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·Review·

Using optogenetics to translate the "inflammatory dialogue" between heart and brain in the context of stress

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Abstract: Inflammatory processes are an integral part of the stress response and are likely to result from a programmed adaptation that is vital to the organism's survival and well-being. The whole inflammatory response is mediated by largely overlapping circuits in the limbic forebrain, hypothalamus and brainstem, but is also under the control of the neuroendocrine and autonomic nervous systems. Genetically predisposed individuals who fail to tune the respective contributions of the two systems in accordance with stressor modality and intensity after adverse experiences can be at risk for stress-related psychiatric disorders and cardiovascular diseases. Altered glucocorticoid (GC) homeostasis due to GC resistance leads to the failure of neural and negative feedback regulation of the hypothalamic-pituitary-adrenal axis during chronic inflammation, and this might be the mechanism underlying the ensuing brain and heart diseases and the high prevalence of co-morbidity between the two systems. By the combined use of light and genetically-encoded light-sensitive proteins, optogenetics allows cell-type-specific, fast (millisecond-scale) control of precisely defined events in biological systems. This method is an important breakthrough to explore the causality between neural activity patterns and behavioral profiles relevant to anxiety, depression, autism and schizophrenia. Optogenetics also helps to understand the "inflammatory dialogue", the inflammatory processes in psychiatric disorders and cardiovascular diseases, shared by heart and brain in the context of stress.

Keywords: stress; inflammatory processes; glucocorticoid resistance; psychoneuroimmunology; psychiatric disorders; cardiovascular disease; neuronal circuits; optogenetics

1 Introduction

Events that disturb or potentially disturb a complex organism's homeostasis or well-being (known as stressors) recruit stress-response systems involving behavioral, cardiovascular, metabolic, and immunological changes that are not mutually exclusive but may act together as one integral event[1]. The interaction between the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenocortical (HPA) axis is essential for adaptation to stress[2]. The pattern and magnitude of a stress response vary with different stressors, and result in different coping strategies. Factors that affect the activation and control of the ANS and the HPA axis include duration of stress exposure (acute *versus* chronic), type of stress (physical *versus* psychological), developmental stage at exposure (early life

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versus adulthood), and the genetic background of the organism[3].

One breakthrough in psychoneuroimmunology in the past two decades was the realization that psychological stress responses are themselves examples of inflammatory responses[1,4]. The central nervous system (CNS) determines the primary profile of an inflammatory response to a stressor through "hardwiring" of sympathetic and parasympathetic innervation of mainly the lymphoid organs[5]. The mechanism of these inflammatory responses lies in the integration of multiple chemical inflammatory messengers and their receptors[3,6]. These inflammatory mediators include small molecules [e.g. free radicals and prostanoids such as prostaglandin E_2 (PGE_2)], neurotransmitters (e.g. noradrenalin, acetylcholine, and serotonin), neuroendocrine peptides (e.g. corticotropin-releasing factor), steroid hormones (e.g. cortisol in humans and corticosterone in rodents), transcription factors [e.g. nuclear factor kappa B (NF-κB)], pro-inflammatory cytokines [e.g. interleukins IL-1β and IL-6 and tumor necrosis factor alpha (TNF-α)], and the anti-inflammatory cytokine IL-10. These inflammatory responses occur both peripherally and within the brain, secondary to or simultaneously with the onset of peripheral inflammatory responses[7-9]. In the context of stress, dysfunction of the neuronal and negative feedback regulation of the HPA axis accounts for prolonged inflammatory responses to stress, under which glucocorticoid (GC) resistance may be a general mechanism[10,11]. Coupled with factors that affect GC availability, these pro-inflammatory cytokines *de novo* would worsen this abnormal modulation and therefore initiate a self-amplifying vicious cycle of inflammatory responses[10]. Moreover, accumulating evidence shows that stress-induced inflammatory responses in early life program vulnerability to later-life stress responses[12, 13], ultimately leading to inflammation-related diseases such as major depression, type 2 diabetes mellitus, and cardiovascular disease (CVD)[14-16]. This review focuses on stress-induced chronic inflammatory processes characterized by hyperactivity of the HPA axis and increased production of pro-inflammatory cytokines dominated by GC resistance[17-19]. Moreover, these inflammatory processes may induce an "inflammatory dialogue" between heart and brain in the context of stress, accounting for the pathogenesis of major depression, CVD, and their co-morbidities. Optogenetics, a new method that combines optical and genetic manipulations, can help to translate this elusive "dialogue". Optogenetics enables the identification of novel therapeutic targets based on neuronal circuit mechanisms in the limbic regions in a top-down manner due to its advantages (cell-type specificity, and high spatial and temporal resolution), and is becoming essential for both basic and clinical research on the common mechanisms in stress-related diseases.

