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Cytokines and hypothalamo-pituitary adrenal axis activation: a now classic of immune-neuroendrcine system interactions

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Abstract

Precise descriptions of neuroendrocrine and immune systems along with a more integrative orientation of research in the life sciences created the conditions in the last quarter of the 20th century to envision interactions between these systems. In particular, it was shown that pro-inflammatory cytokines, such as interleukin-1, produced in response to host detection of bacterial fragments, can activate the hypothalamo-pituitary-adrenal axis, resulting in the release of corticosteroids, which, in turn, have anti-inflammatory effects. Given the existence of the blood-brain barrier that prevents hydrophilic molecules like cytokines to passively enter the brain parenchyma, research efforts have focused on elucidating so-called cytokine-to-brain signaling pathways. The findings obtained indicate that several mechanisms, including prostaglandin-induced neuronal activation, vagal afferents and ascending catecholaminergic brainstem projections, can be involved in hypothalamo-pituitary-adrenal axis activation after systemic interleukin-1 administration depending on, both experimental and physiological, conditions. Most recently, both homeostatic-physiological stressors and emotional-psychological stressors have been proposed to give rise to inflammatory responses suggesting that crosstalk between innate immune mediators and hypothalamo-pituitary-adrenal axis hormones will continue to be at the forefront of research.

Keywords: cytokines, hypothalamo-pituitary axis, stress

Glossary

Cytokines: class of intercellular messenger molecules that can be distinguished from hormones and neurotransmitters (although no absolute separations exist between these categories) based on their mostly local mode of action, biological activity at very low concentrations, and pleiotropic (one molecules having many different effects) and redundant (same effect brought about by different molecules) actions.

Hypothalamo-Pituitary-Adrenal axis: Functional axes formed by the hypothalamus, anterior pituitary and endocrine gland of the adrenal cortex, activation of which typically results in release of Corticotropin-Releasing Hormone (CRH) by the hypothalamus into the portal blood vessel system of the hypophyseal stalk, and that of AdrenoCorticoTropic Hormone (ACTH) by the anterior pituitary and corticosteone by the adrenal cortex in the systemic circulation.

Stress: Although no consensus definition exists because this term is employed in different fields of research, it is safe to say that in psychology stress refers more to a state of an organism, whereas in physiology it is more considered as a response of an organism to threatened homeostasis.

X.1. Introduction: neuroendocrine and immune systems

X.1.1 Post WWII conceptual developments leading to neuroendocrine and immune systems

Although the notions of neuroendocrine and immune systems seem natural to present day scientists and physicians, it took in fact quite some conceptual and experimental work to first describe these systems. As pointed out in chapter 1, an influential vision of neuroendocrine systems was articulated in Geoffrey Harris' 1955 *Neural control of the pituitary gland* book in which he put forward that neuroendocrine neurons could be considered motor or effector neurons (Harris, 1955). He also specified functional criteria for determining if an endogenous substance constitutes a releasing factor at the level of the anterior pituitary. These proposals along with experimental findings led to the idea of functional axes formed by the hypothalamus, the pituitary (anterior and posterior) and endocrine glands, for example of the adrenal and thyroid. Thus, one can encounter mentions of the hypothalamo-pituitary-adrenal (HPA) axis, the hypothalamo-pituitary-gonadal (HPG) axis and the hypothalamo-pituitary-thyroid (HPT) axis from the late 1960s onwards. In the early 1970s, John Porter proposed that "[a] neuroendocrine system consists of a neural cell or cells which secrete into the extracellular fluid a substance which upon reaching other cells modify their behavior" (Porter, 1973), pp. 2-3).

A conceptual view of the immune system was also developed in the 1970s by Niels Jerne. He proposed that "the immune system, even in the absence of antigens … achieves a dynamic steady state as its elements interact between themselves" (Jerne, 1974), p. 383) suggesting possible regulation of immune responses. While much of 20th century research into immunity has focused on antibodies when considering extracellular molecules, another category started to emerge during its last quarter in the form of cytokines. Cytokines can be defined as "cell-surface associated or secreted proteins that interact with specific cell-surface receptors resulting in the mobilization and or modulation of target cells" (Oppenheim, 2018), p. 1). From the point of view of immunology, cytokines correspond to a broadening of the category of interleukins that were viewed as messengers between leukocytes. The very idea of interleukins, and later, cytokines, as intercellular messengers progressively altered the antigen-antibody stimulus-responses vision of the immune system.

