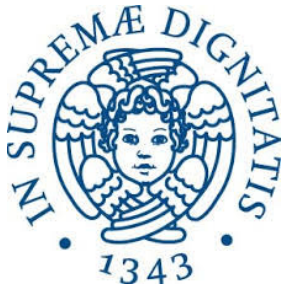

UNIVERSITY OF PISA

RESEARCH DOCTORATE IN NEUROBIOLOGY AND CLINIC OF AFFECTIVE DISORDERS

DEPARTMENT OF PSYCHIATRY, NEUROBIOLOGY, PHARMACHOLOGY AND BIOTECNOLOGIES

THESIS:

Psychoneurobiology of an earthquake:

Brain-Derived Neurotrophic Factor and stress spectrum clinical correlates of a population involved in the L'Aquila (2009) earthquake

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Years 2011-2013

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1. ABSTRACT

Disasters such as earthquakes are events that affect wide populations causing widespread consequences including psychosocial disruption, physical threat, massive psychological stress. Mental health professionals have been increasingly called upon to assist during these acute crises.

Disasters deserve special attention among human traumas because the capacity to traumatize a great many individuals at once, being the most public of traumas, and thus offering unique opportunities to study human response to tragedy on any level. Because natural disasters are random events that expose unselected populations to trauma, they offer unique opportunities for researchers interested in studying subjects 'triggered' to a unique trauma, disentangling confounding issues of pre-existing risk for exposure to traumatic events.

On April 6th 2009, at 3:32 am, an earthquake (Richter Magnitude 6.3) struck L'Aquila, Italy, a town with a population of 72,000 and a 'local health' (i.e. Azienda Sanitaria Locale) of 105,000 inhabitants. L'Aquila earthquake caused the death of 309 people, with more than 1600 individuals injured, among which 200 were severely injured and hospitalized, and 66,000 displaced. Many buildings collapsed in the town of L'Aquila: large parts of it were completely destroyed.

In this thesis psychoneurobiology of stress caused by this traumatic event has been investigated through three studies on Brain-Derived Neurotrophic Factor (BDNF). The BDNF is a key mediator of neuronal plasticity, which stimulate a variety of cellular effects at the structural and functional levels that eventuate in the promotion of survival and differentiation of responsive neurons. Stress has been widely linked with alterations in the expression and functioning of BDNF in both animal and human clinical studies.

The aim of the first study was to investigate plasma BDNF levels in a clinical population who survived to the L'Aquila 2009 earthquake, along with the post-traumatic spectrum that

considers not only full expression of Post-Traumatic Stress Disorder (PTSD) but also subthreshold manifestations such as partial PTSD. To do so a consecutive sample of 37 outpatients referring to the National Mental Health Care Service in L'Aquila for anxiety or affective symptoms after the earthquake, was compared to 15 healthy controls matched for age and gender. Eleven patients were diagnosed as not having PTSD but a different pathological condition that justified the referral and 13 patients respectively were diagnosed as showing Full or Partial PTSD. The subjects without PTSD, but with anxiety or affective disorders, and subjects with full-blown PTSD showed lower BDNF level than subjects with partial PTSD and healthy controls.

The aim of the second study was to investigate the clinical correlates of plasma BDNF levels in a clinical population showing PTSD symptomatology, along with the post-traumatic spectrum. Assessments included: Structured Clinical Interview for DSM-IV Axis-I disorders Patient Version, Trauma and Loss Spectrum-Self Report (TALS-SR) for post-traumatic spectrum symptoms and the Impact of Event Scale Revised (IES-R) for the PTSD symptomatology. Thirteen patients were diagnosed as showing Full PTSD and 13 Partial PTSD. Different relationship pattern of BDNF vs. stress symptoms has been reported in partial and full PTSD samples.

In the third study BDNF modifications in subjects that did not showed psychiatric symptoms or symptoms worsening despite having suffered relevant stress event have been investigated. We hypothesized that, in so far as no stress consequences or relapse/worsening appeared in subjects who suffered relevant stress, any BDNF variation could be due to stress exposure. To do so BDNF plasma levels have been evaluated in subjects who suffered the same stress event, i.e. a clinical and non clinical population exposed to the 2009 L'Aquila earthquake, in comparison to a population not exposed to relevant stress. Statistical difference has been observed for diagnosis factor (clinical samples vs. controls). A trend toward significance was seen for exposition factor (exposed

vs. not exposed subjects); exposition by diagnosis interaction did not reach statistical significance. The exposed clinical sample showed significant higher BDNF level than the not exposed. Lack of statistical difference between exposed and not exposed subjects suggests that no BDNF modification intervened after the stressful event, but: exposed samples show the highest BDNF levels and a trend toward significance was seen; possibility of a ceiling effect, with no possibility of exceed a possible maximum level in the control sample can be considered. Clinical sample shows instead room for stress related BDNF increase. If so a BDNF increase with neuroprotective adaptive aim after stress cannot be excluded.

The findings of these studies add more insight on the mechanisms regulating BDNF levels in response to stress and further proofs on the utility of the distinction of PTSD into full and partial categories along the spectrum approach.

2. Introduction

2.1. When a disaster can aid research

Earthquakes, one of the major natural disasters, are unpredictable, uncontrollable, and may be highly destructive.

The April 6, 2009 L'Aquila earthquake was the strongest seismic event to occur in Italy over the last thirty years with a magnitude of M = 6.3 at 3:32 local time. L'Aquila is a central Italy town with a population of 72,000 inhabitants and a health district catchment of about 105,000 people. This earthquake was the strongest event in a sequence of seismic events that had started a few months earlier (October 2008) and was followed by numerous aftershocks in the next weeks. This number of pre- and aftershocks is likely one of the most catastrophic aspects of this natural disaster, causing fear and expectations of an even more severe earthquake among the residences of the region.

The earthquake caused 309 deaths and considerable damage to structures over an area of approximately 600 km2, including the urban center of L'Aquila and several villages in the mid-Aterno Valley. The seismic event injured more than 1600, 200 of them seriously hurt and hospitalized, and displaced 66,000 people. Five per cent of the population were trapped under the rubble with minor physical consequences, 15% lost at least an acquaintance. Many buildings were damaged; others, especially in the historic centre, were completely destroyed. The earthquake affected an area of approximately 5,000 km^2, significantly damaging most economic activities and public facilities, such as hospitals and schools at all levels. The Italian government gave an official estimate of the cost, in terms of direct economic losses and reconstruction costs, of eight billion Euros.

All residents suffered directly from the disaster to varying degrees, in terms of involvement in the event, e.g. closeness to the epicenter or financial loss as a result of the earthquake, and personal characteristics, e.g. gender or age. The displaced people found accommodation in hotels of the nearby towns, or in tents located in camps near the urban

area. Twelve months after the earthquake, only 25% of the population was able to return to their homes (Stratta et al., 2012; Dell'Osso et al., 2013).

Disasters such as earthquakes are events that affect wide populations causing widespread consequences including psychosocial disruption, physical threat, massive psychological stress. Mental health professionals have been increasingly called upon to assist during these acute crises. Many studies show that disasters and other traumatic events have short- and long-term health consequences, especially as regards mental disorders (Alexander 1996; Carr et al. 1997; Yzermans et al. 2005; Bartels and VanRooyen 2009).

Natural disasters deserve special attention among human traumas because the capacity to traumatize a great many individuals at once, being the most public of traumas, and thus offering unique opportunities to study the human response to tragedy at any level. Because disasters strike randomly, studies of disasters circumvent the limitations of research on trauma to individuals in the community, where risk for traumatic events is confounded with vulnerability to psychopathology (North, 1995). Natural disasters are random events that expose unselected populations to trauma, they offer relevant opportunities for researchers interested in studying subjects 'triggered' to a unique trauma, disentangling confounding issues of pre-existing risk for exposure to traumatic events.

The magnitude and intensity of the L'Aquila earthquake made it a significant subject for the study of mental health effects of trauma because of the profound effects among its survivors, including persons with no pre-disaster psychiatric history (Stratta et al., 2012).

In this thesis the results of a psycho-neurobiological investigation performed on the earthquake survivors are reported. The study toke therefore advantage from the possibility of studying the effect of the same stress event shared from all the subjects recruited.

2.2. Neurobiology of stress

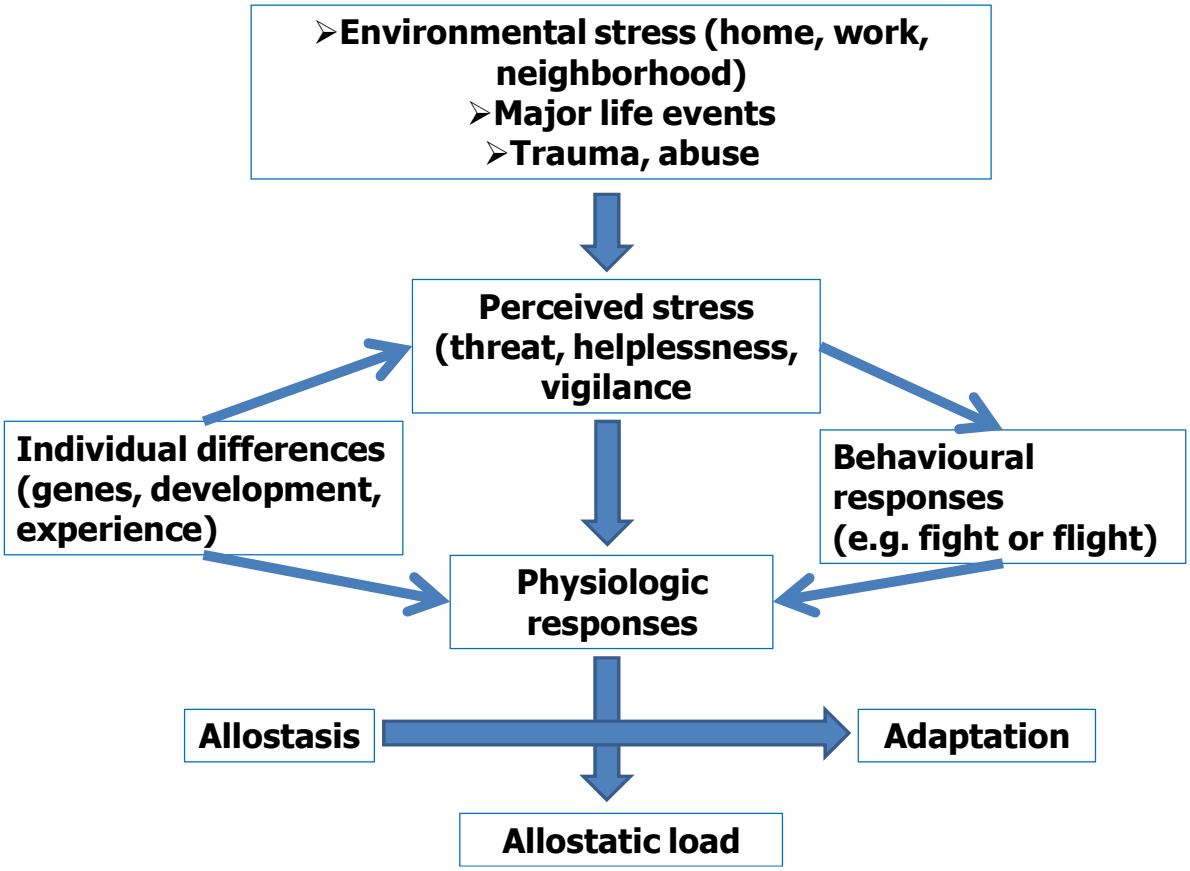
Stress involves activation of neurobiological systems that preserve viability through change or allostasis. Although they are necessary for survival, frequent neurobiological stress responses increase the risk of physical and mental health problems, perhaps particularly when experienced during periods of rapid brain development.

The adaptive physiological response to acute stress involves a process, initially referred to as allostasis by Sterling and Eyer (1988), in which the internal milieu varies to meet perceived and anticipated demand. McEwen (2002) extended this definition to include the concept of a set point that changes because of the process of maintaining homeostasis. The responses to severe stress that promote survival in the context of a life-threatening situation may be adaptive in the short run. However, if recovery from the acute event is not accompanied by an adequate homeostatic response to terminate the acute adaptive response of stress mediators, the deleterious effects on psychological and physiological function, termed the “allostatic load,” occur. The allostatic load is the burden borne by a brain and body adapting to both physiological and psychological challenges. The concepts of allostasis and allostatic load link the protective and survival values of the acute response to stress to the adverse consequences that result if the acute response persists (McEwen and Stellar 1993; McEwen, 1998).

