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Stress-induced sensitization: the hypothalamic-pituitary-adrenal axis and beyond

Xavier Belda [1,2,4] Silvia Fuentes[1,3,4], Nuria Daviu[1,2,4], Roser Nadal[1,3,4], Antonio Armario[1,2,4] *

[1]Institut de Neurociències, [2]Unitat de Fisiologia Animal (Facultat de Biociències), [3]Unitat de Psicobiologia (Facultat de Psicologia), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain and [4]Red de trastornos adictivos (RTA), Instituto de Salud Carlos III, Spain

*To whom correspondence should be addressed:

Dr. Antonio Armario

Unitat de Fisiologia Animal (Facultat de Biociències), Universitat Autònoma de Barcelona,

08193 Cerdanyola del Vallès, SPAIN.

Antonio.armario@uab.es

Phone 34-935811840

Fax 34-935812390

Abstract

Exposure to certain acute and chronic stressors results in an immediate behavioral and physiological response to the situation followed by a period of days when cross-sensitization to further novel stressors is observed. Cross-sensitization affects to different behavioral and physiological systems, more particularly to the hypothalamus-pituitary-adrenal (HPA) axis. It appears that the nature of the initial (triggering) stressor plays a major role, HPA cross-sensitization being more widely observed with systemic or high-intensity emotional stressors. Less important appears to be the nature of the novel (challenging) stressor, although HPA cross-sensitization is better observed with short duration (5–15 min) challenging stressors. In some studies with acute immune stressors, HPA sensitization appears to develop over time (incubation), but most results indicate a strong initial sensitization that progressively declines over the days. Sensitization can affect other physiological system (i.e. plasma catecholamines, brain monoamines), but it is not a general phenomenon. When studied concurrently, behavioral sensitization appears to persist longer than that of the HPA axis, a finding of interest regarding long-term consequences of traumatic stress. In many cases, behavioral and physiological consequences of prior stress can only be observed following imposition of a new stressor, suggesting long-term latent effects of the initial exposure.

Short title: stress-induced sensitization

Key words: ACTH, Corticosterone, Prolactin, Catecholamines, Immune Stressors, PTSD, Immobilization, Electric Shock

Introduction

Exposure to stressors induces a broad and coordinated repertoire of behavioral and physiological responses to enhance probability of survival. In mammals, the two major physiological components of the stress response comprise the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. The activation of the latter results in several physiological responses and the immediate release of catecholamines: noradrenaline (norepinephrine) from sympathetic nerve terminals (and to a lower extent, the adrenal medulla) and adrenaline (epinephrine) from the adrenal medulla. The stress-induced peak of plasma catecholamine concentration is achieved within 1–5 min. Among other effects, sympathetic activation and catecholamines promote hepatic glycogenolysis and increase heart rate and blood pressure.

The initial step in the activation of the HPA axis is activation of parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVN) and the release of corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) and other secretagogues into the portal circulation in the median eminence. These releasing hormones act on the corticotrope cells of the anterior pituitary to stimulate the synthesis and secretion of ACTH, which in turn acts on the zona fasciculata of the adrenal cortex to promote the synthesis and release of glucocorticoids (corticosterone in rats and mice; predominantly cortisol in humans and other mammals). Peak levels of plasma glucocorticoids are achieved 15–30 min after the onset of a brief stressor or even later with exposure to stressor lasting for more than 30 min. A main effect of stress-induced glucocorticoid release is to inhibit the ongoing activation of the HPA axis through negative feedback mechanisms that involve both mineralocorticoid and glucocorticoid receptors (MR and GR, respectively), acting at multiple target areas, such as the medial prefrontal cortex, the hippocampal formation, the PVN and the anterior pituitary (Myers et al., 2012).

However, stress-induced glucocorticoid release has a wide range of additional physiological effects aiming to control for the correct mobilization of resources, to prevent excessive response of the different systems initially activated by stress, and preparing the organism for further stress (Frank et al., 2013; Sapolsky et al., 2000). Importantly, glucocorticoids also play a major role in the elaboration of behavioral strategies to cope with stress, as well as the consolidation of memory regarding the situation (de Kloet et al., 1999).

Sensitization is a simple concept that probably includes a wide range of different underlying processes. In its more general meaning, sensitization is a phenomenon thereby exposure to a particular stimulus triggers a state of hyperresponsiveness to the same or other different stimuli. The triggering stimulus has to be strong and harmful, and the hyper-responsive state can last from some minutes-hours to days (short-term and long-term sensitization, respectively). In order to be more precise, we will use the term sensitization when enhanced responses to the same (homotypic) stimulus are found, and cross-sensitization when enhanced responses to novel (heterotypic) stimuli are observed. Sensitization can affect a particular peripheral physiological system or can affect brain processing, as it is the case of behavioral sensitization. The latter is considered as a primitive form of non-associative learning characterized by a progressive increase in the response to aversive or noxious stimuli after repeated exposure (Rahn et al., 2013). Sensitization and cross-sensitization are considered to underlie a number of physiological and behavioral pathologies including gastrointestinal disorders, chronic pain, post-traumatic stress disorder (PTSD), psychosis and addiction (Overmier et al., 2006; Rahn et al., 2013; Robinson & Berridge, 2008; Ursin, 2014; van Winkel et al., 2008).

