CHAPTER SIX

Emerging evidence for the role of pituitary adenylate cyclase-activating peptide in neuropsychiatric disorders

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Abstract

Stress activates many brain nuclei and causes acute changes in several physiological and behavioral responses to restore homeostasis in affected organisms. While this response is protective, chronic stress exposure causes the sustained activation of these nuclei, leading to maladaptive physiological changes that underlie pathological mood and affective states. Hence, chronic stress may produce anxiety and mood disorders by promoting neuronal plasticity within these stress-responsive nuclei. A growing body of evidence attributes neuropeptide systems in mediating not only the physiological stress response but also pathological states that develop following chronic stress exposure. Recent preclinical data suggest that pituitary adenylyl cyclase-activating polypeptide (PACAP) and its receptors (PAC_1, $VPAC_1$, and $VPAC_2$) play an important role in the behavioral and endocrine responses to stress, as well as in mood and affective disorders. Human studies also point out the significance of the PACAP/PAC_1 receptor system in these disorders. For instance, PACAP through PAC_1 receptor up-regulates the expression of DISC1 (disrupted in schizophrenia 1) and impedes its association with its interacting protein. Interestingly, the DISC1 gene mutation is linked to schizophrenia and depression. Moreover, a link between PACAP blood titer and fear physiology,

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post-traumatic stress disorder (PTSD) diagnosis and symptoms has been reported in heavily traumatized female patients. Additionally, in the peripheral blood, methylation of the gene encoding the PAC_1 receptor is also associated with PTSD. This book chapter describes the emerging evidence that entails PACAP in the stress response and stress-mediated neuropsychiatric disorders.

1. Introduction

Pituitary adenylyl cyclase activating polypeptide (PACAP), a pleiotropic polypeptide, is related to the secretin/glucagon/vasoactive intestinal polypeptide (VIP) family and is present in two biologically active forms, PACAP-38 and PACAP-27.[1] PACAP and VIP have a high resemblance in their primary structure, and both peptides exert a variety of functions through activating G-protein coupled receptors. VIP and PACAP share two common receptors ($VPAC_1$ and $VPAC_2$ receptors) while only PACAP binds with high affinity to PAC_1 receptors.[2]

High to moderate expression of PACAP and its selective (PAC_1) receptor have been reported in the hypothalamic and limbic structures and in other brain regions as well as in peripheral tissues.[3] The broader distribution of PACAP in and outside the central nervous system suggests its involvement in several biological processes, such as regulation of food intake, energy metabolism, body temperature, neuronal survival, and the neuroendocrine and behavioral response to stress.[3–7]

Earlier studies showed expression of PACAP and its receptors in the hypothalamus and adrenal glands. Predominantly abundant innervations of hypophysiotropic neurons by nerve fibers comprising of the neuropeptide PACAP suggests a prominent role of this peptide/receptor system in the control of the hypothalamic-pituitary-adrenal (HPA) axis and stress response (reviewed in Ref. 8). Along this line, PACAP increases the expression of CRH in the paraventricular nucleus (PVN) of the hypothalamus[9–11] and intra-PVN administration of PACAP mimics behavioral changes result from the activation of PVN by CRH or a stressor.[12] Likewise, restraint stress-induced secretion of corticosterone is attenuated in mice lacking PACAP,[13] suggesting that PACAP plays a functional role in the stress response.

While activation of the stress circuit acutely promotes a "fight or flight" response, the prolonged activation of the HPA axis can lead to maladaptive neuroendocrine and behavioral changes. The negative behavioral consequences of chronic stress result from continuous or inappropriate activation

of the stress circuit and continued secretion of stress mediators, leading to improper activation of the fear circuit and the development of anxiety. These mediators also exert anti-rewarding actions, leading to diminished mood and anhedonia as observed in depressed patients. These mediators even overwhelm the sleep-wake cycle that causes insomnia as well as daytime somnolence which are frequently reported in patients with posttraumatic stress disorder (PTSD). Interruption of executive and cognitive functions also could occur as an outcome of continuous activation of the stress circuit.[14] Considering that stress is one of the leading causes of neuropsychiatric disorders, we first discuss the role of PACAP in the stress response followed by its involvement in the pathophysiology of stress-induced mental illnesses.