The importance of acknowledging the protective, as well as the potentially damaging effects of the mediators of stress and adaptation, has led to the introduction of these two terms. “Allostasis,” regards the process of maintaining stability (homeostasis) by active means, namely, by putting out stress hormones and other mediators. On the other hand the “allostatic load” or “overload,” regards the wear and tear on the body and brain caused by use of allostasis, particularly when the mediators are dysregulated, i.e., not turned off when stress is over or not turned on adequately when they are needed (Figure 1).

Figure 1. Role of the brain in

"Allostasis" and "allostatic load" in response to stressors (modified from McEwen, 1998).



The brain is the organ of the body that interprets experiences as threatening or nonthreatening and which determines the behavioral and physiological responses to each situation. Besides the hypothalamus and brain stem, which are essential for autonomic and neuroendocrine responses to stressors, higher cognitive areas of the brain play a key role in memory, anxiety, and decision making. These brain areas are targets of stress and stress hormones, and the acute and chronic effects of stressful experiences influence how they respond (McEwen, 2007).

A number of neurotransmitters, neuropeptides, and hormones have been linked to the acute psychobiological response to stress (Charney, 2004). They have important functional interactions, and mediate the neural mechanisms and neural circuits relevant to the regulation of reward, fear conditioning, and social behavior. The roles of those neurotransmitters, neuropeptides, and hormones that have been shown to be significantly altered by psychological stress are resumed in Table 1.

These mediators of the stress response are not however the only involved. For example, glutamate and neurotrophic factors, such as brain-derived neurotrophic factor, and neuropeptides, such as substance P and cholecystokinin, have to be considered. Longitudinal community- based surveys of successful adaptation to extreme stress should be considered to determine if markers such as these or others can be used to develop a measure of psychobiological allostatic load that will be of predictive value.

Table 1. The neurochemical response to acute stress (modified from Charney, 2004).

Neurochemical	Acute Effects	Brain Regions	Key Functional Interactions	Association With Psychopathology
Cortisol	Mobilized energy, increased arousal, focused attention, fear memory formation, fear learning	Prefrontal cortex, hippocampus, amygdala, hypothalamus	Increases amygdale corticotropinreleasing hormone (CRH), increases hypothalamic CRH	Unconstrained release leads to hypercortisolemia-depression, hypertension, osteoporosis, insulin resistance, coronary vascular disease; overconstrained release leads to hypocortisolemia, seen in some PTSD patients
Dehydroepiandrosterone (DHEA)	Counteracts deleterious effects of high cortisol neuroprotection; has positive mood effects	Largely unknown; hypothalamus	Antiglucocorticoid actions	Low DHEA response to stress may predispose to PTSD and depression and the effects of hypercortisolemia
CRH	Activated fear behaviors, increased arousal, increased motor activity, inhibited neurovegetative function, reduced reward expectations	Prefrontal cortex, cingulate cortex, amygdala, nucleus accumbens, hippocampus, hypothalamus, bed nucleus of the stria terminalis, periaqueductal gray matter, locus coeruleus, dorsal raphe	CRH-1 receptor anxiogenic, CRH-2 receptor anxiolytic, increases cortisol and DHEA, activates locus coeruleusnorepinephrine system	Persistently increased CRH concentration may predispose to PTSD and major depression; may relate to chronic symptoms of anxiety, fear, and anhedonia

Table 1. The neurochemical response to acute stress (continue).

Neurochemical	Acute Effects	Brain Regions	Key Functional Interactions	Association With Psychopathology
Locus coeruleus-norepinephrine system	General alarm function activated by extrinsic and intrinsic threat; increased arousal, increased attention, fear memory formation, facilitated motor response	Prefrontal cortex, amygdala, hippocampus, hypothalamus	Activates sympathetic axis, inhibits parasympathetic outflow, stimulates hypothalamic CRH	Unrestrained functioning of locus coeruleus-norepinephrine system leads to chronic anxiety, hypervigilance, and intrusive memories; some patients with PTSD, panic disorder, and major depression show evidence of heightened locus coeruleusnorepinephrine activity
Neuropeptide Y	Anxiolytic; counteracts the stressrelated effects of CRH and the locus coeruleus-norepinephrine system; impairs fear memory	Amygdala, hippocampus, hypothalamus, septum, periaqueductal gray matter, locus coeruleus	Reduces CRH-related actions at amygdala, reduces rate of firing of locus coeruleus	Low neuropeptide Y response to stress is associated with increased vulnerability to PTSD and depression
Galanin	Anxiolytic; counteracts the stressinduced effects of the locus coeruleus-norepinephrine system; impairs fear conditioning	Prefrontal cortex, amygdala, hippocampus, hypothalamus, locus coeruleus	Reduces the anxiogenic effects of locus coeruleusnorepinephrine system activation	Hypothesized low galanin response to stress is associated with increased vulnerability to PTSD and depression

Table 1. The neurochemical response to acute stress (continue).

Neurochemical	Acute Effects	Brain Regions	Key Functional Interactions	Association With Psychopathology
Dopamine	High prefrontal cortex and low nucleus accumbens dopamine levels are associated with anhedonic and helpless behaviors	Prefrontal cortex, nucleus accumbens, amygdala	Reciprocal interactions between cortical and subcortical dopamine systems	Persistently high levels of prefrontal cortical and low levels of subcortical dopamine activity are associated with cognitive dysfunction and depression; persistently low levels of prefrontal cortical dopamine are associated with chronic anxiety and fear
Serotonin (5-HT)	Mixed effects: 5-HT stimulation of 5-HT2 receptors is anxiogenic; 5-HT stimulation of 5-HT1A receptors is anxiolytic	Prefrontal cortex, amygdala, hippocampus, dorsal raphe	High levels of cortisol decrease in 5-HT1A receptors	Low activity of postsynaptic 5-HT1A receptors may predispose to anxiety and depression
Benzodiazepine receptors	Acute stress down-regulation of cortical benzodiazepine receptors	Prefrontal cortex, hippocampus	May be relationship between decreased 5-HT1A and decreased benzodiazepine receptor function	Decreased cortical benzodiazepine receptors are associated with panic disorder and PTSD

Table 1. The neurochemical response to acute stress (continue).

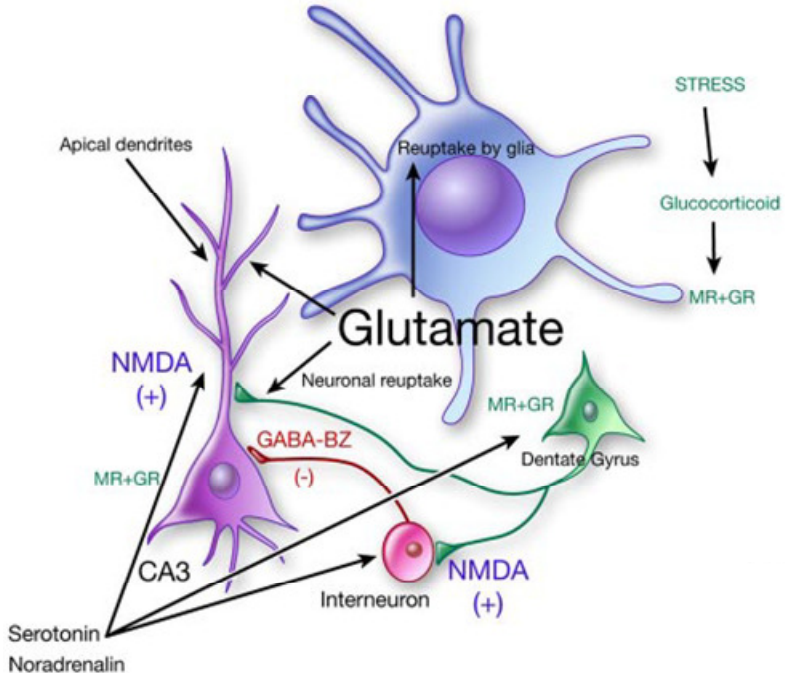
Neurochemical	Acute Effects	Brain Regions	Key Functional Interactions	Association With Psychopathology
Testosterone	Stress-induced decrease in assertive behavior and increase in depression	Hypothalamus	CRH decreases testosterone levels	Decreased CSF testosterone levels are found in PTSD; testosterone supplementation is helpful for depressed men with low testosterone levels
Estrogen	Acute increases in estrogen may dampen hypothalamic-pituitaryadrenal (HPA) and norepinephrine responses to stress	Hypothalamus, hippocampus	Estrogen increases function of benzodiazepine receptors and decreases function of 5-HT1A receptors	Long-term increases in estrogen may down-regulate 5-HT1A receptors and increase risk for depression and anxiety

As a matter of fact another way that stress hormones modulate function within the brain is by changing the structure of neurons. The hippocampus is one of the most sensitive and malleable regions of the brain. The role of this plasticity may be to protect against permanent damage, or it may enhance vulnerability to damage. Whatever the physiological significance of these changes, the hippocampus undergoes a number of adaptive changes in response to acute and chronic stress.

There are many hormonal, neurochemical, and behavioral modulators of neurogenesis and cell survival including estradiol, insulin-like growth factor I (IGF-I), antidepressants, voluntary exercise, and hippocampal-dependent learning (Aberg et al., 2000; Czeh et al., 2001; Trejo et al., 2001). Neurochemical systems that regulate neurogenesis and dendritic remodeling are summarized in Figure 2 and Table 2 and include excitatory amino acids, serotonin, norepinephrine, benzodiazepines, endogenous opioids, neurotrophic factors, such as Brain.Derived Neurotrophic Factor (BDNF), and IGF-I, as well as glucocorticoids.

Exploration of the underlying mechanism for this remodeling of dendrites and synapses reveals that it is not adrenal size or presumed amount of physiological stress per se that determines dendritic remodeling, but a complex set of other factors that modulate neuronal structure (McEwen, 1999).

Fig. 2. Structural plasticity in hippocampus involving synaptogenesis (S), neurogenesis (N), and dendritic remodeling (D) involves multiple neurochemical systems (modified from McEwen, 2007).



Neurochemical systems for structural plasticity

1. Glutamate release and reuptake
2. NMDA receptor activation, blockade
3. Circulating glucocorticoids involving both mineralocorticoid (MR) and glucocorticoid (GR) receptors
4. Serotonin
5. Noradrenalin
6. Endogenous opioids
7. Benzodiazepines
8. BDNF
9. IGF-1, ghrelin, leptin, insulin

Table 2. Adrenal steroid actions on neurochemical systems in hippocampus (McEwan, 2007)

Extracellular glutamate
Adrenalectomy prevents stress-induced rise of extracellular glutamate
Glucortcoids increase extracellular glutamate
Stress induces Glt-1, glutamate transporter
NMDA receptors
Glucocorticoids upregulate NR2A&B (NMDA) receptor subunit mRNA (372)
Calcium currents
Glucocorticoids increase calcium conductances
Glucocorticoids downregulate calcium extrusion pump
Adrenal steroids biphasically regulate voltage-induced calcium currents
5-HT system
Adrenal steroids are required for stress-induced serotonin turnover
Adrenal steroids biphasically regulate 5-HT1A receptors
GABA benzodiazepine receptors
Differential regulation of GABAA receptor subunit mRNA levels by ADX and corticosterone
Opioids
Glucocorticoids reverse ADX decrease in dynorphin in dentate gyrus
Glucocorticoids upregulate preproenkephalin mRNA in hippocampus

Regarding the cellular and molecular mechanisms underlying structural remodeling, adrenal steroids are important mediators of remodeling of hippocampal neurons during repeated stress, and exogenous adrenal steroids can also cause remodeling in the absence of an external stressor (Magarinos et al., 1999; Sousa et al., 2000). The role of adrenal steroids involves many interactions with neurochemical systems in the hippocampus, including serotonin, endogenous opioids, calcium currents, GABA-benzodiazepine receptors, and excitatory amino acids (McEwen, 1999), as summarized in Figure 2 and Table 2 (McEwen and Chattarji, 2004). Central to all of these interactions is the role of excitatory amino acids, such as glutamate. Excitatory amino acids released by the mossy fiber pathway play a key role in the remodeling of the CA3 region of the hippocampus, and regulation of glutamate release by adrenal steroids may play an important role (McEwen, 1999). Chronic psychosocial stress in the tree shrew also downregulated a number of other gene transcripts associated with neurotrophic effects and cytoskeletal plasticity, including neurotrophic factors (Alfonso et al., 2004).

