospective studies and a decreasing focus on cross-sectional studies, although this latter trend was not significant (z score=-0.64, ns). In general, 5HTTLPR studies are more cross sectional and OXT and DOPA studies are more longitudinal; this is congruent with the assumption that the latter genes are implicated in parenting and may play a crucial role in determining developmental pathways related to attachment and behavioral problems

Another criterion that we analyzed was whether studies met the minimum quality criteria in their reporting of the assessment of polymorphisms. Current guidelines (Hewitt, 2012; Johnston C., 2013; Mayo, 2008; Stark & Seneta, 2013; Sullivan, 2007) suggest that the genotyping success rate should be 95% or higher, and that the study should report the HWE, linkage equilibrium or deviations of HWE. Of the 315 articles included in this review, 54 (17.1%) did not report HWE. Most of these studies were earlier studies. Further, there was no association between studies meeting these quality criteria and positive findings concerning GxE, with 77.3% of studies that reported HWE reporting evidence for GxE, and 79.6% of the studies that did not report HWE reporting evidence for GxE, $\chi^2(1, n=315) = 0.18$, ns. Auspiciousness is that the trend is that these studies were decreasing over time ($z = -4.84$, $p > .00$).

Implications of the Social Sensitivity Model

The importance of explaining the pathogenesis of psychopathology by a different model, is that prevention, diagnosis, and treatment of disorders changes. The environmental sensitivity model assumes that if a subject carrying prosocial alleles experiences a negative early environment (insecure attachment and childhood trauma or maltreatment) and subsequently is exposed to environmental stressors (recent past 12 months), and has less social support, he has a greater chance of developing psychopathology that an individual not carrying prosocial alleles. Conversely, if the subject carrying socially sensitive genes is exposed to a positive early environment (secure attachment and no child trauma) and subsequently experiences a positive environment (positive events the last 12 months and greater social support), he has a

lower risk of psychopathology than individuals not carrying these genes. This allows us to hypothesize that patients carrying prosocial alleles will respond better to a treatment involving social interaction and learning, such as psychotherapy. Consistently depressive short allele carriers respond less well to pharmacotherapy than patients carrying the long allele.

Brody (Brody, Beach, Philibert, Chen, & Murry, 2009) evaluated the effects of a family intervention designed to reduce risk behaviors among rural black children at high risk for developing risk behaviors. The intervention of several sessions held at a community center aimed to promote positive parenting practices and increase the propensity of children to follow family rules and set goals for the future. The resulted showed that those classified at "genetic risk" for being short allele carriers were the ones who benefited most from the program. These participants developed substantially less risky activities such as alcohol consumption, drug use, early sexual activity, that individuals genotypically similar but did not participate in the intervention and long allele homozygotes.

Depression and hypothalamic pituitary adrenal axis

The hypothalamic-pituitary-adrenal axis (HHA), is the center of the stress and immune response in mammals (Claes & Nemeroff, 2005). The hypothalamus synthesizes and releases corticotropin-releasing factor (CRF) in response to stress. CRF induces adrenocorticotropin hormone release (ACTH) from the pituitary. And it, stimulates the production of cortisol in the cortex of the adrenal gland (Gutman & Nemeroff, 2003). Axis functioning is assessed by measuring levels of stress hormones: CRF, ACTH, and cortisol. Measuring stress hormones provides an objective way to compare the stress response of individuals with different phenotypes.

There is evidence that the hyperactivity of the HPA axis is a common neurobiological phenomenon in depressed patients. And that this axis hyperactivity is produced by a hypersecretion of CRF. In response to stress, CRF secretion increases, not only in the hypothalamus but also in the central nucleus of the amygdala. Seconds after exposure to stress, CRF secretion rises, resulting in increased cortisol secretion. This response has adaptive acute effects to cope with stress, but if activation of the axis is chronic, it is associated with adverse effects.

Along with CRF, cortisol inhibits growth hormone and the reproductive axis. It also decreases the cellular immune system. Cortisol increases the available energy,

promoting gluconeogenesis, proteolysis, and glycolysis, and increasing insulin resistance. Noradrenergic system activation by CRF in the locus coeruleus increases blood pressure, heart rate, and blood glucose, and decreases the gastrointestinal blood flow. All these adaptations allow the body to respond appropriately to environmental stressors and threats. This is the reaction of escape/attack, which is crucial for the body to properly respond to acute threats, increasing the chances of survival.

In depressed patients, numerous studies since the 60s have shown hypersecretion of cortisol. Hypercortisolemia is considered a marker of status and not a trait, because it tends to normalized in most patients after clinical improvement. Increased cortisol secretion has been related to hypersecretion of CRF. When CRF secretion is increased in the brain, down regulation of CRF receptors is expected. In addition to these findings, an altered sensitivity to endocrine provocateurs test (Challenger test) has been found. Intravenous administration of CRF causes increased ACTH in normal subjects, but in depressed patients the answer is flattened (C. Heim & C. B. Nemeroff, 2001). This is in part secondary to down-regulating of CRF receptors of the anterior pituitary in response to hypothalamic primary hypersecretion of CRF in depressed patients (Heim & Nemeroff, 1999). This would constitute the primary cause of dysfunction in the HPA axis of depressed patients. Another test used to evaluate the functioning of the axis, is the suppression of cortisol and ACTH with Dexamethasone (DEX). In healthy subjects, the secretion of cortisol and ACTH decreases after the intake of DEX. This test has a modest sensitivity in depression (40-50%), but increases in severe, psychotic, with melancholic symptoms depression, mania or schizoaffective disorder (Mello et al., 2007). For its inespecificity for psychiatric disorders, it is a poor diagnostic test, but it has been proposed as a predictive test, because if the suppression of cortisol is not normalized despite the apparent improvement of symptoms of depression, there is a high risk of depressive relapse or suicidal behavior (Ribeiro, Tandon, Grunhaus, & Greden, 1993). One way to increase the sensitivity (80%) of this test is combining it with the DEX-CRF Challenger.

In summary, studies show HPA axis dysfunction with higher concentrations of cortisol in depressed patients, caused in part by a CRF hypersecretion that is not properly suppressed by any feedback system. These neurobiological changes are associated with depressive episodes, but what is interesting is to understand if they are concomitant, consequences or caused by depression. Most data show a trend toward improvement of axis hyperactivity after clinical improvement, which would support the

idea that the changes are concomitant or consequence of depression. But, in a subgroup of patients, the performance of the axis does not normalize after clinical remission (Zobel et al., 2000). The most probable explanation is that some patients have a chronic tendency to hyperactivity of HPA axis, which can be attributed to genetic factors or early experiences of abuse. This dysfunction is aggravated during the depressive episode, and returns to baseline, but not necessarily to normal, after clinical remission.

In chronic activation (Makino et al., 1999), the negative feedback system of glucocorticoids is less effective, possibly due to down regulation of glucocorticoid receptors (GR). Moreover, GR up regulate CRF secretion in the amygdala and increase expression of CRF receptors in the nucleus *paraventricular* (Rivest, Laflamme, & Nappi, 1995). This may explain why in some cases, chronic stress does not lead to a down regulation of the HPA axis, by the inhibitory effect of cortisol, but maintain a hypersecretion of CRF, which contributes to depression.

Christine Heim et.al. (C. Heim et al., 2000) compared ACTH levels among women with history of sexual or physical severe abuse in childhood with women without such a history. For this, she used 4 groups: 1) women with no history of abuse or psychiatric disorder (control), 2) women with current depression who experienced abuse, 3) women without current depression who experienced abuse and 4) women with current depression without history of abuse. Women with history of abuse presented higher ACTH levels than controls. Women with abuse and depression had the highest levels of all groups. These findings provide biological evidence that early environmental adversity may have measurable effect on stress response and by extension, vulnerability to develop psychiatric disorders.

Specific stressors in early life can cause structural changes in the limbic system (hippocampus and other structures) and permanently deregulate the stress response system. It is probable that durable impaired functioning of the HPA axis induced by early trauma depend on various factors, such as critical window of newborns, the nature of the stressor, the presence or absence of support, and genetics. Nemeroff refers to the importance to elucidate these factors.

5HTTPLR Polymorphism and HPA axis

The HPA axis is the centre of immunological and stress response in mammals. As we reviewed, the interaction between stressful life events and 5HTTLPR polymorphism is associated with depression. In response to stress the HPA axis is activated. Studies

show that depression axis activity is increased. Therefore, a potential mechanism by which 5HTTLPR polymorphism may increase the risk of depression is by its impact on the HPA axis.

There is evidence that the 5HTT gene moderates the relationship between life stress and depression, but the mechanism underlying this moderation is still unclear. Some animal studies suggest that a possible mechanism is the construct of stress reactivity. Li (Li et al., 1999) found that rats with a less operative 5HTT gene showed major increases of ACTH in response to stress than controls. Results from a recent meta-analysis suggest that depressed patients have higher cortisol levels after exposure to a stressor compared to not depressed individuals (Burke, Davis, Otte, & Mohr, 2005).

Gotlib (Gotlib, Joormann, Minor, & Hallmayer, 2008) studied 67 healthy girls, 9 to 14 years old. He noted that girls homozygous for the S allele produced and maintained higher levels of cortisol in response to the stressor, compared to girls carrying the long allele.

Figure 11 Daily cortisol curve according to 5HTTLPR

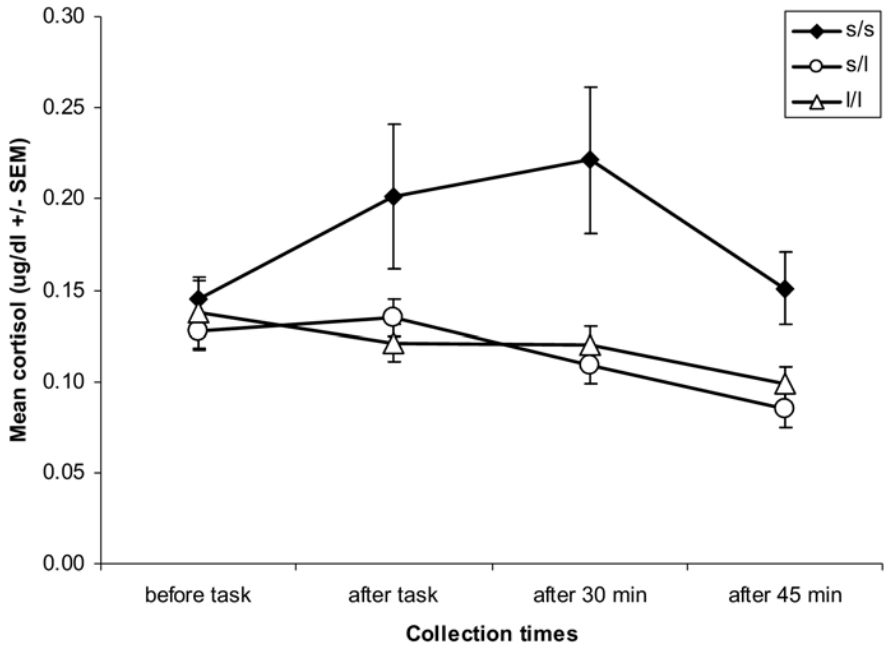


Figure 11 Obtained from: Gotlib, I. H., Joormann, J., Minor, K. L., & Hallmayer, J. (2008). HPA axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry, 63*(9), 847-851. doi: 10.1016/j.biopsych.2007.10.008

Chen (Chen, Joormann, Hallmayer, & Gotlib, 2009) studied the daily curve of cortisol, and observed that girls homozygous for the short allele had higher levels of

cortisol at awakening, but not in the afternoon or at night, compared with long allele carriers. Moreover, Wankerl (Wankerl et al., 2010) studied the daily curve of cortisol (8:00, 12:00, 16:00 and 22:00 hrs) in a group of 130 healthy adults (66 men and 64 women) equally distributed according to the expression of the 5HTTPLR polymorphism. He observed an interaction between sex and polymorphism, SS men, had higher cortisol levels than in other groups.

By contrast, Wust (Wust et al., 2009) studied 216 healthy subjects with Trier Social Stress Test (TSST), and recorded the cortisol awakening curve (CAR) at 30, 45 and 60 minutes immediately after awakening, and a week later exposed them to dexamethasone suppression test and measured ACTH. The levels of cortisol and ACTH in response to stress did not differ between 5HTTLPR gene groups, but he observed a significant association specific by sex, between cortisol at awakening response (CAR) and the presence of the short allele. The SS genotype is associated with a higher CAR in women and with a lower CAR in men. Authors postulate that these sex-specific differences may contribute to gender differences in vulnerability for depression.

Figure 12 Cortisol awakening curve according to 5HTTLPR polymorphism

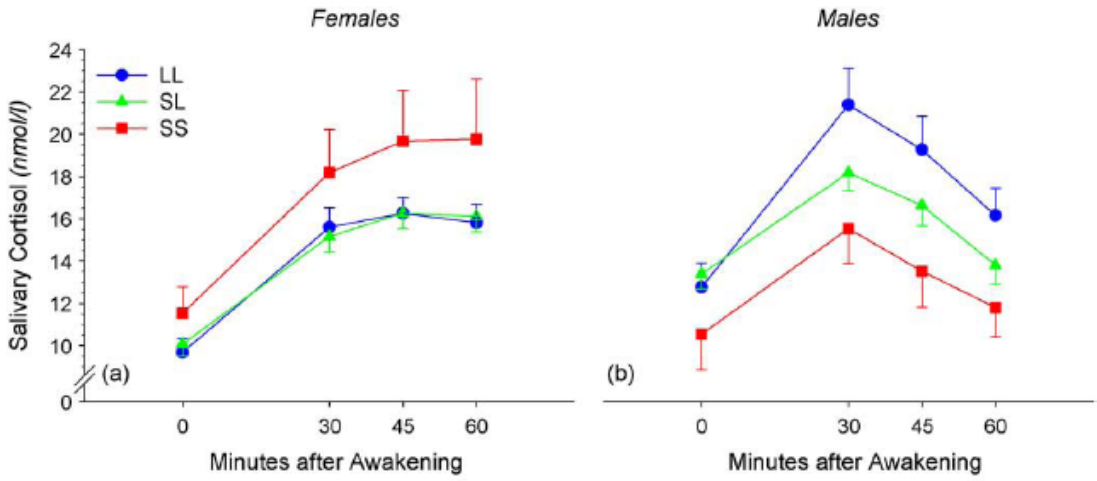


Figure 12 Obtained from: Wust, S., Kumsta, R., Treutlein, J., Frank, J., Entringer, S., Schulze, T. G., & Rietschel, M. (2009). Sex-specific association between the 5-HTT gene-linked polymorphic region and basal cortisol secretion. *Psychoneuroendocrinology, 34*(7), 972-982. doi: 10.1016/j.psyneuen.2009.01.011

Alexander (Alexander et al., 2009) studied 100 healthy men, asked them about stressful events and then exposed them to a stress generator experiment and recorded cortisol levels before, during, and after it. He noted that the short allele carriers with a

history of significant stressful events, showed higher levels of cortisol in response to the stressor, compared with the other groups. Indicating significant GxE interaction in endocrine stress reactivity. No main effect of genotype or life events was observed.

Figure 13 Salivary cortisol response to stressful task in function of 5HTTPLR genotype and stressful life events

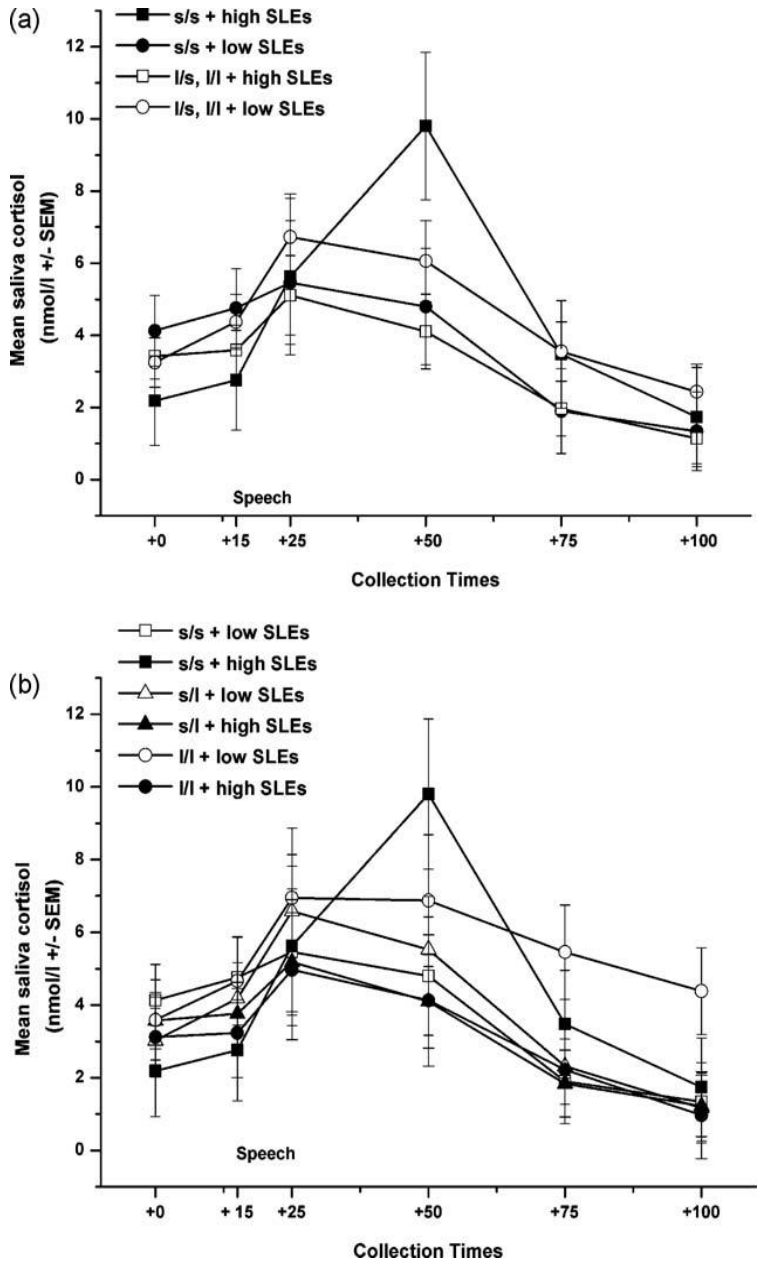


Figure 13 Obtained from: Alexander, N., Kuepper, Y., Schmitz, A., Osinsky, R., Kozyra, E., & Hennig, J. (2009). Gene-environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression. *Psychoneuroendocrinology*, *34*(9), 1294-1303. doi: 10.1016/j.psyneuen.2009.03.017

Another study (Mueller et al., 2011), considering that the GxE interaction may be a function of the age (ie, that this interaction is present in young adult subjects but not in children or elderly), proposed to study the interaction of 5HTTLPR genotype and stressful life events on stress response in subjects of different age groups. A total of 115 children (8-12 years old), 106 young adults (18-31 years old), and 99 other adults (54-68 years old) underwent TSST and structured interviews about stressful life events. Authors observed in both groups of adults, an interaction between the genotype homozygous for the long allele and significantly higher cortisol response to TSST than in individuals with a short allele. Predictably, an interaction between stressful life events and genotype was found, which was only observed in the group of young adults and only when the stressful event had occurred during the first 5 years of life, suggesting that the age and type specific stressful event is important when studying GxE

Figure 14 Cortisol level according to polymorphism and stressful events in the first 5 years of life

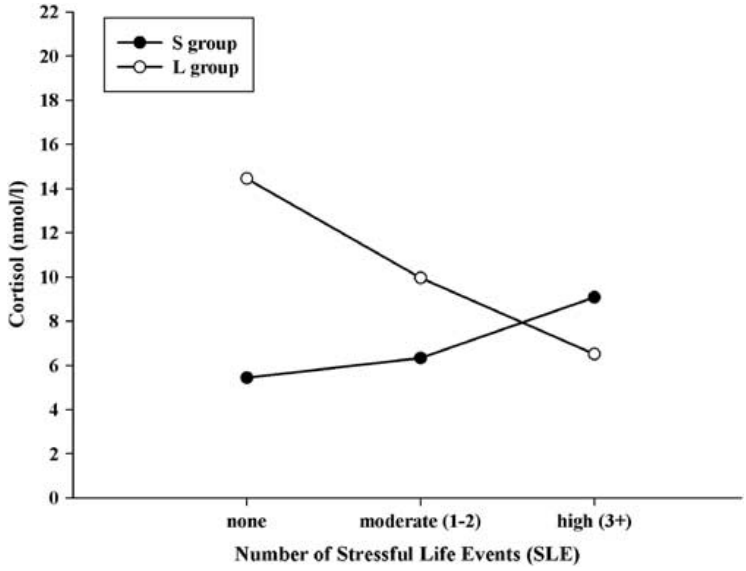


Figure 14 Obtained from: Mueller, A., Armbruster, D., Moser, D. A., Canli, T., Lesch, K. P., Brocke, B., & Kirschbaum, C. (2011). Interaction of serotonin transporter gene-linked polymorphic region and stressful life events predicts cortisol stress response. *Neuropsychopharmacology, 36*(7), 1332-1339.

Interactions between 5HTTPLR genotype and stressors may partly explain why studies on cortisol levels and reactivity that do not consider the history of stressors, present inconsistent findings. For example, Jabbi (Jabbi et al., 2007) and Gotlib (Gotlib

et al., 2008) found higher cortisol response to stressors in S allele carriers. Mueller (Mueller, Brocke, Fries, Lesch, & Kirschbaum, 2010) recently reported that infants homozygous for S allele exhibited higher levels of cortisol in response to a physical stressor. In contrast, Alexander (Alexander et al., 2009) and Wust (Wust et al., 2009) found no differences in cortisol reactivity between 5HTTPLR groups after a stressful task. Contrary to these studies, Mueller (Mueller et al., 2011) reported that the L allele homozygotes exhibit greater cortisol response to a stressor.

However, this reactivity should be analyzed in the context of early stressful life events. Thus, early events and life events are confounding variables that needs to be included in future analyzes of cortisol reactivity depending on the genotype of 5HTTLPR.

In summary, there is considerable scientific evidence to assume that the polymorphism of the SCL6A4 gene is a plastic gene, ie, that is moldable by environmental experiences and therefore confers greater sensitivity to both positive and negative experiences, not only genetic variation that confers increased vulnerability to psychopathology by interacting with negative environmental factors.

Bloss (Bloss, Jeste, & Schork, 2011), in his review Genomics in Psychiatry, refers to this point by suggesting that "the 5-HTTLPR, together with polymorphisms in other genes such as BDNF and CRHR1, are more broadly associated with personal dispositions that are more or less sensitive to environmental surroundings. In terms of treatment for depression, psychological therapy, and antidepressant medications have, on average, comparable efficacy in unselected groups of patients diagnosed with depression. Of importance, however, the potential gene-environment interaction involving 5-HTTLPR suggests that individuals who are more sensitive to environmental stimuli may respond better to psychological treatments than to antidepressant medication. Although this particular gene-environment interaction has been called into question in recent years, these findings illustrate the potential importance of further study of gene-environment interactions in other contexts, as well as the potential implications of such findings for disease treatment and prevention in psychiatry" (Bloss, 2011, p. 155). That is, there is evidence to suggest that certain genes confer different sensitivity to environment and this could explain why some individuals exposed to negative events have negative results, but if the same individuals are exposed to positive events have positive results. Also, that this plasticity would be more marked when environmental events are social in nature. One would expect that the effect of

psychotherapy, as a social and positive interaction, would influence more subjects that are carriers of the short allele.

Moreover, we have found that there are scarce studies that have included neurobiological mechanisms in the study of gene-environment interaction. In addition, most research (except Mueller, 2011) has been designed under the model of diathesis to stress, so it has focused on the interaction of adverse environments with a genotype in predicting depressive symptoms and how this interaction influences the response to stress neurobiologically understood as change in the level of cortisol. Therefore, examining whether cortisol levels are modified, is important to advance in the understanding of the pathophysiology of mood disorders, but if we can also understand how this relationship is in individuals including the exposure to positive experiences, it could be useful for depressed patients, especially for those thought of as the worst prognosis as the most severely depressed women with a history of adverse events and short allele carriers.

This project is a proposal for basic and applied research. Its aim is to determine whether the 5HTTLPR polymorphism interacts with environmental factors and personality under the model of differentiated sensitivity to the environment.

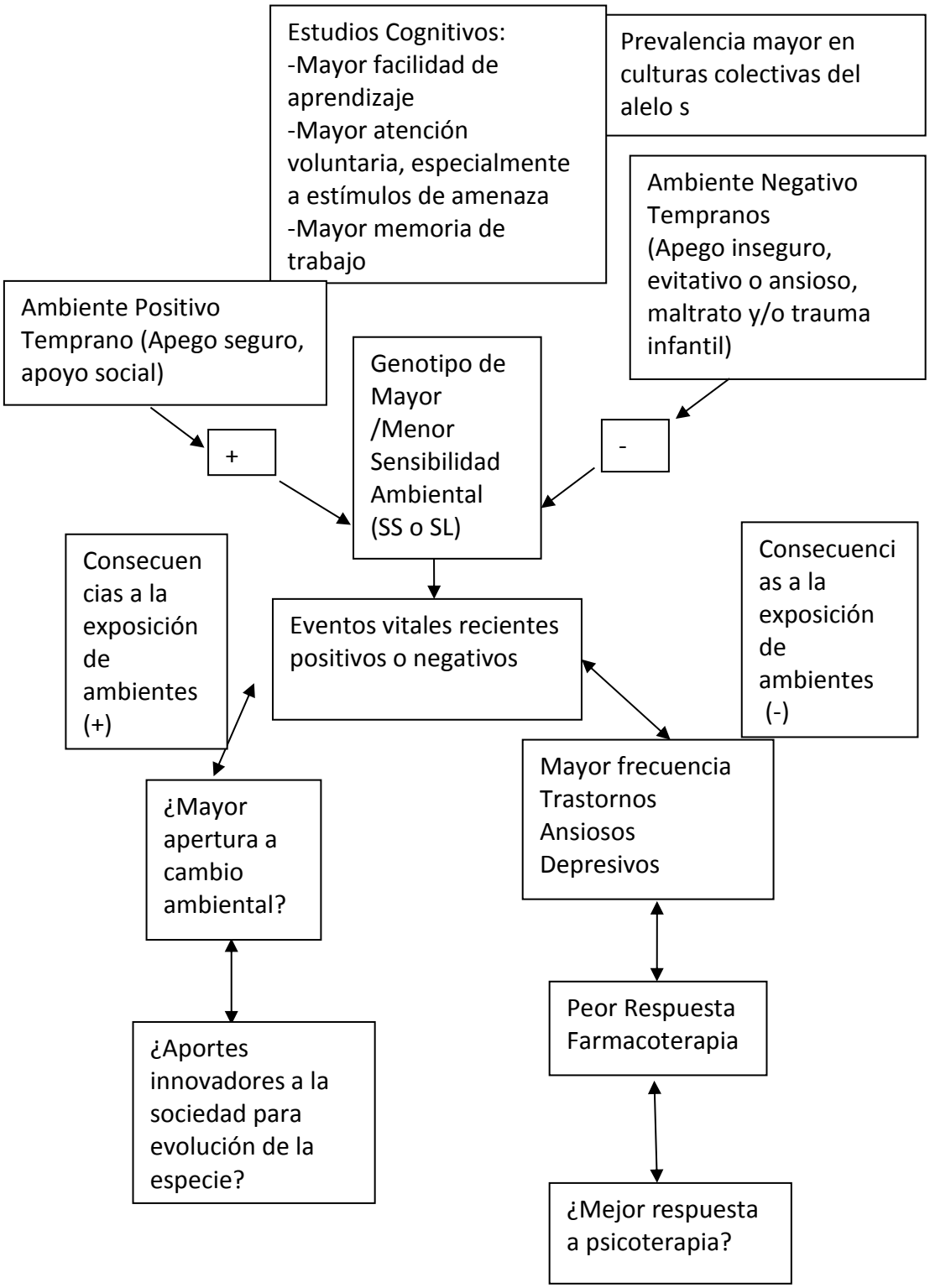
The objectives and hypotheses of the following investigation, center around the question of whether certain genotype moderate the response to the environment, and whether this interaction is mediated by changes in individuals' stress reactivity (understood as changes in cortisol levels in response to stress inducing experiment).

Answering these questions would allow further research to study treatment of depressions more precise and effective.

To answer these questions, we designed a cross sectional *quasi* experimental study. Depression is considered the dependent variable and 5HTTLPR polymorphisms as the independent variable. According to the studies reviewed in the theoretical framework, we consider the environmental aspects, both early and recent, which interact with the short allele genotype for developing depression, as all the studies that have not taken into account these variables have failed to demonstrate the association between the short allele and psychopathology and neurobiological response to stress (cortisol levels). As it is impossible to record all environmental events which a person experiences throughout his life, we decided that as covariates, i.e. factors that may moderate the development of depression, we would include environmental aspects of early development, such as attachment and the history of childhood trauma, social

support, personality configuration and recent environmental events. This latter variable will be measured including positive and negative valence, explicitly, not only to evaluate how the lack of the negative, i.e., no recent negative events has more influence on short allele carriers, but how more positive environments influence short allele carriers, and thus test part of the theory in which we have based our assumptions of a differential sensitivity/response to environment of these individuals.

Figure 15 Summary of selective review of the literature supporting differentiated sensitivity to the environment model



OBJETIVES

General objective:

1. Determine if 5HTTLPR polymorphism (*SCL6A4* gene) and environmental and personality factors interact to predict depressive symptoms, and whether this interaction is associated with neurobiological changes in sensitivity to stress.

Specific Objectives

1.1 Assess whether the genotype interacts with negative environmental factors such as attachment style, the presence of traumatic events in childhood, low social support, and personality factors and recent negative life events to predict depressive symptoms.

1.2. Study whether the genotype interacts with positive environmental factors such as secure attachment, minor trauma history, presence of recent positive events, high social support and personality factors to predict fewer depressive symptoms.

1.3. Determine whether this interaction (GxE) also affects the cortisol levels in response to a stress-inducing experiment.

HYPOTHESIS

Based on the model that presence of the short allele of 5HTTLPR polymorphism confers greater sensitivity to the environment, we hypothesized that:

–Individuals carrying the short allele with more history of early adversity, i.e., alterations in attachment, more history of childhood trauma, less social support, more negative recent events and and predominance of dependent and perfectionist aspects of personality will present higher rates of depressive symptoms.

–Individuals carrying the short allele with a history of secure attachment and lower levels of childhood trauma, and higher levels of positive recent experience, higher social support and lower levels of dependency and autocritic aspects of personality will present less depressive symptoms.

–In short allele carrier's life experiences (positive or negative) will moderate depression rates.

–Short allele carriers will be more sensitive to stress, showing higher levels of salivary cortisol in response to experiment. This will be more pronounced in individuals with higher levels of depression and with more history of negative environment (attachment disorders, history of child abuse, negative recent events, low social support and predominance of dependency and perfectionism/autocritic aspects of personality).

–In turn, individuals carrying the short allele with secure attachment and less history of trauma, more incidence of positive recent events, healthier personality traits and higher social support, will present lower levels of cortisol in response to stress.

METHODOLOGY

General Design Research

To determine whether *the presence of the short allele of the promoter of the serotonin transporter gene interacts with environmental events to develop depression*, and whether this interaction is associated with neurobiological changes in sensitivity to stress (operationalized as changes in levels of cortisol in response to an experimental task), the variables was tested in the study population. To achieve this objective, the variables will be evaluated in one measurement (cross-sectional). This is a quasi-experimental, mixed (analysis between groups and within subjects), unifactorial, quantitative and transversal study. The dependent variables are depressive symptoms and salivary cortisol in response to the experimental test and the independent variable is 5HTTLPR polymorphism. In addition, the interaction between genotype and the following variables in predicting depressive symptoms will be assessed: (1) history of childhood trauma, (2) recent life events, (3) social support, (4) attachment and (5) personality style (anaclitic/introjective). The first four variables are

Participants

The sample consisted of 151 subjects, of which 7 were patients attending an outpatient health mental health of Santiago (Psicomédica), the rest of the sample consisted of volunteers subjects' recruited through contacts via email.