2. PACAP and stress response

Stress can be defined as a condition when homeostasis is threatened, or the organism observes a situation to be threatening. The HPA axis mediates the stress response. Corticotropin releasing factor/hormone (CRF/CRH) has long been known as the key regulator of the HPA axis and plays a crucial role in stress responses.[15] Briefly, activation of the HPA axis by a stressor involves the release of CRH from neurosecretory cells in the PVN of the hypothalamus. CRH travels through the portal circulation to the pituitary and stimulates the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH, in turn, reaches its site of action and acts on the adrenal glands to cause the release of glucocorticoids, which not only mediate the stress response but also act as negative feedback to reduce further activation of the HPA axis.[16] The latter is a biologically protective mechanism that promotes a "fight or flight" response to a stressor. However, the prolonged activation of the HPA axis can lead to maladaptive behavioral changes, such as anxiety and/or depression, in which the negative feedback mechanism may be impaired. Thus, the HPA axis can be the target not only for the stressors but also that of endogenous chemicals that serve as regulators of the stress response and could contribute to the pathophysiology of stress-related neuropsychiatric disorders.

PACAP and its receptors are found in the hypothalamus and adrenal glands. Predominantly abundant innervation of hypophysiotropic neurons by nerve fibers comprising of the neuropeptide PACAP suggests a prominent role of this peptide/receptor system in the control of the HPA axis

and stress response (reviewed in Ref. 8). Indeed, PACAP increases the expression of CRH in the PVN[9–11] and local administration of PACAP into the PVN mimics behavioral changes result from the activation of PVN by CRH or a stressor,[12] showing that PACAP is involved in the stress response. Along this line, restraint stress causes secretion of corticosterone in wild-type mice, a response that is attenuated in mice lacking PACAP.[13] Moreover, repeated stress (2–3 h per day for 7 days) leads to unhabituated corticosterone secretion and this response is reduced in mice lacking PACAP.[17]

Chronic variable stress increase PACAP and PAC_1 receptor transcript in the bed nucleus of stria terminalis (BNST), raising the possibility that PACAP may regulate the stress response and could contribute to the pathophysiology of mood and affective disorders via acting not only on the HPA axis but also on extrahypothalamic brain regions. Indeed, local administration of PACAP into the BNST increases corticosterone secretion[18,19] and induces anxiety-like behaviors.[18] Furthermore, PACAP injection into the central nucleus of amygdala (CeA) mimics the behavioral and neuroendocrine manifestations of stress.[20]

Epinephrine and norepinephrine release from the adrenal medulla represent the cardinal feature of acute stress responses. Neuropeptides are active as transmitters in this circuitry. Remarkably, recent findings in the preceding decade have enhanced our understanding of the perception of the process of neuropeptide–catecholamine interactions in the brain and periphery. A new picture of stress circuitry has emerged, in which catecholamine and neuropeptide systems are intimately interconnected, both centrally and peripherally, during responses to both systemic and psychogenic stress. PACAP modulates the HPA axis in response to acute psychogenic but not systemic stressors, through activation of CRH (reviewed in Ref. 8). PACAP facilitates the biosynthesis of catecholamines in the adrenal medulla.[21] Thus, one other target could be via a local action at the level of the adrenal gland which has not been fully explored and not covered in this chapter.

Recent studies have suggested that action of antidepressants on synaptic plasticity is mediated by their regulatory influence not only upon small-molecule neurotransmitters but also via neuropeptides which may act both as neurotransmitters and as neuromodulators. Neuropeptides and catecholamines act on several circuits in the central and peripheral nervous systems to facilitate systemic and psychological stress responses, as well as enduring adaptive and maladaptive changes to stress. These could lead to survival with resilience, or survival with vulnerability, as manifested in mood and

affective disorders, such as anxiety, depression, PTSD, obesity and eating disorders (reviewed in Ref. 22). A growing body of literature suggests that the PACAP/PAC_1 receptor system may be a potential contributor to neuropsychiatric disorders, which is described below.