2.3. Neurotrophins family

During the development of the central nervous system, neural connections are established and redefined through a series of development programs involving specification of axons and dendrites, growth processes, innervation of target cells, cell death, and synaptogenesis. Many of these developmental processes are regulated by proteins derived from the target cells, which signal in a retrograde manner through long distances from the distal axons to the neuronal bodies. Neurons depend for their survival on these interactions with target cells, which release specific proteins that play a crucial role in these interactions (Leibrock et al., 1989; Zweifel et al., 2005). These specific proteins are called neurotrophins, a family of proteins that include the brain-derived neurotrophic factor (BDNF). The first neurotrophin to be discovered was NGF,about 50 years ago. Several studies demonstrated its involvement in the regulation of neuronal death, both in response to subsequent damage to the nervous system as well as during development (Perez-Polo et al., 1990). The dependence of many neurons on their target cells for normal development and the restricted neuronal specificity of NGF (mainly peripheral nervous system), suggested the existence of other neurotrophic factors. The structure of BDNF was reported in 1990, a neurotrophin that promotes the survival of neuronal populations located in the central nervous system, or neuronal populations directly connected to it (Barde, 1990). In the following years other neurotrophins with similar functions were identified, such as NT-3 (Hohn et al., 1990; Maisonpierre et al., 1990) and NT-6 (Gotz et al., 1994). Neurotrophins are all small, basic, secretory proteins that allow the survival of specific neuronal populations. In its biologically active forms together show about 50% amino acid identity. The genes encoding neurotrophins are expressed not only during development but also in the adult, in a variety of tissues including the central nervous system. Each neurotrophin can signal through two different cell surface receptors: the Trk tyrosine kinase receptor and the p75 neu-rotrophin receptor.

Each Trk receptor is preferentially activated by one ormore neurotrophin:TrkA by NGF, TrkB by BDNF, and TrkC by NT-3. Although the long-term trophic effects of neurotrophins depend on gene regulation, the cytoplasmic effectors activated by neurotrophins also exert a wide range of more rapid actions, including morphogenetic and chemotropic effects on developing neurons, and modulation of neuronal excitability and synaptic transmission(Poo,2001; Chao,2003).

Once the role of the neurotrophins as regulatory factors of neuronal survival and differentiation was properly described, new evidence indicated that they also acted as synaptic modulators. Synaptic activity regulates the synthesis, secretion, and action of neurotrophins. These, in turn, induce changes in the morphology and synaptic efficacy. Thus, the neurotrophins are involved in activity-dependent synaptic plasticity, and with long-term changes in the synaptic connections at a structural and a functional level (Poo, 2001). Neurotrophins seem to be necessary for the maintenance of functional synapses, because their removal produces a reduction in synaptic connectivity between cultured neurons.

2.4. The Brain-Derived Neurotrophic Factor (BDNF)

The Brain-Derived Neurotrophic Factor (BDNF), is a member of the neurotrophin family of polypeptide nerve growth factors, widely expressed in the developing and adult mammalian brain, identified as a key regulator of neuronal development within the central nervous system (Lo, 1996). During development neurotrophins also prevent the death of embryonic neurons (Barde, 1994). Neurotrophins are expressed in almost all neuronal populations in the central and peripheral nervous systems (Huang and Reichardt, 2001; Chao, 2003).

In the central nervous system neurons, BDNF can increase the number of excitatory and inhibitory synapses, regulating axonal morphology, or directly promoting synapse formation. Additionally, BDNF promote the maturation and stabilization of the cellular and molecular components of neurotransmitters release, and this subsequently leads to an increase in the number of functional synapses. It regulates synapse maturation at the morphological, molecular, and functional levels. This role of BDNF in synapses is crucial not only during development but also for synaptic plasticity in the adult (Vicario-Abejón et al., 2002). In addition to the classic effects on neuronal cell survival, it can also regulate axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, long-term potentiation, and synaptic plasticity. Its receptors may act as a point of convergence that might be involved in the integration of many environmental inputs. This can lead to alterations in neuronal circuitry and, ultimately, in behavior. BDNF can also produce long-term changes in the functionality of adult neurons through changes in transcription (Chao, 2003). Activity-dependent synaptic plasticity models, whether in the context of development or in the context of learning and memory, have long postulated the existence of extracellular signaling molecules which reinforce and stabilize the active synapses. The work of many laboratories has shown that BDNF met the two major criteria to be considered a mediator of this type: the production is regulated

by neural activity, and neurotrophic factors themselves have potent effects on signaling properties of target neurons (Lo, 1995).

BDNF is the neurotrophin most widely distributed in the brain with particularly large quantities in the hippocampus and the cerebral cortex (Altar, 1999). It regulates neuronal development/survival, and controls several neurotransmitter systems (Lewin and Barde, 1996). BDNF is produced both by central nervous system cells and by epithelial cells and circulating BDNF is stored by peripheral platelets, those serve as a 'buffer system' for BDNF. BDNF crosses the blood-brain barrier two-directionally, and there is controversial evidence concerning the correlation between central levels and blood levels of BDNF (Karege et al., 2002). Serum levels of BDNF have been found to be 200-fold higher than plasma levels (Rosenfeld et al., 1995); this difference between serum and plasma BDNF may be caused by the amount of BDNF contained by platelets (Fujimura et al., 2002).

In relation to the cellular and molecular biology underlying cognitive functioning, several studies support the conclusion that BDNF plays a key role, given its participation in the processes of synaptic plasticity and learning. An increased expression of BDNF can have a positive effect on the generation of long-term potentiation (LTP) and memory (Lee and Silva, 2009). The role of BDNF in learning and memory was first demonstrated in studies at the cellular and molecular level. It has been shown that BDNF secretion is required for LTP and long-term depression (LTD), which are the cellular mechanisms underlying learning and memory (Poo, 2001; Aicardi et al., 2004). In relation to long-term memory, is noteworthy that BDNF is sufficient to induce the transformation of early phase LTP to late phase LTP, even in the presence of protein synthesis inhibitors (Lu et al., 2005). In animal models, the role of BDNF in cognition has also been shown in studies with BDNF mutant mice that show learning deficits and hippocampal-dependent altered pattern discrimination (Korte et al., 1996; Gorski et al., 2003). Inhibition of the signaling of BDNF also alters long-term memory in animal models (Lu et al., 2005).

The Brain-Derived Neurotrophic Factor (BDNF) in recent years has been proposed as a candidate to explain part of the pathogenesis, development and treatment of a number of psychiatric disorders, including schizophrenia (Galderisi et al., 2005; Carlino et al., 2012; Nieto et al., 2013), mood disorders (Post, 2007; Dell'Osso et al., 2010), anxiety (Kobayashi et al., 2005; Ströhle et al., 2010), eating disorders (Hashimoto et al., 2005; Nakazato et al., 2012; Monteleone and Maj, 2008). Alterations in neurotrophic factors such as BDNF at the protein and gene level may contribute to altered brain development, synaptic disconnectivity, and failures in neuroplasticity, different elements that provide evidence that BDNF may play a role in pathogenesis of these mental disorders, playing a role in the pathophysiology and symptoms.

2.5. BDNF and stress

Smith et al. (1995) first demonstrated the role of neurotrophins in changes related to stress, and reported that stress decreases gene expression of BDNF in the hippocampus CA3 pyramidal cell layers and in the dentate gyrus granule cell layer. Since then, evidence has been produced demonstrating the complex outcome of stress on the BDNF system.

Several authors have investigated the expression of BDNF in animal models that reproduce exposure to adverse life events. The initial observation of Smith et al. (1995) reporting reduced BDNF expression in the rat hippocampus after chronic immobilization stress has been confirmed by several subsequent studies (Nair et al., 2007; Roceri et al., 2002; Vollmayr et al., 2001). Additionally, stress-induced down-regulation of hippocampal BDNF expression was observed not only after physical, but also following psychological stress (Rasmusson et al., 2002).

The susceptibility to such events may depend upon the type and timing of the manipulation. Indeed we have shown that strong stressors, such as maternal separation, can reduce BDNF expression in the rat hippocampus (Roceri et al., 2002), although more protracted manipulations during gestation or the early phase of postnatal life do not produce significant changes on hippocampal BDNF levels. The length of the manipulation appears to be more important than the timing for changes that might occur at cortical level. Prenatal stress as well as repeated maternal separation determine a significant reduction of BDNF levels in prefrontal cortex of adult animals (Fumagalli et al., 2004; Koo et al., 2003; Roceri et al., 2004), whereas a single maternal deprivation did not elicit any change on cortical neurotrophin expression (Roceri et al., 2002). Hence developmental events can have enduring effects on BDNF expression that, based on its role in information processing as well as on cognition, may contribute to the persistent changes on brain function and their relative contribution to psychopathology.

The long-lasting nature of neurotrophin changes appears to be due epigenetic mechanisms. Early maltreatment elicited a reduction of BDNF mRNA levels in prefrontal cortex of adult offspring, which was associated with an increased methylation of the Bdnf gene on specific promoters (Roth et al., 2009). These results could provide a molecular basis for enduring changes in neuronal plasticity that are associated with a reduced ability to turn on the transcription of activity-regulated genes, such as the neurotrophin BDNF.

The expression of BDNF is also significantly affected by stress at adulthood, although the final outcome depends on several variables, particularly the duration of stress and the time elapsed between the end of the stressful experience and the sacrifice. Acute stressors can produce a rapid facilitation of the BDNF system. In fact a significant and transient increase of BDNF mRNA levels has been observed in the rat (Marmigere et al., 2003) as well as in the prefrontal and cingulated cortex of rats (Molteni et al., 2001) and in the frontal lobe of mice (Molteni et al., 2009a) exposed to an acute restraint stress. The synaptic increase of mBDNF in response to the acute stress may be part of a cellular 'defensive strategy' aimed at increasing the pool of the mature neurotrophin that, upon release, might interact with its high affinity receptor TrkB and promote synaptic function and cell survival (Bramham and Messaoudi, 2005; Chao et al., 2006). Interestingly the increased synaptic levels of BDNF after stress are not observed in animal with a compromised function of glucocorticoid receptors (Molteni et al., 2009b), which represent a good model for susceptibility to depression (Ridder et al., 2005), suggesting that indeed rapid changes in BDNF under challenging conditions may reflect an active response strategy to preserve neuronal homeostasis (McEwen, 2008). Moreover, it has been recently demonstrated that glucocorticoids can also activate trkB receptor tyrosine kinases in vivo and in vitro, leading to neuroprotective effects (Jeanneteau

et al., 2008), suggesting a further mechanism of integration between glucocorticoid hormones and neurotrophins.

If the rapid increase of BDNF expression following a single stress may represent a short-term protective mechanism, a prolonged stressful experience may have a detrimental effect on neuroplasticity, an observation supported by the overall negative impact of chronic stress on BDNF expression at hippocampal and cortical level.

Reduced BDNF mRNA levels after different paradigms of chronic stress in rat prefrontal cortex (Fumagalli et al., 2004; Roceri et al., 2004) and in mouse frontal cortex (Fumagalli et al., 2003) have been found. Although the exact mechanism by which prolonged stressful experiences regulate BDNF is still unknown, the involvement of hormonal and neurotransmitter systems, has to be taken into consideration (Joca et al., 2007; Molteni et al., 2009a).

Also chronic stress in adult life can alter the expression of BDNF, and in particular of some of its transcripts, through epigenetic mechanisms. Tsankova and collaborators (2006) have shown that chronic defeat stress down-regulated total hippocampal BDNF mRNA levels. Furthermore a prior history of stress can alter the pattern of changes produced by adult-onset stress on BDNF expression. For example, chronic immobilization stress reduced BDNF levels in the prefrontal cortex of 'normal' rats, while increasing its gene expression in prenatally stressed rats (Fumagalli et al., 2004). Similarly acute stress is not able to alter BDNF in rats previously exposed to MD (Nair et al., 2007; Roceri et al., 2002).

