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**Differential hypothalamic-pituitary-adrenal response to stress among rat strains: methodological considerations and relevance for neuropsychiatric research**

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**Abstract:**

The hormones of the hypothalamic-pituitary-adrenal (HPA) axis and particularly glucocorticoids (GCs) are considered to play a critical role in the behavioral and physiological consequences of exposure to stress. For this reason, numerous studies have described differences in HPA function between different rodent strains/lines obtained by genetic selection of certain characteristics not directly related to the HPA axis. These studies have demonstrated a complex and still poorly understood relationship between HPA function and certain relevant behavioral characteristics. The present review first remarks important methodological considerations regarding the evaluation and interpretation of resting and stress levels of HPA hormones. Then, it presents works in which differences in HPA function between Lewis and Fischer rats were explored as a model for how to approach other strain comparisons. After that, differences in the HPA axis between classical strain pairs (e.g. High and Low anxiety rats, Roman high- and low-avoidance, Wistar Kyoto versus Spontaneously Hypertensive or other strains, Flinder Sensitive and Flinder Resistant lines) are described. Finally, after discussing the relationship between HPA differences and relevant behavioral traits (anxiety-like and depression-like behavior and coping style), a guide for main methodological and interpretative concerns and how to test strain differences is offered.

## 1. INTRODUCTION

### 1.1. The hypothalamic-pituitary-adrenal axis and the stress response

Although there is no fully agreement regarding the definition of stress, we consider one of the most appropriate that proposed by Vigas [1]. He defined stress as the response of the organism, evolved in the course of phylogeny, to agents actually or symbolically endangering its integrity. This definition includes both systemic and emotional stressors. Systemic stressors refer to situations implying real harm or the need for strong metabolic demands to the organisms (systemic stressors), which cannot be solved only with normal homeostatic mechanisms. Emotional stressors include situations that are not harmful on their own but predict a certain possibility of real danger or strong metabolic demand (emotional stressors). These two types of stressors lead to a reactive or anticipatory response, respectively, and are differentially processed by the brain [2]. We prefer the terms systemic-emotional instead of physical-psychological to avoid the mind-body dualism. Exposure to stressors triggers a myriad of biological changes in the organism, but we will mainly focus on the hypothalamic-pituitary-adrenal (HPA) axis.

Glucocorticoids (GCs) are the final hormones of the HPA axis and the most extensively studied stress hormone. The prominent role of GCs in stress research is due to their widespread and critical function in an important set of physiological and behavioral consequences of exposure to acute and chronic stress. However, the response to stress is extremely complex and the activation of the HPA axis with the final release of GCs (cortisol in humans and most mammals, corticosterone in rats and mice) is only one aspect of such response. Consequently, there are other important biological players. Although the present review will focus on the HPA axis, other physiological responses, particularly hormones, will be commented on when appropriate.

Despite the agreement regarding the adaptive role of GCs to cope with stress, the wide range of actions and organs affected by GCs has made it difficult to delineate a clear picture of their role in stress. Nevertheless, considering the most common stressful situation in nature, coping with the presence of a predator, it is unlikely that GCs play a critical role to solve the present situation. The presence of a predator can elicit different behavioral responses depending on the distance to the predator (from long to short): flight, freezing or fight. The flight-fight response might require immediate and intense physical activity, but GC release is only evident 5 min after stress starts, with the maximum at 20-30 min. Therefore, the immediate response to the presence of a predator is critically dependent on sympathetic activation and the release of catecholamines rather than on GCs. GCs might, in turn, be relevant to cope with prolonged (hours) exposure to stressors (gluconeogenesis), to recover from the present situation, and to prepare for future situations [3], including memory of the previously encountered stressor [4].

It is well-known that there are important individual differences in the physiological and behavioral consequences of exposure to stress in all species. However, the precise mechanisms involved in such differences are poorly known. The characterization of individual differences in the neuroendocrine response to stress is an important issue for several reasons. First, some classical stress hormones, particularly those of the HPA axis (ACTH and cortisol/corticosterone) and prolactin, are among the few biological markers that have been demonstrated to be sensitive to the intensity of emotional stressors [5]. Therefore, a greater response might be suggestive of an enhanced vulnerability to stress. Second, stress-induced GCs release is one of the major mediators of the physiological and behavioral consequences of stress. Thus, an inappropriate response (lower or higher than normal) is likely to contribute to the negative consequences of stress [6, 7, 8]. Third, altered neuroendocrine responsiveness might be the reflection of alterations in neurotransmitters and circuits regulating such responsiveness, and these abnormalities might extend to other circuits also involving the same neurotransmitters. Not surprisingly, there has been over decades a marked interest in the possible alterations of the HPA axis in psychiatric diseases, including unipolar and bipolar depression, schizophrenia, anxiety disorders, and posttraumatic stress disorder, although the picture is far from clear (see [9] for review).

To better understand the putative meaning of such differences, a brief outlining of the HPA axis is needed [10, 11]. The key area in the control of the HPA axis is the paraventricular nucleus of the hypothalamus (PVN), particularly the medial parvocellular dorsal subdivision (mpdPVN) where neurons synthesizing the corticotropin-releasing factor or hormone (CRF or CRF) are mainly located. These neurons project to the pituitary portal blood of the median eminence, where CRH is released to reach the anterior pituitary corticotrope cells. CRH acts in corticotrope cells through CRH type 1 receptors (CRHR1) to induce the

synthesis of the ACTH precursor proopiomelanocortin (POMC) and the release of ACTH into the bloodstream. Vasopressin acts on corticotrope cells favoring the effects of CRH on ACTH release. This is of interest because some mpdPVN CRH neurons also co-express vasopressin and the number of double-positive neurons increased after chronic hypersecretion of ACTH caused by either adrenalectomy or chronic stress exposure [12].

ACTH acts through melanocortin type 2 receptors in the cells of the zone fasciculata of the adrenal cortex to induce the synthesis and release of GCs. It is important to note that some factors other than ACTH are capable of activating the adrenal without parallel changes in circulating ACTH release or can modulate adrenal sensitivity to circulating ACTH. The best characterized of such factors is the sympathetic innervation of the adrenal gland, which is presumably involved in the circadian changes in adrenal sensitivity to ACTH and the dissociation between ACTH and corticosterone observed after exposure to prolonged stress [13].

The activity of the HPA axis is subjected to negative feedback by corticosteroids that constrains both resting and stress levels of ACTH [14]. This negative feedback is exerted through the concerted action of mineralocorticoid (type I; MR) and glucocorticoid (type II, GR) receptors acting at the corticotropes and at different brain levels, including the PVN, the hippocampal formation, and the medial prefrontal cortex [14]. Negative feedback involves both non-genomic fast effects and more delayed genomic mechanisms. Although suppression by the synthetic glucocorticoid dexamethasone (DEX) is typically used in most studies in humans and animals, its use has been questioned as its access to the brain is limited by the multidrug-resistant protein 1 (MDR1) that excludes DEX from the brain to a higher extent than natural GCs [15, 16]. Corticosterone is even less excluded than cortisol [15, 16]. As a consequence, the effect of DEX, in contrast to natural GCs, is mainly exerted at the level of the anterior pituitary corticotrope cells.