3. PACAP and anxiety disorders

Anxiety disorders are the most common psychiatric disorder in the USA. Anxiety disorders usually are long-lasting and debilitating and characterized by thoughts and feelings of uncontrollable fear and accompanied by somatic, affective, and behavioral symptoms. PACAP is present in most of the brainstem areas including autonomic sensory and motor system related areas, but also in the centers involved in stress adaptation, such as the ventral tegmental area and locus coeruleus.[7] As stated above, local administration of PACAP in the PVN influences the CRF expression[9] and mimics behavioral changes result from the activation of PVN by CRH or a stressor.[12] Furthermore, intracerebroventricular PACAP administration produces anxiety- and depressive-like behaviors in rodents, effects produced by CRF.[23] On the other hand, PACAP deficiency seems to be protective against the deleterious effects and preventing the chronic activation of the HPA axis.[24]

PACAP and PAC_1 receptors are widely expressed in the CeA and BNST, raising the possibility that this peptide/receptor system is not only involved in the stress response but also in anxiety disorders. Interestingly, fear conditioning and estrogen replacement therapy each is shown to increase PAC_1 receptor gene expression.[25] Furthermore, local administration of PACAP in the CeA is found to be anxiogenic in rats and that the melanocortin receptor 4 (MC4R) system mediates the anxiogenic effects of PACAP.[20] These authors suggested that dysregulation of this neuropeptide system may contribute to the pathophysiology of the anxiety group of disorders.[20] Interestingly, chronic variable stress, which leads to anxiety and depression in rodents, increases PACAP and PAC_1 receptor transcript expression in the BNST and local administration of PACAP in the BNST induces anxiety-like behaviors in rats.[18] On the other hand, continuous infusion of PACAP antagonist (PACAP6–38) in the BNST reduces anxiety-like behaviors induced by chronic variable stress,[18] suggesting that PACAP alterations in the BNST may mediate stress-induced BNST neuroplasticity and that this may be the underlying mechanism of some forms of human anxiety disorders. In line with these observations, mice lacking

PACAP spend more time on the open arms of the elevated plus maze compared to their wild-type controls,[26–28] suggesting that the lack of PACAP is protective against the development of anxiety disorders.

Human studies have also suggested the possible involvement of the PACAP/receptor system in the pathophysiology of anxiety and PTSD.[25] Interestingly, a single nucleotide polymorphism in the PAC_1 receptor gene leads to decreases in amygdala and hippocampus connectivity and may contribute to aberrant fear and anxiety disorders.[29] Together, these studies assign a significant role to neuropeptide systems in the mediation of the stress response, making them potential drug targets for the treatment of stress and anxiety disorders.

4. PACAP and major depression

Major depressive disorder (MDD) is an enduring and life-threatening psychiatric condition characterized by low mood, psychomotor changes, and marked decreased interest or pleasure in most activities, also known as anhedonia (American Psychiatric Association 2013). Major depression is among the leading causes of disability around the globe according to the World Health Organization (WHO, 2012). The lifetime prevalence of depression in the industrialized population ranges between 15% and 25%.[30] It is important to note that patients diagnosed with depression show somewhat different symptoms and the diagnostic criteria themselves are already somewhat contradictory as opposing phenomena might support the same "major depressive disorder" diagnosis (like hyperphagia vs. hypophagia, insomnia vs. hypersomnia or weight gain vs. weight loss). Considering this heterogeneous pathophysiological background, it is not surprising that 30% of patients do not respond to the currently available medications influencing monoaminergic neurotransmission in the brain. The need for new strategies to treat the disease is evident.

PACAP has been a critical neurotransmitter/neuropeptide mediating activation of the HPA and hormonal sympathetic adrenal axes. CRH not only initiates activation of the HPA axis via the release of ACTH from the pituitary but also is released from hypothalamic and extrahypothalamic neurons to feedback on noradrenergic systems driving central to peripheral stress "executive" programs (reviewed in Ref. 22). PACAP and its receptor PAC_1 are widely expressed in several nuclei of the hypothalamus as well as in

various extra-hypothalamic regions including the amygdala, hippocampus, and nucleus accumbens.[7,31,32] Given the importance of these brain regions in motivated behaviors, reward, and the pathophysiology of mental illnesses, PACAP and its receptors may be involved in the pathogenesis of depressive disorders and other neuropsychiatric disorders. Indeed, there is accumulating evidence that the PACAP-ergic signaling plays a vital role in the behavioral and endocrine responses to stress, as well as in synaptic plasticity and neuroprotection.[24,28,33–35]