As a category of intercellular signaling molecules they are distinguished from hormones, in that cytokines often act locally and at much lower concentrations. In addition, cytokines typically have many different many different biological properties (pleiotropism), and, in part, as a consequence, different cytokines may induce similar biological effects (redundancy) (Dinarello, 2007, Oppenheim, 2018). The history of interferons illustrates the changing framework of immunology well in that interferon was initially considered an antiviral molecule, but over time, with other effects and related molecules being discovered, became a prototypical cytokine (Vilcek, 2006, Billiau and Matthys, 2009). Another illustration of categories in immunology changing over time is that of different names

referring to functional effects like lymphocyte-activating factor and endogenous pyrogen that most likely corresponded to one and the same molecule, coined interleukin-1 (IL-1) (Oppenheim and Gery, 1993).

X.1.2. Post WWII tools to study neuroendocrine and immune system components

In the early 1950s, conjugation of the fluorescent marker fluorescein to antibody made it possible to localize antigens and antibodies in tissues (Coons and Kaplan, 1950, Coons et al., 1950, Coons et al., 1951, Coons et al., 1955). Antibody conjugation approaches were subsequently expanded to enzymes, to obtain more permanently stained tissues and to circumvent the problems of fluorophore fading and autofluorescent tissues (Nakane and Pierce, 1967). Not surprisingly, given the peptidergic nature of many hormones, many attempts were undertaken to develop antibody-based detection techniques of these mediators in bodily fluids and tissue extracts (Yalow and Berson, 1960, Utiger et al., 1962, Felber, 1963, Spitzer, 1968). Thus, radioimmunoassays were employed to study the effects of potential hypothalamic releasing factors on pituitary contents of growth hormone or adrenocorticotropin hormone (Rodger et al., 1969, Brazeau et al., 1973, Rivier et al., 1973). A variant of radioimmunoassays in which the radioactive isotope was replaced by an enzyme gave rise to enzyme-linked immunosorbent assays (ELISAs) (Aydin, 2015). Thus, these antibody-based techniques made it possible to refine both biochemical and anatomical approaches. Moreover, In the 1970s, the use of different fluorescent labels also allowed for sorting and concentrating of different cell populations (Bonner et al., 1972, Julius et al., 1972). All of the approaches were further improved after monoclonal antibodies with predefined antigen specificity became available (Koehler and Milstein, 1975).

Other technical developments, of which the impact can hardly be overstated and which have given rise to different technologies, are those of the cloning of genes and the expression of recombinant proteins. Indeed, very few proteins are naturally produced in sufficient quantities and purity to allow for isolation, analysis or calibration (Hartley, 2006). To obtain those quantities, cloning of the gene encoding the protein of interest and its production by cell types different from those naturally expressing the protein is often necessary (Hartley, 2006). It turned out that cytokines, including interferons and IL-1, were amongst the first proteins to be thus produced (Oppenheim and Gery, 1993, Dinarello, 2007). This, in turn, made it easier to generate antibodies and calibration standards for immunoassays. In addition, the cloning of genes encoding for hormones or cytokines allowed for the detection of their expression in tissues by *in situ* hybridization. Naturally, the new techniques were not only employed for a better observation and description of immune and neuroendocrine systems and their components, but also favored the emergence of new tools of intervention. Thus, the classic approaches of lesions of organs or glands or administration of their extracts were enriched by

strategies using neutralizing antibodies, recombinant forms of naturally occurring functional receptor antagonists, such as alpha-helical Corticotropin-Releasing Hormone and IL-1 receptor antagonist, and animals that were deficient for certain genes (for example, knock-out mice).

X.2. Regulation of immune and neuroendocrine systems: cytokine-HPA-axis interactions

X.2.1. Immunophysiology

Although Niels Jerne remarked that the immune and nervous systems share important features like threat detection and memory and "penetrate most other tissues of our body" (Jerne, 1974), p. 387), he also indicated that "they seem to be kept separate from each other by the so-called blood-brain barrier" (Jerne, 1974), p. 387) and that lymphocytes stimulated with antigen *in vitro* "will produce specific antibody molecules, in the absence of any nerve cells" (Jerne, 1985), p. 852). Other immunologists, while embracing the idea of common features between the immune and nervous systems, proposed to move from an antigen-centered to a more organism-centered view of the immune system (Coutinho et al., 1984). These authors, in fact, agreed with Jerne's idea that the immune system is active in the absence of antigenic stimulation, but proposed a more physiological approach with a more important place for *in vivo* studies.