2 Mechanisms underlying inflammatory responses under stress

Psychological stress occurs when environmental demands exceed adaptive capacity[14,20]. Psychological stressors are processed in the limbic forebrain, including the amygdala, hippocampus and prefrontal cortex (PFC). The responses processed by these circuits occur in anticipation of or in reaction to stressful events, on the basis of prior experiences or innate programs[20]. All these parts of the limbic region work in parallel to integrate the activation of the HPA axis and the ANS[2].

Both acute and chronic psychological stress induce a series of inflammatory responses to help an individual survive[1]. In healthy humans, exposure to acute laboratory stressors induces the elevation of markers of innate immunity and a general suppression of adaptive immunity[21]. However, exposure to real-life chronic stressors is associated with a biphasic immune response, partial suppression of cellular and humoral functions accompanied by low-grade, nonspecific inflammation[21]. Psychological stress induces pronounced changes in the innate and adaptive immune responses, which are predominantly controlled by neuroendocrine mediators from the HPA axis and the sympathetic adrenal axis[14]. Psychological stress has also been found to impair vagal tone, resulting from the withdrawal of inhibitory motor vagal input that inhibits pro-inflammatory cytokine release through interaction with the acetylcholine receptor α7 subunit of the nicotinic acetylcholine

receptor[22,23]. Therefore, vagal withdrawal in response to stress may also intensify inflammatory processes.

Increased sympathetic adrenal activity appears to play a major role in immune changes under acute psychological stress[1]. On detection of a homeostatic challenge, the brain activates the sympathetic nervous system (SNS) to release the catecholamines noradrenalin (primarily from the SNS) and adrenalin (primarily from the adrenal medulla). By sympathetic innervation of lymphoid organs and the sympathetic-adrenal axis, through noradrenalin and adrenalin binding with β-adrenoreceptors on the immune cells, and in some subsets through α-adrenoreceptors, the ANS can alter the circulation of leukocyte subpopulations and enhance the production and release of pro-inflammatory cytokines by influencing the functional capacity of innate immunocompetent cells (monocytes/macrophages)[24]. Chronic β-adrenoreceptor stimulation also increases the myocardial gene expression and protein production of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α[25]. All these peripheral pro-inflammatory cytokines in turn influence the inflammatory processes in the CNS[11]. The initial stress-induced increase in inflammatory activity is predominantly mediated by catecholamines; HPA axis activity also participates. GCs exert "permissive" effects on the synthesis of adrenalin and in noradrenalin signaling, as is common in endocrine events[26].

The HPA system is the final common pathway in the mediation of the stress response[2]. Following the initial sympathetic adrenal activity, stress also triggers the activation of the medial parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus, inducing the release of corticotropin-releasing hormone (CRH) and co-secretagogues such as arginine vasopressin (AVP) into the portal vessels of the median eminence. Secretagogues travel through the portal blood to the anterior pituitary where they induce the secretion of adrenocorticotropic hormone (ACTH). Subsequently, ACTH, by means of systemic circulation to the adrenal cortex, induces GC synthesis and secretion[20]. CRH-expressing neurons in the PVN project not only to the median eminence but also to other areas such as the locus coeruleus, through noradrenergic neurons, resulting in noradrenalin release at nerve terminals distributed widely in the CNS, and thereby regulate adrenal innervation and the sensitivity of the adrenal cortex to ACTH through the ANS[27-29]. Yet, activation of the HPA axis resulting in elevation of circulating GCs occurs more slowly than the sympatho-adrenomedullary activation, which ensures an amplified and relatively protracted secretory episode[2]. The coupling of HPA axis activity with the sympathetic mechanisms and other signaling pathways of inflammatory mediators are mainly responses to chronic psychological stress, and they show remarkable individual variability in vulnerability to inflammation-related disease[8,15,30].

3 Integration of peripheral and central neurogenic inflammation in the context of stress

The brain is central to stress responses and plays a crucial role in initiating, organizing, adapting, and restraining inflammatory responses to stress[7,9,31,32]. The CNS hosts processes of inflammatory responses to stress with features similar to those hosted by the peripheral system[26]. The link between stress and inflammatory responses within the CNS and the peripheral system is considered to be a well-organized programming process, as almost all levels of such responses become activated; that is, stress operates on the genetic, mediator, and executive levels, with a corresponding increase in the levels of inflammatory markers.

3.1 Inflammatory signals from periphery to brain Having no classic lymphatic drainage, the brain was considered an immunologically privileged organ separated from the peripheral immune system by the blood-brain barrier (BBB). However, current evidence suggests that extensive communication exists between the CNS and the immune system, accounting for various essential, coordinated stress responses[33]. The brain also houses immune cells, such as macrophages and dendritic cells in the choroid and meninges. When exposed to inflammatory stimuli, parenchymal macrophages (known as microglia) produce pro-inflammatory cytokines and prostaglandins that bind to their receptors on neuronal and non-neuronal brain cells[34].