Intracranial self-stimulation (ICSS) is a useful approach in which electrical activation of specific brain regions is associated with reward. In rodents, it is achieved by the animal performing an operant response, such as lever pressing. An increase in the ICSS threshold is considered as a reflection of deficits in brain reward circuitries, used as a sign of anhedonia, that is a core symptom of major depression and other mood disorders (American Psychiatric Association 2013). Chronic treatment with antidepressant drugs attenuate increases in the ICSS threshold induced by cocaine withdrawal.[36] PACAP, on the other hand, increases ICSS threshold, suggesting that PACAP promotes depressive-like behaviors in rodents.[37] PACAP administration also reduces social interaction and preference for a saccharine solution, which is commonly used as a model of anhedonia. However, it fails to alter immobility time in the forced swim test, an animal model of despair.[37] The changes observed are blocked by the PACAP receptor antagonist, PACAP6-38, suggesting that the PACAP/PAC_1 receptor system may play a functional role in the pathogenesis of depression, particularly the anhedonia and social dysfunction associated with the disease.[37]

While exogenous PACAP appears to promote depression-like behaviors in rodents, conflicting results are reported regarding the role of endogenous PACAP in these behaviors in mice lacking PACAP. Hattori and colleagues[27] reported a slight reduction in immobility time in the forced swim test in mice lacking PACAP, indicating attenuation of depressive-like behaviors in the absence of endogenous PACAP. However, other groups show that mice lacking PACAP express increased immobility time in the forced swim test,[34,38–40] suggesting that endogenous PACAP may serve to protect against the development of depressive-like behaviors in rodents.

Endogenous PACAP, however, promotes depressive-like behaviors in subjects exposed to chronic stress. Thus, early life stress (180-min maternal deprivation) in combination with chronic variable stress induces depressive-like behaviors in wild-type mice, and this behavioral change is found to be

reduced in mice lacking PACAP.[41] Additionally, chronic variable stress is also found to cause reduced depressive-like behaviors in adult mice lacking PACAP compared to their wild-type controls.[42] Moreover, Lehmann and colleagues[24] demonstrate that a 14-day social defeat stress exposure caused a significant increase in PVN *c*-fos expression as well as plasma corticosterone secretion and depression-like behaviors in wild-type mice. These stress-mediated changes are found to be reduced in mice lacking PACAP, suggesting that prolonged activation of the PACAP/PAC_1 receptor system in response to chronic stress may lead to the enduring changes observed in depression.

Human studies underpin the significance of the PACAP/PAC_1 receptor system in mood disorders. For instance, PACAP through PAC_1 receptor up-regulates the expression of the protein DISC1 (disrupted in schizophrenia 1) and radically but momentarily interferes with the interaction of DISC1 with its interacting protein.[43] Interestingly, the DISC1 gene mutation is linked not only to schizophrenia[44] but also to depression.[45,46] Furthermore, Hashimoto and colleagues[40] report on a possible linkage between the PACAP gene and MDD, in which they compare 637 MDD patients versus 967 healthy controls and find that a variant in the PACAP gene is associated with MDD. Additionally, a SNP in the PAC_1 receptor gene leads to a significant interaction between this variant and neighborhood crime for patients suffering from major depression.[47] Interestingly, the PACAP variant gene (rs2856966) is found to be associated with a better treatment outcome to venlafaxine.[48] Taken together, the above studies suggest that the PACAP system may represent a potential target for the development of novel pharmacotherapies to treat depression and possibly other stress-mediated neuropsychiatric disorders.

5. PACAP and post-traumatic stress disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a pervasive and well-known of all the trauma- and stress-related disorders with a twofold greater prevalence in women than men. It affects about 5–10% of the US population and is sparked by exposure to an acute or recurring traumatic event(s). Patients with PTSD, in comparison to trauma-exposed controls, not only exhibit psychological symptoms but also abnormally high conditioned fear responses. This high level of fear may result from a lack of ability to habituate to aversive stimuli, to extinguish or inhibit fear memories, and/or possibly strengthening of the original fear memory.[49–51]

The cardinal feature of PTSD is the dysregulation of the HPA axis, and that the system is altered and hyper-responsive to cortisol feedback. Recent research in PTSD suggests that increased activity of CRH-containing circuits is involved in the pathophysiology of the disease.[52] The neuropeptide PACAP is involved in regulating CRH, a key mediator of the HPA axis,[9] raising the possibility that the PACAP system may be involved in the pathophysiology of PTSD and other stress-related neuropsychiatric disorders.