## **1.2. Studying strain differences in the HPA axis: resting and stress levels: general overview**

Interest in the characterization of individual or strain differences in the activity of the HPA axis has focused on resting levels or, more frequently, on its responsiveness to stressors. Both topics are of potential interest. A nice description of the main aspects to be considered when assessing the activity of the HPA axis can be found in Spencer & Deak's review [11]. Circulating levels of GCs show a marked rhythmicity characterized by both pulsatile secretion and circadian fluctuations. The latter is strongly associated with the daily pattern of activity. Thus, in humans, the highest levels are observed after awakening or in the next hour and the lowest levels in the first sleeping hours, whereas in rats (and mice) low levels are observed at lights on and the highest levels around lights off. The pulsatile and circadian nature of glucocorticoid secretion has important methodological consequences when trying to characterize individual differences. Regarding circadian rhythms, blood levels need to be evaluated at different times of the day to know whether overall secretion is altered or alterations are restricted to specific day times. This is an important issue as it is now acknowledged that a flattened circadian glucocorticoid rhythm might have functional consequences, even if overall secretion is not altered. Pulsatile secretion implies that blood levels might markedly differ within a particular individual from moment to moment. Therefore, a unique sample is far to be representative of individual differences. These problems have been reduced in humans by using the aggregated data of samples taken on various days (e.g. [17]) and the same strategy has been adopted by our laboratory in rats recently [18]. Using this strategy, more representative values are obtained. If strains rather than individual differences are evaluated, aggregated data are not needed, but still obtaining samples at various times across the day would be required to detect overall secretion and possible changes in the circadian rhythm amplitude.

Regardless of the number of samples obtained, a major concern when reviewing available literature is the apparent difficulty to obtain true resting levels of HPA hormones. In our hands, with radioimmunoassay (RIA) procedures, true resting levels of corticosterone in male rats are 10-20 ng/ml at the nadir and 150-200 ng/ml at the peak. An inspection of the literature reveals that most of the studies report values considerably higher, particularly at lights on. Corticosterone levels are extremely sensitive to minor perturbations in the animal room and most animal facilities are not designed to work in stress. It could be argued that all animals or strains are exposed to the same perturbations and therefore, results are representative of subject/strain differences. However, there are multiples—examples of normal resting activity but altered responsiveness to stress. Consequently, we can erroneously interpret strain differences

in stress responsiveness as strain differences in resting HPA activity. Obviously, strains might often differ in both aspects.

The problems to obtain true resting levels of HPA hormones are due to a combination of some critical aspects. First, noise or any other perturbation in the animal room caused by construction near the animal facility [19], by unexperienced animal caretakers in the period preceding the experiment or by unexperienced or poorly trained researchers entering the animal room and touching the cages [20]. Second, the time elapsed between taking the cages and blood sampling: simply moving a cage to another place can alter hormone levels; also touching the cages and taking one animal can alter corticosterone levels in the animals remaining in the cages that would be sampled later [21]. This is particularly relevant in group-housed animals. Finally, the method of blood sampling: true basal levels are obtained by the tail-nick procedure as compared to rapid decapitation [22] if researchers have experience with the procedure. In contrast, all anesthetics except perhaps pentobarbital are known to strongly activate the HPA axis (see [10]) and this should be always avoided.

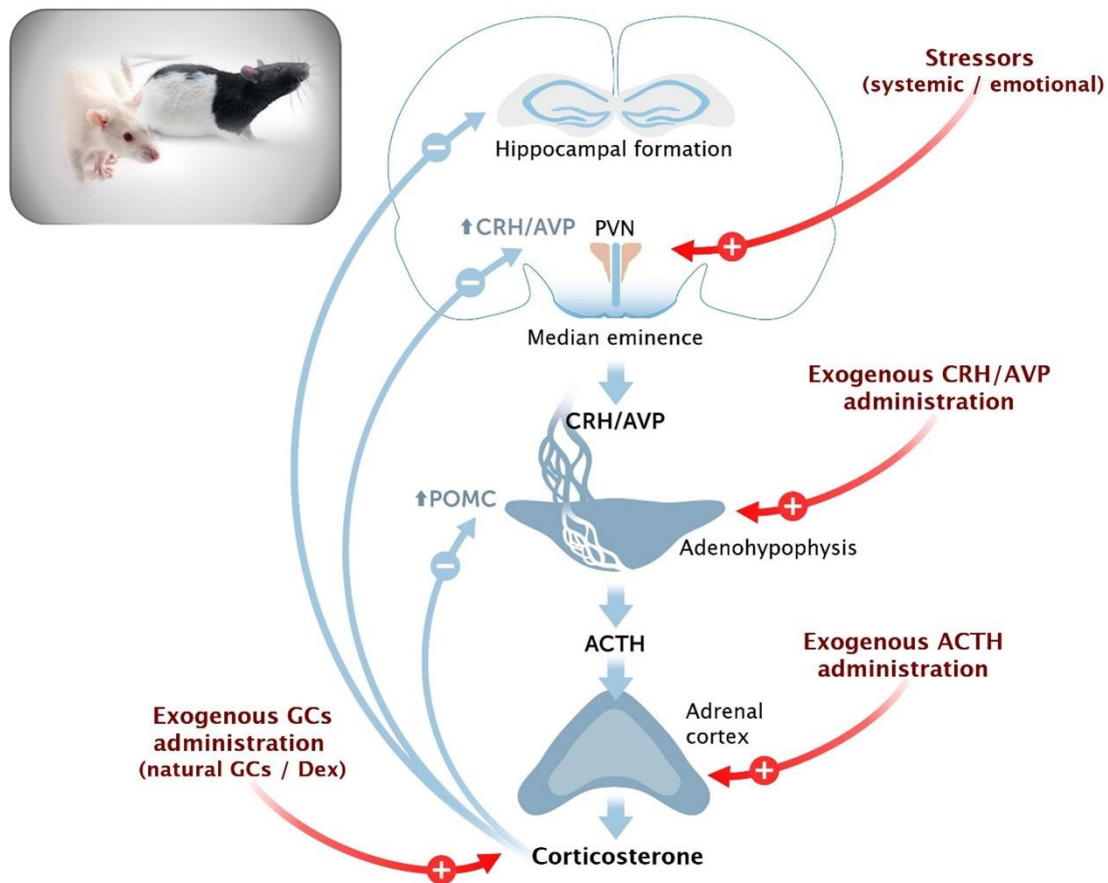
What about possible concerns in the interpretation of the observed subjects/strain differences in HPA responsiveness? Again, the interpretation of the data is very often excessively simple or even partially erroneous for several reasons. It is important to consider the possibility that differences in stress responsiveness are due to vendors rather than to strains when not all animals are from the same vendor or breeding center [23]. Regarding variables measured, it is frequent that comparisons are restricted to blood levels of corticosterone, with the assumption that corticosterone would reflect ACTH release. However, it is well-known that adrenocortical secretion reaches a maximum with intermediate levels of ACTH and therefore is unable to reflect possible differences in ACTH when relatively strong stressors are used [5]. Only when stressors are of low intensity (e.g. open-field exposure) or blood corticosterone levels are followed after the termination of stressor exposure is corticosterone reflecting ACTH release. Moreover, individual or strain differences might exist in adrenocortical sensitivity to ACTH and therefore differences in corticosterone do not necessarily reflect differences in ACTH. Strain differences in other molecules participating in the activity of the HPA axis have been less explored. In this regard, blood levels of transcortin (corticosterone-binding globulin, CBG) are particularly important. CBG levels determine the free levels of corticosterone, considered the biologically active fraction. As measuring free plasma GCs is technically demanding, measurement of total GCs together with CGB can give us an idea of free GCs fraction. An indirect way of detecting possible overall differences in corticosterone is thymus weight, which is very sensitive to circulating levels of corticosterone in rats (e.g. [24]). Recently, hair corticosterone concentration has been pointed out as an integrated measure of free corticosterone levels over periods of a few weeks [25]. Hence, this parameter allows for detect, with a simple measure, possible strain differences [26].