Stress causes damage and atrophy in the neurons in some brain regions, especially the hippocampus (Duman, 2004) in human also. Being an important structure for learning and verbal memory, the hippocampus also plays a pivotal role in the physiopathology of psychiatric disorders. It is known that the hippocampus has connections with the amygdala and prefrontal cortex (MacQueen et al., 2003). On the other hand, the negative modulation effect of the hippocampus on the hypothalamus-pituitary-adrenal stress hormone axis may be responsible for dysregulation of the stress response (Pittenger and Duman, 2007). Due

to stress there may be impairment in hippocampus-dependent memory. These changes can occur due to stress as well as glucocorticoids, and glucocorticoids may cause similar damage in the hippocampus (Sapolsky et al., 1985). In the hippocampus, increasing stress may inhibit neuronal growth (Gould et al., 1992) as well as cause neuronal death (Magarinos et al., 1996).

After exposure to chronic stress, in contrast to reduced hippocampal volume, volume increase of the amygdale has been observed (Frodl et al., 2003). There is not only an increase in volume, but also hyperactivity in the function of the amygdala, as demonstrated in functional imaging studies (Drevets et al., 1992). As the hippocampus is responsible for verbal memory and impairment in verbal memory occurs in hippocampal atrophy, the amygdale is responsible for learning and memory; however, with hyperactivity of the amygdala, amygdala-dependent fear learning increases excessively (Conrad et al., 1999). Moreover, changes in the amygdala do not reverse completely, even after the cessation of exposure to chronic stressor (Vyas et al., 2004). The difference between the effects of stress on the amygdala, and hippocampus and prefrontal cortex is significant. While both acute and chronic stress impairs hippocampal function, reduces the length and complexity of CA3 dendrites, and impairs neurogenesis, they also lead to enhanced amygdala-dependent fear learning, increased length and complexity of amygdalar dendrites, and increased amygdalar volume (Pittenger and Duman, 2007). This shows that stress does not have a unique effect on brain structure and functions, but may have various effects in various brain regions.

2.6. Post-Traumatic Stress Disorder (PTSD)

The development of maladaptive symptoms after exposure to extreme stress has been observed from longtime. Jacob Mendez Da Costa, eminent Philadelphia physician, described an eponymous condition resembling posttraumatic stress disorder (PTSD) among veterans of the American Civil War (Vaisrub, 1975). The relatively high prevalence of this condition among veterans of the Vietnam War (Card, 1987; Long et al., 1996; Beals et al., 2002) was one important impetus for the PTSD research over the last several decades. The diagnosis of PTSD was first included in the third edition of the Diagnostic and Statistical Manual of Mental Disorders III ed. (DSM, APA, 1980) in 1980; since then, significant advances have been made in its, recognition, measurement and management and considerable research effort has been directed towards the etiology, phenomenology, clinical and neuro-biological characteristics, and treatment of PTSD and related and common comorbid disorders (Van Der Kolk et al., 1996; Norris and Riad, 1997). Following this, epidemiological studies were carried out in several countries, which indicated that the lifetime prevalence of PTSD was around 10.1% (Kessler et al., 2012), characterized by high risk of suicidal, abusive and maladaptive behaviours (e.g. dangerous driving, aggressive or self-destructive behaviour, at-risk sexual encounters) (Friedman et al., 2011; Dell'Osso et al., 2013; 2012a).

Over these thirty years diagnostic criteria have modified and evolved from DSM III until the recently published DSM5 (Dell'Osso and Rossi, 2013). The workgroup for Anxiety, Obsessive-Compulsive Spectrum, Posttraumatic, and Dissociative Disorders of the task force for the DSM-5 excluded now PTSD from the Anxiety Disorders, leading to the creation of a new section: Trauma and Stress Related Disorders including all the disturbances whose aethiopathogenesis is related to a traumatic event. Within this section five new diagnoses have been added: Reactive Attachment Disorder, Disinhibited Social Engagement Disorder, Acute Stress Disorder, Other Specified Trauma and Stressor

Related Disorder. This change places the trauma at the centre of a variety of disorders, highlighting the different possible reactions to the events.

2.6.1. DSM diagnostic criteria for PTSD

The traumatic event. In the DSM-III (1980), the Criterion A foresaw the exposition to “a recognizable stressful event that would provoke significant symptoms of illness in almost all individuals”. In the DSM-III-R (1987), the criterion was modified adding that the traumatic experience had to be “outside of the usual human experience” including however also less unusual stressors, such as accident involvement. Further connotation was added in the DSM-IV (1994) with elimination of the statement of a stressor that “would provoke significant symptoms of illness in almost all individuals” and subdivision in two components: Criterion A1, objective, “the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others” and Criterion A2, subjective, “the person’s response involved intense fear, helplessness, or horror”. This choice did not however add more sensitivity and specificity to the diagnosis.

The DSM-5 changed Criteria A1 and A2 into a single Criterion A adopting a restrictive approach for the selection of traumatic events. The Criterion A now foresees the actual exposition to, or threat of, death, severe injury or sexual violence. The event may be either directly experienced, or the person can be direct witness of a traumatic event occurring to others, or become aware that the victim of a traumatic event is a family member or close friend. Testimony does not include exposure via electronic instruments, television, movies or photographs, unless it is linked to work activity.

This further redefinition of the traumatic event in DSM 5 can be the answer to the difficulty in finding the right equilibrium between the need to limit the risk of an excessive number of diagnoses due to the lack of highly specific criteria with diagnostic sensitivity.

Symptomatological criteria

The DSM-IV-TR foresaw a symptomatological triad: reexperiencing (Criterion B), avoidance or emotional blunting (Criterion C) and an increase in arousal (Criterion D).

The validity of this symptomatological clusters structure was not however consistently confirmed. While re-experiencing, avoidance and hyperarousal retain their identity, a further fourth factor, although less defined was suggested by several studies. Several studies indicated emotional blunting (from DSM IV Criterion C), while other ones indicated dysphoria, a combination of a relative emotional blunting and symptoms of hyperarousal.

The DSM-5 integrates these results leading to a symptomatological structure of PTSD of four criteria, Negative alterations in cognition and mood, which derives from the separation of some of the symptoms formerly present in Criterion C of the DSM-IV-TR and with other changes to the other criteria. This new symptomatological cluster (now Criterion D) includes symptoms of emotional blunting of the DSM-IV-TR, emphasizing selected aspects, and two new symptoms, namely pervasive emotional state and persistent negative distorted blame of self or others about the cause or consequences of the traumatic event.

The symptomatological structure of PTSD has gone therefore from three to four clusters, with the separation of Avoidance from Numbing, and the revision of several symptoms in order to better identify the disorder. The addition of aggressive (Criterion E1) and self-destructive (Criterion E2) behaviours, which were not foreseen, but which recent studies have shown are clearly correlated with PTSD: aggressive behaviours often manifest in family and social settings, and self-destructive behaviour, which ranges from substance abuse to maladaptive and suicidal gestures.

Although not revolutionary, the DSM-5 represent however a forward progress in better definition of the disorder.

There is great interest in the effect that proposed DSM-5 criteria will have on the prevalence rate of the disorder. To date only a few studies investigated the impact that the proposed DSM–5 criteria will have on prevalence rates among civilian or veteran samples (Calhoun et al., 2012; Elhai et al., 2012; Forbes et al., 2011). Forbes et al. (2011),

investigating the impact of requiring both active avoidance and numbing symptoms separately, showed a decrease in PTSD prevalence of 1-2% points on data collected using existing DSM-IV-TR criteria, in a sample of 835 traumatic injury survivors 3- and 12-months post-injury. However, in a more recent study, Elhai et al. (2012) found that changes associated with DSM-5 resulted in a slight increase in the observed prevalence rates of the disorder in a non-clinical sample of 585 college students. Calhoun et al. (2012), investigated the concordance of proposed DSM-5 PTSD criteria with DSM-IV classification rules in a sample of 185 participants who were recruited for studies focused on trauma and health, and examined the optimal number of symptoms required for PTSD DSM-5 Criterion D and E. The authors found a stronger concordance to DSM-IV-TR and a better sensitivity and specificity with only 2 symptoms required for Cluster D and E instead of the three actually required by DSM-5.

Recent study examining the concordance between DSM-IV-TR and DSM-5 PTSD criteria and the impact of the proposed DSM-5 criteria on the prevalence rates of PTSD in high-school students attending the last year of high school in the L'Aquila, approximately 10 months after the earthquake on April 6, 2009, showed that DSM-5 seems to lead to a more restrictive approach to PTSD diagnosis due to the introduction of new symptomatological criteria structure (Carmassi et al., 2013).

2.6.2. Future directions for research

Future key priorities for research in the PTSD field are in the area of neurobiology, in order to determine brain mechanisms underlying the possibility of successful response to treatment. The underlying success in treatment can be further elucidated, with the help of a variety of animal models. These studies will likely involve examination of changes in brain receptor, neurotransmitter systems and neuroplastic factors.

Regarding to phenomenology and etiology areas, the validation of the PTSD and acute stress disorder constructs and their distinction from other disorders are important issues as well as to improve the understanding of genetic and other biological contributions to PTSD susceptibility and the complex interrelationships between these factors and life experiences.

Increased knowledge of the PTSD patophysiology of would not only help develop new therapeutic targets and treatments with improved short- and long-term efficacy, but also identifying predictors of treatment response. Finally PTSD needs an operational definition of remission, since the ultimate goal of treatment is not merely reduction of symptoms, but full remission (Nemeroff et al., 2006).

2.7. Post-Traumatic Stress Spectrum

The term *posttraumatic stress disorder* was introduced in an attempt to classify psychiatric sequelae that arise from the experience of severe trauma. But which kind of event can be considered as severe and traumatic to induce a PTSD? Terribly frightening, life-threatening, or otherwise highly unsafe experiences that involved physical harm or the threat of physical harm for self or others can have higher possibility to induce the disorder, but is this always the case? Debate around whether an event can be defined as traumatic has risen controversies, even leading to the suggestion to eliminate the stressor criteria, relying only on the symptomatological aspects of the disorder, as it happens for other mental illnesses.

Numerous studies on clinical characteristics, epidemiology and neurobiology, carried out on persons suffering from PTSD have taken into account not only risk factors, resilience and course of disease, but also the complications of PTSD as well as the subclinical and partial forms, which are neither less relevant or invalidating than the syndrome with complete symptomology (Dell'Osso e tal., 2012; Stratta et al., 2012; Dell'Osso et al., 2011; Dell'Osso et al., 2009).

Individual's vulnerability, involving gene-environment interaction, is a relevant issue that have to be considered for the PTSD onset. Trauma therefore could be a necessary condition but not sufficient to the disorder development, other risk factors such as peri-traumatic dissociation, peri-traumatic negative emotions, social support, likely playing a relevant role. The nature of the trauma, pre-existing psychiatric disorders, available support systems, age at the time of the traumatic experience and other factors predispose patients to a variety of psychiatric responses (Jung, 2004; Silva et al., 2000). Thus in a complex interaction between the nature and severity of trauma and the varying vulnerability of the victim, a spectrum of psychiatric symptoms emerges. Dimensional approaches to PTSD, similarly to what reported for other mental disorders, have been

developed and tested. Moreau and Zisook (2002) first conceptualized a multidimensional approach to PTSD also taking into account the controversies on the definition of the stressor criterion.

Other than a spectrum based on the nature of the trauma, other spectra can be considered: one based on symptom severity (or diagnostic threshold) and another based on potential responses to trauma (Moreau and Zisook, 2002).

With regard to a stressor criteria spectrum, the diagnostic nomenclature initially only recognized severe forms of trauma personally experienced. More recently, however, the person's subjective response and events occurring to loved ones were included. This has greatly broadened the stressor criteria by leading to an appreciation of the range of precipitating stressors and the potential impact of "low-magnitude" events.

Among the severity spectrum, studies that review diagnostic thresholds reveal significant prevalence of PTSD symptoms and impairment that results from subthreshold conditions. Comorbidity patterns suggest that when PTSD is associated with other psychiatric illness, diagnosis is more difficult and the overall severity of PTSD is considerably greater (Figure 3).