Inclusion criteria:

1) Age between 16-65 years

Exclusion criteria:

1) Severe Personality Disorder
2) Post Traumatic Stress Disorder
3) Obsessive Compulsive Disorder,
4) Psychotic Disorder
5) Uncompensated medical disease (i.e., severe anaemia, hypothyroidism, diabetes, adrenal psychopathology).
6) High suicide risk (MINI)

Sample size

The sample size was was calculated considering the literature review, as there is no study using exactly the same variables and measures the effect that the 5HTTLPR genotype has in predicting depressive symptoms, we took into account Taylor´s article (2006), which measured depressive symptoms depending on genotype and a history of family dysfunction (or childhood trauma in our case) and recent life events, used a n = 118 general population. Moreover, studies of changes in cortisol levels pre and post experimental task (Harkness, Stewart, & Wynne-Edwards, 2011; Heim et al, 2000) used a n = 49 and n = 71, respectively.

Procedures

Patients in the clinical sample were contacted when seeking psychotherapeutic attention in health centres that collaborate with the study (Psicomedica). This centres provide treatment for depression in the AUGE/GES programme, the Chilean Health Ministry programme for treating depression. The volunteer subjects joined the study after receiving recruiting information sent by email. With respect to the exclusion criteria for operational reasons, it was decided to apply only the criterion of greater uncompensated medical pathology. Participants were explained the objectives of the study and invited to participate in it by signing the Informed Consent if they agree to participate (see Annexes). Those who agreed to participate completed the study questionnaires in an online platform. Then they were cited to the laboratory at Psicomédica and Universidad del Desarrollo (Centro de Apego y Regulación Emocional, CARE) to perform the experimental test (signal detection task) and collect blood and saliva to determine the genotype and cortisol pre, during and post the experimental task.
The samples were sent keeping the cold chain to the laboratory of the Center for Molecular Biology and Pharmacogenetics at the University of La Frontera, to Professor Luis Salazar, chief of Laboratory.

Variables and instruments

Dependent variable:

- Depressive symptomatology ((BDI-I-A: Beck Depression Inventory)
- Salivary cortisol in response to a stress generating task (equivalent to a university test)

Independent variable:

- Polymorphism of the promoter region of the serotonin transporter gene (5HTTLPR short/long allele)

Interaction variables (environmental and personality):

- Childhood Trauma (CTQ)
- Attachment style (ECR-S[12])
- Recent life events (LEQ positive and negative)
- Social Support (MOS-SSS)
- Depressive experience (DEQ)

Instruments

A *participant record*, which included among others, socio-demographics, routinely used drug and mental health history was built.

In addition, the following instruments are considered (according to variables):

1) For the ***molecular analysis***, DNA will be extracted from blood leukocytes by the method of salting optimized by Salazar (1998). The polymorphism 5-HTTLPR of the serotonin transporter gene (SCL6A4) will be identified by DNA amplification with the polymerase chain reaction (PCR) technique according to previously described conditions by Sanhueza (2011). The possibility of contamination in the molecular analysis will be excluded by the use of reagent controls in each amplification. The correct genotyping of the polymorphisms will be confirmed by repeating random 20% of analyzes previously performed. The agreement must be 100%.

2) ***Saliva cortisol***: The cortisol response curve will be measured in saliva samples in an experimental situation that induces moderate stress equal to the stress generated by a university evaluation (see specific procedures in Annexes N°3). Saliva samples are obtained using the tube system "Salivettes" (Sarstedt, Germany) and stored at - 20°C

until analysis. Cortisol levels will be determined by ELISA, after centrifuging the samples at 3000 rpm for 5 minutes. The accuracy of the determinations of cortisol will be verified using commercial controls.

To determine the conditions of early development, childhood trauma and attachment style will be measured:

3) ***Attachment***: Experience in Close Relationships-Revised (ECR-R) (Fraley, 2000; Brennan, Clark and Shaver, 1998). It is a self-report questionnaire that assess attachment styles. It consists of 36 items, in which people respond the extent to which they describe in Likert format 7 points. The instrument consists of two sub-scales: (1) anxiety associated with attachment and (2) avoidance associated with attachment. A higher score indicates greater anxiety and/or avoidance. Using the averages of each subscale, it can be configured 4 categories (Bartholomew & Horowitz, 1991; Fraley, Waller, & Brennan, 2000): (1) secure attachment (score below average in subscales anxious and avoidant), (2) preoccupied attachment (above the average on anxious subscale and below the average on avoidant subscale), (3) dismissing attachment (anxious attachment below average and avoidant attachment above average) and (4) fearful attachment (anxious and avoidant attachment above average). The original instrument has good internal consistency (Fraley, 2000; Brennan et al., 1998). The instrument has been used in Chilean samples, reaching reliability of .84 for the anxiety scale and of .83 for avoidance scale (Guzman & Contreras, 2012). We will use the shortened version of 12 questions (ECR-S^{12}).

4) ***Child Trauma***: Child Trauma Questionnaire, a retrospective self-Report (CTQ, Bernstein and Fink, 1998), is an instrument that is intended to identify the history of trauma in adolescents and adults. CTQ is composed of 28 items, in which people respond if certain conditions and/or experiences occurred during their childhood. It is answered on a Likert scale of 5 points, the highest score means greater presence of trauma. It consists of 5 different subscales of trauma: physical, sexual, and emotional abuse, and physical and emotional neglect. The questionnaire also includes a scale of denial/minimization, to identify people who under-report trauma. Its convergent validity has been demonstrated with other measurements of history of trauma, it has proven to be a stable measurement over time and is highly sensitive to identify individuals with histories verified trauma (Bernstein, 1997; Bernstein, 1994). This instrument has not been used in Chile, and was translated and piloted to determine the consistency of the items in English and Spanish by our research group (Leighton, C., Botto, A., de la

Cerda, CJ, Quezada J., San Cristobal P.). This instrument consists of 5 subscales (AF: physical abuse, AS: sexual abuse, AE: emotional abuse, NF: NE Physical and neglect: emotional neglect). For each subscale there is a cut-off point that classifies trauma in mild, moderate or severe. Each answer corresponds to a number from 1 to 5 (1 = never, 2 = rarely, 3 = sometimes, 4 = often and 5 = very frequently). It is considered as positive history of trauma those individuals who have at least one subscale with moderate trauma. It was considered as emotional trauma when the sum of the subscales of AE and NE is greater than or equal to 21, physical trauma when the sum of the subscales of AF and NF is greater than or equal to 18 and sexual trauma when the value is moderate (≥ 8).

The corresponding items for each subscale are:

AF: 9, 11, 12, 15 and 17 (ie. "I was so badly beaten by someone in my family that others as a teacher, a neighbour or a doctor, realized").

AS: 20, 21, 23, 24 and 27 (ie "I think I was abused sexually").

AE: 3, 8, 14, 18 and 25 (ie "Some people in my family said hurtful things or insults me").

NF: 1, 2, 4, 6 and 26 (ie "My parents were too drunk or drugged to care for the family").

NE: 5, July 13, 19 and 28 (ie "I felt loved" Item reverse.).

5) To determine ***recent life events*** we used Life Events Questionnaire (LEQ) (Norbeck, 1984). It is an inventory of 82 items, in which the subject marks vital events or changes that have happened over the last year, and should indicate whether the event has been "good" or "bad" and assess the impact on their lives on a scale of 4 points (no impact, some impact, moderate impact, high impact). The instrument is a modification of that developed by Sarason, 1978, which added 9 items of particular relevance for use in women. They are scored: negative, positive, and total events. This instrument has not been used in Chile, was translated and piloted by our research group (Leighton C., A. Botto, San Cristobal P.). Items include questions related to: Health (ei. "Illness or serious personal injury"), work (ei. "Starting a job outside home") studies (ei. "Start or finish school, college or a training program"), residence (ei."Moving to another city, region or country"), love and marriage (ei."Finish a relationship with girlfriend or boyfriend or a commitment"), family and close friends (ei. "Major change in health or behavior of a family member or close friend"), parenting (ei. "Conflicts with your spouse/partner by raising "), personal or social (ei."Important decision regarding immediate future"), financial (ei."Buy things of value (such as TV, car, refrigerator,

etc.)"), crime and legal issues (ei. "Being a victim of a violent crime (rape, assault, etc.)"), and a free space is left for the participant to describe other recent experiences that have had an impact on their lives and were not included in the questions.

6) ***Social support***: will be assessed through self-administered questionnaire Medical Outcome Study Support Social Survey (MOS-SSS) which was developed in the context of a large study on patients with a chronic condition (Sherbourne & Stewart, 1991). The MOS-SSS evaluates the recent appreciation that the subject has on different dimensions of social support: 1) emotional/informational support (expression of affection and empathic understanding as well as guidance and offer of advice and information), 2) instrumental support (provision of material assistance that a person could receive), 3) positive social interaction (availability of people with whom to go out, have fun or get distracted) and 4) emotional support (based on expressions of love and affection). The maximum overall index of social support is 94, with a mean value of 57 and a minimum of 19. The instrument is translated into Castilian and validated in primary care consultant Spanish population (De La Revilla, Moon, Bailon, & Medina, 2005) but has not yet been validated in Chile.

7) ***Configuration of personality (anaclitic/introjective)***: The organization of personality according to anaclitic/introjective polarity will be measured through the questionnaire Depressive Experience Questionnaire (DEQ) developed by Blatt, D'Afflitti, & Quinlan (1976), an self-report instrument that measures two polarities of depressive experience: anaclitic and introjective. The instrument consists of 66 items, where people should indicate the extent to which they are described by the corresponding statement, using a Likert scale of 7 points. Items yield factor scores in three dimensions: dependency, self-criticism, and effectiveness. Subsequently, Blatt, Zohar, Quinlan, Zuroff, & Mongrain (1995), two sides differed in the original dependence factor distinguishing the items that point to the (positive) relational capacity of those that measure the construct dependence. Validity and reliability studies have been developed with non-clinical sample (S. J. Blatt, Quinlan, Chevron, McDonald, & Zuroff, 1982; Zuroff & Mongrain, 1987) and clinical (Klein, Harding, Taylor, & Dickstein, 1988). Blatt et al. (1982) reported Cronbach's alphas of .81, .80 and .72 for scales of dependency, criticism and original efficiency, and Blatt et al. (1995) reported internal consistency of 60-83 in the subscales of relational capacity in different samples. 3 subscales (dependency, self-criticism and efficiency) distinguish four categories of depressive experience: anaclitic (high dependency and low self-criticism), introjective (high self-criticism and low

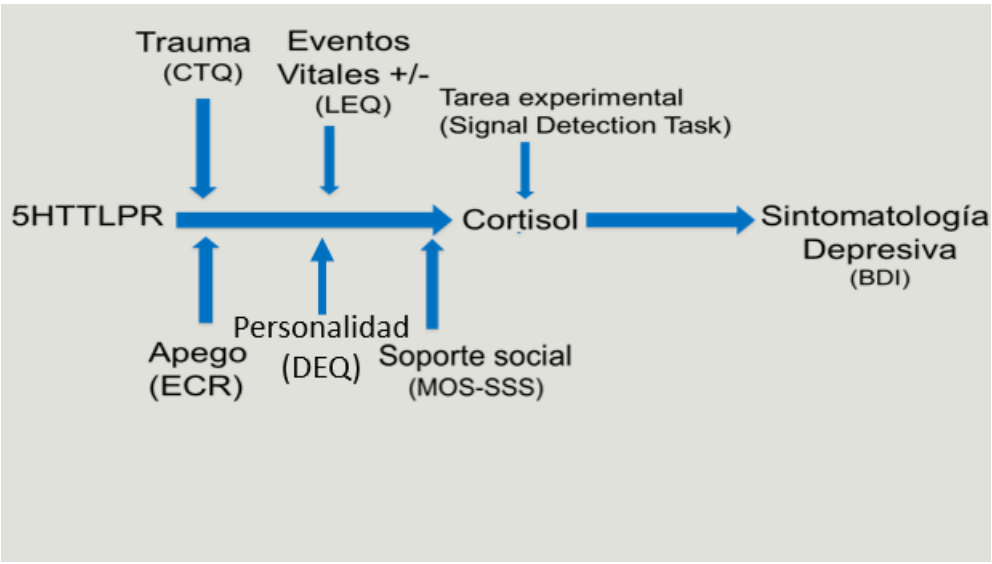
dependence), mixed (high self-critical and highly dependent) and not categorizable (low dependence and low self-criticism).

As a criterion variable, ***depressive symptoms***, were evaluated with:

8) Beck Depression Inventory (BDI-I-A, Beck, 1961; Beck, 1988) is a self-applied instrument designed to assess depressive symptoms in adults and adolescents. It consists of 21 items, which are answered on a 4-point scale (0 to 3). In each of the items, the person has to choose the degree of gravity with which the different symptoms characterized him during the last week. The higher the score, the more symptoms. This instrument has been used in Chile (Alvarado, 2005; Ruiz, 2001, Santander, 2011, among others) and validated by Morales-Reyes I in our country (I-Reyes Morales, 2015). It was used as a cut-off for minimum depression 10 points of BDI, which coincides with the 75th percentile of depressive symptomatology of our sample.

(The scales and questionnaires are attached in Annex)

Figure 16 Summary of the general design and interaction between variables



¿Sensibilidad diferenciada o vulnerabilidad?

Data Analysis

In the first place the presence of outliers was evaluated. To do so an analysis of the distribution of the dependent variable (BDI) was performed according to the method of "labelling rule" (Hoaglin & Iglewicz, 1987; Hoaglin, Iglewicz, & Tukey, 1986). The analysis showed that subjects 147 and 148 were outliers. Both were women who were part of the clinical sample who's BDI score was 56 and 40 respectively. These subjects were excluded from statistical analysis.

Then we performed an analysis deviation from Hardy-Weinberg equilibrium for 5HTTLPR gene. Since there is contradictory data in the literature on whether the S allele has a dominant or recessive effect, we tested with both models (triallelic: Low expression SS, intermediate expression SL and high expression LL, and biallelic: comparing the S allele versus no S allele: SS and SL in a group and LL in another group, and comparing the L allele *versus* no L allele: LL and SL in the same group and SS in another group). We chose to show the results only for the aggrupation that was significant for the interaction. Therefore, for interaction analysis between 5HTTLPR with trauma, social support and recent events, the results are shown with the genes grouped as biallelic, with S dominant (SS/SL and LL) allele, and for the interaction analysis between 5HTTLPR with attachment and depressive experience, genes grouped considering the L allele as dominant (LL / SL and SS).

Sociodemographic characteristics of the subjects and the overall results of separate instruments are then analyzed. Subsequently, the correlation matrix analysis, and linear regression tests to predict depressive symptoms with continuous variables and later an analysis of variance (ANOVA) factorial to assess the interaction between genotype and the variables studied to predict depressive symptoms is performed.

To analyze samples of salivary cortisol we used mixed ANOVA, the within-subject factor was the repeated measures of salivary cortisol levels in response to the stressful task and the between subject factor was the group of allele. Analysis area under the curve, and analysis of cortisol delta was also performed to measure the change in cortisol curve

Finally, classification and regression tree to predict depression was run (CART) (Hodar et al., 2010). Statistical tests have a p-value of .05. Statistical analyzes were performed using SPSS software and R.

RESULTS

151 subjects were studied of which 7 were patients diagnosed with depression and 144 volunteer subjects. Of the total number of subjects only 88 (58.3%) were gentotypified and only 139 responded the BDI; the rest responded some on-line instruments but did not attend to sampling and experiment test. Of the genotypified, 5 were patients with depression and 83 were volunteer subjects. **Table 2** shows the sociodemographic variables and compares genotypified subjects vs non genotypified ones. No significant differences are observed between both groups.

Table 2

Comparison between groups of genotypified vs. non genotypified subjects

Variable	Genotypified	Non genotypified	Significance (p)
BDI	7.11 (5.863)	5.86 (5.114)	.194
Age	22.36 (7.187)	22.2 (7.169)	.895
Sex	H=28 y M=60[a]	H=20 y M=43[a]	.994
Education	EB=0; EM=35; ES=48[b]	EB=1; EM=26; ES=30[b]	.425
Civil status	S=75; CC=7; D=0[c]	S=54; CC=4; D=1[c]	.466
District	SO=5; NO=47; RS=27; R=2[d]	SO=16; NO=15; RS=26; R=2[d]	.000
Occupation	DC=1; E=68; C=3; TA=11[e]	DC=2; E=46; C=2; TA=8[e]	.838
Sample type	MC=5; MNC=83[f]	MC=2; MNC=61[f]	.470

Note:. The values of BDI and age indicate the mean. SD is shown in parenthesis. The values sex, education, district and occupation indicate number of subjects. H=Men; M=Women. EB=Complete primary school; EM=Complete secondary school; ES=Further studies. SO= South East Stgo.; NO= North East Stgo.; RS= the rest of Stgo.; R=regions. DC=house wife; E=student; C=unemployed; TA=employed. MC=clinical sample; MNC=nonclinical sample. S=unmarried; CC=partner/married; D=separated/divorced.

The socio-demographic data of the analyzed sample (n = 88) are shown in **Table 3**. A difference in gender distribution by genotype was found.

Table 3

Sociodemographic data

Variable	5HTTLPR			
	LL	LS	SS	p
Sex	H=7; M=30a	H=17; M=16[a]	H=4; M=14[a]	.009
Age	23.41	21.53	21.94	.565
Education	EB=0; EM=12; ES=22[b]	EB=0; EM=16; ES=16[b]	EB=0; EM=7; ES=10[b]	.479
Civil status	S=30; CC=4; D=0[c]	S=29; CC=2; D=0[c]	S=16; CC=1; D=0[c]	.622
District	SO=2; NO=19; RS=10; R=2[d]	SO=2; NO=15; RS=14; R=0[d]	SO=1; NO=13; RS=3; R=0[d]	.316
Occupation	DC=1; E=28; C=2; TA=3[e]	DC=0; E=26; C=1; TA=5[e]	DC=0;E=14 C=0; TA=3[e]	.751
Sample type	MC=2; MNC=35[f]	MC=3; MNC=30[f]	MC=0; MNC=18[f]	.405
BDI	6.91 (4.693)	7.84 (6.527)	6.18 (6.775)	.628
History of mental health problems	S=11; N=21[g]	S=7; N=25 [g]	S=2; N=15 [g]	.194
History of Depressive episodes	0.65	0.47	0.36	.521
History of psychological treatment	S=15; N=17 [g]	S=11; N=21 [g]	S=8; N=9 [g]	.534
Family history of mental disorder	S=13; N=18 [g]	S=6; N=26 [g]	S=6; N=11 [g]	.128

Note: The BDI values and Age indicate the mean. DS (SD in English) is shown in parenthesis. The values of sex, education, district and occupation indicate number of subjects. H=Men; M= Women. EB=Complete primary school; EM=Complete secondary school; ES=Further studies. SO= South East Stgo.; NO= North East Stgo.; RS= the rest of Stgo.; R=regions. DC=house wife; E=student; C=unemployed; TA=employed. MC=clinical sample; MNC=nonclinical sample. S=unmarried; CC=partner/married; D=separated/divorced

Genetic Analysis

For the 5HTTLPR gen, the success genotyping rate (genotyping exit) was 100%. The genotypic frequency was LL=37 (42%), LS=33 (37.5%) y SS=18 (20.5%). The allelic frequency was S=0.39 y L=0.61. This distribution is not in Hardy-Weinberg' balance (EHW) (X^2=4.005; DF=1; p=.0454). For men the distribution is in EHW (X^2=1,4604; DF=1; p=.226), but for women not (X^2=10,8802; DF=1; p=.0009).

For statistical analysis, 5HTTLPR polymorphism was divided into two and three groups: (SS/ SL and LL) considering the allele S as dominant (SS and SL/LL) considering the S allele recessive and each genotype separately (SS, SL and LL).

The distribution of the average levels between the studied variables according to the genetic polymorphisms are shown on **Table 4** below.

Table 4

Mean average of the variables according to genotypes

		5HTTLPR			
Variable	**Total sample N=88**	**LL N=37**	**LS N=33**	**SS N=18**	**p**
Depression (BDI)	6,59 (5,578)	6.91 (4.693)	7.84 (6.527)	6.18 (6.775)	.628
Child trauma (CTQ)	43,86 (12,234)	41.78 (9.889)	43.66 (10.57)	47.88 (14.89)	.209
Attachment Style (ECR-S[12])	AE=2,87 (1,03) [a] AA=3,37 (1,04) [a]	AE=2.71 (1.18) [a] AA=3.41 (1.14) [a]	AE=2.55 (0.80) [a] AA=3.41 (1.07) [a]	AE=3.23 (1.00) [a] AA=3.26 (1.02) [a]	.115 .901
Recent life events (LEQ)	EVP=7,06 (4,60) [b] EVN=3,96 (4,59) [b]	EVP=7.58 (2.93) [b] EVN=4.48 (4.65) [b]	EVP=8.59 (5.25) [b] EVN=5.07 (5.34) [b]	EVP=6.60 (4.73) [b] EVN=3.31 (3.34) [b]	.344 .495
Social	76,3	79.06	76.93	76.50	.739

support (MOS-SSS)	(13,64)	(11.949)	(13.63)	(13.01)	
Depressive experience (DEQ)	AN=39 (25,8%)[c] IN=9 (6%)[c] MI=25 (16,6%)[c] NC=47(31,1%)[c]	AN=11; IN=2; MI=2; NC=17	AN=6; IN=2; MI=4; NC=17	AN=2; IN=3; MI=0; NC=12	.254

Note: AE=avoidance attachment; AA=anxious attachment; EVP=positive vital events; EVN= negative vital events; AN=anaclitic; IN=introjective; MI=mixed; NO=normal. The value of DEQ corresponds to the number of subjects.

Correlational analysis

In **Table 5**, we present the correlations between the variables included in the study. There is no correlation between the genotype or the alleles and the studied variables. Nor is there any correlation between baseline cortisol and the variables studied. There is a positive correlation between the level of depressive symptomatology and the following variables: recent negative events, child trauma, anxious attachment, level of dependency and self-criticism. The strongest correlation is between the level of depressive symptomatology and recent negative events and self-criticism. There is a negative correlation between the level of depressive symptomatology and social support.

No negative correlation between recent positive events and depressive symptomatology was observed; however, there is a significant negative correlation ($r=-0.547$, $p<0.000$) between the difference in the number of recent positive and negative events measured with LEQ and the depressive symptomatology measured with BDI. I.e., the greater the difference between positive and negative events, lower levels of BDI.

Table 5

Correlation Matrix

		Síntomas Depresivos	5 HTTLPR	Cortisol Basal	Trauma	Eventos Recientes Negativos	Eventos Recientes Positivos	Apoyo Social	Apego Ansioso	Apego Evitativo	Dependencia	Autocritica	Eficacia
Síntomas Depresivos	Correlación de Pearson	1	-,026	-,015	,407(**)	,679(**)	,097	-,334(**)	,442(**)	,055	,432(**)	,645(**)	-,083
	Sig. (bilateral)		,820	,897	,000	,000	,433	,000	,000	,552	,000	,000	,369
	N	139	81	80	119	66	68	117	120	120	119	119	119
5 HTTLPR	Correlación de Pearson	-,026	1	-,014	,196	-,087	,004	-,084	-,059	,107	-,103	,073	-,075
	Sig. (bilateral)	,820		,897	,085	,488	,973	,466	,623	,366	,371	,526	,516
	N	81	88	87	78	66	67	78	73	73	78	78	78
Cortisol Basal	Correlación de Pearson	-,015	-,014	1	-,069	,027	-,102	,136	-,060	,181	-,089	-,070	-,012
	Sig. (bilateral)	,897	,897		,550	,827	,412	,237	,617	,128	,444	,543	,918
	N	80	87	87	77	66	67	77	72	72	77	77	77
Trauma	Correlación de Pearson	,407(**)	,196	-,069	1	,310(*)	,071	-,522(**)	,313(**)	,205(*)	-,012	,515(**)	-,116
	Sig. (bilateral)	,000	,085	,550		,013	,574	,000	,001	,032	,899	,000	,210
	N	119	78	77	120	63	65	116	109	109	118	118	118
Eventos Recientes Negativos	Correlación de Pearson	,679(**)	-,074	,040	,359(**)	1	,351(**)	-,290(*)	,318(*)	-,055	,307(**)	,515(**)	-,046
	Sig. (bilateral)	,000	,533	,740	,002		,004	,015	,010	,664	,010	,000	,708
	N	66	73	72	70	67	66	70	64	64	70	70	70
Eventos Recientes Positivos	Correlación de Pearson	,097	-,050	-,119	,018	,351(**)	1	-,073	,124	-,147	,174	,088	,273(*)
	Sig. (bilateral)	,433	,673	,321	,881	,004		,543	,323	,243	,148	,466	,021
	N	68	73	72	71	66	69	71	65	65	71	71	71
Apoyo Social	Correlación de Pearson	-,334(**)	-,084	,136	-,522(**)	-,259(*)	-,081	1	-,322(**)	-,256(**)	,087	-,535(**)	,203(*)
	Sig. (bilateral)	,000	,466	,237	,000	,039	,516		,001	,008	,353	,000	,029
	N	117	78	77	116	64	66	118	107	107	116	116	116
Apego Ansioso	Correlación de Pearson	,442(**)	-,059	-,060	,313(**)	,276(*)	,155	-,322(**)	1	,101	,413(**)	,428(**)	-,018
	Sig. (bilateral)	,000	,623	,617	,001	,036	,238	,001		,272	,000	,000	,856
	N	120	73	72	109	58	60	107	121	121	108	108	108
Apego Evitativo	Correlación de Pearson	,055	,107	,181	,205(*)	-,045	-,160	-,256(**)	,101	1	-,187	,047	-,106
	Sig. (bilateral)	,552	,366	,128	,032	,739	,221	,008	,272		,052	,632	,274
	N	120	73	72	109	58	60	107	121	121	108	108	108
Dependencia	Correlación de Pearson	,432(**)	-,103	-,089	-,012	,290(*)	,226	,087	,413(**)	-,187	1	,270(**)	,130
	Sig. (bilateral)	,000	,371	,444	,899	,021	,070	,353	,000	,052		,003	,159
	N	119	78	77	118	63	65	116	108	108	120	120	120
Autocritica	Correlación de Pearson	,645(**)	,073	-,070	,515(**)	,493(**)	,166	-,535(**)	,428(**)	,047	,270(**)	1	-,085
	Sig. (bilateral)	,000	,526	,543	,000	,000	,186	,000	,000	,632	,003		,358
	N	119	78	77	118	63	65	116	108	108	120	120	120
Eficacia	Correlación de Pearson	-,083	-,075	-,012	-,116	-,066	,292(*)	,203(*)	-,018	-,106	,130	-,085	1
	Sig. (bilateral)	,369	,516	,918	,210	,607	,018	,029	,856	,274	,159	,358	
	N	119	78	77	118	63	65	116	108	108	120	120	120

** La correlación es significativa al nivel 0,01 (bilateral).

* La correlación es significante al nivel 0,05 (bilateral).

Depressive Symptomatology

Of the total number of subjects (n=151), 139 replied the BDI questionnaire. The average was 6.59 (DS=5.578), the extreme scores were 0 and 27. For females, the average was 6.96 (DS=5.58) and for males, 5.82 (DS=5.56). No differences in gender were found, F (1,137) =1.263; p=0.263. Thirty eight subjects (29 females and 9 males) presented a score higher than the cut-off point established to define minimum depression (score=10) corresponding thus to 27.3% of the sample. No significant differences in gender were observed (X^2=1.804; p=0.179). For the subjects diagnosed with depression who answered the questionnaire (n=5), the mean BDI average was 17.8 (DS=5.63) with extreme scores of 10-25. For the volunteer subjects who answered the questionnaire (n=134), the mean BDI average was 6.17 (DS=5.14) with extreme scores of 0-27. The percentage of subjects with a score higher than the cut-off point established to define minimum depression drops to 24.6%, n = 33, women 28.6%, n = 26 and men 16.3%, n = 7 subjects, $\chi 2$ (1, n = 134) = 2.377, p = .123, ns.

Trauma

Of the total number of subjects (n=151), 120 answered the CTQ and the BDI (79.5%), of these subjects, 82 were females and 38, males. The minimum score was 30 and the highest, 84 with a mean of 43.86 and SD: 12.234. The percentile 85 was 57 points.

We used as general trauma criterion the presence of at least a subscale with moderate to severe trauma. Of the total number of subjects 29.2% (n=35) present a history of positive trauma. Analysing the sample according to sex, we observe that 25.6% (n=21) of the females (n=82) present a history of trauma and that 36.8% (n=14) of the males (n=38) present a history of trauma (X^2 =1.586, p=0.149), no difference between gender. **Table 6** shows the values according to sex detailed in subscales.

Table 6

Percentage of subjects with trauma history

	Trauma	Physical Trauma	Emotional Trauma	Sexual abuse
Female	17.5% (21)	2.5% (3)	17.5% (21)	6.7% (8)
Male	11.7% (14)	3.3% (4)	8.3% (10)	6.7% (8)
Total	29.2% (35)	5.8% (7)	25.8% (31)	13.3% (16)

Note: The absolute number of subjects is shown in parenthesis.

The cut-off points of each subscale are shown on **Table 7**

Table 7

Cut-off points for each trauma subscale CTQ

Abuse Type	Mean	SD	p.85	p.25	p.75	PC mild	PC moderate	PC severe
AF	6.02	2.219	7			8-9	10-12	≥13
AS	5.94	2.416	7			6-7	8-12	≥13
AE	8.61	3.647	12.85			9-12	≥13	
NF	6.63	2.118	9			8-9	≥10	
NE	8.92	3.819	13			10-14	≥15	
CTQ total	43.86	12.234	≥57	≤35	≥48			

Note: AF: physical abuse, AS: sexual abuse, AE: emotional abuse, NF: physical negligence and NE: emotional negligence. PC= cut-off point taken from DiLillo et al. (2006) & Heim et al. (2006)

We estimate the simple lineal regression of the depressive symptomatology based on the history of child trauma and validated the hypothesis that depression is positively related to the history of child trauma. We found that β=.407 was statistically significant F (1,117) =23.198 p<0.000 and therefore accept the hypothesis of the lineal relation between depressive symptomatology and trauma history. For each CTQ unit increase, the level of depressive symptomatology measured by BDI increases 0.197. The value R^2 was 0.165, indicating that 16.5% of the variance of the depressive symptomatology is explained through trauma history. The residue analysis showed that the assumptions of linearity, normality, independence (Durbin-Watson=1.823) are achieved, but no that of homoscedasticity. The analysis of influence was carried out but no significant differences were found in the model.

Univariate analysis of variance (ANOVA) was carried out to study the interaction between the genetic polymorphisms and the history of trauma of depressive symptomatology. The analysis showed is with 5HTTLPR polymorphism groups divided into two groups (SS/SL and LL) considering the S allele as dominant.We analysed the assumptions of ANOVA and found that neither the assumptions of normal distribution

of the depending variable (Kolmogorov-Smirnov=0.000) nor homoscedasticity assumption are complied with (Levene=0.000); however, we assume that the test is robust and allows for the non-compliance of these assumptions.

Table 8 shows the distribution of subjects with and without trauma according to polymorphism.