Another important consideration to characterize individual/strain differences is the type of stressor. If differences in stress responsiveness are the result of brain processing of stressors upstream of the PVN, the type of stressor is clearly relevant, particularly when comparing systemic versus emotional stressors. An example of the critical contribution of the type of stressors is the comparison of Roman strains reported by Gentsch and colleagues [27], who demonstrated higher ACTH response in Roman-low avoidance (RLA) than Roman-high avoidance (RHA) after exposure to relatively mild stressors but not in the case of severe stressors. In general, even within emotional or predominantly emotional stressors, the contribution of qualitative and quantitative aspects of stressors might be relevant.

If two strains markedly differ in basal and/or stress levels of ACTH, this is likely to be the consequence of differences in the inputs to the PVN or the responsiveness of parvocellular neurons synthesizing CRH and/or vasopressin. Expected canonical differences in those strains showing higher ACTH levels are a higher expression of CRH in the mpdPVN and of POMC in the anterior pituitary. If only subtle differences in ACTH levels are observed, it is possible that no differences in CRH and POMC expression are observed.

In some cases, altered function of the HPA axis can be related to alterations in GCs negative feedback. This has been typically tested by injecting DEX and the interpretation of the results is problematic considering that DEX mainly acts at the level of corticotrope cells, not in the brain, as previously commented. Even if natural GCs are used, negative feedback can only be accurately assessed by measuring ACTH not corticosterone, and the precise brain area involved in differential negative feedback cannot be delineated after systemic administration.

Possible differences in the HPA axis have been explored in various strains of rats and mice genetically selected for criteria not related to the HPA axis. In some cases, a pair of strains from a common origin were obtained that dramatically differ in a particular characteristic. It is not our intention to exhaustively review all these pairs of strains but to use some of them to illustrate how to investigate the HPA function. We will focus particularly on HPA hormones and the main hypothalamic secretagogue (CRH) and not on GCs receptors either in the periphery or the brain. A schematic illustration of the HPA axis and its regulation, as well as the main stimuli employed to test differences in this axis, is shown in **Figure 1**. Importantly, we have tried to incorporate most results from the literature in the present review, but our purpose has been to be illustrative rather than exhaustive.



**Figure 1: A schematic view of the HPA axis and how to test functional strain differences**

Possible loci of strain differences in the regulation of the HPA axis, an example of a complex neuroendocrine system. Differences might exist under resting conditions that are likely to be reflected also in response to stressors, but altered response to stressors or other stimuli might exist in absence of differences under resting conditions. Main stimuli used to test differences in the HPA axis are indicated in red. ACTH: adrenocorticotrophic hormone; AVP: vasopressin; CRH: corticotropin-releasing hormone; DEX: dexamethasone; GCs: glucocorticoids; POMC: proopiomelanocortin (precursor of ACTH); PVN: paraventricular nucleus of the hypothalamus.

## 2. LEWIS-FISCHER RATS AS A REFERENCE FOR HOW TO APPROACH STRAIN DIFFERENCES IN THE HPA AXIS

Inbred Lewis rats are mainly characterized by a particular susceptibility to develop experimental arthritis after systemic injection of streptococcal cell wall (SCW) compared with the histocompatible inbred Fischer strain. It was soon realized that the two strains differed in HPA activity and this could be related to the differential susceptibility of Lewis rats to arthritis (see below). Consequently, the two strains have been extensively studied and constitute an example of possible alterations at different levels of the HPA axis. From the recent perspective of possible sex differences, the Lewis-Fischer models are of great interest as



most earlier results were obtained in females, where the differential susceptibility to experimental arthritis is more prominent and was firstly described.

Although no differences in morning basal corticosterone levels between females of the two strains were originally reported [28], when the circadian pattern rather than a single time point was studied, lower corticosterone levels were observed in Lewis versus Fischer around the lights off peak [29, 28].

Importantly, marked differences were found between females of the two strains in ACTH and corticosterone responses to different types of immune (SCW, interleukin-1 $\alpha$ ), systemic (ether) and emotional (open field, restraint, forced swim) stressors, with a lower response in Lewis rats [28, 30]. Similarly, a defective corticosterone response to another immune stressor (endotoxin) was further observed [31]. Impaired HPA response of female Lewis rats was not restricted to typical stressors and can also be observed in response to drugs known to activate the HPA axis through various neurotransmitter receptors, including muscarinic, adrenergic, and serotonergic [28, 32]. The reduced response of Lewis rats to so many different stimuli strongly suggests that the main defect could be at the level of the PVN itself and probably could affect CRH gene expression and release. Supporting this, basal CRH gene expression did not differ between the two strains in the PVN, but a defective response to SCW, IL-1 $\alpha$  and restraint was observed [33, 30] along with a reduced *c-fos* response to water avoidance stress [34]. Another study reported lower levels of basal CRH gene expression in the whole PVN associated with higher levels of vasopressin gene expression [35] and enhanced *in vitro* hypothalamic release of vasopressin [36]. The authors suggested that enhanced vasopressin expression and release could be a compensatory mechanism for reduced CRH activity. However, these results illustrate an important problem regarding the possible role of vasopressin in the control of the HPA axis: the procedures did not distinguish between magnocellular vasopressin neurons that project to the neurohypophysis and parvocellular vasopressin neurons of the mpdPVN, which are those presumable involved in the control of ACTH release. Therefore, the functional meaning of these higher levels of vasopressin in Lewis rats remains unclear.

A main alteration at the level of the PVN and CRH neurons does not preclude other downstream alterations. For instance, reduced *in vivo* ACTH response to exogenous CRH administration was also reported [28, 37], suggesting differences at the level of corticotrope cells in the anterior pituitary. To our knowledge, only one study has explored corticosterone responsiveness to exogenous ACTH administration to detect differences at the level of the adrenal cortex [38]. In that study, a lower lasting rather than lower maximum corticosterone response was observed in both male and female rats Lewis versus Fischer, tentatively suggesting impaired adrenocortical responsiveness in Lewis of both sexes.