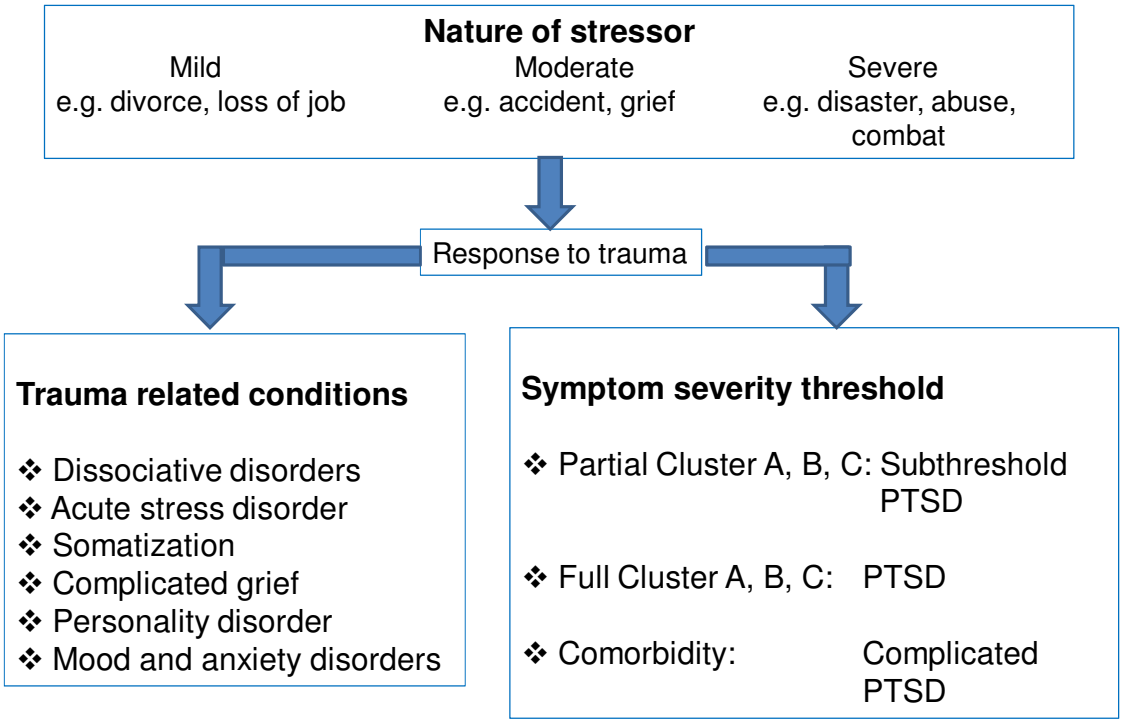
Marshall et al. (2001) reviewed studies that demonstrated that subthreshold PTSD caused by various stressors was as common as full PTSD. Partial PTSD is a chronic and disabling syndrome of trauma victims, subjects with partial PTSD share relevant symptoms with those with the full blown disorder that deserve treatment, confirming other literature observation (Jeon et al., 2007). Subthreshold symptoms may represent a prodrome of the full syndrome or residual symptoms of PTSD in partial remission. Regarding the former possibility, individuals who react to trauma with dissociation are at elevated risk for later developing the full syndrome of PTSD (Yehuda, 1999). For many patients, mild symptoms, such as numbing of affect and irritability limited to the recollection of trauma, persist indefinitely. Even after treatment, many individuals with PTSD continue to suffer from

residual symptoms years after the full syndrome is no longer present (Marshall et al., 2001). Although not yet empirically validated, individuals with residual symptoms may be more susceptible to developing the full syndrome after subsequent traumatic exposure than individuals with full symptom and functional recovery. Further complicating matters, a characteristic course of PTSD is fluctuating symptom severity, with persons meeting full criteria for diagnosis intermittently over the course of many years (van der Kolk et al., 1996).

Given that responses to trauma vary considerably, another possible spectrum includes trauma-related conditions. Traumatic grief, somatization, acute stress disorder and dissociation, personality disorders, depressive disorders, and other anxiety disorders all have significant associations with PTSD. One proposal for classification might include PTSD at the severe end of a spectrum of stress related disorders, with adjustment disorders at the other. The relative rareness of pure PTSD, compared to the presence of more complex forms, suggests that traumatic stress may precipitate a whole host of symptoms and conditions (Yehuda and McFarlane (1995).

Consideration of the severity of symptoms and the range of stressors coupled with the various disorders precipitated by trauma should greatly influence scientific research Moreau and Zisook, 2002.

Figure 3. Spectrum approach to Stress-Related Disorders: Nature of Stressor; Symptom Severity or Diagnostic Thresholds; Response to Trauma (modified from Moreau and Zisook, 2002)



2.8. Spectrum approach to PTSD: the Trauma and Loss Spectrum Questionnaire (TALS-SR)

In the framework of the Spectrum Project (a USA-Italy collaboration), clinicians and researchers of the Department of Psychiatry of the University of Pisa (Italy) developed and validated a questionnaire to assess the "posttraumatic spectrum": the Trauma and Loss Spectrum-Self Report (TALS-SR). Similar to other spectrum measures developed by the Spectrum Project (www.spectrumproject.org), the TALS-SR explores the presence/absence of post-traumatic spectrum that might occur during the lifetime of an individual. The TALS-SR investigates stress response syndromes across three different dimensions: 1) the dimension of the potentially traumatic events, including from extreme to minor ones; 2) the dimension of the peri-traumatic reaction; 3) the dimension of the posttraumatic spectrum symptomatology, including the lifetime occurrence of isolated criterion and non-criterion symptoms and features associated with the DSM-IV-TR diagnosis of PTSD.

The TALS-SR is based on a multidimensional approach to post-traumatic stress reactions that includes a range of threatening or frightening experiences, as well as a variety of potentially significant losses, to which an individual can be exposed. The revised stressor criterion of the DSM-5 specifies that besides witnessing someone's death, learning about the death of a close friend or a relative can be considered to be traumatic when the actual or threatened death has been violent or accidental. In the last decades, increasing efforts were aimed at identifying whether syndromes of unresolved grief are a form of stress response (Maj, 2008; Zisook et al., 2010). A growing literature, in fact provides evidence that a minority of individuals (9-20%) who experience the loss of a close relative or significant other may report symptoms of unresolved grief that are associated with significant distress and impairment, heightened risk for depression, anxiety, alcohol and tobacco consumption, and suicidal ideation. Symptoms include emotions over the

death such as a sense of disbelief, anger, bitterness and preoccupation often associated with distressing intense thoughts, besides intense yearning and longing for the deceased. This condition, named Complicated or Traumatic Grief or, more recently, Prolonged Grief Disorder, is identified by symptoms of both separation and traumatic distress which are distinctive from other Axis I mental disorders, primarily Major Depression (Zisook et al., 2010). Thus, the TALS-SR divides two specific sessions exploring loss events and potentially traumatic events. The second and third dimensions of the TALS-SR explore the spectrum of the peri-traumatic reaction and post-traumatic symptoms, respectively, that may ensue from either type of life events (those that entail exposure to a negative threatening like event and those that entail exposure to a significant loss). These dimensions provide a dimensional approach to the patient's psychopathology by incorporating information related to soft signs, subthreshold symptoms and atypical manifestations that may cause serious distress, as well as a broad array of clinical features associated with trauma and loss events. All these manifestations are commonly seen in clinical populations, but, except for the core or criterion symptoms, are not mentioned in current psychiatric classifications. In line with the new perspectives of the DSM-V also feelings of guilt, anger and shame, besides those of fear, helplessness and horror were added. Somatic and psychic symptoms of anxiety, that might be experienced in the immediate aftermath of the trauma, are also investigated. Consistently with the new proposals of the DSM-5, no distinction is added between acute and post-traumatic stress reactions (Friedman et al., 2011).

The TALS-SR also targets temperamental and personality traits that may constitute risk factors or prodromal symptoms of the disorder. Many researchers have postulated that personal variables (e.g., personality traits) or environmental factors (e.g., level of perceived social support) influence the specific patterns of PTSD expression. Self perception of changes in personality as a result of the traumatic experience is not

uncommon among patients with PTSD. For these reasons the TALS-SR includes a specific domain exploring personality characteristics and risk factors where it is also investigated whether the patient perceived a change in his/her personality after the trauma had occurred. Further, in line with the changes proposed in the new criterion E of the DSM-5 and accordingly with the growing evidence on reckless and maladaptive behaviors occurring in patients with PTSD, the TALS-SR includes a specific session addressing these symptoms (maladaptive coping).

The spectrum approach also allows to identify relevant subclinical comorbidities that may contribute either to the "complex" presentation of PTSD (e.g., the DESNOS) or to the frequent complex behavioral outcomes and complications, such as self-harm behavior and suicidality, also addressed by the DSM-5. By means of a spectrum comorbidity approach we could in fact show the impact of subthreshold mood symptoms on suicidality in patients with PTSD (Dell'Osso et al., 2009), consistently with previous findings in patients with Axis I comorbidity (Simon et al., 2004).

Perhaps no other diagnostic category has gone through as many alterations and permutations as has PTSD. Is not so far the time that many investigators and clinicians considered PTSD a product of malingering or a form of personality disorder (Davidson and Foa, 1991). Over the last 30 or so years, however, the validity of PTSD has become well established and is currently considered one of the most prevalent and disabling psychiatric disorders in civilian and not only in military populations (Yehuda, 1999). An understanding of PTSD and stress-related conditions is in its infancy. This is not surprising given the fact PTSD was not recognized as a distinct diagnostic entity quite recently, in the 1980, by the DSM diagnostic classification. From that time this disorder has undergone continuous change as our understanding of PTSD is refined.

Although consideration of PTSD spectrum has not been included in the DSM 5, the opportunity of further studies is warranted. The dimensional approach is however gaining

support and over the past decade, psychiatrists have proposed a number of such dimensions although they are not used in practice, partly because they are not sanctioned by the *DSM*. The APA claims that the final version of *DSM-5* is a significant advance on the previous edition and that it uses a combination of category and dimensional diagnoses. The previously separate categories of substance abuse and substance dependence are merged into the new diagnosis of substance-use disorder. Asperger's syndrome is bundled together with a handful of related conditions into the new category called autism-spectrum disorder; and OCD, compulsive hair-pulling and other similar disorders are grouped together in an obsessive–compulsive and related disorders category. These changes could help research into common vulnerabilities (Adam, 2013).

Dimensional assessments, such as the spectrum approach developed in the TALS-SR, may give substantial contributions for a better understanding of post-traumatic reaction and thus, for redefining PTSD.

This thesis dissertation wish to be one of these studies.

3. Introduction to the experimental section

The brain-derived neurotrophic factor (BDNF), is a key mediator of neuronal plasticity, which stimulate a variety of cellular effects at the structural and functional levels that eventuate in the promotion of survival and differentiation of responsive neurons (Huang and Reichardt, 2001; . Nagahara and Tuszynski, 2011).

Stress has been widely linked with alterations in the expression and functioning of BDNF in both animal and clinical studies. Animal studies demonstrated that many different types of acute and chronic stress paradigms decrease the expression of BDNF in the hippocampus (Duman and Monteggia, 2006; Andero and Ressler, 2012). Similarly, in human studies, acute and chronic stress has been consistently related to inhibition of hippocampal BDNF synthesis and reduction in BDNF levels (Duman and Monteggia, 2006; Machado-Vieira et al., 2007; Savitz et al., 2007; Rakofsky et al., 2012).

Post-Traumatic Stress Disorder (PTSD) represents the prototypical form of stress-induced mental disorder, but only recently BDNF studies in patients with such a diagnosis have been performed. Results from these studies are however not consistent. Dell'Osso et al. (2009) explored BDNF plasma levels in patients with a DSM-IV-TR diagnosis of PTSD, showing significantly lower levels with respect to healthy control subjects. This result suggests the involvement of this neurotrophic factor in the pathophysiology of PTSD. However, Hauck et al. (2010) found an opposite trend in serum of patients with acute stress disorder (ASD) or PTSD showing higher BDNF levels than controls. These authors suggested that an initial elevation of BDNF in early stages of trauma psychopathology, could be followed by reduction in BDNF levels as reported by Dell'Osso et al. (2009). Despite the changes observed in peripheral BDNF, no significant difference has been found in cerebrospinal fluid BDNF levels between patients with moderate PTSD and healthy controls (Bonne et al., 2011). Differences in treatment history, psychiatric

comorbidities, primary diagnosis, source (i.e. serum vs. plasma or cerebrospinal fluid), and recency of trauma exposure may explain these inconsistent results.

