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Psychobiological Investigation on Sociosexual Behavior in Male: Preclinical and Clinical Studies

Coordinatore:
Chiar.mo Prof. Stefano Parmigiani

Dottorando: Dott. Harold Dadomo

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Introduction

The main purpose of this study was to evaluate through a human and an animal model as certain situations such as the loss of the resource and the ranking can be a situation that induces a series of modification that can bring in some subject to develop certain diseases related by lowering the tone in mood and sexual dysfunction.

In the past, theoretical and empirical approaches to depression have been dominated by investigations targeting the symptomatology and treatment of severe, clinically-significant depressed states, and questions of aetiology have been largely reserved for precipitants of immediate relevance to the depressed individual. While such proximate causes are undoubtedly of central importance to an understanding of depressive phenomena, more recently an expanding body of literature has focused on evolutionary, or functional, explanations. Arguably, Darwinian explanations of depression (e.g., Allen and Badcock, 2003; Bowlby, 1980; Gilbert, 1992; Nesse, 2000; Price et al., 1994; Watson and Andrews, 2002) are of practical and theoretical importance for diverse reasons. An understanding of the role of depressive phenomena in an evolutionary context provides insight into why people experience depression and then is also likely to yield direct, practical implications with regards to treatment (Price et al., 1994; Watson and Andrews, 2002). For this Regarding to this we known that Nesse (1998) has argued that circumstances involving the loss (or the threat thereof) of a reproductive resource (or rank) are likely to shape negative mood states, while circumstances involving the gain (or potential gain) of such resources are likely to shape positive ones.

But recently, For the most part, psychosocially oriented studies have reported an association between ED and depression (araujo 1998, Shabsigh1991), anger (Feldman 1994, Shabsigh 1998), and low score of the personality trait of dominance (Feldman 1994). Empirical investigations have consistently demonstrated a significant relationship between ED and elevated levels of depressive symptoms, low ratings of general happiness, and low self-esteem (Araujo, Durante, Feldman, Goldstein, & McKinlay, 1998; Intili & Nier, 1998; Laumann et al., 1999; Shabsigh et al., 1998).

In this context, the rank theory of depression (known also as defeat theory or resource loss theory) suggests that depression is highly correlated with perceptions of low rank and subordinate status

Rank theory of depression

In the context of this theoretical framework, Price was one of the first that (1969, 1972) observed in animal that the loser of intraspecific conflicts (and then loss of resource) often manifests behaviours such as withdrawal and a sharp decrease in motivation that bears a strong resemblance to behavioural signs of human depression, suggesting that depression is highly correlated with perceptions of low rank and subordinate status. The mechanism that activate this similar depressive state has come to be known as the Involuntary Subordinate Strategy (Allan & Gilbert, 1997; Gardner & Price, 1999; Sloman, Price, Gilbert, & Gardner, 1994) and more recently as the Involuntary Defeat Strategy (IDS; Gilbert, 2000; Sloman, 2000). Therefore, the IDS prompts flight or submissive behaviour after a defeat and, insofar as the individual has been able to escape, obtain help or accept a new social standing, the IDS will have been effective in preventing further losses and is terminated. This leaves the question of how the IDS manifests in humans. In fact Sloman and colleagues (1987, 1994, 2003) argued that when individuals cannot accept their new position, and cannot escape or change the salutation, they are prone to a prolonged and more intense IDS that manifests as clinical depression.

In this regardf Gilbert and Allan (1998) have to explain this used the term ‘entrapment’ to refer to inescapable, negative life situations in humans. These authors have differentiated this construct into external and internal forms of entrapment. External entrapment is defined as a perception of the world that provides motivation for escape and a feeling of being trapped in these negative circumstances. Internal entrapment relates to escape motivation generated by negative thoughts or feelings and the perception that one is stuck with these thoughts and feelings.

Sturman and Mongrain (2005) found that internal and external entrapment accounted for approximately 50% of the variance in self-reported levels of

depression. This would suggest that depressed mood can be characterised in large part by a desire to escape from aversive thoughts/feelings or external circumstances and believing that one is unable to do so.

Social rank theory has also been used to account for anxiety disorders. For example, a

social rank/cognitive model for social anxiety has been proposed, in which socially anxious people are thought to operate within a defence system where frequent social comparison and a readiness to submit predominate (Gilbert, 1989; Trower & Gilbert, 1989; Trower, Gilbert, & Sherling, 1990). Social anxiety functions to increase the sensitivity of the individual to threats and avoid attacks that would result in a loss of status. We should note that in social anxiety the focus is on future attacks whereas depression is the result of past defeat and loss. In fact there is considerable evidence that the essential psychological "theme" for depression is loss (Clark and Beck, 1989) or defeat (Price, 1972; Gilbert, 1992). Then the loss of rank, change the perceived status that would be implicated in the activation of involuntary subordination (Allan and Gilbert 1997). The subject perceive himself as inferior and then behave submissively. In relation to this is well understood that people may find their place in the social hierarchy by comparing themselves to others in terms of rank, social attractiveness and the degree to which one is an insider or outsider (Allan and Gilbert 1995).While basic social comparisons are also seen in other species (i.e. a perception of the self as stronger or weaker) and serve to prevent potentially dangerous conflict, in humans they are used rather extensively and incorporate more sophisticated judgements pertaining to social attractiveness (Allan and Gilbert's 1995). Regard to this, in fact the formulation of social comparison has been linked to submissiveness, depression and other mental disorders (Allan and Gilbert's 1997).

This arguments lead to assert that normative levels of depressed mood represent an evolved, defensive psychobiological strategy associated with a number of fitness-enhancing functions. Gilbert also sees the social environment as critical when attempting to understand the etiology and function of depressed states. Furthermore the models or rank theory interpret the increasing prevalence of major depression in terms of the incompatibility between mechanisms that have evolved during Pleistocene conditions and sociocultural conditions in the modern world (see Allen and Badcock, 2003).

Personality and rank theory of depression

Sturman and Mongrain (2005) provided preliminary support for a revision of social rank theory that would include also personality as a distal risk factor for involuntary subordination. Within the model proposed here, personality determines how defeat is perceived. Defeat may be taken in stride or conversely, it may weigh heavily on an individual. As perceived defeat increases, individuals are more likely to experience involuntary subordination. This response is thought to be adaptive insofar as feelings of inferiority, dysphoria and internal entrapment will cause individuals to cease in their efforts against more powerful opponents. Dysphoria represents the affective component of this defeat strategy, resulting in decreased motivation to continue in a struggle while self-evaluations of inferiority represent a cognitive mechanism that prevents further challenges to dominant others or confident resource-seeking behaviours. Internal entrapment, captures the idea of wanting to escape from this aversive state but not being able to do so, which fits with the notion that one is involuntarily subordinate. Involuntary subordination should increase if one cannot accept the outcome of a contest (Sloman and colleagues, 1987, 1994, 2003). Indeed, the inability to accept defeat may partly distinguish involuntary from voluntary subordination, which does not have negative implications for mood or self-worth (Gilbert, 1992).

Involuntary subordination, entrapment, but also social comparison and self-criticism has been recently connected with vulnerabilities to dysphoria and major depression (Mongrain & Leather, 2006; also see Zuroff, Mongrain, & Santor, 2004 for a review). Dependency generally shows a modest but significant relationship with measures of depressive symptoms while self-criticism is consistently highly associated with depressive symptoms.

Self-efficacy In testing the Depressive Experiences Questionnaire (DEQ), Blatt, D'Affliti, and Quinlan 1976) found a third factor in addition to dependency and self-criticism, which they labelled self-efficacy. Blatt et al. (1976) describe this factor as 'a sense of confidence about one's resources and capacities'. From later writings (Blatt & Shahar, 2004) it is apparent that self-efficacy, as measured by the DEQ is interpreted as a form of resilience. Whereas dependency and self-

criticism have been the focus of many studies on depression, the self-efficacy dimension has generally been ignored until recently (Blatt & Shahar, 2004).

Bandura (1977, 1982), on the other hand, has written extensively on this construct and his Operationalisation. Bandura's conceptualisation of self-efficacy revolves around people's expectations that they will be able to bring about desired outcomes (mastery). Individuals with high self-efficacy will persist in their efforts o succeed even in relatively challenging situations, whereas individuals with low expectations for success will avoid difficult situations (Bandura, 1977, 1982). Regarding the role of personality in social rank theory there are some evidence suggesting that self-criticism may be related to the processes postulated by social rank theory. For example, Santor and Zuroff (1997) have found self-critical women to contest threats to status when outperformed by a friend on a bogus computer task. They were also more likely to withhold praise from their friends if a friend had disagreed with them which was interpreted as retaliation against threats to status. In a ubsequent study, Santor et al. (2000) assigned 36 couples a task and gave them bogus feedback that was either favourable or unfavourable. After unfavourable feedback, self-critical individuals disagreed with their partners more during the assigned tasks, blamed partners more for their joint performance and made fewer suggestions. Therefore, even in a cooperative situation, self-critics were more sensitive to how their team performance affected their own status. Mongrain et al. (1998) examined the interpersonal behaviour of self-critical women and showed that they were rated as more hostile and less loving by their boyfriends and outside observers. Interestingly, self-critics tended to view themselves as submissive in relationship to their partners even though boyfriends or outside observers did not share this opinion. It was suggested that 'derogating and undermining attacks on the self may create internal messages of involuntary subordination' (Mongrain et al., 1998). Gilbert, Birchwood, Gilbert, and Trower (2001) were the first investigators to demonstrate a link between aspects of self-criticism, entrapment and social comparison in a group of depressed subjects. However, these researchers assessed self-critical thoughts, rather than a self-critical personality style and found moderate correlations with feelings of entrapment and poorer social comparisons. Sturman and Mongrain (2005) examined similar variables but using a validated measure of the self-critical personality style (i.e. the DEQ) in a graduate student sample with a prior history

of depression. It was found that highly self-critical people were more likely to engage in poor social comparisons and have feelings of internal entrapment, irrespective of current depressive symptoms. These intrapsychic characteristics of lower perceived status and internal entrapment (not being able to escape from one's negative thoughts) constituted different aspects of a latent variable, interpreted as involuntary subordination. The involuntary subordination latent variable, in turn, accounted for the relationship between self-criticism and the number of major depressive episodes experienced in the past, offering evidence for an evolutionary perspective in explaining the depressive vulnerability of self-critics.

Stress

Notably, Gilbert's (2001) view that clinical depression results from a chronic state of stress where a number of psychobiological defenses are aroused but ineffective defensive psychobiological strategy associated with a number of fitness-enhancing functions.

Stress is usually defined as a state of disturbed homeostasis inducing somatic and mental adaptive reactions, globally defined as "stress response" aiming to reconstitute the initial homeostasis or a new level of homeostasis after successful adaptation, i.e. allostasis (McEwans 2000, de Kloet 2005). There is wide consensus and support from preclinical and clinical data that stress exposure conceivably plays a causal role in the etiology of MD and depression-like disorders (Nestler 2002, Caspi 2003, McEwans 2000, de Kloet 2005). However, no specific mechanism linking stress exposure and stress response to the occurrence of MD has yet been fully elucidated. Growing evidence indicates several classical candidates, including neurotransmitters and neuropeptides, as well as conceptually novel immune and inflammatory mediators as likely intermediate links between stress exposure, depressive symptoms and MD (Duman 2006, Dantzer 2008, De kloet 2005, Anisman 2008, Moreau 2008, Bierhaus 2003, Bartolomucci 2007). Related to the latter, Åsberg and coworkers (Asberg 2009) discuss a potential role for some inflammatory mediators in a cohort of patients under prolonged psychosocial stress in their paper included in

this Collection, providing further epidemiological support (results discussed above in this Overview).

One of the hallmarks of the stress response has long been considered the activation of the HPA axis. Hypothalamic CRH activation is a pivotal signaling molecule in the regulation of the HPA axis in particular and the stress response in general. Therefore, comprehension of the mechanism responsible for the negative feedback regulation of CRH is of paramount importance. In the present Collection, van der Laan and coworkers demonstrates that timing of glucocorticoid receptors (GR) activation determine the effective repression of the cAMP-induced transcription of the CRH gene thus clarifying that *in vivo* a critical time-window may exist for effective repression of the CRH gene and HPA-axis by glucocorticoids.

Knowledge on the functioning of the HPA axis under acute or chronic challenge is also a key to understand the intimate link between stress response and the pathogenesis of depression (Charney 2004). Indeed, in all MD syndromes, a certain degree of HPA axis disturbance is often present, visible either at baseline or with functional tests. Despite the observed changes of HPA regulation are so far not specific for the diagnosis of depression or for any of its clinical syndromes (Holsboer 2008), altered HPA-axis parameter are considered important biomarkers particularly in preclinical studies. Increased circulating hormones such as adrenocorticotropic hormone (ACTH) and cortisol/corticosterone or increased adrenal gland weight are considered biomarkers of stress response in preclinical models (Cryan 2004), including several papers in this Collection (Schule 2009, Touma 2009, Bartolomucci, 2009, Cooper 2009, Ilin 2009, Hunter 2009, Oomen 2009) Despite the bulk of data available, surprisingly, current knowledge has not been yet developed to a point where HPA-axis reactivity can be rationally exploited for targeted drug treatment, as opposed to major achievements of drugs targeting the CRH receptors (Holsboer 2008). Present data offer reliable experimental tools to stimulate future drug discovery programs (Berton 2006).

Also neurobiological findings sustained that stress can cause changes in the 5-HT and CRH–cortisol systems similar to those found in a subtype of depression, and associated with instability of anxiety and aggression regulation (Van Praag, 2004). The fact that stress may cause depression, or phrased probably more accurately, may cause particular depressive features, in particular anxiety and aggression,

seems justified to the fact that clinical depression results from a chronic state of stress where a number of psychobiological defences are aroused but ineffective. Riguardo a ciò, diventa importante analizzare anche gli aspetti comportamentali, correlati con lo stress cronico.

Behavioral Neuroscience

The DSM-IV identifies specific behavioral and cognitive diagnostic criteria for MD patient. Among these, depressed mood, anhedonia, locomotor disturbances and anxiety are accessible for preclinical investigation while others such feeling of worthlessness, thoughts of death or suicide cannot be reliably mimicked in animal models. A number of animal models have been so far developed and validated (Nestler 2002, Bartolomucci 2007, Miczeck 2008, Willner 2002, Koolhaas 1995). In particular, models involving a chronic (i.e. continuous exposure to a threatening stimulus for a significant amount of time, usually weeks) or intermittent (i.e. daily short exposure to a threat for subsequent days) exposure to a negative stressful events can be considered the most effective in modeling MD-associated behavioral and physiological disturbances (but see Koolhaas 1997). In line with this conclusion, the use of chronic or intermittent models of stress (Schweitzer 2009, Bartolomucci 2009, Cooper 2009, Ilin 2009, Hunter 2009) is discussed. Furthermore, the animal models that are increasingly being regarded as the most promising to model etiological factors and key features of MD patients, i.e. i) models in which the threatening stimulus is social in nature [42,46], ii) models in which exposure to stressful stimuli occurs in the early postnatal or juvenile age (Ilin 2009, Oomen 2009).

From a nosological point of view, some behavioral disturbances present in MD diagnostic criteria can be reliably induced and experimentally determined in animals models, including anhedonia (Schweitzer, 2009), locomotor disturbances (Touma 2009, Bartolomucci 2009, Ilin 2009, Collins 2009), anxiety (Ilin 2009, Koolhaas 1997), sleep pattern (Touma 2009) and learning/memory (Ilin 2009, Collins 2009). Of main interest is the experimental evidence [30] that a shift in the circadian rhythm induced by overnight illumination, is the single most important experimental factor influencing intake of a sweetened solution, which is the

currently accepted animal equivalent of anhedonia (Anisman 2005). Furthermore, Ilin and Richter-Levine (2009) show that a daily exposure to a stressful stimulus for 3 consecutive days at juvenile age (named juvenile stress, JS), determine long-lasting behavioral and motivational effects in rats, i.e. increased anxiety, lower exploratory drive and increased learned helplessness. On the contrary, housing JS rats in an enriched environment completely abolish JS-induced behavioral effects. A similar ameliorating effect on stress coping has also been determined by Collins and coworkers (2009), by exposing rats to a different form of environmental enrichment, i.e. home cage presence of a running wheel which induces in rodents spontaneous physical exercise. Exercised rats show increased behavioral coping and reduced anxiety- and depression-like behaviors in the open field test and the forced swimming test when compared to sedentary rats.

"Stress induced depression" (STRID): A New Depression Subtype?

The link between stress and depression is not novel and several authors have aimed at identifying new subtypes of depression based on their functional link with stress exposure (e.g. van Praag 2004, Kendler 1999). Of special interest for this work is to highlight a potentially common depression subtype, named tentatively "stress-induced depression" (STRID) and recently described in Sweden by Åsberg and coworkers (Rydmark 2006). A dramatic increase in the number of workers on long term sick leave was observed between the years 1997 and 2003 (Statistics Sweden, 2004; http://www.scb.se). Studies of consecutive cases with psychiatric diagnoses culled from the databases of two large Swedish insurance companies showed that about 80% of patients met DSM-IV criteria for MD (Åsberg et al, unpublished data). The depression episodes were mild to moderate (MADRS <20), and accompanied by significant working memory impairment (Asberg 2007). Follow-up showed that STRID tended to have a prolonged course, and that the patients often remained in a state of exhaustion after the depressive symptoms had remitted. Typically, the remaining clinical picture was one of deep mental and physical fatigue, disturbed and non-restorative sleep, irritability, perceptual hypersensitivity, emotional liability, and pronounced cognitive disturbances (mainly memory and concentration problems). A closer

examination of the case histories revealed that a majority was clearly induced by psychosocial stress, either at the workplace or often in combination with stress factors in the family. This was confirmed by data obtained in a cohort of almost 5,000 Swedish workers on long term sick leave with a psychiatric diagnosis (Vaetz 2007). Findings are consistent with the life event stress literature showing that specific, enduring work-related stressful experiences contribute to depression (Tennant 2001). From an endocrine standpoint, disturbances of the HPA axis may be distinctive pathophysiological features of this depression subtypes. HPA axis hyper-reactivity has been long known and considered a classical feature of depression, particularly of the severe, melancholic type. An opposite situation, i.e. HPA axis hypo-reactivity was found instead in STRID patients (Rydmark 2006). Beside HPA axis disturbance, the STRID subtype of MD is expected to be coupled to different neurobiological, immunological (Asberg 2009) and metabolic features thus requiring joint forces between preclinical and clinical research.
Overall, the studies presented in this special Collection of PLoS ONE propose an integrated effort on how to move in this direction. Both preclinical and clinical research will be pivotal in clarifying the validity of this new subtype of MD, in improving predictors for treatment response and provide a better basis for genetic studies as well as in stimulating new drug discovery processes.

Animal Models in Studying Stress Related Disease.

Since the majority of stressful stimuli which produce psychopathologies in humans are of a social nature, the study f the consequences of social stress in experimental animal models is considered to be of great interest (Buwalda et al., 005). A number of different animal models for psychopathologies in humans have been developed on the basis of tressful social stimuli, and have been found to have both face and predictive validity (Czeh et al., 2001; Fuchs, ramer, Hermes, Netter, & Hiemke, 1996; Kudryavtseva, Bakshtanovskaya et al., 1991; Kudryavtseva, Madorskaya & akshtanovskaya, 1991; Van Kampen, Kramer, Hiemke, Flugge, & Fuchs, 2002; Van Kampen, Schmitt, Hiemke, & Fuchs, 2000). One of these psychosocial stress models is based on the resident–intruder paradigm, developed initially by Miczek (1979), in which a male establishes a

territory and then defends it against an unknown intruder male through aggressive behavior, thereby establishing a clear relationship of dominance/subordination. Subsequently, numerous variations have been developed on the basis of this model. One of these involves preventing physical contact during the pre-attack and post-attack phases, with the aim of separating the stress produced by the physical attack from the psychosocial or emotional stress produced by the threat of suffering an attack. Thus, in the sensorial contact model, used with rodents and tree shrews, a male is placed in a resident's box, with the two animals separated by a transparent partition, and physical contact is only permitted for a few minutes per day (Bartolomucci et al., 2001; Fuchs et al., 1996; Kudryavtseva, Bakshtanovskaya et al., 1991; Kudryavtseva, Madorskaya et al., 1991). Another model, the Visible Burrow System (VBS), enables researchers to study groups of rats in stress-generating situations of seminatural social interaction. This system consists of a laboratory habitat which includes a series of tunnels and chambers (the burrow system) in addition to an open area where food and water are made available. The mixed groups of rats living in the VBS quickly develop aggressive and defensive behaviors, as well as clear relationships of dominance and subordination (Blanchard et al., 1995, 1998). Recently, through the study of dominance–subordination behavior in animals, another model has been developed which enables researchers to study the disorder of mania or depression. In this model, known as the Dominant Submissive Relationship (DSR), dominance and submission are defined in a competition trial which measures how successful food-deprived rats are at accessing sustenance at the expense of an opponent (Malatynska & Knapp, 2005). When using these social stress models to study human psychopathological disorders, mainly depressive and anxiety-related ones, it is important to bear several points in mind. First, we should remember that depression in humans is defined and diagnosed on the basis of the manifestation of a series of behavioral characteristics over an established period of time. These characteristics may be present in different combinations, although they all lead to a common diagnosis of depression. Thus, the concept of depression itself in humans is in fact an open, rather than homogenous one. As such, so-called animal models of depression cannot, under any circumstances, claim to provide a model for a relatively heterogeneous and open theoretical construct such as depression, which is only operationalizable to a lesser extent; rather, they aim simply to

reproduce the most characteristic behavioral and physiological aspects of human depressive disorders. It is to this type of modeling that the majority of researchers refer when talking about

animal models of depression. These models aim to be of use in painting a more accurate picture of the key physiopathological and behavioral manifestations of the mental disorders to which they are applied (Matthews, Christmas, Swan, & Sorrell, 2005).

Chapter 1:

Sign of Depression in Patients with Erectile Dysfunction

Introduction

The results of several recent epidemiological surveys, show that male sexual dysfunctions (e.g. erectile dysfunction and premature ejaculation) are extremely prevalent in the general population.(Shabsigh 1991, Klein 1996 Araujo, 2000) These sexual disorders are strongly associated with aging and are influenced by a variety of medical, psychiatric, life-style factors and have a significant relationship with mood, state and social determinants (i.e. patterns of interpersonal relationships) and overall quality of life (Morley 1988, Wein 1988, Rosen 1990, Weiss 1991, Rosen, 1991, Laumann, 1994, Mannino 1994, Jønler M, 1995, benet, 1995, Salimpour, 1998). An emerging point of view in the last two decade shows that a combination of psychological (e.g. temperamental and personality trait), physiological (e.g. endocrine profile) and ecological (e.g. social status) variables seem to be the most relevant factors to explain the development of this dysfunctions. Recent epidemiological studies pointed out a relationships between psychometric scores of depression, low dominance and erectile dysfunctions (Shabsigh1991; Feldman 1994, Shabsigh 1998, Araujo 1998) . In this context, the rank theory of depression (known also as defeat theory or resource loss theory) suggests that depression is highly correlated with perceptions of low rank and subordinate status.(Price, 1972; Gilbert, 1992) In fact depressed people exhibit a greter degree of social inhibition in that they perceive themselves as inferior to others and tend to behave submissively, compared to “normal” people (Araujo, Durante, Feldman, Goldstein, & McKinlay, 1998; Intili & Nier, 1998; Laumann et al., 1999; Shabsigh et al., 1998). All these variables are also associated with anxiety, especially social anxiety as a specific stressor (Clark and Beck, 1989) Under this respect in animal species a recurring factor, as the exposure to chronic social stress has been associated with many systemic and behavioural disorders (Sherman et al., 1982, Manuck et al., 1991; Sapolsky, 1994; Cohen and Herbert, 1996, Henn et al., 2002, Malberg and Duman, 2003, Sartorius

et al., 2003, and Takamori et al., 2001). However few experimental and clinical studies in human have been carried out to test this hypothesis (Sturman, 2007) On the contrary animal experimental models , clearly show the behavioural and physiological effects of dfeat and /or losing rank (Bartolomucci, 2003) .More recent studies in mice exposed to chronic social stress indicate that does not necessarily predict subsequent pathological consequences because social factors (i.e. dominance and submission) and individual vulnerability play a jey role in dease development (Bartolomucci 2003, Veselska 2009).