Generally, exposure to acute or chronic stressors is associated with the immunosuppressive aspect of the adap-

tive immune response, especially the proliferative ability of T and B cells *in vitro*; however, these stressors can also activate the innate immune response[35]. The brain monitors peripheral immune responses by several parallel processes: (1) In the neural pathway[36], peripherally produced cytokines from activated monocytes and macrophages stimulate primary afferent nerves, such as the vagus. Vagal afferents project to the nucleus tractus solitarius, from where information is relayed to the ventrolateral medulla, PVN, central amygdala, and bed nucleus of the stria terminalis. (2) In the humoral pathway[37], the macrophage-like cells residing in the circumventricular organs (CVOs) and the choroid plexus generate pro-inflammatory cytokines. As the CVOs lie outside the BBB, these cytokines can enter the brain by volume diffusion through the leaky regions such as the median eminence, organum vasculosum of the lamina terminalis, area postrema, and subfornical organ. (3) The overflowing pro-inflammatory cytokines in the systemic circulation can access the brain through saturable cytokine-specific transport molecules on brain endothelium, and activation of endothelium is responsible for the subsequent release of secondary messengers such as PGE_2 and nitric oxide[38]. (4) Special cytokines such as IL-1 can interact with their receptors located on the perivascular macrophages and endothelial cells of brain venules, resulting in the local production of PGE_2[7].

The immune system – brain communication ultimately leads to the production of pro-inflammatory cytokines by microglia, and they, combined with the inflammatory mediators (such as PGE_2) and reactive nitrogen and oxygen species released from activated microglia, induce mutual activation of astrocytes, thereby amplifying the inflammatory signals within the CNS[39]. NF-κB, an inflammatory signaling molecule, is an essential mediator at the blood-brain interface, and communicates peripheral inflammatory signals to the CNS[33]. Moreover, the whole process requires the convergent action of two events with different temporal niches: the rapid activation of the afferent neural pathway and the slower propagation of the cytokine message within the brain[34].

3.2 GC resistance dominates the profile of the CNS cytokine network under stress Cytokines are soluble bioactive mediators released by various cell types both in the periphery (macrophages and lymphocytes) and in the brain (neurons, astrocytes, and microglia). They are important inflammatory mediators in stress, and function within a complex network either synergistically or antagonistically[9,40]. Complex interactions exist between cytokines, inflammation, and the adaptive responses to maintain homeostasis[41]. Within the brain, cells that produce cytokines, express cytokine receptors, and amplify inflammatory signals (neurons, astrocytes, and microglia)[39], are the substrate of immune signals. These immune signals, including hyperactivation of the HPA axis and CRH coupled with alteration of the metabolism of key monoamines (e.g., serotonin, dopamine, and noradrenalin), play a role in the potent effects of peripheral pro-inflammatory cytokines on pathways involved in the pathophysiology of neuropsychiatric disorders such as depression[35] .

Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, activate the HPA axis at multiple levels (hypothalamus, anterior pituitary, and adrenal cortex) and by multiple mechanisms[42]. This effect is usually attributed to the elevated production of CRH and circulating GCs[43]; however, the underlying mechanisms are yet to be investigated. Cytokines can influence HPA axis function via their effects on negative feedback regulation[17], and impaired negative feedback regulation of the HPA axis has been shown to lead to GC resistance manifested by increased cortisol concentrations following dexamethasone (DEX) administration in a DEX suppression test and a DEX-CRH test[44]. A non-suppressive effect in the DEX-CRH test is also found in patients with flattening of the diurnal cortisol slope and increased evening cortisol concentrations, resulting in adverse behavioral effects such as depression as well as medical disorders including CVD and cancer, with poor outcomes[45,46]. The decreased feedback regulation of the HPA axis by GCs is believed to be partly mediated by alteration of the GC receptor (GR)[44,45].

During chronic inflammatory responses, pro-inflammatory cytokines can incur GC resistance in immunocytes and their cellular targets through a cascade of activation of NF-κB,