The term immunophysiology had started to become used more in the early 1980s to describe immune functions in a particular organ, such as the gut or of relatively ignored cell types like Natural Killer cells (Dobbins, 1982, Oldham, 1983). But a broader vision of immunophysiology also emerged that aimed to "bring the self-regulated immune system into conformity with other body systems … based on the existence of afferent-efferent pathways between immune and neuroendocrine structures" (Besedovsky and Sorkin, 1977), p. 1). Thus, Besedovsky affirmed that neurotransmitters and neuropeptides released by nerve endings and neuroendocrine hormone production and action can constitute "external immunoregulatory signals [super]imposed upon autoregulatory mechanisms" (Besedovsky et al., 1985), p. 750s). Moreover, lymphoid cells were not only shown to express receptors for glucocorticoids and catecholamines, but also synthesize "[c]lassic pituitary hormones [, such as] growth hormone and prolactin [that] appear to have distinct roles as immunomodulators" (Kelley, 1988), p. 2095).

X.2.2. Stress and immunity

In parallel with more physiological visions of the immune system, a perspective emerged that acknowledged the influence of stress on immunity and proposed that this was mediated by neuroendocrine systems. Hans Selye had already shown before WWII that "[e]xposure to general (systemic) stress ["affect[ing] extensive masses of tissue"] initiates a chain of physiologic reactions

[resulting in rapid thymic involution followed by adrenal enlargement], which are essentially similar, irrespective of the particular stress agent employed" (Selye, 1948), p. 186). He next related that "the predominantly emotional stress caused by immobilization" also produced several of these responses (Selye and Fortier, 1950), pp. 153-155). In addition this "neurogenic stress-situation" was found to result in "inhibition of inflammation" (Selye, 1955), p. 124). Given that the anti-inflammatory effects of glucocorticoids have been widely known since the 1950s (Gordon and Katsh, 1949, Glyn, 1998), these findings gave rise to the idea both physiological and psychological stressors could modulate inflammation through the adrenal release of corticosteroid hormones. Further work in the 1960s indicated that a shuttle box stressor to induce electric shock avoidance learning in rodents was also accompanied by hypertrophy of the adrenals and hypotrophy of the spleen and thymus as well as altered susceptibility to various viral infections (Rasmussen, 1969). These animal, along with some clinical, findings then gave rise to the more general hypothesis that "[s]tress and emotional distress may influence the function of the immunologic system via central nervous system and possibly endocrine mediation" (Solomon, 1969), p. 335).

The stress response as a physiological concept that was progressively linked to the HPA axis by Hans Selye between the 1950s and 1970s (Selye, 1950, Selye, 1976). During that time, it had indeed become clear that acute and chronic stress were associated with increased corticosteroid production in animals and humans (Hale et al., 1957, Mason et al., 1961, Treiman et al., 1970, Weiss, 1970, Arguelles et al., 1972, Bassett et al., 1973, Tache et al., 1976). Some authors therefore proposed that "psychosocial processes influence the susceptibility to some infections, to some neoplastic processes, and to some aspects of humoral and cell-mediated immune responses" (Stein et al., 1976), p. 439) and that "[t]hese psycho-social effects may be related to hypothalamic activity, the autonomic nervous system, and neuro-endocrine activity" (Miller, 1977), p. 413). After some debate about how states of mind could give rise to changes in immune responses, the term psychoneuroimmunology was put forward in the early 1980s "to refer to studies of neuroendocrine mechanisms mediating the effects of behavior on immune function – and vice versa" (Ader and Cohen, 1985), p. 103). It is nevertheless interesting to observe how from Selye's initial observations on the effects of systemic stressors on immune organs (even though at that time the thymus was not yet considered to be an immune organ) a progressive shift took place towards a perspective in which neurogenic or psychological stressors affect immune responses through activation of the HPA-axis.

