Chapter 4

Searching for Biological Markers of Personality: Are There Neuroendocrine Markers of Anxiety?

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1. Introduction

1.1. Defining the concepts underlying differences in emotional reactivity

The existence of stable individual differences in cognitive and emotional capabilities both in animals and humans is well-accepted. The theories of personality assume that such individual differences can be categorized and that the richness of individual differences in humans would be the result of the combination of differences in a few underlying personality factors. The most accepted contemporary theory is that of "Big Five" [1] that consider five highest order factors: neuroticism, extraversion, openness, agreeableness and conscientiousness. However, the nature of some of the putative factors is still a matter of dispute in the different theories. Within this framework, the factors extraversion and neuroticism have been associated to the response to positive and negative emotions, respectively. Moreover, it is typically distinguished between personality and temperament, the latter term referring to biological predisposition that is noted early in life and will eventually lead to adult personality [2]. Emotionality may be considered as relatively stable individual characteristic so that subjects labeled as highly emotional will strongly react to emotional stimuli, particularly negative ones. It is of interest to know how high neuroticism subjects react to stressful situations and which are the consequences of such exposure. It has been reported that in response to an adverse event high neuroticism soldiers showed larger increases in psychiatric symptoms than low neuroticism subjects [3], but no differences in the response were observed after controlling for pre-trauma symptoms. These data question the existence of high stress responsiveness in high neuroticism subjects.

In animals, the concept of emotionality is associated with the response to aversive stimuli. On the basis of the study of the behavioral and physiological responses to emotional situa-

tions, it may be concluded that emotional reactivity is clearly multifactorial. For instance, neither behavioral nor physiological responses, all of them presumably related to this concept, follow a uniform pattern when different strains of rats are compared [4]. The obvious conclusion is that emotionality is a complex, multifactorial, concept [4] and that emotional stimuli are probably processed in parallel brain circuits thus resulting in a wide range of associated physiological responses.

For the purpose of the present review we will focus on individual differences in anxiety. This is a particular emotional characteristic that has attracted considerable attention for the important role of anxiety disorders in humans. It is generally distinguished between the concepts of trait and state anxiety. The first refers to a stable predisposition to react with low or high levels of anxiety in response to anxiety-provoking stimuli, whereas the second evaluate the actual reaction to a particular situation. Some classical psychometric test distinguish between both, for instance the trait-state anxiety Spielberger test or STAI [5], trait anxiety being a general predisposition to get higher levels of state anxiety when confronted with aversive situations. The distinction between trait and state anxiety is particularly difficult in animal models, although some authors assumed, in line with the concept in humans, that animals characterized by high levels of trait anxiety should show high levels of anxiety-like behaviour in response to different tests, as it is the case of BALB/c inbred mice [6]. There is no consensus about putative tests that can specifically evaluate differences in trait anxiety in animals. Another important theoretical consideration is the distinction between normal and pathological anxiety, the latter one reflecting merely the extreme of a continuum, or on the contrary qualitative differences with the normal population. This distinction is basically impossible to establish in animal models.

When discussing about animal models, it is important to distinguish between those that involve certain environmental or genetic manipulations aimed to develop high anxious individuals or those aiming at evaluating anxiety-like behaviour in particular individuals. We referred to the latter as tests for anxiety or anxiety-like behaviour. There are different animal tests for anxiety. Some of them involve unconditioned response to aversive stimuli, whereas others imply conditioned responses [6]. Even when unconditioned tests, which usually involve evaluation of the free behaviour of animals, are used there are many instances of dissociation in the outcomes of the different tests when comparing groups of animals [i.e. 7]. This suggests that each test probably evaluate situational-specific components of anxiety. In fact, factorial analysis sometimes supports that putatively underlying factors determining behaviour are likely to differ in great part across tests [i.e. 8,9]. This is important when considering the putative relationship between anxiety and physiological parameters to be discussed later. Nevertheless, marked differences in trait-anxiety, either of environmental or genetic origin, may result in important differences in several different behavioral tests [i.e. 10,11], suggesting partially common underlying factors.

It is now widely-accepted that there are conceptual differences between fear and anxiety in that fear is elicited by precise and temporally defined dangers (the presence of a predator, exposure to well-announced aversive stimuli such as electric shocks), whereas anxiety would be elicited by more diffuse and sustained dangers (contextual fear conditioning,

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predator odours, unpredictable aversive stimuli) [12, 13]. Nevertheless, it is still difficult to be sure whether behaviour of animals in novel environments is related to fear or anxiety. For instance, rats and mice have innate aversion for open spaces, likely to be related to the risks of being predated in such places. Can then we speak about fear (innate predisposition) or about anxiety so far as the open spaces is only potentially nor actually dangerous? This is important as several widely used anxiety tests are based on exposure to novel environments such as the elevated plus-maze (EPM) or the light-dark (or dark-light) tests [14-17]. The EPM consists of a plus-maze elevated over the floor, with two (closed) arms surrounded by walls and other two unprotected. The light-dark apparatus has two compartments, one small and dark and another much greater and illuminated. In the light-dark version we initially put the animals into the illuminated area and measure time spent to entry for the first time in the dark compartment, the number of transitions between light and dark and the time spent in each compartment. In the dark-light version, the animals are introduced into the dark compartment and we measure the latency to enter into the illuminated area and the other measures previously indicated. The EPM and light-dark test are based on the fear elicited in rodents (which are nocturnal animals) by open and illuminated spaces, and the natural tendency of these animals to explore new environments. These two tendencies generated a conflict and we expect that less emotional, fearless or low anxiety animals spend more time in the open arms of the EPM and the illuminated area of the light-dark test. Other animal models are based on the performance in an active avoidance-escape task in a shuttle box. In this task the imminence of a shock is signalled by a specific conditioned stimulus (noise, light; CS) and the animals can learn to avoid the shock (during the CS) or escape from the actual shock by doing a particular active behaviour: jumping from one side to the other. This procedure likely elicits an emotional reaction close to fear. However poor performance in such task is considered to be associated to high anxiety that makes the animals to become immobile and perform poorly. Administration of classical anxiolytic drugs clearly improves performance [i.e. 18]. The extent to which psychological dimensions underlying individual differences are similar in all cases or whether or not we are really detecting differences in anxiety is still an open question.

In addition to the problem of correctly indentifying a particular behavioral trait, there are problems related to the characterization of the physiological profiles associated to such a trait. First, negative emotional situations elicit a wide range of physiological responses and it is important to know whether or not such repertoire of responses is dependent of the particular stimulus or the particular emotion elicited. Until now it has not been possible to conclusively identify physiological response patterns associated to specific emotions. Second, the emotional response to particular situations are greatly influenced by the cognitive processing of the particular stimulus (appraisal) and by coping strategies, that is the behavioral repertoire used for the animals to escape from the source of the aversive experience or to reduce the impact of the situation. Koolhaas [19] considered coping style as a set of coherent behavioral and physiological responses to aversive stimuli. Two different coping styles have been defined: proactive (active) and reactive (passive), characterized by the triggering of active versus passive strategies to cope with aversive situations. The authors considered coping style as independent of emotionality [19,20]. That is, the dimension of active *versus* passive

strategies is considered as orthogonal to emotionality. Nevertheless, coping style can influence the success of the strategy used to face the situation and, indirectly, the behavioral and physiological response to the situation. Therefore, it is difficult to establish putative relationship between physiological variables and emotionality, including anxiety, without knowing other dimensions of personality as coping style.