The term sensitization became extensively used in studies dealing with the long-term consequences of repeated experiences with drugs of abuse, on the basis of the observation that the motor response to a wide range of abused drugs progressively increased after repeated intermittent administration of the drugs (particularly after a period of withdrawal from the

drugs) (Steketee & Kalivas, 2011). This progressive sensitization process is considered by some authors to be in the roots of addiction (Robinson & Berridge, 2008). One of the most relevant topics in sensitization is the specificity of the phenomenon, i.e. whether or not sensitization is observed in response to the same stimulus/drug (homotypic sensitization) or between different stimuli/drugs. In the field of drug addiction, there is evidence for cross-sensitization between different drugs and also between drugs and stress (Kalivas & Stewart, 1991). Cross-sensitization is not surprising, given that the mechanisms involved rely on downstream processes that are shared by both types of stimuli (e.g. the dopaminergic system in stress-drug interaction) (Saal et al., 2003).

In the stress and HPA axis field, sensitization has also been a relevant topic since pioneering work by Dallman and Jones (1973) in the early 1970s. They reported that the endogenous release of corticosterone induced by restraint stress did not modify the adrenocortical response to a subsequent mild stress of intraperitoneal injection when applied several hours later. However, injection of ACTH or corticosterone, which mimicked the corticosterone response to restraint, inhibited corticosterone response to subsequent injection stress. They concluded that stress appears to induce a short-term (hours) facilitation of the HPA response that overcomes negative glucocorticoid feedback. A prediction derived from this hypothesis is that the blockade of glucocorticoid release during the first stress exposure should result in an enhanced response to the second stress, since facilitation is not counteracted by glucocorticoids. This hypothesis has been demonstrated using a single prior exposure to restraint after pharmacological blockade of glucocorticoid synthesis and testing ACTH response to injection stress on the next day (Wong et al., 2000). Although facilitation has been mainly used to explain changes in the HPA response to novel stressors, it might also apply to homotypic stressors. Thus, four brief exposures to immobilization (1 min) on the same day did not alter the ACTH response to the stressor in intact rats, but resulted in facilitation of the ACTH response in adrenalectomized (ADX) rats supplemented with low corticosterone levels in water (ADX+B) (mimicking resting

corticosterone levels) (Andrés et al., 1999). In contrast, more prolonged exposures to immobilization reduced the HPA response to subsequent stressors (Martí et al., 1999). The balance between stress-induced facilitation and negative feedback might explain why controversial results have been found in intact animals using different combinations of stressors (Graessler et al., 1989; Le Mevel et al., 1979); particularly, when elevated levels of corticosterone are found just prior to the second stress (Graessler et al., 1989; Martí et al., 1999).

Whereas the term facilitation is widely used regarding the effects of prior stress on the HPA axis, sensitization has been more extensively used in other behavioral and physiological fields. Since it is difficult to know whether or not sensitization and facilitation are similar terms, the present review will use the terms ‘‘sensitization’’ and ‘‘cross-sensitization’’ as defined before.

Chronic stress-induced sensitization

In general, daily exposure to the same stressor results in a reduction of the response of certain endpoints (mainly plasma levels of ACTH, corticosterone and adrenaline) to an acute session of the homotypic stressor (Martí & Armario, 1998), a process that is usually termed habituation. In some cases, an enhanced corticosterone response to the homotypic stressor was found, which was considered to reflect sensitization (i.e. Natelson et al., 1988). However, we have repeatedly found that repeated exposure to a severe stressor, such as immobilization increases plasma corticosterone levels measured immediately after the stressor despite a marked decrease in plasma ACTH (i.e. Armario et al., 1988a; Márquez et al., 2004). This paradox can be easily explained by a chronic stress-induced increase in maximal capability of the adrenal to secrete corticosterone (Armario et al., 1988a; Ulrich-Lai et al., 2006) and a saturation of adrenal capability with intermediate levels of ACTH (Keller-Wood et al., 1983). If repeated exposure resulted in strong decrease of ACTH with respect to the first exposure, but levels of ACTH are

still capable of eliciting maximal adrenal secretion, reduction of plasma ACTH levels associated with higher levels of corticosterone is to be expected. Indeed, if we consider only the variable more proximal to the brain (ACTH), we would not be able to conclude that sensitization to the homotypic stressor took place.

These arguments do not preclude the possibility that attenuated neuroendocrine habituation or even development of sensitization can occur after repeated exposure to certain stressors. Orr et al. (1990) observed enhanced ACTH and corticosterone responses to daily repeated tail-shocks. More recently, we have compared the habituation of the ACTH response to daily repeated immobilization with that to electric foot-shock using two different (low and high) shock intensities (Rabasa et al., 2011). Although immobilization appears to be a stronger stressor than high intensity footshocks in terms of all classical biological markers, habituation of ACTH was clearly found with immobilization and with the lower intensity foot-shock procedure, but not after daily repeated sessions of high intensity foot-shocks. We argued that high-intensity foot-shocks might cause a strong activation of nociceptive pathways leading to development of sensitization that opposes the expected habituation. A recent paper using a single exposure to foot-shocks followed by daily repeated exposure to the context (fear conditioning) found that some physiological responses to the same stressor in another context were enhanced (heart rate, hyperthermia), whereas that of corticosterone did not (Thompson et al., 2014), illustrating again that sensitization not homogeneously affected all stress-responsive systems. Sensitization of corticosterone response to daily exposure of rats to a cat (potential rat predator) has been reported, although the results are difficult to interpret because ACTH data were not reported (Figueiredo et al., 2003). Moreover, habituation rather than sensitization has been found with repeated exposure to ferret odor (Weinberg et al., 2009).