Table 8

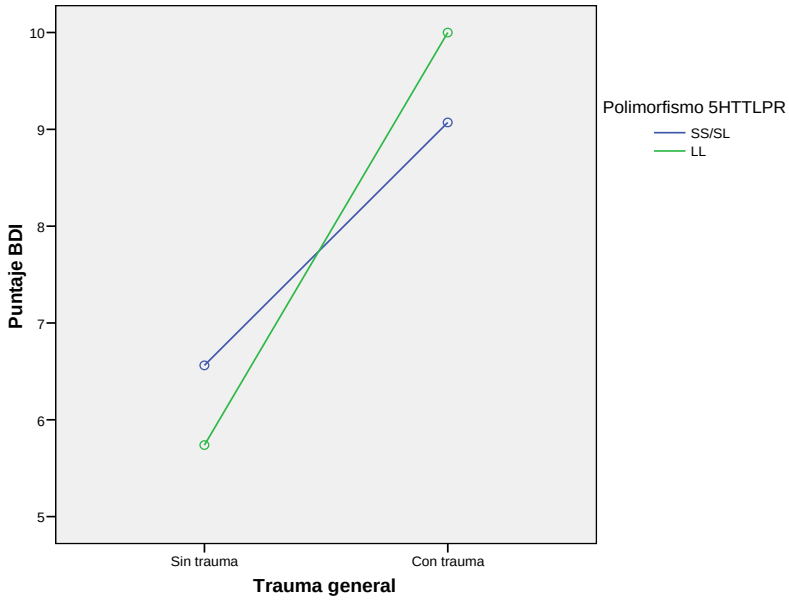
Number of subjects with and without trauma history according to 5HTTLPR polymorphism

Allele	Trauma	Without trauma
SS/SL	14	32
LL	9	23

Factorial ANOVA shows that the effect of general trauma (total CTQ) on depressive symptomatology according to 5HTTLPR polymorphism was not significant (1,74)=0.346, p=0.558, indicating therefore that there is no interaction.

Only the main effect of general trauma on depressive symptomatology was significant F (1,74)=5.171, p=.026. In Figure 17 these differences in the BDI average are observed.

Figure 17 Effect of trauma on depressive symptomatology according to 5HTTLPR polymorphism



If we do the analysis per trauma subscale we did not find a significant interaction between abuse type and 5HTTLPR polymorphism on depressive symptomatology. However, we found a main effect of emotional abuse, $F(1,74) = 4.412$, $p = .039$ and emotional neglect, $F(1,74) = 6.380$, $p = .014$ on depressive symptoms.

Recent life events

Recent negative life events

Of the total number of subjects, (N=151), 72 answered the LEQ questionnaire together with the BDI. Recent negative event was considered when the subject reports that the event effect is "bad" with a "moderate" or "severe" level.

Out of the total number of subjects analysed, 68 (94.3%) presented some kind of negative event during the last year and 62 (86%) presented negative events ranging from moderate to severe, of these 22 were males and 40 females.

The mean of the total number of negative events of any type per person was 6.6 (ds=5.4) and of negative events with moderate to severe effect was 4.54 (ds=4.7). When analysed per gender, the average for males was 3.92 and 4.91 for females $F(1,65)=0.687$, $p=0.410$). According to the type of event, we observed that the subgroup of questions related to "parenting" presented the highest positive answers for negative events with moderate to severe effects during the last year (2.2, SD=1.30), followed by "love and marriage" (1.6, SD=1.67) and by "crime and legal matters" (1.55, SD=1.1).

There is a significant positive correlation between the intensity of depressive symptomatology and the number of negative events with moderate to severe intensity ($r=0.679$, $p<0.000$), the sum of the effect level of negative events ($r=0.659$, $p<0.000$), and the total number of negative events of any intensity ($r=0.666$, $p<0.000$). The highest correlation is with the number of negative events with moderate to severe intensity. The correlation with the total number of negative events of any intensity was lower in comparison with that of moderate to severe negative events; it can therefore be inferred that as events with mild intensity do not have influence on this relation, they will not be considered for the analysis of the effect of recent negative events.

The highest correlation between depressive symptomatology and subtype of recent negative event was with the group of situations related to work ($r=0.891$,

p<0.000), followed by finance (r=0.707, p<0.000), crime and legal matters (r=0.550, p=0.010) and health (r=0.384, p=0.006).

We estimated the simple linear regression of depressive symptomatology and the presence of negative events of moderate to severe intensity during the last year and validated the hypothesis that both variables are positively related. We found that the slope β=0.679 was statistically significant F(1,64)=54.65, p<0.000 and we therefore accepted the hypothesis of linear relation between depressive symptomatology and the presence of negative events of moderate to severe intensity during the last year. For each unit increase of moderate to severe negative events of LEQ, the depressive symptomatology measured by BDI increases 0.86 points. Furthermore, the regression equation predicts that if the subject presents a negative moderate-to-severe event during the last year, it will have a score in the BDI of 4.4. And if the subject presents 8 negative events, the BDI score will be 10.4. The R^2 was 0.461, indicating that 46.1% of the variance in depressive symptomatology is explained by the presence of negative moderate-to-severe events during the last year. The residue analysis showed that the assumptions of linearity, normality, independence (Durbin-Watson=1.604) are met, but not that of homoscedasticity. The analysis of influence was carried out, but no significant differences were found in the model.

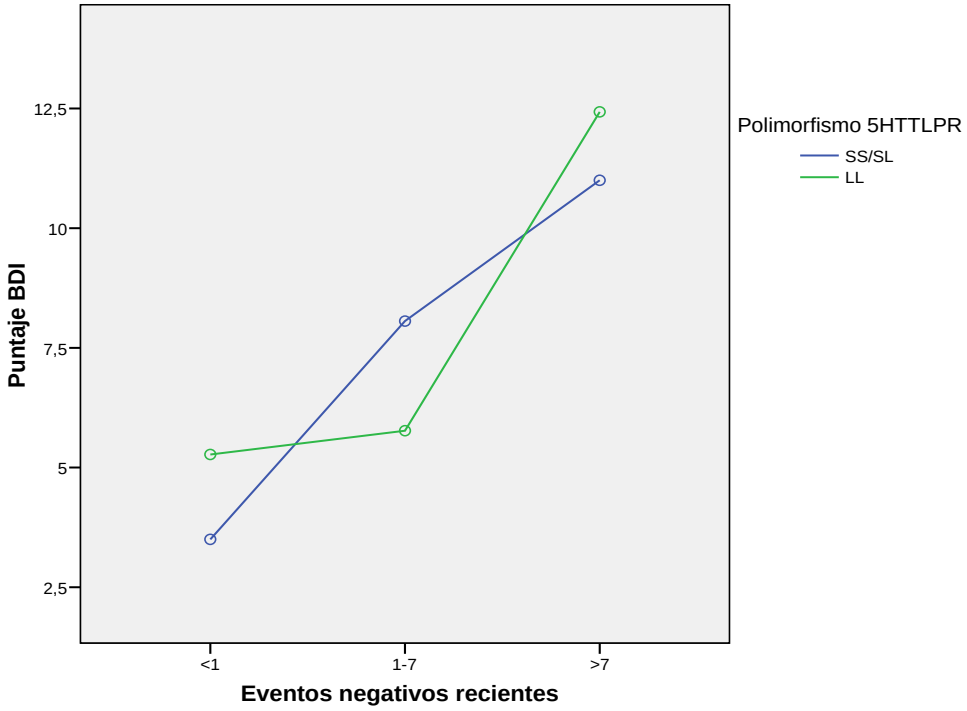
We performed a univariate analysis of variance (ANOVA) to study the interaction between genetic polymorphism and life negative events during the last year over depressive symptomatology. For this purpose we subdivided the results of the LEQ negative events questionnaire in three groups according to percentile: 25 (score <1), 25-85 (score 1-7) y 85 (score >7). We analysed the assumptions of ANOVA and found that the assumption of homoscedasticity (Levene=.01) and the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000) was not met, however, we assume that the test is robust and allows for the non-compliance of this assumption.

The factorial ANOVA shows that the effect of the recent life negative events on depressive symptomatology according to the 5HTTLPR polymorphism was not significant, F (2,64)=1.433, p=.246, indicating therefore that there is no interaction. We observed a main effect of negative life events. The subjects that presented more than 7 negative events scored significantly higher in BDI, F(2,64)=10.930, p<.000.

In Figure 18 we find that subjects carrying the S allele with over 7 recent negative events have an average score of BDI 11.0 and without adverse events have a

mean score of BDI of 3.5, while the LL subjects have an average BDI score of 12.43 when reporting more than 7 recent negative events and a mean score of BDI of 5.27 when no report negative events, but these differences are not significant.

Figure 18 Relation between recent negative events and depressive symptoms according to 5HTTLPR polymorphism



Recent positive life events

Of the total number of analysed subjects, 70 presented some kind of positive event during the last year and 67 (95.7%) presented moderate-to-severe positive events, 23 of them being male and 44 female. A recent positive event was considered as such when the subject reports the event as “good” with an effect level from “moderate” to “severe”.

The average of total positive events of any type per person was 10.94 (ds=4.8) and that of positive events with moderate-to-severe effect was 7.9 (ds=4.18). When analysed per gender, the average for men was 7.67 y 8.02 for women F(1,67)=0.112, p=0.739). According to the type of event, we observed that the subgroup of questions related to “Personal or social” presented the highest average in positive answers for positive events with moderate-to-severe effect during the last year (3.04, ds=1.67), followed by “Love and marriage” (1.91, ds=1.1) and by “Health” (1.33, ds=1.0).

There are no significant correlations among the number of positive events of moderate-to-severe intensity, the total of positive events of any intensity and the sum of intensity levels positive events and depressive symptomatology.

The highest correlation between depressive symptomatology and subtype of moderate-to-severe positive recent event was with the subgroup of situations related to "Health" (r=-0.287, p=0.059, n=44). When the level of effect according to subtype of positive event is considered, the events related to health present a significant correlation of a negative type with depressive symptomatology (r=-0.304, p=0.045, n=44). The higher the positive effect on health, the less is the depressive symptomatology. However, when we analyze the number of positive events independent of their effect, this correlation decreases to r=-0.106, p=0.4, n=65. No significant correlation is observed with the rest of the subtype of events independent of their level of effect.

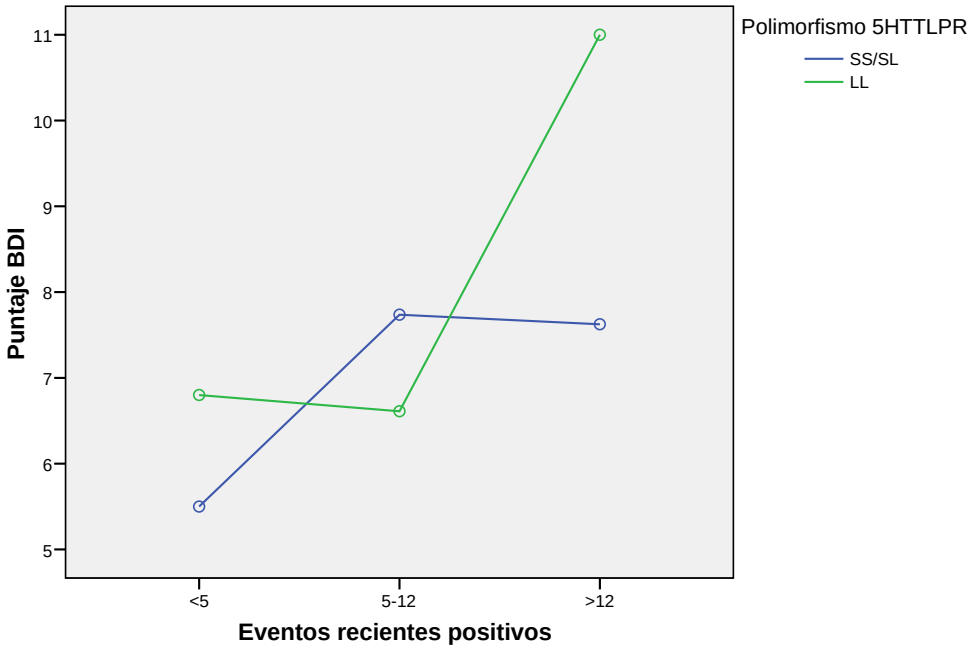
We performed a simple linear regression to predict depressive symptomatology based on the level of effect of the positive events related to health and found a significant regression equation (F(1,42)=4,281, p=0,045), with R^2 de 0,093, which indicates that 9% of the variance of the BDI is explained by the level of positive effect of the recent events related to health. An increase in one unit of intensity in recent positive events related to health, the depressive symptomatology measured by the BDI decreases in 0.647. The residue analysis showed that the assumptions of normality and independence (Durbin-Watson=1.604) are met, but neither those of linearity nor of homoscedasticity. The analysis of influence was carried out, and the regression equation continues to be significant as it excludes the potentially influential cases; however, when analysed excluding the influential cases measured by D de Cook, the model loses significance.

We carried out an ANOVA to study the interaction between genetic polymorphism and the recent positive events on depressive symptomatology. For this purpose we subdivided the results of the positive events of LEQ questionnaire into three groups according to percentile: 25 (score <5), 25-85 (score 5-12) y 85 (score >12). We analysed the assumptions of ANOVA and found that the assumption of homoscedasticity (Levene=0.29) is met, but the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000) is not; however, we assume that test is robust and holds the non-compliance of this assumption.

The ANOVA factorial shows that the effect of the recent positive events on depressive symptomatology according to the5HTTLPR polymorphism, was not

significant, F (2,64)=0.735, p=.483, indicating therefore that is no interaction. We did not observed main effects. Figure 19 shows that the BDI average of SS/SL subjects with more than 12 positive events is lower recent than the average of LL subjects, but this difference is not it is significant (7.63 vs. 11.0 p = 0.370).

Figure 19 Relation between recent positive events and depressive symptoms according to 5HTTLPR polymorphism



Difference between recent positive and negative life events

Finally, we analysed the correlation between negative events and positive of moderate-to-severe intensity and found that there exists a significant positive correlation (r=0.351, p=0.004).

We calculated a simple linear regression to predict depressive symptomatology based on the difference between the number of recent positive and negative events and found a significant regression equation, F(1,63)=26.863, p<0.000), with an R^2 de 0,299, which indicates that 30% of the BDI variance is explained by the difference between the number of recent positive and negative events. The increase in one unit in the difference of positive and negative events decreases in 0.603 points the depressive symptomatology measured by the DBI. The residue analysis showed that the assumptions of normality, linearity and homoscedasticity are met, but not that of

independence (Durbin-Watson=1.495). The analysis of influence and the equation of regression are still significant when excluding the potentially influential cases and influential cases measured by D de Cook.

We carried out an ANOVA to study the interaction between genetic polymorphism and the difference between the number of recent positive and negative life events on depressive symptomatology. For this purpose we subdivided the result of the difference between positive and negative of moderate-to -severe events according to percentile: 25 (score <1), 25-85 (score 1-6) y 75 (score >6). We analysed the ANOVA assumptions and found that it meets the assumption of homoscedasticity (Levene=1.07), but it does not meet the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000); however, we assume that the test is robust and allows for the non-compliance of this assumption.

The factorial ANOVA shows that the effect of the difference between recent positive and negative life events on depressive symptomatology according to the 5HTTLPR polymorphism was not significant, F (2,58)=0.119, p=.888, indicating that there is no interaction. We observed a main effect of the difference. In those subjects that presented a low difference between recent positive and negative events, the average in the BDI score is higher in those that have a high difference, F(2,58)=3.954, p=.025. We analysed the assumptions of ANOVA and found that the assumption of homoscedasticity (Levene=0.107) is met, but the normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000) is not met; however, we assume that the test is robust and allows for the non-compliance of this assumption.

Social support

Of the total number of subjects (N=151), 118 answered the MOS-SSS questionnaire. Of these subjects, 79 were females and 39, males. The results are shown on **Table 9**. The scores do not show significant differences when analysed according to gender. The average of friends and family members considered as close was 7.93 (DS=5.96) showing no significant differences per gender.

Table 9

Results of the MOSS instrument of social support

Values	Highest	Lowest	Mean
Emotional	40	13	31.86 (ds=6.76) (30.5)
Instrumental	20	8	16.12 (ds=3.51) (15.3)
Social interaction	20	8	15.89 (ds=3.18) (15.4)
Affective	15	4	12.42 (ds=2.64) (12)
Global index	95	39	76.3 (ds=13.64) (73.2)

Note: The expected value is indicated in parenthesis according to the original scale (Sherbourne & Stewart, 1991).

The analysis of the correlational matrix shows that there is a negative correlation among the level of depressive symptomatology and the level of global social support (r=-.334, p<.000), emotional support (r=-.330, p<.000), positive social interaction (r=-.254, p=.006) and affective support (r=-.342, p<.000). No correlation with the subscales of instrumental support nor the number of friends and close family members is observed.

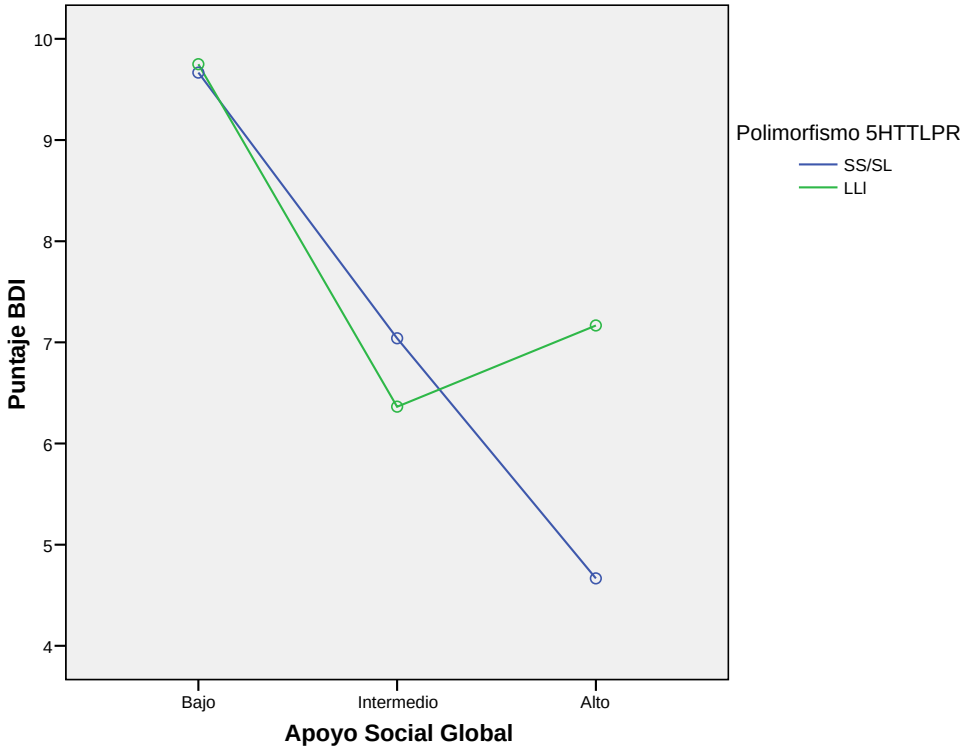
We estimated the simple linear regression of the depressive symptomatology over the level of social support and tested the hypothesis that both variables are related negatively. We found that the slope β=-.334 was statistically significant F(1,115)=14.48, p<.000 and therefore accept the hypothesis of linear relation between depressive symptomatology and level of social support. Per each unit increase in the social support scale, the depressive symptomatology measured by BDI decreases 0.14. The value of R^2 was .112, indicating that 11.2% of the variance in depressive symptomatology was explained by the level of social support. The residue analysis showed that the assumptions of linearity, normality and homoscedasticity are met, but not that of independence (Durbin-Watson=1.465). The analysis of influence was carried out; it was found that when excluding influential cases according to D de Cook, the significance of the model is lost F(1,105)=3.846, p=.053.

A factorial ANOVA analysis was done to analyse the presence of interaction between the genotype and the level of social support on depressive symptomatology. For this purpose the results of the MOS-SSS global questionnaire were subdivided according to percentiles: 25 (score <69, low social support), 25-85 (score 69-90, intermediate social support) y 85 (score >90, high social support). In addition, for the

analysis, we used the average results obtained in the sample both for the global index as well as for the subscales.

The factorial ANOVA shows that the effect of the level of social support on depressive symptomatology according to the 5HTTLPR polymorphism was not significant, F (2,72)=.388, p=.680, indicating that there is no interaction. Main effects were not observed either. The **Figure 20** shows that the carriers of the SS/SL with high levels of social support have lower BDI average score than LL subjects (4.667 vs. 7.167, p = .430), but this difference is not significant. We analysed the assumptions of ANOVA and found that it meets the assumption of homoscedasticity (Levene=.055) but does not meet the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000); however, we assume that the test is robust and allows for the non-compliance of this assumption.

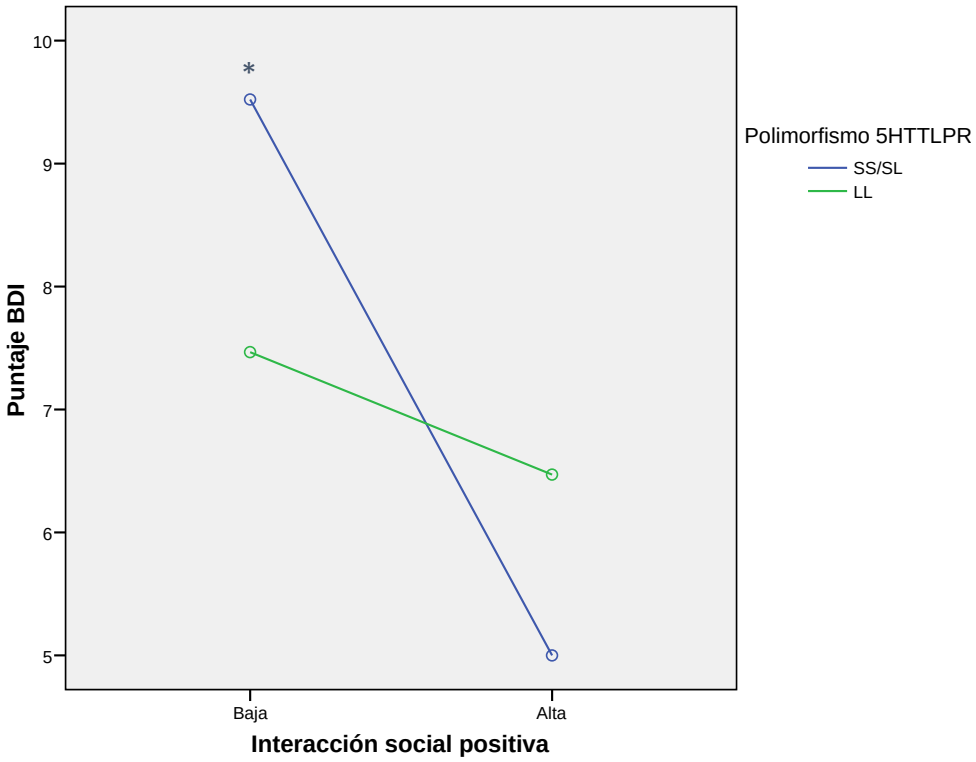
Figure 20 Relation between global social support and depressive symptoms according to 5HTTLPR polymorphism



When analysing per subscales, we found a main effect of positive social interaction on depressive symptomatology. Subjects with high positive social interaction present lower BDI scores, F(1,74)=4.239, p=.043 (**Figure 21**). The figure shows that the SS/SL carriers with low positive social interaction have a BDI average

higher than the SS/SL subjects with high positive social interaction (9,522 vs. 5.0, p = .010). In LL subjects this differences are not observed. We analysed the ANOVA assumptions and found that the assumption of homoscedasticity (Levene=.314) was met, but the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=.000) is not met; however, we assume that the test is robust and allows for the non-compliance of this assumption.

Figure 21 Relation between positive social interaction and depressive symptomatology according to the 5HTTLPR polymorphism



Attachment

Of the total number of subjects (N=151), 120 answered the ECR-S[12] questionnaire together with the BDI. We observed a positive correlation between anxious attachment and depressive symptomatology. In relation to the type of attachment the sample distributed itself in the following way: (1) secure attachment (n=39; 32.2%), (2) preoccupied attachment (n=25; 20.7%), (3) dismissing attachment (n=33; 27.3%) y (4) fearful attachment (n=24; 19.8%). No significant differences were observed between type of attachment and gender (X^2=.374; p=.945).

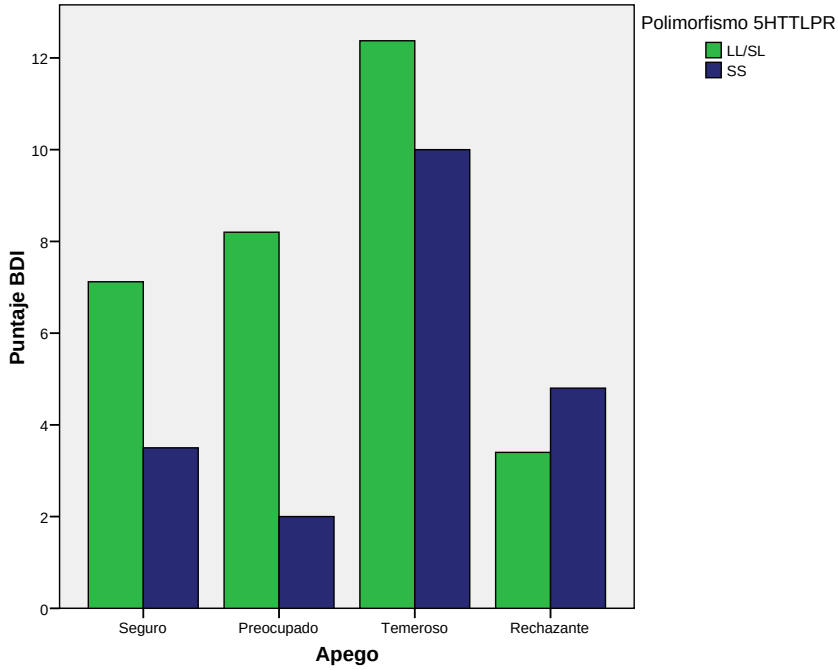
Analysing the simple linear regression of depressive symptomatology over anxious attachment, we validated the hypothesis that both variables are related positively and found that the slope β=.442 was statistically significant F(1,118)=28.63, p<.000; therefore, we accept the hypothesis of linear relation between depressive symptomatology and anxious attachment. Each unit of increase, in the dimension of anxious attachment in the ECR[12] scale, the depressive symptomatology measured by BDI increases 0.4 points. The value of R^2 was .195, indicating that 19.5% of the variance in depressive symptomatology is explained by the level of anxious attachment. The residue analysis showed that the assumptions of linearity, normality and homoscedasticity are met, but not that of independence (Durbin-Watson=1.41). An analysis of influence was carried out and showed that when excluding the potentially influential and influential cases, the model continues to be significant.

We performed a factorial ANOVA to analyse the presence of interaction between the genotype and the type of attachment over depressive symptomatology. For the analysis of the subscales of attachment we used percentiles (<25, 25-75 y >75) and the four categories of attachment (secure, preoccupied, dismissing and fearful). In this case, the serotonin transporter genes they were grouped as SS and SL/LL.

The factorial ANOVA shows that the effect of the type of attachment over depressive symptomatology according to the 5HTTLPR polymorphism was not significant, F (3,64)=.641, p=.592, indicating that there is no interaction. There exists a main effect of the type of attachment. In those subjects with fearful attachment, the depressive symptomatology was significantly higher F(3,64)=3.359, p=.024. We analysed the assumptions of ANOVA and found that the neither assumption of homoscedasticity (Levene=.003) nor the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000) are met; however, we assume that the test is robust and allows for the non-compliance of these assumptions.

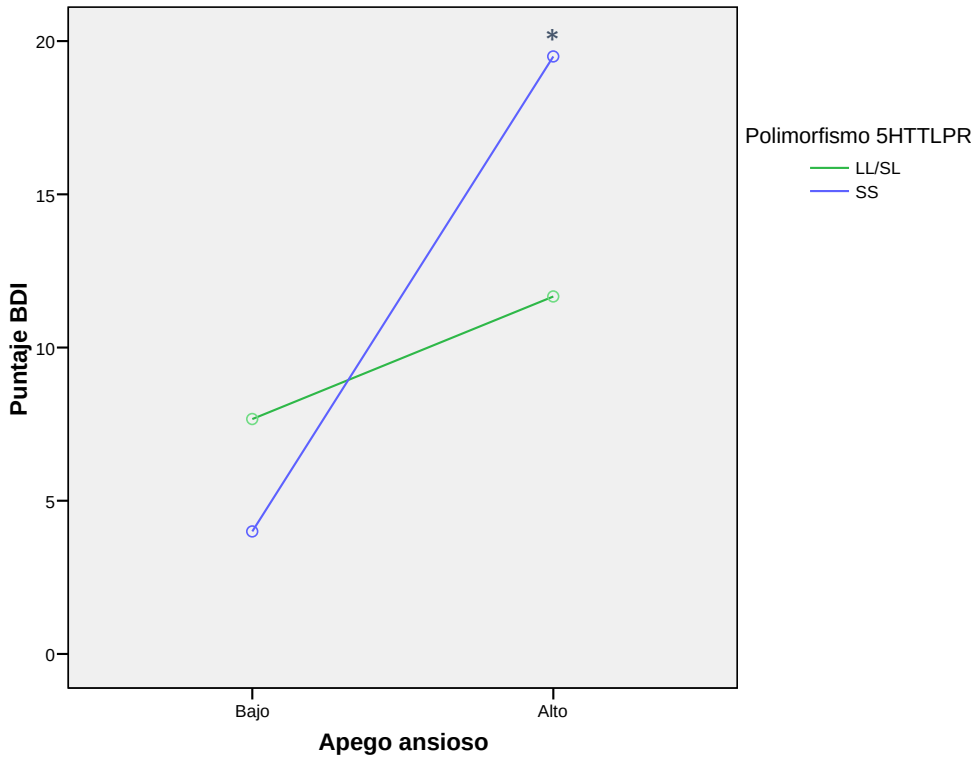
In Figure 22 it is shown that SS subjects have lower BDI scores with secure attachment that subjects LL/SL (BDI = 3.5 versus 7.12, p = .250), but these differences were not significant

Figure 22 Interaction between 5HTTLPR polymorphism and type of attachment on depressive symptoms



When analysing the subscale of anxious attachment according to the extreme percentiles (<25 y >75), we observe an interaction besides the main effect of the type of attachment. The SS subjects with high anxious attachment obtained BDI scores significantly higher as compared to the ones obtained by SS subjects with low anxious attachment, $F(1,32)=3.943$, $p=.056$. This difference is not observed in L carriers subjects. For subjects carrying the S allele, the BDI average with low anxious attachment is 4 and with high anxious attachment is19.5, while for subjects carrying the L allele with low anxious attachment the BDI average is 7.67 and with high anxious attachment is 11.67. In both groups of genes those individuals who have high levels of anxious attachment, show higher levels of depressive symptoms, $F(1,32)=11.336$, $p=.002$. We analysed the ANOVA assumptions and found that the assumption of homoscedasticity (Levene=.697) is met, but not the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000); however, we assume that the test is robust and holds the non-compliance of this assumption. Analyzing avoidant subscale of ECR[12] we found no significant results

Figure 23 Interaction between 5HTTLPR polymorphism and level of anxious attachment on depressive symptoms



Depressive experience

Of the total number of subjects (N=151), 119 answered the DEQ questionnaire together with the BDI. We found a positive correlation between the level of self-criticism, dependency and depressive symptomatology. Regarding the configuration of personality, the sample distributed itself in the following way: anaclitic (n=39; 25.8%), introjective (n=9; 6%), mixed (n=25; 16.6%) and without category (n=47; 31.1%). No significant differences per gender were observed (X^2=1.552; p=.670).