It should be also taken into account that even if we can isolate one particular trait such as anxiety, the final behavioral and physiological responses (measurable outputs) are the result of the activation (or inhibition) of a wide range of divergent brain pathways, each of them putatively influenced by individual characteristics not related to the trait of interest, which may perturb or mask the common influence (trait) on all these variables. For instance, if we evaluate emotional reactivity by the activity of animals in a novel environment, even if two animals experienced the same level of fear/anxiety, the expression of the final measured response (ambulation, rearing) could differ because of different in activity, coping strategies (active or passive) or other traits (i.e. interest for novelty). Available evidence indicates that the genetic control of anxiety appears to be polygenic (as it is the case of other behavioral traits). Similar conclusion applies to the control of certain physiological parameters important for the present issue, as it is the case of the hypothalamic-pituitary-adrenal (HPA) axis [21]. By definition, inbred rats are genetically homogeneous and homozygotic for all genes. This means that every inbred strain has only a particular allele for each gene among the various ones present in the species and that throughout the process of inbreeding, a particular allele of each gene involved in the behavioral trait of interest or in the activity of the HPA axis has been randomly fixed. As it can be assumed that the genes are controlling each particular function in both positive and negative directions, each particular inbred strain could have been fixed a different combination of the alleles involved in the functions of interest. Therefore, it may be theoretically difficult to find a relationship between a behavioral trait and the HPA axis that may apply to other inbred strains or to an outbred population of rats. That is why we will refer only in very specific cases to studies with inbred rats or mice.

1.2. An overview of the HPA axis and other physiological stress markers

The present review will focus on the relationship between anxiety and the sympathetic-medullo-adrenal (SMA) and hypothalamic-pituitary endocrine axes. In the latter case, special attention should be given to the HPA axis and prolactin because they are considered as good biological markers of stress (see below). Activation of the SMA and HPA axes constitute the prototypical physiological responses to stressors in all vertebrates. These two axes have focused great attention in the field of stress for two main reasons [22]. First, the release of SMA and HPA hormones into blood is positively related to the intensity of the stressful situations and therefore they are well-suited to reflect differences among subjects in the degree of emotional activation. Second, activation of the SMA axis have a critical role in the regulation of metabolism and cardiovascular responses and is likely to be important for the development of certain stress-related pathologies (i.e. hypertension). Third, glucocorticoids (cortisol in humans and most mammals; corticosterone in rats and mice), the final output hormone of the HPA axis, has been implicated in a wide range of pathophysiological and

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psychopathological processes, including cardiovascular diseases, immune suppression, altered gastrointestinal function, anxiety disorders, depression and predisposition to drug self-administration. However, It is now well-recognized that stress-induced pathology is not only dependent on the nature and time-schedule of exposure to stressors but on individual differences in vulnerability to them.

The association between the activation of the SMA axis and stress is well-known since the earlier works by Cannon in the first half of the XX century. However, it is now realized that stress exposure also resulted in the activation of certain responses mediated by the parasympathetic nervous system. For instance, changes in intestinal colonic motility and visceral pain sensitivity [i.e. 23-25]. Moreover, the old idea that the SMA axis is activated in an all or none manner is not accepted as there are strong anatomical and functional evidence for a fine tuning of the response of SMA to different stimuli, including stressors [26, 27]. The flexibility of the SMA axis to respond to different stimuli is on the basis of the theories that argue that different emotions in humans can be distinguished by a particular physiological signature, nevertheless, there is not at present unequivocal and precise evidence for such signature [28]. Activation of the SMA axis have been typically evaluated measuring plasma (or urinary) levels of noradrenaline and adrenaline, heart rate (HR), heart rate variability (HRV, a measure of parasympathetic cardiac activity), diastolic and systolic blood pressure (DBP; SBP) and electric skin conductance. Plasma levels of adrenaline derived almost totally from the adrenal medulla, whereas plasma noradrenaline derived in part from the adrenal medulla but mostly from the activity of sympathetic nerves in all body. It is well-established that both plasma adrenaline and noradrenaline increases in response to emotional stressors, but the former better reflects the intensity of emotional stressors [29]. As circulating adrenaline is the main factor controlling stress-induced hyperglycaemia, it is not surprising that plasma glucose is a marker of stress intensity under moderate to strong stressful conditions [29].

The HPA axis is a complex and dynamic system whose regulation has been very well-characterized in the last decades [30]. The main brain locus of control of the HPA axis is the paraventricular nucleus of the hypothalamus (PVN). The PVN is a complex nucleus with two main types of neurons and several subdivisions. Big (magnocellular) neurons are located in the PVNm and synthesize the neurohypophyseal hormones oxytocin and vasopressin (VP), sending axons directly to the neurohypophysis. Small (parvocellular) neurons are concentrated in the PVNp and send axons to the median eminence to release ACTH secretagogues into the pituitary portal blood. Among such secretagogues, the corticotropin releasing factor (hormone) (CRF or CRH) is considered to be the most important in that it controls both synthesis and release of the adrenocorticotropic hormone (ACTH) and other peptides derived from pro-opiomelanocortin (POMC) in anterior pituitary corticotrope cells. Among the other ACTH secretagogues, VP appears to play a prominent role, acting synergistically with CRH to increase the release (but not the synthesis) of ACTH. In the PVNp appears to be two different populations of CRH neurons, one co-expressing and another one non-coexpressing VP. Interestingly, persistent or repeated activation of the HPA axis is accompanied by an increase in the number of CRH neurons coexpressing VP in the PVNp, suggesting a more

prominent role of VP in those situations associated to hyperactivity of the HPA axis. CRH in the anterior pituitary acts through CRH type 1 receptors (CRH-R1), whereas VP acts through AVP1b receptors. In addition to the above considerations, it should be taken into account that the contribution of CRF, VP and other secretagogues to the release of HPA hormones appears to be dependent on the particular type of stressor.

When the animals are exposed to stressful situations ACTH is promptly released (a few minutes), reaching a maximum between 5-10 minutes after a brief exposure to stressors or between 15-30 minutes with more prolonged exposures. Plasma levels of ACTH may well reflect a wide range of stressor intensities provided that samples are taken at appropriate times after the initial exposure to the stressor [29]. If exposure to a stressor lasts only a few minutes, maximal ACTH levels are achieved in a period of 5-10 minutes, then declining. If exposure to the stressor continues and it is relatively severe, the ACTH response is maintained for about 1 h but not more, and, therefore, plasma levels of ACTH are no longer a reflection of stressor intensity. One critical point regarding stress-induced adrenocortical secretion is that the maximum is reached with relatively low levels of ACTH so that plasma levels of glucocorticoids are only a good reflection of ACTH release with low intensity stressors. In fact, differences in plasma levels of corticosterone immediately after exposure to relatively severe stressors (i.e. footshock, restraint, immobilization) reflect more the maximal capability of the adrenal to secrete glucocorticoids, which is related to the adrenal weight [i.e. 31], rather than the circulating levels of ACTH, thus leading to a frequent misinterpretation of the results.

