What can we know from pituitary-adrenal hormones about the nature and consequences of exposure to emotional stressors?

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Abstract

Exposure to stress induces profound physiological and behavioral changes in the organisms and some of these changes may be important regarding stress-induced pathologies and animal models of psychiatric diseases. Consequences of stress are dependent on the duration of exposure to stressors (acute, chronic), but also of certain characteristics such as intensity, controllability and predictability. If some biological variables were able to reflect these characteristics, they could be used to predict negative consequences of stress. Among the myriad of physiological changes caused by stress, only a restricted number of variables appears to reflect the intensity of the situation, mainly plasma levels of ACTH and adrenaline. Peripheral hypothalamic-pituitary-adrenal (HPA) hormones (ACTH and corticosterone) are also able to reflect fear conditioning. In contrast, the activation of the HPA axis is not consistently related to anxiety as evaluated by classical tests such as the elevated plus-maze. Similarly, there is no consistent evidence about the sensitivity of the HPA axis to psychological variables such as controllability and predictability, despite the fact that: (a) lack of control over aversive stimuli can induce behavioral alterations not seen in animals which exert control, and (b) animals showed clear preference for predictable versus unpredictable stressful situations. New studies are needed to re-evaluate the relationship between the HPA axis and psychological stress characteristics using ACTH instead of corticosterone and taking advantages of our current knowledge about the regulation of this important stress system.

Key words: stress, hypothalamic-pituitary-adrenal axis, biological markers, stress intensity, fear-conditioning, anxiety, controllability, predictability

Introduction

Since the pioneering work of Walter Cannon describing the fight-flight reaction and the characterization of the stress concept by Hans Selye, it is well-accepted that in all vertebrates stress resulted in the release of glucocorticoids and catecholamines (noradrenaline and adrenaline) into the bloodstream. Cannon focused on the sympathetic nervous system and catecholamines, whereas Selye focused on the adrenal cortex and glucocorticoids. Although there are some differences among vertebrates regarding the precise routes for the release of these two types of hormones, in mammals they are described as the hypothalamic-pituitary-adrenal (HPA) and sympatho-medullo-adrenal (SMA) axes. Details about the influence of acute and chronic stress on the SMA axis have been extensively reviewed (Kvetnansky et al. 2009) and will be only occasionally discussed here. Stress-induced activation of the HPA axis is the result of stimulatory signals arriving at the paraventricular nucleus of the hypothalamus (PVN). In the PVN there are parvocellular neurons that mainly synthesize corticotropin-releasing factor (hormone) (CRF or CRH) and other peptides as well (i.e. vasopressin, VP) and send axonal projections to the pituitary portal blood of the external zone of the median eminence (ME). Upon activation, these neurons release CRH, VP and other secretagogues, which reach the corticotrope cells of the anterior pituitary (AP) to activate the synthesis and release of the adrenocorticotropic hormone (ACTH). Circulating levels of ACTH act on the zona fasciculata of the adrenal cortex to stimulate the synthesis and secretion of glucocorticoids. In most mammals, including humans, the main glucocorticoid is cortisol, but there are also some levels of corticosterone. In rodents, the predominant glucocorticoid is corticosterone, and in the particular case of rats and mice they have only corticosterone.

The concept of stress has markedly changed since Selye's initial definition as any agent that can alter homeostasis. Stress may be defined as a state of emergence created in the organism either by exteroceptive and interoceptive stimuli that profoundly alter homeostasis in a way that cannot be solved by specific homeostatic physiological mechanisms, or by stimuli that have no direct impact on homeostasis but have a reasonably probability to be followed by a real challenge to homeostasis (Vigas 1984). The first type of stimuli (i.e. hypovolemia, hypoglycaemia) belongs to the category of systemic (physic) stressors, whereas the second type (i.e. novelty, uncertainty, innate fear, learned fear) is

categorized as emotional (psychological) stressors. The above definition is compatible with that proposed in a very recent review, which in addition considers that rather than the pure physiological response, which may reflect arousal, unpredictability and uncontrollability should be considered as critical factors for a situation to be considered as stressful (Koolhaas et al., 2011).

Activation of the HPA axis in response to systemic stressors is the result of quite specific brain pathways that have relatively direct connections to the PVN. In contrast, PVN activation in response to emotional stressors follows a more tortuous route and can involve a wide range of telencephalic regions, including medial prefrontal cortex, hippocampal formation, lateral septum, different subregions of the amygdala complex and the bed nucleus of stria terminalis and a set of hypothalamic nuclei that have direct projections to the PVN (Herman et al. 2003). Some extensively used laboratory stressors have, to some extent, both systemic and emotional components. For instance, forced swim involves physical exercise and results in hypothermia if temperature of water is clearly below 36ºC (i.e. Dal-Zotto et al. 2000; Porsolt et al. 1979). Restraint in tubes is not likely to involve important systemic components, but several methods of immobilization (IMO) can have systemic components as a result of local inflammation. Obviously, restraint in cold water involves an important thermoregulatory component and tailshocks or footshocks nociceptive and local inflammatory components.

HPA activation as a marker of stress intensity

The available data suggest that mixed or pure emotional stressors (hereafter referred to as emotional stressors) elicited not only the release of the catecholamines and HPA hormones, but also of prolactin, an hormone that is sensitive to emotional or predominantly emotional stressors, but appears to respond only to certain systemic stressors (Martí and Armario 1998). Emotional stressors can differ in several dimensions such as the type and the intensity of the emotion that is experienced, the degree of control over the aversive situation and the degree of predictability. The aim of the present review is reevaluate old data and incorporate new data to better understand the information we can get from the HPA axis about stressors. When appropriate, comparisons will be done with other physiological variables.