When analysing the simple linear regression of level of self-criticism over depressive symptomatology, we validated the hypothesis that both variables are positively related and found that the slope β=.645 was statistically significant F(1,117)=83.353, p<.000; therefore, accepted the hypothesis of linear relation between depressive symptomatology and level of self-criticism according to DEQ. Every unit of increase in the DEQ self-criticism dimension, increases in 3.42 points the depressive symptomatology measured by BDI. The value of R^2 was .416, indicating that 41.6% of the variance in depressive symptomatology is explained by the level of self-criticism. The residue analysis showed that the assumptions of linearity, normality and

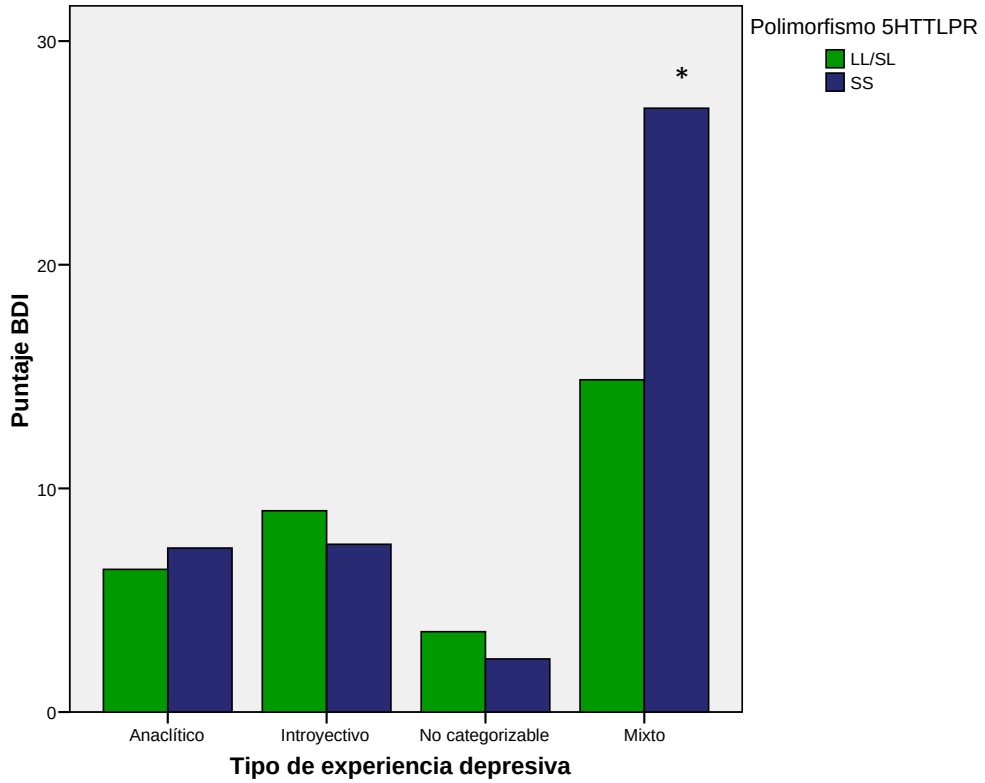
homoscedasticity were met, but not that of independence (Durbin-Watson=1.48). The analysis of influence showed that when excluding the potentially influential and influential cases, the model continues to be significant.

When analysing the simple linear regression of the level of dependency over the depressive symptomatology, we validated the hypothesis that both variables are positively related and found that the slope β=.432 was statistically significant F(1,117)=26.817, p<.000; therefore accept the hypothesis of linear relation between depressive symptomatology and the level of dependency according to DEQ. Every unit of increase in the dependency of DEQ, increases in 2.18 points the depressive symptomatology measured by BDI. The value of R^2 was .186, indicating that 18.6% of the variance in depressive symptomatology is explained by the level of dependency. The residue analysis showed that the assumptions of linearity, normality, homoscedasticity and independence (Durbin-Watson=2.125) are met.

The factorial ANOVA was carried out to analyse the presence of interaction between the genotype and the type of depressive experience on depressive symptomatology. For the analysis of the depressive experience we will use the 4 resulting categories of the DEQ (anaclitic, introjective, mixed and no category) and the percentiles (<25, 25-75 y >75) of the subscales of dependency, self-criticism and efficacy.

The factorial ANOVA shows that the effect of the type of depressive experience on depressive symptomatology according to the 5HTTLPR polymorphism was significant F (3,70)=3.560, p=.018, indicating that there is interaction. Those SS subjects exhibiting depressive mixed type experience obtained significantly higher BDI scores than LL/SL subjects, F (1,70) = 9.793, p = .003 (**Figure 24**). In addition, a main effect of the type of depressive experience was observed. The subjects with mixed depressive experience category obtain significantly higher BDI scores than the anaclitic and introjective category and the latter have significantly higher scores than non categorisable category F(3,70)=25.691, p<.000. We analysed the ANOVA assumptions and found that neither the assumption of homoscedasticity (Levene=.037) nor the assumption of normal distribution of the dependent variable (Kolmogorov-Smirnov=0.000) are met; however, we assume that the test is robust and holds the non-compliance of these assumptions

Figure 24 Interaction between 5HTTLPR polymorphism and type of depressive experience over depressive symptoms



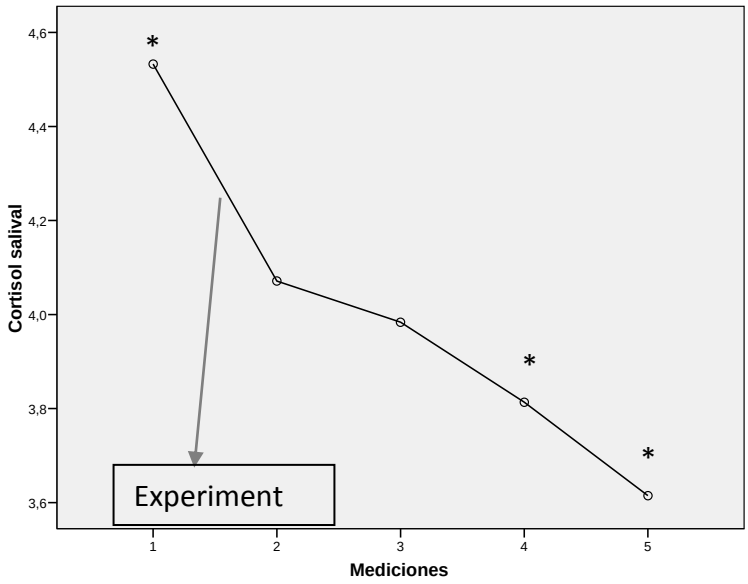
When analysing per subscales, we found that the subjects who present higher levels of dependency, obtain higher BDI scores, $F(2,75)=3.552$, $p=.034$, and that the subjects with higher levels of self-criticism obtain higher BDI scores , $F(2,75)=22.063$, $p<.000$. We did not found interaction between the 5HTTLPR polymorphism and the level of dependency or self-criticism of depressive experience scale over depressive symptomatology. The subscale of efficacy did not showed any significant relations or differences.

Cortisol curve analysis

An analysis of variance (ANOVA) for repeated measures was conducted to evaluate the variation in salivary cortisol before the experiment (measure 1, basal), at the end of the task (measure 2), within 10 minutes of the end of the task (measure 3), within 20 minutes of the end of the task (measure 4), and within 30 minutes after application of experimental task (measure 5). We found a significant effect of time on the level of cortisol, Wilks Lambda $(4,84) = 0.566$, $F (4,84) = 16.086$, $p <.000$, showing a

significant decrease in the level of salivary cortisol among all measures except between the second and third.

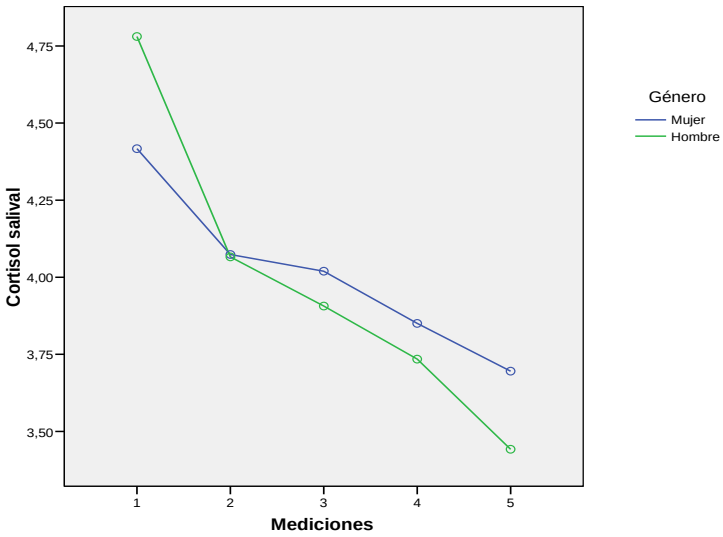
Figure 25 Salivary cortisol variation over time



Cortisol curve and gender

A mixed analysis of variance (ANOVA) was performed to evaluate the variation of salivary cortisol before, during and after the experimental task controlling by gender. We found no significant interaction between gender and cortisol curve, Wilks Lambda (4,83) =. 914, F (4,83) = 1.953, p = .109.

Figure 26 Salivary cortisol variation over time according to gender



Cortisol curve, gender and 5HTTLPR polymorphism

A mixed analysis of variance (ANOVA) was performed to evaluate variation salivary cortisol before, during and after the experimental task by gender of the subjects. We found no significant interaction between gender, 5HTTLPR polymorphism and cortisol curve, Wilks Lambda (4,81) =. 956, F (4,83) =. 927, p = .453.

Figure 27 Salivary cortisol variation over time in SS/SL polymorphism 5HTTLPR carriers according to gender

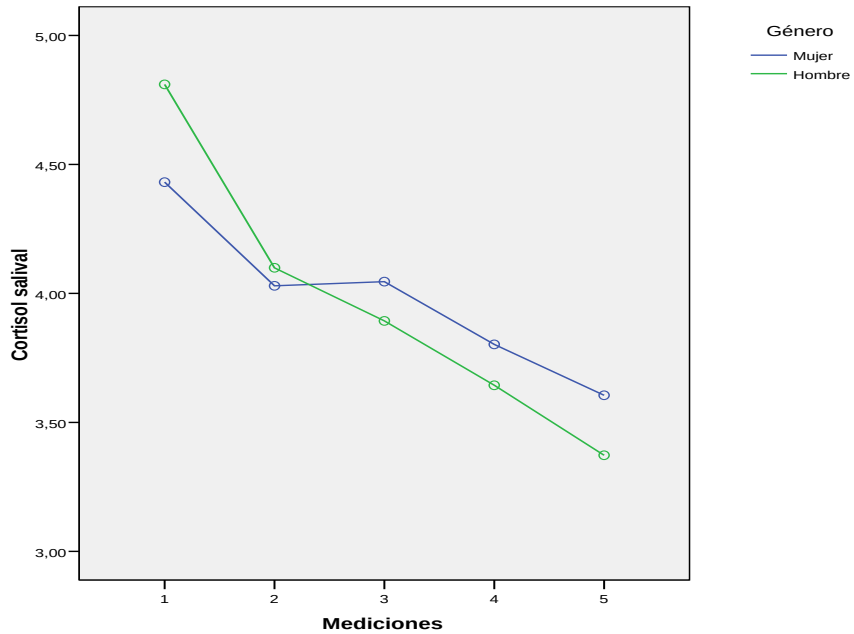
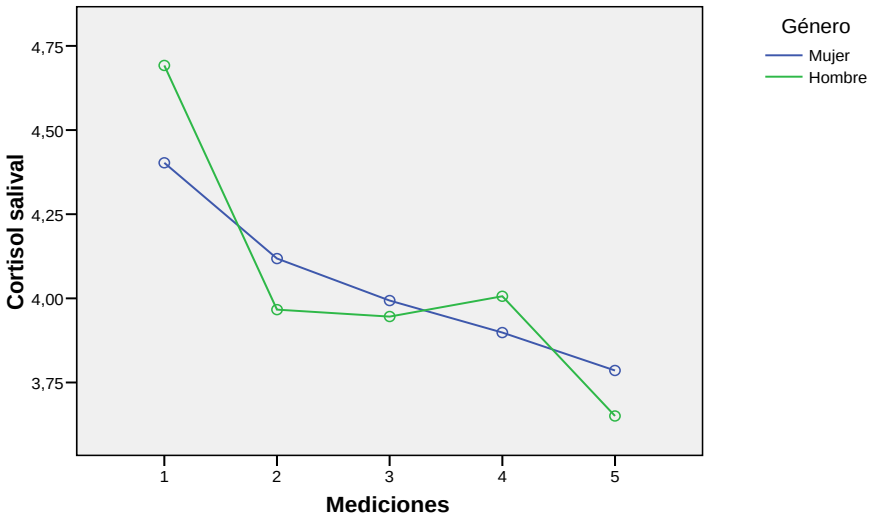


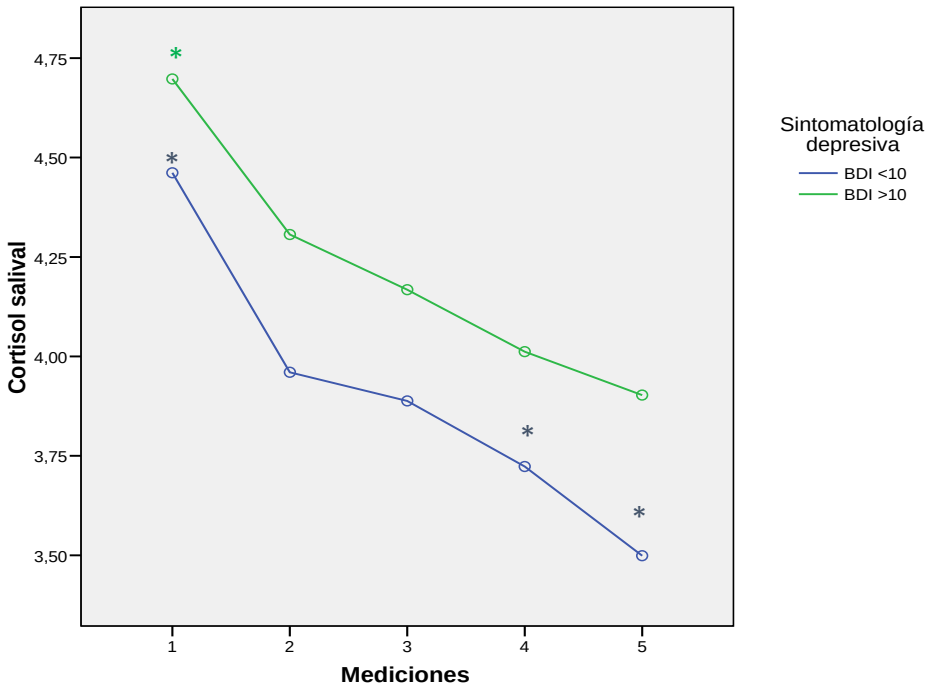
Figure 28 Salivary cortisol variation over time in LL polymorphism 5HTTLPR carriers according to gender



Cortisol curve and depressive symptoms

A mixed analysis of variance (ANOVA) was performed to evaluate variation of salivary cortisol before, during and after the experimental task based on depressive symptoms. We found no significant interaction between depressive symptoms and cortisol curve, Wilks Lambda (4,76) =. 983, F (4,76) =. 335, p = 0.853. But it is observed that the curve of subjects with lower depressive symptoms show significant differences between all measures of cortisol, except between 2 and 3 measure, Lambda Wilks (4,76) = 0.610, F (4,76) = 12,158, p <.000, whereas in subjects with higher depressive symptoms, significant differences are only observed between 1 and 2 measure, ie the decrease of the cortisol curve is slower in subjects with higher depressive symptomatology, the curve is flatter, Wilks Lambda (4.76) = 0.822, F (4,76) = 4.125, p = .004.

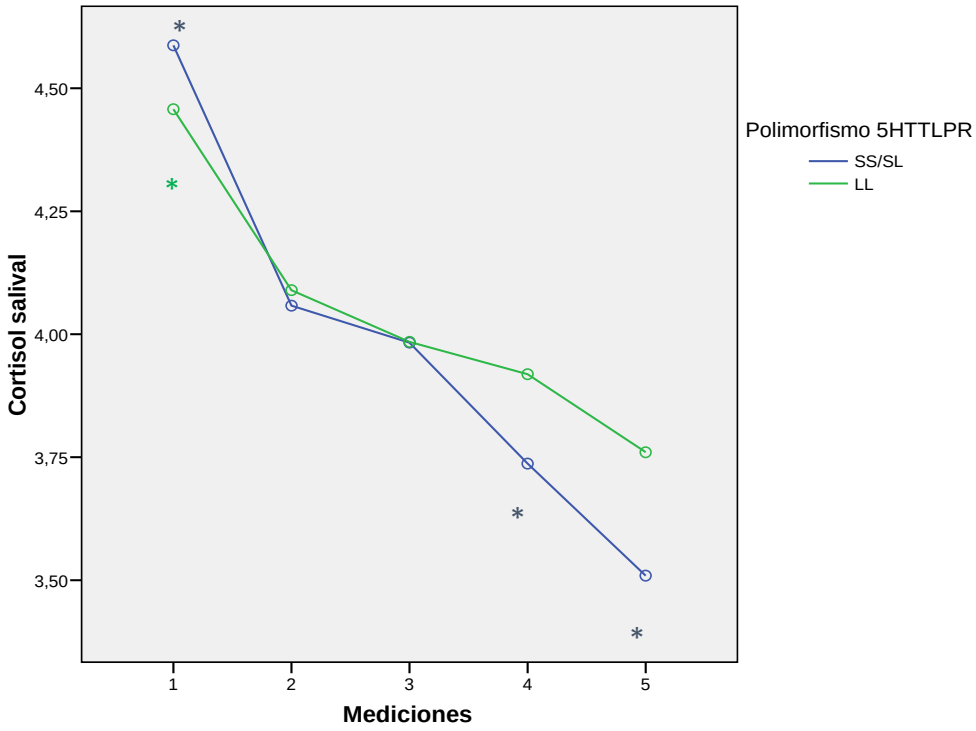
Figure 29 Salivary cortisol variation over time according to depressive symptoms



Cortisol curve and 5HTTLPR polymorphism

A mixed analysis of variance (ANOVA) was performed to evaluate the variation of salivary cortisol before, during and after the experimental task according to 5HTTLPR polymorphism. We found no significant interaction between genotype and time, but a main effect of time on the level of cortisol Wilks Lambda (4,83) =. 581, F (4,83) = 14,968, p <.000. Analysing by genotype we found that the SS/SL subjects show significant differences in all measures except between the second and third, Wilks Lambda (2,83) =. 610, F (4,83) = 13,279, p <.000. Regarding the LL, we observed significant differences only between the first and second measures, Wilks Lambda (4,83) =. 834, F (4,83) = 4.116, p = .004. I.e. the curve flattens.

Figure 30 Salivary cortisol variation over time according to 5HTTLPR polymorphism



Cortisol curve, depressive symptoms and 5HTTLPR polymorphism

Regarding depressive symptoms, 5HTTLPR polymorphism and cortisol curve, no double or triple interaction was observed. Between cortisol and polymorphism, Wilks Lambda (4.74) = 975, F (4,74) = 467, p = .760; between cortisol and depressive symptoms, Wilks Lambda (4,74) =. 980, F (4,74) =. 375, p = .926; and between cortisol, depressive symptoms and 5HTTLPR polymorphism, Wilks Lambda (4.74) = .990, F (4,74) =. 181, p = .948. When analysing the decrease in curves, it is observed that the SS / SL subjects with lower depressive symptoms show significant differences between all measures except between 2 and 3 measurement Lambda Wilks (4,74) =. 674, F (4,74) = 8.932, p <.000, while the SS / SL subjects with major depressive symptoms (BDI> 10) have only significant difference between 1 and 2 measurement, Wilks Lambda (4,74) =. 851, F (4,74) = 3.230, p = .017. In LL subjects with lower depressive symptomatology significant difference is observed only between 1 and 2 measure Lambda Wilks (4,74) =. 830, F (4,74) = 3.791, p = 0.007, while in the higher depressive symptomatology no significant differences were observed between cortisol measurements, Wilks Lambda (4,74) =. 947, F (4,74) = 1.032, p = .397.

Figure 31 Salivary cortisol variation over time in SS/SL polymorphism carriers based on depressive symptomatology

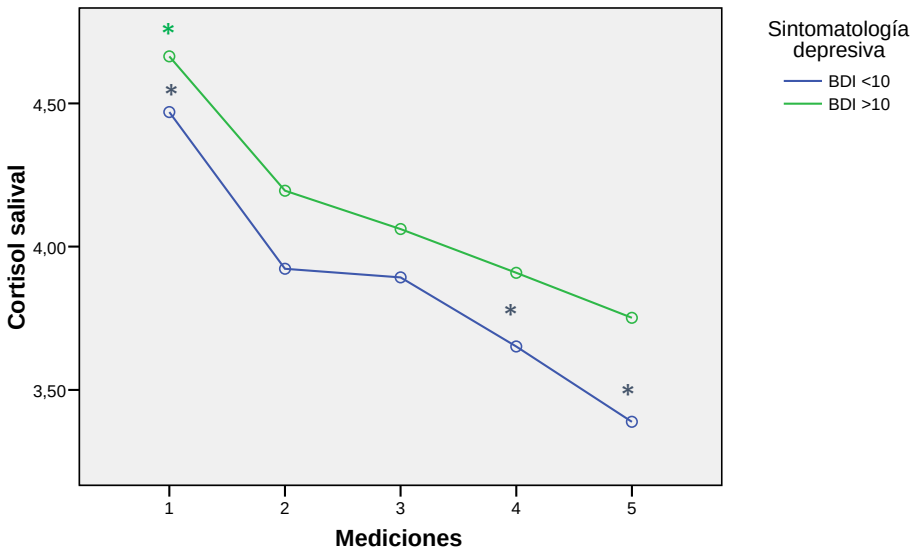
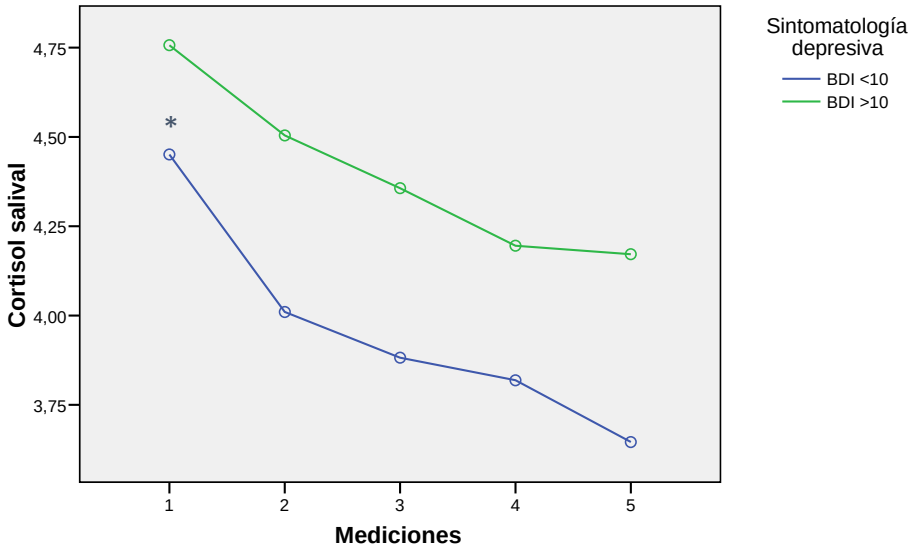


Figure 32 Salivary cortisol variation over time in LL polymorphism carriers based on depressive symptomatology

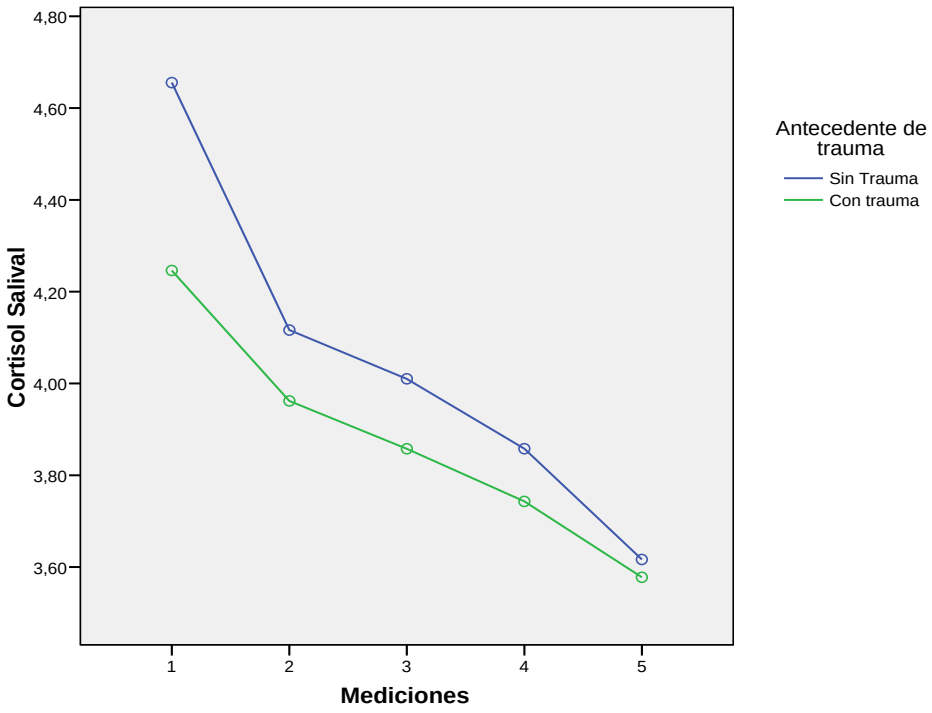


Cortisol curve and trauma

Analysing the history of trauma we did not observe an interaction between history of trauma and cortisol curve, Wilks Lambda (4,73) =. 969, F (4,73) =. 580, p = .678. But we found that subjects without trauma showed significant differences in all measures except between the second and third, Wilks Lambda (4,73) =. 579, F (4,73) = 13.250, p

<.000. While in subjects with a history of trauma we did not found significant differences between measures of salivary cortisol in time, Wilks Lambda (4,73) =. 859, F (4,73) = 3.003, p = .024. I.e. the curve of subjects with trauma is flatter, decrease slower than in subjects without a history of trauma.

Figure 33 Salivary cortisol variation over time according to history of trauma



Cortisol curve, trauma and 5HTTLPR polymorphism

With respect to the history of trauma, polymorphism and cortisol curve, no double or triple interaction was observed. Interaction between 5HTTLPR polymorphism and cortisol curve, Wilks Lambda (4.71) = 969, F (4,71) = 568, p = .686; between cortisol and a history of trauma, Wilks Lambda (4,71) =. 963, F (4,71) =. 610, p = .678 and between cortisol, history of trauma and polymorphism 5HTTLPR Wilks Lambda (4.71) =. 981, F (4,71) =. 348, p = .844. When analysing the decrease in curves, we observed that the differences between the measures of cortisol in the group without trauma remain in both polymorphisms, and disappear in the group with trauma in both polymorphisms, i.e. trauma flattens cortisol curve in both polymorphisms.

Figure 34 Salivary cortisol variation over time of SS/SL polymorphism carriers according to the antecedent of trauma

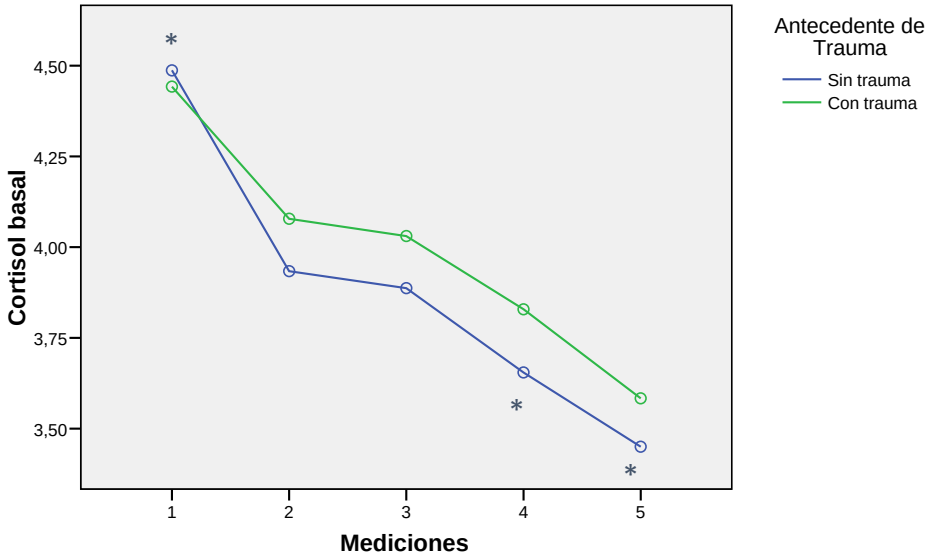
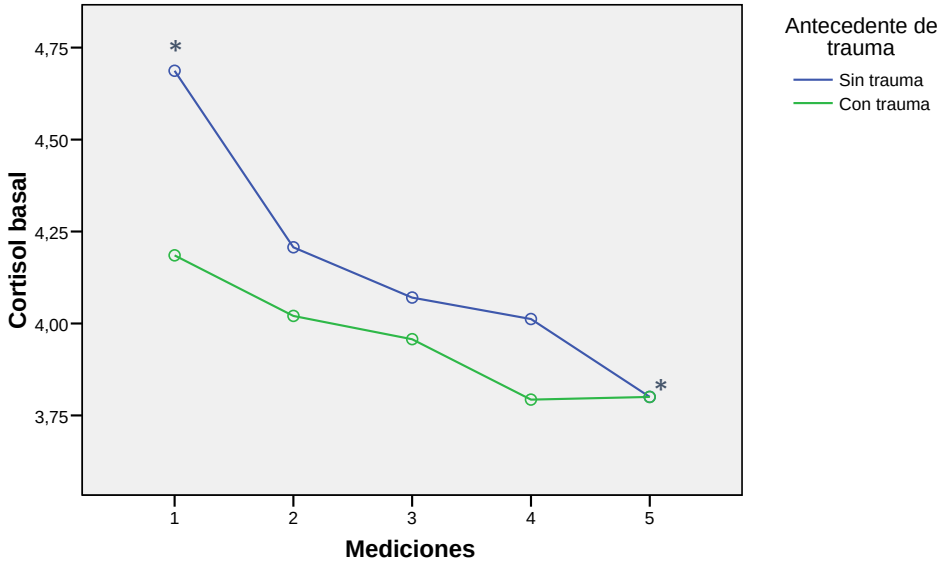


Figure 35 Salivary cortisol variation over time of LL polymorphism carriers according to the antecedent of trauma

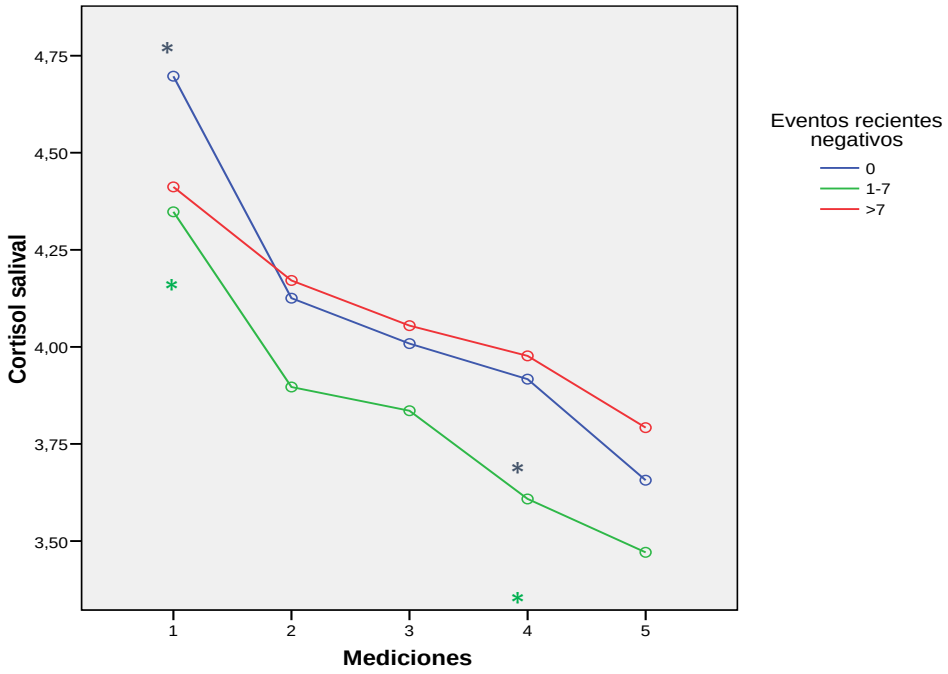


Cortisol curve and recent negative life events

Analysing by recent life events no interaction was observed between the number of recent negative events of moderate to large effect and the cortisol curve, Lambda Wilks (8,148) =. 950, F (8,148) =. 484, p = .866. But we found that subjects with fewer recent

negative events have more significant differences between measures of cortisol in time, without recent negative events Lambda Wilks is (4,74) =. 701, F (4,74) = 7.90, p <.000, and for those with between 1 and 7 recent negative events Lambda Wilks is (4,74) =. 749, F (4,74) = 6.189, p <.000. Subjects with more than 7 recent negative events (> p85) did not showed significant differences between measures of salivary cortisol in time, Wilks Lambda (4,74) =. 903, F (4,74) = 1.987, p = .105. I.e. the cortisol curve of those with more recent negative events is slower, is flatter.