The aim of this clinical and experimental study was designed to test the hypothesis that loss of resource and/or rank is a critical factor in the development of sexual dysfunction accompanied by depressive state.

Materials and methods

Subjects

clinical sample

For this study, 36 patients with a chief complaint of functional erectile dysfunction (ED), were recruited from the andrological outpatient unit of the Parma hospital "Ospedale Maggiore". This unit is composed by dual specialty team consisting by an andrologist and psychometric and clinical-psychological unit. The patients has at least 18 years old (mean age 39 $\pm$ 5 SEM) heterosexual, and in an ongoing, stable, monogamous, relationship for at least 6 months (average 36 months $\pm$ 9.5 SEM) before the medical history tacking. They report a clear history, of recurrent ED with a sudden onset on average of 9 months before the medical examination ($\pm$2 month SEM). The patients report no difficulties in achieving and maintaining erection during masturbation. However during sexual intercourse they report no erectile difficulties in vaginal penetration but with the loss of the erection few minutes after penetration. No symptom of premature and/or delayed ejaculation and orgasm (i.e. climax) problems were reported.

All the patients report morning and/or nocturnal erection but without full tumescence. In a subjective 0-10 scale of erectile rigidity the current firm was scored in average 4 in all patients ($\pm$0.8 SEM).

All patients had normal puberty onset at the age of 13th and height in the normal range for the specific age and the examination, report a normal body structure (mean height 172 cm SD ±10 cm; mean weight 78 kg SD ±8 Kg). normal blood pressure and BMI.

The clinical assessment reveals that, in the same period, the patients began having difficulties in arousal and sexual desire, expressed by a decreased interest in sex engagement (sexual activity) with the partner and/or sexual interest towards other women. In life span they reported brief periods (1 or 2 week) of ED with other sexual partners. All subject reported a consistent decrease of energy, motivation and activity.

Exclusion criteria

The main exclusion criteria regarded: the presence of current axis I psychiatric disorder, including substance abuse or dependence including alcohol or cigarettes smoking; current use of any antidepressant medication; abnormal serum hormone levels (i.e., prolactin, testosterone, or thyroid); history of major hematologic, renal, or hepatic abnormalities; Untreated diabetes, retinitis pigmentosa, previous history of pelvic genital, perineal, spinal cord injury, head trauma or any other neurological diseases; peripheral vascular or cardiovascular disease, history of cryptorchidism, surgery or radiation therapy and/or particular procedures related to genitourinary or gastrointestinal malignancy; signs of testis varicocele or fibrosis in corpum cavernosum, andrectable foreskin, gynecomastia, prostate abnormality or any other physical disease that may account for clinical ED.

Control subjects

A total of 30 control subjects, selected from a random sample consisting of students and employees at the University of Parma, were included in this study. The mean age was 39.1 years (SD=6.7) and they were ongoing in a stable relationship on average from 32 months (± 8 ES). They gave a history with no ED, and no symptom of premature and/or delayed ejaculation and orgasm (i.e. climax) problems were reported. The patients report morning and/or nocturnal erection and in a subjective 0-10 scale of erectile rigidity the current firm was scored 9 in all patients.

The case records of the subjects registered were meticulously maintained with all the details including socio-demographic and clinical variables being entered at the time of assessment.

Procedure

In the first examination was made the medical history tacking, clinical and genital examination, to exclude or recruit patients and control subjects (see below for details). ED presence and status of health was evaluated by experienced medical clinician. At the end of this session the study purpose and protocol were clearly explained to the participants. Only subjects who agreed to participate in the study were assessed. To guarantee subjects privacy, an alphanumeric code was used for individual identification. No specific instruction was provided to the subject on their diagnosis, to reduce the potential for bias during psychological test and ethological session.

Subsequently, the subjects were exposed to psychological investigation, were the behavior of each participant was videotaped (see below for details).

Finally, a Psychometric test sessions for trait-personality evaluation was used, were the patients completed four standardized test (see below). The presentation of the tests to each subject were balanced for order and sequence to avoid effects connected to these variables.

Psychological Investigation

Psychological ED status was evaluated by experienced or trained clinicians using a semi structured clinical interview according to DSM-IV-TR criteria (SCID). The use of the SCID made it possible to exclude the presence of current or past psychiatric disorder and to standardize the content of interviews. the patients who show other psychopathology related to axis I and II DSM IV-TR disorders or medical and psychosocial/lifestyle risk factor (i.e. DSM IV-TR axis III and IV) were excluded to the study.

Ethological assessment:

Ethological data were collected during the SCID diagnostic interview. The interview was conducted by a clinical psychologist who was unaware of the subject's score on the psychometric test. Two clinical psychologists participated in the study as interviewers; both were men between ages of 30 and 40 years. Each of them interviewed 37 subjects.

The subject's non-verbal behavior during SCID diagnostic interview was quantified by means of the Ethological Coding System for Interviews (ECSI). The ECSI used in the present study is a revised version of Grant's ethogram, specifically designed for clinical interviews (Grant, 1968; Troisi, 1999). Non-verbal behavioral patterns act as social signals and reflect mood and intentions to behave in certain ways (Dixon and Fisch, 1998). This version of ECSI includes 37 different patterns, mostly facial expressions and hand movements. The ECSI was specifically designed for measuring non-verbal behavior during stress interviews by combining behavior patterns described in published human ethograms. The interview was videotaped with a camera adjusted so that the subject's face and trunk were in full view. Subsequently, a trained observer examined the videotape and scored the subject's behavior according to a one-zero sampling method (Troisi, 1999). Each video recording (lasting 300 s) was divided into 20 successive 15-s sample intervals and the observer recorded whether a certain behavioral pattern had occurred or not, in each sample interval. The score for each pattern was expressed as the proportion of sample intervals (%) during which that pattern occurred as to the total number of sample intervals. The 37 behavioral patterns were then grouped in eight behavioral categories, each reflecting a different aspect of the subject's emotional and social attitude (see Troisi, 1999 for details on the 37 behavioral patterns and grouping criteria): (1) eye contact, which expresses attention and involvement and is considered an essential component in a dyadic interaction; (2) affiliation, i.e. a set of patterns which consists of facial expressions and head movements, which invite social interaction and reflect interpersonal attitude, tending to reassure and increase attachment; (3) submission, i.e. patterns used to appease the interviewer and prevent or inhibit hostile responses; (4) flight, which includes non-verbal behaviors aimed at cutting off sensory systems from incoming social stimuli perceived as stressful or

adverse; (5) assertion, which includes facial expressions and head movements that signal low-level aggression and hostility; (6) gesture, which includes hand movements that accompany, illustrate and, accentuate the verbal content of utterances—this behavioral category reflects the subject's communicative efforts in social interactions and is a reliable index of global psychomotor activity; (7) displacement, which includes behavioral patterns that consist of movements which are focused on one's own body or addressed to manipulation of external objects; and (8) relaxation, which consists of behavioral patterns indicative of a low level of emotional arousal. The score of a given behavioral category was expressed as the sum of the percentages of all the behavioral patterns belonging to it. Before the beginning of the study, the observer was trained in order to reach an adequate level of inter-observer reliability (i.e. a k coefficient of at least 0.70 for each behavioral pattern). The assessment of inter-observer reliability was based on a sample of 30 interviews, which did not include the ones with the subjects of this study.

Psychometric Test Sessions for Trait-Personality Evaluation

Under the supervision of two psychologists all subject completed the following sequence of Italian versions of four auto-administration psychometric questionnaires.

Cognitive Behavioural Assessment tab.4 (CBA 2.0)

The CBA 2.0 (Bertolotti, 1990) includes a series of questionnaires investigating broad issues of potential clinical interest, identifying possible areas dysfunctional in the present life of the subject. In this study were chosen only tab.4 to investigate the life span events in patients' life. previous clinical experience suggested that patients who recently had experienced "a social defeat (e.g. lost of job and/or social prestige and position" . On this bases we decided to modify the question 4.21 that refers to relevant life changes (such as lost of job and impairment of economical resources) in the last 12 months by including the additional questions concerning the the time of the event (i.e. when expressed in

months). This was done to analyse the loss of status/resource with the time of onset of sexual disfunction (see the Beck depression …)

Beck Depression Inventory (BDI).

The BDI (Beck et al., 1961)is a 21-item self-report rating inventory that measures symptoms, characteristic, attitudes and severity of depression and corresponds with the DSM-IV diagnosis of depressive disorder. Responses to items for the present study covered the 'past two weeks, including today'. Responses on the BDI items range from 0 to 6 with higher values indicating higher severity. The total score can be divided into categories reflecting severity of depression. Accordingly, the minimal range of depression is 0–13, the mild range is 14–19, the moderate range is 20–28 and the severe range is 29–63.

At the end of the test we asked to subjects if and when the symptoms compare, in months.

International Index Of Erectile Function (IIEF)

The IIEF (Rosen et al., 1997) is a 15-item, 5-point Likert-type, self-administered measure assessing different areas of male sexual functioning. A principal component analysis identified five factors: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction.

The measure allows the calculation of specific indices for each dimension as well as a total sexualfunction index (calculated through the sum of the specific dimensional indices), with higher scores indicating greater levels of sexual functioning (sexual desire: 2-10, erectile function: 1-30, orgasmic function: 0-10, intercourse satisfaction: 0-15, overall satisfaction: 2-10, total: 5-75).

At the end of the test we asked to subjects if and when the symptoms compare, in months.

Statistical Analysis

We analyzed the differences between the patient and control group for the scores of psychological measures (i.e. BDI, IIEF and CBA) and the scores of ethological

sampling using respectively two-tailed Mann-Whitney tests and Student's t-test where appropriate.

Secondary, we divided the patients into two groups, Mild depressive symptoms (MDS) and no depressive Symptoms (NDS) according to their BDI score to fall above or below 11 respectively. These two groups were compared using two-tailed Mann-Whitney tests for the scores of psychological measures (BDI, CBA 2.0, IIEF) and with Student's t-test in the scores of ethological sampling.

Scores of ethological sampling were expressed as mean $\pm$ SEM. Scores of psychological measures were expressed as median and 25 to 75 percentiles. Spearman-Brown correlation coefficient was used to assess relationships between psychological, and ethological scores. Statistical significance for all tests was set at $p<0.05$. All statistics were performed using Statistica 6.0 software package (StatSoft, Inc.,Tulsa, OK, USA).

Results

Psychological Traits in Patient and Control Group.

The BDI score significantly differ between the patients ands controls group ($U_{36,40}$=56,10 $P<0.001$; figure 1) indicating that patients show a moderate depressive symptoms. As expected IIEF showed that Patients are significantly lower (i.e. the higher the score, the highest is sexual functionality) than controls in total score ($U_{36,40}$=52,50 $p<0.001$; figure 2), and in three out of five sub-factors: in fact the control groups had a significant better erectile function ($U_{36,40}$=226 $P<0.001$) and sexual desire ($U_{36,40}$=249 $p<0.01$), than patients group, but overall a better overall satisfaction ($U_{36,40}$=165 $p<0.001$) in sexual intercourse. (figure 2 and 3)

These data according to test range value suggest that patients show a moderate range of ED… On basis of BDI results we divided the sample group into two groups depending on their BDI total score. The first group, composed by 20 patients, showed a BDI score that range from 0 to 11, and then for this psychometric instrument they are classified as people with no depressive symptoms (i.e. NDS). Instead, the second group of 16 patients showed a range

value that came from 11 to 16, and in agreement with the categories of the BDI is named MDS. (Figure 5).

Finally CBA 2.0 revealed that the proportion of subjects loosing their job in the 12 months before their visit to the andrological clinic was higher in MDS (11/16) compared to NDS (1/20). (Chi^2=8.16; Fisher p<0.005) Revelated by question n° 4.21 of CBA 2.0.

In IIEF, NDS group showed a higher score then MDS group ($U_{20,16}$=67,50; p<0.01; figure 6). Indicating a better sexual and erectile function in NDS group. We may also observe that 15/16 MDS patients, show that in average 1.8 months (1,4 SEM) after losing their job, compared the firs symptoms of ED. And consequently after 3.1 (1,6 SEM) months after first ED symptoms compared the first depressive symptoms.

Ethological Profiles

The analysis of ethological profile essentially revealed that the group of patients scored significantly higher in displacement behavior (t=2,80; p<0,05) and flight behavior (t=2,73; p<0.05) respect to controls. On the contrary, the control group showed significantly higher occurrence of eye contact (t=-5; p<0.001; figure 4).

In addition, MDS patients scores significantly higher in displacement behavior (t=-2,48; p<0,05) respect to NDS (figure 7).

Correlating Ethological and Psychometric Parameters

According to the above reported data which show that the patients group showed higher scores indicative of subclinical depression when compared to controls subjects, we analysed the correlations between psychological traits and ethological variables.

In patients group, we found a negative correlation between Eye Contact and intercourse satisfaction (R=-0.38; p<0.05; figure 8) and a positive correlation between Displacement and total score of BDI (R=0.44; p<0.05; figure 9). No significant correlation were find in controls group.

According to the above reported data which show that the MDS group had higher depressive scores, respect to NDS group and a different behavior patterns of interaction, we analysed the correlations between psychological traits and Ethological data. In MDS group, we find a positive correlation between Submission measured with ECSI and respectively BDI total score ($R=0.64$; $p<0.05$; figure 10)

Conclusions

The comparison between ED and control subjects as far as behaviour (i.e. ethological analysis ECSI), psychometric mood depressive status (BDI) and erectile dysfunction (measured as IIEF) clearly shows that erectile dysfunction is accompanied by decrease in sexual desire , low mood and alteration of interpersonal behaviour which are characteristic of depressive status (Troisi) Compared to control the ED subjects show a greater BDI score variability which allowed a subdivision of ED subjects (according to the BDI classification) into two different categories namely MDS (mild depressive symptoms: BDI scores between 12 and 16) and NDS (no depressive symptoms; BDI scores between 0 and 11) subjects. It is worth noting that although there are difference between MDS and NDS in BDI (with the first showing a trend to reach the cut off score of a real pathological depressive state) from a clinical point of view all the Ed subjects fall into the range of normality .Interestingly 70% of MDS (cf (vs) 10% of NDS) subjects are people that lost their job in the last twelve months.

In this respect The ethological analysis pointed out that the displacement behaviour (as an index of ambivalent motivation based on anxiety) is positively correlated with the BDI scores (i.e. higher is displacement behavior higher is the value of depressive symptoms) in the ED subjects. Interestingly MDS subjects show higher levels of displacement behavior compared to NDS patients. This data corroborate the idea that the ethological methods can be clinically used to distinguish between normal and pathological subjects (Troisi et 1989, 1998)

Moreover The ED dysfunction is more severe in MDS than NDS. Consequently the data show a clear relationships between the loss of job, low mood state and erectile dysfunction severity. In fact These symptoms are higher in (MDS)

patients who are subjects that reported an important resource loss (i.e. they lost their job and decreased their financial resource and/or social status) in the last 12 months. Noteworthy The temporal sequence of events i.e losing the job followed (approximately three months later) by ED symptoms and finally(approximately five months after losing the job) the appearance of depressive symptoms suggests that the key and triggering event is the job loss which probably leads to a decrease in the subjective perception of self esteem.

These findings tend to support the hypothesis of resource loss or rank theory of depression that predict that depressive symptoms should manifest in subjects that feel to have lost their rank (i.e. expressed as loss of job, economical value, and than self esteem). Indeed we observed that the lowering of mood, the erectile dysfunction and sign of submissive and avoidance behaviors (i.e. displacement behaviours) are significantly elevated in patients that lost their job. Moreover the temporal kinetic clearly suggest that this effect is not a casual. In fact only after three months of the loss of job event all subjects exhibited symptoms of ED, followed (five months later) by depressive symptoms. Essentially Our data indicate that a) the loss of job is the critical life event and that from that moment the symptoms severity increases with time probably due to the incapability of the subject to psychologically cope with the adverse situation and b) the rank loss is also one of the possible determinant, of the functional erectile dysfunction.

In this perspective the ED may have a Psychological cause and this symptoms precede the depressive state. From the clinical point of view the ED may be an important sign of an appetitive-consummatory dysregulation that, if persistent, might lead to major depression (Sloman 2003, 2006). Hence ED of psychological origin might not be only a simple sexual dysfunction, but an uncomfortable antechamber of a much more serious disorder, like major depressive disorder.

These data stimulate a further hypothesis concerning the individual vulnerability in that it remains open the question why some people do not develop either ED and/or depression Symptoms as a consequence of psychological “defeat” (loss of resource and/or rank) and if these differences are based on diverse genetically determined coping strategies.

Chapter 2:

Psychobiological Characterization of Male with Erectile Dysfunction and Theory of Rank.

Introduction

Empirical investigations have consistently demonstrated a significant relationship between erectile disfunctions (ED) and elevated levels of depressive symptoms, low ratings of general happiness, and low self-esteem (Araujo, Durante, Feldman, Goldstein, & McKinlay, 1998; Intili & Nier, 1998; Laumann et al., 1999; Shabsigh et al., 1998, Townley, 2009).

In this context, the rank theory of depression describe how the losing of rank subsequent lead to depressive state (Coulehan, 1988; Ruiz-Doblado; 1999; Araujo & McKinlay, 2000, Monroe and Hadjiyannakis, 2002).

In the previous section (see chapter 1), in a population of patients with ED, we determined that depressive symptoms were more severe when compared to a control population and that this was particularly true, in those who lost their jobs a few months earlier. The conclusion was that those who have lost their job (i.e. lowering social rank), are subject to a more stressful situation; which can lead to an increase in chronic stress and then to a dysregulation of defensive systems including erectile dysfunction. However, a question which remained unanswered in that study is: if loss of job (i.e. rank) a cause or consequences of depression and ED? In other words, do people who loose their job have any peculiar temperament trait which may determine or associate with job loss, depression and ED?

Materials and Methods

Subjects:

clinical sample

For this study, 95 consecutive patients with a chief complaint of functional ED, were recruited from the andrological outpatient unit of the Parma hospital "Ospedale Maggiore". This unit is composed by dual specialty team consisting by an andrologist and psychometric and clinical-psychological unit.

For the hope of this work the clinical sample were divided into 2 groups depending of the response to question 4.21 of CBA 2.0, that identified the subjects who lose their job 12 month before the clinical examination (i.e. Losers) composed by 26 subjects and who don't lose the job (i.e. no losers) composed by 69 subjects. All patients has at least 18 years old (losers mean age 36 $\pm$ 5 SEM; no losers mean age 35 $\pm$ 4 SEM), heterosexual, and in an ongoing, stable, monogamous, relationship for at least 6 months (losers mean 29,7 months $\pm$ 9.5 SEM, no loser mean 33,3) before the medical history tacking. They gave a clear history, of recurrent ED with a sudden onset on average of 9 months before the medical examination ($\pm$5 month SEM for both). The patients report no difficulties in achieving and maintaining erection during masturbation. However during sexual intercourse They report no erectile difficulties in vaginal penetration but with the loss of the erection few minutes after penetration. No symptom of premature and/or delayed ejaculation and orgasm (i.e. climax) problems were reported.

All the patients report morning and/or nocturnal erection but with not full tumescence. In a subjective 0-10 scale of erectile rigidity the current firm was scored in average 4,2 in all patients ($\pm$0.5 SEM).

All patients had normal puberty onset at the age of 13th and height in the normal range for the specific age and the examination, report a normal body structure, normal blood pressure and BMI.

The clinical assessment reveals that, in the same period, the patients began having difficulties in arousal and sexual desire, expressed by a decreased interest in sex engagement (sexual activity) with the partner and/or sexual interest towards other women. In life span they reported brief periods (1 or 2 week) of ED

with other sexual partners. All subject reported a consistent decrease of energy, motivation and activity.

The Exclusion criteria

For patients group was the presence of current axis I psychiatric disorder, including substance abuse or dependence including alcohol or cigarettes smoking. Current use of any antidepressant medication. Abnormal serum hormone levels (i.e., prolactin, testosterone, or thyroid); or history of major hematologic, renal, or hepatic abnormalities. Poorly controlled diabetes, retinitis pigmentosa, previous history of pelvic genital, perineal, spinal cord injury, head trauma or any other neurological diseases. Peripheral vascular or cardiovascular disease, history of cryptorchidism, surgery or radiation therapy and/or particular procedures related to genitourinary or gastrointestinal malignancy. Signs of testis varicocele or fibrosis in corpum cavernosum, andrectable foreskin, gynecomastia, prostate abnormality or any other physical disease that may better explain the ED.

Control subjects

A total of 50 nonpatients (i.e. control subjects), selected from a random sample of male employees on a university campus, were included in this study. The mean age was 39.1 years (SD=6.7) and they were ongoing in a stable relationship on average from 32 months ($\pm$ 8 ES). They gave a history with no ED, and no symptom of premature and/or delayed ejaculation and orgasm (i.e. climax) problems were reported. The patients report morning and/or nocturnal erection and in a subjective 0-10 scale of erectile rigidity the current firm was scored 9 in all patients.

The case records of the subjects registered were meticulously maintained with all the details including socio-demographic and clinical variables being entered at the time of assessment.

Procedure

In the first examination was made the medical history tacking, clinical and genital examination, to exclude or recruit patients and control subjects. ED presence and status of health, was evaluated by experienced or trained medical clinician. At the end of this session the study purpose and protocol were clearly explained to the participants. Only subjects who agreed to participate in the study were assessed. To guarantee subjects privacy, an alphanumeric code was used for individual identification. No specific or more instructions were in place for providing feedback to the subject on his diagnosis, to reduce the potential for bias during psychological test and ethological session. In the second session the subjects were exposed to psychological investigation and psychometric test sessions for trait-personality evaluation. In this session the patients completed four pen and pencil standardized test. The presentation of the tests to each subject were balanced for order and sequence to avoid effects connected to these variables. (see below for details).

Psychological Investigation

ED status was evaluated by experienced or trained clinicians using a semi structured clinical interview according to DSM-IV-TR criteria (SCID). The use of the SCID made it possible to exclude the presence of current or past psychiatric disorder and to standardize the content of interviews. the patients who show other psychopathology related to axis I and II DSM IV-TR disorders or medical and psychosocial/lifestyle risk factor (i.e. DSM IV-TR axis III and IV) were excluded to the study.

Under the supervision of two psychologists all subject completed the following sequence of Italian versions of four auto-administration psychometric questionnaires.

Psychometric test Sessions for Trait-Personality Evaluation

CBA 2.0. (cognitive behavioural assessment 2.0) 4.21 question

The CBA 2.0 (Bertolotti, 1990) includes a series of questionnaires investigating broad issues of potential clinical interest, identifying possible areas dysfunctional in the present life of the subject. In this study were chosen only tab.4 to investigate the life span events in patients' life. previous clinical experience suggested that patients who recently had experienced "a social defeat (e.g. lost of job and/or social prestige and position" . On this bases we decided to modify the question 4.21 that refers to relevant life changes (such as lost of job and impairment of economical resources) in the last 12 months by including the additional questions concerning the the time of the event (i.e. when expressed in months). This was done to analyse the loss of status/resource with the time of onset of sexual disfunction (see the Beck depression …)

The Temperamental and Character Inventory (TCI)

Designed by Cloninger (1994) the TCI is a trait questionnaire test, measuring four temperamental dimensions: Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD) and Persistence (PER); as well as three character dimensions: Self Directedness (SD), Cooperativeness (Cop) and Self-Transcendence (ST).

The 16 Personality-Factors-C (16 PF-C)

Designed by Cattell (1959), the 16 PF is a classic trait questionnaire test. The C form was used as that version is most used in Italy (Grava and Bressani, 1968). This test measure 16 personality factors (16 PF) which are named by alphabets letters e.g emotional stability (*C*), insecurity (*O*), self-confidence (*Q2*), and so on. Each factor was scored in a scale ranging from 9 to 1. For example a score of 1 for *C-trait* means a very low emotional stability whereas a score of 9 means a high emotional stability.