On the basis of the above, two major points should be considered in evaluating the impact of a stressor on the HPA axis. Firstly, measurement of circulating levels of glucocorticoids at a time shorter than 15 minutes after initial exposure to stress is non-appropriate to reflect the actual impact of a stressor on adrenocortical secretion because maximum levels are achieved nearly and beyond this time point. Secondly, plasma levels of glucocorticoids are not a reflection of stressor intensity above a certain level of intensity, which usually lies within low to moderate range. In the rat, exposure to a relatively stressful novel environment is probably the situation above which glucocorticoids hardly can detect actual anterior pituitary activation. Although, plasma glucocorticoids levels just after stress did not reflect ACTH levels, the follow-up of their plasma levels for a period of time after the termination of stress can reflect the initial ACTH release and therefore should be used in those cases where there is no possibility to directly measure ACTH.

Glucocorticoids release by stress exerts a wide range of actions in the body, both peripherally and centrally. These effects are exerted through genomic and non-genomic processes [32, 33]. Genomic effects of glucocorticoids are exerted through two well-characterized receptors: mineralocorticoid (MR, type I) and glucocorticoid (GR, type II) receptors. The non-genomic receptors are still uncharacterized at the molecular level, but are likely to be located in plasmatic membrane. Regarding the regulation of the HPA axis, one major function of glucocorticoids is to exert a negative feedback to reduce initial activation of the HPA axis. This negative feedback [34] is exerted at different levels: at the anterior pituitary, at the PVN and at other key brain areas such as the hippocampal formation and the prefrontal cortex

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[30]. The negative glucocorticoid feedback controls both normal resting activity of the HPA axis and the response to stressors. Since a defective negative feedback can markedly alter HPA functioning, there are classical tests for the efficacy of such feedback that use exogenous administration of natural or synthetid glucorticoids. In humans, it is extensively used the administration of the synthetic glucocorticoid dexamethasone (DEX) in the so called suppression DEX test. However, the validity of this test has been questioned by the fact that DEX, which easily penetrated the brain, is excluded from the brain by the multi-drug resistant protein P-glycoprotein [35]. Therefore, depending on the dose DEX mainly acts at the pituitary and only to a limited extent within the brain.

The HPA axis shows both circadian and pulsatile rhythms [36]. In addition to its biological meaning, the existence of a pulsatile secretion of ACTH and corticosterone is an important concern when only one sample is taken as it could not be representative of the actual secretion. Regarding circadian rhythm, maximum activity is observed around the awakening time. Maximum levels of plasma glucocorticoids are associated in all animals and humans to the start of the active period, being observed just around lights off in rats and mice and just after sleep in humans. Although the circadian rhythm affects both ACTH and glucocorticoids, the amplitude is much greater for the latter than for ACTH due to an increase in adrenal sensitivity to circulating ACTH [37]. In humans, there is a sharp increase in the first 30 minutes after awakening (called the cortisol awakening response, CAR) followed by a progressive decline over the day [38, 39]. Both in animals and humans, proper evaluation of the HPA axis requires taking several samples over the day.

Measurement of plasma levels of ACTH and corticosterone under resting (basal) conditions and after exposure to stress is the simplest approach when studying the functionality of the HPA axis. It is important to note that altered responsiveness of HPA hormones to stress can be observed with normal resting levels, but increased responsiveness to stressors may eventually result in increased resting levels of plasma glucocorticoids. However, these measures are very often insufficient for a deeper understanding of HPA differences between individuals or between different physiological or pathological conditions. Other classical measures include the evaluation of: (a) adrenal responsiveness to ACTH by administering exogenous ACTH and measuring plasma levels of cortisol or corticosterone; (b) adrenocorticotrope cell responsiveness to CRH and VP by exogenous administration of these neurohormones and measurement of plasma levels of glucocorticoids and preferable of ACTH; (c) the integrity of negative glucocorticoid feedback mechanisms, usually by given DEX. More recently, the combined DEX-CRH test has gained considerable interest, although the biological processes underlying this test are not well-understood. In animals, we can obviously use a wide range of additional approaches, but the most used are the evaluation of the brain expression of those neuropeptides directly related to the regulation of the HPA axis. If some subjects respond more to stress, it is assumed that they will ideally show enhanced PVN expression of CRH and/or VP, enhanced AP expression of the POMC gene, increased adrenal weight and perhaps higher resting levels of plasma glucocorticoids and reduced efficacy of negative glucocorticoid feedback. This is a typical pattern after exposure of animals to chronic severe

stressors [40]; however, it is realistic to assume that this whole pattern would be rarely found in humans.

Individual differences in some of the components of this complex biological system may oppose to the expected results, complicating the interpretation of the results. For instance, a highly emotional rat or mouse strain may be characterized by a physiological defect in the HPA axis (i.e. defective CRH production, reduced adrenocortical responsiveness to ACTH) that would act in the opposite direction to emotionality thus cancelling the differences in particular hormonal output. This is the case of inbred Lewis rats. They are considered as highly emotional [4], but are also characterized by a defective HPA system thus resulting in reduced ACTH and corticosterone response to a wide range of stressors (i.e. 41, 42). Therefore, if we expect higher HPA activation in these emotional animals (a hypothesis that is not necessarily true), defective HPA function could mask the expected higher HPA response. This problem is particularly important when comparing inbred animals.

In addition to the HPA axis, all anterior pituitary hormones (growth hormone, GH, thyrotropin stimulating hormone, TSH, prolactin, luteinizing hormone, LH, and follicle-stimulating hormone, FSH) have been extensively studied regarding stress and psychopathology. However, in recent decades, the interest focused on the HPA axis and to lower extent in prolactin. Prolactin is a stress-responsive hormone that is regulated by two hypothalamic mechanisms [43]. One involves a potent and tonic inhibitory control by a population of dopaminergic neurons located in the arcuate nucleus that send axons to the pituitary portal blood (tuberoinfundibular system). The other involves one or several prolactin releasing factors (PRFs). There are several candidates as PRFs, including oxytocin and VP, but there is no still agreement about the actual PRF. It is likely that during stress, prolactin release is the consequence of the reduction of dopaminergic inhibitory signals and the increase in stimulatory inputs. Although the precise role of prolactin during stress is not known, there is evidence that peripheral prolactin has access to the brain through prolactin receptors and can exert anxiolytic and anti-stress effects [44].

1.3. Are the intensity and nature of the stressor important for characterizing individual differences?

Which are the objectives of characterizing individual differences in responsiveness to stressors? One important purpose is to associate altered physiological responsiveness to pathological conditions: i.e., increased cortisol response to stressors may underlie immune suppression. Another one is to establish whether or not certain individuals or psychopathologies are characterized by an altered sensitivity to stressors. In the latter case, we assume that the chosen physiological variable is able to distinguish between hypo- or hyper-responsive subjects. However, to accomplish this goal we need to demonstrate first that these variables are able to reflect the intensity of stressors and that the results are relatively unaffected by the type (quality) of stressor. In animals, on the basis of neuronal activation as revealed by c-fos and lesion experiments it appears that those stressors having a predominant emotional component (i.e. electric shock, restraint, immobilization, exposure to predator or predator odors) activate the HPA axis following telencephalic pathways, whereas stressors

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having a predominantly physical component (endotoxin, cytokines, hemorrhage) act primarly at the level of the brainstem, brainstem nuclei sending stimulatory signals to the PVNp [45, 46]. In fact, recent studies suggest that is likely that each particular stressor can have a particular brain activation signature, thus leading to differential adaptive behavioral and physiological responses and pathological consequences [47]. Nevertheless, it has been demonstrated in rats and mice that in response to predominantly emotional stressors, plasma levels of adrenaline, noradrenaline, ACTH, corticosterone (under certain conditions) and prolactin reflect, under appropriate conditions, the intensity of stressors [29]. In contrast, whereas circulating levels of some other anterior pituitary hormones (GH, TSH, LH) are altered by stress in animals and humans [i.e. 48-51], there is no evidence that they are sensitive to the intensity of stressors. In rats, we have found a very consistent correlation between the ACTH or corticosterone response to different novel environments [52, 53], whereas no correlation at all when comparing the response to a novel environment and to a much more severe stressor such as immobilization (unpublished). Whether or not the critical factor for the lost of correlation is the markedly different intensity of the two stressors or the qualitative differences among them is unclear.