When intuitively thinking about qualitatively different emotional stressors, it is clear that they can greatly differ in the intensity of emotional reaction the animals can experience. It is not the same if we are exposed to an unknown environment than if we are directly exposed to a predator or we are receiving a painful stimulus. How can we get information about the particular dimension of the intensity of stress experienced under all those circumstances? We need to find reliable biological markers of the intensity of emotional stressors as such information may be critical considering that the negative impact of stress is likely to be related to its intensity. Obviously, this does not rule out that certain emotional situations can result in negative behavioral and physiological consequences not merely related to the dimension of intensity, but rather to particular psychological dimensions of the stressors.

One main concern when comparing different emotional stressors in order to find reliable biological markers is to be sure that changes in these particular biological variables are related to intensity and not to other dimensions. Some decades ago, Hennessy and Levine (1978) designed an experiment in mice that avoid the above problem. They introduced progressive changes in the normal environment of the animals, each containing the preceding changes, thus resulting in the following stressful situations: (1) picking up mice and putting them in cages similar to their regular ones, but with clean sawdust and without food and water; (2) picking up mice and putting them in their regular cages without sawdust, and (3) picking up mice and putting them in an empty glass jar (very different from the home cages). They found a progressive increase in plasma corticosterone when evaluated 15 min after the initial perturbation. Using a similar approach in rats we were able to confirm the graded increases in plasma corticosterone (Armario et al. 1986) and to demonstrate a parallel pattern of plasma prolactin. Quite interestingly, other anterior pituitary hormones responded to all stressful situations with increases (luteinizing hormone, thyroid stimulating hormone) or decreases (growth hormone), which were always independent of the intensity of the stressful situations (Figure 1). Using very brief exposures to footshocks of increasing intensities, Natelson et al (1981) demonstrated that peak plasma noradrenaline and, more particularly, adrenaline, were sensitive to the intensity of footshocks. Using the same stressor, similar results were obtained with plasma levels of corticosterone and prolactin (Kant et al. 1983). All the above data demonstrated that among the wide range of physiological variables that respond to stress, only a few appear to be appropriate markers of the intensity of emotional stressors.

However, the paper by Kant et al. (1983) also indicated that a plateau of plasma corticosterone and prolactin was reached with the highest footshock intensities. Regarding corticosterone, this was due to the saturation of adrenocortical synthesis with relatively low levels of ACTH (Keller-Wood et al. 1983). That means that plasma corticosterone cannot be used as an appropriate marker of stress intensity when stressors are from intermediate to severe intensities (Figure 2). This fact has been almost ignored in the literature of stress when studying how HPA activation can reflect different characteristics of stressors. Although it is better to simultaneously measure both ACTH and corticosterone, if only the latter hormone is measured, it is critical to follow plasma levels of corticosterone after the termination of exposure to the situation (i.e. García et al. 2000). Indeed, with appropriate sampling times, post-stress corticosterone levels may distinguish between different initial ACTH responses to stressors.

Even if plasma ACTH is measured, severe stressors can result in saturation of corticotrope cells to release ACTH and, therefore, will be unable to reflect stressor intensity when measured immediately after the stressors. This was the case in one study comparing high intensity footshock and IMO (Márquez et al. 2002). When ACTH (and corticosterone) was measured just after 1 h of exposure to the stressors, its levels where similar, but the follow-up of the post-stress levels clearly revealed a slower return to resting levels with IMO as compared to footshock, suggesting that IMO was stronger than footshock. To rule out that this was a particular behavior of the HPA axis not reflecting differences in intensity, we also evaluate other stress markers, including prolactin, glucose and the changes in food intake over the days following stressor exposure. All parameters followed the same pattern strongly supporting that the two stressors differed in intensity.

Fear conditioning and HPA activation

The finding that HPA activation appears to be related to the intensity of stressors prompted us to study whether HPA activation was able to reflect fear conditioning. In classical tests of fear conditioning, animals are exposed to a particular environment (footshock chamber) for a few minutes and then they received some footshocks (unconditioned stimulus, US), either unsignalled or preceded by a specific originally neutral cue (i.e. sound or light). This results in the association of the shock chamber and the specific neutral cue (conditioned stimuli, CS) with the aversive stimulus, thus inducing context and cue

fear conditioning, respectively. Testing for contextual fear conditioning consists of merely exposing the animals to the shock context without shocks, and comparing behavioral (usually freezing) and physiological responses in shocked animals and non-shocked controls (having the same previous experience with the context without shocks). However, the observation of higher levels of freezing or physiological activation in previously shocked rats is not necessarily indicative of the actual development of fear conditioning. We have to rule out that differences were not due to a non-specific sensitization of the HPA response to any novel environment caused by previous shock exposure. Evidence for long-lasting (days) sensitization of the HPA axis after a single exposure to severe stressors has been consistently reported (Belda et al. 2008; Gagliano et al. 2008; Johnson et al. 2002). Surprisingly, previous data did not distinguish between footshock-induced fear conditioning and footshock-induced sensitization (i.e. Campeau et al. 1997; Cordero et al. 1998; Goldstein et al. 1994; Van de Kar et al. 1991).