Recent efforts have been oriented to explore the prevalence rates and impact of not only Axis I-DSM-IV-TR PTSD in individuals exposed to a traumatic event but also of partial or subthreshold forms. The concept of partial PTSD was introduced for those subjects who fulfill only a subset of the DSM-IV criteria (B, C and D) for PTSD (Weiss et al., 1992; Stein et al., 1997; Marshall et al., 2001; Breslau et al., 2004; Mylle and Maes, 2004; Hepp et al., 2005). More recently, dimensional approaches to PTSD have also been developed that conceptualize post-traumatic stress reactions as consisting of three main dimensions: the nature of the stressor, the possible responses to trauma and the symptom severity (Dell'Osso et al., 2008; 2009a)

The spectrum approach represents an important challenge to the PTSD construct, allowing to identify relevant subclinical comorbidities that may contribute either to the "complex" presentation of PTSD or to the frequent behavioral outcomes and complications, such as self-harm behavior and suicidality. Studies on partial PTSD agree in reporting significantly less symptoms severity and functional impairment than in patients with full-blown disorder, but significantly more than in no-PTSD subjects, with an associated need for treatment (Stein et al., 1997; Dell'Osso et al., 2011).

In this thesis psychoneurobiology of stress in L'Aquila earthquake survivors has been investigated through four related studies on Brain-Derived Neurotrophic Factor (BDNF):

1. : Plasma Brain-Derived Neurotrophic Factor in earthquake survivors with full and partial Post-Traumatic Stress Disorder. In this study the plasma BDNF levels in a clinical population who survived to the L'Aquila 2009 earthquake, along with the post-traumatic spectrum that considers not only full expression of Post-Traumatic Stress Disorder (PTSD) but also subthreshold manifestations such as partial PTSD has been investigated. The results of this study has been published (Stratta et al., 2013a).

2. Clinical correlates of Plasma Brain-Derived Neurotrophic Factor in full and partial Post-Traumatic Stress Disorder. In this study the clinical correlates of plasma BDNF levels in a clinical population showing PTSD symptomatology, along with the post-traumatic spectrum has been investigated.

3. What does late BDNF increase mean? A pilot comparison study of clinical and non clinical samples exposed and not exposed to the earthquake. In this study the BDNF modifications in subjects that did not showed psychiatric symptoms or symptoms worsening despite having suffered stress event, compared to subjects not having suffered relevant stress, have been investigated.

4. Study 1: Plasma Brain-Derived Neurotrophic Factor in earthquake survivors with full and partial Post-Traumatic Stress Disorder

The aim of the study is to investigate plasma BDNF levels in a clinical population who survived to the L'Aquila 2009 earthquake, along with the post-traumatic spectrum that considers not only full expression of PTSD but also subthreshold manifestations such as partial PTSD by means of dimensional assessment.

4.1. Methods

4.1.1. Subjects

A consecutive sample of 37 outpatients (24 women and 13 men; mean age±SD: 45.7±11.7 years) referring for anxiety or affective symptoms after the earthquake, were recruited at the National Mental Health Care Service (NMHCS) facilities in L'Aquila. The recruitment was performed about two years after the traumatic event (July – December 2011).

Exclusion criteria were the following: current or lifetime diagnosis of organic mental disorder, schizophrenia, schizophreniform or other psychotic disorders, substance-related disorders, uncontrolled or severe medical conditions. At the time of BDNF evaluation all patients were treated with low doses of benzodiazepines and / or antidepressants as stated by Ethical Committee. No patients were treated with antipsychotics or mood stabilizers.

Fifteen healthy control subjects with no current or lifetime DSM-IV-TR mental or use of psychotropic medication, age and gender matched were enrolled from general population of L'Aquila exposed to the 2009 earthquake (10 women and 5 men; mean age±SD: 45.1±11.9 years).

The Ethics Committee of the Azienda Sanitaria Locale of L'Aquila approved all recruitment and assessment procedures. All subjects included provided written informed

consent, after receiving a complete description of the study and having the opportunity to ask questions.

4.1.2. Clinical assessment

The assessment included: the Structured Clinical Interview for DSM-IV Axis-I disorders Patient Version (SCID-I/P, First et al., 1995). The SCID-I/P was administered to patients by a psychiatrist (PS) trained and certified in the use of the instrument.

4.1.3. BDNF evaluation procedure

Venous blood samples were taken in the morning (between 8:00 and 9:00 am) to avoid diurnal variations of BDNF levels (Piccinni et al., 2008). Blood was drawn into EDTA-coated tubes that were kept on ice, centrifugated at 2000 × g for 10 min for separating plasma from cells and supernatant was stored at −80 °C until the analysis. To measure the amount of total BDNF, acidification and subsequent neutralization of the samples were followed before proceeding with the enzyme-linked immunosorbent assay (ELISA) protocol, according to manufacturer's instruction (Promega, Wallisellen, Switzerland). The well-plates were coated with anti-BDNF monoclonal antibody and incubated at 4 °C for 18 h. The plates were then incubated in a blocking buffer for 1 h at room temperature, then samples were added. The samples and BDNF standards were maintained at room temperature under shaking for 2 hours, followed by washing with the appropriate buffer. The plates were successively incubated with anti-human BDNF polyclonal antibody at room temperature for 2 h, washed and incubated with anti-IgG antibody conjugated to horseradish peroxidase for 1 h at room temperature. The plates were incubated in peroxidase substrate and tetramethylbenzidine solution to produce a colour reaction. The reaction was stopped with 1 M HCl. The absorbance at 450 nm was measured with a microplate reader (iMark, Microplate reader, Bio Rad Laboratories) to determine BDNF values that are expressed as pg/ml.

4.1.4. Statistical analyses

Due to the relatively small size of the samples and scarce gaussianity of variables, non parametric statistic was chosen. Kruskal-Wallis Test for comparisons among n samples and Mann-Whitney Test for post hoc planned analyses between two samples were used. All analyses yielding a $p < 0.05$ were considered significant.

4.2. Results

By means of SCID interview, 13 patients were diagnosed as showing Full PTSD and 13 patients as showing Partial PTSD (age 49.9±10.2, 10 women and 3 men and age 44.4±13.6; 9 women and 4 men respectively); 11 patients (age - mean±SD - 42.3±10.3; 5 women and 6 men) were diagnosed as not having PTSD but a different pathological condition that justified the referral (3 subjects with depressive episode, 5 with depressive episode in bipolar disorder, 3 with panic attack). No differences in sex distribution (X^2=2.75, df=2, NS) or age (F=1.49, df=2,34, NS) were observed in these diagnostic categories.

Non parametric comparisons among the four samples of subjects exposed to the earthquake showed significant difference for BDNF (Table 1). Post-hoc planned Mann-Whitney Test reported that subjects with full-blown PTSD and subjects without PTSD, but with anxiety or affective disorders, have lower BDNF level than subjects with partial PTSD and healthy controls. Although not significant, a trend toward higher BDNF levels in subjects with partial PTSD than controls is also shown.

Table 1. Comparison of BDNF plasmatic levels (pg/ml, mean±SD) among patients categorizations (no PTSD, Partial PTD, Full PTSD) and healthy subjects, although exposed to the earthquake.

Subjects	**Mean**	**Standard Deviation**
No PTSD (n=11)	3650.64	1648.23
Full PTSD (n=13)	3473.31	2073.22
Partial PTSD (n=13)	6180.93	2348.30
Healthy subjects (n=15)	6004.81	4100.28

Kruskal-Wallis Test X^2=18.76, df = 3, p=.0005

Post hoc planned Mann-Whitney Test:

No PTSD vs. Full PTSD Z=.67, NS

No PTSD vs. Healthy subjects Z=2.31, .05

Full PTSD vs. Healthy subjects Z=2.60, p<.01

No PTSD vs. Partial PTSD Z=3.10, p<.005

Full PTSD vs. Partial PTSD Z=3.51, p<.0005

Partial PTSD vs. Healthy subjects Z=1.63, p=.10

4.3. Discussion

We report that patients with full but not Partial PTSD show reduced BDNF peripheral levels. The present results are in agreement with previous findings by Dell'Osso et al. (2009). In addition, the BDNF levels in patients with Partial PTSD can be in line with Bonne et al. (2011) who reported no significant difference between patients with moderate PTSD severity and healthy controls. On the other hand, an integration of this report with Hauck et al. (2010) findings and hypotheses is possible. In fact, our patients with Partial PTSD, whose evaluation has been made two years after the traumatic event, show the highest BDNF values, thus strengthening the hypothesis that BDNF level could increase in the close aftermath of the trauma and then progressively decrease. In addition, patients reported in the study by Hauck et al. (2010) with higher BDNF and more recent stress had also less severe symptoms, so that they could be assimilated to our subjects with partial PTSD.

Thus, not only the closeness to the trauma could lead to a BDNF increase as Hauck et al. (2010) hypothesized, but the kind of PTSD (i.e. partial and full-blown PTSD) also has to be considered. As BDNF peripheral level might parallel changes occurring at cerebral level, its trend to increase in plasma of patients with partial PTSD could be interpreted as a neuroprotective reaction to the stress and to the fully expression of PTSD. If so, in Partial PTSD only an initial BDNF increase could happen, followed by BDNF normalization over time. This hypothesis is confirmed by Dell'Osso et al. (2009) finding in full-blown PTSD patients of no significant difference between plasma BDNF levels of subjects who had experienced the trauma less or more than one year before the time of assessment.

Alternative or integrative hypothesis of these data can be that BDNF level reflect PTSD severity, i.e. the severer the patient's illness, the lower the biological parameter as previously observed in patients with depressive disorder (Dell'Osso et al., 2010a). As a matter of fact our evaluation has been made two years after the traumatic event. Although

speculatively, we may hypothesize that our patients with partial PTSD had full PTSD in the aftermath of the earthquake, although did not required psychiatric intervention likely due to contextual reasons that could change the perception of the disorder (Holzinger et al., 2011); the only partial improvement of the disorder lead therefore these patients to the clinical observation. The normal level of BDNF of these patients could be the neurobiological expression of the clinical improvement. This hypothesis is consistent with previous reports from our research group of BDNF increase only in patients showing clinical improvement after antidepressant or electroconvulsive therapy (Piccinni et al., 2008; 2009).

Interpretation of our results should keep in mind some limitations of the study. The first limitation is represented by the relatively small sample size, not allowing further subgrouping, e.g. for gender, although no different distribution is observed among the clinical groups; however all the patients recruited were on similar low dosage of drugs and no patient presented comorbid psychiatric diagnoses that might have affected the results.

The important issue of the source of sample (serum, plasma, CSF) must be considered regarding the possible relationship between BDNF and PTSD and the different interpretations of results. BDNF is found in both human serum and plasma, with serum reporting even 200-fold higher than plasma levels due to the amount stored in circulating platelets (Rosenfeld et al., 1995). Plasma BDNF therefore may be considered a more reliable marker of neurotrophin activity.

BDNF is synthesized as a precursor protein (pro-BDNF) and then processed into two isoforms (truncated-BDNF and mature BDNF) (Carlino et al., 2012). Due to their well different biological functions they are able to elicit, their evaluation as well as they reciprocal ratios could add further insight on the meaning of BDNF on stress reactions. This issue also should be object of research. Several factors other than neurotrophins can influence the possibility of having stress spectrum symptoms: among these personality

characteristics (Campbell-Sills et al., 2006), social support (Ozbay et al., 2008), number and degree of concurrent stressors, genetic polymorphisms (Frielingsdorf et al., 2010; de Quervain et al., 2012), as well as other variables representing the neurobiological basis of the stress-related psychopathology (Charney, 2004). This is object of our ongoing research.

It is not clear whether abnormal BDNF function is a marker of PTSD itself, or a marker of a neural vulnerability to PTSD, as hypothesized for mood disorders. [36] Based on our results it is reasonable to hypothesize that lack of BDNF response to stress could increase vulnerability to PTSD.

Finally, our observations of a lower BDNF level in subjects with PTSD as well as in the mixed sample with anxiety and affective disorder, suggest the existence of a common pathophysiological mechanism; it is also possible that the high rates of comorbidity that exist among many of these disorders may account for the inability of plasma BDNF level to discriminate between these pathologic conditions.