p38 mitogen-activated protein kinase, and signal transducer and activator of transcription 5[47], thereby translocating GR from the cytoplasm to the nucleus and inhibiting GR-DNA binding through nuclear protein-protein interaction[47]. Cytokines can also influence GR expression by decreasing GRα (the active form of the receptor) and increasing GRβ (a relatively inert isoform)[18]. At the hypothalamic level, this cytokine-dependent GC resistance pathway might be the underlying mechanism for the reduced ability of GCs to downregulate the production of CRH[34]. Yet, at the peripheral (monocytes/macrophages) and central innate (monocytes/macrophages and microglia) immune systems, the inhibitory effect of GCs on cytokine production and action decreases and gives way to a feed-forward cascade of increased production of pro-inflammatory cytokines[34]. In the brain, these increased inflammatory responses *de novo* result in downregulated inhibitory feedback of GCs on CRH, thereby intensifying the stress-response system[11]. Moreover, CRH can directly enhance the production of pro-inflammatory cytokines in macrophages[48]. The hypersecretion of CRH through the projection from CRH-expressing neurons in the PVN to noradrenergic neurons in the locus coeruleus results in noradrenalin release, ultimately leading to the elevation of SNS outflow[27,28]. Catecholamines released from sympathetic nerve endings, acting through α- and β-adrenergic receptors on macrophages, then enhance the release of pro-inflammatory cytokines in plasma; within the brain, the β-adrenergic receptors on microglia fulfill this role[8]. Under chronic stress, GC resistance also accounts for T-cell dysfunction, manifested as decreased inhibitory responsiveness on proliferation *in vitro*, redistribution *in vivo*, and a shift in the Th1/Th2 cytokine (pro-/anti-inflammatory) ratio (significantly lower IL-10 levels and a higher IL-6/IL-10 ratio), thereby leading to chronic or non-resolving inflammation[35,41,42]. Recent developments in immunology concerning the role of special T-cell subsets in the regulation of inflammation, and the relative expression and function of T_{reg} and Th17 cells are of special relevance to Th1 and Th2 cell function[35]. The overall effects of T-cell dysfunction may be a potential source of chronic inflammation[35]. Unrestrained pro-inflammatory cytokines, once gaining access to the CNS and interacting with the cytokine network through GC resistance, influence virtually every aspect of brain function, including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and neuronal circuits with cytokine receptor expression-rich regions that regulate mood, memory, and decision-making, including the limbic and reward circuits (striatum), hypothalamus, and PFC[34,39]. The whole profile of the inflammatory processes relevant to stress adaptation (involving behavioral, cardiovascular, metabolic and immunological responses) may be a common mechanism underlying psychiatric disorders such as depression, metabolic disease and CVD.

Optogenetics is a novel method that has advantages including millisecond-scale optical control of defined small-scale events occurring in specified cellular populations. Therefore, it is considered a breakthrough in neuroscience and has already been applied in various studies to modulate synaptic plasticity and control neuronal activity that releases various neurotransmitters and modulators. For instance, optogenetic tools have been used to induce long-term potentiation and long-term depression in research on the synaptic plasticity involved in addiction[49], to selectively stimulate action potentials in ventral tegmental area dopaminergic neurons in freely behaving mammals[50], to detect the co-release of glutamate and acetylcholine from the medial habenula to the interpeduncular nucleus by selectively activating the cholinergic neurons in the former[51], and to test the function of raphe obscurus serotonergic neurons as central respiratory chemoreceptors[52]. With new tools allowing control over diverse cellular events, optogenetics has shown great potential in revealing the bi-directional communication between inflammation and stress-related diseases.

4 The "inflammatory dialogue" between heart and brain in the context of stress

It is a long-held belief that there is a bidirectional association of some types of psychiatric disorder and heart diseases with stress[53-55]. For example, the prevalence of co-morbid depression in patients with coronary heart dis-

ease (resulting from chronic inflammation in the vascular wall) is three times higher than that in the general population[56]. In patients with chronic heart failure, this prevalence might be even higher[57]. Meanwhile, depression is also recognized as a major contributor to subsequent cardiac events and even death[58]. Converging evidence from both experimental and epidemiological studies shows that increased concentrations of circulating cytokines, such as IL-1β, IL-6, and TNF-α, can be detected not only in patients with depression but also in patients with CVDs such as ischemic heart disease, heart failure, arteriosclerosis, and hypertension[59-61]. Understanding the mechanisms underlying the link between the co-morbidity of depression and CVD is of urgent concern to improve public health, despite their complexity and heterogeneity[53]. Factors that are common to both mood and cardiovascular regulation may help reveal the mechanisms. These include corticotropin-releasing factor, the HPA axis, the renin–angiotensin–aldosterone system, pro-inflammatory cytokines, and the central neurotransmitter systems, as well as their interactions in the context of stress[54]. In the CNS, as discussed above, microglia are the main sources of pro-inflammatory cytokines resulting from communication between immune system and brain. Furthermore, the interactions between microglia and neurons have been shown to play a key role in some neuroinflammation-related brain diseases, yet the underlying mechanisms are not fully understood due to the previous lack of cell type-specific approaches[62,63]. Fortunately, optogenetics has made it feasible to explore this field due to its advantages of cell-type specificity and high spatial and temporal resolution.