International Index Of Erectile Function (IIEF)

The IIEF (Rosen et al., 1997) is a 15-item, 5-point Likert-type, self-administered measure assessing different areas of male sexual functioning. A principal component analysis identified five factors: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction.

The measure allows the calculation of specific indices for each dimension as well as a total sexual

function index (calculated through the sum of the specific dimensional indices), with higher scores indicating greater levels of sexual functioning (sexual desire: 2-10, erectile function: 1-30, orgasmic function: 0-10, intercourse satisfaction: 0-15, overall satisfaction: 2-10, total: 5-75).

Beck Depression Inventory (BDI).

The BDI (Beck et al., 1961)is a 21-item self-report rating inventory that measures symptoms, characteristic, attitudes and severity of depression and corresponds with the DSM-IV diagnosis of depressive disorder. Responses to items for the present study covered the 'past two weeks, including today'. Responses on the BDI items range from 0 to 6 with higher values indicating higher severity. The total score can be divided into categories reflecting severity of depression. Accordingly, the minimal range of depression is 0–13, the mild range is 14–19, the moderate range is 20–28 and the severe range is 29–63.

At the end of the test we asked to subjects if and when the symptoms compare, in months.

Procedure of Statistical Analysis

Kruskal-Wallis ANOVA median test, was used to compare the personality traits among the three groups, (i.e. losers, no losers, control) followed by Mann-Whitney post hoc analysis. Spearman-Brown correlation coefficient was used to assess relationships between personality, and hormonal values. Personality trait were expressed as median and 25 to 75 percentiles. Statistical significance for all tests was set at $p<0.05$. All statistics were performed using statistica 6.0 software package (StatSoft, Inc.,Tulsa, OK, USA).

Results

Psychological Trait in Losers, no Losers and Control Group

The proportions of ED patients who lost their job (as specified in 4.21 question of CBA 2.0) is higher in subject that has a range score between 12-16 in BDI score, the also called mild depressive symptoms subject (MDS) respect no depressive symptoms (NDS; i.e. the subject that ranged between 0-11 in BDI score) subject (figure 1).

The losers subject was composed by 26 subject, the no losers subject by 69 subject).

The BDI score was significantly higher in losers respect to no losers and control group (*Kruskal Wallis* rank sum test, $p<0.0001$; Post Hoc $Z_{26,69}=3,49$; $p<0.005$ for no losers. Post Hoc $Z_{26,50}=6.38$; $p>0.000005$ for controls; figure 2).

As expected IIEF showed that controls group is significantly higher (i.e. the higher the score is the highest functionality) than do losers group and no losers group, in total score (*Kruskal Wallis* rank sum test, $p<0.0001$; Post Hoc $Z_{50,26}=9,65$; $p<0.00001$ for losers. Post Hoc $Z_{50,69}=5,40$; $p>0.00001$ for no losers; Figure 3). and this means that control subject decalre toh ave a normale sexual behavior respet patinets group.

As far as personality traits are concerned, we observed that Controls showed higher score in NS respect both losers and no losers group (*Kruskal Wallis* rank sum test, $p<0.0001$; Post Hoc $Z_{50,26}=3,95$; $p<0.001$ for losers. Post Hoc $Z_{50,69}=3,29$; $p>0.005$ for no losers; figure 4). Similarly, controls showed lower HA trait when compared to losers and no losers groups (*Kruskal Wallis* rank sum test, $p<0.0005$; Post Hoc $Z_{50,26}=3,12$; $p<0.01$ for losers. Post Hoc $Z_{50,69}=3,41$; $p>0.005$ for no losers; figure 5).

Testosterone in Losers, no Losers and Control Group

Somewhat surprisingly, the loser group had a significant higher level of Testosterone in front of controls group ($F_{2,134}$= 4,29 $p<0.05$; Post Hoc $p<0.05$), no significant differences was revealed with no losers group (Figure 6).

Correlating Hormonal and Psychometric

According to the data described in the previous sections, Losers group had a higher depression score, as well as high testosterone respect to controls and no losers subjects. We investigated the correlations between psychological traits and biological data.

When all subjects are considered altogether, the level of testosterone correlate negatively with BDI score ($R=-0.18$; $p<0.05$; Figure 7). Analysing the three groups separately we found that the negative correlation accounts for the data of the controls ($R=-0.48$; $p<0.0005$; Figure 9) and surprisingly, of the Losers group ($R=-0.41$; $p<0.005$; Figure 8) but not for the no losers group. Indeed losers showed higher T and higher BDI when compared to Controls, therefore whichever the mechanism linking the level of this steroid hormone to BDI this may not represent the causal factor explaining the profile of the Losers.

Conclusion

This work indicate that the patients that lost their work show more signs indicative of a possible depression than patients who didn't lost their job. Interestingly all patients, irrespective of losing or not the job (i.e. a resource) are equal in Harm avoidance (HA), Novelty Seeking (NS) Ineterational index of erectile function (IEF) but they differ from controls. Patients who lost the resource show elevated testosterone (T) compared to controls and that T correlate negatively with BDI score.

Although our data corroborate the negative correlation between T and BDI (Kaplan 1987, Pope 2003,Adali 2008) if one analyse in details the levels of

testosterone between losers and no losers it appears that the losers have higher values of T compared to control. We can suggest two possible explanations:

1) we has in face a rare case of alteration regarding aromatase activity as been already show (Zirilli, 2008, Rochira 2007, Lanfranco 2008).

2) Some studies show the that T level may be increased at initial stages of acute stress (Rivier 1991, Wingfield 2003), and in organism that have (i) greater experience in winning in agonistic competitions and/or (ii) had acquired a dominant status in a population hierarchy (Sapolsky 1986, 1989, Buck 2003). Taking this into account, an author suggested that the T rise is due to the increased sensitivity of testicles to LH. This increase of sensitivity is a result of the activation of the sympathetic nervous system, predominantly NE type of responding. This type of responding is a characteristic of dominant animals in a social hierarchy; only in these subjects is frequently observed this increase of T (Chichinadze 2008).

Then probably losing the job for these patients, and than the loss of status may activate a diverse coping strategy that enhance NE response, responsible of T rise. But to test this hypothesis we must study the temperamental trait.

Regard to this, NS and HA value differentiate only DE subject respect to controls, but they are not able to differentiate the losers, from no losers.

This results clearly show that the group of patients, losers and no losers, it should be considered from temperamental the point of view, as a single group and then, we'll analyze the temperamental profiles, looking for a specific vulnerability to DE. Regarding to this, Novelty seeking trait in ED patients, is lower than controls. this mean that this group may be described, as less exploratory, less prone to find satisfaction in social exploration and than more related to familiar environement (Cloninger, 1994).

Elevated HA (as the ED patient display) are usually described as pessimistic worries, apprehensive, fearful, insecure, tend to anticipate harm and failure, passive and tend to be shy in social interactions (Cloninger, 1994). In literature, this trait is well defined, because high levels of HA was reached in many psychopatology from personality disorder (Pecorari, 2008), anxiety disorder (Calvo 2008) mood disorders (Farmer 2009, Trouillet, 2008) or as well as addiction (Evren, 2008) and was already linked in the work-related stress and the expression of depressive symptoms (Jurado, 2005). This demonstrate that HA is

related to risk-related behaviors, and the people that has a higher score (a score that ranges from 60 to 80) tend to react in fearfulness way against a wide range of treath (Pujol, 2002). This may be a good hypothesis to explain also the NE effect on T.

Finally we can find that this study fail its first target that is to reach a differences in temperamental and personality profile between losers and no losers, ED patients; but indicated that ED patients (losers and no losers togheter) probably has a specific temperamental vulnerability (higher HA and lower NS). Than we may say that; if is true that losing a job, and then the loss of social rank, is one of the most stressogenic event for human (Keller 1994, Mandal 2008, Friedman 2008, Amital 2008) that characterize some depressive states (Pennebaker 2007, Morey 1997) but is already true that this may be supported by temperamental vulnerability and this in turn activate a specific coping strategy that affect NE and HPG axis.

Chapter 3:

Relationships between Personality Traits and Hormonal Responses in Male Karate practitioners

Introduction

Martial arts (e.g. Judo) have been used as a model of competitive aggression to study the neuroendocrine response in agonistic behaviour in humans since they appear to provide close parallels for the dyadic encounters commonly studied in rodents and infra-human primates (Archer, 2006; Parmigiani et al., 2006; Suay et al., 1999; Salvador et al. 2003). The martial arts originated from combat techniques applied in overt and unritualized aggressive confrontations. In particular, two distinct varieties of martial art combat activity exist, namely real fights (Randori in Judo, Kumite in Karate, etc.) and highly ritualised dyadic fights (Kata). Real fights are simple combat whereas Kata is designed to study the form of attack, defence and counter-attack strategies needed to achieve the best style in terms of both psychological (i.e. mental) control and the energetic cost of fighting. Although Kata is not real combat, it is a psychological "social stressor" because the members of the dyads perform alternately (with one attacking and the other being a sort of passive "sparring partner") essentially, the same series of attacks that illustrate the athlete's respective abilities. Such psychological training will develop an individual mental attitude that may influence coping in challenging situations. It follows that we cannot study agonistic activity in such fighters without taking into consideration both individual psychology and the characteristics of different agonistic contexts. Indeed, psychological (i.e. cognitive) perceptions and interpretation of the contest situation may have more impact on hormonal responses than winning or losing. In fact, testosterone (T), and cortisol (C) appear to play important roles in the expression and regulation of competitive aggression (Archer, 1991; Bartolomucci et al. 2006;Brain 1994;

Dxson, 1980; Mazur, 1976). Social encounters elicit hormonal changes but the nature of the physiological responses are strongly dependent on their outcome (in terms of victory or defeat) in a variety of species (Archer, 1988; Brain, 1990; Bartolomucci et al., 2005; 2006). Variations in plasma C, and T in symmetric (i.e. matched in terms of body weight and fighting ability) dyadic encounters in male Judo participants, studied both in Randori and Kata contests, showed that T increased only during the former whereas C increased significantly in both (Parmigiani et al., 2006).

The T response appears to depend on the competition context (particularly whether a real fight is involved), individual character and the participant's evaluation of the likelihood of winning or losing the match. In contrast, C release appeared to follow any generally stressful physical activity.

In essence, results from previous studies suggested an intriguing correlation between psychological (the so-called 'personality traits') and biological (i.e. hormonal responses) factors in response to specific contexts (Parmigiani et al., 2006; Salvador et al., 2003). Personality traits are thought to reflect genetic and epigenetic influences and are responsible for relevant lasting differences between individuals and describe how individual subjects tend to perceive the world and react in predictable ways to environmental and social stimuli (Allport, 1960; Cattell, 1981, Cloninger, 1999; Eysenck, 1951; Spielberger, 1972). Subsequently, the trait describes the predisposition to react and to behave predictably in their life (Allik., 2005, Hampson and Goldberg., 2006).

To test this potential link between personality traits and hormonal responses in martial arts we studied karate athletes because in karate the Kumite contest (a real fight) is a dyadic encounter whereas the highly ritualised Kata is an individual (i.e. solitary) performance in front of a mirror whilst "imagining" an opponent. It was thought that this might well offer a better opportunity to analyse the individual personality traits and specific responses to a situation as it avoids the confounding effect of reacting to an (albeit passive) opponent.

For the aim of the study we analysed : a) the endocrine responses in Kata and Kumite sessions; b) winners and losers in pre and post-agonistic fighting activities and c) the correlation between pre and post-competition physiological changes with the outcome of psychometric tests.

Materials and Methods

Subjects

Twenty-four healthy male fighters aged 18-44 years (mean 26.75 SD=7.98) who had practiced Karate in competition and had an average of 8-10 hours of training per week took part in this study. Body weights were between 68 and 92 kg (mean 74 SD=5.5) and BMI ranged between 19 to 24. Their technical abilities ranged from brown to black belt. The subjects were recruited from two Sports Clubs in the Parma area of Italy. All subjects provided informed consent before participating in the study, previously approved by the ethics Committee of the School of Medicine of the University of Parma.

Procedure

The study purpose and protocol were clearly explained to the participants. In order to avoid confounding effects on the hormonal levels, all participants were drug free and had no endocrine and psychological (i.e. mood) disorders at the time of the study. Only athletes who agreed to participate in the study were assessed. To guarantee subject privacy, an alphanumeric code was used for individual identification.

With the help of two Karate masters, subjects were allocated to dyads (with each member coming from a different sports club to avoid familiarity). Dyads were, as far as possible, matched for weight, technical ability and BMI in order to achieve symmetrical contests. Each participant was subjected first to a symmetric Kumite (an actual fight) session, followed after four weeks by a Kata (highly ritualised fighting) session (see later for details). Both sessions were carried out between 18:00 and 21:00 hours (local time) on a “tatami” (i.e. the training and fighting ground where the Kumite and/or Kata take place) with a dojo unfamiliar with either Karate participant (to avoid the so-called ‘resident effect’ on fighting motivation and outcome). Blood was collected from each member of the dyad (see later) before and after the Kumite and Kata sessions. Furthermore, two weeks after the Kumite session, a battery of questionnaires to measure personality trait was presented to the subjects.

Kumite Session

Every Karate competitor took part in a single symmetrical Kumite confrontation with a maximum duration of 3 minutes. Using the international rules of the World Karate Federation, the two masters alternately refereed and judged the fights. Although Kumite is a free fight, it is a regulated sporting activity in which ritualization is always the rule (i.e. the hits in terms of kicks and punches should stop before hitting the target and thus the most dangerous and deadly techniques are banned). The winner was the athlete who successfully delivered the greater numbers of hits to the opponent and thus achieved the higher score.

Kata Session

In the Kata there is a peculiar situation in which the athlete performs alone in front of a mirror rather than confronting an opponent. This is a simulation of combat consisting of a sequence of highly ritualised and stereotyped patterns of fighting. The timings, locations and judges were the same as those used in the Kumite session.

Hormonal Analysis

Two 10 ml venous blood samples were taken from the antecubital vein of the forearm, 10 minutes before (pre) and 10 minutes after (post) the Kumite and Kata session. The blood samples were centrifuged to obtain serum which was then frozen at -20°C until assay. Titres of C was made by enzyme-linked immuno-absorbent assay (Quantikine HS, R&D Systems Europe Ltd UK). Total T was determined by Radioimmunoassay I^{125} (RIA-Pantec SRC, Torino, Italy). All samples were treated in duplicate in the same assay and in a randomized order. The intra- and inter-assay variation coefficients were 3.9 and 9.00 % for C; 3.9 and 6.2 % for total T. The sensitivities were 2.5 ng/ml for C, 0.07 ng/ml for total T.

Psychometric Test Sessions for Trait-Personality Evaluation.

All competitors completed together in an unfamiliar room the following sequence of Italian versions of four auto-administration psychometric questionnaires. To avoid confusion between the abbreviations used in the literature to specify hormones and traits (e.g. C for Cortisol and C for stability trait), the trait and state names are written in italics (e.g. *C-trait* instead of C)

The Temperamental and Character Inventory (TCI)

Designed by Cloninger (1994) the TCI is a trait questionnaire test, measuring four temperamental dimensions: Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD) and Persistence (PER); as well as three character dimensions: Self Directedness (SD), Cooperativeness (Cop) and Self-Transcendence (ST).

The 16 Personality-Factors-C (16 PF-C)

Designed by Cattell (1959), the 16 PF is a classic trait questionnaire test. The C form was used as that version is most used in Italy (Grava and Bressani, 1968). This test measure 16 personality factors (16 PF) which are named by alphabets letters e.g emotional stability (*C*), insecurity (*O*), self-confidence (*Q2*), and so on. Each factor was scored in a scale ranging from 9 to 1. For example a score of 1 for *C-trait* means a very low emotional stability whereas a score of 9 means a high emotional stability.

The State-Trait Anxiety Inventory-Y

STAI-Y is a classic test that measures the state-anxiety as well as anxiety as a personality trait.

Statistical Analysis

Hormonal measures in Kumite and Kata tests were analysed using a 2-way-ANOVA for repeated measure, followed by Tukey's HSD post hoc test. Trait personality data were analysed using two-tailed Mann-Whitney tests. Correlations

were performed using Spearman-Brown. Delta value were calculated with the following formula: [[(value after/value before)*100]-100] to give the net differential value.

Results

Hormonal Responses to Kumite and Kata Sessions

The data for this section are shown in Table 1.

T increased only between the pre- and post-competition phases (treatment x time $F_{1,46}$=10.65 p<0.005; Post Hoc p<0.005), and we observe a significant differences in T between pre and post kumite and kata situations (time $F_{1,46}$=10,65; P<0.005). The post-competition level of T in Kumite was found to be greater than the post-competition level of the Kata (p<0.05).

In C level too, we observe an increase only between pre and post Kumite phase (treatment x time $F_{1,46}$=16.74 p<0.0005; Post Hoc p<0.0005) and kumite and kata situation induce a huge C increase between pre and post (time $F_{1,46}$=39.77 p<0.00001). The post-competition level of C in Kumite session was found to be greater than the post-competition level in Kata (p<0.0005).

The Kumite activity induced a greater delta-T ($U_{24,24}$=175.0, p<0.05) and delta-C than Kata ($U_{24,24}$=131.0, p<0.001).

Hormonal Titres in Winners and Losers in Kumite Contests

The data for this section are shown in Table 2.

C levels increased between the pre- and post-competition phases in winners and losers (treatment x time $F_{1,22}$=31.71 p<0.00005) but The C level was higher in losers in pre (p<0.05) and in post-competition level (p<0.05).

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Personality Trait in Winners and Losers

The relevant data are shown in Figure 1. The 16 PF-C scores for motivational distortion (i.e. the so-called social approval effect which measures the subject's tendency to "lie" to alter the perceived social image) revealed that such an effect was minimal, and the test results reliable.

The analysis essentially revealed that losers had greater sensitivity (expressed by the *I-trait* $U_{12,12}$=19 $p<0.005$), and insecurity (expressed by the *O-trait* $U_{12,12}$=14 $p<0.0005$) scores than winners. The greater insecurity of losers is also confirmed by their lower value (compared to winners) of emotional stability (expressed by the *C-trait* $U_{12,12}$ =16 $p<0.001$) and self-confidence (expressed by the *Q2-trait*; $U_{12,12}$=16 $p<0.05$).

The TCI analysis (see Figure 2) showed that losers score significantly higher than winners in three out of seven personality trait, namely harm avoidance (*HA*; $U_{12,12}$=19 $p<0.005$), reward dependence (*RD*: $U_{12,12}$=15 $p<0.0005$), and persistence (*PER*: $U_{12,12}$=34 $p<0.05$) . Winners scored higher in one personality trait namely novelty seeking (*NS*; $U_{12,12}$ =9 $p<0.0005$). This suggests that, compared to losers, winners tend to be more exploratory (see the higher *NS* value) and less risk averse (see the lower value of *HA* as an index of risk assessment). Losers were also significantly higher than winners on trait anxiety as measured by STAI-Y ($U_{12,12}$=9,5 $p<0.0005$).

Correlating "Biological" and "Psychological" Factors

According to the above reported data which show that the agonistic activity such as Randori is the only one activating significant hormonal responses (C), we analysed the correlations patterns between personality traits and basal level (i.e. pre-competition level) of C in winner and losers.

Independently of the competition outcome, a significant positive correlation ($r=0.52$ $p<0.01$) was found between the pre-competition level of C and the *I-trait* (i.e. an index of emotionality) measured using the 16 PF-C test, HA (Harm avoidance; $r=0.50$ $p<0.05$) and RD (Reward Dependence; $r=0.48$ $p<0.05$). Pre

competition level of C was negatively correlated with NS (Novelty Seeking; r=-0.44 $p<0.05$).

In contrast, only losers showed pre-competitive C levels that were positively correlated (see Figure 3) with *I-trait* parameter (r=0.73 $p<0.01$) and (see figure 4) with RD (r=0.88 $p<0.001$).

Conclusion

The main objective of the present study was to assess whether responses of Karate fighters in two different contexts (namely a real and highly ritualised fight) could be used to link an individual's "biological" and "psychological" variables. The most intriguing result of the present study concerns the psychometric analysis showing that winners have higher levels of *novelty seeking* (NS) and lower levels of *harm avoidance* (HA) than do losers. Moreover, as indicated by the O-*trait and C-trait* scores in the 16-PF losers showed more *emotional instability* and *insecurity* as far as social relationships compared to winners. These data have parallels with studies of animal aggression (e.g. rats and mice) as far as victory/defeat, dominance, submissive status, and genetic differences in aggression levels are concerned (Parmigiani et al., 1999). In fact, dominant individuals are less fearful and show reduced levels of *risk assessment* and more intense *exploratory activity* of the environment whereas submissive individuals show exactly the reverse (Bartolomucci et al., 2005). Moreover, dominant animals show reduced levels of *anxiety* compared to submissive counterparts (Bartolomucci et al., 2005; Blanchard et al., 2001; Blanchard and Blanchard, 2003; Koolhaas et al., 1999; Parmigiani et al., 1999). These findings tend to support the above suggestion, derived from the psychometric analysis, that losers are more anxious as well as more defensive than winners. Indeed, high levels of *anxiety* are characteristic of defensive types of aggression such as those described both in humans and animal models of agonistic behaviour (Blanchard & Blanchard, 1977; Blanchard et al. 1978; Brain, 1981). Blanchard et al (1997) have clearly shown that human defensive behaviour show parallels to fear and anxiety related defence patterns of non-human mammals and that a high anxiety trait may be associated with alterations of systems involved in risk assessment and

defensive threat/attack. Indeed clinical studies point out that individuals with high levels of shyness and anxiety are characterized by high levels of harm avoidance and this is particularly evident in anxiety disorders (Chatterjee et al., 1997; Faytout et al. 2007; Marteinsdottir et al., 2003; Stein et al., 2001)

As far as the "biological" variables (i.e. hormones) T increased only during 'real' agonistic activity (i.e. Kumite), suggesting that the activation of Hypothalamic-Pituitary-Gonadal (HPG) axis and consequent release of T is dependent on the competitive context. In contrast, the Hypothalamic-Pituitary-Adrenocortical (HPA) axis was activated in both situations albeit with the increase of C being greater in a real fight. This suggests that C is not strictly dependent on the agonistic activity and the emotional and cognitive perception of the outcome of fighting rather reflecting the general stressfulness of situations. The C data are very similar to those found in a previous study in Judo fighters (Parmigiani et al., 2006). It is also interesting to note that in the Judo study the losers showed a greater release of T whereas, in this present study, no such difference was found. The fact that, in the Judo study the losers showed higher levels of T than the winners, might be related to Judo being a wrestling activity with intense physical contact providing the two contestants with greater opportunities to evaluate their respective fighting abilities. On the basis of game theory predictions on animal conflict (Maynard Smith and Price, 1973), small variations in fighting ability (i.e a dyadic confrontation between contestants matched for size and technical ability) are likely to result in more intense attack by the eventually defeated individual. Animal studies have also clearly shown, differences (both in terms of neuroendocrine responses and behaviour) between victory and defeat when a single fighting episode is used and/or when dominants are contrasted with submissive in a grouped situation (Bartolomucci et al., 2005; Bartolomucci, 2005; Brain, 1981; Koolhaas et al., 1999; Parmigiani et al., 1998; 1999). Interestingly, the endocrine data corroborate the psychometric analysis that losers are more anxious than winners. In fact although the C levels were positively correlated with the *I trait* (an index of emotionality) of the 16-PF in both winners and losers only in losers the C levels showed a significant increase before and after the agonistic fight (i.e. kumite). This finding suggests that C production in an impending challenging situation is linked, not only to the context, but probably to a specific individual's coping strategies.

Moreover C levels were positively correlated with TCI test dimensions such as *RD* a trait indicative of elevated fearfulness and insecurity which were greater in losers than winners. It seems that the personality of winners, is characterized by security and reduced fear.