In humans, despite the extensive human literature on stress, there have been few attempts to establish which physiological variables may be sensitive to the intensity of emotional stressors. Callister [54] used two tests (a modified Stroop colour word test and mental arithmetic task) each with different levels of difficulty over one unique session and observed progressive increases in the perceived stress in function of the difficulty; in contrast, HR was independent and DBP and SBP promptly achieved a plateau with relatively low levels of intensity. Therefore, there is negative evidence for a relationship between HR and level of stress and limited evidence regarding blood pressure. In our own work we compared in Medicine female students the anxiety, cortisol, prolactin and glucose responses to two exams (Psychology and Physiology) that were known to induce different levels of anxiety [55]. As expected, state anxiety increased in response to both exams as compared to a regular day, but anxiety was greater with Physiology. The response to plasma cortisol was low, but in the same direction, whereas prolactin not only increased with respect to the routine day, but the increase was greater with Physiology than Psychology exam. In another study, salivary cortisol appears to reflect the degree of stress when assessed in different situations during military survival training [56]. These data support the hypothesis that biological stress markers are likely to behave similarly in humans and rodents. Interestingly, despite the parallel behaviour of state anxiety, cortisol and prolactin, no significant correlation was observed between the variables in our work [55], suggesting parallel but in great part independent regulation. The Trier social stress test (TSST) is an extensively used psychosocial stress that includes public speech and evaluation [57]. Subjects classified as high or low responders in function of the ACTH and cortisol responses to the TSST did not differ in their HR, adrenaline or noradrenaline responses [58]. This suggests that classification of subjects was based more on a specific functional difference in the regulation of the HPA or on individual differences in stress responsiveness that only affected the HPA axis, not reflecting a general stress hyper-responsiveness.

In sum, the available results are not suggestive of a stressor-independent pattern of response of the HPA axis and other variables that could unequivocally characterize individuals. That is, individual differences in physiological responsiveness to stressors are not only depending on certain characteristics of the individuals, but also on the particular stressor used as a challenge. Interestingly, attention should be paid as to how subjects can experience different emotional reactions to the same stressful situations. Thus, it was observed in healthy subjects a differential emotional response (evaluated by facial expression) to a mental arithmetic task that translated to a differential cardiovascular and salivary cortisol response [59]. In contrast, self-reported emotional experience did not contribute to such differential physiological response.

2. Neuroendocrinology of anxiety in humans

2.1. General considerations

In evaluating the neuroendocrinology of anxiety we can take some critical points into consideration. First, is there any relationship between state anxiety and certain hormones in response to some acute aversive situations? Second, is there any relationship between trait anxiety in a non-pathological population and resting or stress levels of hormones? Third, are resting or stress levels of hormones altered in pathological anxiety?

It is well-known in humans that exposure to acute stress can induce physiological (including hormonal) changes and increased anxiety, with a pattern quite similar to that observed in animals. However, there are numerous inconsistencies in the literature regarding the response of cortisol or prolactin to stressors. This is likely to be due to our poor knowledge on the dose-response relationship between stressor intensity and the elicited physiological and anxious responses in humans. The characterization of the dose-response curves of stressor intensity and physiological variables is critical for three main reasons. First, we can identify which physiological variables are actually sensitive to the intensity of stressors, thus ruling out those which are not. Second, we need to know which range of intensity of stressors can be appropriately evaluated using a particular variable. For instance, we know that in rodents plasma corticosterone is useful for low to intermediate intensity stressors but not for the intermediate-severe intensities, whereas the opposite is true for plasma glucose. Third, if the physiological response is well-characterized, this can help to objectively place any experimental stressful situation within the stress scale. Finally, and importantly, if we are using experimental situations eliciting a modest (or a very high) physiological response, the characterization of individual differences should be theoretically more difficult. This is particularly critical when the experimental conditions only elicited an extremely low, if any, response as appear to be the case in an important number of papers [for review, see 60].

In analyzing the literature about individual differences in responsiveness to stressful laboratory tasks, it is important to consider the importance of pre-task hormone levels. It has been repeatedly observed that some physiological markers of stress are elevated by the anticipation of the task rather than by the task itself. This sometimes leads to misin-

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terpretation of the results as a reduced response to the task. In fact, anticipatory anxiety and physiological response may be indicative of high rather than reduced responsiveness to putative stressful situations.

2.2. Neuroendocrinology of anxiety in healthy subjects

Unless otherwise stated, differences in trait or state anxiety were evaluated with the well-characterized STAI. We will comment first data regarding state anxiety and then trait anxiety.

Although numerous studies have demonstrated increases in both state anxiety and some physiological parameters in response to stressful situations, only few studies reported correlation between them. In an important number of studies correlation between state anxiety and some hormones was low or absent, suggesting that despite the apparent parallelism, underlying factors are likely to differ. In response to anticipation of surgery a significant correlation was observed between state anxiety and cortisol, but not prolactin [61]. In contrast, no association between anxiety and the increases in cortisol, prolactin or TSH levels were observed after parachute jumping [50]. In our own work with exam stress, no significant correlation was found between state STAI anxiety and plasma cortisol or prolactin levels [55]. Similarly, in a speech task, some correlations were found between certain physiological parameters (HR, BP, noradrenaline, cortisol), but not between them and state anxiety [62]. Pottier et al [63] observed in medical students that consultation in an unfamiliar ambulatory setting caused more anxiety (as evaluated by the STAI and a visual analog scale, VAS) and salivary cortisol response than consultation in a familiar (in-hospital) setting, but no correlation was found between the two measures. Similarly, VAS anxiety did not appear to predict changes in cortisol or HR response to the TSST test in young males whereas perceived stress did [64]. A study with arithmetic stress observed significant correlation between state anxiety and salivary α-amylase, but not cortisol or chromogranin-A [65]. Salivary α-amylase and chromogranin-A both reflect SMA activation, but it is possible that salivary α-amylase represents a specific component of SMA activation more closely related to anxiety than other SMA markers and cortisol. In contrast to most of the previous results, a study evaluating in surgeons the physiological and STAI response to 54 different surgical procedures (some of them not perceived as stressful) observed significant correlations between STAI and HR or salivary cortisol, and between HR and cortisol [66].