Are the same strain differences observed in males? Whereas the above data in female rats are quite consistent, differences between males of the two strains are less obvious, although the overall data fit with results in females. Male Lewis and Fischer rats were first compared by Dhabhar and colleagues [39] who found lower basal corticosterone levels in Lewis than Fischer in most times of the circadian rhythm, although levels were also higher in Fischer when compared with outbred Sprague-Dawley (SD) rats. Differences in the circadian rhythm between Lewis and Fischer were later confirmed [40, 41]. However, in those studies where only basal ACTH and/or corticosterone levels were assessed at a single time point in the morning, no differences were usually observed (e.g. [42, 43, 44]). Despite no strain differences in morning basal levels, a lower HPA response to forced swim [43, 45], restraint [44, 46, 47], transfer in a bucket [48], noise [49], or morphine [50] has been found in Lewis than Fischer. In contrast, no differences in basal or stress corticosterone levels were found between Lewis, Fischer, SD and Brown-Norway rats in response to foot-hocks [51], although the negative results could be due to adrenocortical saturation of corticosterone synthesis. This saturation can explain why, in response to restraint, lower corticosterone response of Lewis versus Fischer was only noted during the post-stress recovery period [52]. Fischer rats appear to be characterized by a more sustained HPA activation after exposure to acute restraint stress for 4 h or after daily repeated restraint, compared with both Lewis and SD rats [46].

In accordance with data in females, Lewis and Fischer males do not appear to differ in basal CRH gene expression in the PVN [41, 53]. We are not aware of any study about PVN CRH gene expression after acute stress in males of these strains, but chronic immobilization (IMO)-induced increase in CRH gene expression was similar in the two strains [41]. More recently, Ergang and associates [54] compared males exposed or not to a 3 days restraint stress protocol with sacrifice at 2 h after the last stressor and studied various parameters related to central aspects of the HPA axis. Although this protocol does not allow to distinguish

between the protracted consequences of prior restraint and that of the last restraint exposure, no differences between Lewis and Fischer rats were observed in PVN CRH or vasopressin gene expression

In some studies where both males and females were simultaneously studied, a lower HPA response to forced swim, restraint or a novel environment was observed in Lewis than Fischer rats of the two sexes [43, 55, 56]. In contrast, Gomez-Serrano et al [57] also studied both sexes and reported lower corticosterone response to endotoxin (at 2 h) in Lewis than Fischer females, but no differences in males. Spinedi et al [42] did not observe any difference between Lewis and Fischer of the two sexes in ACTH response to insulin-induced hypoglycemia or ether, but did observe a reduced response to immune stimuli (e.g. endotoxin), though restricted to females. That altered PVN function is more dramatic in female and male Lewis rats is supported by the finding that, in contrast to the reduced *c-fos* expression after stress observed in the PVN of Lewis vs Fischer females, no differences in *c-fos* response to footshock or IL-1 $\beta$  has been found in males [58]. In sum, the lower HPA response to stress of male Lewis compared with male Fischer rats might not be so general as that of females.

Interestingly, to our knowledge, only one laboratory has measured CBG levels in these two strains and also in SD rats [39, 44], but they did not include females. After exposure to restraint stress, CBG levels were higher in Fischer than Lewis, but despite these higher CBG levels, free corticosterone levels were still higher in Fischer than in the other strains, with no differences between Lewis and SD. These results point again to the idea that Fischer rats are in the extreme of high HPA responsiveness to stressors.

When looking at possible overall indexes of HPA activity, results indicate a lower relative adrenal weight and higher relative thymus weight in both male and female Lewis rats compared to Fischer rats [28, 59, 41, 46, 53, 60], confirming lower HPA activity in Lewis. Although the integrity of negative GCs feedback has been assessed in only one study, it appears that Lewis and Fischer do not differ in sensitivity of ACTH and corticosterone response to DEX in stress conditions. However, blockade of corticosterone synthesis increased ACTH in Fischer and other strains but not in Lewis [61]. This supports the hypothesis of impaired capability of the HPA axis of Lewis to stimulatory inputs rather than enhanced negative feedback. In fact, no differences in GR or MR expression in the hippocampal formation have been found between the two strains [41].

To demonstrate that Lewis and Fischer are likely to be in the extreme of differential HPA responsiveness, comparisons with other strains are very useful. Both male and female Lewis rats showed lower ACTH and corticosterone responses to forced swimming than Fischer, but also than other inbred strains such as Brown-Norway, spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) [43, 45]. The impaired ACTH and corticosterone response to stress (novel environment) of female Lewis rats is also observed compared with two other strains (Brown-Norway and Dark Agouti) [62]. In males, it has been reported reduced ACTH response to various stressors (tail-cut and sampling, open-field, IL-1 $\beta$ , ether-surgery), with low or null differences in corticosterone [63]. In addition, reduced CRH gene expression in the PVN under basal conditions and impaired *in vitro* adrenocortical responsiveness to ACTH were found, suggesting also defective adrenocortical function. When compared with SD rats, Lewis also showed reduced ACTH [64] and corticosterone responses [46, 65, 47] to restraint. Reduced corticosterone response to amphetamine was also observed [65]. The reduced HPA response to restraint in Lewis compared with SD is compatible with a reduced *c-fos* response in the PVN [66]. In the latter study, strain differences in *c-fos* expression were dependent on the particular brain area, with a reduced response being observed only in a subset of the areas studied, for instance, the PVN and the medial prefrontal cortex, whereas in others (locus coeruleus) even a higher response was found.

The above data indicate that defective HPA function is a characteristic of Lewis rats as compared with some other rat strains and not only with Fischer. However, some studies also suggest that Fischer rats might be characterized by certain HPA hyperactivity as compared with other inbred rat strains (e.g. [43, 67]). Comparing male Fischer and SD, the former showed higher corticosterone response to restraint, but differences were greater after chronic restraint, suggesting also impaired adaptation [39, 44, 46, 68].

### **3. HPA ACTIVITY IN STRAINS OR LINES DIFFERING IN EMOTIONALITY AND COPING**

#### **3.1. Maudsley rats**

Maudsley reactive and non-reactive (MR, MNR) strains were selected on the basis of emotional reactivity (mainly defecation rate) to open-field exposure, the MRN being the one most deviating from the outbred population [69, 70]. The two strains differ in other behaviors, and enhanced anxiety (elevated plus maze, EPM) and immobility (forced swim) have been observed in MR [71] associated with lower acoustic startle response [72]. The two strains do not differ either in basal levels of corticosterone or their responsiveness to an open-field, restraint or forced swim [73, 74]. More recently, the lack of differences between the two strains was confirmed by measuring both ACTH and corticosterone responses to an open-field or inescapable foot-shocks [75]. Interestingly, all the above results were obtained from colonies maintained in the USA. In the only study using MR and MNR rats bred outside the USA (i.e. from the Queen Mary Univ. London), greater ACTH but normal corticosterone response to 30 min restraint was observed in MR than MNR rats [76]. The reasons underlying these differences are unclear, but genetic drift or animal facility breeding differences could contribute.