Figure 36 Salivary cortisol variation over time according to recent negative events



Cortisol curve, recent negative life events and 5HTTLPR polymorphism

Respect to the number of recent negative events, polymorphism and cortisol curve, no double or triple interaction was observed. Interaction between 5HTTLPR polymorphism and cortisol showed a value of Wilks Lambda of (4.71) = 968, F (4,71) = 591, p = .670; between cortisol and number of recent negative events, Wilks Lambda (8,142) =. 950, F (8,142) =. 464, p = .880 and between cortisol, number of recent negative events and 5HTTLPR polymorphism Wilks Lambda (8,142) = .966, F (8,142) =. 308, p = .962. Analysing the differences in decrease of cortisol curves, in the SS/SL group, subjects with more than 7 negative events do not differ on cortisol measures, Wilks Lambda (4,71) =. 936, F (4,71) = 1.207, p = .316, while subjects with less than 7 negative events

show differences between measures of cortisol (subjects without recent negative events: Wilks Lambda (4,71) =. 779, F (4,71) = 5.026, p = .001; subjects that reported between 1-7 recent life events: Lambda Wilks (4 , 71) =. 773, F (4,71) = 5.215, p = .001). In the LL group, subjects that reported more than 1 recent negative event do not show significant differences on cortisol measures (for 1-7 recent negative events: Wilks Lambda (4,71) =. 933, F (4,71) = 1.275, p = .288 and for more than 7 recent negative events: Wilks Lambda (4,71) =. 931, F (4,71) = 1.315, p = .273), but if they do not report recent negative events, there are significant differences between cortisol measures: Wilks Lambda (4.71) = .861, F (4,71) = 2.868, p = .029. Although cortisol curve for LL subjects with more than 7 recent negative events has higher values of cortisol that subjects without the antecedent of recent negative events, these differences are not significant (**figure 38**).

Figure 37 Salivary cortisol variation over time in SS/SL polymorphism carriers according to recent negative events

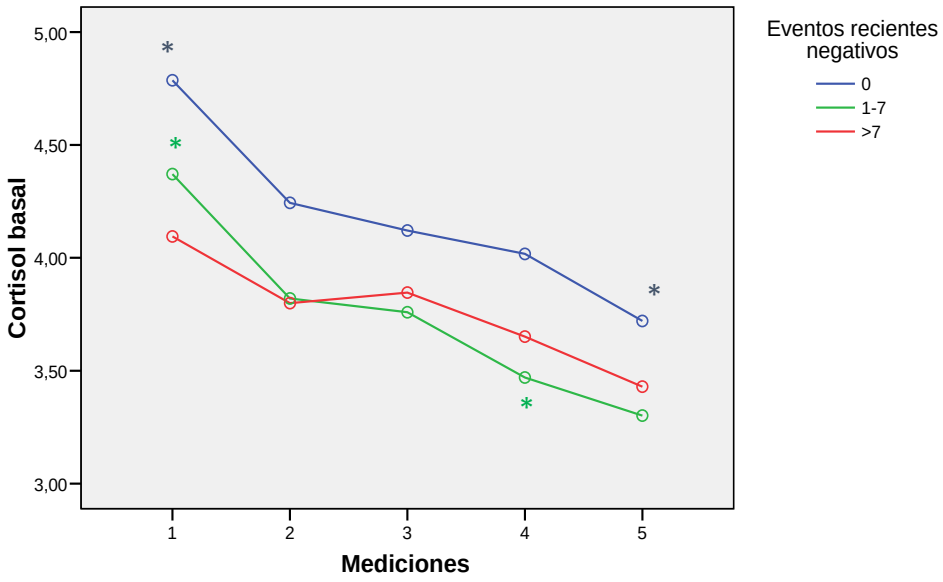
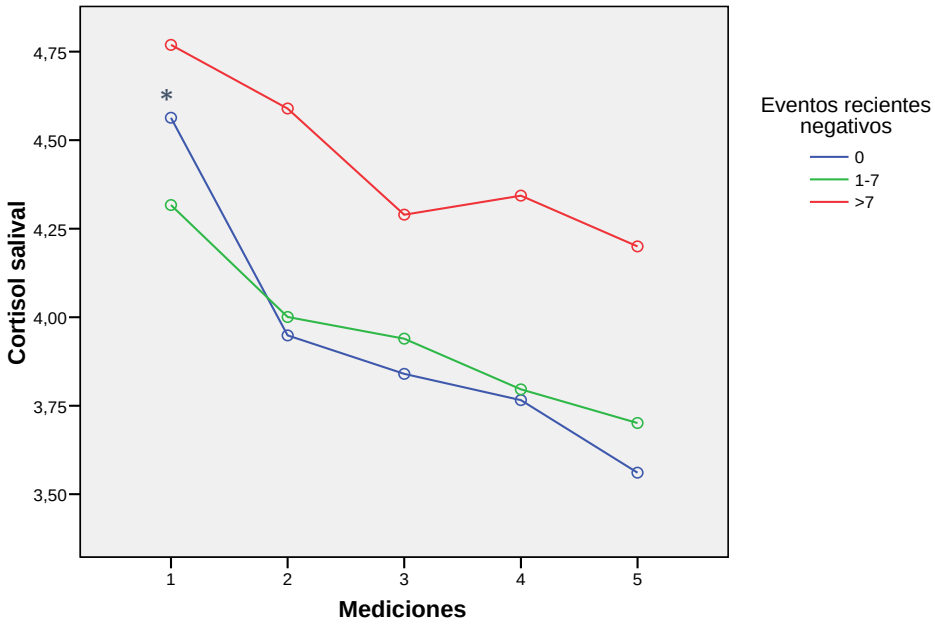


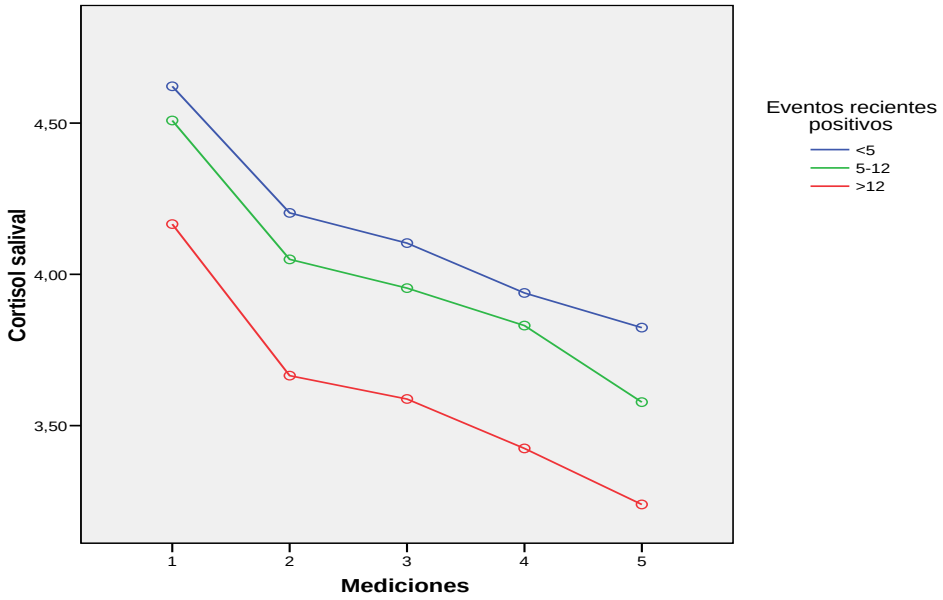
Figure 38 Salivary cortisol variation over time in LL polymorphism carriers according to recent negative events



Cortisol curve and recent positive events

Analysing by recent positive life events no interaction was observed between the number of recent positive events of moderate to large effect and the cortisol curve, Lambda Wilks (8,148) =. 981, F (8,148) =. 178, p =. 994. But the figure shows that subjects with more recent positive events present lower cortisol levels, but this difference is not significant. The decrease in cortisol curve has no difference between the groups of more or less positive events.

Figure 39 Variation in salivary cortisol over time according to the number of recent positive events



Cortisol curve, recent positive events and 5HTTLPR polymorphism

Regarding the number of recent positive events, polymorphism and cortisol curve, no double or triple interaction was observed. Between 5HTTLPR polymorphism and cortisol measures, Wilks Lambda (4.34) = 966, F (4,34) = 297, p = .878; between cortisol and number of recent positive events, Wilks Lambda (4,34) =. 976, F (4,34) =. 206, p = .933; and between cortisol, number of recent positive events and polymorphism, Wilks Lambda 5HTTLPR (4,34) =.902, F (4,34) =.463, p = .463. We observed in SS/SL group a significant difference in cortisol mean between individuals who have less than 5 and more than 12 positive events (F (1,37) = 4.486; p = .041), this difference is not observed in the LL group (F (1,37) ==, 135; p = 0.716). The S-carriers with <5 positive events have an average of 4.326 cortisol and subjects with more than 12 positive events have an average of 3.414. Analysing where are these differences, we observed significant cortisol mean differences on measure 2 (p = .054), 4 (p = .015) and 5 (p = .022). No difference in the rate of descent of the curve between groups of polymorphisms or number of recent positive events were observed.

Figure 40 Salivary cortisol variation over time in the SS/SL polymorphism carriers group according to the number of recent positive events

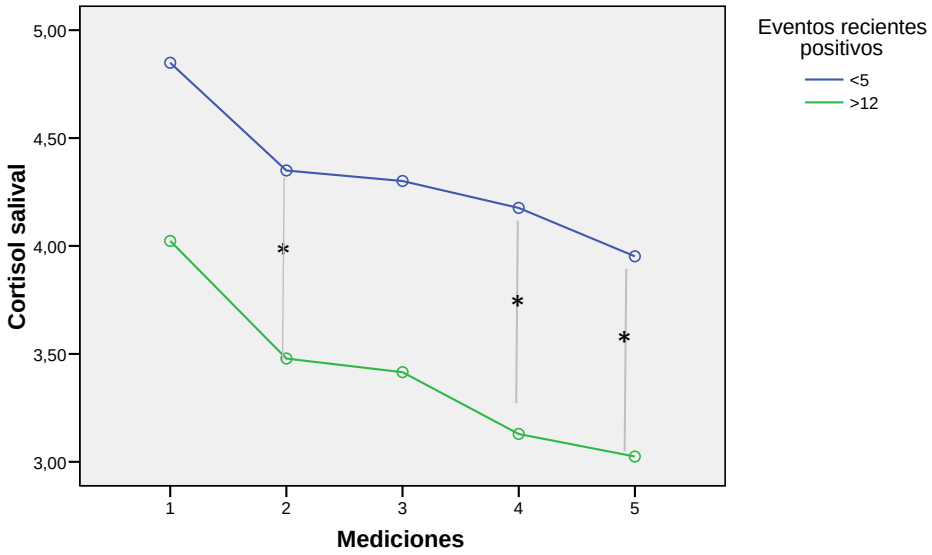
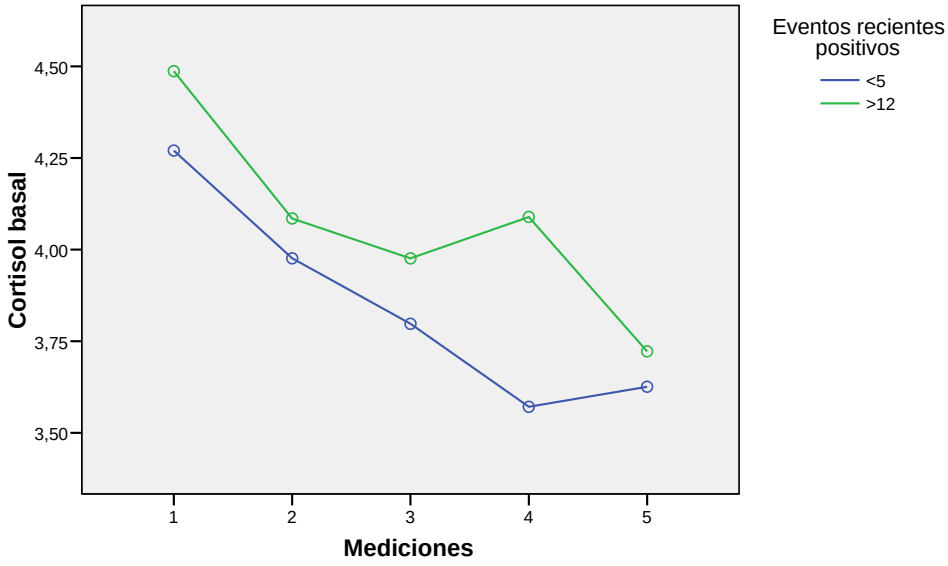


Figure 41 Salivary cortisol variation over time in the LL polymorphism carriers group according to the number of recent positive events

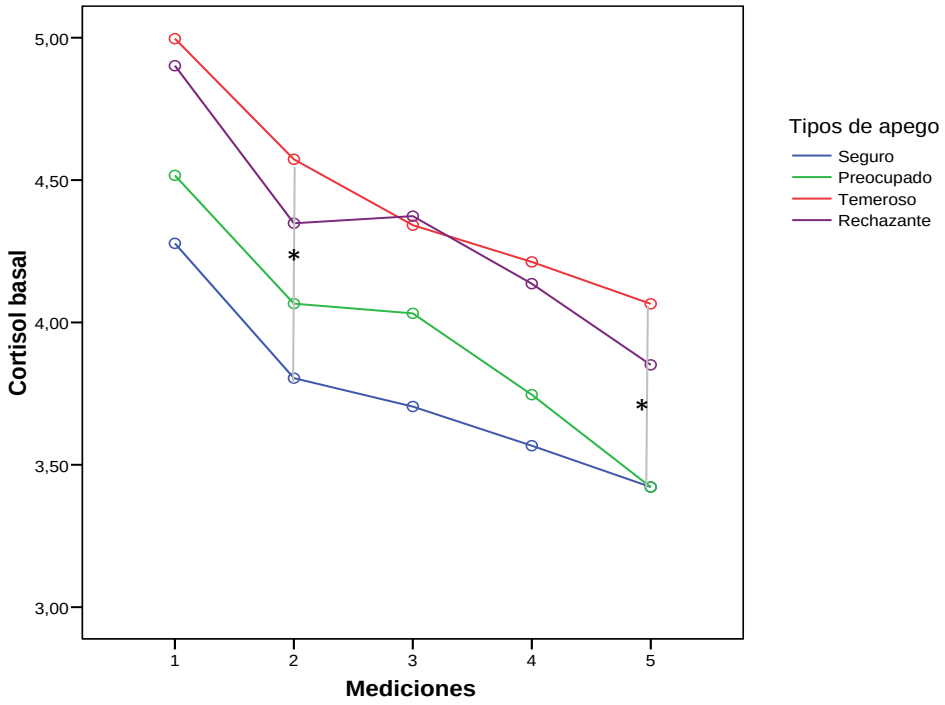


Cortisol curve and attachment

No interaction was observed between the type of attachment and cortisol curve, Wilks Lambda (12, 174.911) =. 915, F (12, 174.911) =. 500, p = .913. No difference was observed in the rate of descent of the curve of cortisol in the 4 types of attachment. As shown in figure 42, secure attachment shows lower levels cortisol, although this

difference is only significant when comparing secure attachment with fearful attachment in the 2 and 5 cortisol measure (p = .036 and .047, respectively).

Figure 42 Salivary cortisol variation over time depending on the type of attachment



Cortisol curve, type of attachment and 5HTTLPR polymorphism

About the type of attachment, polymorphism and cortisol curve, no double or triple interaction was observed. Interaction between cortisol and 5HTTLPR polymorphism, Wilks Lambda (4.62) = 958, F (4,62) = 672, p = .614; between cortisol and type of attachment, Wilks Lambda (12, 164.382) =. 893, F (12, 164.382) =. 596, p = .844; and between cortisol, type of attachment and 5HTTLPR polymorphism, Lambda Wilks (12, 164.382) =. 808, F (12, 164.382) = 1.150, p = .327. The intersubject test shows a significant interaction between 5HTTLPR polymorphism and type of attachment (F(3,65)=3,046; p=.035). The average values of cortisol for secure attachment are significantly lower than those of the fearful and dismissing attachment (p = .025 and .042, respectively). Moreover, in the SS/SL group is a significant difference between the cortisol average for preoccupied attachment and fearful attachment (p = .049), with no other significant differences observed. In the LL group, a significant difference

between the cortisol average for secure and preoccupied attachment (p = .09) and fearful attachment (.034), and dismissing attachment (p = .017) were observed.

Comparing the groups of polymorphism and attachment type, we observed that in the preoccupied attachment group, SS/SL subjects had a lower cortisol average than LL subjects (F (1,65) = 6.442, p = .014). Analysing this difference, it appears that the SS/SL group has significantly lower average of cortisol than the LL group in measure 2, 3, 4 and 5. There is no difference in the rate of descent of the curves of cortisol according to the type of attachment and polymorphism.

Figure 43 Salivary cortisol variation over time SS/SL polymorphism carriers group according to the type of attachment

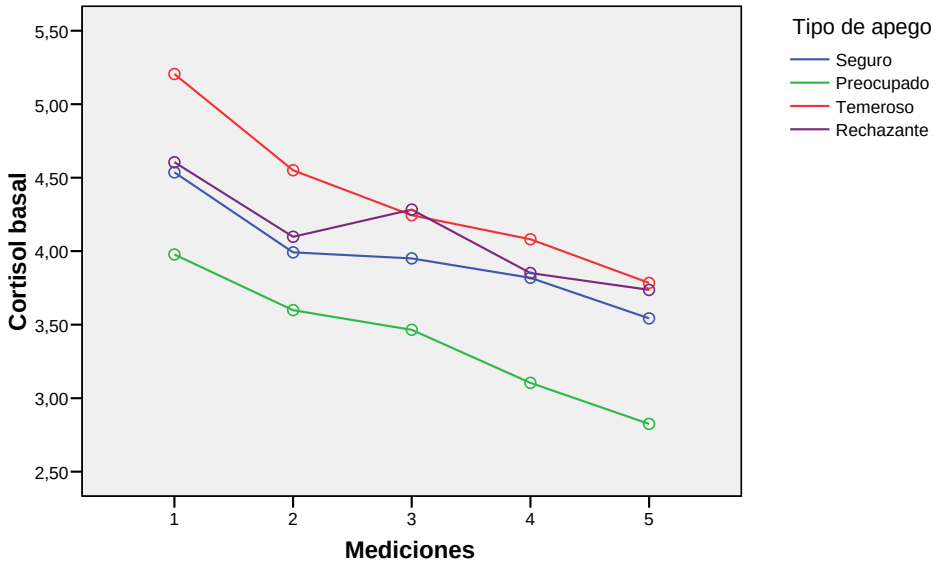
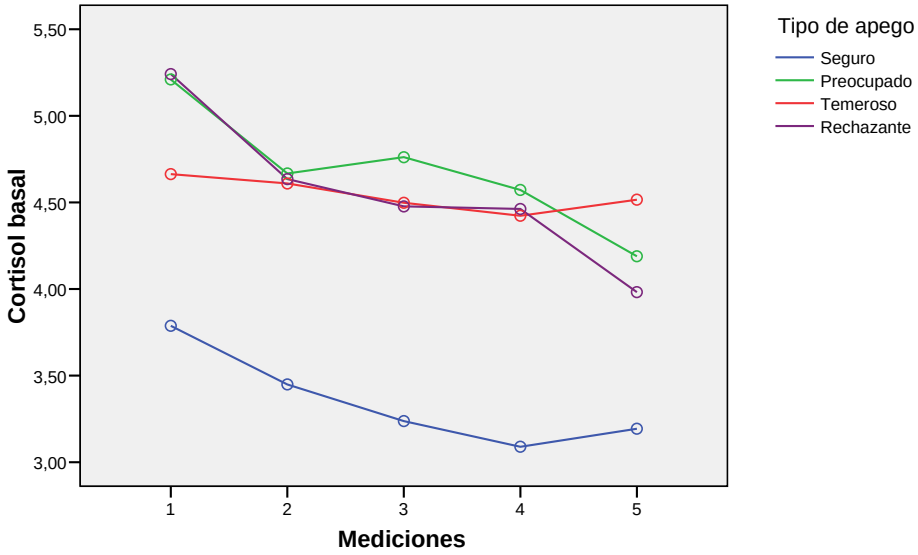


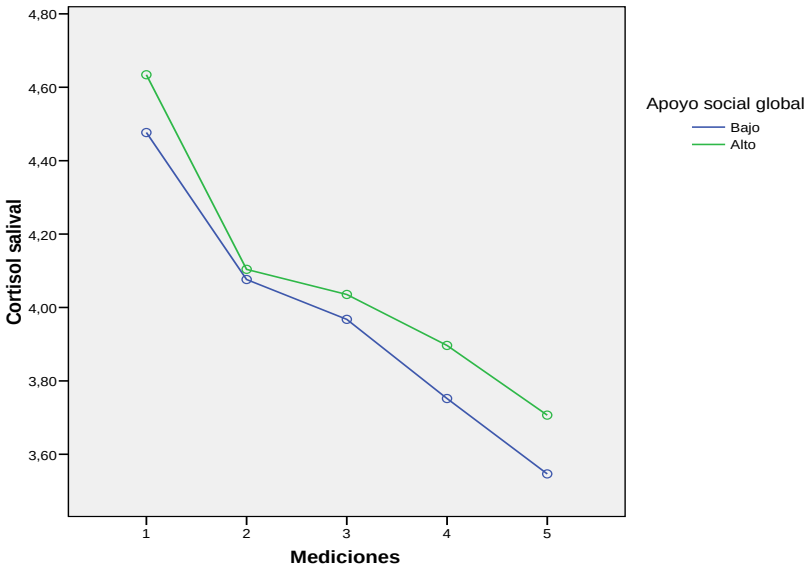
Figure 44 Salivary cortisol variation over time LL polymorphism carriers group according to the type of attachment



Cortisol curve and social support

A mixed analysis of variance (ANOVA) was performed to evaluate the variation on salivary cortisol before, during and after the experimental task according to global social support. We found no significant interaction between social support and cortisol curve.

Figure 45 Salivary cortisol variation over time according to global social support



Cortisol curve, global social support and 5HTTLPR polymorphism

Regarding social support, polymorphism and cortisol curve, no double or triple interaction was observed. Interaction between cortisol and 5HTTLPR polymorphism, Wilks Lambda (4.71) = 972, F (4,71) = 514, p = .726; between cortisol and social support, Wilks Lambda (4,71) =. 968, F (4,71) =. 590, p = .671 and between cortisol, social support and 5HTTLPR polymorphism, Wilks Lambda (4.71) = 0.965, F (4,71) =. 638, p = 0.637. No differences between the averages of cortisol or the rate of decrease of cortisol curves was observed.

Figure 46 Salivary cortisol variation over time in SS/SL polymorphism carriers group according to the level of social support

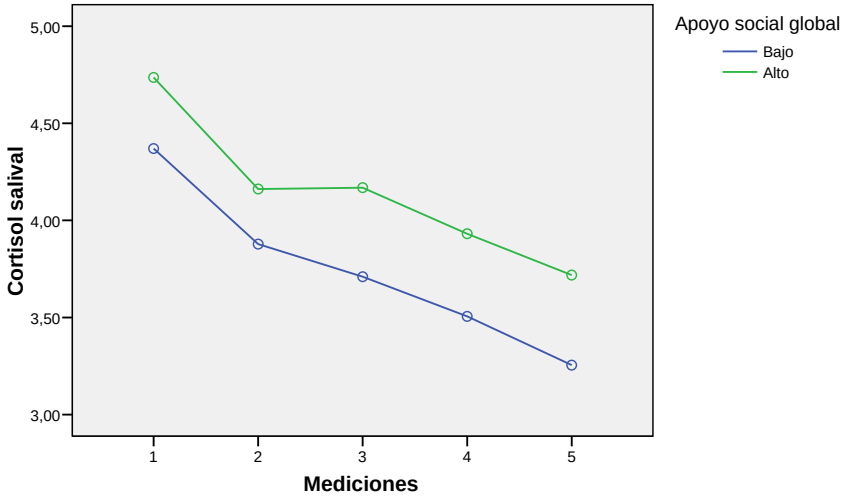
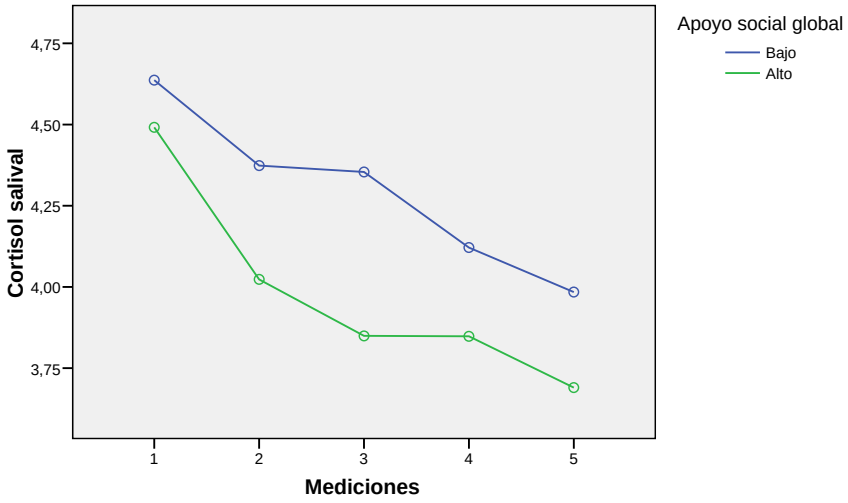


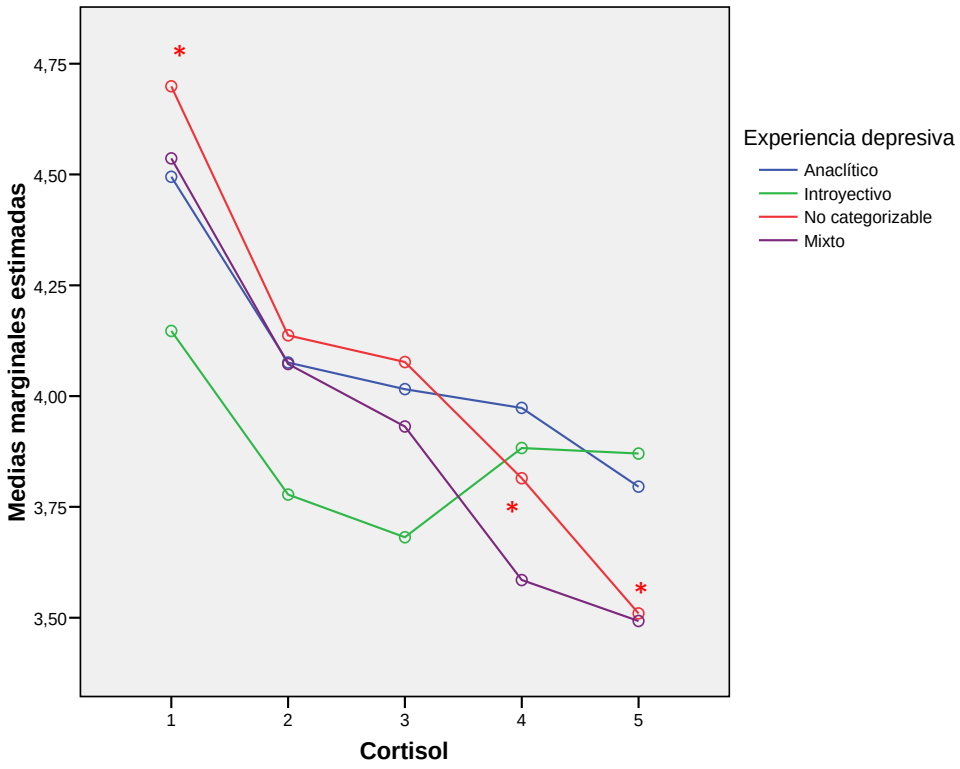
Figure 47 Salivary cortisol variation over time in LL polymorphism carriers group according to the level of social support



Cortisol curve and depressive experience

A mixed analysis of variance (ANOVA) was performed to evaluate the variation on salivary cortisol before, during and after the experimental task according to the depressive experience. We found no significant interaction between depressive symptoms and the curve of cortisol, Wilks Lambda (12,188,140) = 0.792, F (12188140) = 1.448, p = 0.147. But it is noted that the curve of subjects with not categorizable type of depressive experience differ significantly between all cortisol measures, except between 2 and 3 measurement, Lambda Wilks (4,71) =. 607, F (4, 71) = 11,502, p <0.000, while in the rest of the subjects, these significant differences are not observed, ie the decrease is slower, the curve is flatter, (for introjective group: Lambda Wilks (4 , 71) =. 953, F (4,71) =. 870, p = .487, for anaclitic group: Wilks Lambda (4,71) =. 850, F (4,71) = 3.130, p = .020 and for mixed group: Lambda Wilks (4.71) = 771, F (4,71) = 5.263, p = .001).

Figure 48 Salivary cortisol variation in time according to the type of depressive experience



Cortisol curve, depressive style and 5HTTLPR polymorphism

Regarding the type of depressive experience, polymorphism and cortisol curve, no double or triple interaction was observed. Interaction between cortisol and 5HTTLPR polymorphism, Wilks Lambda (4,67) = 963, F (4,67) = 647, p = .631; between cortisol and type of depressive experience, Wilks Lambda (12,177.557) =. 792, F (12,177.557) = 1.360, p = .189 and between cortisol, depressive experience and polymorphism 5HTTLPR, Wilks Lambda (12,177.557) =. 871, F (12,177.557) =. 795, p = .665. No differences between the averages of cortisol or the rate of decrease of the cortisol curves was observed.