Then, we performed an experiment (Daviu et al. 2010) in which rats were exposed to a chamber for 3 min and then received 3 shocks or exposed for the same amount of time to the chamber without shocks (controls). Six, 7 and 12 days later, animals were exposed for 15 min to one of three typical novel environments that strongly differed from the shock context and on day 13 they were again exposed to the shock context. In all novel environments shocked animals showed a transient hypoactivity as compared to controls, but the HPA response was similar in both groups, ruling out a non-specific sensitization of the HPA response to any mild stressful situation. In contrast, after exposure to the shock context, previously shocked animals showed the expected high levels of freezing, associated with a greater HPA response than controls. To further demonstrate the specificity of fear conditioning-induced HPA activation, we took advantage of the immediate shock deficit. This means that animals shocked immediately after being exposed to a chamber do not acquire contextual fear conditioning (i.e. Fanselow 1990), likely because they had not time to acquire appropriate information about the context prior to the first shock. We then used three groups of rats and only 1 shock to induce fear conditioning. The typical shock group received the single shock 3 min after being exposed to the chamber, whereas the immediate shock group received the shock immediately after the introduction in the chamber. When tested days later in a novel environment, the HPA response was similar in all three groups, whereas during re-exposure to the shock context only the typical shock group, but not the immediate shock group, showed high levels of freezing

and a greater HPA response than controls (Daviu et al., 2010). Thus, the data clearly demonstrated that the HPA axis is sensitive to contextual fear conditioning.

The sensitivity of the HPA axis to aversive conditioning is not restricted to shock. Conditioning also appears when rats are exposed in a specific environment (context) to a piece of cloth impregnated with cat fur odor. In addition to eliciting an avoidance of the area where the cloth is and hipoactivity during exposure to the situation, these animals showed the same type of behavior when exposed again some days later to the same context including a clean piece of cloth (Blanchard et al. 2001; McGregor and Dielenberg 1999), suggesting that conditioning to odor-induced anxiety had developed. We then studied simultaneously behavioral and HPA response to three different conditions: (a) during exposure to cat fur odor, (b) during exposure, some days later, to a completely different novel environment, and (c) during aversive conditioning testing (exposure to the same original odor context, but now with a clean cloth without odor). The results demonstrated the expected behavioral pattern (hypoactivity and avoidance of the cloth area) and a specific activation of the HPA axis during exposure to the cat fur odor as compared to the mere exposure to the same environment (controls). Nine days later, a higher HPA response was observed in the cat odor group when re-exposed to the cat odor context, but a normal response was observed when exposed to a different environment two days before exposure to the context (Muñoz-Abellán et al. 2009). The latter results rule out a non-specific sensitization of the HPA axis and give support to the capability of the HPA axis to reflect conditioning to aversive stimuli.

It is important to note that the parallelism between plasma levels of ACTH and fear conditioning is not observed when samples are taken just after a 5 min test in the context re-exposure session (Figure 3). This is the typical testing time in behavioral studies about shock-induced fear conditioning. The reason for the dependence on testing time is unclear. A 5 min period is enough to markedly activate the ACTH release in response to different stressors (i.e. De Souza and Van Loon 1982; Kovacs and Sawchenko 1996) and therefore it is unlikely that this period was too short to properly activate the HPA axis. Although plasma levels of ACTH are a fast response to stressors, a few minutes are needed to release enough amount of the hormone into the blood to be consistently detected. We then hypothesize that plasma levels of ACTH just after the 5 min exposure to the situation are mainly a reflection of all previous procedures (i.e. transporting the cage, handling the rat) rather than of the recognition of the potentially dangerous

environment. Therefore, more time is needed in order for ACTH to reflect the enhanced fear associated to the situation.

Anxiety and HPA activation

All these data clearly demonstrate that HPA activation is able to reflect, under appropriate conditions, fear conditioning. However, is the HPA axis sensitive to spontaneous or experimentally-induced changes in anxiety? We firstly studied the influence of spontaneous individual differences in anxiety. To this end, a group of non-selected adult male rats was tested in the elevated plus-maze (EPM), a classical test for anxiety, and classified them into low and high anxiety (LA, HA) by the median of the time spent in the open arms of the EPM. Time in open arms is the parameter that in our hands better reflects anxiety-like behavior in the EPM. It is intuitively assumed that the greater the anxiety the higher should be the plasma levels of hormones in response to the EPM and other similar novel environments. Despite clear differences between LA-HA rats in the time spent in the open arms of the EPM no differences were found in either ACTH or corticosterone responses to different novel environments (Márquez et al. 2006). In another set of animals we directly compared the behavioral and hormonal response to the EPM in LA-HA, and we observed marked differences in anxiety associated again to a similar ACTH response (Figure 4). In rats genetically selected in function of the open arm time, lower ACTH and corticosterone responses in low-anxiety behavior (LAB) versus high-anxiety behavior (HAB) lines were only observed after forced exposure to the open arms of the EPM (Landgraf et al. 1999), whereas no differences were found after exposure to the regular EPM or to forced swim (Keck et al. 2003; Liebsch et al. 1998). Even a higher HPA response to social defeat was found in LAB rats (Frank et al. 2006). Therefore the HPA axis is unable to consistently reflect trait differences in anxiety as evaluated in the EPM. In fact, it has been also reported in humans a lower cortisol response to stressors in high anxiety subjects (Duncko et al. 2006; Jezova et al. 2004). It is clear that the relationship between HPA activation and anxiety is far from being adequately characterized.

Sensitivity of the HPA axis to controllability and predictability

In addition to intensity, there are some factors related to stress that may play critical roles on the consequences of stress: controllability and predictability. Control over stress means that we have the possibility to avoid the aversive situation or at least to escape from it. The classical design to characterize the effect of control (or lack of control) per se and differentiate this factor from the effect of the aversive stimulus is the triplet firstly used by Jay M Weiss. In a classical experiment, a group of rats was loosely restrained and exposed to tailshocks, but they could learn to avoid or escape from the shock making a specific behavior with their nose. Thus, they could acquire control over the initiation/termination of shocks (master). A second group was wired in series with the first group so that they received exactly the same amount of shock, with the critical difference that they had not control at all over the shock (yoked). A third control group was only exposed to the restrainer. Using this procedure, body weight loss and gastric ulceration were found to be greatly reduced in the master as compared to the yoked group (Weiss 1968).