X.2.3. Communication between immune and neuroendocrine systems

During the 20th century, the idea of multicellular organism displaying a division of labor of "a society of cells" that was orchestrated by intercellular communication (Reynolds, 2017) was progressively

rendered more concrete with the notions of hormones, neurotransmitters and later cytokines as soluble secreted mediators. In parallel, and regarding organ functions, the metaphoric image of the brain "as governor" in which it is considered "as the executive control center of the body" also gained traction (Fuller, 2014), p. 100). This image was, in part, reinforced by the very idea of neuroendocrine systems, which attribute an important role to the hypothalamus in controlling pituitary-endocrine gland axes (see chapter Y). From the 1970s onwards, emerged a perspective in which peripheral immune responses activate hypothalamic neurons and "bring about changes in hormone levels," for example increased corticosteroid concentrations, which, in turn, may "suppress[] a potentially harmful expansion of lymphoid tissue" and "prevent accumulation of macrophages in the delayed hypersensitivity reaction" (Besedovsky and Sorkin, 1977), p. 4, p. 7, p. 9). In such a perspective, the hypothalamus would receive afferent information of ongoing peripheral immune responses and activate efferent pathways in the form of the HPA-axis resulting in corticosteroid-mediated inhibition of immune responses as some form of feedback loop.

X.3. How cytokines and the HPA-axis interact

X.3.1. Cytokines can interact with the HPA-axis at different levels

In 1977, Besedovsky argued that "[I]t is a rational assumption that the primary link between the immune and the neuroendocrine system is effected by one or another of the multiple events known to follow immunization" and proposed that "chemical mediators" produced by immune cells after stimulation "may influence the endocrine target glands either directly or more likely via hypothalamus-hypophysis" (Besedovsky and Sorkin, 1977), p. 6). Interleukins or cytokines, as soluble polypeptides released by activated immune cells, were obvious candidates to constitute such mediators. Interestingly, in the 1980s, it was shown that IL-1 can stimulate pituitary mRNA expression and secretion of adrenocorticotropin hormone (ACTH) as well as hypothalamic production of corticotropin-releasing hormone (CRH) (Berkenbosch et al., 1987, Bernton et al., 1987, Brown et al., 1987, Sapolsky et al., 1987). If other cytokines, like IL-6 or tumor necrosis factor-alpha, produced after exposure of host cells to bacterial fragment also increase corticosteroid release, IL-1beta seems the most potent one (Besedovsky et al., 1991, Dunn, 1992, Matta et al., 1992). Although it has been shown that IL-1 can induce corticosteroid secretion by acting directly on the adrenal (Andreis et al., 1991), this cannot explain why increases in plasma corticosterone after peripheral IL-1 administration require an intact pituitary and action of CRH (Berkenbosch et al., 1987, Gwosdow et al., 1990, van der Meer et al., 1996). These findings indicate that although a cytokines may act at different levels of the HPA-axis, activation at the level of the hypothalamus seems necessary in the case of rise in circulating corticosteroid concentration after systemic IL-1 administration.

X.3.2. Mechanisms mediating IL-1-induced HPA-axis activation

But if one supposes that activation of the HPA-axis by peripheral IL-1 involves CRH neurons in the hypothalamus, then one would need to explain how such a response would circumvent the blood-brain barrier (BBB) that prevents water-soluble polypeptides like cytokines to passively leave the blood and act in the brain parenchyma. Given that, in rodents, a functional blood-brain barrier is absent in the hypothalamic median eminence (Gross, 1992), IL-1 decreases median eminence contents of CRH (Berkenbosch et al., 1987, Watanobe et al., 1991), IL-1 type 1 receptor mRNA expression in the median eminence (Cunningham et al., 1992, Yabuuchi et al., 1994) and intra-median eminence administration of IL-1ra attenuates IL-1-induced adrenocorticotropin hormone secretion (Matta et al., 1993), the action of circulating IL-1 on CRH-containing terminals in the median eminence would be one obvious mechanism explaining IL-1-induced HPA-axis activation.