#### **3.2. High and Low anxiety-related behavior lines (HAB, LAB)**

HAB and LAB rats were selected from Wistar on the basis of open arm avoidance in EPM and this also resulted in HAB rats being less active in novel environments and more passive in the forced swim test (FST) [77]. This strongly suggests that HAB rats are both more anxious and prone to adopt passive strategies than LAB rats. Interestingly, whereas no ACTH or corticosterone differences were found in response to the EPM or the FST [77], greater HPA and prolactin responses were observed after forced exposure to the open arms of an EPM [78], suggesting that is forced exposure to the open space that caused the greater response. In a further study, greater ACTH and corticosterone levels in response to CRH in DEX-treated rats (DEX+CRH test) were found in HAB together with greater vasopressin PVN expression [79]. However, in response to social defeat, greater rather than lower HPA response was found in LAB vs HAB rats [80]. These findings are of major relevance as they illustrate two major points: first, anxiety is not necessarily related to increased HPA responsiveness to stressors; and, second, exposure to different types of emotional stressors is important when characterizing individual/strain differences.

#### **3.3. Performance in two-way active avoidance tasks (TWAA)**

##### **3.3.1. Syracuse rats**

Syracuse high and low avoidance (SHA, SLA) rats originated from Long-Evans rats and differ not only in TWAA, but also in other behaviors: SLA showed normal open-field activity, improved passive avoidance and greater conditioned emotional response [81, 82, 83]. SLA showed higher adrenal weight and adrenal cortex and medulla size [84, 85, 86]. In both sexes, SLA showed higher adrenal and probably higher basal corticosterone levels, but a similar response to ether exposure (although adrenal content was higher in SHA) [85, 83]. Given that only the response to a high-intensity systemic stressor was studied and that ACTH response was not measured, no firm conclusions can be drawn, although greater HPA activity associated with low avoidance cannot be disregarded.

##### **3.3.2 Hatano rats**

Hatano high and low avoidance rats (HAA, LAA) derived from SD [87]. The LAA rats of both sexes have very poor avoidance performance that does not improve over the days and HAA rats, in addition to a good performance in the TWAA task, are much more active in a running-wheel and an open-field [87, 88]. In both sexes, the adrenal weight was found to be higher in HAA than LAA and the ACTH response to the task was also clearly higher in HAA, with no differences in corticosterone [89]. In a further study, ACTH response to another emotional stressor (restraint) was markedly higher in HAA males, whereas changes in corticosterone were complex but not different overall [90]. In the latter study, the higher ACTH response was not a reflection of generalized neuroendocrine hyperresponsiveness as prolactin response to restraint was lower in HAA. However, whereas the data regarding HPA response to restraint was further replicated, no differences in prolactin were found [91]. In the most recent study with these strains, the neuroendocrine response to the first and the last of three TWAA sessions was assessed [92]. HAA showed greater ACTH but lower corticosterone responses on days 1 and 3. The greater ACTH response was consistent with

previous studies, but the lower corticosterone response was not. If consistent, data might be suggestive of an impaired adrenocortical responsiveness to ACTH in HAA rats despite their higher adrenal weight.

### **3.3.3. Roman high and low avoidance rats**

Roman high and low avoidance (RHA, RLA) lines were generated by Bignami [93] in Italy on the basis of performance in TWAA. It has been later demonstrated that these lines also differ in several behavioral characteristics, RLA being more anxious and emotional and more prone to adopt passive coping strategies [94]. Characterization of the neuroendocrine response to stress in these lines was firstly conducted in animals maintained in Driscoll's lab in Switzerland. In a seminal and elegantly designed study, Gentsch et al [27] showed that RLA and RHA lines did not differ in basal levels of classical stress markers (ACTH, corticosterone, prolactin or glucose). However, RLA showed a greater endocrine response when exposed to relatively mild stressors (saline injection, novel cage, open-field), but not to more severe stressors such as ether, immobilization or inescapable foot-shocks (except greater prolactin levels after foot-shocks). In contrast, no differences in the glucose response to any stressor were found. A further study confirmed the greater HPA response of RLA rats to mild stressors in addition to a greater *in vivo* ACTH response to exogenous CRH [95]. However, relative adrenal weight has given inconsistent results [96, 95]. In sublines derived from Switzerland stock but bred in Bordeaux higher HPA response to an open-field in RLA was only observed at certain ages, whereas consistently higher prolactin response was found at all ages [97]. It thus appears that the greater stress sensitivity of RLA rats is better observed with prolactin. Interestingly, neonatal handling decreased HPA and prolactin stress responsiveness of RLA rats and did not affect RHA rats, thus eliminating the typical higher neuroendocrine responsiveness of RLA rats [98].

Are there differences in the HPA axis at the level of the PVN? Enhanced corticosterone response to an open-field in RLA was associated with normal CRH gene expression, but enhanced vasopressin expression in the mpdPVN [99]. This is of interest as vasopressin gene expression in the mpdPVN typically increases when the HPA axis is chronically more active [12]. Taking advantage of the inbreeding process carried out in these lines, we performed a characterization of the HPA axis in RHA-I and RLA-I rats [100]. We found no differences in basal levels of ACTH or corticosterone, but a higher HPA response to mild stressors in RLA rats. In addition, we also detected a higher *Crh* gene expression in the PVN, suggestive of a more active HPA axis, with no differences in adrenal weight or the expression of corticosteroid receptors (GR or MR) in critical brain regions, including the PVN. A further study in these inbred animals has confirmed the higher HPA and prolactin responses to a novel environment of RLA versus RHA rats and showed that the pattern of the former rats was similar to that of genetically heterogeneous stock [101]. Overall, the data suggest lower neuroendocrine responsiveness to stress in RHA rats.

## **3.4. HPA ACTIVITY IN RAT STRAIN/LINES SHOWING DEPRESSION-LIKE BEHAVIOR**

### **3.4.1. Wistar Kyoto rats**

WKY and SHR derived from Wistar rat ancestor in Kyoto, but they have been obtained by two independent inbreeding processes [102, 103]. Interest was initially focused on SHR as a putative animal model of essential hypertension and some studies in this regard have compared SHR with WKY, whereas other studies compared SHR with outbred Wistar or other strains. From a behavioral perspective, they are of interest as putative animal models of attention deficit and hyperactivity disorder (SHR) [104] and depression (WKY) [105]. WKY rats were initially characterized by extreme passive behavior in the FST and for being resistant to antidepressants (e.g. [106, 107, 108]).

There is ample evidence for enhanced sympathetic activity and stress-induced catecholamine release under stress conditions in SHR compared with WKY and other normotensive rat strains [109] and this will not be discussed here. Importantly, when SHR and WKY have been studied together with WK-HA (hyperactive) and WK-HT (hypertensive), greater plasma catecholamine response to stress is associated with hyperactivity and not hypertension [110]. In the present review, we will focus on alterations in the HPA axis in WKY as compared with SHR and other strains [110]. Since most studies comparing WKY and SHR have been done in males, specific references to sex will be made only when females or the two sexes were used.

Inconsistent results have been reported regarding relative adrenal weight, with greater [111, 112, 113, 114, 115] or similar [116, 117, 118, 119, 120] weight in male SHR compared with WKY. In a study with both

sexes and three different ages in adult rats, no differences were generally observed in adrenal weight, whereas, in contrast, consistently reduced thymus weight was found in SHR [121]. Suggesting hyperactivity of the HPA axis in SHR versus WKY. However, when compared WKY with Brown-Norway or SD rats, there is no evidence of lower adrenal weight in WKY [122, 123].