Another critical factor is the degree of predictability of the occurrence of the aversive stimuli. This can be modified introducing irregularly spaced exposure to aversive stimuli, with or without signaling their occurrence. Most studies have been carried out using exposure to brief (a few seconds) and intermittent electric shock as the aversive stimulus. In addition, some of them have modified simultaneously controllability and predictability. Predictability of irregular shocks can be enhanced by introducing a warning signal before shocks. Interestingly, it turned out to be also important the introduction of a signal after the shocks. This type of post-shock signal is considered as a relevant feedback indicating that behavior was effective to avoid/escape shocks. Consequently, this signal acts to indicate a safety period, in that shock will not follow for a period of time after the signal (Seligman and Mayer 1970). It has to be noted that most typical protocols of shock-induced fear conditioning use a single or a few shocks so that factors such as predictability are difficult to study.

The influence of controllability on HPA response is conflicting. Using a variant of the triplet used by Weiss that does not allow the rats to avoid but only to escape from tailshocks, Maier's laboratory has described an important number of consequences of exposure to shock (when evaluated typically 1-3 days

after shock) that only appear in the inescapable shock (IS, yoked) group, but not in the escapable (ES, master) group (the learned helplessness paradigm, LH). For instance, IS impaired escape in a shuttle-box, reduced social behavior and enhanced conditioning (Maier and Watkins, 2005). Despite the markedly behavioral consequences of exposure to ES versus IS, the activation of the HPA axis was exactly the same in both groups as measured by plasma levels of ACTH and corticosterone just after stress and during the post-stress period (Maier et al. 1986). Other labs studying the influence of control on HPA response relied only on plasma corticosterone, which is not appropriate as previously discussed. The lack of differences in the HPA axis between yoked and master rats using the classical LH procedure has been further observed in other studies analyzing different aspects of the HPA axis. Thus, exposure to IS has been reported to induce long-lasting (days or a few weeks) sensitization of the HPA response to further stressors and resistance to dexamethasone-induced negative feedback, but the effects were similar after exposure to ES (O'Connor et al. 2003). When foot-shocks instead of tailshocks were used, again the degree of control did not affect either the peripheral or central (CRF and AVP gene expression) components of the HPA response (Helmreich et al. 1999). Using another stressor, forced swim, no differences were again observed between yoked and master rats in a procedure that allows escaping from the water, but not avoiding it (Drugan et al. 2005).

Quite interestingly, it has been found, using the IS-ES paradigm, that plasma corticosterone levels were the same after IS than after ES; but when dexamethasone (DEX) was given 2 h prior to the shock sessions, the reduction of corticosterone levels was higher in ES than IS rats (Haracz et al. 1988), suggesting an impaired efficacy of negative feedback in IS rats. There are at least two possible explanations. First, it could be assumed that differences in ACTH actually existed between the two shocked groups receiving vehicle 2 h before shocks that were not reflected in corticosterone because of the ceiling in adrenocortical secretion. After DEX, the decrease in ACTH could have been enough for corticosterone levels to reflect circulating ACTH. However, the results by Maier et al. (1986), who did not find differences in ACTH or corticosterone among the two groups, do not give support to this hypothesis. An interesting alternative possibility is that ES and IS are differentially processed within the brain, with a more important participation of areas sensitive to negative glucocorticoid feedback (i.e. medial prefrontal cortex). In fact, It is known that medial prefrontal cortex is critical to reduce the negative impact of stress when controllability is available (Amat et al. 2005). A recent work has been

unable to demonstrate altered negative DEX feedback in ES versus IS rats (Helmreich et al. 2008), but the dose of DEX used was higher than optimal to evaluate differences in negative feedback.

Other results in rats suggest that controllability may affect the HPA axis under certain conditions, particularly when avoidance and not only escape from shock is possible. For instance, Tsuda and Tanaka (1985) observed, using an active avoidance/escape task, that plasma corticosterone levels did not differ in yoked as compared with master rats after a single 21 h stress session; however, after 5 days of pre-training with the same task, the master group did not further respond to the stressor with an increase in corticosterone levels, whereas yoked rats maintained a high corticosterone response. Similarly, greater corticosterone response to an avoidance/escape task was observed in rats after 3 daily sessions (Kant et al. 1992). Indeed, the above data support a critical influence on the HPA axis of the possibility to avoid rather than escape from the aversive situation.

An earlier study in dogs had shown that control over shocks reduced plasma cortisol response (Dess et al. 1983). It is possible that cognitive differences among species may contribute to explain the discrepancies. If cognitive capabilities are important, which is the influence of controllability on HPA responsiveness in non human primates and humans? One early study in rhesus monkeys did not observe differences in plasma cortisol between animals having control over noise and those who had not, but loss of control in those animals having previous experience of control did increase cortisol (Hanson et al. 1976). In humans, lack of control in a highly demanding mental arithmetic task increased cortisol response (Peters et al. 1998), but no influence of controllability is more frequently observed (Breier et al. 1987; Isowa et al. 2006). In this regard, one important limitation of human studies is that, in most cases, laboratory stressors are not of enough intensity to induce a marked activation of the HPA axis so that it is extremely difficult to detect the influence of underlying psychological factors such as controllability.