Thus the psychometric data suggest that winners and losers are characterized by having different expressions of personality traits. As symmetric confrontations were used in this study, these differences in personality may reasonably be ascribed to individual character (i.e. traits) rather than a generalized response to the context (i.e. personality states). Animal data supports the view that individual differences in responses to agonistic or stressful situations reflect genetically-programmed differences in coping strategies (Koolhaas et al., 1999; Salvador, 2005). Personality traits are thought to be based on either genetic and/or epigenetic (i.e. developmental) factors. Numerous studies indicate a heritable basis of human personality traits across different personality models (Cloninger et al. 1993; Ebstein et al., 2002; Savitz and Ramesar, 2004). About 50% of variation in most personality traits may be attributed to genetic influences. Indeed, individuals using different coping strategies (e.g. introverts and extroverts) may show varied bio-psychological responses to specific situations (Zuckermann, 1993, Gaab, 2005).

The correlations between "biological" and "psychological" factors suggest that psychometric tests can predict hormonal responses in specific contexts (in this case, a competitive situation). The reverse may also be true (i.e. the hormonal responses may be used to infer the personality trait of an individual and the likely response in a specific situation). In this connection a clinical study of stress response, in healthy individuals, reported that harm avoidance (a behaviour modulated by anxiety) was positively related to plasma C concentrations. Thus showing a clear associations of temperamental traits and HPA function (Abelson et al., 2006, Tyrka et al. 2006). These data are important for sports psychology and sports science in general. In fact the combination of biological and psychological measures can be used to characterize the athlete in terms of attitude and performance. It follows that psychobiological studies of martial arts can increase our understanding of the power of "mind" to modulate neurochemical responses and consequently be used in such agonistic sports activities as a form of

personality training to enhance individual coping strategy in challenging situations.

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Chapter 4:

Serotonin-Transporter-Linked Polymorphic Region (5HTTLPR) and Temperamental Trait, in Patients With Premature Ejaculation.

Introduction

Premature ejaculation (PE), is characterized by the loss or absence of ejaculatory control, a marked distress or interpersonal difficulty and a short intra-vaginal ejaculatory latency time (IELT). This is the most common male sexual dysfunction that can substantially impact a patient's - and his partner's - quality of life (QoL) (CIT)

Related to this has been observed that serotonin (5-HT) has an inhibitory role on ejaculation, throughout brain descending pathways. Indeed, antidepressant selective serotonin reuptake inhibitors (SSRIs) are currently prescribed to cure premature ejaculation (Waldinger 2003).

It is important to note that 5-HT is not only involved in sexual behavior, but also in other behaviors related such those related to : a) aggression and in particular impulsiveness (Zuckerman, 1994, Olivier, B. 1995; Miczek,K.A. 1998; Fish. E.W. 1999; Lyons, W.E. 1999; Lesch, K.P. e Merschdorf, U. 2000; Chiavegatto, S.2001; Hennig, 2004), and b) behavioural traits such as for example neuroticism (Lesch et al, 1996; Jacob et al, 2004) and depression (Merschdorf, 2000; Caspi et al, 2003).

In particular the polymorphism within the promoter region of the the 5-HT Transporter (5-HTTLPR) has been associated with personality traits such as anxiety- depression and aggressiveness (Munafò et al., 2003; Sen et al., 2004).

This gene has two alleles i.e. a) the so called long allelic variant (L) that is associated with higher affinity and activity of the RNA polymerase, high 5HTT mRNA and protein and consequently a greater rate of serotonin reuptake from the

synaptic cleft when compared with the b) the short allelic variant (S) (Lesch et al., 1996; Greenberg et al., 1999) whose limited transcription rate results in lower uptake of serotonin (Hranilovic et al., 2004). Up to now no study directly investigate the association between l or s allele with sexual disorders. In this study we aimed at investigating possible association between the 5HTTLPR allele, personality traits and general sexual parameters in a population of secondary PE patients.

Materials and Methods

Subjects:

clinical sample

For this study, 32 patients with a chief complaint of premature ejaculation (PE) were recruited from the andrological outpatient unit of the Parma hospital "Ospedale Maggiore". This unit is composed by dual specialty team consisting by an andrologist and psychometric and clinical-psychological unit.

The patients have a history of secondary PE with a sudden onset on average of 1,1 years before the medical examination (+ 4 months SEM). The patients report ejaculatory problems only during sexual intercourse but not during masturbation. No symptom of erectile dysfunction were reported.

All patient were male gender with at least 18 years old (mean age 39,6 ± 6 SEM), heterosexual, and in an ongoing, stable, monogamous, relationship for at least 6 months (mean 38,5 months ± 6 SEM) before the medical history tacking. They reported a normal puberty onset at the age of 13th, regular statural growth (mean height 172 cm SD ±10 cm; mean weight 78 kg SD ±6 Kg) and normal body structure (BMI).

The Exclusion criteria

Patients with the following manifestations were excluded from this study.

a)the presence of current axis I psychiatric disorder, including substance abuse or dependence including alcohol or cigarettes smoking. B) Current use of any antidepressant medication. C Abnormal serum hormone levels (i.e., prolactin,

testosterone, or thyroid); or D) history of major hematologic, renal, or hepatic abnormalities. Poorly controlled diabetes, retinitis pigmentosa, previous history of pelvic genital, perineal, spinal cord injury, head trauma or any other neurological diseases. Peripheral vascular or cardiovascular disease, history of cryptorchidism, surgery or radiation therapy and/or particular procedures related to genitourinary or gastrointestinal malignancy. Signs of testis varicocele or fibrosis in corpum cavernosum, andrectable foreskin, gynecomastia, prostate abnormality.

All subjects provided informed consent before participating in the study, previously approved by the ethics Committee of the School of Medicine of the University of Parma.

Procedure:

First Examination of the Patient and Selection Session

the first examination consisted of medical history taking, clinical and genital examination, to exclude or recruit patients. PE presence and status of health, was evaluated by experienced or trained medical clinician. At the end of this selection session the study purpose and protocol were clearly explained to the potential participants.

According to the informed consent approved by the ethical Comitee Only subjects who agreed to participate in the study were assessed. To guarantee subjects privacy, an alphanumeric code was used for individual identification. No specific or more instructions were in place for providing feedback to the subject on his diagnosis, to reduce the potential for bias during psychological test and ethological session. In the second session the subjects were exposed to psychological investigation and psychometric test sessions for trait-personality evaluation. In this session the patients completed four pen and pencil standardized test, and small amount of skin near to fingernail to extract Genomic DNA is made (see below for details). The presentation of the tests to each subject were balanced for order and sequence to avoid effects connected to these variables. (see below for details).

Psychological Investigation:

PE status was evaluated by experienced or trained clinicians using a semi structured clinical interview according to DSM-IV-TR criteria (SCID-II). The use of the SCID-II allowed to exclude the presence of current or past psychiatric disorder and to standardize the content of interviews. the patients who show other psychopathology related to axis I and II DSM IV-TR disorders or medical and psychosocial/lifestyle risk factor (i.e. DSM IV-TR axis III and IV) were excluded to the study.

Under the supervision of two psychologists all subject completed the following sequence of Italian versions of four auto-administration psychometric questionnaires.

Psychometric Test Sessions for Trait-Personality Evaluation.

The Temperamental and Character Inventory (TCI)

Designed by Cloninger (1994) the TCI is a trait questionnaire test, measuring four temperamental dimensions: Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD) and Persistence (PER); as well as three character dimensions: Self Directedness (SD), Cooperativeness (Cop) and Self-Transcendence (ST).

The 16 Personality-Factors-C (16 PF-C)

Designed by Cattell (1959), the 16 PF is a classic trait questionnaire test. The C form was used as that version is most used in Italy (Grava and Bressani, 1968). This test measure 16 personality factors (16 PF) which are named by alphabets letters e.g emotional stability (*C*), insecurity (*O*), self-confidence (*Q2*), and so on. Each factor was scored in a scale ranging from 9 to 1. For example a score of 1 for *C-trait* means a very low emotional stability whereas a score of 9 means a high emotional stability.

Index of Sexual Satisfaction (ISS)

The ISS is a specific scale of 25 item developed as an unidimensional self- rate 7 point likert-scale with the aim of assessing problems in sexual satisfaction.

5HTT

Genomic DNA was extracted and purified by menas of the AquaPure Genomic-DNA Kit (Bio-Rad Laboratories), from small amount of skin obtained by delicate scratching near fingernail fixed in absolute ethanol and later extracted

Polymorphisms of serotonin transporter *5-HTT* were investigated using specific primersfor *5-HTTLPR* according to Gelernter et al. (1999) , they were respectively:

forward 5'-ATGCCAGCACCTAACCCCTAATGT-3', reverse 5'-GGACCGCAAGGTGGGCGGGA-3'. PCR amplification was performed in a final volume of 20 µl consisting of 30 ng of genomic DNA, dNTPs 0.2 mM, 10 pmol of forward and reverse primers, Mg2+ 1.5 mM, 1x reaction buffer and 1 U of GoTaq DNA Polymerase (Promega, Madison Wi). Amplification conditions for *5-HTTLPR* were an initial step of denaturation at 94°C for 10 min and subsequent 35 cycles including 1 min at 94°C, 1 min at 66°C and 1 min at 72°C. Different alleles (short, s and long, l) were separated on 2.5% agarose gel electrophoresis followed by ethidium bromide staining and visualized under UV light. The different alleles were determined using the GeneRuler 100 bp DNA ladder (Fermentas, Hanover Md).

Statistical Analysis

Personality trait were expressed as median and 25 to 75 percentiles. Kruskal-Wallis ANOVA median test, was used to compare the personality traits among the three groups, followed by Mann-Whitney post hoc analysis. All statistics were performed using statistica 6.0 software package (StatSoft, Inc.,Tulsa, OK, USA).

Results

Personality Trait in L/L, L/S and S/S subjects

The S/S subjects has a coopertiveness and inconstant (-G) trait higher respet to L/S.Instead the L/L has their first sexual intercorse at a younger age and a higher sexual satisfaction respect L/L. Harm avoidance (HA) temperamental trait is higher in L/S than L/L.

The relevant data are shown in Figure 1. The 16 PF-C scores for motivational distortion (i.e. the so-called social approval effect which measures the subject's tendency to "lie" to alter the perceived social image) revealed that such an effect was minimal, and the test results reliable.

The personality analysis essentially revealed that S/S subjects had a low self emotional controls and difficulties in complying with the rules (expressed by G trait: *Kruskal Wallis* rank sum test, $p<0.05$; Post Hoc $Z_{14,8}=2,68$; $p<0.05$), than L/S subject .

The S/S subjects resulted to be more motivated in social relationship and more altruistic respect L/S as described by higher level of cooperation trait (CP) (*Kruskal Wallis* rank sum test, $p<0.05$; Post Hoc $Z_{14,8}=2,86$; $p<0.05$)

For this traits L/L subjects showed a range value similar to L/S subject, but probably the limited numbers of subjects don't revealed significant differences.

The temperamental analysis revealed that L/L are more exploratory (see the novelty seeking-NS) and less risk averse (see harm avoidance HA as an index of their risk assessment tendency) in social environment compared to L/S (expressed by HA trait: *Kruskal Wallis* rank sum test, $p<0.05$; Post Hoc $Z_{14,10}=2,54$; $p<0.05$). For this traits L/L subjects showed a range value similar to L/S subject, but probably the limited numbers of subjects don't revealed significant differences.

Sexual Behavior in L/L, L/S and S/S Subjects

The most relevant results in this section is that in S/S subjects, the age of their first sexual intercourse is significantly higher respect only to L/L (*Kruskal Wallis* rank sum test, $p<0.05$; Post Hoc $Z_{10,8}=2,82$; $p<0.05$). But in the total score of Index of Sexual Satisfaction S/S is significantly lower than L/L subjects only (*Kruskal Wallis* rank sum test, $p<0.05$; Post Hoc $Z_{10,8}=3,42$; $p<0.005$).

Conclusion

The S/S subjects has a coopertiveness and inconstant (-G) trait higher respet to L/S.

Instead the L/L has their first sexual intercorse at a younger age and a higher sexual satisfaction respect L/L. Harm avoidance (HA) temperamental trait is higher in L/S than L/L.

Although the number of patients examined is limited the data of this study suggest that there are not substantial differences among the three group of patients as far as their temperamental traits on the basis of their genetic polymorphism

The only differences detected were related to S/S who showed a lower value of the trait (-G) than L/S subjects. In the Cattell's personality model the factor G measures the orientation to rules, procedures, and social expectations, and to a considerable extent it is a measure of responsibility and dutifulness (Cattel 2001). Therefore these subjects has not developed moral standards and conventional self-control rules and their behaviours may be considered by others people unpredictable as far as for example the expression of aggression (Cattell 2001, Lee 2005). According to this interpretation the S/S could be subjects who have more difficulty in complying with the rules and procedures (Cattell 2001).

Regarding to this description clinical literature (i.e. that contrary to our case is referring to pathological subjects) show that S/S subject as more violent and disturbed, as far as the expression of aggression, than L/S (Munafò et al., 2003; Sen et al., 2004) this polymorphism has been associated with various psychiatric conditions (Reif et al. 2007). In fact, regarding the 5-HTPLPR polymorphism,

several studies have found a relationship between S/S polymorphism and higher level of violence, alcoholism and behaviors linked to antisocial personality, impulsivity (Däderman and Lidberg, 2002; Goodman and New, 2000; Larsson et al., 2007; Lesch and Merschdorf, 2000), mental disorders (Lidberg et al., 2000), including borderline personality disorders (BPD) (Reif and Lesch, 2003).

Moreover the frequency of only one short S allele 5-HTT promoter polymorphism, seems to be higher in male individuals with conduct disorder, aggressiveness and ADHD (Cadoret et al., 2003). In our case inconstancy (G-trait) is not a diagnostic criteria but indicate this findings.

In connection to this trait, Harm avoidance has been identified as a trait that predisposes to the vulnerability of different psychopathologies.

In this case is higher in L/S than L/L individuals and this confirm other findings that see that the S allele seems to confer susceptibility to a temperamental profile of high novelty seeking and low harm avoidance, (Gerra et al., 2005; Hallikainen et al., 1999; Sander et al., 1998;), impulsive-Sensation Seeking (Pascual et al., 2007) and an association with violence (Reif et al. 2007). Nevertheless, there are some discrepancies in regard to this type of results (Ebstein, 2006). For instance, there has been no supporting evidence of the association of the 5-HTTLPR and impulsive aggressive personality traits with cocaine addicts (Patkar et al., 2002). However, no differences were found in any allelic variation between borderline personality disorder (BPD) patients and control subjects (Pascual et al., 2008). Also in this case we found that our L/S or S/S individuals show only a premature ejaculation and then express only a vulnerability to control some aspect of sexual behaviors, but no ones show a conduct disorders, substance abuse disorder, impulsive or aggressive related disorders. This result may be explained by taking into account the cooperation trait (CP). In fact this trait has been shown to be protective against depression (Kusnoki et al, 2000), to have a negative correlation with BDI (depressive symptoms) (Farmer 2003), and in clinical trial we see that low cooperativeness is related to the presence of a clinically defined personality disorder, (Cloninger et al, 1993; Svrakic et al, 1993; Mulder & Joyce, 1997; Casey &Joyce, 1999;Mulder et al, 1999), and moreover was find an important positively correlation between cooperation and prefrontal cortical activity, as was measured by PET (Semple 1993).

An intriguing data might be the age of the first sexual intercourse of the these PE patients. In fact the L/L had their first sexual intercourse at a younger age than S/S. Moreover the sexual satisfaction index revealed that L/L have a higher sexual satisfaction with respect to S/S. Regarding this last findings we known that PE was a common problem, associated with significant effects on sexual functioning and satisfaction (waldinger) but the differences between different polymorphism of 5HTTLPR in expression of sexual satisfaction is not clear.

Regard to this we known that administration of 5-hydroxytryptophan, the direct precursor of 5-HT, 5-HT itself and 5-HT releasers, such as MDMA and fenfluramine, has been shown to inhibit sexual behavior (Ahlenius et al. 1980; Dornan et al. 1991; Foreman et al. 1992; Gonzales et al. 1982). Altogether these findings suggest that a decrease in 5-HT neurotransmission may be involved in facilitation, on the other hand antidepressants of the selective serotonin reuptake inhibitor class (SSRIs, including Prozac and Zoloft) impair ejaculatory/orgasmic function and frequently inhibit erectile function and sexual interest or pleasure as well [135,136].

Conversely, decreases in serotonergic activity, due either to lesions of cell bodies in the raphe nuclei [139–141] or to synthesis inhibition [142,143], (Albinsson Serotonergic Modulation of Sex and Aggression 35 et al. 1996) and 5-HT depletion (Tagliamonte et al. 1969) facilitate sexual behavior male copulatory behavior, and satisfaction. But regard to this we known that SS is linked with a low production in 5-HT then the question is why do we observe this phenomenon? More clearly why a low dose of 5-HT is linked with a poor sexual satisfaction?

Because sexual satisfaction is linked as reward activity to mesolimbic pathway that is comprised of cell bodies that originate in the ventral tegmental area (containing DA neurons known as the A10 group) and projects mainly to the limbic system through mesolimbic pathway. This pathway is associated with feelings of reward and desire (hull) and this region is rich of 5-ht 5-HT1A, 5-HT1B, 5-HT2A, 5-HT3 and 5-HT4 receptors, act to facilitate DA release (Alex, 2007). Poor in 5ht level is probably linked with a poor mesolimbic DA modulation (Jiang, 1990) and a then a low pleasure in sexual satisfaction.

Finally we may underline that S/S polymorphism has been associated with various psychiatric conditions

Such complex interactions between genetic variation in the 5-HT circuitry and certain environmental factors point to the complex causation of aggressive and violent behaviors, which are definitively not only dependent on the functioning of the serotoninergic system (Miczek et al. 2007) in fact a recent study (Reif et al. 2007) showed an association between the short allele 5HTT genotype and violence, but only in those patients with an adverse childhood environment.

It is important to note that the literature reported above, as already mentioned, refers to psychopathological subjects, while our data are collected from people who are not pathological either from physiological or psychological viewpoint. In essence our data suggest that 5HTTLPR polymorphism is not predictive or associated with the expression of the secondary PE in that all. the three genetic variant can be vulnerable to the onset of secondary premature ejaculation. Indeed these subject were people that had normal ejaculatory response and started to exhibit this ejaculatory disturbance in certain circumstances that we have not properly evaluated in this study. Secondary PE is not classified as disease but as a "disturbance" and we might suspect that this symptoms could be, in certain circumstances, an " alarm bell" indicative of a possible vulnerability to mood disorders? Our data can also stimulate new perspective on the clinically reported link between personality traits and 5HTTLPR. It is debatable if differences in genetic constitution (S/S, S/L, L/L) and psychopathological personality traits is or not linked. The apparent lack of any significant association in the present study may suggest the hypothesis these polymorphism is not predictive of vulnerability to develop a specific psychopathological disease in response to social environment, but how the genetic trait, affects the behavioural response, of the individual to disease. The question is therefore open if SS and LL different in relation to their response to social factors and stress.

Chapter 5:

Biophysiological Response to Chronic Sildenafil Treatment During a Mouse Model of Chronic Psychosocial Stress.

Introduction:

Summary Social stress is a major factor in the aetiology of several psychopathologies, with individuals greatly differing in vulnerability. The development of appropriate animal models of social stress is, thus, a major challenge of modern bio-medical research. Kind of stimulus, duration, intensity and predictability of the stressor applied are all relevant factors determining the development of a chronic stress state (Natelson et al., 1988; Mormede et al., 1988; Koolhaas et al., 1997; Stefanski and Engler, 1998). Indeed, models of social stress are currently considered the best available models of human psychopathological disorders, including major depression (Blanchard et al., 1995; van Kampen et al., 2002). Regard to this our model of social stress (Bartolomucci et al., 2001) has offered the opportunity to investigate whether territory ownership (being resident in a territory) and social status (becoming dominant or subordinate), as a result of their resident intruder interactions interact in the stress-induced behavioural (Bartolomucci et al. 2004), immune (Bartolomucci et al. 2003), endocrine (Batolomucci, 2001) and physiological (Bartolomucci et al., 2001, 2003b, c) alterations, and then is considered a model with a good face validity in studying depressive state.

Nonetheless, a major goal of depression research from a biomedical or medical perspective is to develop pharmacological interventions suitable to control mood disturbance in individuals.

Regard to this some recent studies have localized PDE-5 in diverse areas of the brain and this provides for the possibility of central nervous system (CNS) effects of sildenafil (i.e. PDE-5 inhibitor) administration on aggression and on mood. In fact several direct effects of sildenafil administration on the CNS have been reported in both rodents and humans. For example, sildenafil is capable of centrally altering copulatory behavior in rats, (Philips 2000, Ferrari 2002) aggressive behavior, (Hotchkiss, 2003) or increases sympathetic nerve activity in humans (Giuliani 2002), these studies suggest that increased levels of cGMP, in addition to potential decreases in NO levels in the CNS, profoundly drive elevated aggression (Nelson 2006).

Aim of the present study was whether and how the administration of sildenafil can change social behavior (i), agonistic (ii) and sexual (iii), in subordinate subject, in a context of chronic psychosocial stress.

The choice of subordinates in this model is not casual, in fact usually in this model, we observe that the male that loss status and becoming subordinate, show no attacks behaviors and display only submissive behaviors as upright posture, flight behavior, and squeaking vocalization. In this regard it is very rare see the subordinates to counterattack dominant again.

Matherials and Methods

Animals

Subjects were 67 adult males Swiss CD-1 mice from an outbreed stock originally obtained from Charles River Italia (Calco, Italy). Mice were born and reared in a colony room at the University of Parma at 20 2_C in a 12-hr light–dark cycle (lights on 0700–1900). After weaning (25–28 days of age) they were housed in same-sex- groups of siblings (4–7 per cage) in Plexiglas cages (45 _ 25 _ 20 cm) with wood shaving bedding. The cages were changed weekly. All animal experimentation was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/EEC) and approved by the Italian Institute of Health.

Procedure

At the end of the 5-7 days of individual housing, male mice were individually marked and housed in pairs in 40×23×15-cm cages with wood chips, food and water ad libitum (Day 0). At day 7, 50 animals start the Chronic psychosocial stress procedure (see below for details), and the remaining 16, where used as controls animals, were individually housed and no stress procedure was performed.

After the first week of stress procedure (Day 7) rank differences was settled and then dominant or subordinate status, in both resident and intruders animals, were find (see stress procedure for more details). From day 8 to day 42, we injected adult male mice i.p. with either saline vehicle 10 mg/kg of sildenafil, thrice weekly for 5 weeks (then n=12–15 dose for animal at all) (Hotchkiss, 2005). During this period, the injection were made immediately after the stress procedure. The animal treated with sildenafil were respetively 6/12 for resident subordinate, 6/12 for intruder subordinate and in control subject 8/17 animal were treated; the remaining were treated with a saline solution. Animals were weight-ranked at the beginning of the study to ensure similar starting body masses between groups. Subsequent the end of chronic psychological stress procedure (Day 42) a sexual behavior test was performed (see below for details).

Preparation of Dosing Solution

Dosing solutions were prepared following methods similar to those previously reported (Behn 2001). Briefly, sildenafil citrate (50 mg) tablets were ground into a fine powder using a morter and pestle. The resulting powder was then mixed with saline and passed through 40 Am filter paper. The resulting solution was kept chilled at 4°C. Dosing solutions were brought to room temperature 2h (i.e. 900 h) prior to injections.

Chronic Psychosocial Stress

The procedure has been originally described by Bartolomucci et al. (2001). Adult male mice to be used as resident animals, were individually housed in Plexiglas cages (45 _ 25 _ 20 cm) for one week to allow the establishment of individual territories. Intruder mice were group-housed males whose body weight was either equal or lower than that of their resident mouse. Throughout the study food and water were available at libitum to all experimental mice. On day 1, each resident mouse received an intruder mouse and the two animals were allowed to interact freely for 10 mins. In order to prevent injuries, the social interaction was interrupted if fighting escalated (when the dominant persistently bit the opponent). After the interaction, the two animals were separated by means of a perforated polystyrene- metal partition, which allowed continuous sensory contact but no physical interaction. The partition bisected the cages diagonally in two symmetrical compartments. The partition was removed daily (for a total of 21 days) in an unpredictable moment between 0900 and 1200 hours.