Male WKY compared with SHR have been shown to have lower [124, 125, 126], similar [127, 43, 41, 61] or higher [128] basal levels of corticosterone release. Hashimoto et al [126] showed lower corticosterone levels in male WKY despite similar ACTH levels. Age might contribute to discrepancies as similar basal corticosterone levels were observed in WKY and SHR between 4 and 12 weeks and transiently higher levels at 16 weeks in WKY that normalized again at 20 weeks [129].

CRH gene expression in the PVN has been found to be either similar in WKY and SHR [41] or lower in WKY and Wistar compared with SHR [130]. Nevertheless, differences were more evident in the latter study after exposure to acute restraint stress. No differences in POMC gene expression in the anterior pituitary have been observed in one study [131], whereas in another POMC gene expression was higher in WKY than SHR [132]. It seems that available studies did not offer a clear picture of the HPA function at anterior pituitary and PVN levels.

Results are also controversial regarding the response to stressors. After ether exposure, the corticosterone response of WKY has been shown to be higher [128], similar [119] or lower [133] compared with SHR. In the latter study, WKY, SHR and outbred Wistar rats were included and lower ACTH and corticosterone responses of both WKY and Wistar versus SHR were found, with similar response in WKY and Wistar at all ages. Interestingly, differences were more marked in very young rats and progressively vanished, with no differences in 16-week-old rats [133]. These results are suggestive of a critical contribution of age with the higher responsiveness of SHR with respect to WKY and other strains being better observed at young ages. However, Hashimoto et al [126], observed higher ACTH and corticosterone responses to ether or hemorrhage in 7-week-old WKY than SHR, associated with a lower corticosterone response. This higher ACTH response was accompanied by a lower ACTH responsiveness to exogenous CRH and a similar response to exogenous vasopressin, suggesting that differences might be related to higher stress-induced CRH release, but lower adrenal responsiveness to ACTH in WKY than SHR. Although results are puzzling, they illustrate the importance of measuring both ACTH and corticosterone and checking responsiveness of the corticotrope cells and the adrenal cortex.

The picture is not clearer with more emotional stressors. After immobilization, similar corticosterone levels were found in WKY and SHR in one study [124], and lower levels in WKY in another one [134]. A lower corticosterone response was also observed in WKY after a brief exposure to foot-shocks [135]. However, the corticosterone response elicited by daily sessions of TWAA involving foot-shocks did not differ between the two strains [136]. A similar ACTH response to forced swim (both sexes) and tail-shocks (males) has been observed [43, 61], although corticosterone levels were higher in males but not females after forced swim in WKY [43]. This suggests enhanced adrenal responsiveness in WKY males, which is in contrast to Hashimoto et al [126]. Regarding the response of the two strains to exogenous DEX administration, a similar reduction of the HPA response to tail-shocks was found [61], with no evidence of altered negative GCs feedback.

In sum, the comparison of the activity of the HPA axis in WKY and SHR has resulted in a complex picture, with no consistent overall differences in contrast to the well-characterized hyperreactivity of catecholamines. The characterization of the HPA function in WKY compared with strains other than SHR can give us a clearer picture of the putative particularities of WKY.

When the circadian ACTH and corticosterone pattern were compared in WKY and Wistar, similar levels were found during lights on, but during the lights off greater ACTH and corticosterone levels were found in WKY [137]. Accordingly, higher POMC expression in the anterior pituitary was observed in WKY versus Wistar [138]. In the same study, *in vitro* basal ACTH release was higher in WKY, but the response to CRH and vasopressin was lower, consonant with lower CRHR1 binding and expression in the anterior pituitary. All these data suggest that corticotrope cells of WKY might have higher intrinsic activity but lower response to hypothalamic inputs than Wistar. Unfortunately, HPA response to stress was not assessed in the same study, but higher ACTH response to cold-restraint stress has been reported in WKY compared with Wistar, associated with a similar corticosterone response [137]. PVN CRH gene expression does not

appear to be different from some other inbred strains [41] or from FIS and SD rats [139], but in another study higher expression was found versus SD [140]. In accordance with the latter results, WKY showed similar basal levels of ACTH as SD and Lewis, but greater ACTH levels than SD during the recovery period after being exposed to 60 min restraint, whereas Lewis showed, as expected, a lower response than the other two strains [64]. In the same line, similar basal levels but greater ACTH and corticosterone responses to forced swim were found in WKY than in SD [141]. Of note, a study by Redei et al [142] compared WKY with Fischer and Wistar after manipulating corticosterone levels by adrenalectomy with or without exogenous corticosterone supplementation (Sham, ADX, ADX+C), in order to study the contribution of corticosterone to water-restraint stress-induced ulcers. Lower corticosterone levels were found in ADX+C WKY than Fischer and Wistar, suggesting enhanced steroid metabolism. This is an aspect that has not been basically studied and can contribute to explain discrepancies between ACTH and corticosterone.

To our knowledge, responsiveness of WKY to acute GCs manipulations has been tested in only two studies [142]. In a first study, no differences in ACTH and corticosterone response to tail-shocks or in the response to DEX-induced negative feedback or pharmacological adrenalectomy were found in WKY compared with SHR and Fischer [61]. In the second study, no differences in DEX-induced suppression of basal ACTH were found between WKY and SD, whereas impaired suppression was apparent with corticosterone [141], which could be explained by enhanced corticosterone metabolism.

In addition to the few studies involving both sexes, only two additional studies have explored differences in HPA hormones in WKY and SHR females, both under basal conditions. A study compared female WKY and SHR together with SD and no differences in corticosterone were found [143]. In adolescent females, corticosterone levels of WKY were lower than that of SHR [144], suggesting a contribution of age to the strain differences, as reported in males.

### **3.4.2. Flinders sensitive and resistant lines**

Flinders sensitive and resistant lines (FSL, FRL) were genetically selected on the basis of the response to muscarinic drugs and were found to differ in coping behavior in the FST and other behaviors, with FSL showing depression-like characteristics [145]. Some studies have compared both lines and others FSL with SD as controls. Baseline levels of corticosterone did not differ between FRL and FSL, but the response to the muscarinic cholinergic agonist arecoline was higher in FSL [146], in accordance with the criteria used for genetic selection. Later, Owens et al [147] observed lower basal ACTH but normal corticosterone in FSL. However, no differences in basal corticosterone levels were reported between FRL and FSL either in unstressed controls or after exposure to chronic unpredictable stress (CUS), despite the expected increase in basal corticosterone after CUS [148]. In response to noise stress, reduced corticosterone was found in FSL than FRL, whereas similar corticosterone levels were observed in the two lines after restraint or colorectal distension under restraint [149]. Although ACTH levels were not measured, the lower corticosterone response to a mild stressor is suggestive of a reduced HPA responsiveness to stress in FSL versus FRL. Urinary excretion of corticosterone during 24 h exposure to metabolic cages is similar in the two lines [150].