The hypothesis that certain particular characteristics of systemic stressors can not only impede habituation but rather cause sensitization after daily repeated exposure is supported by other

data showing reduced plasma catecholamine response to daily repeated immobilization, but enhanced response to daily exposure to trauma (rotation in the Noble-Collip drum) (Kvetnansky et al., 1984). Similarly, whereas repeated exposure to forced swim at 34 ºC elicited habituation of both noradrenaline and adrenaline responses, daily swim exposure to 18 and 24 ºC did not cause habituation of adrenaline and the response of noradrenaline was enhanced rather than reduced (Konarska et al., 1990). These data are supported by our recent results demonstrating that relatively low temperature of water (24 versus 36 ºC) interfere with the habituation of the HPA axis to forced swim (Rabasa et al., 2013).

More typically, enhanced HPA response has been described in animals with a prior history of chronic stress when facing novel (heterotypic) stressors (cross-sensitization), although controversial results have been obtained (Dallman, 1993; Martı´ & Armario, 1998). The reasons for these discrepancies are not entirely clear, but some general pattern emerges. When plasma levels of ACTH rather than that of corticosterone have been evaluated (for the reason explained above), cross-sensitization has been found in response to a variety of stressors (saline injection, restraint, ether) after chronic exposure to continuous or intermittent cold (Bhatnagar & Dallman, 1998; Bhatnagar & Meaney, 1995; Hauger & Aguilera, 1992; Ma & Morilak, 2005; Pardon et al., 2003; Sakellaris & Vernikos-Danellis, 1975), with some exceptions (Akana et al., 1996; Bhatnagar et al., 1995). Chronic administration of hypertonic saline also resulted in HPA sensitization to a different stressor (ether) (Kiss & Aguilera, 1993). Therefore, prior chronic exposure to systemic stressors appears to induce HPA cross-sensitization.

With stressors having lesser systemic components (i.e. noise, predator odor, restraint or immobilization on boards), there are studies reporting a normal response to heterotypic stressors (Armario et al., 1986, 1988a; Babb et al., 2014; Ferland et al., 2014; Spiga et al., 2009; Weinberg et al., 2009), and others showing an enhanced response (Hauger et al., 1990; Heydendael et al., 2011; Lachuer et al., 1994; Ma et al., 1999). In a few cases, a reduced

response has also been reported after repeated exposure to restraint or immobilization (Chen et al., 2008; Mansi & Drolet, 1997; Martí et al., 1994). Interestingly, some reports using ADX rats or ADX rats supplemented with low corticosterone pellets (mimicking resting levels) (ADX+B) have observed chronic-stress-induced cross-sensitization of the ACTH response that were not found in intact rats (Akana et al., 1996; Martí et al., 1994). It is possible that, at least with predominantly emotional stressors, the eventual consequences of prior chronic stress might depend on two opposite processes: stress-induced HPA facilitation and glucocorticoid-induced inhibition by negative feedback. In conclusion, HPA cross-sensitization appears to be more likely with certain systemic stressors and with high-intensity (predominantly) emotional stressors, but individual/strain differences might explain why under apparently similar conditions sensitization can develop or not.

Chronic-stress-induced cross-sensitization also affects the central and peripheral catecholaminergic systems (see Kvetnansky et al., 2009 for an extensive review about these systems). Whereas habituation of the plasma noradrenaline and, more particularly, adrenaline responses to a daily repeated stress is observed with typical laboratory stressors (immobilization, restraint, foot-shock), the response to novel heterotypic stressors is consistently increased (Konarska et al., 1989a,b; Kvetnansky et al., 1984). Similarly, sensitization of both adrenaline and noradrenaline has been observed in response to an acute immobilization after chronic administration of lipopolysaccharide (endotoxin), an immune stressor (Moncek et al., 2003), and chronic cold (Kvetnansky, 2004).

The pattern is somewhat different when looking at the adrenomedullary expression of enzymes involved in catecholamine synthesis: tyrosine-hydroxylase (TH), the first and rate synthesis limiting enzyme, and phenylethanolamine-N-methyltrasferase (PNMT), the enzyme responsible for the synthesis of adrenaline. Prior exposure to chronic immobilization increased resting levels of TH and PNMT mRNA, while slightly reducing the response to acute immobilization and

completely blocking the response to heterotypic stressors of different nature (Kvetnansky et al., 2003; Kvetnansky, 2004; McMahon et al., 1992; Viskupic et al., 1994). Basic maintenance of responsiveness to the homotypic stressor (immobilization) is associated with marked changes in the induction of transcription factors from acute to chronic immobilization (Sabban & Serova, 2007). It is unclear whether lack of responsiveness to heterotypic stressors is related to the already high resting levels of mRNA and protein levels of enzymes or the choice of challenging stressors of lower intensity than immobilization. In contrast to the effects of immobilization, chronic exposure to cold potentiated the increase in TH and PNMT mRNA levels caused by various acute heterotypic stressors (Kvetnansky, 2004), again revealing the particular characteristics of chronic cold.

Chronic stress-induced cross-sensitization of anterior pituitary hormones other than ACTH has been poorly studied. Prolactin is of interest because it is as sensitive to the intensity of stressor as ACTH (Armario et al., 2012). There is one report revealing cross-sensitization of prolactin release using daily cage transfer or handling as the chronic stressors, with exposure to the opposite stressor used to test the heterotypic response (Dobrakokova & Jurcovicova, 1984). In contrast, studies using more prolonged and/or severe stressors (i.e. noise, restraint, immobilization, foot-shock) to induce or test sensitization indicated normal or reduced response to heterotypic stressors (Armario et al., 1986, 1988b; Kant et al., 1985; van Raaij et al., 1997). Since glucocorticoids exert an inhibitory effect on stress-induced prolactin release (Martí & Armario, 1998), it is possible that daily glucocorticoid release acts to impede the expression of any stress-induced sensitization, if present at other levels.