During the social interaction offensive and defensive behaviors of the animals were recorded and their social status was determined as follows: the chasing and biting animal was defined as 'Dominant', while the mouse displaying upright posture flight behavior and squeaking vocalization was the 'Subordinate'. Accordingly, four behavioral categories were distinguished within the stress group: (i) Resident Dominants (RD), (ii) Resident Subordinates (RS), (iii) Intruder Dominants (InD), (iv) Intruder Subordinates (InS). According to Bartolomucci et al. (2001) all dyads that were considered for subsequent analysis: i) developed a dominance/ subordinate relationship after the second day of interaction; ii) maintained a stable hierarchy over the stress phase.

Behavioral Analysis

Interactions of males during psychosocial stress model were scored by a trained observer by means of a series of chronometers. During the interaction, the numbers of attacks of subordinate with sildenafil (SS) and subordinate with saline (SF) was quantified and further analyzed as dependent measures. For the hope oh

this work to compare the amount of aggression between SS and SF, the mean of frequency of attacks for every week were computed.

Sociosexual Behavior

On Day 43 all male were used to evaluated the behavioural effects of this social stress procedure on dominant sociosexual interactions. The test was conducted between 1500 and 1700 h in a separate room where the males were exposed to an unfamiliar adult female in an unfamiliar transparent cage. Two cameras were placed immediately above and below this cage, to allow filming and identify all the stages of sexual behavior. The observations of sexual behavior were carried out for 30 min.

The home cages were moved to the strees room, 12 min before starting the sexual behavioural test .

The data collection method used was continous sampling for a 30-min period: (Altman, 1974). Behavioural data were collected according to van Oortmerssen's (1971) ethogram. Data analysis was based on the following behavioural categories for all males: (1) exploration (EXP): sniffing the cage, sniffing with the nose up in the air, rearing, attention posture, and digging. (2) grooming, divided in self grooming (SG): wiping, licking, nibbling its own fur and scratching movements and allo grooming (AG), when all these behavior was made to female. (3) affiliation: sniffing the female's body (SB), sniffing the females's muzzle (SM); plus (4) sexual: anogenital sniffing (SAG), mounts and attempts to mount, genital self-grooming, intromission, ejaculation. (5) attack.

Each male of the pair participated in the experiment only once.

For every behavior we take latency, total duration, and the number of times it is done. Tapes were scored by a trained observer using a specific software (The Observer, Noldus, The Netherland).

Again, the oestrous condition of the females was ascertained and only the females in oestrous or proestrous conditions were used.

Statistical Analysis

In the comparison with the 3 categories dominant (Dom), subordinate (SF), subordinate treated with sildenafil (SS), Control subjects (CF) and Control subject trated with sildenafil (CS) we used a ANOVA followed by duncan post hoc test.

Body weight was analyzed by means of 2-way ANOVA (between factor: treatment; within factor: weeks). Duncan's test was used for post hoc analysis when a significant F was obtained; otherwise, Tukey's HSD test, which may be used also in case of nonsignificant F (Wilcox, 1987), was used.

median of Frequency of the attacks displayed by SS and SF mice were compared by means of Mann-Withney U test conducted on the median of each week this because the variance in days 1–7 was null, thus making impossible to be analyzed with ANOVA.

Data were analyzed by means of Statistical 6.0 (Stat-Soft, Tulsa, OK, USA).

Results

Sociosexual Behavior

In figure 1 The latency to mount is significantly higher in SF respect SS ($F_{3,38}$=3.64; $p<0.05$; Post Hoc $p<0.05$), CF (Post Hoc $p<0.05$) and CS (Post Hoc $p<0.05$). But there's no differences between SS, CS and CF, this means that sildenafil normalized the effect of subordination, lowering the latency to mount to level of control subjects.

Body Weight

An interesting differences emerged when considering the body weight of the two categories of subordinate animals SS and SF under stress (treat x time: $F_{5,115}$=2,44; $p<0.05$). In detail, SS show a significant increased body weight at he last week ($p<0.05$) respect SF as see in figure 3.

On the other hand, SS mice showed a reduction of delta body weight when compared to SF mice after 4 weeks of stress phase. No differences were observed between CF and CS.

Behavior During the Daily Fight

As evident in Figure 4, till the day 7 all subordinate animals show no attack behavior. But at the start of sildenafil treatment (day 7) the SS show an increase in numbers of subject that counterattack stably during the weeks, so that, at 4^{th} week of sildenafil treatment, we noted, a significant differences in numbers of attacks ($U_{13,11}$=33; $p<0.05$) and in numbers of subject that counterattack (Chi^2=6.77; Fisher 2t $p<0.05$). No differences in frequency of attacking behavior were observed between the dominant males that live with SS or SF males. All these data could indicate that the SS males, and than sildenafil treatment, is effecting on aggressive behavior.

Conclusion

In Latency to mount SF are higher respect the others group. After 4 week of treatment the frequency of attacks, in subject treated with sildenafil, show an increase in number of attacks and numbers of subject that counterattack.

In this connection, also body weight, shows after 4 week of treatment that the weight of subordinate subject treated with Sildenafil (SS) is significantly lower than the counterpart (SF).

These results show that after the administration of Sildenafil, the subjects began to express a number of counterattacks. So much so that after 4 weeks, the difference between the number of attacks in males treated (SS), has become significantly higher, respect the number of attacks in respective subordinate ones treated with saline (SF).

This finding is important if we consider that this model of chronic psychosocial stress, once an animal becomes submissive, very rarely is prone to attack again.

In this case, almost 50% of animals (6 / 13) came to fight back permanently.

A similar increase in the levels of aggression in fact was also observed in other models of aggression who used sildenafil. These models have revealed how the administration of sildenafil may increase the number of attacks (Nelson et al 1995). The hypothesis, that may explain this effect, concerned the induced accumulation of cGMP by sildenafil, and subsequent negative feedback of cGMP on NO production (Hotchksiss 2004; Nelson 2004). In fact increased concentrations of cGMP might, over time, feedback on nNOS to reduce the production of NO (Canteros, 1996; Jeremy 1997, Moreland, 1998; Marmor, 1999). In recent studies, inhibition of NOS enzyme elicited antidepressant-like behavioural effects in several animal experiments (Harkin et al., 1999; Jefferys and Funder, 1996; Da Silva et al., 2000; Yildiz et al., 2000a,b) suggesting that NO plays an important role in these behavioural responses (Harkin et al., 1999; Jefferys and Funder, 1996; Yildiz et al., 2000a,b).

For this reason, the effect of sildenafil on aggression, during administration of the drug, would be directly dependent on its effect on cGMP levels. But we may specified that the direct action of sildenafil on this pathway is at the level of the PDE-5 enzyme, which prevents the breakdown of cGMP, not cGMP production per se (Hotchksiss 2004).

Other study in vivo show that the elevation of cGMP was observed to be positively correlated with increased aggression in mouse, dogs and humans (Angel, 1976, Brown, 1981; Kantak, 1984). The differences between this and others works, is that we observe the increase of aggression, and the modification of sexual behavior, that suggest us, that probably this drug may increase the agonistic motivational system.

In this connection an other important result in this work is that sildenafil, act also on sexual behavior, in specific lowering the latency to mount. The latency to mount, is the amount of time that has passed, since the beginning of each sexual experimental session, to the first mounting behavior. In this case only SF subject shows higher latency in this behavior, suggesting a presence of an alteration in sexual seeking behavior only in subordinate animals. Regard to this other study shows impairment sexual performance in subordinate mice (D'Amato 2001), or different hormonal, physiological and behavioural profiles in subordinate respect to dominant or control ones But in this model we find also that SS show a latency to mount lower respect SF, but not different respect CS or CF. This tell us that

sildenafil do not modify the sexual behavior per se, (in fact in control subject we don't observe any differences), but modify the latency to mount only in subject exposed by social defeat, and then only in subject that had loss their rank. Previous works in literature, show a correlation between the indirect inhibition of NO production through sildenafil, and the increase of sexual behavior in rats, confirming the sexual stimulant activity of sildenafil (1996; Melis et al., 1996; Du and Hull, 1999, Ottani 2002). or an increasing of persisting mounting behavior in study using male nNOS $^{-/-}$ mice (Nelson 1995). But here we see that the sildenafil act on agonistic system, normalizing the submission-effect of subordinate males. In fact only subordinate males SF show a longer latency of mount respect SS, CF and CS.

The last result of this work concern the effect of sildenafil on the weight loss of subordinates. To explain this data we lack the measure of food intake, or locomotory activity. Respect to this, a recent study that use this psychosocial stress model (Bartolomucci et al., 2004, 2005), has observed that, in male mice, both social status and territory ownership are not factors associated with food intake, but they are linked to individual vulnerability to chronic psychosocial stress-induced effects on body weight and fat deposition. This suggest that, in male mice, the loss of resources and social status, actually interact to drive the effects of stress toward dysmetabolism. In this study the SS, show a lower decrease in body weight respect SF, and the fact that only SS show this effect, suggest that the lowering is linked to agonistic behaviors. In fact the same effect is not present in CF and CS subject.

These results suggest that sildenafil does not directly lower the weight of the animals, but act on the motivational systems involved in the modulation of competitive behavior, social status determination and territory ownership, that has been demonstrate to be linked to weigh gain.

In conclusion we can say that sildenafil, changes the agonistic behavior, and some behavioral and physiological aspects (weight loss) in connection with the status of dominance. At this point, it might be interesting to investigate the effects of this drug, not in a context of chronic stress, but in a context of middle stress: that makes it possible to observe not only the number of attacks, but also the possible reversion of dominance, something that this model did not permit.

In fact, the key feature of this model is to ensure through a cohabitation and a sensory contact, a constant psychosocial stress, and thus, after a few days of cohabitation, to maintain the dominance hierarchy gained. This may explain also the lack of other, more significative evidence, in aggression and dominance. Perhaps a model of middle stress, could lead to study the effect of modulation of sildenafil on competitive behavior, so as to include the possible reversion of dominance. Therefore, the effect this could have, consequently on the weight and on the others variables related behavior.

Chapter 6

Biophysiological Response to Chronic Sildenafil Treatment During Siblings Cohabitation

Introduction

The results of experiment presented in the previous chapter, demonstrates that sildenafil may enhance the inhibition of aggressive behaviour, body weight gain and sexual disturbances in subordinate mice under chronic psychosocial stress..

However, chronic psychosocial stress (Bartolomucci et al., 2005) represent an extreme social challenge for male mice with high face and construct validity as a model for human depression (Bartolomucci 2005), thus not being representative for a non clinical population. Furthermore we previously demonstrated that housing in groups of siblings represent the ideal housing for male mice (Bartolomucci et al., 2001, 2003). In sibling group aggression is present but never escalate and social status rarely have a major impact on mice behaviour and physiology. This model may represent a model for a normal, non clinically depressed population in which a certain degree of socio-sexual disorders may manifest.

Accordingly, the aim of this study was to chronically inject sildenafil in dyads of sibling male mice and investigate aggression, socio sexual behaviour and general metabolic parameters.

Materials and Method

Animals

Subjects were 72 adult males Swiss CD-1 mice from an outbreed stock originally obtained from Charles River Italia (Calco, Italy). Mice were born and reared in a colony room at the University of Parma at 20 2_C in a 12-hr light–dark cycle (lights on 0700–1900). After weaning (25–28 days of age) they were housed in same-sex- groups of siblings (4–7 per cage) in Plexiglas cages (45 _ 25 _ 20 cm) with wood shaving bedding. The cages were changed weekly. All animal experimentation was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/EEC) and approved by the Italian Institute of Health.

Procedure

When tree months old, males were housed in pairs. Food and water were always present ad libidum

On day zero and seven days afterwards rank differences (i.e. dominant or subordinate status) were determined by direct observation for 30 minutes after the normal cleaning procedure consisting of weekly changing the home cage . Body weight was determined as well.

After this baseline phase, 18 subordinate mice were randomly selected as experimental animals, while 18 were used as control animals.

From day 8 to day 42, we injected each subordinate with sildenafil 10 mg/kg dissolved in saline (see below) or saline only thrice weekly for 5 weeks (then n=12–15 dose for animal at all) (Hotchkiss, 2005).

Dominants were also treated with saline only.

Preparation of Dosing Solution

Dosing solutions were prepared following methods similar to those previously reported (Behn 2001). Briefly, sildenafil citrate (50 mg) tablets were ground into a

fine powder using a morter and pestle. The resulting powder was then mixed with saline and passed through 40 Am filter paper. The resulting solution was kept chilled at 4°C. Dosing solutions were brought to room temperature 2h (i.e. 900 h) prior to injections.

Behavioral Analysis

Interactions of males during cohabitation were scored by a trained observer by means of a series of chronometers, for 30 minutes. During this observation session, the numbers of attacks of subordinate with sildenafil (SS), subordinate with saline (SF) and dominant with saline (DF) was quantified and further analyzed as dependent measures. For the hope oh this work, to compare the amount of aggression between SS and SF, the mean of frequency of attacks for every observation session (i.e. a time for week during the change of litter) were computed.

Sociosexual Behavior

On Day 43 all male were used to evaluated the behavioural effects of this social stress procedure on dominant sociosexual interactions. The test was conducted between 1500 and 1700 h in a separate room where the males were exposed to an unfamiliar adult female in an unfamiliar transparent cage (misure). Two cameras were placed immediately above and below this cage, to allow filming and identify all the stages of sexual behavior. The observations of sexual behavior were carried out for 30 min.

The home cages were moved to the strees room, 12 min before starting the sexual behavioural test .

The data collection method used was continous sampling for a 30-min period: (Altman, 1974). Behavioural data were collected according to van Oortmerssen's (1971) ethogram. Data analysis was based on the following behavioural categories for all males: (1) exploration (EXP): sniffing the cage, sniffing with the nose up in the air, rearing, attention posture, and digging. (2) grooming, divided in self

grooming (SG): wiping, licking, nibbling its own fur and scratching movements and allo grooming (AG), when all these behavior was made to female. (3) affiliation: sniffing the female's body (SB), sniffing the females's muzzle (SM); plus (4) sexual: anogenital sniffing (SAG), mounts and attempts to mount, genital self-grooming, intromission, ejaculation. (5) attack.

Each male of the pair participated in the experiment only once.

For every behavior we take latency, total duration, and the number of times it is done. Tapes were scored by a trained observer using a specific software (The Observer, Noldus, The Netherland).

Again, the oestrous condition of the females was ascertained and only the females in oestrous or proestrous conditions were used.

Statistical Analysis

In the comparison with the 3 categories dominant (DF), subordinate (SF), subordinate treated with sildenafil (SS), we used a ANOVA followed by Duncan's post hoc test.

Body weight was analyzed by means of 2-way ANOVA (between factor: treatment; within factor: weeks). Duncan's test was used for post hoc analysis when a significant F was obtained; otherwise, Tukey's HSD test, which may be used also in case of nonsignificant F (Wilcox, 1987), was used.

median of Frequency of the attacks displayed by SS and SF mice were compared by means of Mann-Withney U test conducted on the median of each week this because the variance in days 1–7 was null, thus making impossible to be analyzed with ANOVA.

Data were analyzed by means of Statistical 6.0 (Stat-Soft, Tulsa, OK, USA).

Results

Sociosexual behaviour

As was for the previous experiment the only parameter affected by social status was the latency to mount the female. The latency to mount was significantly higher in SF respect to SS ($F_{2,60}$=3.24; p<0.05; Post Hoc p<0.05) and DF (Post Hoc p<0.05). Accordingly sildenafil normalize the behavioural difference between dominant and subordinates. The detailed behavioural analysis did not showed others sexual abnormalities nor any other effect of sildenafil It is important to clarify that only a 10% of males for each category completed intromission and ejaculation.

Body weight

An interesting differences within emerged when considering the body weight, in fact all animals, weight decrease with time (time: $F_{6,204}$=10,039; p<0.000001) within time. But if we consider the weight of the animals in detail, SS show a significant decrese in body weight after two (Post Hoc; P<0.05) and four (Post Hoc; P<0.05) weeks, as we see in figure 2. On the other hand, SS mice showed a reduction of delta body weight when compared to SF after few days.

Behavior during the daily fight

As evident in Figure 3, at the start of sildenafil injection the numbers of attacks increase dramatically in SS respect SF, during treatment and this effect is significative especially after two (treatment x time $F_{8,272}$=1,93; post hoc p<0.05) and five weeks (treatment x time $F_{8,272}$=1,93; post hoc p<0.05). moreover we note also a tendency respectively after three (Post Hoc=0.0632) and four weeks (Post Hoc p=0.0642). Respect to this we show that DF and SS animals don't show any differences in numbers of attacks. This is an important finding that tell us as the

rank of SS animals changed after the administration of the drug. Regarding to this, we see that the numbers of animals that counterattack stabily in SS group reached the sum of 8/18 respect SF group, were 4 animals counterattack sporadically and only one counterattack with a such staility (Chi^2=10.60; Fisher 2t $p<0.005$). All these data could indicate that the SS males, and than sildenafil treatment, is effecting on aggressive behavior.

Discussion

The results of this study confirm the findings of previous one.

The number of attacks that we see in subjects taking sildenafil, are extremely high compared to the attacks of control subjects. Furthermore, there are more subjects who counterattack among those taking the drug than among those who do not.

In relation to this, it's important to underline that the effect observed in this study, is due in predominantly way to two factors: the model used, it is not a true stress model, but it's a simple cohabitation between siblings, which is acknowledged to be the lesser psychosocial stress situation for mus musculus.

The second factor is the number of attacks. In this case we have observed, for a period of about thirty minutes after the change of sawdust, and not for 10 minutes as in the classic model of psychosocial stress. The situation of cohabitation between siblings, it is well understood to be a situation with a minimum social stress. Also the definition of dominance, it is not so clear and precise, like we usually observed in the model of chronic psychosocial stress, because usually the counterattacks are not so rare. Then enter these characteristics, this situation is more sensitive to the possibility, if sildenafil act on aggressive behavior, look at how best when this happens and when. In this regard, we see that sildenafil, after the first week of treatment, beginning to have an important effect on the number of attacks. This is a confirmation in using this model. In fact the previous work, showed an almost immediate increase in the number of attacks, but this number reached significance only after 4 weeks. This suggest that sildenafil produces effects on the behavior after a few consecutive treatments, and that the effect depends on the general level of activation, ie stress. in this case, we may see how

sildenafil showing an important effect when the stress level is low, and a less marked when the stress level is high.

With regard to sexual behavior we observed the same effect look at previous work. In this case, we see that the latency to mount, in the subject who use sildenafil, is significantly lower respect the subject with saline.

Interestingly, the conservatism of this result because, in all behaviors observed, only the latency of mounting is shown to be sensitive to the drug, In fact the others sexual behavior examinees were not significative. The reason may due to the fact that the males were sexually naïve. [[[But the decision to use naïve males is made in relation to reason that naïve males hadn't experience in sexual behavior, and then, if the drug affect really on sexual behavior, we may affirm more convincingly that the drug involve CNS.]]]

In fact for each category only 60% of males completed a regular mounting behavior. And only a 10% of males for each category completed intromission and ejaculation. Again this confirms the results seen in previous studies, suggested that action on the appetizing behavior therefore its involvement in CNS.

The effect of sildenafil, on weight gain, in this case is very similar to that seen previously. The important difference, relates to the trend of growth, that in this case is negative, both for the DF, than for SS. In SF, however, the situation changes, showing how the weight appears to be highest respect SS already after two weeks. This additional data, says that sildenafil clearly exerts an effect on weight, but this effect is not so high to lower the weight steadily.

Finally we conclude that sildenafil may positively change not only competitive behavior, but also with aspecific sexual behavior (latency to omount) and physiological variable (weight loss) and in connection to this how that these three diverse variable are correlated with rank.

In this regard have been made many assumptions about how sildenafil can achieve a similar effect. In that regard, as we know it is acting indirectly as inhibitor of NO, and this action is achieved through the direct PDE-5 inhibition and followed by the subsequent accumulation of cGMP. Alternatively, accumulation of cGMP has the potential to negatively feedback on upstream mechanisms involved in nitric oxide (NO) production. Recent evidence have shown that the reduction of NO levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal NO in the neurobiology of stress and

depression (Joca and Guimaraes, 2006). In fact nitric oxide (NO) plays a significant neuromodulatory role in the CNS. Any pharmacological manipulation of NO pathway may be considered as a novel therapeutic approach for the management of CNS disorders, more so for mental depression (Heiberg et al., 2002).

Chapter 7

General Conclusions

On the basis of sexual selection theory (Darwin, 1871) that states that certain evolutionary traits (Morphological, physiological and behavioural traits) can be explained by intraspecific competition (i.e. intrasexual as direct competition between members of the same sex – usually the male-and intersexual selection as the result of mate choice) I present in this thesis an attempt to re-evaluate in evolutionary perspective the ontogenesis of certain type of psychopathology that impact the individual such as sexual dysfunction and/or mood disorder. This has been done by means of a) human models derived from clinical observation on psychologically based sexual dysfunction and sport agonistic competition and b) animal model based on chronic social stress concerning the acquisition and/or loss of social status and the expression of sexual behaviour.

Following the evolutionary hypothesis that one of the major "trigger" of depression is loss of resource (Clark and Beck, 1989) or social defeat (Price, 1972; Gilbert, 1992), in the first clinical study involving andrological patients with sexual disorders (i.e. erectile dysfunction -ED) we have observed that there is a clear cut relationships between the loss of resource expressed as recent loss of job (i.e. loss of social status and self esteeme) and the severity of mood lowering and ED. In fact the lowering of mood, the erectile dysfunction and sign of submissive and avoidance behaviors (i.e. displacement behaviours) are significantly elevated in these patients compared to the other that have not lost the job. Regarding to this we may underline how the patients that lost their rank, showed a higher displacement behavior (i.e., self-directed behaviors such as self-touching, scratching, and self-grooming). These behavior is a reliable ethological indicator of increased emotional and physiological arousal (Troisi 2000) during a face to face interaction. Subsequently, the loss of rank may have changed the perception of the social status that would be implicated in the activation of involuntary subordination (Allan and Gilbert 1997).

The subject perceive himself as inferior and then behave submissively. In relation to this is well understood that people may find their place in the social hierarchy by comparing themselves to others in terms of rank, social attractiveness and the degree to which one is an insider or outsider (Allan and Gilbert 1995). While basic social comparisons are also seen in other species (i.e. a perception of the self as stronger or weaker) and serve to prevent potentially dangerous conflict, in humans they are used rather extensively and incorporate more sophisticated judgements pertaining to social attractiveness (Allan and Gilbert's 1995). Moreover our results show an other important finding, that is the temporal kinetic about the appearance of symptoms. Only after three months of the loss of job event all subjects exhibited symptoms of ED, followed (five months later on average) by depressive symptoms, clearly suggest that this effect is not casual. In this perspective the ED may have a psychological cause and this symptoms precede the depressive state. This tell us that this phenomenon may be ascribed to the stress induced depression theory. In fact we known that the loss of job or job changes, financial problems, disruptions of old relationships are the more intense stressor, or triggers event in our species (Paykel 2003, Asberg 2006,2008). This events in fact may activate distress related behaviours like involuntary depressive states that may lead to real major depression (Gilbert 2001). But why before the depressive symptoms we observe ED?

The response is lead to the mechanism that linked rank and submission to sexual behavior. In fact all animal species that organize themselves in groups, relations of dominance are established and maintained through agonistic behavior. In fact the members of a social system are characterized by differences in social status (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993). Thus, subjects are constantly exposed to social relationships of dominance and subordination, since they result in marked differences regarding access to resources, like sexual partner. In fact, submission is linked to a low access to sexual resource. Accordingly, our explanation show us that the loss of rank in human, like the loos of work activate an involuntary submissive state, that also involves disorders of sexual behavior.

Other findings in the literature show that prior to manifest symptoms of emotional nature, some individuals have showed signs of ED. But in that regard, only a few studies show this kinetic time. for example a recent study shows that the

population of drug abusers manifest ED (La pera 2003). From the clinical point of view then, ED may be an important sign of an appetitive-consummatory dysregulation that, if persistent, might lead to major depression (Sloman 2003, 2006). In fact, many specific works tell us that the depression is often cited as another variable associated with this troubling in sexual dysfunction. (Buvat et al. 1990, Bozmann 1991, feldmann 1994, Araujo 1998, Shabsig 1998, Laumann, Paik, & Rosen, 1999, Melman & Gingell, 1999, Seidman & Roose, 2000, Goldestein 2004).