In some other studies, FSL has been compared with typical strains (e.g. SD) and results are puzzling. Inconsistencies were observed within the same laboratory [151, 152]: presumably basal levels of ACTH and corticosterone were lower in FSL in one study [151], whereas in another ACTH did not differ and corticosterone levels were higher in FSL [152]. In pre-weanling rats, FSL showed slightly higher ACTH response to exposure to a novel cage or an adult rat, but corticosterone response was similar [153, 143, 144]. Thiele et al [154] observed higher basal corticosterone levels in males FSL than males SD and the same trend in females, whereas in another study with males no differences were observed [155]. However, Thiele et al [154] compared FSL rats bred in their center with SD rats purchased from Charles-River, which is in general non-appropriate. Finally, Krokas et al [156] studied corticosterone levels in male and female FSL and SD rats 20 min after behavioral testing (open-field and EPM) on the day after chronic vehicle or citalopram administration: higher corticosterone levels were observed in male FSL versus SD vehicle-treated rats that disappeared in citalopram-injected rats, whereas no difference was observed in females. The origin of the two strains was not reported.

### 3.5. RATS GENETICALLY SELECTED FOR STRESS CORTICOSTERONE RESPONSIVENESS

It is quite surprising that genetic selection of low or high HPA responsiveness to stressors has only recently been approached by Sandi's lab. They exposed young rats for three days (postnatal days 28-30) to two relatively mild stressors daily and used plasma corticosterone on the last day to classify rats in low, intermediate and high responders (LR, IR and HR, respectively). Several males and females from each group were crossed and exposed to the same procedure over generations to establish the three lines. Lines did not differ in relative adrenal weight or basal corticosterone levels (at nadir or peak of the circadian rhythm), but did in corticosterone response to restraint stress [157]. Lines also differed in cardiovascular regulation with higher heart rate and basal vagal tone in both LR and HR compared with IR [158]. HR rats appear to be slightly more anxious (EPM) and prone to adopt passive strategies in the FST than LR, but showed clearly higher offensive behavior [159]. In addition, the impact of juvenile stress exposure was quite similar, except that offensive behavior was increased in LR but not HR rats. Differences have also been observed regarding strategies used for spatial learning (Morris water maze), with overall higher long-term memory in HR [160]. Whether physiological and behavioral differences between lines are causally linked to altered corticosterone responsiveness to stress remains to be studied. Nevertheless, in order to delve into the contribution of constitutive differences in the HPA axis on behavior and the consequences of exposure to stress, it would be of great interest to develop other lines differing in HPA axis activity.

## 4. OVERALL DISCUSSION

In-depth evaluation of strain/lines differences in the HPA is scarce. Essential aspects to consider are the characterization of the circadian pattern of corticosterone, the measurement of stress levels of both ACTH and corticosterone and the report of the relative weights of adrenal glands and thymus, which can provide us with important complementary information.

### 4.1. Methodological concerns

Although individual studies characterizing the HPA axis in different rat strains/lines are necessarily limited in scope, a point of major relevance in any study is whether obvious methodological concerns are detected that can lead to misinterpretation of data. In addition to the difficulties in reporting true basal levels of ACTH and corticosterone, probably the most common drawback of the available literature data, a major concern when comparing inbred strains is whether or not they can be obtained from the same breeding center. Important details that might differ between centers and hence affect the results are the number of pups per mother, the time of weaning, the characteristics of the home cages (and possible enrichment) and the degree of perturbations in the animal rooms by laboratory routines. A second major concern is that most published results have characterized strain differences only in males and data in females are scarce. The exception to this rule is the Lewis and Fischer rats in which earlier studies were done in females and a high number of publications are available with both sexes.

### 4.2. Answers to critical questions on the basis of experimental evidence

At present, there is no evidence for a genetic selection resulting in high overall reactivity to emotional stressors deduced from the responsiveness of several classical neuroendocrine stress systems. Classical physiological markers of stressor intensity included plasma levels of ACTH (and corticosterone with some limitations), catecholamines (adrenaline, noradrenaline), glucose, as a surrogate of adrenaline release, and prolactin [5]. In most cases, higher responsiveness of a particular strain/line is restricted to one or two of them. **Table 1** summarizes the results obtained comparing stress-sensitive variables other than ACTH and corticosterone in some relevant rat strains.

The best example of a dissociation between different stress markers is the pair SHR-WKY. Whereas consistent hyperreactivity to stress in terms of adrenaline and noradrenaline is found in SHR (e.g. [124, 134]), differences in the reactivity of the HPA axis are controversial and no differences in the prolactin response to stress have been found [43]. A second example is the Lewis-Fischer pair, in which the defective HPA response to stress detected in Lewis was not observed with prolactin [43]. In the only available study regarding plasma catecholamines, lower response to IMO was found in Lewis compared with Fischer [161], suggesting a parallelism with the HPA axis.

To our knowledge, the most marked differences in the HPA axis are found in outbred Long-Evans rats as compared with some other outbred or inbred strains, including Fischer [162]. Long-Evans also appear to have a greater prolactin response to stressors than Wistar [163] and higher levels of enzymes involved in catecholamine synthesis in the adrenal compared with SD, suggestive of enhanced CA responsiveness. When considering another pair of strains widely studied, Fischer and Lewis, clearly lower levels of ACTH after acute restraint were observed in Lewis, in parallel with modest but significantly lower plasma adrenaline responsiveness [164]. However, no differences between Lewis and SD were found in the prolactin response to IMO despite the expected lower corticosterone response in Lewis [65].

Is HPA responsiveness independent of the type of emotional stressor? This is a question difficult to answer as most studies focused only on one single stressor. However, it is likely that the type of stressor is important. In an outbred population of rats, individual differences are consistent when stressors are of similar intensity (exposure to novel environments), but are not when comparing stressors greatly different in intensity (e.g. immobilization versus novel environment) [165, 166, 167]. If this applies to genetically-selected animals, particular characteristics of stressors might be relevant and should be tested when describing the stress phenotype of particular rat strains. Some data supporting an important role of the type of stressor follows. First, in RHA-RLA rats, higher ACTH and prolactin responses were observed in RLA in response to relatively mild stressors but not to more severe stressors [96]. Second, in HAB-LAB rats, higher ACTH and prolactin responses were observed in HAB after forced exposure to the open arms of the EPM, but not after free exploration of the EPM, and social defeat resulted in lower ACTH response in the more anxious HAB rats (*see above*).

**Table 1: Comparison of resting and stress levels of biological parameters in selected strain pairs.**

Strain pairs	Plasma catecholamines		Glucose		Prolactin	
	Basal	Stress	Basal	Stress	Basal	Stress
<b>Lewis-Fischer</b>	E ↓ NE = [161]	E ↓ NE ↓ [161]	= [43]	↓ [43]	= [43]	= [43]
<b>Lewis-SD</b>	E ↓ NE = [168]	E ↓ NE ↓ [168]	ND	ND	= [65]	= [65]
<b>HAB-LAB</b>	ND	ND	ND	ND	↑ [78]	↑ [78]
<b>RLA-RHA</b>	ND	ND	= [27, 169]	= [27]	= [27, 97, 101, 170, 171]	↑ [27, 97, 98, 101, 170, 171]
<b>WKY-SHR</b>	See [109]	See [109]	= [43]	↓ [43]	= [43, 172], ↑ [173]	= [43]
<b>FSL-FRL</b>	ND	ND	ND	ND	= [150]	↑ [150]

Differences are indicated by symbols, always using as the reference the first strain or the pair. ND=not determined.