Although chronic-stress induced sensitization and cross-sensitization can obviously affect peripheral physiological response to stressors, the brain is likely to be critically involved. Evidence for neurochemical homotypic sensitization was reported by Anisman and Sklar (1979), who observed hypothalamic noradrenaline depletion after a brief foot-shock session in

mice previously exposed to a long footshock session the day before, but not in stress-naïve controls. Cross-sensitization of serotonin and noradrenaline responses to a superimposed acute stressor (immobilization on boards) was also demonstrated several decades ago in rats chronically restrained in plastic tubes (Adell et al., 1988). Furthermore, several groups have obtained evidence for development of sensitization of certain neurochemical systems after chronic stress. Most studies have been focused on the noradrenergic and dopaminergic systems, using microdialysis to evaluate neurotransmitter release. The role of chronic stress on the dopaminergic system is extremely complex and out of the scope of the present review, but it is of note that several studies have demonstrated that prior chronic stress enhances dopamine release in response to novel stressors in the medial prefrontal cortex and the nucleus accumbens (Cuadra et al., 2001; Di Chiara et al., 1999).

Using microdialysis, Nisenbaum et al. (1991) firstly demonstrated that continuous exposure of rats to cold (4 ºC) for 3–4 weeks resulted in enhanced hippocampal noradrenaline synthesis and release in response to tail-shock. With the same chronic stressor, cross-sensitization of the dopamine and noradrenaline response to tail-shock was also observed in the medial prefrontal cortex (Gresch et al., 1994). In the latter report, dopamine response was also studied in striatum and nucleus accumbens, with no evidence for cross-sensitization, indicating that the phenomenon was region-specific. It is important to note that cold has a strong systemic component and would not be a representative stressor, particularly when using continuous exposure to the stressor (see above discussion of the effects of cold on the HPA axis and peripheral catecholamines). Thus, the same authors reported that medial prefrontal cortex noradrenaline release induced by an acute session of tail-shocks was not observed after chronic intermittent (4 h/day) exposure to cold or after continuous exposure to foot-shock (Jedema et al., 1999). Nevertheless, chronic intermittent cold can induce cross-sensitization under certain conditions. This stressor did sensitize the noradrenaline response to an acute immobilization in

the bed nucleus of the stria terminalis, but only in Wistar Kyoto rats and not in Sprague-Dawley rats (Pardon et al., 2003).

The effects of prior chronic stress on the transcriptional response of locus coeruleus (LC) catecholamine synthesis enzymes to homotypic and heterotypic stressors are poorly known. In addition, basal levels of enzymes prior to the last challenging stressor have not always been assessed, thus confounding the interpretation of the results (i.e. Makino et al., 2002). The issue is further complicated by the fact that the contribution of post-transcriptional mechanisms appears to be more relevant in the LC than in the adrenal medulla (Osterhout et al., 2005; Sun et al., 2004). Nevertheless, when basal levels of enzymes prior to the last stress exposure have been assessed, daily repeated immobilization resulted in enhanced resting levels of TH, with reduction of the acute response to the homotypic stressor and normal response to heterotypic stressors (Rusnak et al., 2001). Accordingly, after daily repeated exposure to a relatively mild stressor (air-puff)-reduced TH gene expression in response to the homotypic stressor has been observed in WKY and SHR rats (McDougall et al., 2005).

The critical role of stressor type is also supported by studies on stress-induced changes in electrophysiological activity of LC. Whereas chronic continuous cold has been found to exert a slight enhancement or null effect on spontaneous firing rate of LC neurons (recorded in anesthetized rats on the day after the last exposure) (Jedema et al., 2001; Mana & Grace, 1997), the responsiveness of LC neurons to superimposed physical stressors was increased (Mana & Grace, 1997). These effects are associated with a potentiated stimulatory response to CRH (Jedema et al., 2001) and reduced sensitivity to negative feedback mediated by a2 receptors (Jedema et al., 2008). However, after daily repeated social defeat reduced firing rate has been reported in resting conditions, associated with a higher l opioid tone (Chaijale et al., 2013). Unfortunately, the response to other stressors was not studied.

Recent evidence implicates the LC and noradrenergic systems in PTSD-like effects of a single exposure to stress. Thus, exposure of mice to a single shock followed by situational reminders caused long-term changes in anxiety-like and social behavior in susceptible but not resilient animals. Behavioral changes were associated with enhanced activation of the LC and prevented by pharmacological blockade of the noradrenergic system (Olson et al., 2011). After exposure to the single prolonged stress model for PTSD (consisting of exposing the animals to three different stressors on the same day), George et al. (2013) demonstrated a long-lasting reduction of spontaneous electrophysiological activity of LC neurons after, but enhanced response to a noxious stimuli. Additional studies with other acute and chronic stress models are needed to clarify the impact of stress on LC neuronal activity and its functional consequences.

In conclusion, there is evidence that chronic stress can, under certain conditions, induce cross-sensitization of the sympathoadrenomedullary (SAM) and HPA axes in response to heterotypic stressors, but the effect does not generalize to other neuroendocrine systems. Central sensitization of monoamines is also sometimes observed, although it is unclear the extent to which chronic exposure to stressor having a predominant emotional component are able to induce cross-sensitization of brain noradrenaline release. More consistent evidence appears to link long-term effects of acute stressors with altered noradrenergic activity. Finally, important regional differences as well as individual/strain differences in the development of cross-sensitization are expected.