Another hypothesis emerged after it was shown that microinjection of the toxin 6-hydroxydopamine into the catecholaminergic fiber bundle connecting the brainstem to hypothalamus reduces IL-1-induced increases in hypothalamic CRH mRNA and plasma corticosterone (Chuluyan et al., 1992, Parsadaniantz et al., 1995). This along with the repeated observation that peripheral IL-1 administration stimulates hypothalamic noradrenalin turnover, both in the paraventricular hypothalamus containing CRH cell bodies and in the median eminence in which CRH neurons terminate (Dunn, 1988, Kabiersch et al., 1988, MohanKumar and MohanKumar, 2005) indicated that brainstem catecholaminergic projections to the hypothalamus play an important role in IL-1-induced activation of the HPA-axis. In addition, prostaglandin synthesis was shown to mediate IL-1-induced activation of the HPA-axis as administration of a cyclooxygenase inhibitor can attenuate increased hypothalamic noradrenalin turnover and plasma corticosterone after peripheral injection of this cytokine (Dunn and Chuluyan, 1992). This pharmacological finding was soon followed by the demonstration of cyclooxygenase-2 induction associated with brain endothelial cells in response to peripheral IL-1 injection (Cao et al., 1996, Lacroix and Rivest, 1998). Not surprisingly, these different findings gave rise to intense research aimed at testing various hypotheses of how peripheral IL-1 can activate hypothalamic CRH neurons resulting in HPA-axis activation.

In these endeavors, some groups chose to use IL-1 as a stimulus while others preferred to administer bacterial lipolysaccharides (LPS) fragments as an inducer of IL-1 and other pro-inflammatory cytokines in mononuclear leukocytes. In doing so, the former used a robust well-characterized and progressively standardized single cytokine, while the latter allowed for the possibility of different pro-inflammatory cytokines to be produced and interact and preserved a potential role for monocytes and macrophages in immune-to-brain signaling. With respect to macrophages, it was shown that peripheral LPS

administration induces IL-1 beta production, not only in liver Kupffer cells, but also in brain circumventricular organs, like the median eminence, lacking a functional blood-brain barrier (Chensue et al., 1991, van Dam et al., 1992). Given that vagal nerve fibers in the liver can bind and react to IL-1 (Niijima, 1996, Goehler et al., 1997), these findings raise the possibility that paracrine actions of IL-1beta, either on peripheral nerves or in the median eminence, could also play a role in activation of the HPA-axis.

The results of several, mostly lesion- and pharmacology-based, intervention strategies indicate that prostaglandin-dependent activation of catecholaminergic brainstem projections to hypothamamic CRH neurons underlies peripheral IL-1-induced HPA-axis activation (Ericsson et al., 1997). Interestingly, vagal afferent fibers in the brainstem terminate close to catecholaminergic neurons (Sumal et al., 1983) and subdiaphragmatic vagotomy attenuates adrenocorticotropin hormone responses after intraperitoneal, but not necessarily intravenous, administration of IL-1beta (Kapcala et al., 1996, Ericsson et al., 1997, Wieczorek and Dunn, 2006). Regarding the role of mononuclear leukocytes, it was shown early on that elimination of macrophages by administration of dichloromethylene-diphosphonate-filled liposomes prevents increases of adrenocorticotropin hormone and corticosterone in response to low doses of bacterial LPS (Derijk et al., 1991). However, and more surprisingly, this same intervention increases hormonal responses after chronic peripheral IL-1beta infusion, suggesting that endogenous macrophage-derived mediators exert an inhibitory effect on IL-1-induced HPA axis activation (van der Meer et al., 1996). The brain contains *bona fide* macrophages, in addition to immunocompetent microglia, in areas without a functional blood-brain barrier, such as the meninges and circumventricular organs as well as in perivascular spaces (Williams et al., 2001); (Prinz et al., 2021). Interestingly, eliminating brain macrophages by intracerebroventricular administration of these dichloromethylene-diphosphonate-filled liposomes attenuates acute adrenocorticotropin hormone and corticosterone responses after peripheral IL-1beta injection, but increases them in response to bacterial LPS (Serrats et al., 2010). Taken together, these findings indicate that different mechanisms can underlie and modulate peripheral IL-1-induced activation of the HPA-axis and that the actual involvement of such mechanisms may dependent on the conditions, both experimental and physiological.