In conclusion, the above results did not reveal a consistent positive relationship between state anxiety and physiological response to stressors. One theoretical explanation for the inconsistencies may be explained by the type of data incorporated to the measurement of correlation. If we include data corresponding to different stressful situations differing in intensity and, therefore, in the magnitude of the response of certain variables (i.e. anxiety and cortisol), obviously both variables would increase in parallel. Consequently, a positive correlation should be observed (Fig. 1). In contrast, if we consider only the same data corresponding to each particular stressful situation, no correlation could be observed. In addition, there are other possibilities to explain this lack of consistent relationship. Firstly, failure to find association may be due to methodological problems such as the clearly different dy-

namics of each variable that make it very difficult to design experiments optimizing all variables. Secondly, physiological variables may capture specific psychological processes, only some of them being more specifically related to measures of anxiety. Finally, dissociation may exist between subjective and physiological measures of emotion. For instance, invasive cardiologists showed increased anxiety response when they adopted a secondary assistant (teaching) than a primary operator (autonomous) role, but this subjective state was not associated to higher HR and salivary cortisol responses [67].

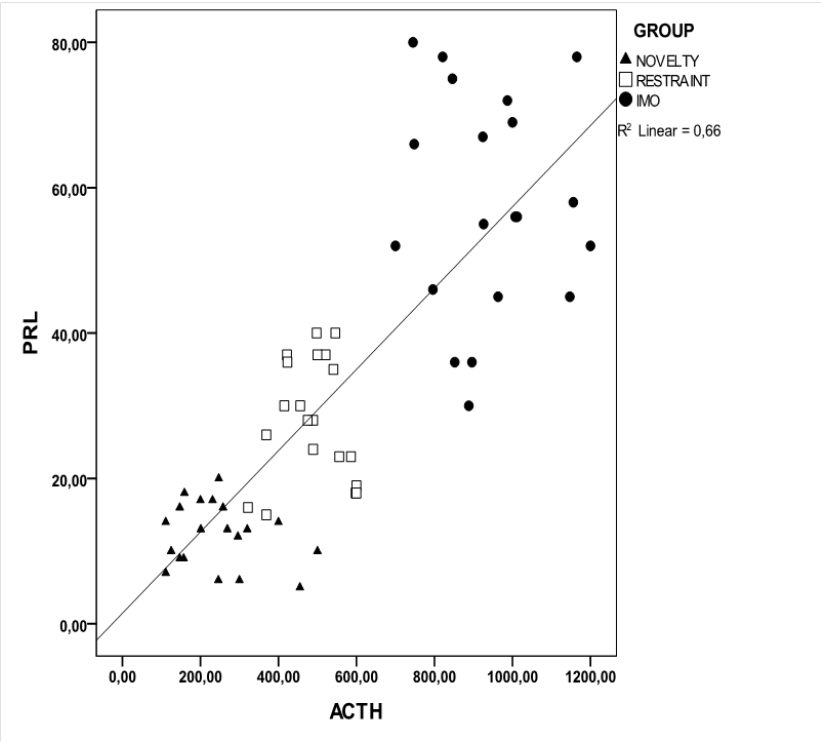

Figure 1. Correlation between two physiological measures (ACTH and prolactin, PRL) in a simulated response to three stressors of different intensity: a novel environment, restraint in tubes, and immobilization on boards (IMO). It should be noticed than when all samples are considered there is a positive statistical significant correlation between the two hormones, whereas no correlation at all was found when only samples corresponding to the same stressor were studied. This can explain inconsistencies in the literature regarding correlations between physiological variables and between them and state anxiety.

Regarding trait anxiety, there is negative evidence for an association between trait anxiety and salivary cortisol response to a speech task or the TSST in adult males [68. 69]. In a study that compared the response to the TSST of controls and patients with chronic atopic disease, the lack of relationship between trait-anxiety and salivary cortisol was confirmed and extended to plasma levels of ACTH [70]. Similarly, no relationship was found between trait

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and state anxiety and salivary amylase and cortisol responses to TSST or electrical stimulation either in males or females [71]. Surprisingly, some authors have reported a negative rather than positive relationship between trait anxiety and stress responsiveness. Healthy subjects classified as highly anxious showed a diminished salivary cortisol response to an unpleasant film as compared to low anxiety subjects [72]. This result has been extended in two studies showing lower plasma ACTH, cortisol, prolactin, adrenaline and noradrenaline in response to psychosocial stress (public speech) in anxious versus non-anxious subjects [73, 74]. Moreover, similar results were obtained using the Hospital Anxiety and Depression Scale that evaluated BP, HR and salivary cortisol responses to a combined (Stroop test, mirror-tracing and speech) psychosocial stressor [75]. The above data thus suggest a negative rather than positive relationship between neuroendocrine markers and trait anxiety, although neurobiological underpinnings are unknown.

The relationship between trait anxiety and resting activity of the HPA axis has also attracted attention. There is no association with basal salivary evening cortisol [76] or the cortisol response to the DEX+CRH test [77]. However, trait anxiety appears to affect the circadian rhythm of salivary cortisol in military men under free-living conditions, those with high trait anxiety displaying less pronounced decreased from early morning to mid-morning [78]. In post-pubertal adolescents, high trait anxiety resulted in higher evening salivary cortisol with no differences in morning levels [79]. Taken together, trait anxiety may be associated to a dysregulation of circadian resting cortisol levels, particularly the decline over the waking period, although there are discrepancies in the details. Studies measuring ACTH are needed to discern between ACTH-dependent or ACTH-independent dysregulation.

Interestingly, in response to a stressful video (corneal transplant) where higher and faster increased was observed in saliva α-amylase than in cortisol, a significant positive correlation was observed between trait anxiety and α-amylase, but not cortisol [80]. A recent report in children exposed to 3 consecutive stressors (including performance and peer rejection) confirmed the positive relationship of trait anxiety (measured by the revised children's manifest anxiety scale) and baseline or stress levels of α-amylase [81]. Considering the previously discussed positive relationship between α-amylase and state anxiety, this parameter offers promising results in studies of anxiety.

2.3. Neuroendocrinology of anxiety disorders

The relationship between anxiety disorders and basal (non-stress) levels of classical stress hormones is not clear. There are different types of anxiety disorders, as defined by the DSM-IVR [82]: Panic attacks, agoraphobia, social phobia, obsessive-compulsive disorder, post-traumatic stress disorder and generalized anxiety disorder (GAD). We will focus mainly in GAD on this aspect as an example among the different anxiety disorders.

Measures of urinary cortisol give inconsistent results, whereas higher catecholamine content appears to be more consistent in patients (see review of earlier works in [83]). Plasma prolactin was found to be normal in early studies [83] and this was further confirmed [84]. Cerebrospinal fluid (CSF) levels of CRH are considered as an index of overall activity of brain CRH neurons, including those neuronal CRH populations not directly related to the regula-

tion of the HPA axis. It appears that CSF CRH levels are not altered in GAD, suggesting normal brain CRH function [85]. In addition to the inconsistencies of early studies, data from some recent studies using salivary cortisol do not offer a clearer picture. In late-life GAD, increased resting levels of salivary cortisol were observed at several times in the morning but not the evening and the levels were positively related to the severity of anxiety [86]. In accordance, slightly higher awakening levels of cortisol were observed in a sample of patients with anxiety disorders, the effects being particularly significant in those with panic disorder with agoraphobia and those showing comorbidity with anxiety and depression [87]. In contrast, lower CAR was observed in another study with a large cohort of older adults with several types of anxiety disorders when compared to healthy controls [88]. No differences were observed at other times. Another study with middle-age people suffering from GAD showed no differences from controls either in the CAR or in the daily pattern of cortisol, despite higher levels of α-amylase [89]. Whether or not the inconsistencies are due to the age of patients or confounding factors is not known, although the latter concern should be taken into account considering the usually small magnitude of the effects. Quite interestingly, decreased levels of hair cortisol were recently observed in GAD patients despite no changes in salivary cortisol over the day under resting conditions [90]. As hair cortisol represents the integration of cortisol release over periods of months, the results support a negative relationship between GAD and HPA activity. It is unclear whether these patients show reduced response to daily stressors (and therefore, less release of cortisol) rather than reduced resting activity. This hypoactivity of the HPA axis does not appear to be a general characteristic of all anxiety disorders. Thus, slightly alterations in circadian and pulsatile secretion of cortisol and to a lesser extent in ACTH was reported in panic patients, with overall higher levels as compared to controls and increased amplitude of cortisol pulses [91].