Acute stress-induced HPA sensitization

In the past decades, a great interest has been generated for the long-term consequences of a single exposure to stress in animal models for PTSD in order to understand biological mechanisms underlying this pathology (Armario et al., 2008). In animal models of PTSD, sensitization appears to be a crucial phenomenon to explain enhanced responsiveness to both

stimuli associated with the trauma and other types of stressors. In some experiments, electric shocks were used for both inducing and expressing sensitization, with shocks given in the same manner (i.e. foot-shock) or in different body regions (i.e. tail-shock versus foot-shock or foot-shock versus probe-shock). In the latter cases, it is difficult to know whether the animals can perceive the stimulus as distinct.

Behavioral sensitization has been demonstrated after exposure to a session of several shocks in rats and after a single shock in mice (i.e. Servatius et al., 1995; Siegmund & Wotjak, 2007; van Dijken et al., 1992). Shocked animals showed an enhanced freezing response to superimposed stressors, such as sudden interruption of a background noise or sudden appearance of a noise burst (Siegmund & Wotjak, 2007; van Dijken et al., 1992, 1993), and an enhanced startle response (Servatius et al., 1995). In our laboratory, we have demonstrated that a single exposure to immobilization can transiently enhance anxiety, but when such an effect apparently disappeared after 10 days, a brief superimposed session of shocks markedly increased anxiety as measured with the elevated plus maze, whereas the same shocks had no effect in stress-naive rats (Belda et al., 2008). These data strongly indicate that animals are still more susceptible to novel stressors, but the long-lasting effect of prior severe stress could not be evident unless a new challenge has to be faced. Similarly, a single inescapable foot-shock or tail-shock session has been demonstrated to enhance foot shock-induced fear conditioning for several days after the inescapable footshocks (Baratta et al., 2007; Rau et al., 2005). In some cases, the impact of a prior stress is only observed after an incubation period of several days (typically one week), suggesting some kind of slowly progressing phenomenon (Pamplona et al., 2011). There is also evidence for incubation of fear after exposure to single prolonged stress (Knox et al., 2012; Koda et al., 2007; Takahashi et al., 2006; Wang et al., 2008). Thus, it appears that some behavioral consequences of stressors reach a maximum on the next hours or days whereas in other cases such consequences progressively increase over time (Figure 1). Interestingly, when simultaneously studied, behavioral sensitization to further stressors outlasted HPA sensitization

(Belda et al., 2008). Nevertheless, this does not preclude that some components of the HPA axis or other biological systems activated in response to the stressor might lead to long-term changes in gene expression and consequently in behavior.

Caggiula et al. (1989) and van Dijken et al. (1993) were the first to report that a single session of foot-shocks was able to induce long-lasting (10–14 days) enhancement of ACTH or corticosterone response to a short exposure to another brief shock session or to a novel environment, respectively. In parallel, several reports appeared regarding long-term effects of single exposure to systemic stressors on HPA responsiveness. Although the present review focuses on the long-term effects of emotional stimuli, we will make a brief mention of some relevant data on systemic stimuli. Tilders' lab was also the first to report that a single cytokine (IL-1b) administration in rats was able to induce long-term sensitization of the HPA response to a variety of challenging stressors including IL-1b (Schmidt et al., 1995), amphetamine (Schmidt et al., 1999, 2001), novel environments and foot-shocks (Schmidt et al., 1995, 2001, 2003). When the time-course of sensitization was studied, it was found that the effect of IL-1b on HPA responsiveness to a novel environment reached a maximum of 11–22 days after the administration of the cytokine and vanished at 42 days (Schmidt et al., 2003). After these initial studies with IL-1b, Anisman's lab published a series of papers studying the long-term effects (between 24 h and 28 days) of a single administration of other immune stimuli including endotoxin and the cytokine tumor necrosis factor-a (TNF-a). They studied not only the response of the HPA axis, but also changes in sickness behavior and neurochemistry (Anisman et al., 2003; Hayley et al., 2003). With respect to the HPA axis, the time-course of the effects appears to show a relatively high level of variability depending on both the particular nature of the initial immune stimuli and the challenging systemic stressor, but evidence for homotypic and heterotypic sensitization was found that in some cases enhanced with the time elapsed between the two exposures. Taken together, it appears that exposure to systemic stressors, in contrast to emotional stressors, can induce sensitization of the HPA response to both the homotypic and the

heterotypic stressors and this effect progressively enhances over time (see Armario et al., 2004). In contrast, emotional stressors would induce sensitization of the response to heterotypic stressors, particularly when the challenging stressors have immune components.

Regarding emotional stressful stimuli, the data obtained so far point to a long-lasting sensitization of the HPA response when animals are exposed to a heterotypic stress. After a long-term sensitization of the HPA axis with a single exposure to a moderate session of foot-shocks was described (van Dijken et al., 1993), we demonstrated that a single prior exposure to immobilization resulted in a reduced HPA response to the homotypic stressor, but an enhanced response to a different (forced swim) stressor, particularly evident in both cases during the post-stress period (Martí et al., 2001). Later on, long-lasting HPA sensitization of the response to heterotypic stressors was observed in Maier's lab using the inescapable tail-shock procedure typical of the learned helplessness paradigm. They observed that a single session of shocks caused sensitization of the HPA response to emotional (pedestal) and systemic (endotoxin, 10 µg/kg) stressors (Johnson et al., 2002a, 2003; O'Connor et al., 2004). Although the effect on exposure to the pedestal was studied only after 24 h (Johnson et al., 2002a; O'Connor et al., 2004), sensitization to endotoxin was already observed on the day after shock and lasted for at least 10 days, disappearing at 21 days (Johnson et al., 2002a). Having control over the aversive experience (comparing the effects of escapable and yoked inescapable tail-shocks) does not modify long-term sensitization of corticosterone response to other emotional stressor, such as restraint (Weinberg et al., 2010). Interestingly, the same lab also reported that prior exposure to one tail-shock session can induce sensitization (priming) of the immune response to endotoxin that might be mediated by glucocorticoid released during tail-shocks (Frank et al., 2010, 2012; Johnson et al., 2002b, 2003).