5. Study 2: Clinical correlates of Plasma Brain-Derived Neurotrophic Factor in full and partial Post-Traumatic Stress Disorder

In the first study on L'Aquila (Italy) earthquake survivors, using a sample categorization based on a dimensional approach of the disorder symptomatology, we found reduced BDNF level in subjects with full blown PTSD but not in those with partial PTSD (Stratta et al., 2013).

The aim of the present study is to investigate the clinical correlates of plasma BDNF levels in a clinical population showing PTSD symptomatology, along with the post-traumatic spectrum that considers not only full expression of PTSD but also subthreshold manifestations, such as partial PTSD, by means of a dimensional assessment.

5.1. Methods

5.1.1. *Subjects*

A consecutive sample of 26 outpatients (19 women and 7 men; mean age±SD: 47.15±12.12 years) referring for post-traumatic symptoms after the 2009 earthquake, were recruited at the National Mental Health Care Service (NMHCS) facilities in L'Aquila. The recruitment was performed about two years after the traumatic event (July – December 2011). Eleven consecutive patients (age - mean±SD - 42.3±10.3; 5 women and 6 men) diagnosed as not having PTSD but a different pathological condition that justified the referral (3 subjects with depressive episode, 5 with depressive episode in bipolar disorder, 3 with panic attack) were also evaluated.

Exclusion criteria were the following: current or lifetime diagnosis of organic mental disorder, schizophrenia, schizophreniform or other psychotic disorders, substance-related disorders, uncontrolled or severe medical conditions. At the time of BDNF evaluation all patients were treated with low doses of benzodiazepines and / or antidepressants as

stated by Ethical Committee. No patients were treated with antipsychotics or mood stabilizers.

5.1.2. Cinical assessment

The assessment included: the Structured Clinical Interview for DSM-IV Axis-I disorders Patient Version (SCID-I/P) (First et al., 1995); the Trauma and Loss Spectrum-Self Report (TALS-SR) for assessing post-traumatic spectrum symptoms related to the April 2009 earthquake (Dell'Osso et al., 2009); the Impact of Event Scale Revised (IES-R, Weiss and Marmar, 1997) for the PTSD symptomatology.

The SCID-I/P was administered to patients by a psychiatrist (PS) trained and certified in the use of the instrument. On the basis of the SCID interview Full and Partial PTSD distinction has been made. A diagnosis of partial PTSD was assessed when criteria B and or C or D for DSM-IV were satisfied (Stein et al., 1997), while full blown PTSD subjects meet all the disorder criteria.

Subjects were also asked to complete the symptomatological domains of the TALS-SR, referring to the last week condition. The TALS-SR explores a range of post-traumatic spectrum symptoms comprising emotional, physical and cognitive responses to the trauma, including re-experiencing it, avoidance and numbing, and arousal symptoms. According to the aims of the present study, subjects were asked to fulfill domains IV and over, referring to symptoms that occurred after the earthquake exposure. Domain IV (Items 59–76) includes a range of emotional, physical and cognitive responses to this event. Domain V (Items 77–85), Domain VI (Items 86–97) and Domain VIII (Items 106–110) include re-experiencing, avoidance and numbing, and arousal symptoms respectively, Domain VII (Items 98–105) targets maladaptive coping.

The IES-R is a widely-used scale with excellent psychometric properties, which assesses intrusion, avoidance and hyperarousal symptoms that characterize stress response syndromes during the week preceding the evaluation.

The Ethics Committee of the Azienda Sanitaria Locale of L'Aquila approved all recruitment and assessment procedures. All subjects included provided written informed consent, after receiving a complete description of the study and having the opportunity to ask questions.

5.1.3. *BDNF evaluation procedure*

The procedure utilized in this study was the same of the study 1.

5.1.4. *Statistical analyses*

Due to the small size of the samples and scarce gaussianity of variables, non parametric statistic was chosen. Kruskal-Wallis Test for comparisons among n samples and Mann-Whitney Test for post hoc planned analyses between two samples were used. Spearman's rho for correlation analysis and chi-square test were also used. Spearman's rho for correlation analysis and Fisher r-to-z transformation for comparison between correlations were also used. T test for independent samples was used for between group age comparison. All analyses yielding a $p < 0.05$ were considered significant.

5.2. Results

By means of SCID interview, 13 patients were diagnosed as showing Full PTSD and 13 patients as showing Partial PTSD (age 49.9±10.2, 10 women and 3 men and age 44.4±13.6; 9 women and 4 men respectively). No differences in sex distribution (X^2=.65, df=1, NS) or age (t=1.17, df=24, NS) were observed.

Regarding to BDNF levels, as seen in the study 1, non parametric comparisons among the four samples of subjects exposed to the earthquake showed significant difference for BDNF (Table 1). Post-hoc planned Mann-Whitney Test reported that subjects without PTSD, but with anxiety or affective disorders, and subjects with full-blown PTSD have lower BDNF level than subjects with partial PTSD and healthy controls. Although not significant, a trend toward higher BDNF levels in subjects with partial PTSD than controls is also shown.

Table 1. Comparison of BDNF plasmatic levels (mean±SD) among patients categorizations (no PTSD, Partial PTD, Full PTSD) and healthy subjects, although exposed to the earthquake.

Subjects	**Mean**	**Standard Deviation**
No PTSD (n=11)	3650.64	1648.23
Full PTSD (n=13)	3473.31	2073.22
Partial PTSD (n=13)	6180.93	2348.30
Healthy subjects (n=15)	6004.81	4100.28

Kruskal-Wallis Test X^2=18.76, df = 3, p=.0005

Post hoc planned Mann-Whitney Test:

No PTSD vs. Full PTSD Z=.67, NS No PTSD vs. Partial PTSD Z=3.10, p<.005

No PTSD vs. Healthy subjects Z=2.31, .05

Full PTSD vs. Healthy subjects Z=2.60, p<.01

Full PTSD vs. Partial PTSD Z=3.51, p<.0005

Partial PTSD vs. Healthy subjects Z=1.63, p=.10

Patients with PTSD (full or partial) showed higher score in IES-R and TALS-SR score than subjects without PTSD. No significant difference was seen between partial of full PTSD (Table 2).

Table 2. Comparison of IES-R scores and TALS-SR domains among patients categorizations (no PTSD, Partial PTD, Full PTSD); values are expressed as mean±SD.

	No PTSD (n=11)	**Full PTSD (n=13)**	**Partial PTSD (n=13)**	**Kruskal-Wallis Test (df=2)**
IES-R				
Intrusions [a]	.71±.72	2.15±.89	1.60±.92	X^2=11.99 p<.005
Avoidance [b]	.95±1.05	1.75±.75	1.36±.80	X^2=6.95 p<.05
Hyperarousal [c]	.85±.89	1.97±.81	1.68±.96	X^2=9.88 p<.01
Total score [d]	.84±.77	1.96±.67	1.54±.81	X^2=11.05 p<.005
TALS-SR				
Domain IV Reaction to losses or upsetting events	6.30±4.60	8.92±2.14	8.27±3.56	X^2=2.55 NS
Domain V Re-experiencing [e]	.80±1.40	3.58±1.80	3.27±2.00	X^2=13.14 p<.001
Domain VI Avoidance and numbing [f]	2.10±2.59	4.50±2.25	4.00±2.58	X^2=7.06 p<.05
Domain VII Maladaptive coping	.70±1.79	.42±.49	.73±.92	X^2=1.61 NS
Domain VIII Arousal [g]	1.50±1.56	2.42±1.66	3.09±1.75	X^2=5.40 p=.07

Post hoc planned Mann-Whitney Test:

[a] No PTSD vs. Full PTSD Z=3.29, p<.001	No PTSD vs. Partial PTSD Z=2.27, p<.05	Full PTSD vs. Partial PTSD Z=1.49, NS
[b] No PTSD vs. Full PTSD Z=2.53, p<.01	No PTSD vs. Partial PTSD Z=1.51, NS	Full PTSD vs. Partial PTSD Z=1.33, NS
[c] No PTSD vs. Full PTSD Z=3.05, p<.005	No PTSD vs. Partial PTSD Z=2.27, p<.05	Full PTSD vs. Partial PTSD Z=.75, NS
[d] No PTSD vs. Full PTSD Z=3.17, p<.005	No PTSD vs. Partial PTSD Z=2.15, p<.05	Full PTSD vs. Partial PTSD Z=1.44, NS
[e] No PTSD vs. Full PTSD Z=3.13, p<.005	No PTSD vs. Partial PTSD Z=3.27, p<.001	Full PTSD vs. Partial PTSD Z=.29, NS
[f] No PTSD vs. Full PTSD Z=2.50, p<.05	No PTSD vs. Partial PTSD Z=2.02, p<.05	Full PTSD vs. Partial PTSD Z=.62, NS
[g] No PTSD vs. Full PTSD Z=1.35, NS	No PTSD vs. Partial PTSD Z=2.27, p<.05	Full PTSD vs. Partial PTSD Z=1.17, NS

When correlations among variables were considered, no significant correlations were seen in the sample with PTSD symptoms (partial + full PTSD) and that with partial PTSD (Table 2). When we analyzed the sample with full-blown PTSD, a significant correlation emerged between BDNF level and TALS-SR IV domain (reaction to losses or upsetting events): (rho=.59, p<.05). The comparison of this correlation with that of the partial PTSD sample showed significant difference (Table 2).

Table 2. Correlations (Spearman rho) between BDNF levels and TALS domains in full blown and partial PTSD subjects.

	Plasmatic BDNF		Correlation comparison[a]
TALS-SR	Full PTSD	Partial PTSD	z
TALS Domain IV	0.59*	-0.44	2.58**
TALS Domain V	-0.05	-0.09	0.09
TALS Domain VI	-0.23	-0.21	0.05
TALS Domain VII	0.06	-0.31	0.57
TALS Domain VIII	0.19	-0.25	0.13

* p<0.05; ** p<0.001

[a] Fisher r-to-z transformation

5.3. Discussion

We observe that patients with full PTSD show reduced BDNF peripheral levels than those with partial disorder; in the previous related report on this clinical sample, full blown PTSD subjects only showed lower BDNF level than healthy control sample (Study 1 and Stratta et al., 2013).

In front of this difference however, full blown and partial PTSD patients showed no different symptomatology as evaluated by TALS-SR. The result suggests that subjects with partial PTSD share relevant symptoms with those with the full blown disorder that deserve treatment, confirming other literature observation (Jeon et al., 2007).

We found no correlation between BDNF levels and severity of symptoms in the total clinical sample (i.e. partial and full PTSD), in agreement with other observations (Berger et al., 2010; Bonne et al., 2011; Dell'Osso et al., 2009a; Hauck et al., 2010). Intriguingly enough, when the two samples were distinguished, the full blown PTSD sample showed a significant correlation between BDNF and stress symptoms: differently than in the partial PTSD sample, BDNF level strongly and positively correlated with TALS-SR IV domain (i.e. higher reaction to losses or upsetting events, higher BDNF: rho=.59).

Although speculatively we hypothesize that this correlation could reflect a failure to cope stress, i.e. a 'blunted' stress-induced increase of the BDNF level. This 'blunted' BDNF response to stress could unveil and explain the counterintuitive association. On the other hand, in subjects with partial PTSD a stress-induced BDNF level increase is likely but, when the upper limit is reached, a sort of ceiling effect could cover possible correlations.

Alternative or integrative hypothesis for interpretation of these data can be that the correlational pattern of full blown PTSD subjects is a feature of a phase that precede improvement, being the observed positive correlation a stress-induced effort of a BDNF increase.

The shared variance between BDNF level and the TALS domain, is not high, although not negligible. As a matter of fact we examined the relationship of BDNF with symptomatology only. Several other factors than neurotrophins can influence the possibility of modulating stress spectrum symptoms: among these personality characteristics, (Campbell-Sills et al., 2006) social support, (Ozbay et al., 2008) number and degree of concurrent stressors, genetic polymorphisms, (de Quervain et al., 2012; Frielingsdorf et al., 2010) as well as other variables representing the neurobiological basis of the stress-related psychopathology (Charney, 2004). These issues deserve further research.

This is the first report on PTSD symptom correlates using a post-traumatic spectrum perspective. It is not clear whether abnormal BDNF function is a marker of PTSD itself, or a marker of a neural vulnerability to PTSD, as hypothesized for mood disorders (Hashimoto, 2010). Based on our results it is reasonable to hypothesize that lack or 'blunted' BDNF response to stress could increase vulnerability to PTSD.