4.1 Brain cytokines act as executors in the association between CVD and depression Numerous clinical studies have shown increased cytokine concentrations in the blood of patients with hypertension, arteriosclerosis, heart failure, and ischemic stroke[59-61,64]. Earlier studies revealed that central infusion of pro-inflammatory cytokines such as IL-1β and TNF-α leads to significant hemodynamic and neurohormonal responses (such as increased arterial blood pressure) that are typical of CVDs, sympathetic activity, and the synthesis of renin, aldosterone and vasopressin[65,66]. Recent studies further showed that myocardial infarction induces the production of cytokines in the hypothalamus, which modulate neurotransmission in the PVN during the subsequent process of heart failure, resulting in elevated sympathoexcitation[67-69]. However, chronic central block of TNF-α in a rat model of heart failure returns the concentrations of several neurotransmitters in the PVN to control levels, while retaining the elevation of renal sympathetic nerve activity[70]. Moreover, increases in the central concentrations of anti-inflammatory cytokines (such as IL-10) by cerebroventricular gene transfer ameliorates the hemodynamic and humoral indices of heart failure in the infarcted rat[71]. In another study, the central infusion of IL-1 receptor antagonist (IL-1ra) reduces the magnitude of the pressor response to acute stress in healthy rats[72]. More recently, experiments suggested that cytokines as neuromodulators exert their action on cardiovascular control through interaction with the brain angiotensin system: pretreatment with either IL-1β or TNF-α enhances the pressor response to the central infusion of angiotensin II[73]; the pressor and dipsogenic effects of angiotensin II in mice require the presence of TNF-α[74]; blockade of NF-κB in brain ameliorates the development of AngII-induced hypertension, and reduces the expression of angiotensin II type 1 receptors in the heart and the PVN[75].

Major depression is associated with increased levels of inflammatory cytokines in the blood, cerebrospinal fluid, and various brain regions[76,77]. Elevated pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are positively related to individual symptoms of depression, including fatigue, cognitive dysfunction, and impaired sleep, as well as a factor of treatment resistance[78]. Among them, IL-1β and TNF-α play important roles in the pathology of depression[34,39]. For example, infusions of IL-1β or TNF-α into wild-type mice evokes depressive behavior[79], whereas mice lacking caspase-1 (an enzyme essential for IL-1 synthesis) display reduced "sickness behavior"[80]. Furthermore, deletion of genes for TNF-α receptors in mice has anti-depressive effects.

The pathophysiological mechanisms linking depression with inflammation include the modulation of synaptic

plasticity, dysregulation of neuroendocrine function, and abnormal metabolism of neurotransmitters involved in mood regulation, such as serotonin, dopamine, and noradrenalin[35]. In a rat model of depression in response to chronic mild stress, the development of anhedonia was reported, accompanied by dysfunction of the HPA axis and increased expression of IL-1β and TNF-α in the hypothalamus, pituitary, and plasma[81]. Similar disturbances have also been found in rats with post-infarct heart failure. Furthermore, in infarcted rats, peripheral inhibition of TNF-α attenuates the symptoms of anhedonia[82] and decreases the sympathetic drive and angiotensin receptor type 1 expression in the brain[83].

Of note, pro-inflammatory cytokines are also important modulators of the hormonal and behavioral components of the stress response[7]. Under stress stimuli, increased IL-1β mRNA and/or protein expression occurs not only in peripheral tissues but also in the brain (e.g., hypothalamus and hippocampus). IL-1β also plays a key role in the activation of the HPA axis, while central infusion of IL-1ra reduces the circulatory response to the stressor[84]. Cytokines are likely to act as "executors" in the pathogenesis of some CVDs and depression associated with stress[85].

4.2 HPA axis dysregulation underlies the association between CVD and depression The HPA axis promotes survival and adaptation to stress by increasing vascular tone and ensuring energy availability through its end products such as cortisol in humans and corticosterone in rodents; moreover, it plays a pivotal role in the stress response[86]. One of the main roles of GCs in the stress response is to prime the metabolic, autonomic, psychological, hemostatic, and cardiovascular components in preparation for environmental stress stimuli[87]. The permissive role of GCs enhances the vascular and metabolic effects of other stress hormones, such as catecholamines, glucagon, and angiotensin II, through enhancement of not only the expression of α1 and angiotensin II receptors but also the binding capacity of β-adrenergic receptors[88-90]. Another role of GCs is performing suppressive-like actions on inflammation, cellular proliferation, and tissue repair to avoid "overshooting" and self-injury or circulatory collapse[87,91]. The third role of GCs is inducing insulin resistance in muscle by partitioning of body composition. Long-term exposure of tissues to GCs due to chronic activation of the HPA axis may result in specific conditions such as major depression, metabolic syndrome and even CVDs associated with negative GC effects[86].

A variety of related CVD risk factors such as abdominal obesity, hypercholesterolemia, hypertriglyceridemia, hypertension and glucose intolerance, are associated with dysregulation of the HPA axis[92]. Excessive GCs affect specific cardiovascular risk factors, including body composition, plasma lipoprotein and carbohydrate metabolism, endothelial function, oxidative stress, vascular tone, inflammation and tissue repair, and emotional dysregulation. Increasing clinical evidence shows the presence of hypercortisolism, flattening of diurnal cortisol slope, and increased evening cortisol concentrations in patients with coronary artery disease (CAD)[93], and HPA axis dysfunction has been implicated in its pathogenesis[46,86].