Supporting our preliminary results using forced swim as the heterotypic stressor (Martí et al., 2001), we have repeatedly observed that prior exposure to a single immobilization session

induces sensitization of the HPA response to novel environments that lasts for around one week (Belda et al., 2008; Gagliano et al., 2008; Muñoz-Abellan et al., 2008). It is unclear whether qualitative aspects of triggering stressors, rather than their intensity or duration, are responsible for long-lasting HPA sensitization. We then exposed rats to 2 h of immobilization, 2 h of severe foot-shock (2 mA, 6 s, one per min), or to a brief session of three mild shocks (0.5 mA, 3 s, one per min). Seven days after that, these rats, together with appropriate controls were exposed for 5 min to an open-field and sampled immediately after. As it can been seen in Figure 2, sensitization of the ACTH response was observed after previous exposure to immobilization and severe footshocks, but not after a brief session of mild foot-shocks. These results support the hypothesis that long-lasting HPA sensitization is mainly dependent on the intensity and/or duration of the triggering stressor rather than on its particular nature. As HPA sensitization can be induced by both acute and chronic exposure to a particular stressor, it is important to know whether stress-induced sensitization changes when animals are daily exposed to the same triggering and challenging stressors. After daily repeated exposure to immobilization (1 h) and to an open-field (5 min) for one week, sensitization of ACTH response was basically maintained, with a slight decline of ACTH response associated to a modest increase in corticosterone response (Daviu et al., 2014). It is possible that corticosterone sensitization after chronic stress could be associated to an extra-ACTH regulation that remains to be studied.

The mechanisms underlying acute stress-induced HPA sensitization are unclear at present. Previous studies using tail-shock as the inductive stressor demonstrated that HPA sensitization was associated with a reduced efficacy of negative glucocorticoid feedback (O'Connor et al., 2003). More recently, O'Connor et al. (2004) showed, using the same paradigm, that the greater ACTH and corticosterone response to a novel acute stressor 24 h later were accompanied by increased activation of the PVN (enhanced *cfos* expression and CRH mRNA levels) and the anterior pituitary (enhanced *cfos* and pro-opiomelanocortin gene expression). This strongly suggests that sensitization took place in the brain rather than peripherally. Although

sensitization could be secondary to the lower efficacy of glucocorticoid negative feedback, it was also observed in ADX animals (O'Connor et al., 2004), indicating that glucocorticoids are not needed to induce or express HPA sensitization. Therefore, sensitization is likely to be the result of a brain sensitization process independent of glucocorticoids. That glucocorticoids play no role in stress-induced sensitization was confirmed in our lab using immobilization as the inductive stressor and two different approaches to block or reduce the effects of glucocorticoid released during stress: the inhibition of glucocorticoid synthesis with metyrapone (MET) and blockade of GR with the antagonist mifepristone (Belda et al., 2012).

There are several reasons to consider CRH as a possible candidate for the induction of sensitization. First, there is evidence that CRH is involved in the induction and/or expression of stress-induced locomotor sensitization to amphetamine, acting centrally (Cador et al., 1993; Cole et al., 1990). Second, sensitization of ethanol withdrawal-induced reduction of social interaction was potentiated by prior exposure to two restraint stress sessions one week apart, an effect that was prevented by the administration of a CRH type 1 receptor (CRHR1) antagonist prior to stress (Breese et al., 2004). Third, CRHR1 receptors are involved in the development of stress-related visceral hyperalgesia (Larauche et al., 2012). Finally, repeated administration of urocortin into the basolateral amygdala causes long-lasting increases in anxiety-like behavior, probably acting through CRHR1 receptors as CRHR2 receptors have not been detected in this area (Rainnie et al., 2004). We then tested whether CRHR1 receptors are important for immobilization-induced HPA sensitization by given the selective antagonist R121919 prior to immobilization. The drug did not block sensitization (Belda et al., 2012), thus tentatively suggesting that CRHR1 receptors are not involved in this process. It will be of interest to determine whether CRHR1 receptors are important for the expression of HPA sensitization, and whether CRHR2 also plays a key role in the process.

Despite the consistent effect of single exposure to severe stressors to cause HPA sensitization, it appears that not all stressors have this property. Taking advantage of our previous data demonstrating that high doses of MET can act itself as a pharmacological stressor (Rotllant et al., 2002), we further studied whether prior MET administration can induce similar HPA sensitization as severe stressors. We found negative evidence (Belda et al., 2012), suggesting that HPA sensitization is not an universal consequence of exposure to all stressor-like agents, even if they are severe.

Another question is whether sensitization can affect other stress-sensitive neuroendocrine systems. When animals are exposed on two consecutive days to hemorrhage, sensitization of HPA response as well as the adrenaline response has been observed (Lilly et al., 1982, 1983, 1986). In contrast, repeated exposure to noise on the same day resulted in a (homotypic) reduction of corticosterone, adrenaline and noradrenaline response that is likely to be due to short-term habituation to an emotional stressor, as the behavioral response was also reduced (De Boer et al., 1988). Unfortunately, the response to a heterotypic stressor was not studied. We have analyzed plasma prolactin levels in several situations where HPA sensitization was found, examining the influence of prior immobilization on the prolactin response to a 5-min open-field exposure on the following day. As shown in Figure 3, prior exposure to 2 h immobilization caused a modestly enhanced prolactin response to the open-field in one experiment and no significant effect in another similar experiment. It is thus clear that unlike the HPA axis, the prolactin response to heterotypic stressors is not consistently sensitized by prior acute severe stressors. This inconsistency is similar to that achieved after chronic stress. However, in this case we could demonstrate that the lack of consistent acute stress-induced prolactin sensitization is not due to a constraining effect of glucocorticoids released during exposure to immobilization, as blockade of GR at this time had no effect on subsequent prolactin response to the open-field the following day.