Unfortunately, there are scarce studies on the comparison of the response to stress of GAD patients as compared to controls. In adolescents with GAD, increases in ACTH, GH and prolactin (but not noradrenaline, adrenaline and cortisol) were found in the phase of anticipation to the task in GAD patients but not in controls [92]. In contrast, no response to the task was observed.

Phobic subjects offer an interesting model for the study of the relationship between behavioral reaction to the situation and the concomitant physiological response. Severe anxiety was reported in patients with phobia to insects and small animals after forced exposure, whereas no changes were found in prolactin [93]. In a further study, increases in HR, blood pressure and plasma levels of adrenaline, noradrenaline, cortisol and GH were reported, although the increases in state anxiety were stronger and did not correlate to physiological responses [94]. The strong dissociation between subjective behavioral arousal and cortisol response to spider phobia was confirmed in another study comparing phobics and healthy controls [95]. Driving phobics as compared to controls showed increased anticipatory anxiety and cortisol response to driving, with further increases in anxiety but not cortisol during driving [96]. Moreover, no significant correlation was found between anxiety and cortisol in phobic subjects. Less clear is the response of social phobia patients to social stimuli. Salivary cortisol response to the TSST was similar in social phobic adolescent girls than in controls [97]. In

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contrast, in another study, children with social phobia showed greater trait anxiety (measured by the STAI for childrens, STAI-C) and also greater state anxiety and cortisol responses to a public speaking task than controls [98]. In the latter study, trait anxiety was positively related to cortisol, but it was not described whether both control and patients, which already differed in trait anxiety, were included in the same analysis. In children with social phobia, exposure to an adapted TSST resulted in higher baseline and TSTT-induced anxiety (scales for Iconic self-assessment of anxiety in children) than controls [99]. In physiological terms, baseline HR was higher and the response to the stressor lower in patients as compared to controls, whereas salivary cortisol and α-amylase response tended to be lower. Finally, a study comparing healthy controls, social phobia and post-traumatic stress (PTSD) patients showed higher salivary cortisol response to the TSST in social phobics as compared to controls and PTSD [100]. The authors also reported a positive correlation between cortisol response to the TSST and avoidance of angry faces in social phobics but not in controls. Taken together, all those data suggest at least a lower physiological than subjective response to the phobic situations.

Perhaps the strongest evidence for dissociation between subjective and physiological responses comes from patients with panic disorder. These patients have been studied during spontaneous panic attacks, after pharmacological provocation of panic attacks or in response to different types of stressors. During spontaneous attacks, despite strong subjective anxiety and physiological signs, changes in HR were not strong and changes in hormones (noradrenaline, adrenaline, GH and cortisol) were low and inconsistent, being the increases in prolactin the most consistent [83, 101]. When agoraphobic subjects were exposed to the phobic situation to trigger a panic attack, most of them experienced panic attacks while control subjects did not [102], but only the HR was higher in patients than in controls, whereas other measures (i.e. blood pressure, cortisol, prolactin or GH) did not differ. There are several pharmacological manipulations (i.e., lactate, CO2 inhalation, cholecistokinin-4, pentagastrin, doxapram or meta-chlorophenylpiperazine, m-CPP) that have been demonstrated to induce panic attacks only in a few healthy subjects, whereas they strongly induce panic attacks in almost all panic patients. This experimentally controlled approach has been extensively used to compare the physiological response (including GH, prolactin and cortisol) of panic patients and control subjects, but the results are difficult to interpret because of the effects of these manipulations on physiological variables. For instance, m-CPP is a serotoninergic drug that can pharmacologically induce the release of cortisol and GH. If the greater panicogenic effect of the drug on panic patients is paralleled by a greater cortisol and GH release [103], this can be interpreted as a parallelism between the subjective state and hormones, but also as a putative sensitization of brain serotoninergic pathways controlling these hormones in panic patients. Nevertheless, the overall conclusion is again that there are no parallelism between the strong anxiety- and panic-inducing effects of these manipulations in panic patients as compared to controls and the physiological response [104-111].

Finally, some studies aimed at characterizing the physiological response to stressors in panic patients. Fully remitted, medication-free panic patients exposed to a mild psychological stressor showed a clear anticipatory DBP response and a greater cortisol response

to the stressor as compared to a normal population [112]. In another study, in response to public speaking, anticipatory anxiety developed in medication-free symptomatic patients as compared to normal subjects, whereas the anxiety response to the actual stressor was lower [113]. Salivary cortisol showed an anticipatory response, with no further response to the stressor [114], whereas a permanently higher (anticipatory) skin conductance was observed in patients that did not further respond at all to the stressor [113]. No differences were observed in HR, DBP and SBP. The anticipatory plasma or salivary cortisol responses were not detected in a study using the TSST as the stressor that nevertheless showed markedly reduced plasma and saliva cortisol responses in panic patients as compared to controls, associated to a normal HR response [115]. In a very recent report using mild shocks as the stressor, the anxiety and salivary cortisol and α-amylase response was studied in panic patients as compared to controls [116]. Then, patients were treated with the benzodiazepine anxiolytic alprazolam and classified as responder and non-responder to the therapy. When the two groups of patients and controls were retrospectively compared, it was found a similar anticipatory increase in anxiety in the two groups of patients as compared to controls, but an anticipatory increase in α-amylase (but not in cortisol), only in those panic patients who further responded to the therapy with alprazolam. The similar state anxiety response of responders and non-responders accompanied by a differential anticipatory cortisol and α-amylase response demonstrates again the dissociation between subjective and physiological measures.

Table 1 summarizes the relationship between anxiety and the neuroendocrine response to stressors in healthy people and with anxiety disorders. The experimental data indicate a lack of parallelism between subjective state or trait anxiety and neuroendocrine response to stressors in healthy subjects. In fact, there is some evidence for a negative relationship between trait anxiety and physiological response to stressors. Regarding anxiety disorders, a negative relationship is frequently observed in panic and GAD patients, and a lack of association in social phobia.