Available genetic data suggest that PACAP/PAC_1 receptor expression and signaling may be integrally involved in regulating the psychological and physiological responses to traumatic stress and variants of PACAP or PAC_1 receptor gene may be potential contributors of PTSD. For example, in highly traumatized female but not male subjects, there seems to be a strong sex-specific association of PACAP blood levels and PAC_1 receptor gene variants with fear discrimination, PTSD diagnosis, and symptoms.[25] The sex-specific association occurs within a single-nucleotide polymorphism (rs2267735) that is located on the so-called estrogen response element within the PAC_1 receptor gene that mediates the regulation of PAC_1 receptor gene by the sex hormones.[25] In line with these human data, Mercer and colleagues report that fear conditioning and estradiol additively increase the expression of the PAC_1 receptor gene in mice.[53] In line with this, in women with low levels of estradiol, the CC allele is associated with reduced PAC_1 receptor gene expression which is linked to higher signs and symptoms of PTSD.[53] These data suggest that perturbations in the PACAP/PAC_1 receptor system are regulated by estrogen and are involved in abnormal fear responses underlying PTSD. Additionally, these sex-related differences in PACAP signaling during emotional learning could provide novel targets for the treatment of PTSD.[54]

Functional magnetic resonance imaging of patients with moderate to high trauma revealed a higher reactivity of limbic structures, such as the amygdala and hippocampus, in patients with this genotype. Likewise, there was decreased connectivity of the amygdala and hippocampus in these individuals,[55] suggesting that this gene alteration may contribute to aberrant fear, which is a hallmark of PTSD and anxiety disorders.[29] Moreover, decreased hippocampal activity is observed in female subjects carrying the PAC_1 variant.[56] Considering that genetic risk factors may account for up to 30–40% of the heritability of PTSD, the above findings suggest that perturbations in the PACAP/PAC_1 receptor system are regulated by estrogen and are involved in abnormal fear responses underlying PTSD. Additionally, these sex-related differences in PACAP signaling during emotional learning could provide novel targets for the treatment of PTSD.[54]

6. PACAP and schizophrenia

Schizophrenia is a severe and debilitating neuropsychiatric brain disorder that affects 1% of the population. It is equally prevalent across people irrespective of ethnicity, race, and nationality across the globe and is equally prevalent in men and women. Its cause is due to the interaction of several abnormal genes with environmental factors. The course of schizophrenia is variable, with some individuals experiencing exacerbations and others remaining stable but chronically ill.[57] Schizophrenia is not only a neurodevelopmental disorder but also neurodegenerative disorder.[58] There have been several reviews recently of various aspects of PACAP as well as its role in neurodevelopmental disorders.[59] It has been proposed that PACAP may play a role in mental diseases and that regulation of the PACAPergic signals could be a potential treatment for schizophrenia considering schizophrenia is not only neurodegenerative but also neurodegenerative disorder.[60] PAC_1 receptor gene alternative splicing could contribute to the risk of developing schizophrenia, not only by affecting embryonic neurogenesis but also by impairing proliferation and differentiation of adult stem cells.[61] Accordingly, a reduction in the amount of hippocampal neural stem cells is reported in post-mortem schizophrenic brains,[62] raising the possibility that this could explain the decrease in hippocampal volume and the correlation with several cognitive deficits observed in schizophrenic patients.

PAC_1 is a G-protein-coupled receptor encoded by *Adcyap1*, which is a potential schizophrenia susceptibility gene in rodents and humans. Variations of the PACAP and/or PACAP receptor genes may be a genetic risk factor for psychiatric disorders including schizophrenia-like phenotype in mice.[63] Studies have shown that PACAP-deficient mice exhibit behavioral and neurophysiological abnormalities including emotional lability, depression-like behaviors, and memory impairments that are ameliorated with atypical antipsychotic drugs. For example, Ago and colleagues[38] show that mice lacking PACAP display reduced performance in the object recognition test. Likewise, PACAP heterozygous mice exhibit deficits in sensorimotor gating, as measured in the prepulse inhibition paradigm following the administration of an aversive stimulus, i.e., administration of a $5HT_2$ receptor agonist.[64] However, an earlier study reports no change in prepulse inhibition in mice lacking PACAP compared to their wild-type controls.[27] These authors also show more social interaction, unlike previous studies, with a mild performance deficit in working memory in mice lacking PACAP.[27]

Human studies, however, have implicated the PACAP/PAC_1 receptor system in schizophrenia. For instance, the PACAP gene allele that is over-expressed in patients with schizophrenia is found to be associated with reduced hippocampal volume and poorer memory performance.[63] Likewise, PACAP through PAC_1 receptor up-regulates the production of the protein DISC1 and drastically but briefly reduces interaction of DISC1 with its interacting protein[43] and may alter neurite outgrowth. Interestingly, DISC1 gene mutation was linked to schizophrenia.[44] Likewise, stathmin1, which induces abnormal axonal arborization, may be a linkage for the involvement of PACAP in schizophrenia, as the expression of stathamin 1 is upregulated in the brain of mice lacking PACAP as well as in patients with schizophrenia (reviewed in Ref. 60). These findings suggest that it is possible that neural development via these two proteins is disturbed in schizophrenic patients and PACAP may be involved in this process.