Conclusions

Exposure to both acute and chronic stressors induces sensitization of certain behavioral and physiological responses to further stressors. The type of stressor, particularly whether they are systemic or emotional, could be critical to observe sensitization, especially when the response to the homotypic stressor is studied. In general, sensitization: (a) is dependent on the particular characteristics of the triggering and challenging stressors (nature, duration, intensity); (b) affects only a restricted subset of peripheral physiological processes and centrally mediated behavioral and physiological responses; (c) is better observed in response to novel heterotypic stressors; (d) in very restricted number of cases appears after an incubation period (days to weeks). The heterogeneity of the phenomenon precludes its consideration as a unitary process. We suggest some avenues to a better understanding of this phenomenon. For instance, in the case of homotypic stressors, it would be of particular interest to delineate the characteristics of the stimuli that determine the development of adaptation versus sensitization and the behavioral or physiological responses affected. Simultaneous evaluation of several different behavioral patterns or physiological systems could contribute to identify those systems more prone to sensitize. Finally, individual differences play a major role in the development of sensitization, but it is likely that some individuals are more susceptible to sensitization of particular behaviors or physiological systems, rather than showing a generalized susceptibility to develop sensitization in all systems.

Declaration of interest

The authors declare no conflicts of interest. They alone are responsible for the whole content of this manuscript.

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FIGURES

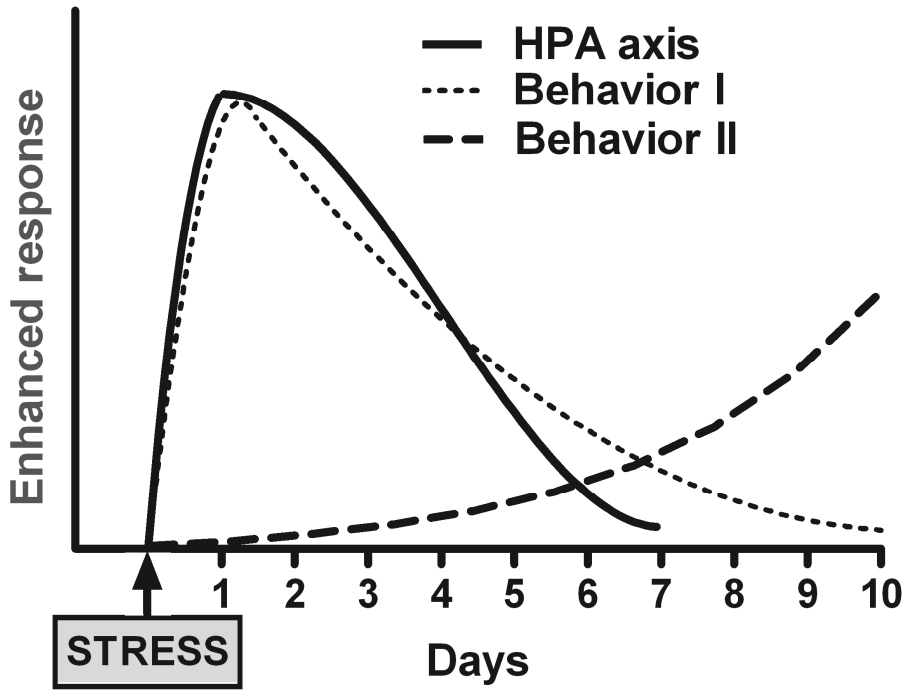

Figure 1. Exposure to severe emotional stressors can induce HPA and behavioral sensitization. HPA sensitization is maximally observed on the day after the stressors and can persist for up to 2 weeks depending on the characteristics of the stressor and the individual susceptibility (solid line). Behavioral consequences of stressors belong to two categories: (i) one that showed a maximum very soon after stressor exposure to progressively vanishing after that (Behavior I; dotted line); and (ii) another that are fully manifested long after stressor exposure showing some kind of incubation process (Behavior II; dashed line). Note that behavioral sensitization can be unmasked by superimposing a brief stressor, appears to be longer lasting than HPA sensitization and is usually reflected in different types of tests related to fear conditioning and anxiety-like behavior (i.e. elevated plus-maze, acoustic startle response).