Although previous results in rats generally demonstrated no effect of control over shocks on HPA response to the situation, particularly in rats, we reasoned that the effect of control to reduce HPA activation may appear after the sense of control has consolidated due to repeated experience with the same situation. We then designed an experiment with three groups of rats, one group was exposed to an escape task (ES) in a shuttle-box, using footshock as the aversive stimulus (60 shocks per session, 0.5

mA, 15 sec, with an interval between shocks of 30 sec), whereas another group of rats was yoked animals (IS) and a third control group was merely exposed to the apparatus without receiving shocks. A strong activation of the HPA axis was found with ES and IS as compared to controls, but no differences emerged between the former groups even after repeated exposure to the situation for 10 days (unpublished). Therefore, our results confirmed the lack of effect of control over shocks on the magnitude of activation of the HPA axis. Similar results have been reported by Mormede et al. (1988), who exposed animals to 10 daily sessions of avoidance/escape task in a shuttle-box.

Predictability and safety signals

The role of predictability of aversive stimuli has deserved considerable attention in the literature. It has been repeatedly demonstrated that animals prefer predictable as compared to unpredictable exposure to shocks and other aversive events (Badia et al. 1979), probably because they can engage in appropriate anticipatory responses, thus resulting in less pathological effects. In striking contrast, physiological studies have yielded conflicting results, with no clear theoretical explanation for the discrepancies. There are several putative reasons for this controversial data. First, the number of independent variables manipulated was high. Second, all the studies have evaluated plasma levels of corticosterone as the output variable. Finally, markedly different experimental protocols have been used to study predictability, some of them involving sessions lasting for 24-48 h (Weiss et al. 1971a) and other several short daily sessions (i.e. Hennessy et al. 1977). It is also important to note that the capability of the HPA axis to reflect the intensity of stressors is good with short-term (10-60 min) exposure, but it is unclear whether HPA axis is a good marker under longer lasting (several hours) situations. Changes in plasma ACTH and corticosterone after long-lasting sessions of stress (several hours) are difficult to interpret because of the progressive return of these hormones, particularly ACTH, to resting levels despite the persistence of the situation (i.e. Hauger et al. 1988; Rivier and Vale 1987).

When animals are repeatedly exposed to shocks, we can use a regular or an irregular interval between shocks. Regular intervals imply some kind of temporal prediction about the occurrence of shocks, whereas irregular shocks do not. In addition, when using relatively short sessions, we can maintain a fixed number of shocks in every daily session or change the number of shocks the animals will receive each

day, obviously maintaining on average the same number of shocks with the two procedures (Seligman and Meyer 1970). This latter protocol appears to be relevant because with a fixed number of shocks the animals can predict that after receiving the expected number of shocks no further shock will be received and they enter into a *safe period*. More frequently, we can introduce some specific cue predicting the imminence of shocks (i.e. a tone) or, on the contrary, the occurrence of a period without shocks (safety signal in Weiss's terminology). This safety signal provides the animals with a relevant feedback about the effectiveness of its behavior to terminate the shocks.

In one report, Bassett et al. (1973) studied the influence of the shock intensity and the degree of predictability on plasma corticosterone response to footshock. Predictability was modified by regular versus irregular exposure to shocks and by introducing a warning signal before either regular or irregular shock protocols. In the absence of warning signal, irregular shocks tended to enhance corticosterone response. Surprisingly, the introduction of the warning signal resulted in an enhanced corticosterone response, irrespective of the degree of regularity, although the effect was more marked with the irregular schedule. This high response to signaled-irregular shock exposure was not reduced by the possibility to escape even after 4 daily sessions. In contrast 5 or 24 h of exposure to non-signaled tailshocks resulted in higher plasma corticosterone levels and greater ulceration than signaled shocks (Weiss 1970). Similar results were obtained after 19 h of exposure to footshocks (Tsuda et al. 1989). Signal after shock is also important as Weiss (1971b) demonstrated that exposure for 19 h to irregular tailshocks resulted in high level of stomach ulceration that was not different in master and yoked rats. However, when a signal was introduced after each avoidance/escape of master rats, ulceration strongly diminished in master but not yoked rats. Similar results were observed with corticosterone. He interpreted the results to indicate that the signal after shocks acted as a relevant feedback that behavior was successful to terminate shock. This signal is then predicting a safety period without shock. In conclusion, the studies on the influence of predictability on HPA hormones do not allow us to identify a clear pattern of response in function of the degree of predictability, despite the fact that predictability can improve preparedness to withstand the aversive situation and reduce pathology.

Conclusions

In conclusion, activation of the HPA axis appears to be quite sensitive to the intensity of the stressors within a wide range of intensities if ACTH is measured. This range is clearly lower if only corticosterone immediately after the stress exposure is evaluated. HPA activation is also sensitive to shock and predator-odor fear conditioning. In contrast, the HPA axis is not consistently related to trait anxiety as evaluated in classical tests such as the EPM. Similarly, HPA activation does not appear to be able to reflect some critical qualitative aspects of aversive situations such as the degree of control and predictability. Nevertheless, considering the renewed interest for the potential importance of controllability, predictability and safety signals regarding the impact of stressors (Christianson et al. 2008; 2011; Koolhaas et al., 2011), more attention should be paid in the future to re-evaluate the neuroendocrine consequences of stress predictability and related psychological factors. New experimental designs are welcome for a more thoroughly evaluation of the influence of these factors.

Acknowledgements

This work was supported by grants from Ministerio de Ciencia e Innovación (SAF2008-01175), Instituto de Salud Carlos III (RD06/0001/0015, Redes Temáticas de Investigación Cooperativa en Salud, Ministerio de Sanidad y Consumo), Plan Nacional sobre Drogas and Generalitat de Catalunya (SGR2009-16).