Chronic Psychosocial stress is a major factor in the aetiology of several psychopathologies, with individuals greatly differing in vulnerability. Nonetheless, a major goal of depression research from a biomedical or medical perspective is to develop pharmacological interventions suitable to control mood disturbance in individuals.

Regard to this some recent studies have localized phosfodiesterase (PDE)-5 in diverse areas of the brain (cit) and this allows the possibility to explore the pharmaceutical potential of different drugs acting on this enzyme, such as sildenafil (i.e. PDE-5 inhibitor). In particular it is of interest to explore how sildenafil may affect aggression and on mood. The results on our animal data shows that the chronic administration of sildenafil may increase the number of attacks and reducing the latency to mount at least in subordinate males under chronic psychososcial stress. . In connection with this "agonistic effect" another result show that sildenafil act on the weight loss of subordinates. These findings suggest that, in male mice, the loss of resources and social status, interact to drive the effects of stress toward dysmetabolism while sildenafil may counteract these effects.

Another hypothesis we tested in our clinical sample is that there is a personality tratit associated with job loss.

But Regard to this, we fail the target that is to reach a differences in temperamental and personality profile between losers and no losers, in ED patients; but this indicated that ED patients (losers and no losers togheter) probably has a specific temperamental vulnerability (higher HA and lower NS). Than we may say that; if is true that losing a job, and then the loss of social rank, is one of the most stressogenic event for human (Keller 1994, Mandal 2008, Friedman 2008, Amital 2008) that characterize some depressive states

(Pennebaker 2007, Morey 1997) but is already true that this may be supported by temperamental vulnerability.

In fact in literature, this trait is well defined, because high levels of HA was reached in many psychopatology from personality disorder (Pecorari, 2008), anxiety disorder (Calvo 2008) mood disorders (Farmer 2009, Trouillet, 2008) or as well as addiction (Evren, 2008) and was already linked in the work-related stress and the expression of depressive symptoms (Jurado, 2005). This underline that HA is related to risk-related behaviors, and the people that has a higher score (a score that ranges from 60 to 80) tend to react in fearfulness way against a wide range of treath (Pujol, 2002).

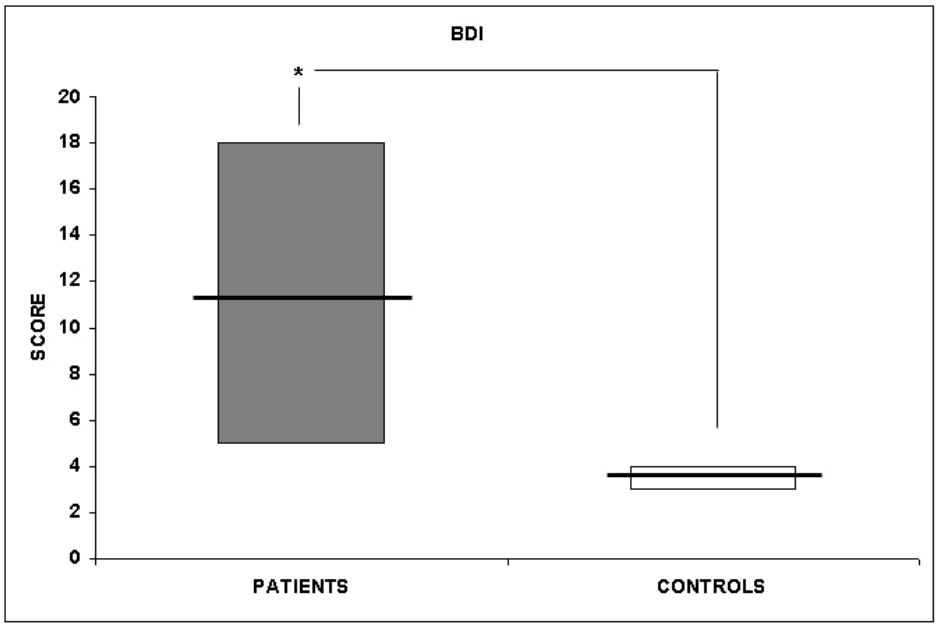
Then we known that personality or temperamental traits characterize sexual behavior, but we also known the relation between sexual behavior and agonistic behavior. Regard to this was interesting to study the connection between agonistic behavior and temperamental trait and we used a martial art model, that is well understood and used because is very similar to animal model on aggression for studying the effect of dominance and subordination.

And regard to this the study on aggression shows a similar result. In fact the agonistic behavior in a single confrontation, karate athlete showing that winners have higher levels of *novelty seeking* (NS) and lower levels of *harm avoidance* (HA) than do losers. These findings tend to support the idea that the subject that lose a confrontation are more anxious as well as more defensive has characteristized of defensive types of aggression. But more important result in this case is that C production is more elevated in losers just before the Contest, showing that an impending challenging situation is linked, not only to the context, but probably to a specific individual's coping strategies that predispose some individual to lose.

Figures and Tables

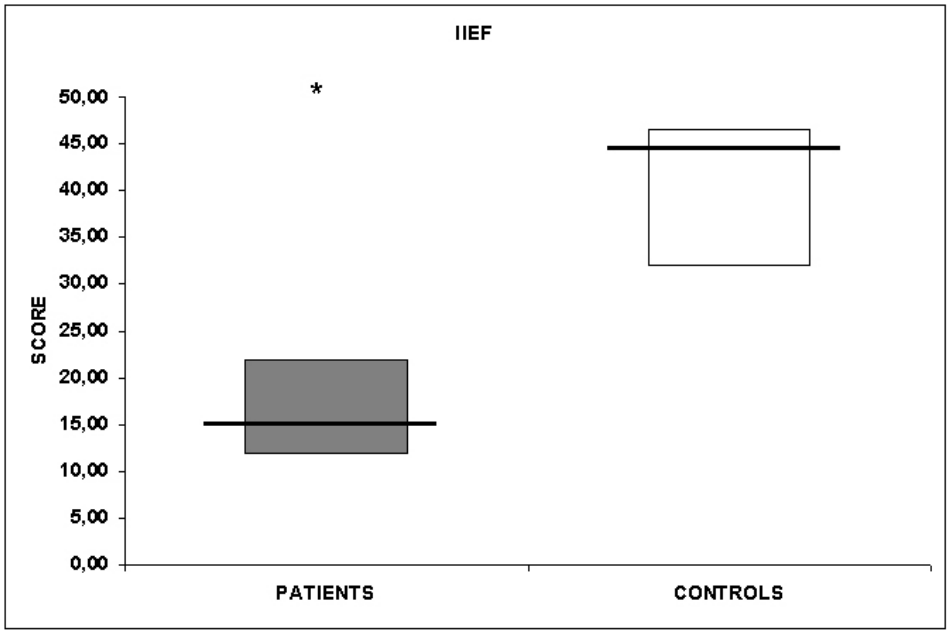
Chapter 1

Figure 1.



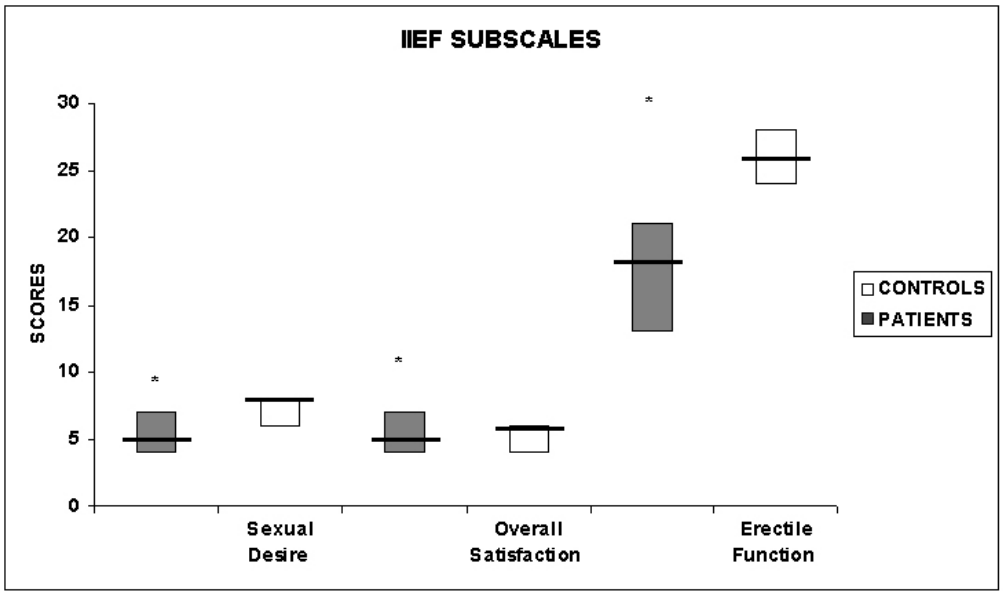
Data are express as the median ± 25-75% confidence level for significant traits: *significant difference (see text for statistical levels).

Figure 2.



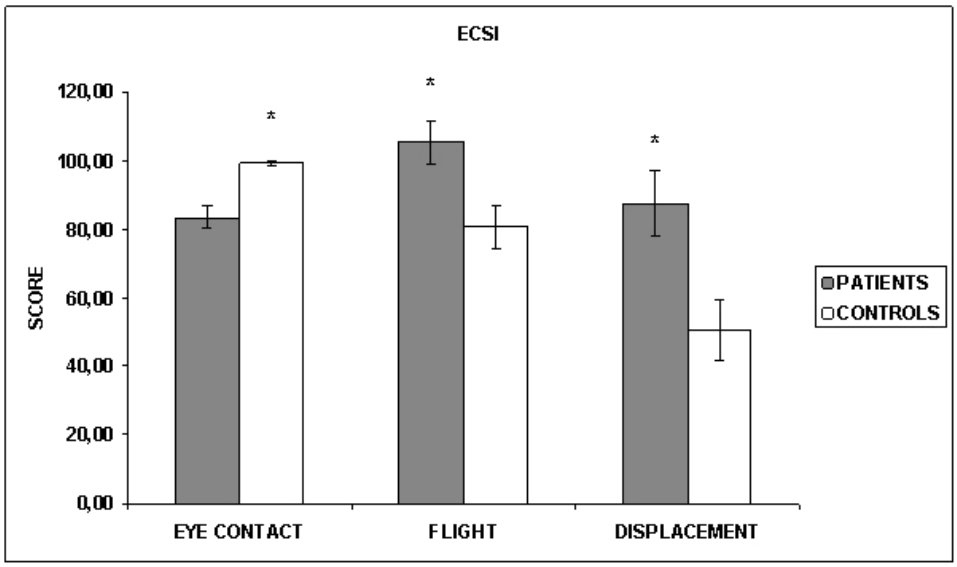
IIEF: Data are express as the median ± 25-75% confidence level for the significant traits:*significant difference (see text for statistical levels).

Figure 3.



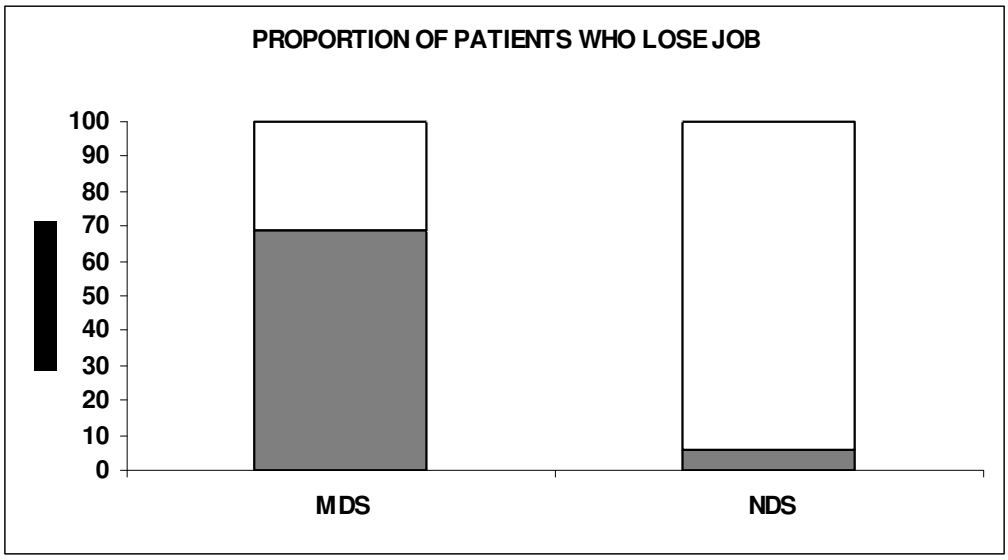
IIEF subscales: Data are express as the median ± 25-75% confidence level for the significant traits: *significant difference (see text for statistical levels).

Figure 4.



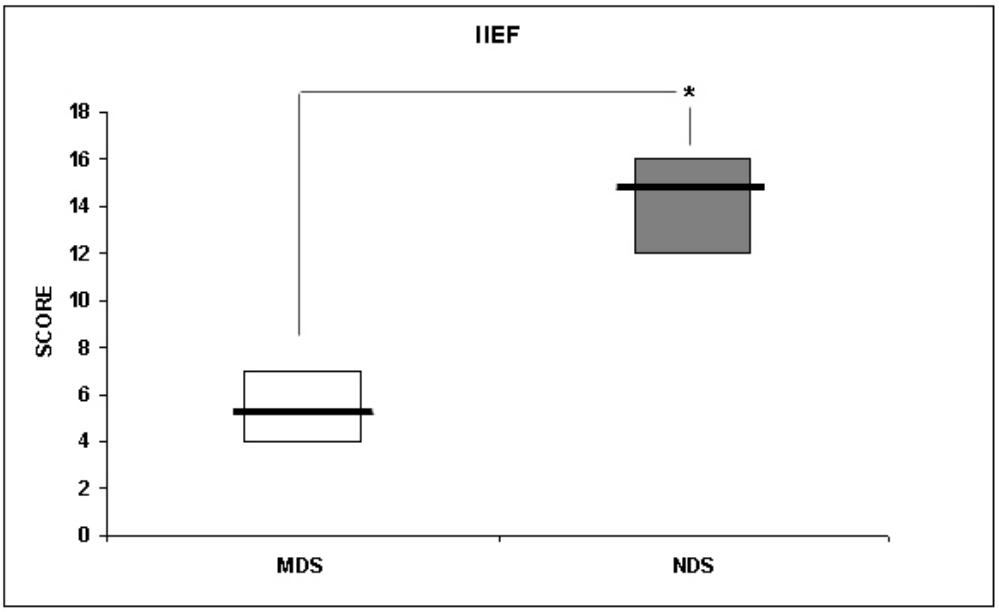
ECSI: Data are express as the mean ± SEM. See methods for the procedure to calculate the scores on the ethological categories. *significant difference (see text for statistical levels)

Figure 5.



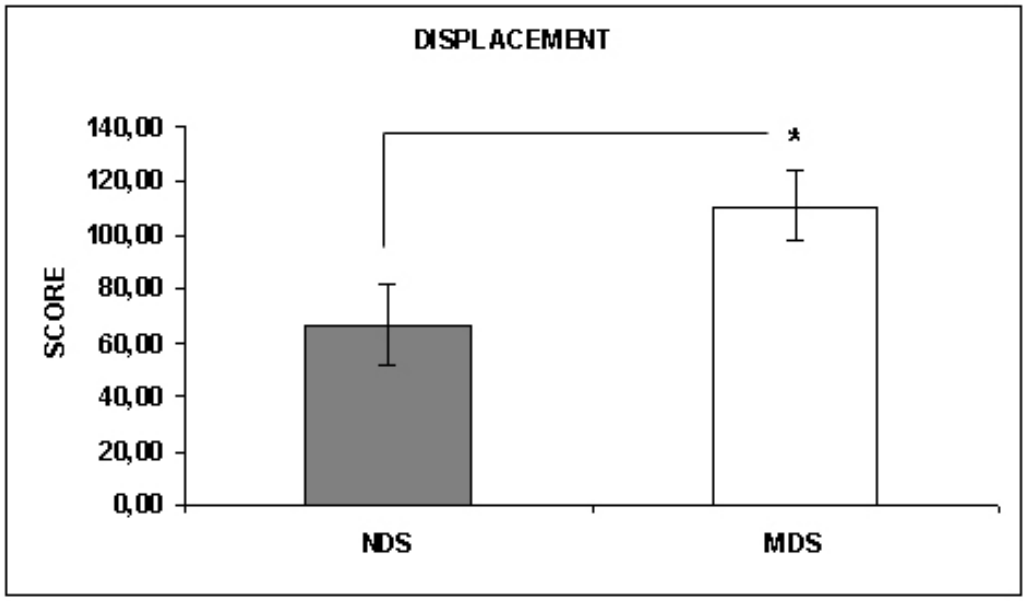
Proportions of patients who lose job. In grey the percentage of patients who lose the job in 12 month before, respectively in NDS and MDS group. see text for details.

Figure 6.



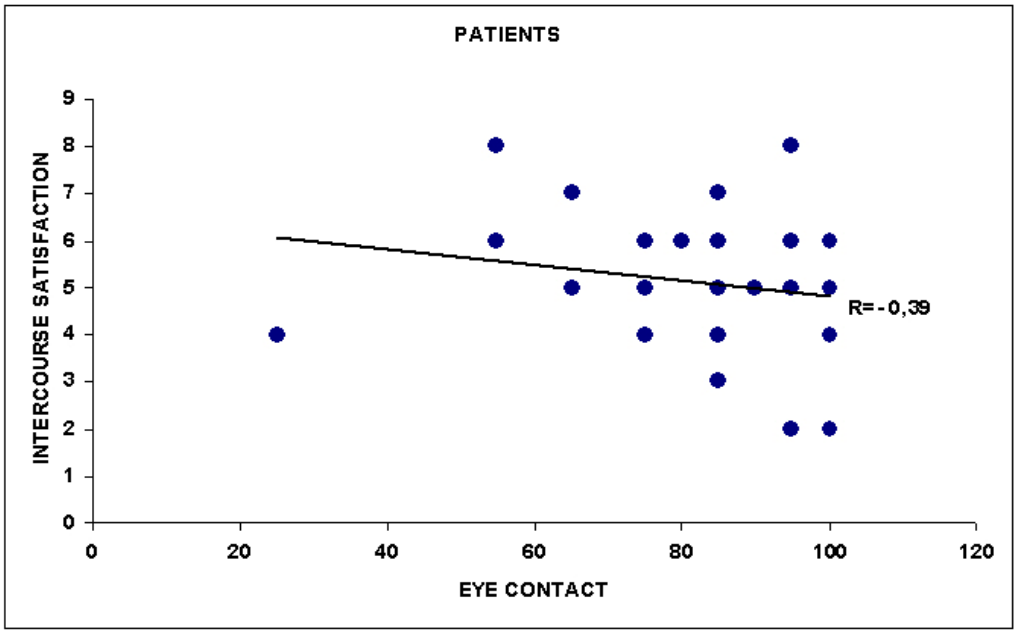
IIEF in MDS and NDS patients: Data are express as the median $\pm$ 25-75% confidence level for the significant. *significant difference, see text for statistical levels.

Figure 7.



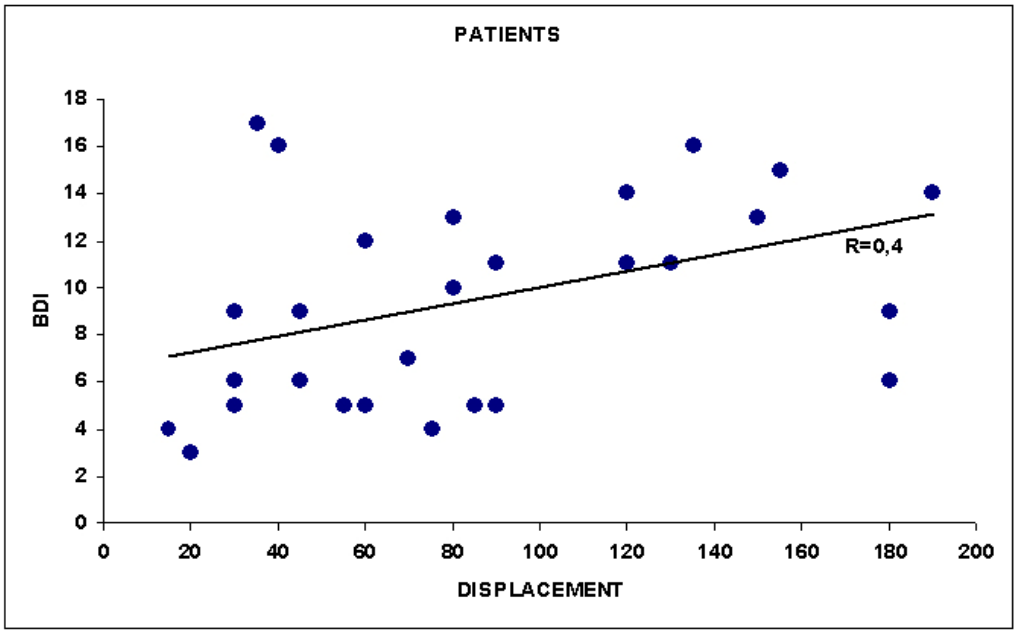
See methods for the procedure to calculate the scores on the ethological categories. Data are expressed as mean ± SEM*significant difference (see text for statistical levels).

Figure 8.



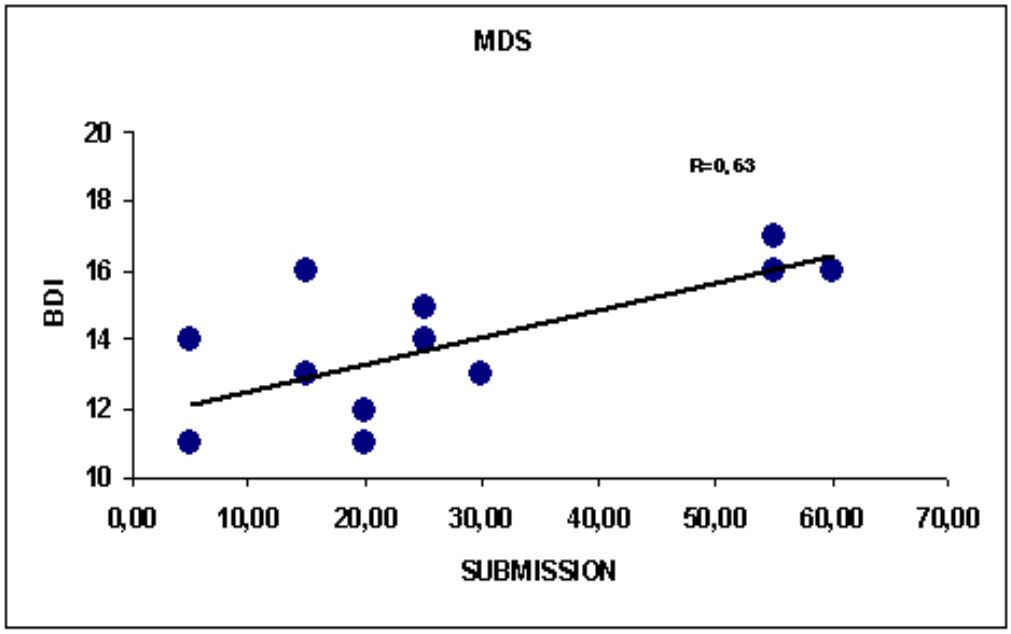
Correlations between IIEF sub-trait score and measure of Eye contact in patients group. see text for statistical levels

Figure 9.