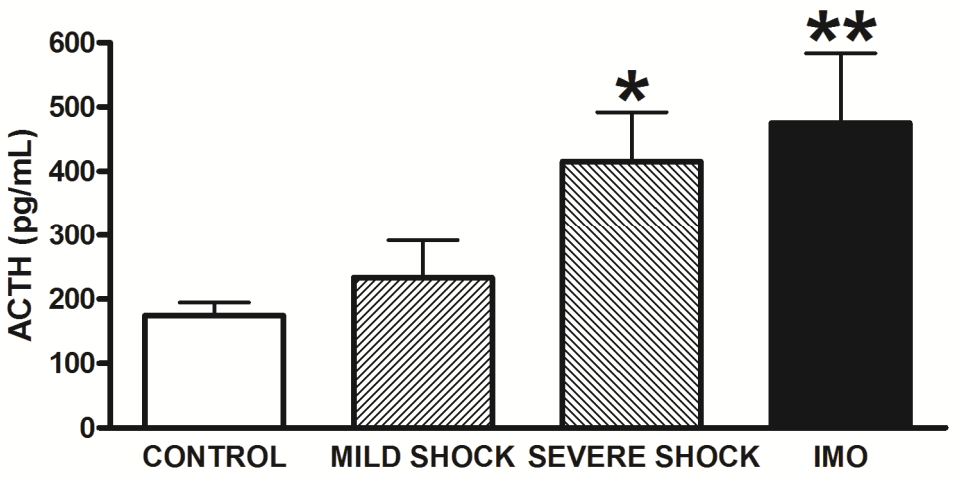

Figure 2. Long-lasting HPA sensitization is dependent on the intensity and/or duration of exposure to the triggering stressor. Adult male rats remained undisturbed or were exposed to a brief 5 min session of low intensity foot-shocks (3 x 3 s scrambled, AC current, shocks of 0.5 mA), a prolonged (120 min) session of high intensity foot-shocks (120 x 6 s scrambled, AC current, shocks of 2.0 mA) or 120 min of immobilization on boards (IMO). Seven days later all animals were exposed for 5 min to an open-field and immediately blood sampled. Means and SEM are represented (n¼8 per group). *p<0.05, **p<0.01 versus control.

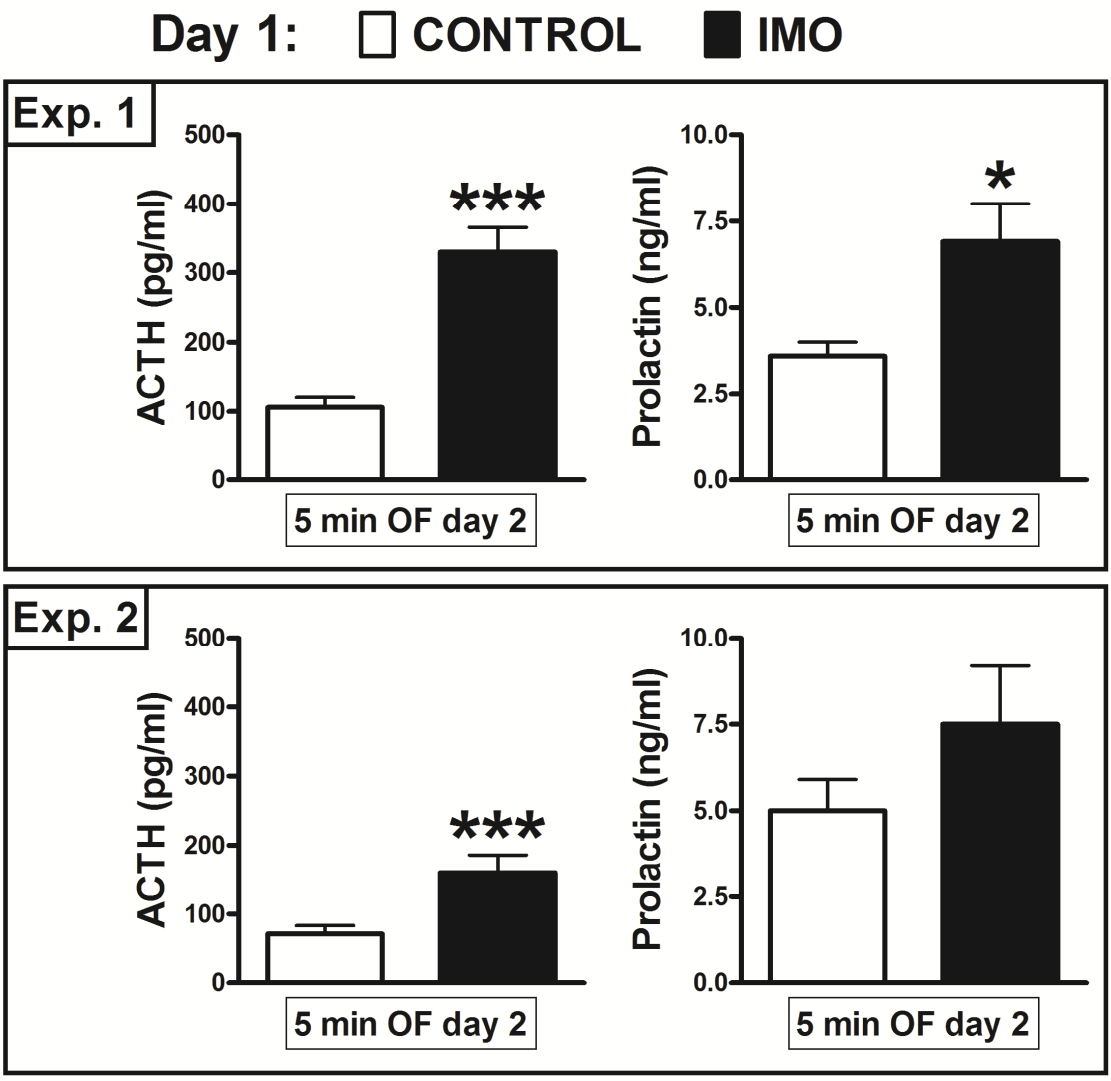

Figure 3. Prior acute stress induces null or weak prolactin sensitization. In contrast to the consistent sensitization of the HPA response to a brief heterotypic stressor caused by a single exposure to immobilization (IMO, 2 h) on the day before (Belda et al., 2012), prolactin response is only slightly enhanced, the results being statistically inconsistent among the different experiments. Panels A and B present results from representative experiments that always show consistent HPA sensitization and inconsistent prolactin sensitization. Means and SEM are represented (n = 10–12 per group). *p<0.05, ***p<0.001 versus control.