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Legends

Fig. 1. Schematic representation of two patterns of hormonal response to stressful situation differing in intensity. Animals were left undisturbed (controls, C) or exposed to different mild stressful situation with progressive increases in the level of environmental perturbations (from 1 to 4). Whereas plasma levels of corticosterone increases in function of the intensity of the stressful situation, plasma levels of luteinizing hormone (LH) responded to stress, but independently of the intensity of the situation. Original data can be seen in [Armario et al. 1986].

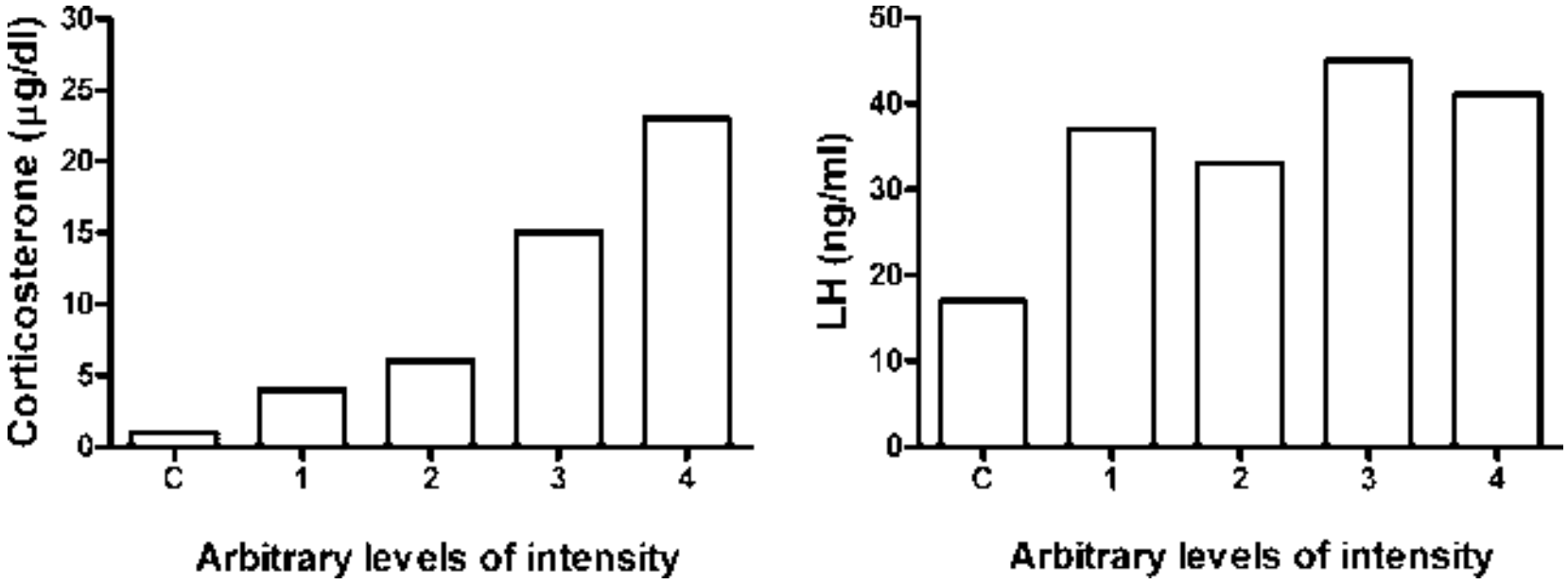
Fig. 2. Schematic representation of the response of several physiological variables to stress differing in intensity. Levels of intensity are progressively increased from the left to the right. Note that the curve representing each variable has a specific shape, indicating different levels of sensitivity to the intensity of stressors and a saturation of the response at different levels of intensity. Particularly noteworthy is the saturation of the corticosterone response with relatively low levels of stressor intensity.

Fig. 3. ACTH response is not sensitive to context fear conditioning using the typical 5 min exposure to the context. Animals were exposed to a particular chamber without receiving electric footshocks (controls) or after 30 footshocks (1.5 mA, 3 sec, 1 per min). When exposed 8 days later to the same chamber for 5 min, controls rats showed low levels of freezing, whereas previously shocked rats showed high levels of freezing (left panel). Despite this marked differences in behavior, ACTH levels were similar in both control and shocked rats (right panel). This lack of sensitivity of ACTH to fear conditioning is due to the short period of exposure to the context as ACTH reflects context fear conditioning when exposure to the context last for 15 min [Daviu et al. 2010]. Means and SEM (n=16) are represented. *** $p < 0.001$ vs controls.