X.4. Outstanding questions and perspectives

Most recently, intervention strategies have often consisted of using genetically-modified organisms, for example in experiments with knock-out mice or optogenetics. Many of the conclusions drawn from old intervention strategies have been confirmed with these newer techniques, including differences between the mechanisms mediating the effects of IL-1 and LPS on the HPA-axis. Thus, the lack of effect

of administration of IL-1ra on bacterial LPS-induced increases in plasma ACTH and corticosterone was corroborated in mice genetically-deficient for the IL-1 type 1 receptor (Dunn, 2000, Matsuwaki et al., 2017). It is, indeed, important to consider the results of different lines of evidence when addressing a research question in the life sciences as each single line or approach, and even the latest technology, is associated with particular limitations and biases (Munafo and Davey Smith, 2018). In this context, it is also important to keep in mind that the use of genetically-modified organisms may have less utility in addressing the role of metabolites, cells or particular structural organizations. For example, when addressing the question of which cell types mediate the neuroendocrine or other effects of IL-1, most recent studies have used promotor-specific Cre-Lox recombinant mice. But one of the major bottlenecks of such approaches is the specificity of the promoters used since specific promotors may not yet be widely available for all cell types and the specificity of promoters that have already been used may be questioned (Chaskiel et al., 2021).

Another important issue related to interpretation of experimental findings, is that many regulatory processes in biology are functionally redundant. Thus, it is important to keep in mind that a regulatory response that appears to be top-down in the sense that it involves entities at a perceived higher level of organization, typically the brain, influencing lower-level entities does not exclude other forms of regulation. So, even though, it has been shown that in the case of systemic administration of IL-1, corticosterone release depends, in large part, on the hypothalamus (Berkenbosch et al., 1987, Gwosdow et al., 1990, van der Meer et al., 1996), this does not mean that every time plasma corticosterone concentrations increase that the full HPA-axis is active. Indeed, IL-1 can induce corticosteroid secretion by acting directly on the adrenal where its signaling receptor is expressed (Andreis et al., 1991, Engstrom et al., 2008). Therefore, an increase in circulating glucocorticoid concentrations under certain inflammatory conditions or after administration of some molecule alone cannot be taken to reflect HPA-axis activation or indicate interactions between neuroendocrine and immune systems as such. In addition, the existence of functionally-redundant regulatory mechanisms at different levels of perceived organization raise questions regarding how these interact and in which contexts they are mostly activated.

Beyond the progressively unravelling of mechanisms mediating the effects of cytokines on the HPA-axis and *vice versa*, it is important to point out that some conceptual shifts have occurred in the process. Back In 1977, Besedovsky and Sorkin wrote that “the possibility that the immune response itself could bring about changes in hormone levels has not been previously considered” (Besedovsky and Sorkin, 1977), p. 4). Interestingly, some fifteen years later several paper titles referred to “infection as stressor” or “immune system-mediated stress response” (Eskay et al., 1990, Dunn, 1993). Indeed, while Selye in the mid-20th century did certainly consider infection as a systemic or physiological stressor, subsequent research interests had progressively moved to studying the effects of so-called

neurogenic, emotional or psychological stressors on immune responses. Considering infection as a systemic, homeostatic or physiological stressor led several groups in the 1990s to compare the brain circuits involved in activation of the HPA-axis by these two broad categories of stressors. Thus, systemic, homeostatic or physiological stressors including hypoglycemia and peripheral IL-1 injection, are thought to activate the HPA-axis through ascending catecholaminergic brainstem projections, whereas stimulation of the HPA-axis neurogenic, emotional or psychological stressors, such as restraint or foot shock, seem to involve forebrain circuits (Herman et al., 1996, Li et al., 1996, Herman and Cullinan, 1997, Sawchenko et al., 2000). Interestingly, most recently, both homeostatic-physiological stressors and emotional-psychological stressors have been proposed to give rise to inflammation (Konsman, 2019). In this respect, it is certainly going to be important in the future to specify subcategories of inflammation (Meizlish et al., 2021).

X.5 Key references

Besedovsky et al., *Journal of Immunology,* 1985. This review provides important insights into immune-neuroendocrine interactions.

Dinarello, *European Journal of Immunology,* 2007. This review, by one of the main actors in the field, gives a historical overview of the coming into being of cytokines as a class of intercellular messengers.

Herman & Cullinan, *Trends In Neurosciences,* 1997 and Sawchenko et al., *Progress in Brain Research,* 2000. These two review articles make the case that distinst categories of stressor mobilize different brain circuits that conerge in the hypothalamus to activate the hypothalamo-pituitary-adrenal (HPA) axis.

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