Figure 49 Salivary cortisol variation in time in SS/SL polymorphism carriers group according to the level of social support

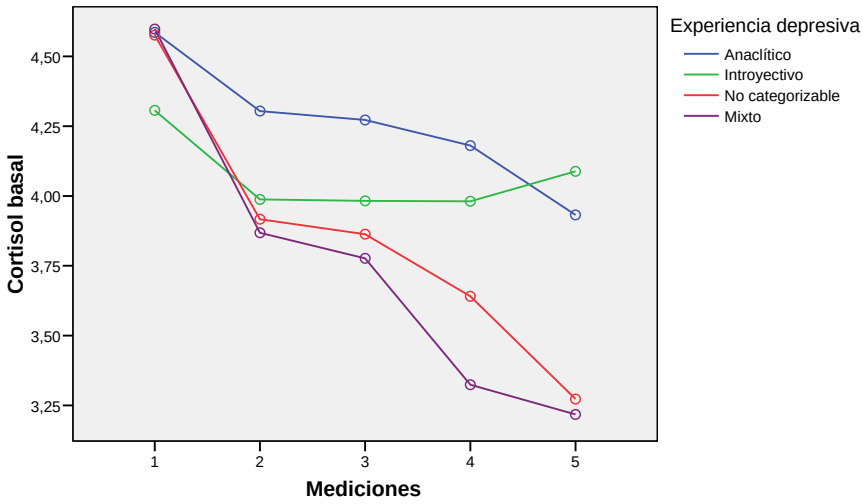
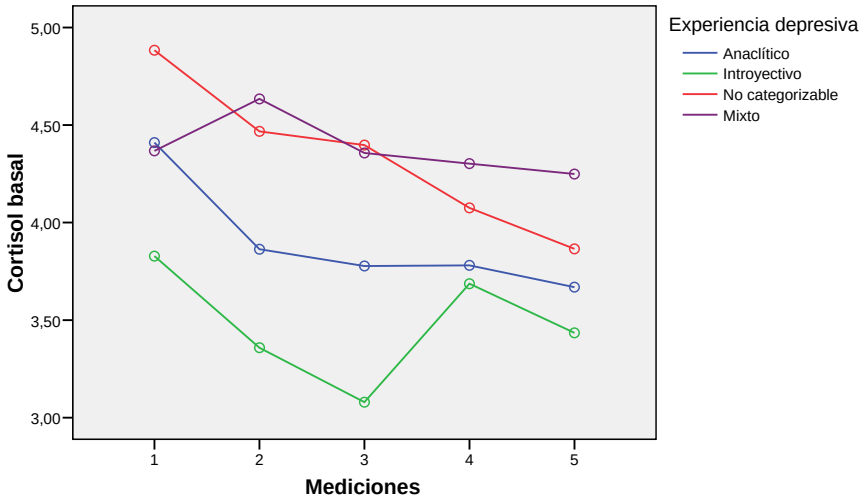


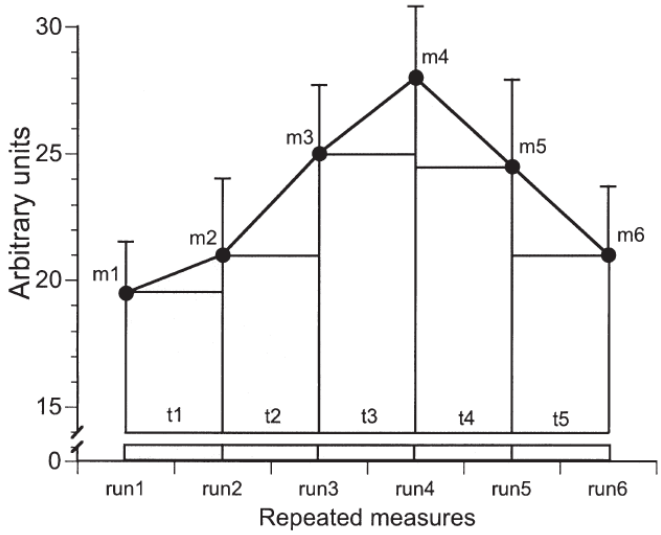
Figure 50 Salivary cortisol variation in time in LL polymorphism carriers group according to the level of social support



Area under the curve of cortisol

We calculate the area under the curve of cortisol, using the trapezoid method described by Pruessner (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). That is, the area under the curve of cortisol with respect to the ground (AUCg, Cortisol response curve with respect to ground), as an indicator of the total release of cortisol during the experiment. The figure shows how the calculation is done

Figure 51 Calculation of area under the curve



Note Obtenaid from Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology, 28(7), 916-931.

We performed a correlation between AUCg and the variables studied and found no significant correlation between AUCg curve cortisol and depressive symptoms, 5HTTLPR polymorphism, and environmental and personality variables included in the study. In general, the AUCg cortisol curve was not different by gender, 5HTTLPR polymorphism, or for environmental and personality variables. **Table 10** shows the value of AUCg cortisol curve for each category of variable.

Table 10

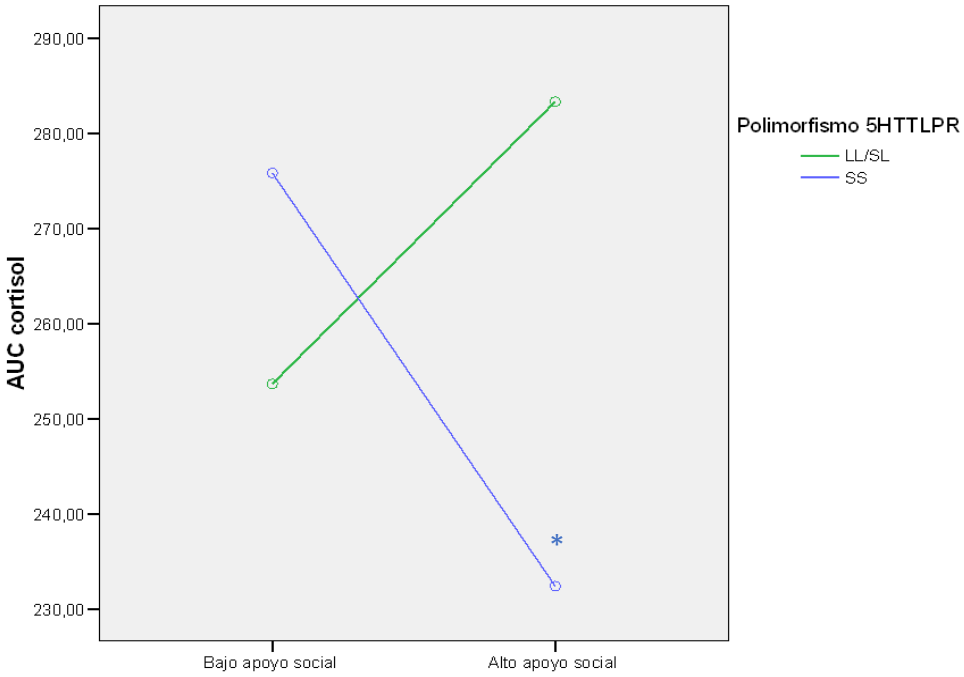
Area under the cortisol curve

		AUCg Cortisol Mean (DS)	F(df)	Significance
5HTTLPR	SS	255.17 (85.7)	.317 (1,75)	p=.575
	LL/SL	266.61 (70.91)		
Gender	Female	255.28 (67.32)	2.17 (1,75)	p=.145
	Male	281.319 (84.3)		
Trauma	Low	259.36 (68.66)	.397 (2,65)	p=.531
	High	271.2 (66.0)		
Recent Negative Events	Low	268.46 (75.28)	.328 (2,66)	p=.721
	Intermediate	254.32 (63.89)		
	High	265.54 (62.3)		
Recent Positive Events	Low	268.666 (72.25)	.678 (2,66)	p=.511
	Intermediate	263.28 (69.37)		
	High	240. 50 (48.94)		
Anxious Attachment	Low	269.99 (76.6)	.183 (2,59)	p=.833
	Intermediate	261.14 (71.97)		
	High	274.09 (72.88)		
Social support	Low	258.24 (75.80)	.643 (1,65)	p=.426
	High	271.26 (57.13)		
Depressive Experience	Not categorizable	272.29 (74.3)	.473 (3,64)	p=.702
	Anaclitic	254.65 (60.51)		
	Introjective	243.45 (45.5)		
	Mixt	270.44 (67.59)		

We conducted a factorial ANOVA between AUCg, 5HTTLPR polymorphism, and environmental and personality variables.

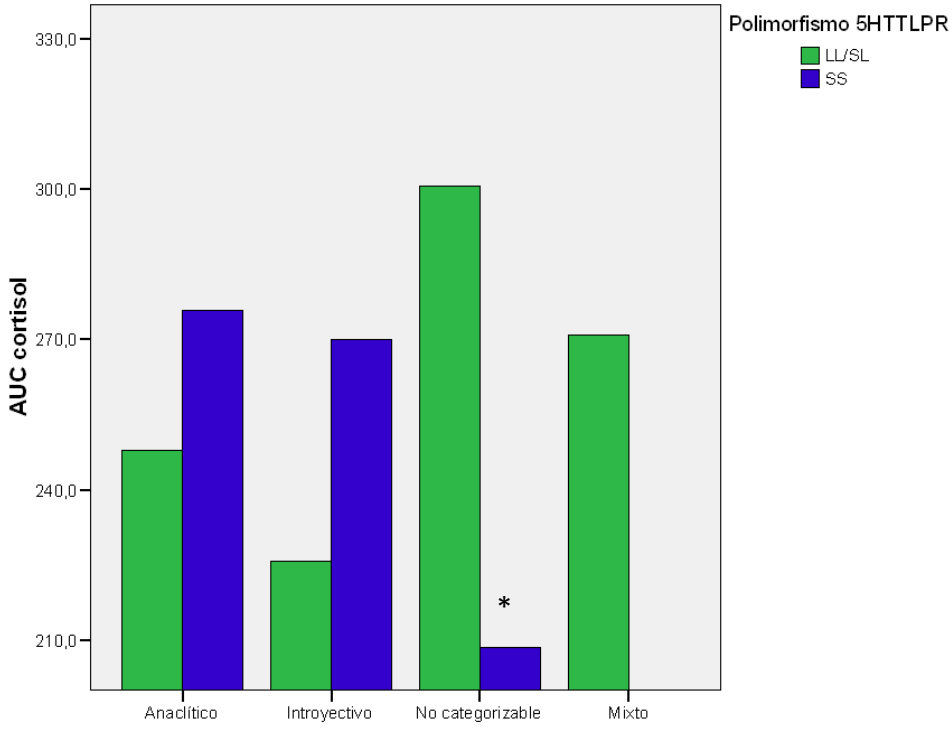
An almost significant interaction between social support and 5HTTLPR polymorphism on AUC average was observed, F (1,63) = 3.614, p = .062. With high social support, the SS group has a significantly smaller area under the curve (p = .043) than L allele carriers. No differences were observed between genotype groups with low social support. No significant differences in the genotype group with high or low social support are observed.

Figure 52 Interaction between 5 HTTLPR polymorphism and level of social support on the area under the curve of salivary cortisol



When analysing the area under the curve, the interaction between 5HTTLPR polymorphism and depressive experience, we found a significant interaction F (2,61) = 5.335, p = .007. In the category not categorizable (low self-criticism and dependency) of depressive experience, SS group has a significantly minor area under the cortisol curve tan L carriers. No significant differences were observed in the other categories of depressive experience.

Figure 53 Interaction between 5 HTTLPR polymorphism and type of depressive experience on the area under the curve of salivary cortisol



We found no other significant interactions or main effects of the variables included the study over the area under the cortisol curve.

Cortisol Delta

As an indicator of change of cortisol released during the experiment we calculate the cortisol delta, i.e., the difference between the last measured cortisol and baseline cortisol. We observed no correlation between cortisol delta and the variables included in the study. Nor we observe interactions between environmental variables and personality and 5HTTLPR polymorphism to predict delta cortisol.

Classification and Regression Tree

A classification and regression tree was constructed by using the "Classification and Regression Trees" method (CART), (Hodar et al., 2010) to predict the categoric variable "depressive symptomatology" (BDI > or < 10 points) using as explanatory

variables the genotype (5HTTLPR polymorphism), personality and the "environmental" variables (**Figure 54**).

The tree finds the best question regarding the value of each explanatory variable. The model indicates which variable can be used sequentially and which is the cut-off point that best classifies. The variables are selected looking for the combination (variable and coefficient) where the offspring nodes (branches) obtain less impurities (Gini index). The whole process is repeated for each node till the final nodes are reached (leaves), for which a new classification does not significantly improve the prediction significantly.

In the figure, the node (circle) contains the n and the probability of being depressed (P (BDI> 10), and in the branches is written the question, and when the branch ends we arrive at a terminal leaf that goes in square.

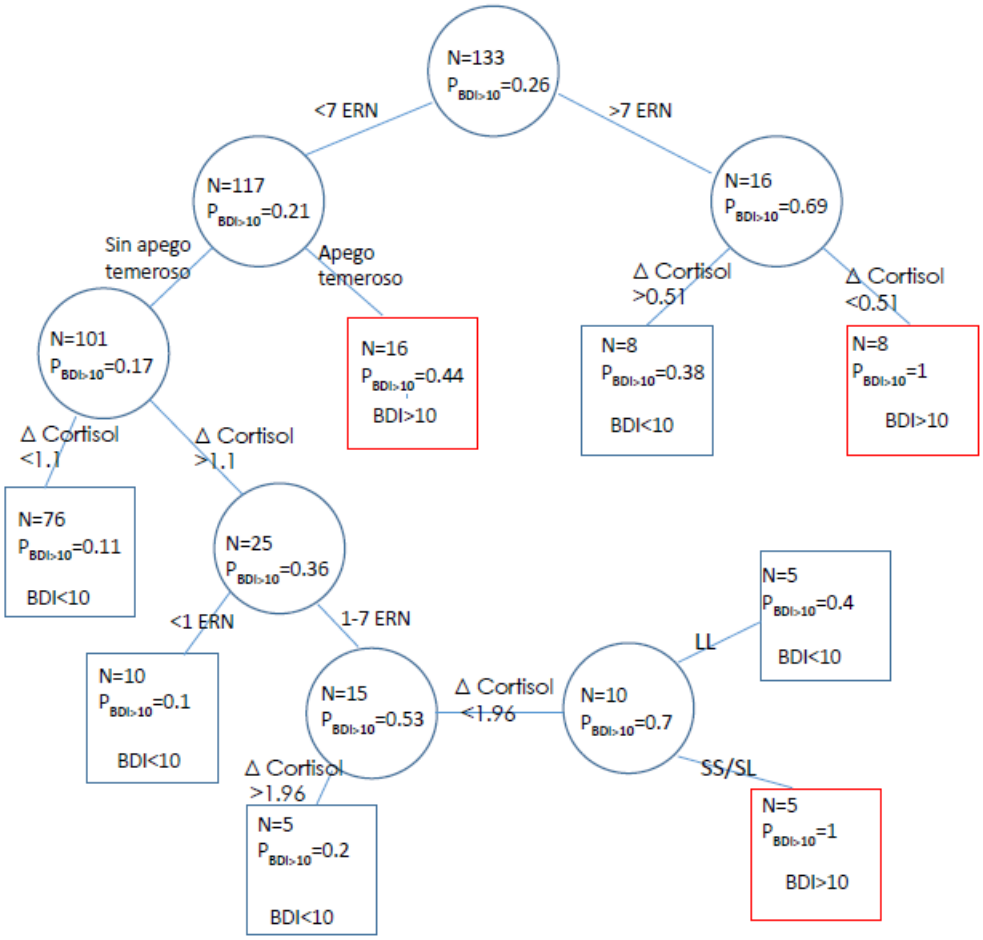
This tree has a general accuracy: .842, and sensitivity to detect depression is: .457. The specificity for depression are: .98.

We found that the variable more than 7 recent negative life events in addition to <.51 delta cortisol presented the highest prediction of depressive symptomatology (BDI>10).

Furthermore having less than 7 negative life events, but fearful attachment type also predicts depressive symptoms (BDI> 10) but with a lower probability (.44).

It is observed that being carrier of the S allele is of risk for depressive symptoms (BDI> 10) when subjects have had between 1 and 7 recent events, not fearful attachment and cortisol delta between 1.1 and 1.96. Increasing the likelihood of having a BDI> 10, from 0.4 to 1, although this difference is not significant, X^2 (1, N = 10) = 4.29, p = 0.17, ns.

Figure 54 Classification and Regression Tree to predict depression



Summary of results

General

- 151 subjects were studied of which 7 were patients diagnosed with depression and 144 volunteer subjects. Of the total number of subjects, 88 (58.3%) were gentotypified and 139 responded the BDI (28 male, 60 female, mean age=22.4±7.99). No significant differences are observed between genotypified and not genotypified group.
- The genotypic frequency was LL=37 (42%), LS=33 (37.5%) y SS=18 (20.5%). The allelic frequency was S=0.39 y L=0.61. This distribution is not in Hardy-Weinberg' balance (EHW)
- There is no significant differences in the average of the studied variables between genetic polymorphisms groups.
- There is no correlation between the genotype or the alleles groups and the studied variables. Nor is there any correlation between baseline cortisol and the variables studied. There is a positive correlation between the level of depressive symptomatology and the following variables: recent negative events, child trauma, anxious attachment, level of dependency and self-criticism. The strongest correlation is between the level of depressive symptomatology and recent negative events and self-criticism. There is a negative correlation between the level of depressive symptomatology and social support.
- 27.3% of the total sample (n=38 subjects, 29 females and 9 males) presented a score higher than the cut-off point established to define minimum depression (BDI>10). The percentage of volunteer subjects with a score higher than BDI>10 drops to 24.6%, (n = 33, women 28.6%, n = 26 and men 16.3%, n = 7 subjects), No significant differences are observed between gender groups.
- 29.2% (n=35) present a history of positive childhood trauma. No difference between genders.
- 86% (n=62) presented negative events during last year of moderate to severe intensity. The mean of number of negative events with moderate to severe effect was 4.54 (ds=4.7), without significant differences in the reported by male and females.
- 95.7% (n=67) presented moderate-to-severe positive events during last year. The average of total positive events with moderate-to-severe effect was 7.9 (ds=4.18), without significant differences between gender.

- The average of friends and family members considered as close was 7.93 (DS=5.96) showing no significant differences per gender.
- In relation to the type of attachment, the simple showed 32.2% of secure attachment (n=39) and 67.8% of the simple presents insecure attachment (preoccupied attachment, dismissing attachment or fearful attachment). No significant differences were observed between type of attachment and gender.
- Regarding the configuration of personality, the sample distributed itself in the following way: anaclitic n=39 (25.8%), introjective n=9 (6%), mixed n=25 (16.6%) and without category n=47 (31.1%). No significant differences per gender were observed
- We found that the experimental task was not stressing as the cortisol curve showed a significant decrease in the level of salivary cortisol among time, but without presenting a pick.

Summary of the results of the interaction analysis of environmental and personality factors with 5HTTLPR polymorphism to predict depressive symptoms

- Trauma: that there is no interaction of trauma on depressive symptomatology according to 5HTTLPR polymorphism. Only the main effect of general trauma on depressive symptomatology was significant. Subjects with a history of childhood trauma have higher depressive symptoms.
- Recent negative life events (RNLE): There is no interaction between recent negative events on depressive symptomatology according to the 5HTTLPR polymorphism. But, we observed a main effect of negative events. Subjects that presented more than 7 negative events scored significantly higher in BDI.
- Recent positive life events (RPLE): There is no interaction between recent positive events on depressive symptomatology according to the 5HTTLPR polymorphism. We did not observed main effects of the genotype or positive events groups. That is, reporting more positive events over the past year is not associated with the levels of depressive symptomatology in this study. There was also no significant negative correlation with the depressive symptomatology score.
- Delta RPLE-RNLE: To analyse if there is a relation between the number of positive and negative events, we analyse the difference between both variables. We did not found an interaction, but we found a main effect of the difference. In those subjects that presented a low difference between recent positive and

negative events, the average in the BDI score is higher in those that have a high difference.

- Social support: When analysing per subscales, we found a main effect of positive social interaction on depressive symptomatology. Subjects with high positive social interaction present lower BDI scores. The figure shows that the SS/SL carriers with low positive social interaction have a BDI average higher than the SS/SL subjects with high positive social
- Attachment: When analysing the subscale of anxious attachment according to the extreme percentiles (<25 y >75), we observe an interaction besides the main effect of the type of attachment. The SS subjects with high anxious attachment obtained BDI scores significantly higher as compared to the ones obtained by SS subjects with low anxious attachment. This difference is not observed in L carriers subjects (LL/LS). In both groups of genes those individuals who have high levels of anxious attachment, show higher levels of depressive symptoms.
- Depressive experience: There is interaction between the effect of the type of depressive experience on depressive symptomatology according to the 5HTTLPR polymorphism. Those SS subjects exhibiting depressive mixed type experience obtained significantly higher BDI scores than LL/SL subjects. In addition, a main effect of the type of depressive experience was observed. Subjects with mixed depressive experience category obtain significantly higher BDI scores than the anaclitic and introjective group and the latter have significantly higher scores than non categorisable group.

Summary of the results of the cortisol curve analysis and environmental and personality factors according to the 5HTTLPR polymorphism

- Cortisol: We found a significant effect of time on the cortisol level, showing a significant decrease of salivary cortisol level throughout the measures.
- Depressive symptoms: We found no significant interaction between depressive symptoms and cortisol curve. But we observed that the curve of subjects' higher depressive symptoms, the decrease of the cortisol curve is slower, the curve is flatter, than in subjects with lower depressive symptoms.
- Polymorphism 5HTTLPR: We found no significant interaction between genotype and time, but we found that the SS/SL subjects show faster decrease curve than LL subjects.

- Depressive symptoms and 5HTTLPR polymorphism: no double or triple interaction was observed. When analysing the decrease in curves, it is observed that the SS/SL subjects with lower depressive symptoms show a faster decrease of the curve than SS/SL subjects with major depressive symptoms (BDI> 10) and LL subjects with low or high depressive symptomatology.
- Trauma: We did not observe an interaction between history of trauma and cortisol curve. But we found that subjects with trauma showed a flatter curve, slower decrease curve.
- Trauma and 5HTTLPR polymorphism: With respect to the history of trauma, polymorphism and cortisol curve, no double or triple interaction was observed. When analysing the decrease in curves, we observed that trauma flattens the cortisol curve of both polymorphism groups.
- Recent negative life events: No interaction was observed between the number of recent negative events of moderate to large effect and the cortisol curve. But we found that subjects with more than 7 RNLE (>p85), the cortisol curve is slower, is flatter, than in those with less RNLE.
- Recent negative life events and 5HTTLPR polymorphism: no double or triple interaction was observed. Analysing the differences in decrease of cortisol curves, in the SS/SL group, subjects with more than 7 negative events shoed flattened curves compared to SS/SL subjects with less than 7 negative events. In the LL group, subjects that reported more than 1 recent negative event show flattened cortisol curves.
- Recent positive events: Analysing by recent positive life events no interaction was observed between the number of recent positive events of moderate to large effect and the cortisol curve.
- Recent positive events and 5HTTLPR polymorphism: no double or triple interaction was observed. We observed in SS/SL group a significant difference in cortisol mean between individuals who have less than 5 and more than 12 positive events, this difference is not observed in the LL group. The S-carriers with more than 12 positive events have a lower cortisol mean (3.414) than individuals who have less than 5 positive events (4.326). No difference in the rate of descent of the curve between groups of polymorphisms or number of recent positive events were observed.

- Attachment: No interaction was observed between the type of attachment and cortisol curve. No difference was observed in the rate of descent of the curve of cortisol in the 4 types of attachment. As shown in figure, secure attachment shows lower levels cortisol.
- Attachment and 5HTTLPR polymorphism: no double or triple interaction was observed. The intersubject test shows a significant interaction between 5HTTLPR polymorphism and type of attachment. The mean values of cortisol for secure attachment are significantly lower than those of the fearful and dismissing attachment. Moreover, in the SS/SL group is observed a significant difference between the cortisol average for preoccupied attachment and fearful attachment, with no other significant differences observed. In the LL group, a significant difference between the cortisol average for secure attachment and preoccupied, fearful and dismissing attachment was observed. Comparing the groups of polymorphism and attachment type, we observed that in the preoccupied attachment group, SS/SL subjects had a lower cortisol average than LL subjects. There is no difference in the rate of descent of the curves of cortisol according to the type of attachment and polymorphism.
- Social support: We found no significant interaction between social support and cortisol curve.
- Social support and 5HTTLPR polymorphism: no double or triple interaction was observed. No differences between the averages of cortisol or the rate of decrease of cortisol curves was observed.
- Depressive experience: We found no significant interaction between depressive symptoms and the curve of cortisol. But it is noted that the curve of subjects with not categorizable type of depressive experience decreases faster than the curves of the other type of depressive experience.
- Depressive style and 5HTTLPR polymorphism: Regarding the type of depressive experience, polymorphism and cortisol curve, no double or triple interaction was observed. No differences between the averages of cortisol or the rate of decrease of the cortisol curves was observed.

Summary of results of Cortisol AUC and environmental-personality variables:

- An almost significant interaction between social support and 5HTTLPR polymorphism on AUC average was observed. With high social support, the SS group has a significantly smaller area under the curve ($p = .043$) than L allele

carriers. No differences were observed between genotype groups with low social support. No significant differences were observed in the genotype group with high or low social support.

- When analysing the area under the curve, the interaction between 5HTTLPR polymorphism and depressive experience, we found a significant interaction. In the category not categorizable (low self-criticism and dependency) of depressive experience, SS group has a significantly minor area under the cortisol curve tan L carriers. No significant differences were observed in the other categories of depressive experience.

DISCUSSION AND CONCLUSIONS

Regarding the limitations of the thesis project, given that the studied population is not at Hardy-Weinberg equilibrium (HWE) for the 5-HTTLPR polymorphism, the results obtained cannot be extended to the Chilean population. HWE states that the genetic composition of a population remains in balance only if natural selection or any other factor are not active and if no mutation occurs. That is, the deviation from equilibrium can be explained when these assumptions have been violated in the study sample. These could include non-random mating in the sample (inbreeding, assortative mating, small size population), mutation—which could have a subtle effect on allele frequencies—and migration, which could affect balance. In this case, the alteration of the HWE might be due to the sample size and migration.

Along with impacting the HWE of the sample, the low number of subjects studied can affect the power to obtain the desired results, as the outcome of the interaction between gene and environment, if minor, will require a greater number of subjects for observation. The difficulty in recruiting clinical samples did not allow us to make a comparative study between patients with depression and healthy controls, thus increasing the power of the study by raising the chances of finding the sought interactions.

In regards to the sample, it is important to highlight that the patient group showed no subjects with SS genotype, which can partly lead to a bias in the results. Additionally, the sample is constituted of students from eastern communes in Santiago, for the most part, which is an unrepresentative sample of the Chilean population. Previous studies on the Chilean population found an S allele frequency of .58-.61 (Sanhueza, Herrera, Salazar, & Silva, 2011; Silva et al., 2010), while American-European populations show an S allele frequency of .40-.45, and the East Asian population shows a frequency of .70 to .80. As previously discussed, (Eyheramendy, Martinez, Manevy, Vial, & Repetto, 2015), genetic markers of the ancestry of Chileans has a gradient depending on the geographical location in which the individual resides. It could be thought that for the 5-HTTLPR polymorphism the same gradient could be seen, as the study sample contains an overrepresentation of the L allele frequency expected for the Chilean population. This sample may represent a population with more European ancestry than the rest of Chile, so the presence of L allele is higher than the

expected for the average Chilean population (allelic frequency of this sample: S = .39 and L = .61).

Another limitation is that the instrument used to measure depression (BDI) only evaluates depressive symptoms and does not make depression diagnoses. So subjects evaluated with a score greater than 10 might not correspond to patients with clinical depression. Moreover, the other instruments used to assess environmental and personality variables are self-report scales, which, as previously discussed (Uher & McGuffin, 2010), may present problems due to a recall bias or temperamental factors, which can be influenced by genetic factors. In other words, it is possible that they are not evaluating the "external environment" but rather the "perception of the environment," failing to pass the correlation rGE test. That is, a gene determines how the person perceives the environment rather than interacting with the environment to produce a new result.

Despite the project's limitations, the following are some of the interesting results we discovered:

We found that almost a quarter of the sample (24.6%, n = 33) presents minimal depression (BDI> 10), regardless of the clinical sample. This is a fairly high figure considering that the national health survey shows that 17.2% of the population presented depressive symptoms in the last year (8.5% in men and 25.7% in women). The national health survey revealed that, when increasing the number of years of education of subjects, the prevalence of depressive symptoms decreased, reaching 11.8% in subjects who had studied for over 12 years (Ministerio de Salud de Chile). Our sample corresponds mostly to the group that has studied for over 12; hence, 24.6% is very high compared to 11.8%.

As for the environmental and personality variables included in the study and prediction of depressive symptoms, we found that recent negative events, childhood trauma, anxiety attachment, and the level of dependency and self-criticism are directly correlated with the BDI score. The strongest correlation is between the level of depressive symptoms and recent negative events and self-criticism. In turn, the level of social support correlates indirectly with the BDI score.

Of the total number of subjects, 29.2% (n = 35) presented a history of trauma. No significant differences between genders were observed. The frequency of trauma found is in line with the figures previously described in national (Crempien, Rojas, Cumsille, & Oda, 2011; Florenzano et al., 2002) and international (Iffland, Brahler,

Neuner, Hauser, & Glaesmer, 2013) literature. Similarly, subjects with a history of childhood trauma had higher depressive symptoms.

Most participants reported recent positive and negative life events of moderate to large effect over the past year. The average of negative events with moderate to large effect was 4.54 (SD = 4.7), with no differences between those reported by women and men. Subjects who had more than seven negative events had significantly higher BDI scores. Moreover, to present more than eight recent negative events was associated with having a BDI score above the cutoff of minimal depression.

The average of recent positive events with moderate to large effect was 7.9 (SD = 4.18); results reported no difference between men and women. It should be noted that subjects reported nearly twice as many positive events than negative ones. No main effect or interaction between the genotype and recent positive events were observed. That is, reporting a higher number of positive events over the past year is not associated with levels of depressive symptoms in this study. Also, there was no significant negative correlation between positive events and the depressive symptoms observed.

Concerning the relation between positive and negative recent events, subjects that present a low difference between positive and negative recent events (i.e., that reported almost the same number of positive events than negative events) show a higher average of depressive symptoms than those who show a high difference (i.e., either a lot of positive events or few negative events). So, it could be hypothesized that the influence of positive events on depressive symptoms depends on the number of adverse events reported, as negative recent events have a direct effect on depressive symptoms, while positive recent events do not. However, when the number of positive events is significantly higher than that of negative ones, the BDI scores drop.

Regarding social support, the average number of close relatives and friends was 7.93 (SD = 5.96), with no significant differences by gender.

About a third of the sample showed secure attachment (32.2%, n = 39), while 67.8% of the sample showed insecure attachment. Subjects with fearful attachment presented higher levels of depressive symptoms.

Relating to personality configuration, the sample was distributed as follows: anaclitic n = 39 (25.8%), introjective n = 9 (6%), mixed n = 25 (16.6%), and non-categorizable n = 47 (31.1%). Those subjects with mixed type of depressive experience have scored significantly higher than anaclitic and introjective, and the latter have significantly higher BDI scores than non-categorizable.

There is no correlation between genotype or allele and the variables studied. This is significant as it can be concluded that, whatever the allele, the 5-HTTLPR polymorphism is not associated with presenting higher or lower depressive symptomatology, nor a particular environment or particular depressive style.
There is also no relation between baseline cortisol and the variables studied. That is, there is no direct relation between the level of cortisol and polymorphism, or the environmental variables, or the depressive symptomatology observed.

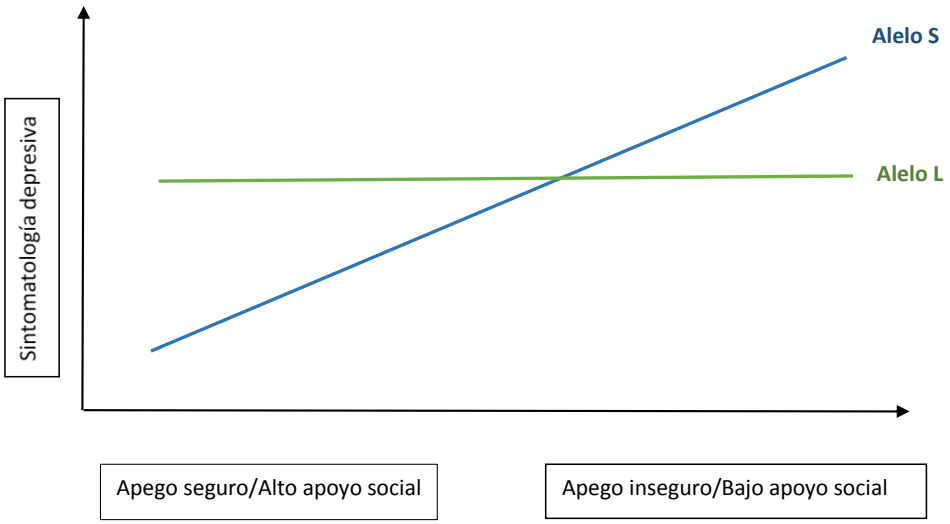
This makes it possible to pass the rGE test and continue with GE interaction studies.