In major depression, the phenotype of HPA axis dysregulation is similar to that in CAD patients, resulting in either chronically excessive secretion of cortisol or the flattening of diurnal cortisol slope and increased evening cortisol concentrations due to GR resistance[34,39,43,45,94].

Although further investigations are warranted, current research findings suggest a dominant role for the HPA axis in the association between CVDs and depression[53]. HPA axis dysregulation not only influences cardiovascular risk factors in depressed patients but is also implicated in the pathogenesis of CAD and thus has been used to predict death in the depressed male CAD patient[95]. Moreover, a previous study showed that the severity of depressive symptoms is significantly related to the incidence rate of metabolic syndrome, partially mediated by HPA axis function, as reflected by urinary cortisol levels[96]. In addition, cortisol might be another risk factor for depression and CVD, and common polymorphisms related to altered HPA axis function might increase the risk of both depression and CVD[97,98].

Taken together, when psychological stress-related information is conveyed to the brain, the limbic regions that

subserve higher brain functions related to cognition process it and determine the phenotype of the stress response, by regulating the activity of the HPA axis and the ANS in a top-down manner, on the basis of genetically predisposed early-life experiences. In the context of chronic stress, the elevation of CRH levels resulting from impaired negative feedback regulation of HPA function (caused by the effect of pro-inflammatory cytokines on GC receptors) elevates the outflow of the SNS (through CRH-expressing neurons projecting from the PVN to the locus coeruleus) and thus produces more pro-inflammatory cytokines, intensifying the stress-response system both peripherally and within the brain[11]. Moreover, vagal withdrawal in response to stress might also intensify the inflammatory processes. The chronic inflammatory processes under conditions of stress, characterized by a hyperactive HPA axis and increasing production of pro-inflammatory cytokines dominated by GC resistance, can induce the development of stress-related diseases such depression and CVD. Optogenetics may be a potent tool for future research focusing on the neuronal circuits involved in the inflammatory mechanism of interaction between heart and brain (Fig. 1).

5 Optogenetics: a potent tool in neuroscience to explore higher-order brain functions

In neuroscience, understanding the system-level processes governing higher-order brain functions, such as emotion, perception, and cognition, is a long-term pursuit. Yet, neither current pharmacological (limited by temporal resolution) nor electrophysiological (lack of cell type-specific targets) techniques fully match the speed and heterogeneity of the neuronal system. By combining the use of light and genetically encoded light-sensitive proteins, optogenetics allows cell type-specific targeted, fast (millisecond-scale) control of precisely defined events such as gain or loss of function in biological systems, even in freely-moving animals. It is also a suitable tool for the analysis of the global phenotype of dysfunctional circuits[99,100]. Thus, optogenetics offers a potent perturbational tool for understanding the causal link between neural activity patterns and behavior underlying psychiatric disorders such as depression, anxiety, autism and schizophrenia[101-103].

Optogenetics involves two main classes of single-component microbial opsins that are widely used in neuroscience. The first class consists of gain-of-function opsins such as channelrhodopsin (ChR). A recently developed variant is ChR2, which is a cation channel activated by blue light, resulting in depolarization and even action potentials at the cellular level with or without ensuing synaptic currents[104,105]. The second class comprises the loss-of-function halorhodopsin (HR), a fully-developed variant of which is *Natronomonas pharaonis* HR, a chloride pump

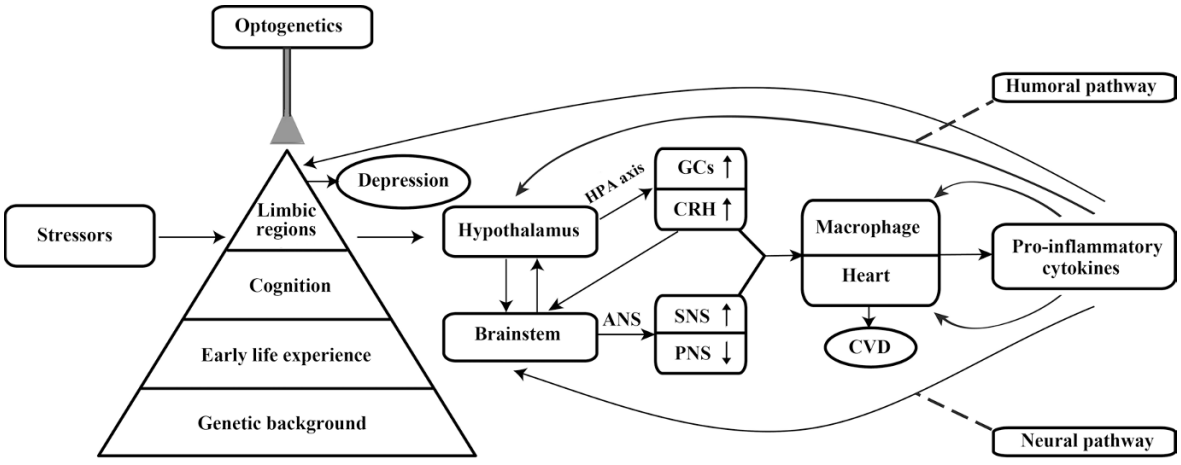

Fig. 1. Schematic of inflammatory responses to chronic stress. See text for details. ANS, autonomic nervous system; CRH, corticotropin-releasing hormone; CVD, cardiovascular disease; GCs, glucocorticoids; HPA axis, hypothalamic–pituitary–adrenocortical axis; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

activated by yellow light, resulting in hyperpolarization at the cellular level[106].