Not only the PACAP/PAC_1 receptor system but also the PACAP/$VPAC_2$ signaling is suggested to be associated with mental illnesses. Indeed, a duplication of the chromosome that encodes the $VPAC_2$ receptor is found to be linked to schizophrenia.[65] Thus, restoring the changes induced by the PACAP/PAC_1/$VPAC_2$ signaling may be a novel approach to treat schizophrenia.

7. Concluding remarks

The PACAP/PAC_1 receptor system has been implicated in several neuropsychiatric disorders. Previous studies have shown alterations in anxiety and depression in mice lacking PACAP or its receptor compared to their wild-type controls. However, considering that compensatory developmental changes may occur in knockout mice, further studies using pharmacological tools and other approaches are needed to fully characterize the role of the PACAP/PAC1 receptor system in these disorders. Currently, the lack of selective small-molecule PAC1 receptor antagonists represents a challenge. However, attempts have been made to develop such small molecules.[66]

PACAP has the potential to be neuroprotective and regulate mood and affective states via multiple mechanisms. Earlier studies have demonstrated that PACAP is associated with the expression of tyrosine hydroxylase, which is the rate-limiting enzyme in the catecholamine synthesis. This can be significant in the monoamine theory of depression. Likewise, studies have shown that PACAP can increase the expression of brain-derived neurotrophic factor (BDNF), which is involved in neurogenesis and neuronal survival.

PACAP is thus well positioned to alter mood and be involved in the pathophysiology of depression and related disorders. Furthermore, studies have shown that PACAP receptors are also found on macrophages and immune cells. Thus, PACAP has the potential to impact neurological and immunological pathways via altering the function of these cells. Future studies can be directed to address these unexplored areas of research.

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References

1. Miyata A, Arimura A, Dahl RR, et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun*. 1989;164(1):567–574.
2. Cazillis M, Gonzalez BJ, Billardon C, et al. VIP and PACAP induce selective neuronal differentiation of mouse embryonic stem cells. *Eur J Neurosci*. 2004;19(4):798–808.
3. Ghatei MA, Takahashi K, Suzuki Y, Gardiner J, Jones PM, Bloom SR. Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. *J Endocrinol*. 1993;136(1):159–166.
4. Reglodi D, Kiss P, Horvath G, et al. Effects of pituitary adenylate cyclase activating polypeptide in the urinary system, with special emphasis on its protective effects in the kidney. *Neuropeptides*. 2012;46(2):61–70.
5. Reglodi D, Kiss P, Szabadfi K, et al. PACAP is an endogenous protective factor-insights from PACAP-deficient mice. *J Mol Neurosci*. 2012;48(3):482–492.
6. Reglodi D, Tamas A, Koppan M, Szogyi D, Welke L. Role of PACAP in female fertility and reproduction at gonadal level—recent advances. *Front Endocrinol (Lausanne)*. 2012;3:155.
7. Vaudry D, Falluel-Morel A, Bourgault S, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev*. 2009;61(3):283–357.
8. Mustafa T. Pituitary adenylate cyclase-activating polypeptide (PACAP): a master regulator in central and peripheral stress responses. *Adv Pharmacol*. 2013;68:445–457.
9. Agarwal A, Halvorson LM, Legradi G. Pituitary adenylate cyclase-activating polypeptide (PACAP) mimics neuroendocrine and behavioral manifestations of stress: evidence for PKA-mediated expression of the corticotropin-releasing hormone (CRH) gene. *Brain Res Mol Brain Res*. 2005;138(1):45–57.
10. Grinevich V, Fournier A, Pelletier G. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on corticotropin-releasing hormone (CRH) gene expression in the rat hypothalamic paraventricular nucleus. *Brain Res*. 1997;773(1–2):190–196.
11. Stroth N, Liu Y, Aguilera G, Eiden LE. Pituitary adenylate cyclase-activating polypeptide controls stimulus-transcription coupling in the hypothalamic-pituitary-adrenal axis to mediate sustained hormone secretion during stress. *J Neuroendocrinol*. 2011;23(10):944–955.