3. Emotionality, anxiety and neuroendocrine markers in selected rat lines

3.1. Selection on the basis of defecation rate: Maudsley reactive (MR) and Maudsley non-reactive (MRN) rats

The first genetic selection of a putative emotional strain of rats used the criterium of defecation rate in a novel, stressful, environment (an open-field) and led to the characterization of high defecation rate (MR) and low defecation (MRN) lines [see 117]. This selection also resulted in lower activity in the open-field of MR as compared to MNR rats, thus supporting the hypothesis that emotional animals would display a lower level of activity in a stressful, environment. However, it soon became evident that the relationship between defecation rate and activity in the open-field was more controversial than previously assumed and of much lower magnitude than that of defecation. In addition, not consistent differences have been observed in other anxiety test, including the EPM, the acoustic startle response (ASR), the

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light-dark test and the shock-induced conditioned suppression of appetitive operant task [118-121] perhaps related to the existence of two different stocks of rats (UK and USA). Unfortunately, there only two reports comparing the HPA response in the two strains: Abel et al [122] found no differences in plasma corticosterone levels after 10 minutes of exposure to an open-field or to forced swimming. However, Kosti et al. [123] observed greater ACTH response to restraint in MR vs MNR, despite no differences in plasma corticosterone. This apparent discrepancy is likely to be due to increased corticosterone responsiveness to ACTH in MNR. Therefore, MR and MNR, which differ in some aspects of emotionality but not clearly in anxiety-like behaviour, did appear to show differences in HPA function.

Population	Physiological system					
	SMA		HPA		PRL	
Healthy subjects						
State anxiety	≈ *		≈		≈	
Trait anxiety	≈ / ↓		≈ / ↓		≈ / ↓	
Anxiety disorders						
GAD	↓		A / ↓		A / ↓	
Phobia	phobic Ss	others	phobic Ss	others	phobic Ss	others
	↓	?	↓	?	↓	?
Social phobia	≈	?	≈	?	?	?
Panic	panic attack	others	panic attack	others	panic attack	others
	↓	A / ↓	↓	A / ↓	↓	?

≈ : no correlation or approximately normal response (* except α - amylase, see main text)

↓: reduced, at least with respect to subjective anxiety

A : anticipatory response

Ss: stimuli

? : not tested

PRL : prolactin

Table 1. Relationship between normal or pathological anxiety and physiological response to stress.

3.2. Selection on the basis of the EPM: high anxiety and low anxiety rats (HAB, LAB)

The only specific selection process aiming at selecting two strains of rats strongly differing in their performance in the EPM, the most widely used test for anxiety, has resulted in HAB and LAB rats, the former displaying very low levels of exploration of the open arms of the plus-maze [124]. In addition, HAB rats spent less time in light and make less number of transitions in a dark-light test, and also spent less time in the social interaction test [125], confirming differences in anxiety. It is important to note that HAB rats are less active in the forced swimming test [124, 126], a classical test to evaluate antidepressants [127], which pre-

sumably evaluates passive-active coping strategies [128]. Therefore, HAB rat appear to be prone to use passive coping strategies and to depression-like behavior.

HAB showed greater ACTH and corticosterone responses than LAB, mainly when the animals are forced to remain in the open arms (more stressful than the closed arms) of the EPM [129], but not when they can freely explore both open and closed arms [124]. Moreover, no differences were observed in the ACTH and corticosterone responses to forced swim, despite differences in behaviour [124]. Surprisingly, HAB rats showed lower ACTH response than LAB to social defeat [130], demonstrating that differences in responsiveness to stress was dependent on the particular type of stressor used. Therefore, extreme differences in anxiety, evaluated by the EPM, only resulted in consistent differences in the HPA response to situations similar to those that serves as criteria for selection. When exposed to other situations, the results can markedly change. These data are very important because they suggest that individual differences in HPA responsiveness to stress are critically dependent on the type of stressor used.

HAB-LAB rats likely represent the most complete characterization of genetic differences in the HPA axis. In several reports it has been demonstrated enhanced VP gene expression in the PVN, affecting both magnocellular and parvocellular subdivisions [131]. In another report, enhanced PVN CRH expression was also observed [132]. These data suggest increased drive to the corticotrope cells, what is supported by an enhanced POMC gene expression in the anterior pituitary [133]. No differences were observed in CRH-R1 in the anterior pituitary, whereas there were increases in CRH-R2 (the other type of CRH receptor) and V1b receptors in the HAB rats [134]. It is quite possible that VP is responsible for the enhanced ACTH response to the DEX+CRH test in HAB rats [131], as the ACTH response to the mere administration of exogenous CRH was normal [135] and there are no differences between lines in the expression of GR in the anterior pituitary [131, 133]. Although most of the above described changes in the central aspects of the HPA axis may be better ascribed to depression-like rather than anxiety-like behavior, administration of an VP receptor antagonist in the PVN normalize anxiety-like behaviour of HAB rats [134]. This strongly suggests that enhanced PVN VP expression plays a critical role in anxiety.

The data regarding the PVN and the anterior pituitary would suggest increased drive to the gland and a generalized greater ACTH response to stress in HAB rats. However, this is not the case as reported above. A greater adrenal gland is associated in a normal population of rats with greater maximal corticosterone secretion [31]. Therefore, the increased adrenal cortex size of HAB rats is compatible with a greater maximal corticosterone secretion. In fact, HAB rats showed a normal ACTH response to endotoxin accompanied by a greater corticosterone response [136], which is likely to be maximal secretion under these conditions.

3.3. Selection on the basis of active avoidance performance

Several pairs of rat lines have been obtained on the basis of performance in passive or active avoidance tasks in a shuttle-box, using electric footshock as the aversive stimulus. Some, but not all, of these strains appears to differ in emotionality, particularly in fear/anxiety, but is should be taken into account that even if they actually differed in anxiety, also could differ in other

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traits (i.e. novelty-seeking or depression like behavior) that may affect the neuroendocrine response. These caveats should be taken into consideration in the discussion that follows.

The outbred Roman high avoidance (RHA) and Roman low avoidance (RLA) rats were obtained by genetic selection on the basis of performance in a two-way active avoidance task [see 20]. Most of the behavioral and endocrinological studies have been obtained in different substocks of the swiss sublines (RHA/Verh, RLA/Verh) and later by inbred RHA and RLA strains. It was soon realized that the two lines differed not only in active avoidance, but also in terms of emotionality, the RLA rats being more emotional than RHA rats. Subsequent research has demonstrated that the two lines differ in several important behavioral traits, including coping style and impulsivity [20]. The lines differ in some tests of anxiety more markedly than in others, being particularly relevant the inconsistencies regarding the EPM [137].

There have been some discrepancies regarding the responsiveness of the HPA axis to stress in these strains. In 1982, Gentsch et al. [138] firstly reported that RHA/Verh rats showed lower ACTH, corticosterone and prolactin responses to mild stressors (i.e. novel environments) than RLA/Verh rats, but the differences disappeared with stronger (i.e. ether stress, footshock, restraint) stressors. However, inconsistent differences were observed in when the lines were maintained in another laboratory [139, 140]. The study by Walker et al [141] is one of the most complete characterizations of differences in the HPA axis between the two lines. Unfortunately, the results are extremely difficult to interpret. Thus, it was found in RHA as compared to RLA rats: (a) higher adrenal weight; (b) higher basal levels of ACTH accompanied by normal corticosterone levels; (c) no differences in ACTH levels after 10 minutes of exposure to a novel environment or ether (10 minutes), despite an enhanced anterior pituitary response to exogenous CRH administration; (c) a lower corticosterone response to stressor despite the normal levels of ACTH and the increased adrenal weight. In addition, a higher number of GR in the pituitary along with higher MR levels in the hippocampus was found in RHA rats. The higher number of GR in the anterior pituitary may have contributed to the reduced ACTH response to CRH, whereas the higher MR in the hippocampus could be expected, if any, to reduce ACTH response to stress, which was not the case (in absolute terms) in their paper. In further reports, the early findings of increased ACTH and corticosterone responsiveness of RLA rats to novel environments were confirmed [137, 142]. Moreover, RLA rats showed normal levels of CRF mRNA, but increased levels of VP mRNA in the PVNp [142], a pattern observed in situations characterized by a chronic hyperactivity of the HPA axis. At first glance, the latter results suggest that HPA axis of RLA may be generally more responsive to stress than RHA, thus resulting in increased VP gene expression in the PVN. However, one could expect a greater relative adrenal weight in RLA as a consequence of the cumulative impact of higher ACTH response to daily events, but the opposite has been repeatedly found [139, 141, 143]. The possibility remains that the greater adrenal weight of RHA vs RLA rats is a compensatory mechanisms to maintain appropriate adrenocortical secretion despite some defect at the level of the adrenal.