Genetic-based strategies that use optogenetics to deliver opsins to distinct neuronal populations can be classified into two types[99,100]: one uses cell type-specific promoters to drive transgene expression, and the other is through a recombinase-dependent system if the promoters are too large for the viral vector or if optimal opsin expression is unattainable; in the latter case, opsin expression is restricted to cells carrying Cre recombinase when delivered to transgenic rodents expressing Cre. By stereotaxic injection, the viral vector carrying the opsin gene under the control of cell-specific promoters can be delivered to target regions. Then, through an optical-neuronal interface composed of permanent optical fiber implants that deliver light from a laser or from a light-emitting diode directly targeting the opsin-expressing region, gain- or loss-of-function can be evoked in awake, freely moving rodents by means of a light pulse[107].

Limbic regions that are responsible for regulating HPA axis and ANS stress responses, including the amygdala, hippocampus, and medial PFC (mPFC), interact with circuits responsible for memory, emotion, and reward systems and are also relevant to the development of psychiatric illnesses, as revealed by the use of optogenetics in animal models of such diseases[108]. For example, the amygdala has been implicated in the neuronal circuitry of anxiety disorders; yet, it is challenging to fully elucidate the highly heterogeneous population of functionally distinct subnuclei within the amygdala and their complex interconnectivity. By targeting basolateral amygdala (BLA) neurons, optical stimulation of BLA terminals projecting to the central nucleus of the amygdala have anti-anxiety effects in mice, and these effects are reversible during optical inhibition of the same pathway[102]. This study highlights the excellent precision of optogenetics in studying highly complex microcircuits, and may help identify key target regions for future treatments. Optogenetics has also shown potentials in studies of depressive behaviors in mice susceptible to chronic social defeat stress: optogenetic stimulation of the mPFC resulting in antidepressant effects suggests that lower PFC activity mediates depressive-like behaviors[101]. Using the reversible loss-of-function of optogenetics based on cell type-specific targeting of CA1 excitatory neurons and temporal precision in a mouse model of contextual fear conditioning, one study on remote memories showed that the hippocampus not only encodes and temporarily stores memories (held by current theory) but is also persistently involved in real-time remote memory recall through the dynamics of retrieval strategies that involve both the anterior cingulate cortex and CA1[109].

In summary, these findings and the ground-breaking progress in optogenetics presented here can serve as a basis for future studies exploring the role of specific neuronal populations in cognitive and neuropsychiatric processes, and enable temporally, genetically, and spatially resolved dissection of the underlying neuronal circuits[100,109].

6 Translational implications of the "inflammatory dialogue" between heart and brain in the context of stress

The neural control of stress is a complex process involving the integration of actual and potential outcome information. Circuits in the limbic forebrain process psychological stressor-related information and determine the response in a top-down manner, in anticipation of or in reaction to stressful events on the basis of prior experiences or innate programs[2,110]. The top-down strategy may likely depend on higher cognitive brain areas and their downstream effects on many processes[31]. Therefore, borrowing concepts and tools from emotion and cognitive neuroscience is necessary for understanding the interaction of the neuroendocrine and immune systems underlying the inflammatory responses to psychological stressors[1]. This will help to elucidate the hierarchical, temporal, and spatial communication patterns linking the brain, the stress-perceiving system, and the neuroendocrine and peripheral immune responses to acute and chronic psychological stress in different diseases[111]. It is important to identify the basic psychological mechanisms associated with the relevant brain regions and signaling, which are subsequently processed through neuroendocrine efferent pathways

to immunocompetent cells in the peripheral system[112]. Dysregulation of the HPA axis is known to be involved in inflammatory response-related diseases under stress, such as metabolic disease, CVD and depression. Moreover, HPA axis pathology is closely associated with altered activation or volumes of the hippocampus, amygdala, and PFC[32]. Thus, appropriate initiation and cessation of HPA axis stress responses are vital for both homeostasis and adaptation to adverse events. Strategies to control the HPA axis stress response in a top-down manner through limbic circuits are important for both basic research and clinical practice[19]. Fortunately, with the emergence of optogenetics, these are no longer impossible tasks in the study of the nervous system.