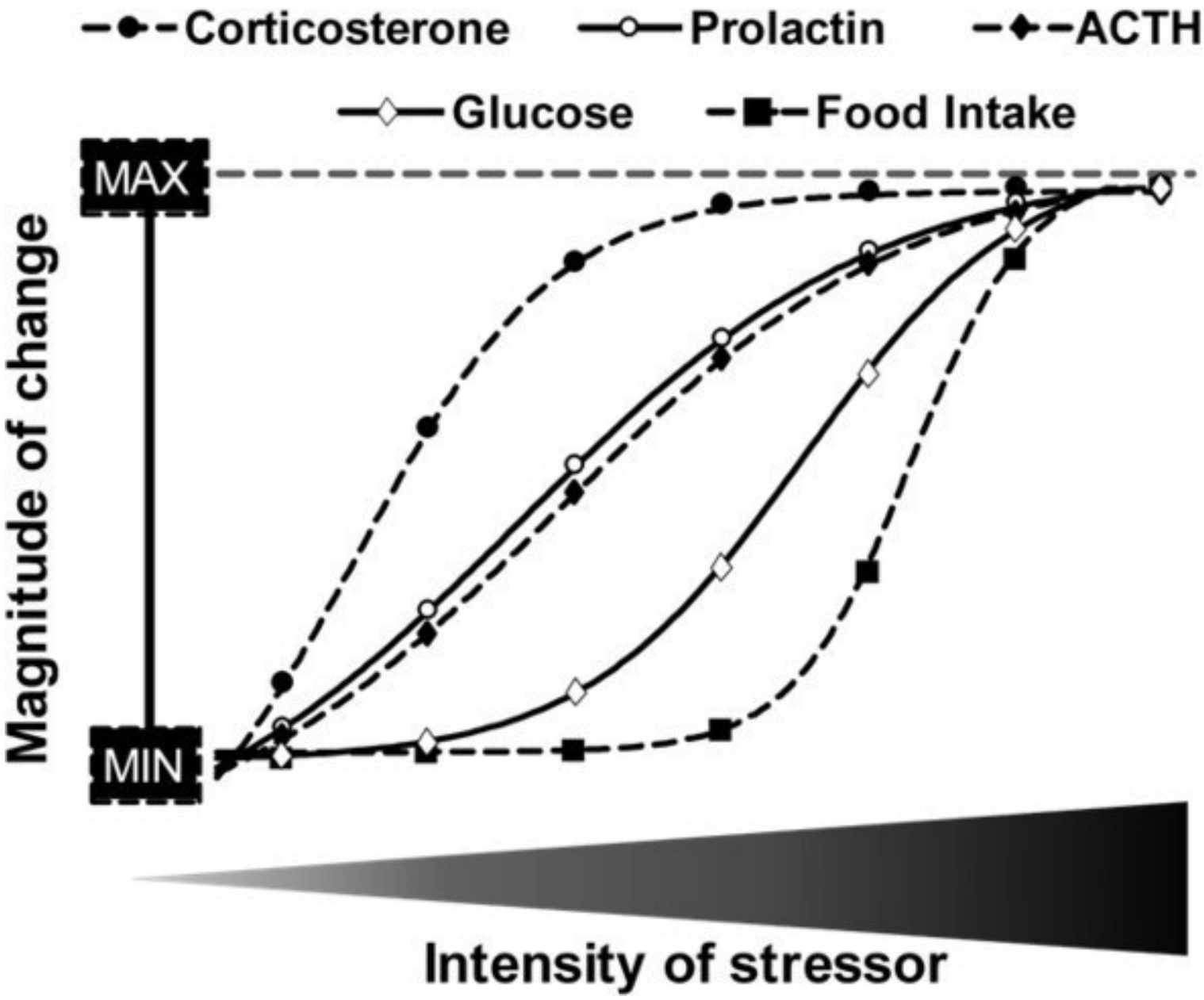
Fig. 4. ACTH response did not reflect levels of anxiety in the elevated plus-maze. Time spent in the open arms of the elevated-plus-maze (EPM) in a normal population of adult male rats classified in function of the median in low-anxiety (LA) and high-anxiety (HA). In the right panel can be seen that ACTH response to the EPM did not differ between LA and HA rats. Means and SEM (n=9) are

represented (unpublished data from C. Márquez, R. Nadal and A. Armario). ** $p < 0.01$ vs LA, NS: non significant.