Takeaways regarding the interaction between the 5-HTTLPR polymorphism and environmental factors:

First, S allele carriers with high social support present less depressive symptoms than S allele carriers with low social support. These differences are not observed in L allele carriers. Despite these findings, these results are not enough to account for a thorough sample of the differentiated sensitivity model, as the difference between the BDI scores of both S and L allele carriers would have to be greater at both ends of social support. This means that SS/SL subjects with high social support would have had to present BDI scores significantly lower than LL subjects with high social support and that the SS/SL subjects with low social support would have had to show significantly higher BDI scores than LL subjects.

Moreover, we found that SS subjects with low anxiety attachment showed less depressive symptoms than SS subjects with high anxious attachment. This difference was not observed in L allele carriers. These findings, as we explained, fail to exemplify the differentiated sensitivity model because we would need significant differences between S and L allele carriers, which exist but are not significant.
These findings reveal that there is a tendency for S allele carriers to be more influenced by negative (low social support and high levels of anxiety attachment) and positive environments (high social support and low anxiety attachment) than L allele carriers; these factors affect their results in a more negative or more positive way according to the environment to which they were exposed.

Figure 55 Representation of the differentiated sensitivity model in the interaction between the 5-HTTLPR polymorphism and the attachment style and social support in predicting depressive symptoms



Regarding personality style, it could be concluded that being homozygous for the short allele of the 5-HTTLPR polymorphism confers vulnerability to depression when presenting a mixed personality organization.

As for the response of cortisol to the experimental task, we found that subjects who carry the L allele or present a history of trauma, more depressive symptoms, more recent negative events, and a mixed, anaclitic or introjective depressive experience presented slower or flatter curves. S-carriers with lower depressive symptoms presented a descending, faster curve than S subjects with depressive symptoms and L subjects with or without depressive symptoms. Moreover, subjects with secure attachment presented a lower cortisol average.

According to our analysis, the interaction between the 5-HTTLPR polymorphism and social support and the level of anxious attachment to predict depressive symptoms was not associated with changes in cortisol response to the experimental task. This may be due, in part, to the low number of subjects and to the fact that the task was not stressful enough, so biological stress reactivity was not properly evaluated. And differences observed in literature are found mainly in cortisol's response to stress and not in basal cortisol measurements. However, when assessing the total release of cortisol during the experiment with an area under the curve of cortisol

method, we found a quasi-significant interaction between social support and the 5-HTTLPR polymorphism. In a high social support group, SS genotype participants have a significantly smaller cortisol area under the curve ($p = .043$) than L allele carriers with high social support. No differences of cortisol AUC were observed between genotype groups with little social support. That is, S allele homozygous subjects with high emotional support showed lower cortisol release during the experiment, which could be associated with S allele carriers with high social support showing the lowest level of depressive symptoms of the sample (BDI = 5) and S allele carriers with low social support showing the highest BDI scores of the sample (9,522).

Interestingly, we found no direct association between the level of depressive symptoms, social support or genotype and the amount of cortisol released during the experiment. However, we did observe that subjects with SS genotype and high social support release less cortisol and have less depressive symptoms. In other words, the S allele likely behaves as a more environmentally sensitive allele, presenting better results (less depressive symptoms) when exposed to positive environments (greater social support).

Moreover, when analyzing the interaction between the 5-HTTLPR polymorphism and depressive experiences (personality configuration), we found a significant interaction in the area under the curve. In the category of non-categorizable depressive experience (low self-criticism and dependency), SS subjects' cortisol area under the curve is significantly lower than that of L carriers. Contrary to what occurs with social support, in the case of depressive experience, we noticed that for SS subjects, a mixed depressive experience (high levels of dependency and self-criticism) gave way to depression, while we did not observe an association between a non-categorizable depressive experience with lower levels of depression. Thus, we conclude that the short allele in interaction with this variable leads to vulnerability but not to greater environmental sensitivity. However, in terms of neurobiological reaction to stress (operationalized as the release of cortisol during the experiment), we could assume that this genotype grants greater sensitivity to positive environments, since we found that SS subjects with non-categorizable depressive experience (low dependency and self-criticism) showed a lower cortisol release during the experiment.

In the classification and regression trees, we observed that the most important variables to discriminate between subjects with and without depression are the presence of more than 7 recent negative life events, followed by a flat cortisol curve (delta <.51 ng / ml),

which leads to a high probability of being in the group with depression. It could be theorized that these participants are subjects with low neurobiological reactivity, which in literature is generally associated with the effects produced in the HPA axis by chronic stress (C. Heim & Binder, 2012b). Moreover, for subjects that have <7 NRE (negative recent events), the variable that best discriminates depressive subjects is the type of attachment, i.e., fearful (high anxiety and avoidance) versus non-fearful. For subjects who do not have a severely altered attachment and show <7 NRE, delta cortisol is again the variable that best discriminates between subjects with and without depression, but at a different cut-off point.

Subjects who have a moderate cortisol delta (<1.1 ng/ml), are those with lower probability of depression (n = 76, $P_{BDI>10}$ .11). Moreover, subjects with <1 NRE also have a low probability of having depression (n = 10, $P_{BDI>10}$ .10). For those who report between 1 and 7 NRE, the delta cortisol variable is again the discriminatory tool; when the delta cortisol is very high (> 1.96) the probability of depression is low (n = 5, $P_{BDI>10}$ .20), but when the delta cortisol is moderate (between 1.1 and 1.96 ng/ml), the 5-HTTLPR genotype is the variable that discriminates. S carriers are more likely to have depression when neurobiological reactivity is moderate.

Unlike the vulnerability model, the differentiated sensitivity to the environment model is a model that includes an evolutionary perspective, which considers the potential disadvantages and advantages of individual differences.

This evolutionary perspective may be better able to explain the observation that many of the genetic variants included in studies of GxE candidate genes in psychiatry are "common" variants (i. e. have a high frequency in the general population).

If there were genetic variants associated exclusively with an increased risk for the development of psychopathology in the presence of adversity, it could be expected that the frequency of these genes would decrease over time (and that the genetic variants associated with resilience would increase). However, this has not been observed, many of these variations are very frequent.

It is thought that this type of genetic variation could allow faster adaptation to environmental changes and favour the reproduction of the species.

We will end this discussion with, what we think are, the most important conclusions from the systematic review and thesis project. We have organized the discussion of the findings around six major areas that we believe emerge from the thesis. For each of these areas, we also discuss guidelines for future research.

1. Minimum quality assessment of genes and environment

First, this review clearly shows that the past decades have witnessed a marked increase in the number of GxE studies. Importantly, it is also evident that the quality of studies in this area is clearly growing, as demonstrated by the relative increase in numbers of experimental and prospective studies, as well as the growing quality of genotyping in more recent studies. This trend needs to continue, as only well-conducted prospective and experimental studies have the potential to truly increase our knowledge of the role of GxE in explaining vulnerability/sensibility for psychopathology and the mechanisms involved (see Table 11, point 1). A specific difficulty for the retrospective assessment of environmental factors in GxE studies is that participants' memories of events may be influenced by genes, and that these same genes may influence their personality and behavior. This implies that some retrospective environmental measures may be confused with disorder-relevant genes and so cannot pass the test of rGE (Moffitt et al., 2005) (see Table 11, point 2). As noted, one of the major knowledge gaps in the study of mental disorders concerns how an environmental factor external to the person "gets under the skin" to result in a given behavior or mental disorder. Experimental designs and studying the effect of GxE on neurobiological systems promise to allow us to better understand how environmental and biological factors interact to shape human behavior. Unfortunately, despite the large number of studies including 5HTTLPR polymorphism, most studies to date have neglected to reproduce the GxE interactions in experimental designs.

2. Differential susceptibility vs diathesis stress

Second, in line with a number of meta-analyses in human and animal research, the majority of GxE studies reported positive results, which were found in around 60 to 80% of studies, (depending on the gene studied); this was the case regardless of whether positive or negative outcomes were focused upon. These findings provide support not just for the role of GxE in human behavior, but specifically for social susceptibility rather than vulnerability theories (see Table 11, point 3). As explained earlier, social susceptibility models contend that there are differences between individuals in susceptibility to environmental influences, with some individuals being far more affected than others by both negative and positive contextual conditions. So, one would expect that GxE evidence would be found for both positive and negative circumstances. In contrast, in the vulnerability model, one would expect evidence for GxE only in interaction with negative circumstances. Even though the first study of GxE under the

social susceptibility model dates back to 2006 (Bakermans-Kranenburg & van Ijzendoorn, 2006), by 2010 only four studies had been published(Bakermans-Kranenburg, Van Ijzendoorn, Mesman, Alink, & Juffer, 2008; Bakermans-Kranenburg, Van, Pijlman, Mesman, & Juffer, 2008; Oreland, Nilsson, Damberg, & Hallman, 2007; Sheese, Voelker, Rothbart, & Posner, 2007). Although the consistency of positive findings may be interpreted as congruent with the social susceptibility hypothesis, this could also be a result of publication bias (Turner, 2013). We cannot ignore that there is a publication bias, most of the articles published in scientific journals are those with positive results, so if we are interested on finding the percentage of positive results we will find around 70% of positive results for all investigations, but if the case was that we cannot trust what is published, we cannot be sure of the evidence for scientific statement, as the usefulness of psychotherapy or psychotropic drugs. More research is needed in this area, therefore, and future studies should include both positive and negative environments and outcomes, rather than a focus on one type of environment or outcome alone, as is typical of most current studies in this area.

3. Candidate gene versus general indices of vulnerability/susceptibility genotypes

Third, over half of the studies on GxE have focused on 5HTTPLR. It is clear that there are good reasons for this, as there is good evidence from both animal and human studies that this gene is implicated in susceptibility for environmental influences (Barr et al., 2004; Christine Heim & Nemeroff, 2001; C. Heim & Nemeroff, 2002; Homberg, Molteni, Calabrese, & Riva, 2014; Lindell et al., 2012; Spinelli et al., 2007; Vergne & Nemeroff, 2006),. However, as pointed out by others (Dick et al., 2015), the frequency of the genes studied may not necessarily mean that these genes are the most promising, but might partly be a consequence of early positive studies, as for 5HTTPLR. Similarly, although there is a clear scientific rationale for the role of genes related to oxytocin in parenting, as studies have suggest that the oxytocin system plays an important role in social affiliation, the "popularity" of some genes in certain areas (e.g., stress sensitivity versus parenting) may also be partly to do with the fact that early studies of these genes reported positive findings. It may now be time to take stock of the field and reconsider some of these foci. For instance, although most studies of the 5HTT gene have focused on its influence on depression, increased stress reactivity probably characterizes many types of psychopathology, such as borderline personality disorder or post-traumatic stress disorder, for instance(van Zuiden et al., 2015; Wingenfeld & Wolf, 2015; Yehuda et al., 2014). In addition, extensive research in both humans and animals has

demonstrated structural and functional relationships between the stress system and the reward/affilation system(Pizzagalli, 2014). Furthermore, a known association between a particular genetic polymorphism and a disorder can nominate a gene for a GxE hypothesis, but the absence of such an association does not in itself disqualify a gene (Moffitt et al., 2005) In this context, recent work concerning the mapping of the human genome presents another exciting development that needs to be incorporated into future research on GxE. Hence, an exclusive focus on the influence of specific genes in specific disorders or behaviors may be misguided. Instead, genotyping of an array of genes as index of social susceptibility or a polygenic risk score is likely to be more appropriate when studying complex human behaviors (Dick et al., 2015) (see Table 11, point 4). Hence, in line with the Research Domain Criteria matrix of the US National Institute of Mental Health (see Table 11, point 5), it may be time to adopt a spectrum approach that cuts across disorders and behaviors, rather than to focus on specific disorders, specific behaviors or specific outcomes (T. Insel et al., 2010; T. R. Insel, 2014). It appears that biological findings for mental disorders are relatively non-specific; most genetic findings and neural circuitry maps appear to link to many different syndromes (Conway, Slavich, & Hammen, 2014). Until recently, relatively few studies have addressed the question of whether several disorders may share important etiological factors. A transdiagnostic view, considering a more etiologically based approach, is in line with an increasingly comprehensive body of research in genetics, neuroscience, behavioral and evolutionary science that has transformed understanding of how the brain produces adaptive behavior and the ways in which normal brain functioning may become disrupted (Luyten & Blatt, 2013). As noted by many (Dick et al., 2015; Duncan & Keller, 2011), such studies will necessitate large samples. The fact that other trends besides scientific arguments are driving some of the research on GxE is also exemplified by the finding that approximately four times as many articles have focused on 5HTTPLR as on the second most studied gene, BDNF. Serotoninergic alleles (5HTTLPR), predominantly, have been studied with regard to their interaction with early and negative events to predict depression, in longitudinal or cross-sectional studies in adults. Only recently have studies concerning this polymorphism begun to focus on its interaction with positive events, and its underlying neurobiology.

In contrast, dopaminergic alleles have been investigated in studies that address how the genes' interaction with early positive and negative events predict changes in

social behavior, in longitudinal or experimental studies in children or adolescents. Yet, these genes may also be important in terms of their interaction with life events in the prediction of psychopathology, particularly as these genes may play a key role in the regulation of the reward system, which has been implicated in depression and substance abuse disorders, for instance (Auerbach, Admon, & Pizzagalli, 2014; Bogdan & Pizzagalli, 2006; Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008; Whitton, Treadway, & Pizzagalli, 2015). This further suggests that it may be time for research to move away from candidate genes toward general indices of vulnerability/susceptibility genotypes (Dick et al., 2015).

4. Neurobiological mechanisms involved in GxE should be included in studies

Fourth, most research to date has focused on psychopathology and social behavior; It may now be time to shift more toward the study of the mechanisms involved in GxE. Future studies should routinely include a focus on mechanisms, rather than focusing on GxE alone (see Table 11, point 6). For example, studying if the carriers of plastic alleles are more sensible to experience by having a more reactive hypothalamic-pituitary-adrenocortical axis, that is more susceptible due to epigenetic modification on specific brain areas (Gotlib et al., 2008; Hunter, Gagnidze, McEwen, & Pfaff, 2015)

5. Need for life time perspective

Fifth, more studies in children and adolescents are needed (see Table 11, point 7). Developmental neuroscience has shown that there are periods of increased plasticity of the brain throughout development. During such periods, experiences may have profound programming and organizing effects on the brain (Andersen et al., 2008; Rice & Barone, 2000). These critical periods refer to time windows where expected experiences are necessary for a particular brain function to develop normally. However, during such times of heightened plasticity, the brain may also be particularly sensitive to negative or positive experiences (C. Heim & Binder, 2012b). These critical windows are directly relevant to early prevention and intervention strategies. It may be the case that GxE has a greater impact on children and young adults, while in older adults the influence of the environment is less dependent on genetic variance.

6. Cultural and ethnic variables should be included on GxE studies

Sixth, most of the GxE studies covered in this review have focused on the early environment, and on negative environments in particular. This focus is clearly warranted in view of findings concerning the “programming” of stress and other neurobiological systems by early adversity (C. Heim & Binder, 2012a; Lupien,

McEwen, Gunnar, & Heim, 2009). Yet, as the number of studies findings evidence for GxE in interaction with positive environments compared to negative environmental factors, future studies might do well to simultaneously focus on interactions with both positive and negative environments (see Table 11 point 8). Indeed, if a potentially disadvantageous gene variant is maintained at a high prevalence, this might imply that natural selection has not been able to eliminate the variant because its effects on the phenotype are expressed only under certain environmental conditions, and/or perhaps even because it confers an advantage under particular environmental conditions (Moffitt et al., 2005). The importance of including recent and positive events in GxE studies is that transforming the environment into a positive one, whether at personal level (i.e. by encouraging prosocial behaviors and psychotherapy interventions), or at political level (i.e. by lobbying for a wider, more positive environment for populations), could have positive outcomes, especially for more sensitive individuals (Bakermans-Kranenburg & van, 2015).

Further, most studies to date focus on discrete events. However, there is good evidence to suggest that more chronic stressors and broader environmental factors, such as cultural minority status, social disadvantage and sociocultural factors more generally, may influence GxE (see Table 11, point 9). This may be particularly relevant as there is a clear cultural bias in GxE studies, with almost 90% of studies to date focusing on North American and European populations. Given the potential of gene–culture interactions and even gene–culture co-evolution (Laland, Odling-Smee, & Myles, 2010), it is surprising that only a small minority of studies has been conducted in other geographical regions such as Latin America, Africa or Asia, particularly as many cultures within these regions are traditionally seen as more interdependent– and thus individuals within these cultures are more susceptible to environmental factors such as social support and interaction. Therefore, cross-cultural studies are needed. This is all the more needed as the prevalence of social susceptibility polymorphisms may vary greatly among different populations; this may reflect a process of natural selection of gene–culture co-evolution, such that genes that serve survival and adaptation in a given culture are selected for. Researchers in the field of cultural neuroscience have argued that maybe the different beliefs, values and practices of different cultures may influence the selection of genes and interact with genetic variables to regulate human brain and behavior (Chiao et al., 2010; Laland et al., 2010). These models suggest that cultural influences may dramatically affect the rate of change of allele frequencies in response to

selection (Laland et al., 2010). For instance, social susceptibility genes (5HTTLPR, OPRM1, MAOA) have been shown to be more prevalent in collectivistic cultures (Chiao & Blizinsky, 2010), and collectivistic values have been found to moderate the prevalence of depression, for instance, in these cultures (Way & Lieberman, 2010). Hence, the same polymorphism may interact in different ways in different populations, and therefore it may not be possible to generalize across different populations. Because of this, caution is needed when attempting to interpret findings on GxE; this is even more the case because these studies are also limited in terms of the types of environmental factors they have studied. Finally, the strong overlap in studies, with only 160 original samples included in this study, and most samples/studies originating in the US and Western Europe, are a reason for concern, and we strongly emphasize the need for caution in drawing conclusions concerning GxE effects.

7. Need for studies of response to psychosocial interventions

The importance of including recent and positive experiences in GXE studies, is that transforming the environment to a positive, either on a personal level (e.g., promoting pro-social behaviors or psychotherapy), or on a political level (e.g. exerting pressure for more extensive positive environments for people), could have very positive results, especially for sensitive subjects (Bakermans-Kranenburg & van, 2015) (Table 11, item 9). Scant research has evaluated the response to psychotherapy based on 5HTTLPR polymorphism and the results so far have been mixed. First, (Bryant et al., 2010) in patients with post-traumatic stress disorder (PTSD) diagnosis showed, against expectations, that individuals carrying the short allele had poorer response to cognitive behavioral therapy than patients homozygous for the long allele. One possibility is that, although the cognitive system of short allele carriers is more malleable, this malleability in turn causes a set of changes that in the case of PTSD are deeply rooted for life. Another study (Kohen et al., 2011) on the response to psychosocial interventions in depressed patients post cerebrovascular accident (CVA). 101 patients with depression post CVA, were randomized to antidepressant treatment and usual care (n = 53) or 9 sessions of psychosocial intervention plus antidepressant treatment. Variables associated with prediction of response to antidepressants and post-CVA depression as age, gender, severity of CVA, CVA hemisphere, severity of depression based Hamilton Rating Scale-Depression (HRSD), history of depressive episodes, level of social support and adherence to antidepressants were controlled for. Response to treatment was asses by 17 HRSD. Findings showed that younger patients responded better to treatment in

both groups. Patient's homozygote for short allele, presented a larger effect that patients homozygous for the long allele in response to psychosocial treatment (Figure 56).

Figure 56 Interaction between 5HTTLPR genotype and response to treatment according to percentage of decrease in 17 HRSD

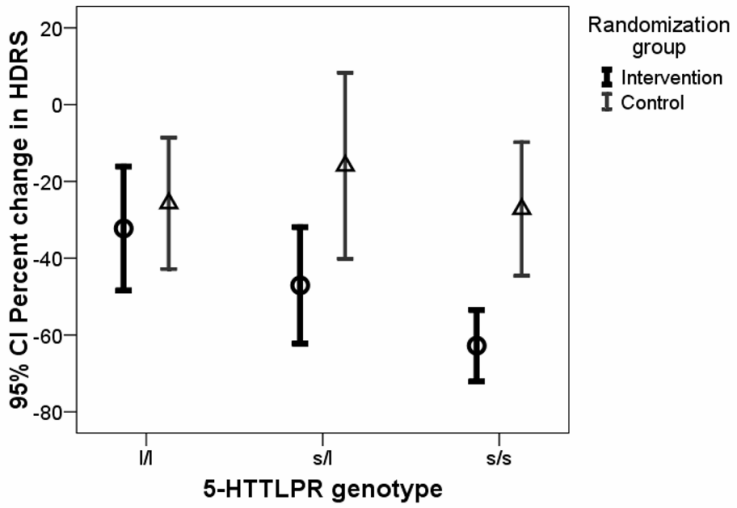


Figure 56 Obtained from: Kohen, R., Cain, K. C., Buzaitis, A., Johnson, V., Becker, K. J., Teri, L., Mitchell, P. H. (2011). Response to psychosocial treatment in poststroke depression is associated with serotonin transporter polymorphisms. *Stroke; a Journal of Cerebral Circulation, 42*(7), 2068-2070.

Moreover, Bockting (Bockting, Mocking, Lok, Koeter, & Schene, 2013) found no difference in preventing recurrence/relapse in a group of 180 adults with recurrent major depression who were randomly assigned to psychotherapeutic treatment with cognitive behavioral therapy. In both groups (SS vs SL/LL) decreased with treatment relapse was observed, but no interaction was observed with genotype.

Figure 57 Prevention of relapse/recurrence in response to cognitive behavioral psychotherapy based polymorphism 5HTTLPR

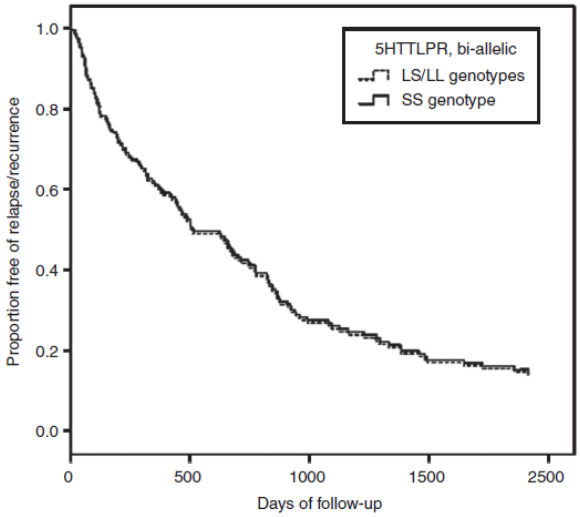


Figure 57 Obtained from Bockting CL, Mocking RJ, Lok A, Koeter MW, Schene AH.Therapygenetics: the 5HTTLPR as a biomarker for response to psychological therapy? Mol Psychiatry. 2013 Jul;18(7):744-5. doi: 10.1038/mp.2012.92. Epub 2012 Jul 3.

Eley (Eley et al., 2012) studied the response to cognitive behavioral therapy of 359 British and Australian children (aged 6-13 years) with anxiety disorder. They assessed symptoms, pre- and post-treatment and at six months follow up. Founded a higher percentage of carriers SS without a diagnosis of anxiety disorder at follow-up, than in L allele carriers group (Figure 58).

Figure 58 Response to cognitive behavioral therapy in anxious children based on 5HTTLPR polymorphism

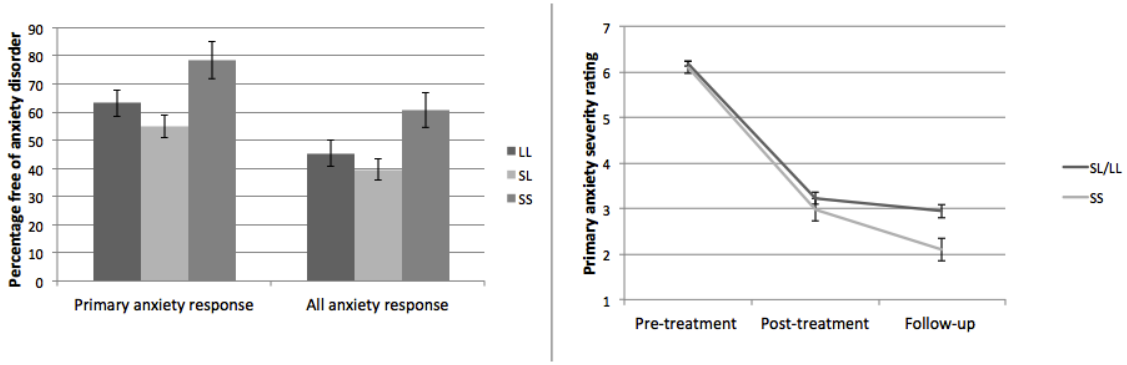


Figure 58 Obtained from Eley, T. C., Hudson, J. L., Creswell, C., Tropeano, M., Lester, K. J., Cooper, P., Collier, D. A. (2012). Therapygenetics: the 5HTTLPR and response to psychological therapy. *Mol Psychiatry, 17*(3), 236-237. doi: 10.1038/mp.2011.132

The following table summarizes the recommendations for future research on the interaction of genes and environment

Table 11

Recommendations for future research

1. 1. Need for standardized genotyping technique in order to make data from different studies comparable. Minimum quality criteria: study reports a genotyping success rate of 95% or higher; and the study reported Hardy Weinberg Equilibrium (HWE), linkage equilibrium or deviations of HWE.
2. Need for standardized assessment of environment, with more attention to GxE and rGE
3. Future research should incorporate "differential susceptibility" or "plasticity" models in order to measure not only the presence/absence of disease or environmental stress, but also the "positive" side of variables such as the presence of subjective well-being or adequate social support.
4. Need to move away from candidate genes to general indices of vulnerability/susceptibility genotypes
5. Need for a transdiagnostic approach, congruent with the Research Domain Criteria approach that would lead to understanding the ways (pathways) in which GxE occur.
6. Need for more studies on the neurobiological mechanisms involved. Although we can continue to lean on findings from animal studies, we need to move to human research.
7. Need to broaden scope in terms of samples and environments (including culture and developmental context). This will necessarily lead to longitudinal studies.
8. Since there is evidence for gene–culture interaction in the prediction of social behavior, future studies should incorporate variables that measure cultural aspects, such as individualism/collectivism or ethnicity
9. Given evidence that these genes are prosocial, suggesting the possibility of environmental influences, further studies are needed in response to psychosocial interventions

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ANNEXES

Annexes 1: Thesis instruments

Experience in Close Relationships

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

Proyecto Fondecyt
Postdoctorado Nº3120109

N. Folio:	Grupo:

ECR-S[1][2]

Las siguientes afirmaciones se refieren a como usted se siente en las relaciones cercanas. Nos interesa saber cómo vive usted ***generalmente*** las relaciones con personas significativas para usted y con las cuales tiene un alto grado de intimidad; y no sólo lo que le está ocurriendo en una relación particular actual.

Responda a cada afirmación indicando en qué grado está usted de acuerdo o en desacuerdo con ella.

NOTA ACLARATORIA: El siguiente cuestionario utiliza la palabra "**INTIMIDAD**" como un elemento importante de las relaciones cercanas **Esta *intimidad* incluye distintos aspectos como por ejemplo: la cercanía emocional y física, la comunicación, el compromiso mutuo, la privacidad, etc.**

Marque con una X el número que mejor represente su respuesta de acuerdo a la escala que se presenta a la derecha de cada afirmación.

	Totalmente en desacuerdo	Bastante en desacuerdo	Un poco en desacuerdo	Ni desacuerdo / ni acuerdo	Un poco de acuerdo	Bastante de acuerdo	Totalmente de acuerdo
Ítem	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. Me ayuda mucho recurrir a las personas cercanas a mí en épocas de crisis.							
2. Necesito que las personas cercanas a mi me reafirmen constantemente que me quieren							

[1]Wei, M., Russell, D. W., Mallinckrodt, B., & Vogel, D. L. (2007). The experiences in Close Relationship Scale (ECR)-Short Form: Reli8ability, validity, and factor structure. *Journal of Personality Assessment, 88,* 187-204.
[2]La presente versión ha sido traducida y adaptada para los propósitos específicos del Núcleo Milenio Intervención Psicológica y Cambio en Depresión, de indagar sobre relaciones cercanas más allá de las relaciones de pareja.

	Totalmente en desacuerdo	Bastante en desacuerdo	Un poco en desacuerdo	Ni desacuerdo / ni acuerdo	Un poco de acuerdo	Bastante de acuerdo	Totalmente de acuerdo
3. Quiero acercarme afectivamente a las personas que quiero, pero a la vez pongo distancia entre nosotros.							
4. Creo que las personas que quiero no quieren tener tanta intimidad emocional conmigo como a mí me gustaría.							
5. Recurro a personas importantes para mí para muchas cosas, por ejemplo cuando necesito consuelo y tranquilidad							
6. A veces mi deseo de excesiva intimidad asusta a la gente.							
7. Intento evitar establecer demasiada intimidad con las personas cercanas.							
8. Pocas veces me preocupa la idea de ser abandonado.							
9. Frecuentemente converso sobre mis problemas y preocupaciones con personas cercanas.							
10. Me siento frustrado/a si las personas que quiero no están disponibles cuando las necesito.							
11. Me pongo nervioso/a cuando alguien cercano a mi logra demasiada intimidad emocional conmigo.							
12. Me preocupa que el otro no se interese por mí tanto como yo me intereso por él/ella.							

Depressive Experience Questionnaire

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

ID Participante	
Fecha	

DEQ[1]

A continuación se presentan una serie de frases que describen características y rasgos personales. Lea cada una de ellas e indique con una cruz el grado en que estas características lo describen en un rango del 1 al 7 ("totalmente en desacuerdo" a "totalmente de acuerdo"). Si usted se muestra neutral o indeciso, marque el número 4 ("neutral o indeciso").

1	2	3	4	5	6	7
Totalmente en desacuerdo			Neutral o indeciso			Totalmente de acuerdo

1.	Fijo mis metas y objetivos lo más alto posible	1	2	3	4	5	6	7
2.	Sin el apoyo de los que están cerca de mí, me sentiría desamparado/a	1	2	3	4	5	6	7
3.	Tiendo a sentirme satisfecho/a con mis metas en vez de aspirar por metas más altas	1	2	3	4	5	6	7
4.	A veces me siento muy grande y otras veces muy pequeño/a	1	2	3	4	5	6	7
5.	En mis relaciones íntimas, nunca siento celos	1	2	3	4	5	6	7
6.	Necesito urgentemente de cosas que solamente otras personas me pueden dar	1	2	3	4	5	6	7
7.	Usualmente me da la sensación que no cumplo mis propias metas o ideales	1	2	3	4	5	6	7
8.	Siento que siempre uso todo mi potencial	1	2	3	4	5	6	7
9.	La poca estabilidad en las relaciones humanas no me afecta	1	2	3	4	5	6	7
10.	Si fracaso al intentar lograr expectativas, me siento sin valor	1	2	3	4	5	6	7
11.	Muchas veces me siento desamparado	1	2	3	4	5	6	7
12.	Rara vez me preocupo de que me critiquen por cosas que haya hecho o dicho	1	2	3	4	5	6	7
13.	Hay una diferencia considerable entre lo que ahora soy y cómo me gustaría ser	1	2	3	4	5	6	7

[1] Zuroff, Quinlan & Blatt, (1990); traducido al español por Añez y Paris (2000); adaptado para Chile por Rosti & Dagnino (2010).