Correlations between BDI total score and measure of Submission in patients group. see text for statistical levels

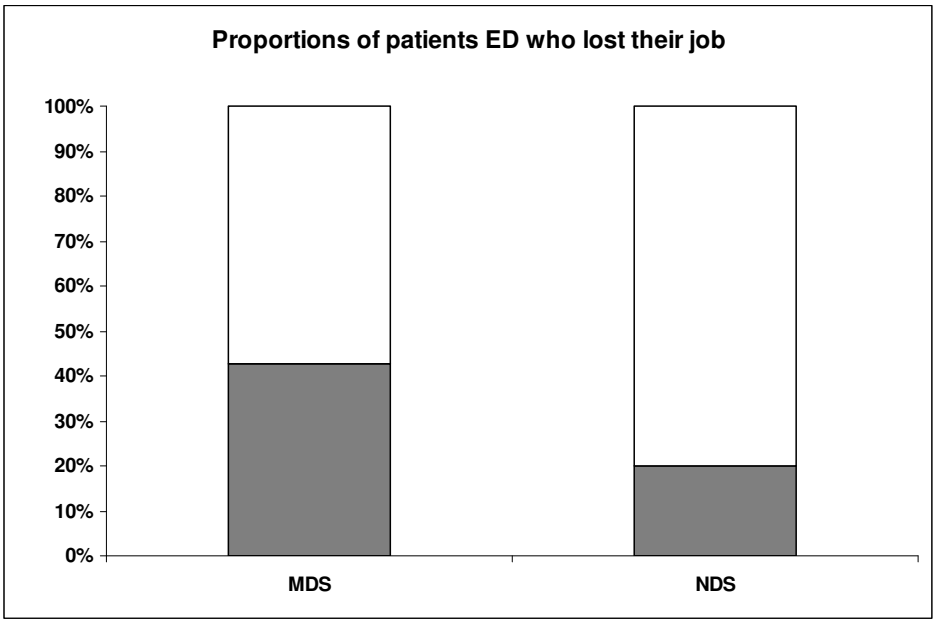
Figure 10.



Correlations between BDI total score and measure of Displacement in MDS patients group. see text for statistical levels.

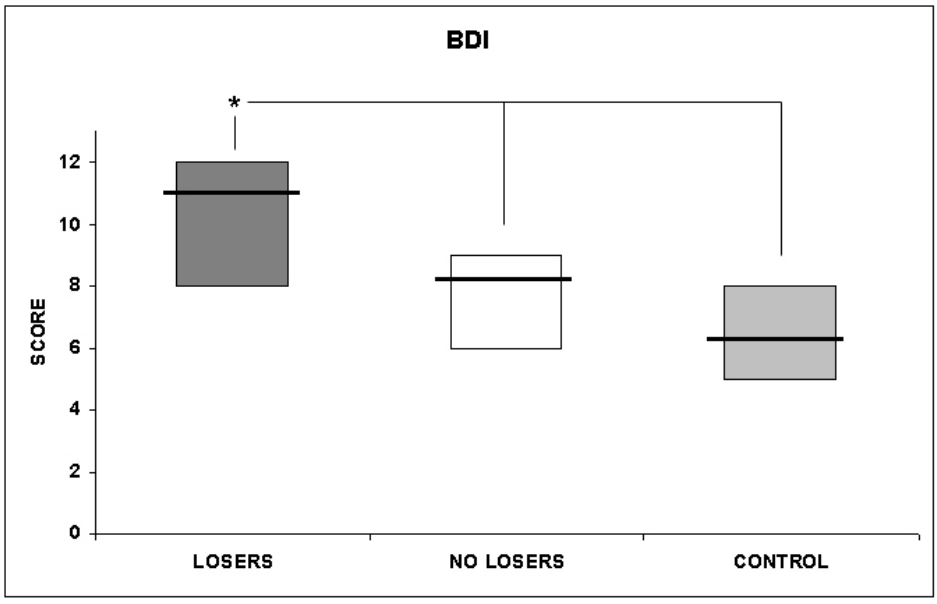
Chapter 2

Figure 1.



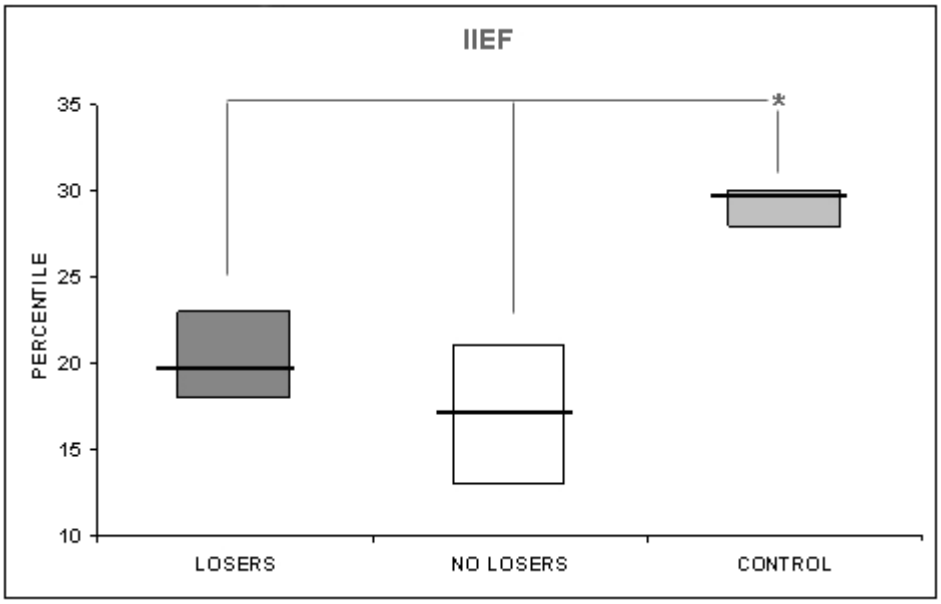
In grey the percentage of patients who lose the job in 12 month before, respectively in NDS and MDS group. *significant difference (see text for statistical levels).

Figure 2.



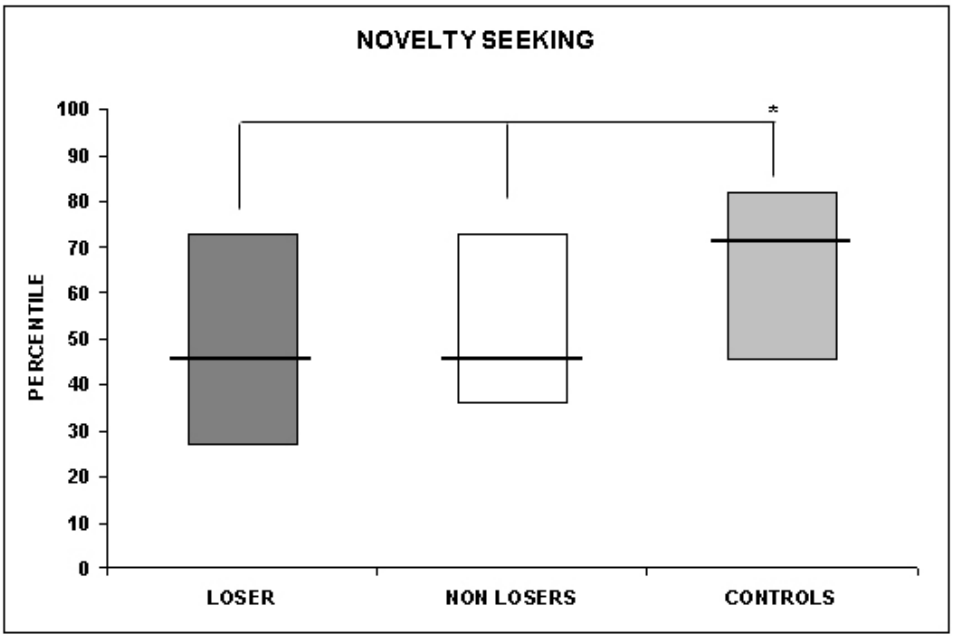
BDI Score. Data are express as the median $\pm$ 25-75%. *significant difference (see text for statistical levels).

Figure 3.



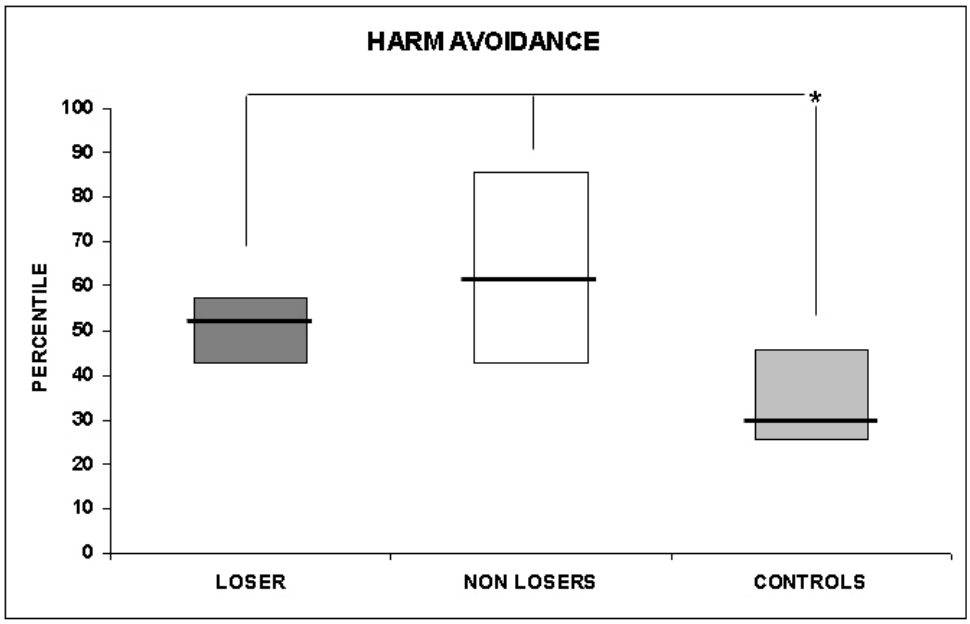
IIEF Score. Data are express as the median ± 25-75% confidence level for the significant traits.*significant difference (see text for statistical levels).

Figure 4.



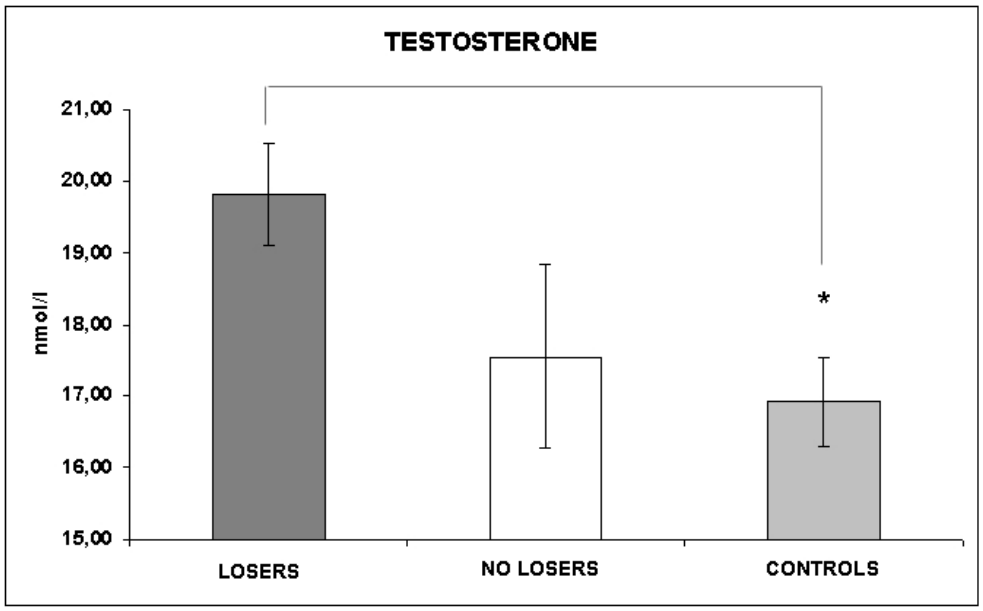
NS score. Data are express as the median ± 25-75% confidence level for the significant traits. *significant difference (see text for statistical levels).

Figure 5.



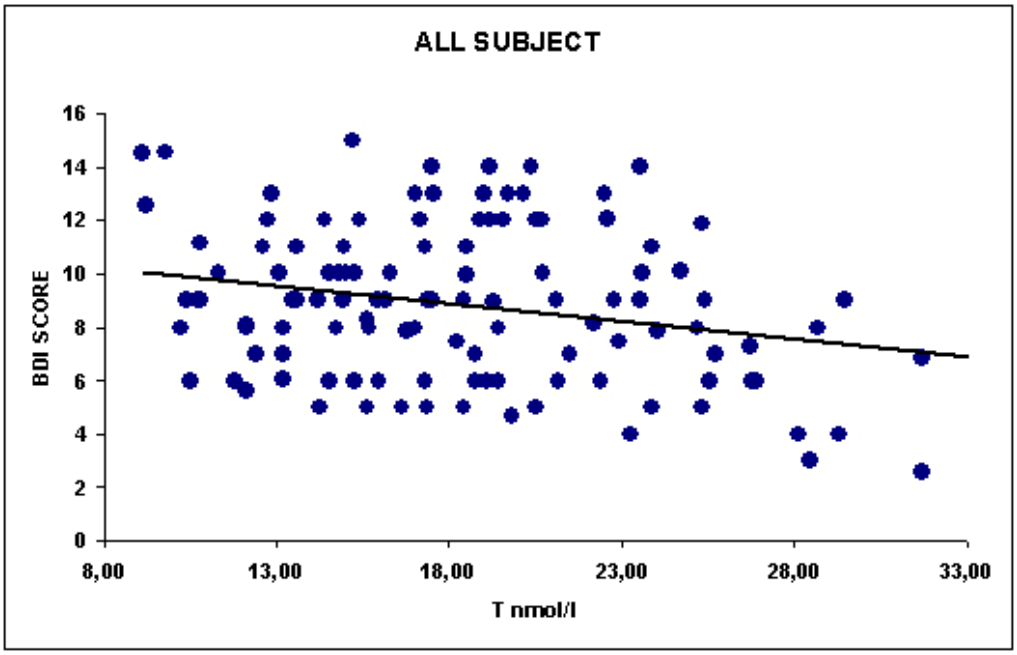
NS score. Data are express as the median ± 25-75% confidence level for the significant traits. *significant difference (see text for statistical levels).

Figure 6.



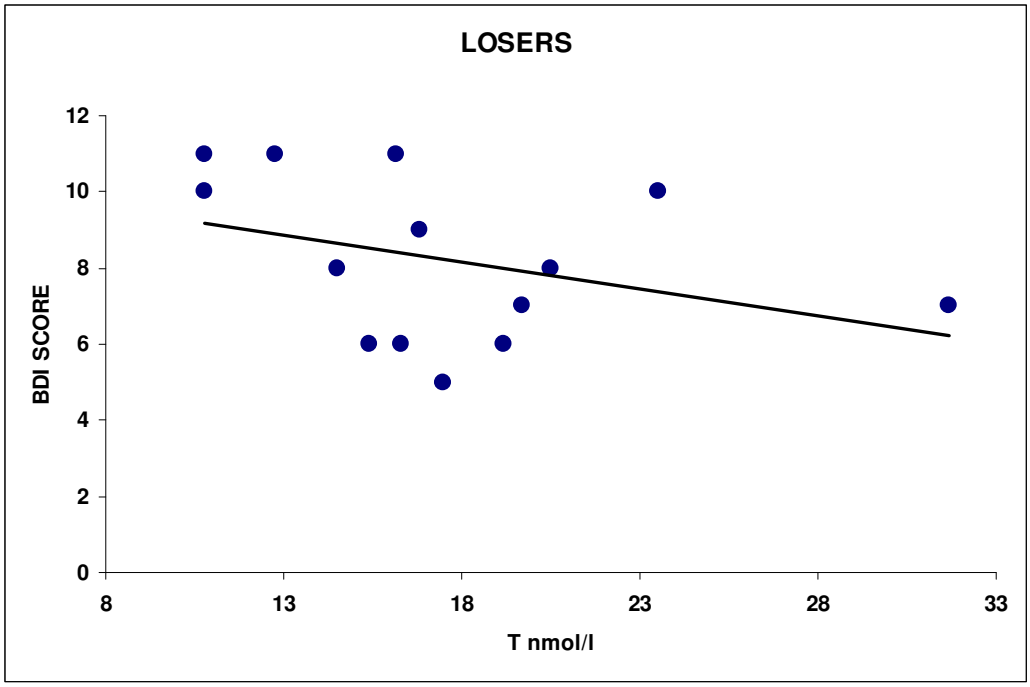
T levels. Data are expressed by mean ± SEM. *significant difference (see text for statistical levels).

Figure 7.



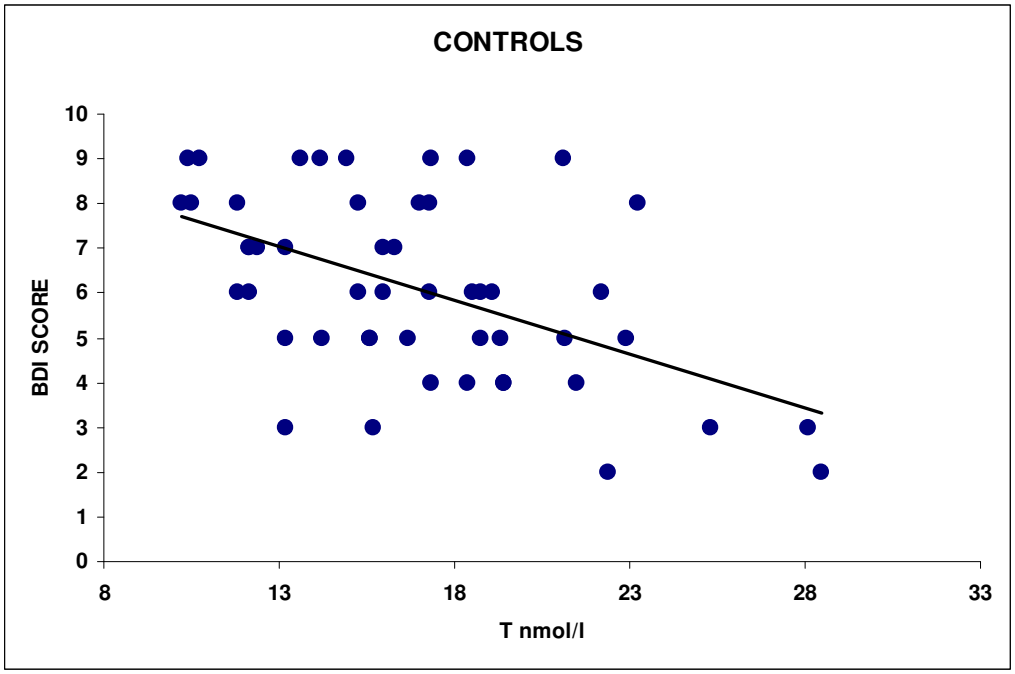
Correlation between T level and BDI score in all subjects.

Figure 8.



Correlation between T level and BDI score in Losers.

Figure 9.



Correlation between T level and BDI score in Control.

Chapter 3

Table 1

		Kumite	*Kata*
Testosterone ng/ml	Pre	3.95 (0.41)	3.90 (0.33)
	Post	4.46 (0.49)*#	4.03 (0.36)
	Delta T	13.47 (4.84)#	2.77 (2.13)
Cortisol ng/ml	Pre	108.36 (9.41)	103.40 (11.56)
	Post	162.80 (11.09)*#	115.00 (12.03)
	Delta C	73.36 (16.16)#	16.48 (5.26)

Mean hormones levels (± SE) in pre and post Kumite and Kata sessions.
*significantly different from pre-competition level.
#Significantly different from Kata (see text for statistical levels).

Table 2

		Winners	*Losers*
Testosterone ng/ml	Pre	4.15 (0.68)	3.75 (0.46)
	Post	4.76 (0.81)	4.16 (0.57)
	Delta T	15.93 (7.80)	11.02 (6.03)
Cortisol ng/ml	Pre	88.58 (11.43)	128.14 (12.98)#
	Post	141.23 (16.60)*	184.37 (15.49)#*
	Delta C	91.81 (28.19)	54.90 (15.33)

Mean hormones levels (± SE) of Winners and Losers in the Kumite session.
*Significantly different from pre-competition level
#Significantly different from winners (see text for statistical levels)

Figure 1

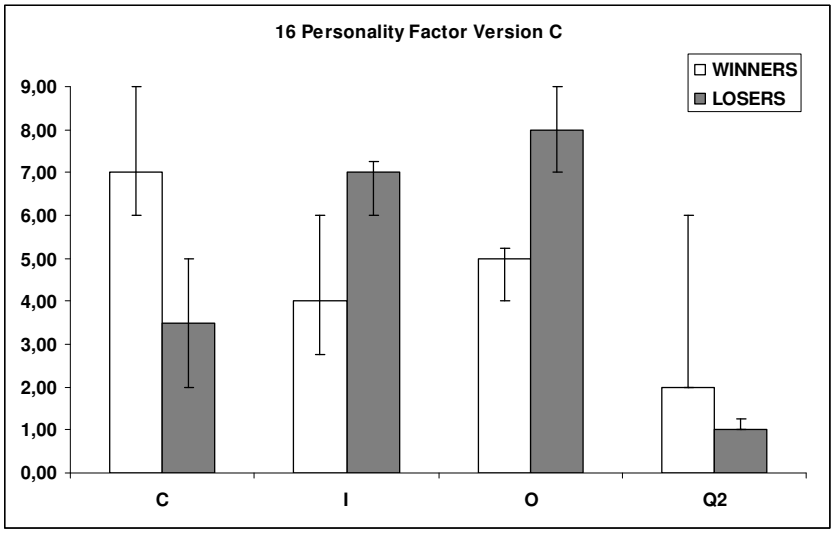


Figure 1. Winner and Loser scores in the 16 Personality Factor version C.

Data are express as the median ± 25-75% confidence level for significant traits.

Figure 2

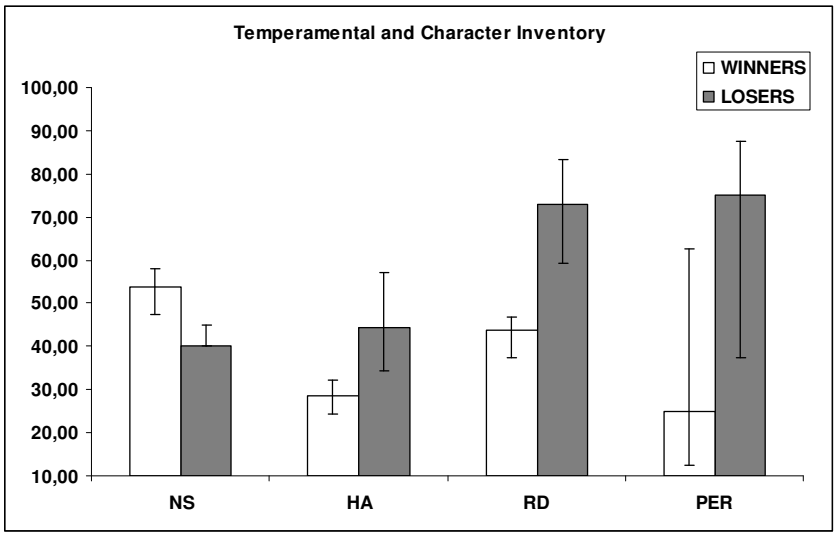


Figure 2. Harm Avoidance, Novelty Seeking, Reward Dependence and Persistence in winners and losers.

Data are express as the median ± 25-75% confidence level for significant traits.

Figure 3

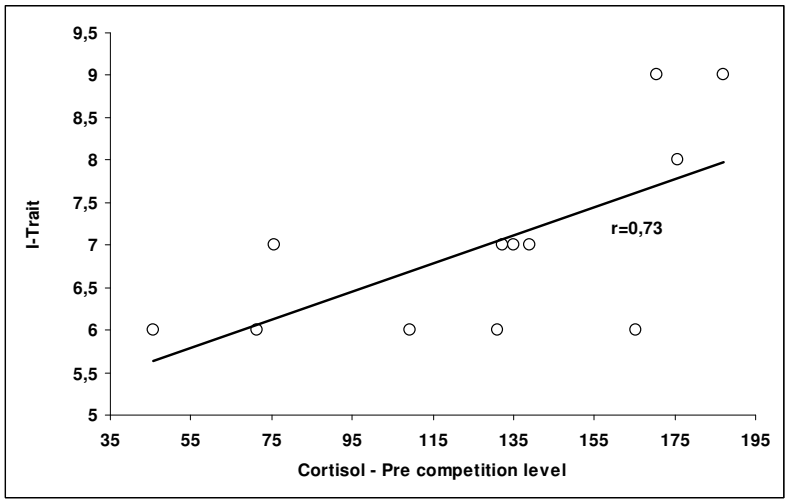


Figure 3. Correlations between pre-competition levels of Cortisol and I-trait in Losers.