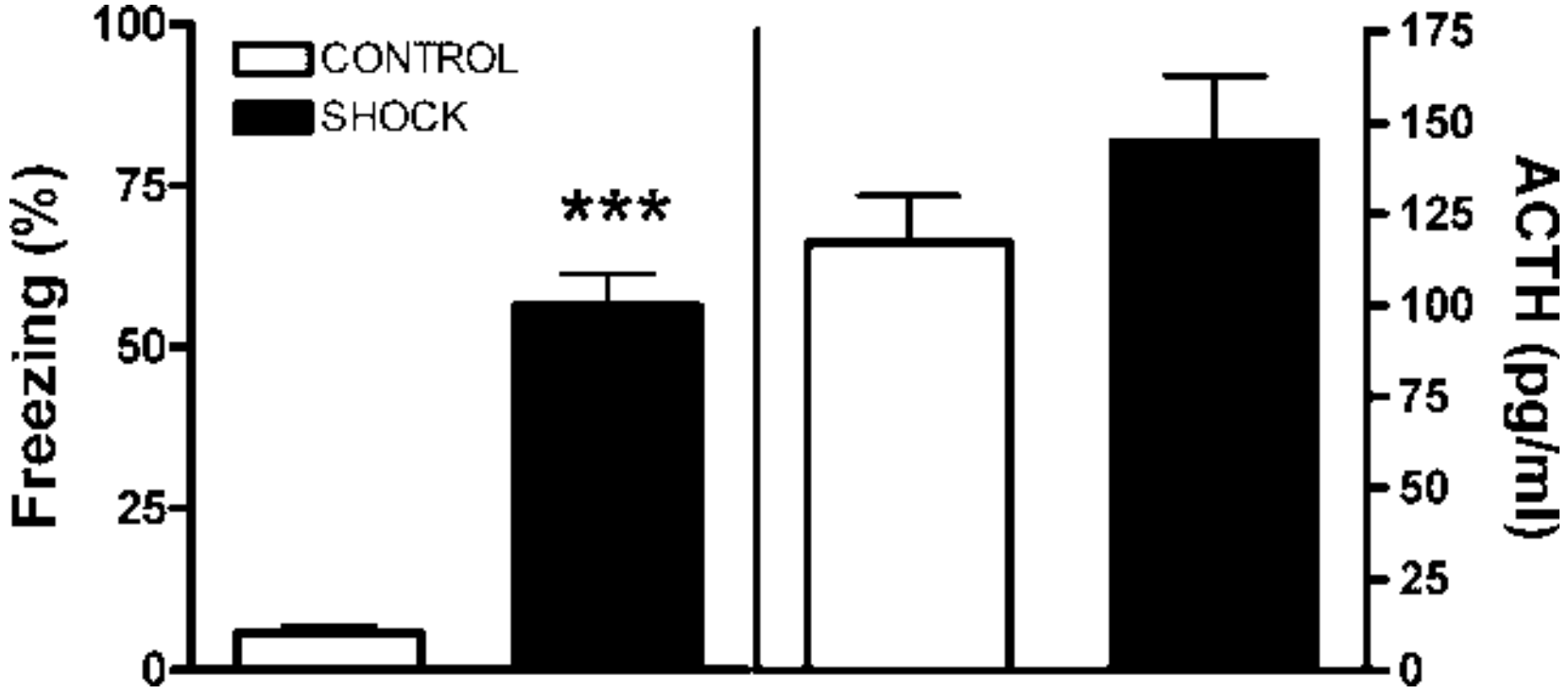
Figure


Corticosterone (µg/dl)
30
25
20
15
10
5
0
C
1
2
3
4
Arbitrary levels of intensity
LH (ng/ml)
50
40
30
20
10
0
C
1
2
3
4
Arbitrary levels of intensity

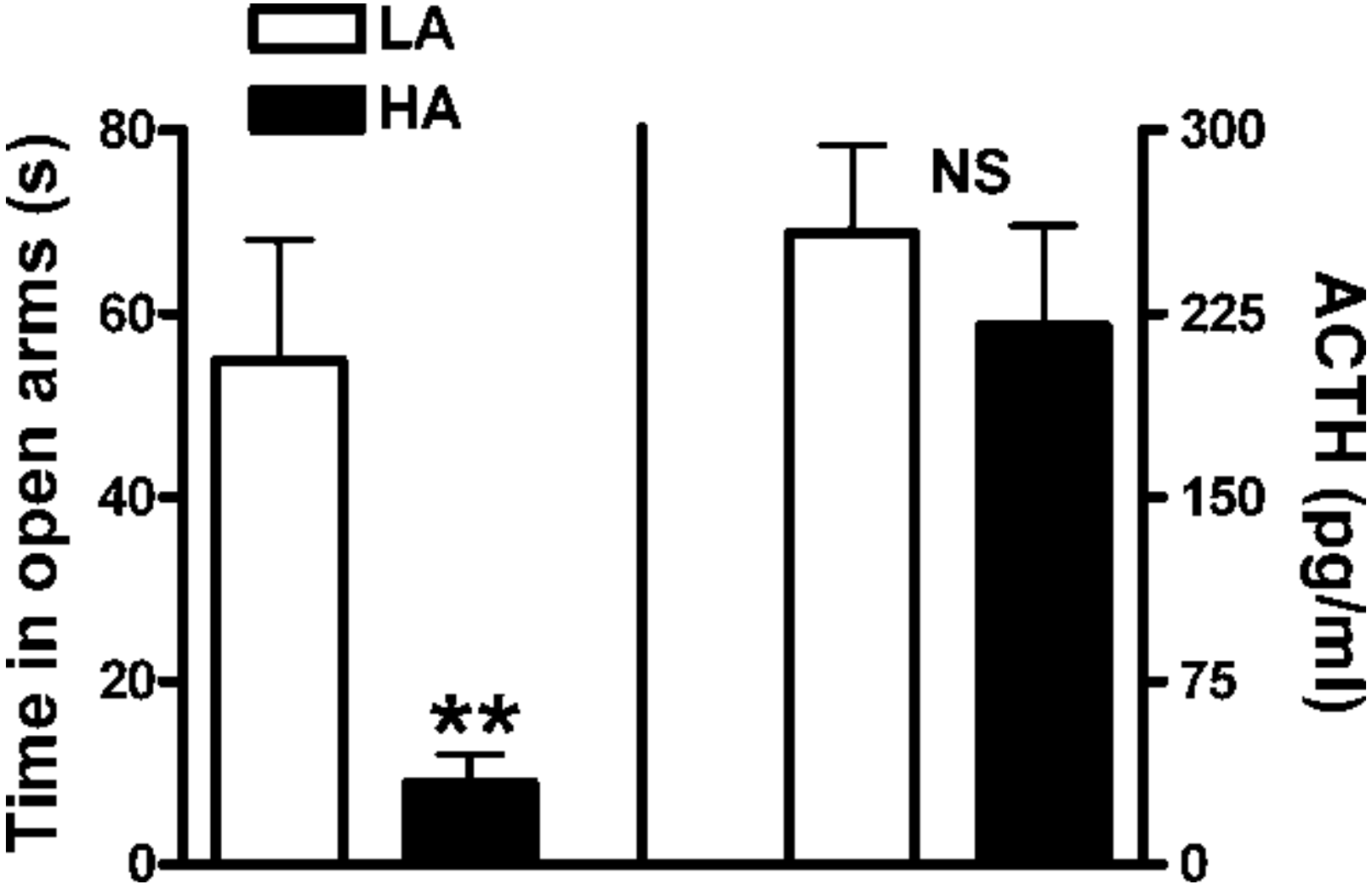
Figure


Corticosterone
Prolactin
ACTH
Glucose
Food Intake
MAX
MIN
Magnitude of change
Intensity of stressor

Figure


CONTROL
SHOCK
***
Freezing (%)
100
75
50
25
0
ACTH (pg/ml)
175
150
125
100
75
50
25
0

Figure


LA
HA
Time in open arms (s)
80
60
40
20
0
**
NS
300
225
150
75
0
ACTH (pg/ml)