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

ID Participante	
Fecha	

1	2	3	4	5	6	7
Totalmente en desacuerdo			Neutral o indeciso			Totalmente de acuerdo

14.	Disfruto de la competencia fuerte	1	2	3	4	5	6	7
15.	Siento que tengo muchas responsabilidades que cumplir	1	2	3	4	5	6	7
16.	Hay momentos durante los cuales me siento vacío	1	2	3	4	5	6	7
17.	Tiendo a no estar satisfecho/a con lo que tengo	1	2	3	4	5	6	7
18.	No me importa si no alcanzo las metas que otros esperan de mí	1	2	3	4	5	6	7
19.	Me angustia cuando me siento solo/a	1	2	3	4	5	6	7
20.	Si perdiera un amigo muy cercano sentiría como si estuviera perdiendo una parte de mi mismo/a	1	2	3	4	5	6	7
21.	Estoy seguro/a que los demás me aceptarán sin importar cuantos errores haya cometido	1	2	3	4	5	6	7
22.	Me resulta difícil romper una relación que me hace sentir infeliz	1	2	3	4	5	6	7
23.	Con frecuencia pienso acerca del peligro de perder a alguien cercano a mí	1	2	3	4	5	6	7
24.	Las personas esperan mucho de mí	1	2	3	4	5	6	7
25.	Cuando estoy con otros tiendo a devaluarme y presentarme negativamente	1	2	3	4	5	6	7
26.	No me preocupa mucho cómo los otros reaccionan conmigo	1	2	3	4	5	6	7
27.	No importa lo cercana que puede ser la relación entre dos personas, siempre habrán inseguridades y conflictos	1	2	3	4	5	6	7
28.	Soy muy sensible a las señales de rechazo de los otros	1	2	3	4	5	6	7
29.	Es importante para mi familia que yo triunfe	1	2	3	4	5	6	7

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

ID Participante	
Fecha	

		1 Totalmente en desacuerdo	2	3	4 Neutral o indeciso	5	6	7 Totalmente de acuerdo
30.	A menudo siento que he desilusionado a los demás	1	2	3	4	5	6	7
31.	Si alguien me enoja se lo dejo saber	1	2	3	4	5	6	7
32.	Constantemente trato, a veces con esmero, de agradar y ayudar a personas cercanas a mí	1	2	3	4	5	6	7
33.	Tengo muchos recursos internos (habilidades, fortalezas)	1	2	3	4	5	6	7
34.	Me es muy difícil decir "no" a mis amistades	1	2	3	4	5	6	7
35.	Nunca me siento realmente seguro/a en una relación íntima	1	2	3	4	5	6	7
36.	Me siento constantemente diferente. A veces me siento extremadamente bien, otras veces me siento muy mal, como si fuese un/a fracasado/a	1	2	3	4	5	6	7
37.	A menudo me siento amenazado/a cuando las cosas cambian	1	2	3	4	5	6	7
38.	Aunque la persona más cercana a mí se fuera, yo podría continuar solo/a	1	2	3	4	5	6	7
39.	Uno tiene que esforzarse continuamente para ganar el amor de otras personas: esto es, el amor debe ganarse	1	2	3	4	5	6	7
40.	Soy muy sensible a los efectos que mis palabras y mis acciones tienen sobre los sentimientos de otras personas	1	2	3	4	5	6	7
41.	A menudo me culpo por las cosas que he dicho o he hecho a otra persona	1	2	3	4	5	6	7
42.	Soy una persona muy independiente	1	2	3	4	5	6	7
43.	A menudo me siento culpable	1	2	3	4	5	6	7
44.	Pienso que soy una persona muy completa, con muchas facetas diferentes	1	2	3	4	5	6	7

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

ID Participante	
Fecha	

1	2	3	4	5	6	7
Totalmente en desacuerdo			Neutral o indeciso			Totalmente de acuerdo

45.	Me preocupa mucho ofender o herir a alguien muy cercano a mí	1	2	3	4	5	6	7
46.	La ira me asusta	1	2	3	4	5	6	7
47.	Lo importante no es "quién eres", lo que cuenta son "las cosas que has logrado"	1	2	3	4	5	6	7
48.	Triunfe o fracase, me siento bien conmigo mismo/a	1	2	3	4	5	6	7
49.	Fácilmente puedo dejar mis sentimientos y problemas de lado y puedo concentrarme totalmente en los sentimientos y problemas de otra persona	1	2	3	4	5	6	7
50.	Si alguien a quien yo le tengo afecto se enoja conmigo, tendría temor que él/ella me abandonara	1	2	3	4	5	6	7
51.	Me siento incómodo/a cuando me dan responsabilidades importantes	1	2	3	4	5	6	7
52.	Después de pelear con un/a amigo/a, siento que debo hacer las paces lo más pronto posible	1	2	3	4	5	6	7
53.	Me es muy difícil aceptar mis propias debilidades	1	2	3	4	5	6	7
54.	Es más importante que yo disfrute de mi trabajo a buscar que mi trabajo sea aprobado por otros	1	2	3	4	5	6	7
55.	Después de discutir me siento muy solo/a	1	2	3	4	5	6	7
56.	En mis relaciones con los demás me importa mucho lo que otros me puedan aportar	1	2	3	4	5	6	7
57.	Rara vez pienso acerca de mi familia	1	2	3	4	5	6	7
58.	Con mucha frecuencia, mis sentimientos hacia alguien cercano a mí varían, hay veces en las que me siento muy molesto/a, y hay otras veces en que siento solo amor por esa persona	1	2	3	4	5	6	7
59.	Lo que hago y digo afecta mucho a quienes me rodean	1	2	3	4	5	6	7

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ID Participante	
Fecha	

1	2	3	4	5	6	7
Totalmente en desacuerdo			Neutral o indeciso			Totalmente de acuerdo

60.	A veces siento que soy especial	1	2	3	4	5	6	7
61.	Me crié en una familia muy unida	1	2	3	4	5	6	7
62.	Estoy muy satisfecho conmigo y mis logros	1	2	3	4	5	6	7
63.	Espero mucho de aquellos con quienes me relaciono	1	2	3	4	5	6	7
64.	Tiendo a criticarme mucho	1	2	3	4	5	6	7
65.	El estar sola/o no me molesta en lo absoluto	1	2	3	4	5	6	7
66.	Muy frecuentemente me comparo con metas y estándares	1	2	3	4	5	6	7

Beck Depression Inventory

NÚCLEO MILENIO
INTERVENCIÓN PSICOLÓGICA
Y CAMBIO EN DEPRESIÓN

ID Participante	
Fecha	

BDI

En este cuestionario aparecen varios grupos de afirmaciones. Por favor, lea con atención cada una. A continuación, señale cuál de las afirmaciones de cada grupo describe mejor cómo se ha sentido **DURANTE ESTA ÚLTIMA SEMANA, INCLUIDO EL DIA HOY**. Rodee con un círculo el número que está a la izquierda de la afirmación que haya elegido. Si dentro de un mismo grupo, hay más de una afirmación que considere aplicable a su caso, puede marcarla también. **Asegúrese de leer todas las afirmaciones dentro de cada grupo antes de efectuar la elección.**

A	F
0. No me siento triste 1. Me siento triste 2. Me siento triste continuamente y no puedo dejar de estarlo 3. Ya no puedo soportar esta pena	0. No siento que esté siendo castigado/a 1. Me siento como si fuese a ser castigado/o 2. Siento que me están castigando o que me castigarán 3. Siento que merezco ser castigado/a
B	**G**
0. No me siento pesimista, ni creo que las cosas me vayan a salir mal 1. Me siento desanimado/a cuando pienso en el futuro 2. Creo que nunca me recuperaré de mis penas 3. Ya no espero nada bueno de la vida, esto no tiene remedio	0. No estoy decepcionado de mí mismo/a. 1. Estoy decepcionado de mí mismo/a. 2. Estoy muy descontento/a conmigo mismo/a 3. Me odio, me desprecio
C	**H**
0. No me considero fracasado/a 1. Creo que he tenido más fracasos que la mayoría de la gente 2. Cuando miro hacia atrás, sólo veo fracaso tras fracaso 3. Me siento una persona totalmente fracasada	0. No creo ser peor que otras personas 1. Me critico mucho por mis debilidades y errores 2. Continuamente me culpo de todo lo que va mal 3. Siento que tengo muchos y muy graves defectos
D	**I**
0. Las cosas me satisfacen tanto como antes 1. No disfruto de las cosas tanto como antes 2. Ya nada me llena 3. Estoy harto/a de todo	0. No tengo pensamientos de hacerme daño 1. Tengo pensamientos de hacerme daño, pero no llegaría a hacerlo 2. Siento que estaría mejor muerto/a o que mi familia estaría mejor si yo me muriera 3. Me mataría si pudiera
E	**J**
0. No me siento culpable 1. Me siento culpable en bastantes ocasiones. 2. Me siento culpable en la mayoría de las ocasiones. 3. Todo el tiempo me siento una persona mala y despreciable	0. No lloro más de lo habitual 1. Ahora lloro más de lo normal 2. Ahora lloro continuamente, no puedo evitarlo 3. Antes podía llorar, ahora no lloro aunque quisiera

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K	Q
0. No estoy más irritable que normalmente 1. Me irrito o enojo con más facilidad que antes 2. Me siento irritado/a todo el tiempo 3. Ya no me irrita ni lo que antes me irritaba	0. No me canso más de lo normal 1. Me canso más fácilmente que antes 2. Cualquier cosa que hago me cansa 3. Estoy demasiado cansado/a para hacer nada
L	**R**
0. No he perdido el interés por los demás 1. Me intereso por la gente menos que antes 2. He perdido casi todo mi interés por los demás 3. Los demás no me importan en absoluto	0. Tengo el mismo apetito que siempre 1. No tengo tan buen apetito como antes 2. Ahora tengo mucho menos apetito 3. He perdido totalmente el apetito
M	**S**
0. Tomo mis decisiones como siempre 1. Estoy inseguro/a de mi mismo/a y evito tomar decisiones 2. Ya no puedo tomar decisiones sin ayuda 3. Ya no puedo tomar decisiones en absoluto	0. No he perdido peso últimamente 1. He perdido más de 2 kilos 2. He perdido más de 5 kilos 3. He perdido más de 8 kilos Estoy bajo dieta para adelgazar: SI NO
N	**T**
0. No me siento con peor aspecto que antes 1. Me preocupa que ahora parezco más viejo/a o poco atractivo/a 2. Creo que se han producido cambios permanentes en mi aspecto que me hacen parecer poco atractivo/a 3. Creo que tengo un aspecto horrible	0. No estoy más preocupado/a por mi estado de salud que lo habitual 1. Estoy preocupado/a por problemas físicos como dolores, molestias, malestar de estómago, o estreñimiento 2. Estoy preocupado/a por mi salud y me es difícil pensar en otra cosa 3. Estoy tan preocupado/a por mis problemas de salud que soy incapaz de pensar en otra cosa
O	**U**
0. Puedo trabajar tan bien como siempre 1. Tengo que hacer un esfuerzo especial para iniciar algo 2. Tengo que obligarme mucho para hacer algo 3. Soy incapaz de hacer algún trabajo	0. No he notado ningún cambio en mi atracción por el sexo 1. Estoy menos interesado/a en el sexo que antes 2. Actualmente me siento mucho menos interesado/a en el sexo 3. He perdido todo mi interés por el sexo
P	
0. Duermo tan bien como siempre 1. Me despierto más cansado/a por la mañana 2. Me estoy despertando una o dos horas más temprano de lo habitual y no puedo volver a quedarme dormido/a 3. Me despierto varias horas más temprano todas las mañanas y no logro dormir más de 5 horas	

Subtotal Página 1	
Subtotal Página 2	
Total	

Cuestionario de eventos vitales

Cuestionario de Eventos Vitales (CEV, LEQ)[1,2]

Nombre_____
Fecha_____

Instrucciones

A continuación se enumeran una serie de eventos, que pueden provocar cambios en las vidas de aquellos que los experimentan.
Encierre en un círculo los acontecimientos que le han ocurrido en su vida durante el último año y encierre en un círculo si los consideró buenos o malos.
Muestre lo mucho que el evento afectó a su vida con un círculo en el número apropiado, que se corresponda con la afirmación (0 = sin efecto, 1 = efecto leve, 2 = efecto moderado, 3 = gran efecto).
Si usted no ha experimentado un evento en particular en el último año, déjelo en blanco.
Por favor, mire toda la lista antes de empezar para tener una idea del tipo de eventos que se le pedirán que califique.

Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			
A SALUD		Sin efecto	Efecto leve	Efecto moderado	Gran efecto
1. Enfermedad o accidente personal grave	Bueno Malo	0	1	2	3
2. Cambio importante o significativo en los hábitos alimenticios.	Bueno Malo	0	1	2	3
3. Importante cambio en los hábitos de sueño	Bueno Malo	0	1	2	3
4. Importante cambio en el tipo o la cantidad de recreación usual	Bueno Malo	0	1	2	3
5. Tratamiento dental mayor	Bueno Malo	0	1	2	3
6. (Mujer) embarazo	Bueno Malo	0	1	2	3
7. (Mujer) Aborto	Bueno Malo	0	1	2	3
8. (Mujer) Inicio de la menopausia	Bueno Malo	0	1	2	3
9. (Mujer) Dificultades mayores con métodos anticonceptivos					

Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			
B. TRABAJO		Sin efecto	Efecto leve	Efecto moderado	Gran efecto
10. Dificultades para encontrar un trabajo	Bueno Malo	0	1	2	3
11.Inicio de un trabajo fuera del hogar	Bueno Malo	0	1	2	3
12. Cambio de trabajo	Bueno Malo	0	1	2	3
13. Cambio en las horas o las condiciones de trabajo	Bueno Malo	0	1	2	3
14. Cambio en sus responsabilidades en el trabajo	Bueno Malo	0	1	2	3
15. Problemas en el trabajo con su empleador o compañeros de trabajo	Bueno Malo	0	1	2	3
16. Reajustes mayores o significativos en la empresa	Bueno Malo	0	1	2	3
17. Ser despedido del trabajo	Bueno Malo	0	1	2	3
18. Jubilar del trabajo	Bueno Malo	0	1	2	3
19. Tomar cursos por correspondencia/online o estudiar en casa para potenciar su trabajo	Bueno Malo	0	1	2	3
C. ESTUDIOS					
20. Iniciar o terminar la escuela, la universidad o un programa de formación	Bueno Malo	0	1	2	3
21. Cambiar de colegio, universidad o programa de formación	Bueno Malo	0	1	2	3
22. Cambio de carrera o de especialidad académica	Bueno Malo	0	1	2	3
23. Problemas en la escuela, la universidad o en el programa de formación	Bueno Malo	0	1	2	3

Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			
D. RESIDENCIA		Sin efecto	Efecto leve	Efecto moderado	Gran efecto
24. Dificultades para encontrar una vivienda	Bueno Malo	0	1	2	3
25. Cambio de residencia dentro de la misma comuna o ciudad	Bueno Malo	0	1	2	3
26. Traslado a otra ciudad, región o país	Bueno Malo	0	1	2	3
27. Importante cambio en sus condiciones de vida (arreglos en el hogar/vecindario o deterioro de su hogar/vecindario)	Bueno Malo	0	1	2	3
E. AMOR Y MATRIMONIO					
28. Inicio de una nueva relación cercana y personal	Bueno Malo	0	1	2	3
29. Se comprometió	Bueno Malo	0	1	2	3
30. Problemas con novia/a o pololo/a	Bueno Malo	0	1	2	3
31. Terminar con una novia o novio o con un compromiso	Bueno Malo	0	1	2	3
32. (Hombre) Embarazo de esposa o novia	Bueno Malo	0	1	2	3
33. (Hombre) Aborto de esposa o novia	Bueno Malo	0	1	2	3
34. Casarse o iniciar una convivencia	Bueno Malo	0	1	2	3
35. Cambio en la cercanía/intimidad con su pareja	Bueno Malo	0	1	2	3
36. Infidelidad	Bueno Malo	0	1	2	3
37. Problemas con los suegros	Bueno Malo	0	1	2	3
38. Separación del cónyuge o pareja debido a un conflicto	Bueno Malo	0	1	2	3
39. Separación del cónyuge o pareja por motivos de trabajo, viajes, etc	Bueno Malo	0	1	2	3
40. Reconciliación con su cónyuge o pareja	Bueno Malo	0	1	2	3
41. Divorcio o separación permanente	Bueno Malo	0	1	2	3
42. Cambio en el trabajo de su esposo o pareja (inicio de trabajo, despido, cambio de trabajo, jubilación, etc)					

Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			
F. FAMILIA Y AMIGOS CERCANOS		Sin efecto	Efecto leve	Efecto moderado	Gran efecto
43. Nuevo integrante de la familia (por nacimiento, adopción, familiar que se traslada a vivir con uds. etc)	Bueno Malo	0	1	2	3
44. Hijo o miembro de la familia sale de casa (por matrimonio, para asistir a la universidad, o por alguna otra razón)	Bueno Malo	0	1	2	3
45. Cambio importante en la salud o el comportamiento de un familiar o un amigo cercano (enfermedad, accidentes, problemas de drogas o disciplinarios, etc)	Bueno Malo	0	1	2	3
46. Muerte del cónyuge o pareja	Bueno Malo	0	1	2	3
47. Muerte de un hijo	Bueno Malo	0	1	2	3
48. Muerte de un familiar o amigo cercano	Bueno Malo	0	1	2	3
49. Nacimiento de un nieto	Bueno Malo	0	1	2	3
50. Cambio en el estado civil de sus padres	Bueno Malo	0	1	2	3
G. CRIANZA					
51. Cambio en la organización del cuidado de los niños	Bueno Malo	0	1	2	3
52. Conflictos con su esposo/a o pareja por la crianza	Bueno Malo	0	1	2	3
53. Conflictos con los abuelos del niño (o con otra persona importante) acerca de la crianza	Bueno Malo	0	1	2	3
54. Asumir toda la responsabilidad de la crianza como un padre soltero	Bueno Malo	0	1	2	3
55. Discusiones/peleas por la tuición con su ex cónyuge o pareja	Bueno Malo	0	1	2	3
Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			

H. PERSONAL O SOCIAL		Sin efecto	Efecto leve	Efecto moderado	Gran efecto
56. Gran logro personal	Bueno Malo	0	1	2	3
57. Decisión importante con respecto a su futuro inmediato	Bueno Malo	0	1	2	3
58. Cambio en sus hábitos personales (vestimenta, estilo de vida, ocio, etc)	Bueno Malo	0	1	2	3
59. Cambio en sus creencias religiosas	Bueno Malo	0	1	2	3
60. Cambio en sus creencias políticas	Bueno Malo	0	1	2	3
61. Pérdida o daño de sus bienes	Bueno Malo	0	1	2	3
62. Viaje por vacaciones	Bueno Malo	0	1	2	3
63. Hizo un viaje, no por vacaciones	Bueno Malo	0	1	2	3
64. Cambio en reuniones familiares	Bueno Malo	0	1	2	3
65. Cambio en sus actividades sociales (discoteques, películas, reuniones, etc.)	Bueno Malo	0	1	2	3
66. Ha hecho nuevos amigos	Bueno Malo	0	1	2	3
67. Discutió con un amigo	Bueno Malo	0	1	2	3
68. Adquirió o perdió una mascota	Bueno Malo	0	1	2	3
I. FINANCIERO					
69. Gran cambio en las finanzas (los ingresos aumentan o disminuyen)	Bueno Malo	0	1	2	3
70. Compra cosas de valor (tales como la televisión, auto, refrigerador, etc.)	Bueno Malo	0	1	2	3
71. Realiza una compra importante o un préstamo hipotecario (para comprar una casa, negocio, o propiedad, etc.)	Bueno Malo	0	1	2	3
72. Ha sido embargado o ha tenido problemas con una hipoteca o un préstamo	Bueno Malo	0	1	2	3
73. Dificultades para obtener un crédito	Bueno Malo	0	1	2	3
Evento	Tipo de Efecto	Nivel de efecto de los sucesos en su vida			
J. CRIMEN Y ASUNTOS LEGALES		Sin efect	Efecto leve	Efecto modera	Gran efecto

		o		do	
74. Ser asaltado o víctima de usurpación de identidad	Bueno Malo	0	1	2	3
75. Ser víctima de un delito violento (violación, asalto, etc)	Bueno Malo	0	1	2	3
76. Ha estado involucrado en un accidente	Bueno Malo	0	1	2	3
77. Ha estado involucrado en una demanda legal	Bueno Malo	0	1	2	3
78. Ha estado involucrado en una falta o delito menor (multas de tránsito, alteración del orden público, etc)	Bueno Malo	0	1	2	3
79. Ha tenido problemas judiciales que lo llevan a ser arrestado o a la cárcel	Bueno Malo	0	1	2	3
K. Otras experiencias recientes que han tenido un impacto en su vida. Enumérelas y califíquelas	Bueno Malo	0	1	2	3
80. _____	Bueno Malo	0	1	2	3
81. _____	Bueno Malo	0	1	2	3
82. _____	Bueno Malo	0	1	2	3

Cuestionario MOS de apoyo social

CUESTIONARIO M.O.S
PARA INVESTIGAR APOYO SOCIAL

Las siguientes preguntas se refieren al apoyo o ayuda de que Ud. dispone:

1. Aproximadamente, ¿Cuántos amigos íntimos o familiares cercanos tiene Ud.? (Personas con las que se encuentra a gusto y puede hablar acerca de todo lo que se le ocurre)

Escriba el nº de amigos y familiares | | |

La gente busca a otras personas para encontrar compañía, asistencia, u otros tipos de ayuda. ¿Con qué frecuencia dispone Ud. de cada uno de los siguientes tipos de apoyo cuando lo necesita?
Marque con un círculo uno de los números de cada fila:

	Nunca	Pocas Veces	Algunas veces	La mayoría de veces	Siempre
2-Alguien que le ayude cuando tenga que estar en la cama.	1	2	3	4	5
3-Alguien con quien puede contar cuando necesita hablar.	1	2	3	4	5
4-Alguien que le aconseje cuando tenga problemas.	1	2	3	4	5
5- Alguien que le lleve al médico cuando lo necesita.	1	2	3	4	5
6-Alguien que le muestre amor y afecto.	1	2	3	4	5
7-Alguien con quien pasar un buen rato.	1	2	3	4	5
8-Alguien que le informe y le ayude a entender una situación.	1	2	3	4	5
9-Alguien en quien confiar o con quien hablar de si mismo y sus preocupaciones.	1	2	3	4	5
10-Alguien que le abrace.	1	2	3	4	5
11-Alguien con quien pueda relajarse.	1	2	3	4	5
12-Alguien que le prepare la comida si no puede hacerlo.	1	2	3	4	5
13-Alguien cuyo consejo realmente desee.	1	2	3	4	5
14-Alguien con quien hacer cosas que le sirvan para olvidar sus problemas.	1	2	3	4	5
15-Alguien que le ayude en sus tareas domésticas si está enfermo.	1	2	3	4	5
16- Alguien con quien compartir sus temores y problemas más íntimos.	1	2	3	4	5
17- Alguien que le aconseje cómo resolver sus problemas personales.	1	2	3	4	5
18- Alguien con quien divertirse.	1	2	3	4	5
19- Alguien que comprenda sus problemas.	1	2	3	4	5
20-Alguien a quien amar y hacerle sentirse querido.	1	2	3	4	5

Este Test, permite investigar 4 dimensiones de Apoyo:

1.- Apoyo Emocional / Informacional:

La expresión de afecto y comprensión empática, así como la guía y oferta de consejos e información.

Preguntas: 3-4-8-9-13-16-17-19

2.- Apoyo Instrumental:
La provisión de ayuda material que pueda recibir la persona.

Preguntas: 2- 5-12-15

3.- La Interacción social positiva:
La disponibilidad de personas con las cuáles poder salir, divertirse o distraerse.

Preguntas: 7 –11 –14 – 18

4.- Apoyo Afectivo:
Las expresiones de amor y de afecto.

Preguntas: 6 – 10 – 20

VALORES	MAXIMO	MINIMO	MEDIO
Emocional	40	8	24
Instrumental	20	4	12
Interacción Social	20	4	12
Afectivo	15	3	09
Indice Global	95	19	57

El **índice global de Apoyo Social** se obtiene sumando los 19 items.

El **Apoyo Social es Escaso** cuando el Indice es inferior a 57 puntos.
Habrá **Falta de Apoyo Emocional** cuando la puntuación sea menor a 24.
Habrá **Falta de Apoyo Instrumental** cuando la puntuación sea menor a 12.
Habrá **Falta de Interacción Social** cuando la puntuación sea menor a 12.
Habrá **Falta de Apoyo Afectivo** cuando la puntuación sea menor a 9.

Child Trauma Questionnaire

CTQ [1]

Mientras iba creciendo…

	Nunca	Rara vez	Algunas veces	Frecuentemente	Muy frecuentemente
1. No tenía suficiente para comer					
2. Yo sabía que había alguien para cuidarme y protegerme					
3. Algunas personas de mi familia me decían cosas como "estúpido/a", "flojo/a", o "feo/a"					
4. Mis padres estaban demasiado borrachos o drogados como para cuidar de la familia					
5. Había alguien en mi familia que me ayudaba a sentirme importante o especial					
6. Tenía que usar ropa sucia					
7. Me sentía amado/a					
8. Alguna vez pensé que mis padres desearon que yo jamás hubiese nacido					
9. Alguna o algunas personas de mi familia me pegaron tan fuerte que tuve que ver un doctor o ir al hospital					
10. No hubo nada que haya querido cambiar de mi familia					
11. Algunas personas de mi familia me pegaban/golpeaban tan fuerte que me dejaban marcas o moretones					
12. Era castigado con un cinturón, una palo, un cuerda o algún otro objeto duro					
13. Las personas en mi familia nos cuidábamos lo unos a los otros					
14. Algunas personas de mi familia me decían cosas hirientes o insultos					
15. Yo creo que fui maltratado físicamente					
16. Tuve una infancia perfecta					
17. Fui tan fuertemente golpeado/a por alguien de mi familia que otras personas, como un profesor, un vecino o un médico, se dieron cuenta					
18. Yo sentía que alguien en mi familia me odiaba					
19. Las personas en mi familia se sentían cercanas entre ellas					
20. Alguien intentó tocarme en una forma sexual, o trató que yo lo/la tocara					
21. Alguien me amenazó con hacerme daño o decir mentiras acerca de mí a menos que yo hiciera algo sexual con el o ella.					
22. Yo tenía la mejor familia del mundo.					
23. Alguien intentó que yo hiciera cosas sexuales o que viera cosas sexuales					
24. Alguien me acosaba /incomodaba					
25. Yo creo que fui maltratado emocionalmente					
26. Había alguien para llevarme al doctor si lo necesitaba					
27. Yo creo que fui sexualmente abusado/a					
28. Mi familia era una fuente de fuerza y apoyo					

[1]Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., Zule, W. (2003). Development and validation of a brief screening version of the childhood trauma questionnaire. *Child Abuse & Neglect, 27*(2), 169-190. doi: 10.1016/S0145-2134(02)00541-0 Adaptado para Chile por Leighton, C.; Botto, A.; De la Cerda C.J.; Undurraga, C.

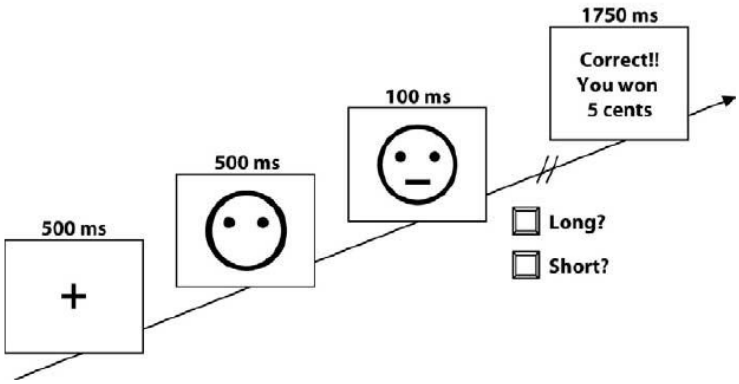
Annexe 2: Experimental Task summary

Procedimiento experimental "Curva de Cortisol en Respuesta a Tarea Estresante"

Control de estímulos y respuesta	Se utilizara una estación de trabajo para el registro de respuestas conductuales y la presentación de los estímulos experimentales a través del programa E-prime 2.0
Verificación de la manipulación afectiva	Para evaluar la manipulación afectiva de las condiciones del estudio se utilizará la versión "en este momento" de la versión en español del Positive & Negative Affect Schedule (PANAS). Consta de 20 ítems que describen diferentes sentimientos y emociones, los cuales se presentan en una escala de Lickert de 5 puntos que va de menor a mayor grado de aceptación
Tarea experimental	Se utilizará la tarea detección de señal, Signal Detection Task (SDT). Es una tarea conductual de 300 ensayos dividido en 3 bloques de 100 ensayos. Su duración es de 20 a 25 minutos y es ejecutada bajo un programa de refuerzamiento diferencial que permite la evaluación de modo objetivo de la propensión de modular la conducta en base a refuerzos previos. En la SDT, los participantes deben elegir entre dos opciones que están relacionadas a diferentes probabilidades de refuerzo. Una de las opciones es desproporcionadamente reforzada (E+), mientras que la otra no (E-). Es decir, los participantes no pueden inferir cual es la respuesta más ventajosa basados en el resultado de un ensayo único, y deben considerar los refuerzos previos para optimizar sus elecciones. El desempeño conductual se analiza utilizando la teoría de detección de señal calculando el sesgo de respuesta hacia el estimulo mas reforzado y la discriminación total. Además de los tiempos de respuesta y aciertos. (Pizzagalli 2008)
Procedimiento	Los participantes completaran en forma individual la DST. Se instruirá a los participantes las características de la tarea (identificar el estímulo lo mejor posible) y se les explicará que deben ganar el máximo de dinero posible, el cual le será entregado al final de la tarea. Cada sujeto completará la tarea 2 veces utilizando los lineamientos del grupo de Pizzagalli; una condición neutral y una condición de estrés psicosocial en el cual el sujeto recibirá durante la tarea varios feedback negativo de su performance. De este modo en la condición neutral el participante recibirá un feedback de un buen rendimiento en la tarea (>70% correctas) mientras que en la condición de estrés se le indicará continuamente que su performance esta dentro del 40-20% de los peores rendimientos de participantes pasados. Este procedimiento confiablemente induce ansiedad en los participantes en la condición de feedback negativo (Bogdan, 2006). Inmediatamente antes de comenzar y al terminar cada una de las tareas, para ambas condiciones se evaluará el nivel de afecto negativo experimentado, para verificar la manipulación afectiva.
Estrategia de análisis	1. Respuesta conductual: La reducción del sesgo de respuesta (variable principal de interés) se realizará mediante la formula: log b=1/2 log

	(E+correcto*E-incorrecto/E+incorrecto*E-correcto). Así un sesgo de respuesta elevado deriva de un número alto del producto de identificaciones correctas de un E+ por identificaciones incorrectas del E-. Para explorar los efectos y/o interacciones de grupo, bloques y condiciones se realizará, entre otros, un ANOVA de modelo mixto con un factor intersujeto de grupo (depresivo vs. control) y factores intrasujeto de bloque (1,2,3) y condición (neutral vs. estrés) sobre las mediciones de sesgo de respuesta. Adicionalmente se realizará un análisis de tendencia sobre las medidas de sesgo de respuesta para explorar el patrón que adopta en el tiempo.

Tarea de detección de señal:



Esta tarea esta destinada en medir la sensibilidad al estrés de los individuos, (y se supone que en depresivos esta estaría aumentada). Se instruye a los participantes sobre la tarea y se les explica que el objetivo es lograr ganar la mayor cantidad de dinero posible. La tarea consiste en 300 ensayos divididos en 3 bloques de 100 ensayos, con cada bloque separado por un break de 30 segundos. Cada ensayo se inicia con la presentación de un asterisco por 500 ms al medio de la pantalla que sirve como punto de fijación. Luego aparece una cara sin boca, después de 500 ms aparece una boca corta (11,5 mm) o larga (13 mm) por 100 ms. La cara sin boca permanece en la pantalla hasta que se realice la respuesta.

Se les pide a los participantes que identifiquen que tipo de boca se presentó utilizando la tecla z o / del teclado. Para cada bloque se presento el mismo número de veces cada boca en una secuencia pseudo azarosa, sin presentar mas de 3 veces seguidas el mismo estímulo. Además se hace un reforzamiento asimétrico luego de algunas respuestas positivas. Se mide la precisión de respuesta y tiempo de respuesta antes y después del refuerzo negativo. (Pizzagalli, D. Jahn, A. & O'Shea, P, 2005)