12. Norrholm SD, Das M, Legradi G. Behavioral effects of local microinfusion of pituitary adenylate cyclase activating polypeptide (PACAP) into the paraventricular nucleus of the hypothalamus (PVN). *Regul Pept*. 2005;128(1):33–41.
13. Tsukiyama N, Saida Y, Kakuda M, et al. PACAP centrally mediates emotional stress-induced corticosterone responses in mice. *Stress*. 2011;14(4):368–375.
14. Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. *Annu Rev Physiol*. 2005;67:259–284.
15. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science*. 1981;213(4514):1394–1397.
16. Vyas S, Rodrigues AJ, Silva JM, et al. Chronic stress and glucocorticoids: from neuronal plasticity to neurodegeneration. *Neural Plast*. 2016;2016:6391686.
17. Jiang SZ, Eiden LE. Activation of the HPA axis and depression of feeding behavior induced by restraint stress are separately regulated by PACAPergic neurotransmission in the mouse. *Stress*. 2016;19(4):374–382.
18. Roman CW, Lezak KR, Hartsock MJ, et al. PAC1 receptor antagonism in the bed nucleus of the stria terminalis (BNST) attenuates the endocrine and behavioral consequences of chronic stress. *Psychoneuroendocrinology*. 2014;47:151–165.
19. Lezak KR, Roelke E, Harris OM, et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) in the bed nucleus of the stria terminalis (BNST) increases corticosterone in male and female rats. *Psychoneuroendocrinology*. 2014;45:11–20.
20. Iemolo A, Seiglie M, Blasio A, Cottone P, Sabino V. Pituitary adenylate cyclase-activating polypeptide (PACAP) in the central nucleus of the amygdala induces anxiety via melanocortin receptors. *Psychopharmacology (Berl)*. 2016;233(17):3269–3277.
21. Stroth N, Kuri BA, Mustafa T, Chan SA, Smith CB, Eiden LE. PACAP controls adrenomedullary catecholamine secretion and expression of catecholamine biosynthetic enzymes at high splanchnic nerve firing rates characteristic of stress transduction in male mice. *Endocrinology*. 2013;154(1):330–339.
22. Eiden LE. Neuropeptide-catecholamine interactions in stress. *Adv Pharmacol*. 2013;68:399–404.
23. Dore R, Iemolo A, Smith KL, Wang X, Cottone P, Sabino V. CRF mediates the anxiogenic and anti-rewarding, but not the anorectic effects of PACAP. *Neuropsychopharmacology*. 2013;38(11):2160–2169.
24. Lehmann ML, Mustafa T, Eiden AM, Herkenham M, Eiden LE. PACAP-deficient mice show attenuated corticosterone secretion and fail to develop depressive behavior during chronic social defeat stress. *Psychoneuroendocrinology*. 2013;38(5):702–715.
25. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature*. 2011;470(7335):492–497.
26. Hashimoto H, Shintani N, Tanaka K, et al. Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). *Proc Natl Acad Sci USA*. 2001;98(23):13355–13360.
27. Hattori S, Takao K, Tanda K, et al. Comprehensive behavioral analysis of pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice. *Front Behav Neurosci*. 2012;6:58.
28. Otto C, Martin M, Wolfer DP, Lipp HP, Maldonado R, Schutz G. Altered emotional behavior in PACAP-type-I-receptor-deficient mice. *Brain Res Mol Brain Res*. 2001;92(1–2):78–84.
29. Stevens JS, Jovanovic T, Fani N, et al. Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. *J Psychiatr Res*. 2013;47(10):1469–1478.
30. Kessler RC, Berglund P, Demler O, et al. The epidemiology of major depressive disorder: Results from the National Comorbidity Survey Replication (NCS-R). *JAMA*. 2003;289(23):3095–3105.