#### 4.3. Is HPA response to stress related to anxiety?

The results obtained with HAB-LAB rats nicely demonstrated that there is no obvious relationship between anxiety-like characteristics derived from the EPM and HPA responsiveness to stressors. These data are in accordance with our data in outbred rats [165]. Interestingly, differences between the two lines were dependent on the type of emotional stressor, introducing major concerns regarding simple characterization of trait-like differences in HPA responsiveness. Thus, forced open arm exposure did result in a greater hormonal response in HAB, suggesting specific fear to open areas, rather than generalized stress



hyperresponsiveness. Perhaps even in outbred rats, those showing lower open arm entries during EPM exposure will show higher response with forced exposure.

Interpretation of good versus poor performance in the TWAA is difficult because of the contribution of several factors. Evidence obtained in RHA-RLA rats has demonstrated a major contribution of anxiety to impair TWAA acquisition but also of coping style, with passive coping predisposing to freezing, which in turn is detrimental to engaging in the active behavior needed for avoidance. Since in these rats RHA showed greater neuroendocrine responsiveness to stressors (at least when they are of relatively low intensity), high anxiety and passive coping appear to be associated with increased neuroendocrine stress responsiveness. What is the conclusion derived from Hatano lines? These rats were selected by TWAA performance, but those rats showing higher levels of freezing were eliminated during the process of genetic selection [89]. In these rats, HAA appear to be more anxious than LAA rats, this increased anxiety contributes to their better avoidance performance. HAA also showed a greater HPA response to stress, the data favoring a positive relationship between anxiety and HPA responsiveness, at least within animals that are prone to active coping. This might suggest that enhanced HPA responsiveness might be a characteristic of high anxiety, regardless of coping style. However, it is possible that high-anxiety passive copers could manifest enhanced HPA responsiveness to mild stressors whereas enhanced HPA responsiveness would still be observed after exposure to more severe stressors in high-anxiety active copers.

#### **4.4. FST and coping**

Behavior in the FST does not appear to be related to anxiety and might instead reflect coping style [174]. A critical question is whether passive coping in the TWAA task (i.e. freezing) is related to passive coping in the FST. Although correlation studies have not been done, results in RHA-RLA and NIH rats suggest a parallelism between freezing behavior in the TWAA context and immobility during the first 5 min of the FST [101].

The HPA axis does not appear to be related to coping behavior in the FST. This was the main conclusion achieved by comparing several different inbred rat strains [43, 45]. This is nicely supported by Redei and collaborators' studies with WKY sub-strain differing in immobility in the FST [175, 176]. Since WKY does not appear to be completely inbred, the authors selectively bred WKY for low and high immobility in the FST, but basal or restraint stress corticosterone levels were similar in the two substrains [175]. A similar conclusion was reached using F2 of WKYxFIS, concluding that depressive-like behavior in the FST and HPA function (basal or stress corticosterone and adrenal weight) were dissociated [176].

#### **4.5. Guide to explore individual or strain differences in the HPA**

There appears to be a general agreement about the importance of characterizing individual differences in critical physiological and behavioral traits. Comparison of outbred populations of rodents is a good approach, but exploitation of available outbred and inbred strains could allow us to establish or rule out important relationships between different aspects of behavior or between relevant physiological aspects and behavior. However, advances in the field also require one to be aware of methodological problems and reject excessively simple explanations. We have summarized in *Tables 2* and *3* both methodological considerations and their implications as well as a recommended guide to better interpret experimental data about the HPA axis. We hope this could be of value to those interested in this relevant endocrine system.

**Table 2: Methodological concerns.**

Consideration	Implications
In rats and mice resting levels of corticosterone in the morning hours are about 10-20 ng/ml (1-2 µg/dl) when measured by radioimmunoassay (RIA) or enzyme-immunoassay (ELISA). Most published studies present values between 2- and 10-fold higher.	Values usually reported in published studies are indicative of stress associated with blood sampling and do not reflect true HPA resting levels.
Under certain conditions (circadian rhythm, acute stress prolonged for several hours, chronic stress) corticosterone levels do not reflect ACTH levels.	If we consider ACTH the primary response to a stressor, measuring only corticosterone can lead to erroneous interpretations.
Strain or individual differences in the HPA response might differ between systemic and emotional stressors.	Results cannot be extrapolated from one type of stressor to another.
Genetic selection could have resulted in altered responsiveness of one particular system rather than general emotional reactivity to stressors.	Strain differences in the HPA response to emotional stressors are not necessarily indicative of a more general endocrine response.

**Table 3: Looking for the main locus of individual/strain differences in stress responsiveness.**

Aspects to be considered	Observations
<b>1. Animals differ in a general construct of emotional reactivity</b>	<p>If strains differ in emotional reactivity, differences are expected to be in the same direction in all or most of the physiological responses that are sensitivity to the intensity of stressors.</p> <p>If this is true, differences should be observed in the physiological response to emotional but not systemic stressors.</p>
<b>2. Animals differ in the activity of a particular physiological system</b>	<p>Genetic selection affects a wide range of genes related to different functions. If the selected genes have impact in a particular physiological system (e.g. HPA axis), we can observe differences in this particular system but not in other stress-related systems (e.g. prolactin).</p> <p>We cannot infer from a particular system that the two strains differ in responsiveness to stress, as the differences are likely to be restricted to a particular system.</p>
<b>3. Which is the locus of differences in a particular system?</b>	<p>Suppose we detect differences in corticosterone response to stress between two strains. As discussed previously, the critical locus might be at different levels of the HPA axis:</p> <ol style="list-style-type: none"> <li>a) Processing of inputs arriving at the PVN and the corresponding release of CRH and others ACTH secretagogues.</li> <li>b) Responsiveness of corticotrope cells to hypothalamic stimulatory factors.</li> <li>c) Responsiveness of adrenocortical cells to ACTH.</li> <li>d) Altered corticosterone metabolism.</li> <li>e) Altered sensitivity to negative glucocorticoid feedback at any level.</li> </ol>
<b>4. Sensitivity of tissues to circulating corticosterone</b>	<p>This can be linked to changes in corticosteroid receptor expression or to others independent cell characteristics.</p> <p>Differences cannot be extrapolated from one cell or tissue to others</p>

## CONCLUSION

The activity of the HPA axis and its main output, GCs, is considered to be critical for coping and adaptation to stress and it has been associated with a number of psychiatric diseases, including anxiety and depression. Consequently, attention has been devoted to the characterization of the HPA function in rodent strains differing in particular physiological or behavioral aspects. However, the eventual consequences of the described differences are unclear. The present review of selected rat strains shows that previous approaches on this subject have been incomplete and plagued by methodological problems. Therefore, available data are very often controversial. Nevertheless, the overview of all these data strongly suggests that there is no simple relationship between HPA activity and anxiety-like behavior, depression-like behavior and coping style. We need new perspectives about the putative role of the HPA axis in these extremely relevant traits and neuropsychiatric diseases.

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Not applicable

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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