Interpretation of our results should keep in mind however some limitations of the study. The first limitation is represented by the relatively small sample size, not allowing further subgrouping, e.g. for gender, although no different distribution is observed among the clinical groups. The subjects were being treated with drugs (benzodiazepines or antidepressants) that might affect the results; however all subjects were on similar low dosage and no patient presented comorbid psychiatric diagnoses. Other limitation is the use of a self-report instrument in the PTSD symptoms detection. Moreover there is more than one definition of partial PTSD in the literature; consistency however in results of epidemiological studies has been observed (Mylle and Maes, 2004).

6. Study 3: What does late BDNF increase mean? A pilot comparison study of clinical and non clinical samples exposed and not exposed to the earthquake

Brain-Derived Neurotrophic Factor (BDNF) is among the most important within the neurotrophin family in cerebral synaptic plasticity and cognitive functions regulation. It is highly sensitive to environmental factors and its increase enhances neurogenesis, neurite sprouting, electrophysiological activity with beneficial effects on cognitive functions such as learning and memory (Bekinschtein et al., 2008; Murray and Holmes, 2011). Alterations in the BDNF expression and functioning has been widely linked with stress both in animal and clinical studies (Duman and Monteggia, 2006; Andero and Ressler, 2012; Machado-Vieira et al., 2007; Savitz et al., 2007; Rakofsky et al., 2012).

From a teleological perspective, synaptic modifications can be an adaptive process to adequate the organism to stress events, being BDNF a relevant modulator (Poo, 2001). It is precisely in the case of stressful events that greater information processing is needed in order to identify opportunities and possibilities to overcome the encountered difficulties. If the process is successful, the rapid BDNF capacity of performing cerebral modifications could avoid unwanted stress consequences and pathologies (McEwen, 2012; McEwen et al., 2012). On the other hand if no such neurotrophin and synaptic plasticity modifications append, pathological stress consequences could be result (Calabrese et al., 2009).

In this study we aimed to investigate BDNF modifications in subjects that did not showed psychiatric symptoms or symptoms worsening despite having suffered relevant stress event. We hypothesized that, in so far as no stress consequences or relapse/worsening appeared in subjects who suffered relevant stress, any BDNF variation could be due to stress exposure. To do so we considered subjects who suffered the same stress event, i.e. a clinical and non clinical population exposed to a natural catastrophe (i.e. the 2009 L'Aquila earthquake) in comparison to a population not exposed to relevant stress. Because natural disasters are random events that expose unselected populations

to trauma, they offer unique opportunities for researchers interested in studying subjects 'triggered' to a unique trauma, disentangling confounding issues of pre-existing risk for exposure to traumatic events.

6.1. Methods

6.1.1. Subjects

Within a study on the predictivity of clinical and neurobiological factors on stress spectrum symptomatology (Stratta et al., 2013), here we considered healthy control subjects and depressed/anxious patients exposed to the earthquake and healthy control subjects and depressed/anxious patients not exposed to stress events.

These populations were: 1) fifteen healthy control subjects with no current or lifetime DSM-IV-TR mental or use of psychotropic medication exposed to the L'Aquila 2009 earthquake (10 women and 5 men; mean age±SD: 45.1±11.9 years); 2) a consecutive sample of 11 outpatients (age - mean±SD - 42.3±10.3; 5 women and 6 men), 8 with depressive episode and 3 with panic attack. These subjects were diagnosed before the earthquake and did not show worsening or relapse after the event. The control subjects shared with the clinical sample the stress due to the earthquake. The recruitment was performed about two years after the traumatic event (July – December 2011) at the National Mental Health Care Service facilities in L'Aquila; 3) a consecutive sample of 10 outpatients (age - mean±SD 52.3±12.3; 5 women and 5 men) diagnosed as having depressive or panic attack disorders recruited at the Department of Clinical and Experimental Medicine of the University of Pisa; 4) thirty-seven healthy control subjects from Pisa (23 women and 14 men; mean age±SD 33.1±7.4 years), These two latter samples had not been exposed to the L'Aquila earthquake and exposition to traumatic events has been excluded.

At the time of BDNF evaluation all patients were treated with low doses of benzodiazepines and / or antidepressants as stated by Ethical Committees. No patients were treated with antipsychotics or mood stabilizers.

The Ethics Committees of the Azienda Sanitaria Locale of L'Aquila and of the Azienda Ospedaliero - Universitaria of Pisa approved all recruitment and assessment procedures. All subjects included provided written informed consent, after receiving a complete description of the study and having the opportunity to ask questions.

6.1.2. Procedures

All subjects were assessed using the Structured Clinical Interview for DSM-IV Axis-I disorders Patient Version (SCID-I/P) (First et al., 1995). The Clinical Global Impression (CGI) measuring severity of illness was also used.

The procedure utilized in this study for the BDNF evaluation was the same of the study 1.

6.1.3. Statistical analyses

In order to control the gaussianity, BDNF values were log transformed. One-way and two-way Analysis of Variance (ANOVA) have been used. Main effects were further analyzed with post hoc planned t-test analysis. Chi square and Pearson's r correlation were also used. All analyses yielding a *p*-value less than .05 were considered significant.

6.2. Results

No significant difference has been seen in gender distribution across the four sample (X^2=5.7, d.f. 3, NS). Different instead was the age (1-way ANOVA F=14.9, d.f. 3, p<.0005), but not significant correlation with BDNF was found. No difference for CGI severity of illness between exposed and not exposed clinical samples subjects (L'Aquila vs. Pisa) was found. No differences were observed between subjects with depression or panic attack disorders and these subjects were therefore considered in the same clinical samples.

BDNF plasma levels for the four recruited samples are reported in Table 1. Statistical difference has been observed for diagnosis factor (clinical sample vs. controls F=15.2; p<.0005). Exposition factor (subjects exposed vs. not exposed to the earthquake F=2.9; p=.09) or exposition by diagnosis interaction (F=1.53; NS) did not reach instead statistical significance. Post hoc planned t-test showed significant difference between exposed and not exposed clinical samples (t=2.16, df 19, p<,05).

Table 1. Comparison of BDNF plasmatic levels (pg/ml, mean±SD) and age between depressed / anxiety (dep/anx) patients and healthy subjects, exposed or not exposed to the earthquake.

Subjects	**plasmatic BDNF**	**Age**
dep/anx exposed (n=11)	3650.6±1648.2	42.2±10.2
dep/anx not exposed (n=10)	2495.0±1335.9	52.3±12.3
controls exposed (n=15)	6004.8±4100.2	46.3±10.6
controls not exposed (n=37)	5526.2±2659.4	33.1±7.4

two-way ANOVA for plasmatic BDNF after natural logarithm transformation with exposition and diagnosis as factors and age as covariate

exposition factor F=2.9; p=.09

diagnosis factor F=15.2; p<.0005

exposition by diagnosis interaction F=1.5; p=.22

6.3. Discussion

The lack of global significant difference between the exposed and not exposed subjects indicate the acceptation the null hypothesis that no BDNF modification intervened after the stressful event.

Some considerations however have to be made. Although not significantly, the exposed samples show the highest levels in absolute BDNF values with a trend for the exposition factor (p=.09) and significant difference has been found between the two clinical samples.

The possibility of a ceiling effect cannot be excluded: it could be true for the healthy control sample, with no possibility of exceed a possible maximum level. If a ceiling effect could explain findings in controls, this is not the case for the clinical sample where the significant difference between exposed and not exposed samples is the double in size. Although BDNF decrease in subjects with affective disorders as well as panic disorder is a well reported finding (Lee et al., 2007; Piccinni et al., 2008; Pandey et al., 2010; Sözeri-Varma et al., 2011; Kobayashi et al., 2005; Ströhle et al., 2010) further confirmed by our data, room however for a stress related increase seems to be possible.

As BDNF peripheral level might parallel changes occurring at cerebral level, its trend to increase in plasma could be interpreted as a neuroprotective reaction to the stress. On the basis of these considerations, although speculatively, the higher BDNF levels of L'Aquila clinical and control samples than not exposed samples could be a residual 'signal' of the suffered stress and of a BDNF neuroprotective reaction with an adaptive aim.

Some limitations should be considered in interpreting the results of the current study. The sample recruited is not large, but sufficient for a pilot study. We are aware that the control sample we studied is biased toward an exclusion of any symptom. By the same way the BDNF increase could be a correlate of resiliency. A further limitation is the

selection of comparison groups in a quasi experimental design where the samples are not equated prior to manipulation of the independent variable (i.e. earthquake exposure). Our evaluation has been made two years after the traumatic event; an initial BDNF increase therefore may have occurred in the aftermath of the stressful event, followed by BDNF normalization over time.

Globally these findings add more insight on the mechanisms regulating BDNF levels in response to stress, delineating a landscape somewhat more complex than what could be expected from the somewhat inconsistent literature on PTSD alone (Dell'Osso et al., 2009; Hauck et al., 2010; Bonne et al., 2011; Stratta et al., 2013).

7. Conclusions

From the observation of these studies, the role of BDNF as a versatile protein, key regulator of pathways which identify and responds to emotionally salient stimuli emerges. This is in line with previous results in literature (Rakofsky et al., 2012), but some interesting cues however can be added.

The kind of the correlational patterns with stress related symptoms evaluation, as well as the comparison between subjects hit or not by a significant stressful event suggest that BDNF can be considered as a protective factor to stress.

Different is the meaning of a protective vs. a compensatory mechanism in the stress literature (Luthar, 2006; Friborg et al., 2009). Protective mechanisms are dynamic developmental processes generated through successful engagement with adversities. On the other hand compensatory mechanisms involve resources that operate irrespective of stress levels.

Along a protective model therefore resources are activated and adapt themselves in the face of adversity. This model can be consistent with our observations, BDNF operating in a dynamic process wherein individuals display positive adaptation despite experiences of significant adversity or trauma.

Although speculatively, BDNF can be hypothesized as operate within an ‘immunity’ like process that can allow the person to overcome relevant salient stress.

This ‘immunity’ terminology, relating to somatic disorders, are now commonly used in mental health research and clinical practice as a general term for the state of being not susceptible or resistant to a specific threat to wellbeing. The possibility of an immunity model can be considered in relation to mental well-being, involving psychological and biological mechanisms induced by significant stress. In this sense BDNF can be considered the protagonist of a compliant and dynamic system, such as an immune system that subsists in absence of a microbes’ attack, but reacts when is the case

increasing its coping abilities. The lack of possibility of an increase of BDNF level in front of a significant stressor would expose to the possibility of stress-related harms, such as PTSD.

This immunity model has been recently considered at the basis of the resilience to stress (Davidov et al., 2010). The resilience approach can help to understand the constellation of interacting biological, psychological, social factors protecting from adversities (Bennett, 2008). Resilience involves intrinsic and extrinsic processes of successful adaptation to adversity. Because of the crucial importance of gene-environment interactions with various epigenetic, 'plasticity genes' and 'meaning change' mechanisms relating to resilience, a wide range of research strategies spanning psychosocial and biological methods is needed.

Risk and protective processes operate at genetic, neurobiological, individual, and social levels, and do not act in isolation. The understanding of how these elements work together to promote resilience will advance by incorporating measures of endophenotypes and intermediate phenotypes using a system perspective to elucidate pathways from neurobiology to the coping to stressful events (Reich et al., 2010).

BDNF is a candidate resilience element, associated with the ability to be adaptable and to cope flexibly with stress. Insight into biological factors underpinning resilience to stress may open new avenues for prevention and treatment of stress-related disorders (Charney, 2004). Studies on the relationships between neurotrophic factors and resilience are therefore needed (Taliaz et al., 2011).

Application of a 'general immunity model' as a common framework to stress research in mental health can help to clarify underlying mechanisms and challenges, which contribute to our understanding of health in general and mental health in particular. Such a common framework would help in the discussion of stress reaction in terms of multi-level defense barriers from transfer of adaptive (health) to maladaptive (disorder) reactivity, and a

balance of biological, psychological and social interactive effects for developing an adaptive trade-off between tolerance and sensitivity to stress.

Globally the findings of this thesis can add more insight on the mechanisms regulating BDNF levels in response to stress, delineating a landscape somewhat more complex than what could be expected from the previous work (Dell'Osso et al.,2009). Moreover these data point at the utility of the distinction of PTSD into full and partial categories and to the spectrum approach to mental disorders.

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