As shown above, the use of optogenetics to identify novel mechanistic-based therapeutic targets in limbic regions has yielded promising findings. Chronic inflammatory processes under conditions of stress, which are characterized by hyperactivity of the HPA axis and increasing production of pro-inflammatory cytokines dominated by GC resistance[18], is another field that awaits extensive investigation using optogenetics. Limbic brain structures modulate anticipatory HPA responses to stress and are logical candidates for the neural and feedback regulation of the HPA axis with respect to prior experiences based on learning, emotion, and memory[2]. Moreover, GRs in the hippocampus and cortical output circuits play an important role in inhibition of anticipatory HPA axis stress responses through GC effects[2]. Since GCs have cell type-specific effects and different cell types express variable amounts of mineralocorticoid receptors and GR when exposed to variable hormone levels due to regulation[26], traditional endocrinological and pharmacological approaches are limited. Therefore, genetic approaches using cell-specific manipulation of regulatory proteins should be considered. In this respect, optogenetics may provide a necessary and sufficient method for future research directed at determining how GCs act on different cell types and in brain regions (including limbic structures) that produce such profoundly different immunomodulatory outcomes at the system,

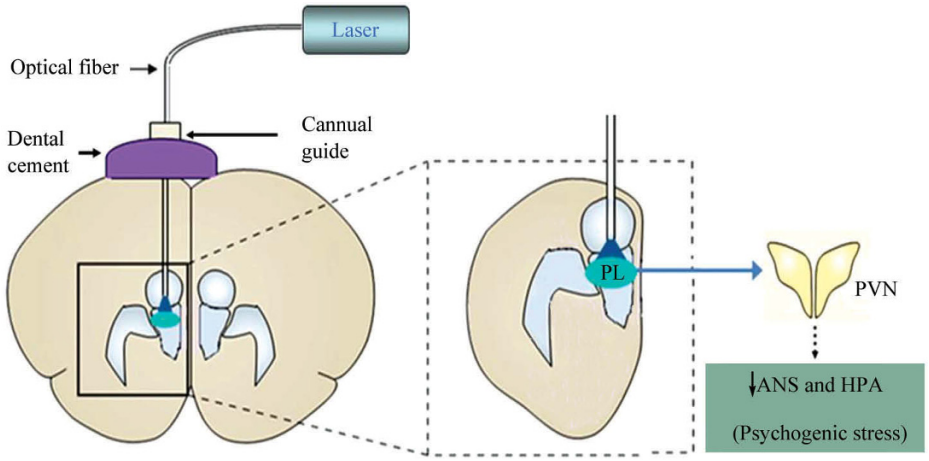

Fig. 2. The medial prefrontal cortex (mPFC) is a potential target for optogenetic perturbation in the context of stress. The mPFC functions as a principal limbic structure in initiating and coordinating the psychogenic stress response. In humans, mPFC dysfunction is linked to stress-related disease such as depression. The prelimbic cortex (PL), one of the most important subregions of the mPFC in response to psychogenic stress, preferentially inhibits the autonomic nervous system (ANS) and hypothalamic–pituitary–adrenocortical (HPA) axis via its glutamatergic projection to inhibitory paraventricular nucleus relays. With the optogenetic perturbational system, opsin genes can be delivered to glutamatergic neurons in the PL by stereotaxic virus injection under the control of calcium/calmodulin-dependent protein kinase IIα (CaMKIIα) promoter. The optical fiber is then implanted through a guide cannula above the PL to set up an optical-neural interface. After expression of the opsin proteins, photostimulation is available to fulfill the role of gain- or loss-of-function in freely moving rodents. The readout systems, including behavior tests, cardiovascular parameters and biochemical indexes in plasma such as cytokines, GCs and catecholamines, help to reveal the causal links between neuronal activity and stress responses.

cellular, and molecular levels. The use of optogenetics involves modulating the HPA axis in response to stress in a top-down manner through limbic regions involved in cytokine effects on behavior (Fig. 1). The strategy is based on concepts borrowed from emotion and cognitive neuroscience[19] and might further identify targets for therapeutic intervention in the future (Fig. 2). All these efforts will help to fully elucidate the common background of depression and CVD.

7 Conclusion

In chronic inflammatory responses to psychological stress, the interaction between cytokines and the HPA axis results in GC resistance, which leads not only to hyperactivation of the HPA axis (characterized by elevation of GCs and CRH), but also to a self-amplifying vicious cycle of inflammatory responses. Moreover, GC resistance further intensifies the stress-response system both peripherally and centrally, involving behavioral, cardiovascular, metabolic, and immunological responses that consist in the common background of depression and CVD. This inflammatory process also represents a potential "dialogue" between heart and brain. Optogenetics, with advances of cell-type specificity and high spatial and temporal resolution, aims to translate this elusive dialogue to identify novel therapeutic targets within the limbic regions in a top-down manner. Optogenetics will be essential for both basic and clinical research that explores the common mechanisms in stress-related diseases, for the benefit of public health.

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