Genetic analysis of cosegregation of different behavioral and physiological variables in these lines has allowed to conclude, in accordance with the inconsistency of the HPA data, that prolactin, but not the variables related to the HPA axis, is probably related to differences in active

avoidance [143]. Even if RLA are characterized by a greater HPA reactivity, the possible influence of behavioral traits other than anxiety on the HPA axis should not be disregarded.

After inbreeding (RHA-I, RLA-I), we have reported normal resting levels of ACTH and corticosterone, but increased response of the two hormones to a novel environment [144]. Enhanced PVN CRH gene expression, but unaltered VP expression in PVNp and PVNm, was also observed in RLA-I versus RHA-I. Quite interestingly, enhanced CRH expression in the RLA-I rats was found in a brain area, the dorsolateral division of the bed nucleus of stria terminalis (BST). As the BST has been repeatedly implicated in the control of anxiety [13], our data suggest that extra-PVN changes in CRH gene expression may participate in some of the behavioral differences between the two strains.

Syracuse Low and Syracuse High avoidance (SLA, SHA) rats, have been also selectively bred on the basis of their behaviour in an active avoidance task (see [145]). Again, SLA and SHA rats appear to differ in emotionality. Thus, SLA rats defecate more in an open-field and show faster learning of a passive avoidance task and more fear conditioned suppression of appetitive instrumental behaviour than SHA, but no differences were observed in sensitivity to shock or activity. Unfortunately, it is not known whether they differ in anxiety as evaluated by the EPM. In accordance with their greater emotionality, SLA rats show a greater glucose response to an open-field [146]. However, SLA rats are characterized by modestly lower corticosterone response to ether stress, but much lower adrenal corticosterone content, as compared with SHA [147]. Similar results were observed after exogenous CRH administration [148]. Quite surprisingly, reduced adrenal corticosterone levels occur despite greater relative adrenal weight and greater size of adrenal cortex in SLA rats [148, 149]. The most likely explanation is that RLA showed a defective adrenocortical responsiveness to ACTH that tended to be compensated by increased adrenal mass. Unfortunately, ACTH levels were not measured in any experiment.

In conclusion, the comparison of the neuroendocrine characteristics of RLA-RHA and SLA-SHA is limited by the lack of information regarding the last pair of lines. Nevertheless, the available information does not reveal a homogenous pattern. Accordingly, in mice, the best performed studied compared several inbred strains of mice in several test for anxiety (EPM, ASR and hyponeophagia) and in basal and stress levels of corticosterone [150]. Whereas a good correlation among the strains was observed with the three tests of anxiety, no correlation was found between anxiety-like behaviour and corticosterone. These data support conclusions in rats.

4. General conclusions

The overall conclusion of the present review is that the physiological response does not reflect concomitant changes in objective anxiety as evaluated by classical tests in laboratory animals or self-reported measures in humans. There are several reasons that can explain such dissociation and the sometimes controversial results. First, the uncertainty about the underlying psychological or behavioral traits of interest and the way we can evaluate them.

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Second, the use of animal lines differing in more than one trait, making difficult to separate the contribution of anxiety from that of other traits. Third, the different dynamics of the behavioral processes and the physiological variables measured. Four, the possibility that others, still not characterized, biological parameters may be more appropriate as biological correlates of anxiety. Finally, there are uncertainties about the relationship between subjective reports of anxiety and the biological response to aversive stimuli.

In laboratory animals, the classical approach has been the selection of the animals in function of particular criterium or test, assuming that this identifies the particular trait of interest, anxiety for the present discussion. However, it is unrealistic to assume that the selection of animals on the basis of one single test can really identify one particular trait. In addition, the experimental evidence strongly indicates that these animals also differ in other different traits, making it difficult to isolate anxiety for other traits. For instance, HAB-LAB rats not only differ in anxiety but also in depression-like behavior [124]. Similarly RLA and RHA rats also differ in impulsivity [137].

The most widely used physiological responses are those related to the SMA and the HPA axis, in addition to other hormones such as prolactin. The different indices greatly differ in terms of the time needed to reflect changes in the environment. Cardiovascular changes (i.e. HR, blood pressure) can rapidly change in one minute, plasma levels of adrenaline and noradrenaline is also very fast and their half-life is very short, thus resulting in the possibility of marked changes in periods of 5 minutes. Plasma levels of anterior pituitary hormones are released very fast (a few minutes), but half-life is longer than that of catecholamines (between 5 and 30 minutes or more, depending on the particular hormone). Finally, changes in plasma or salivary cortisol are relatively slow, with maximum no more than 15-30 minutes after the initial exposure to the situation. Thus, the dynamics of the response is important when considering the influence of cognitive processes in the regulation of the emotional response to the situation.

Although more elaborated endocrinological studies may help to elucidate some controversial results, it is important to look at other physiological variables. For instance, a recent study observed lower plasma levels of nesfatin-1, a recently characterized satiety molecule, in GAD patients [151]. Immunological markers are currently studied regarding stress and personality factors. In one interesting paper in a large population of men and women, anxiety positively correlated to levels of certain inflammatory markers (C-Reactive Protein, interleukin-6, Tumor Necrosis Factor-α and fibrinogen) [152]. Characterization of putative inflammatory markers of anxiety requires further studies.

In humans, psychological traits are complex constructs that involve top-down cognitive processes. In contrast, physiological response to aversive situations is likely to be reflexive in nature at least initially. It is possible that both processes are relatively independent. Rapid attention and responding to putatively threatening stimuli is a characteristic of several anxiety disorders and healthy people with high neuroticism or trait-anxiety [153]. In a very interesting study, preconscious and conscious attention biases to emotional stimuli were evaluated in subjects exposed 4 and 8 months later to a laboratory stressor or to examination, respectively [154]. Preconscious negative bias processing was a better predictor of cortisol response than self-reported neuroticism, trait-anxiety or extraversion.

Another additional problem when addressing human data is the limitation of the information we can obtain from typical laboratory stressors. First, emotional processing of stressors may be complex and dependent on the particular nature of the situation. Anxiety disorders may be associated to a differential processing of certain categories of stressors but not all stressors and therefore information obtain from exposure to standard stressors may be limited and different depending on the particular type of anxiety disorder. Second, laboratory stressors tend to be of lower intensity that some real-life stressors and it is unclear whether or not we can extrapolate the results from one type to the other.

Even if we can identify physiological variables related to pathological anxiety, an important concern is whether these variables are the consequence of the pathology or a predisposing factor. In the last year particular attention has been paid to this problem, but it is still an important drawback when analyzing published data.

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