Figure 4

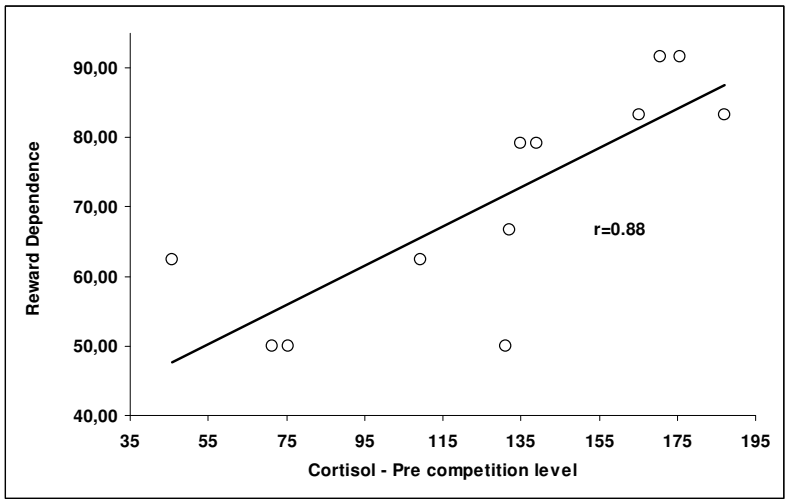
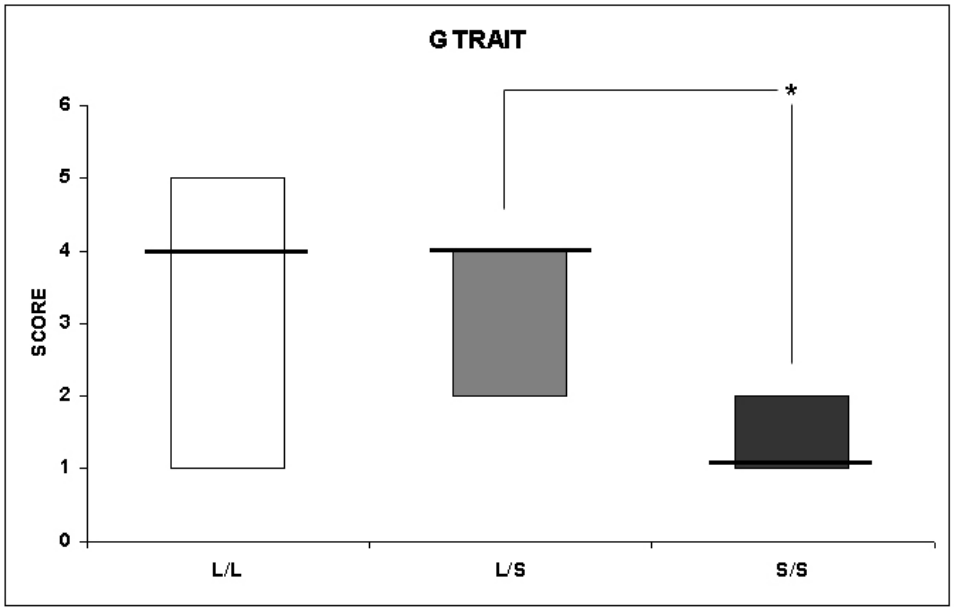


Figure 4. Correlations between pre-competition levels of Cortisol and Reward Dependence in Losers.

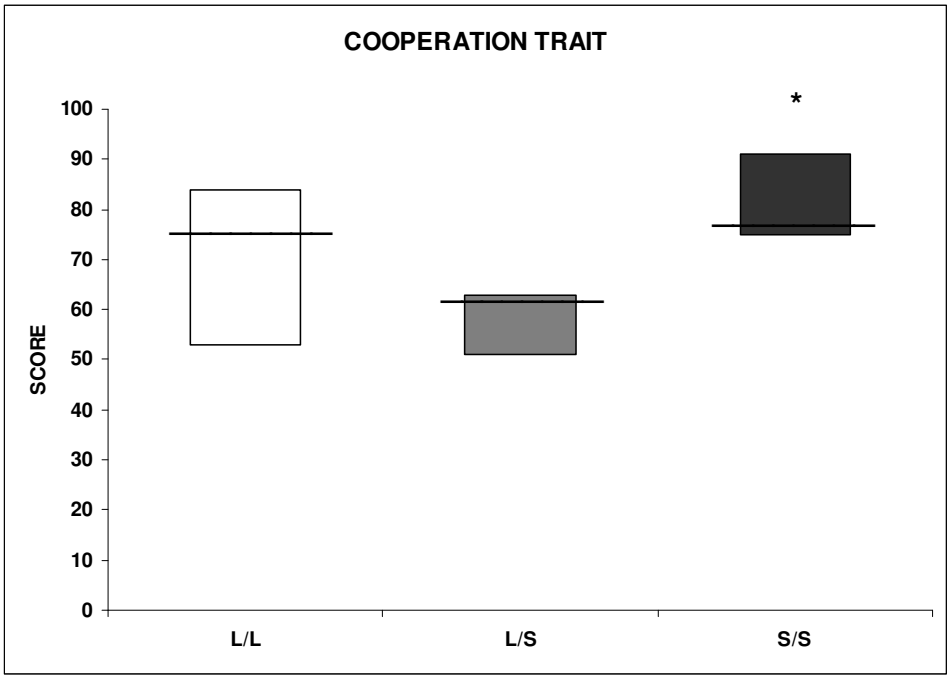
Chapter 4.

Figure 1.



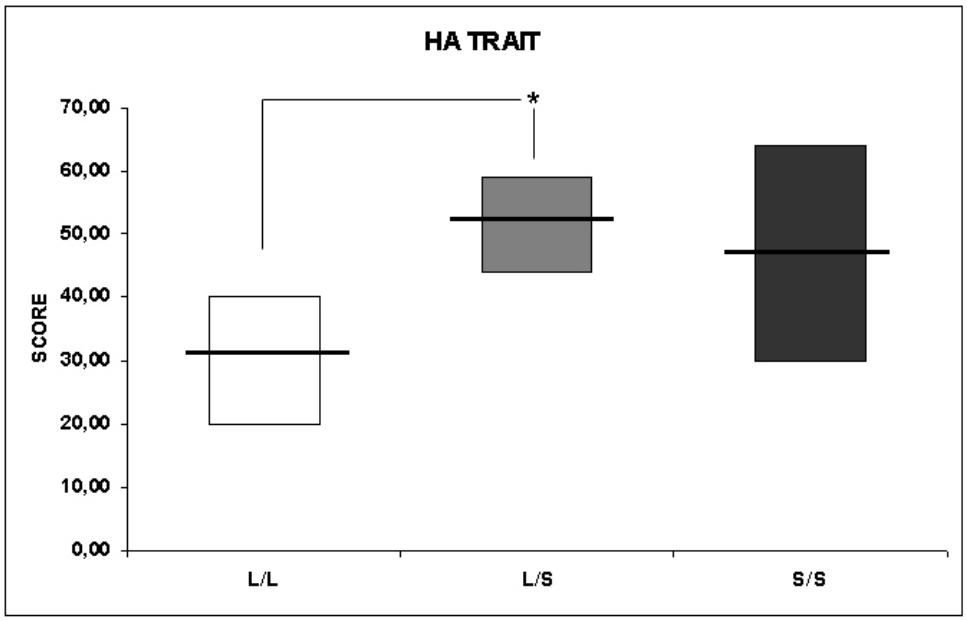
G score. Data are express as the median ± 25-75% confidence level for the significant traits. *significant difference (see text for statistical levels).

Figure 2.



CP score. Data are express as the median ± 25-75% confidence level for the significant traits: *significant difference (see text for statistical levels).

Figure 3.

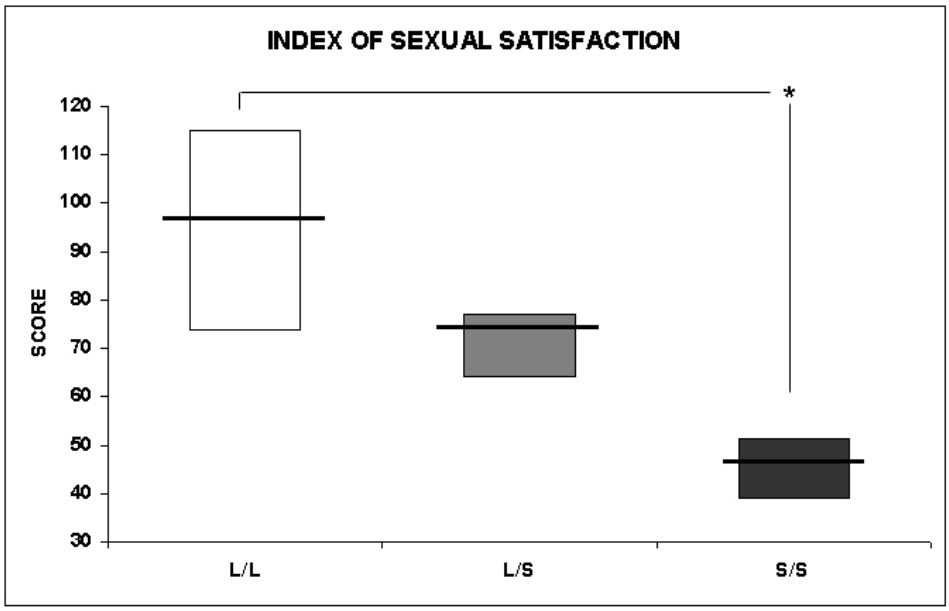


HA score. Data are express as the median ± 25-75% confidence level for the significant traits: *significant difference (see text for statistical levels).

Figure 4.

Age of first intercourse score. Data are express as the median ± 25-75% confidence level for the significant traits: *significant difference (see text for statistical levels).

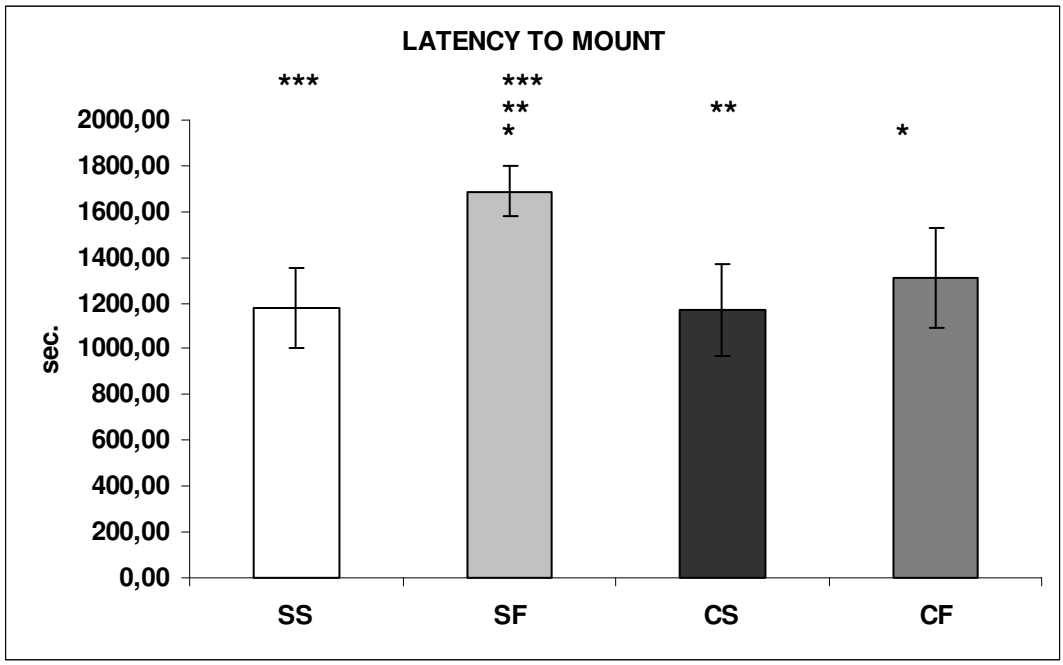
Figure 5.



ISS score. Data are express as the median ± 25-75% confidence level for the significant traits: *significant difference (see text for statistical levels).

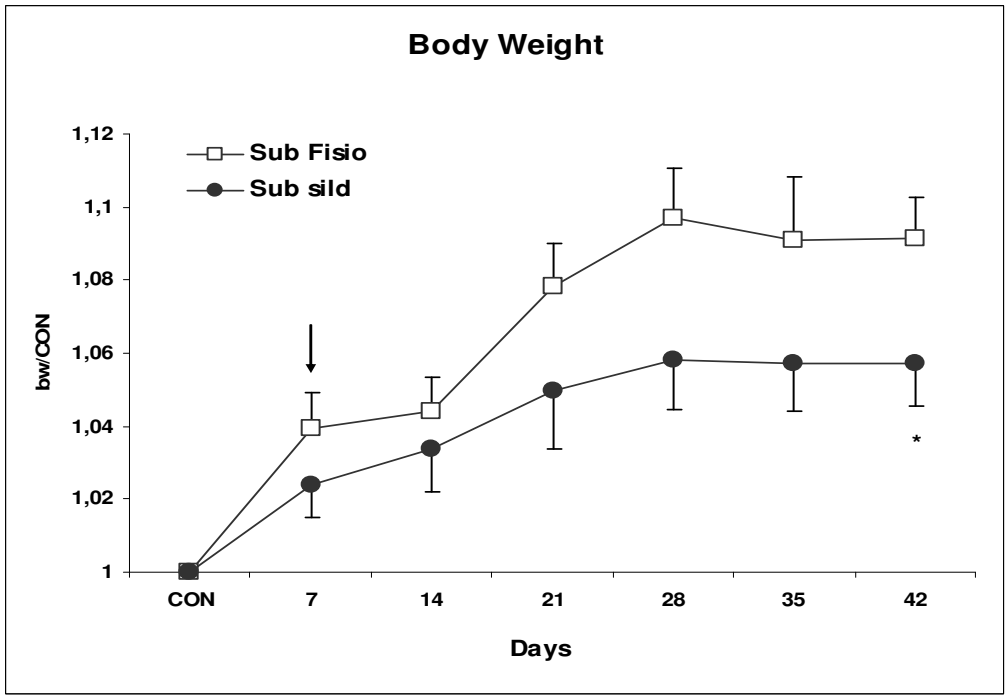
Chapter 5

Figure 1.



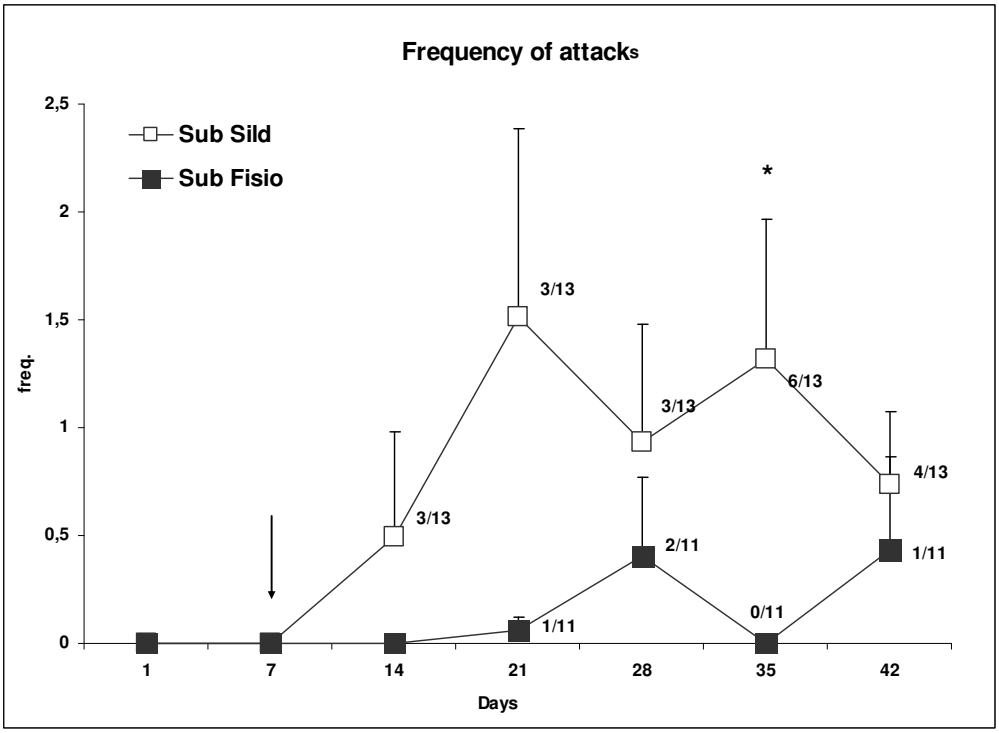
Data are expressed by mean ± SEM. *significant difference (see text for statistical levels).

Figure 2.



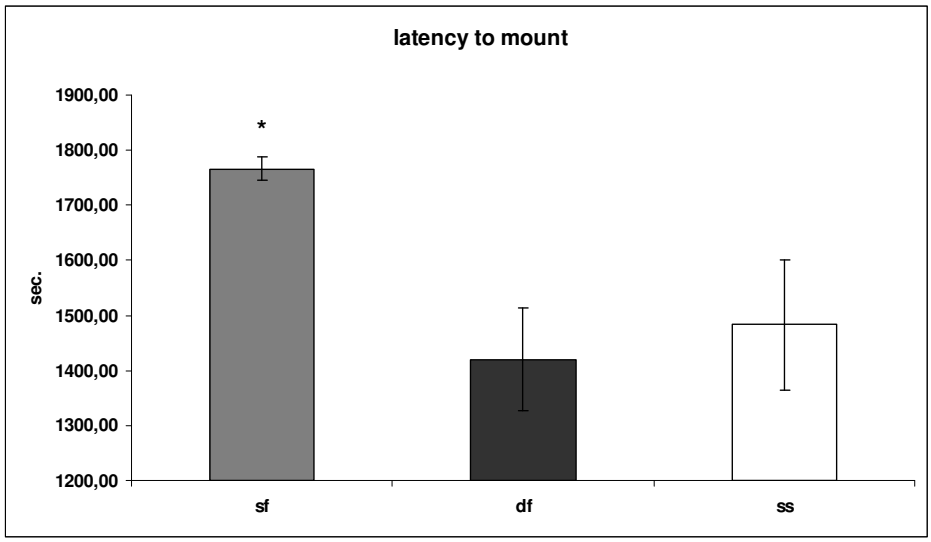
Data are expressed by mean ± SEM. *significant difference (see text for statistical levels). The arrow indicate the start of sildenafil treatment

Figure 3.



Data are expressed by mean ± SEM. *significant difference (see text for statistical levels). The arrow indicate the start of sildenafil treatment

Figure 1



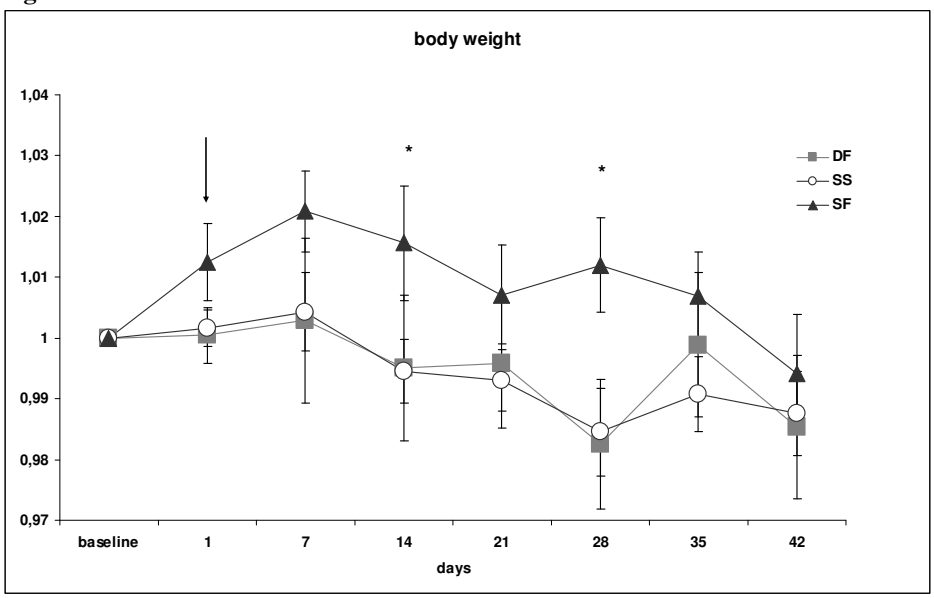
Data are expressed by mean ± SEM. *significant difference (see text for statistical levels).

Table 1

		mounting behavior		intromission		ejaculation	
numbers	sf	0,79	± 0,43	0,00	± 0,00	0,00	± 0,00
	df	2,48	± 0,84	0,33	± 0,23	0,19	± 0,15
	ss	1,24	± 0,62	0,10	± 0,10	0,10	± 0,10
latency	sf	**1766,35 ***	± 20,36	1800,00	± 0,00	1800,00	± 0,00
	df	**1420,10 ***	± 93,24	1613,85	± 106,98	1649,73	± 94,29
	ss	**1483,07 ***	± 118,13	1786,34	± 13,66	1794,74	± 5,26
total duration	sf	4,23	± 2,26	0,00	± 0,00	0,00	± 0,00
	df	17,03	± 6,76	3,73	± 2,77	1,01	± 0,78
	ss	15,34	± 8,31	0,59	± 0,59	0,33	± 0,33

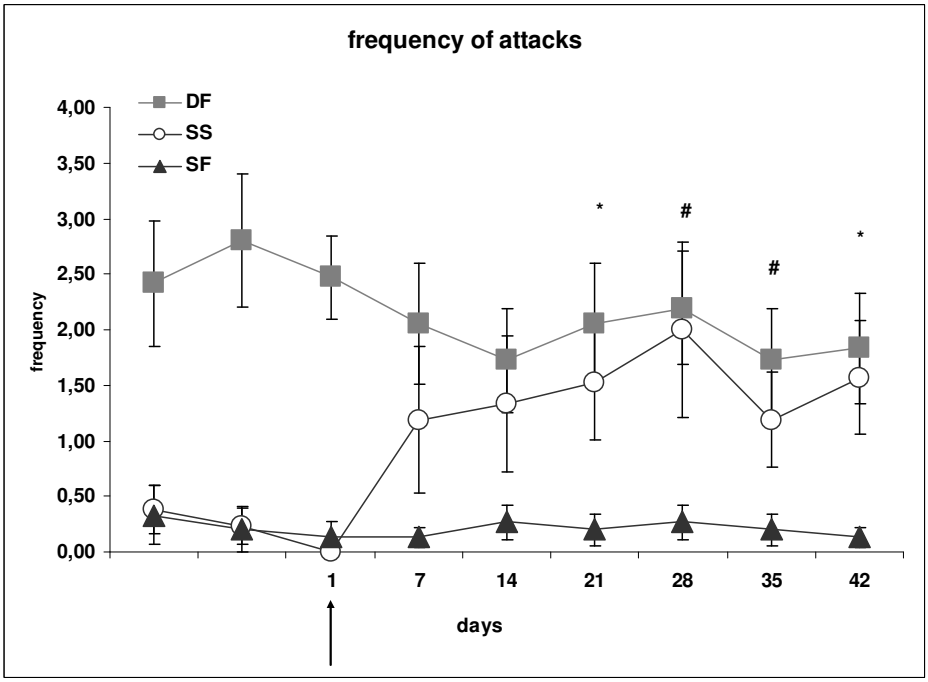
Numbers of sexual behaviors observed. *significant difference (see text for statistical levels).

Figure 2.



Data are expressed by mean ± SEM. *significant difference (see text for statistical levels). The arrow indicate the start of sildenafil treatment

Figure 3.



Data are expressed by mean ± SEM. *significant difference (see text for statistical levels). The arrow indicate the start of sildenafil treatment.

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