31. Joo KM, Chung YH, Kim MK, et al. Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. *J Comp Neurol*. 2004;476(4):388–413.
32. Piggins HD, Stamp JA, Burns J, Rusak B, Semba K. Distribution of pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactivity in the hypothalamus and extended amygdala of the rat. *J Comp Neurol*. 1996;376(2):278–294.
33. Hammack SE, May V. Pituitary adenylate cyclase activating polypeptide in stress-related disorders: data convergence from animal and human studies. *Biol Psychiatry*. 2015;78(3):167–177.
34. Hashimoto H, Hashimoto R, Shintani N, et al. Depression-like behavior in the forced swimming test in PACAP-deficient mice: amelioration by the atypical antipsychotic risperidone. *J Neurochem*. 2009;110(2):595–602.
35. Stroth N, Holighaus Y, Ait-Ali D, Eiden LE. PACAP: a master regulator of neuroendocrine stress circuits and the cellular stress response. *Ann N Y Acad Sci*. 2011;1220:49–59.
36. Markou A, Hauger RL, Koob GF. Desmethylimipramine attenuates cocaine withdrawal in rats. *Psychopharmacology (Berl)*. 1992;109(3):305–314.
37. Seiglie MP, Smith KL, Blasio A, Cottone P, Sabino V. Pituitary adenylate cyclase-activating polypeptide induces a depressive-like phenotype in rats. *Psychopharmacology (Berl)*. 2015;232(20):3821–3831.
38. Ago Y, Hiramatsu N, Ishihama T, et al. The selective metabotropic glutamate 2/3 receptor agonist MGS0028 reverses psychomotor abnormalities and recognition memory deficits in mice lacking the pituitary adenylate cyclase-activating polypeptide. *Behav Pharmacol*. 2013;24(1):74–77.
39. Gaszner B, Kormos V, Kozicz T, Hashimoto H, Reglodi D, Helyes Z. The behavioral phenotype of pituitary adenylate-cyclase activating polypeptide-deficient mice in anxiety and depression tests is accompanied by blunted c-Fos expression in the bed nucleus of the stria terminalis, central projecting Edinger-Westphal nucleus, ventral lateral septum, and dorsal raphe nucleus. *Neuroscience*. 2012;202:283–299.
40. Hashimoto R, Hashimoto H, Shintani N, et al. Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder. *Neurosci Lett*. 2010;468(3):300–302.
41. Farkas J, Kovacs LA, Gaspar L, et al. Construct and face validity of a new model for the three-hit theory of depression using PACAP mutant mice on CD1 background. *Neuroscience*. 2017;354:11–29.
42. Kormos V, Gaspar L, Kovacs LA, et al. Reduced response to chronic mild stress in PACAP mutant mice is associated with blunted FosB expression in limbic forebrain and brainstem centers. *Neuroscience*. 2016;330:335–358.
43. Hattori T, Baba K, Matsuzaki S, et al. A novel DISC1-interacting partner DISC1-binding zinc-finger protein: implication in the modulation of DISC1-dependent neurite outgrowth. *Mol Psychiatry*. 2007;12(4):398–407.
44. Millar JK, Wilson-Annan JC, Anderson S, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet*. 2000;9(9):1415–1423.
45. Blackwood DH, Muir WJ. Clinical phenotypes associated with DISC1, a candidate gene for schizophrenia. *Neurotox Res*. 2004;6(1):35–41.
46. Blackwood DH, Pickard BJ, Thomson PA, Evans KL, Porteous DJ, Muir WJ. Are some genetic risk factors common to schizophrenia, bipolar disorder and depression? Evidence from DISC1, GRIK4 and NRG1. *Neurotox Res*. 2007;11(1):73–83.
47. Lowe SR, Pothen J, Quinn JW, et al. Gene-by-social-environment interaction (GxSE) between ADCYAP1R1 genotype and neighborhood crime predicts major depression symptoms in trauma-exposed women. *J Affect Disord*. 2015;187:147–150.

48. Cooper AJ, Narasimhan S, Rickels K, Lohoff FW. Genetic polymorphisms in the PACAP and PAC1 receptor genes and treatment response to venlafaxine XR in generalized anxiety disorder. *Psychiatry Res*. 2013;210(3):1299–1300.
49. Jovanovic T, Norrholm SD, Blanding NQ, et al. Impaired fear inhibition is a biomarker of PTSD but not depression. *Depress Anxiety*. 2010;27(3):244–251.
50. Jovanovic T, Norrholm SD, Blanding NQ, et al. Fear potentiation is associated with hypothalamic-pituitary-adrenal axis function in PTSD. *Psychoneuroendocrinology*. 2010;35(6):846–857.
51. Jovanovic T, Ressler KJ. How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *Am J Psychiatry*. 2010;167(6):648–662.
52. Dunlop BW, Rothbaum BO, Binder EB, et al. Evaluation of a corticotropin releasing hormone type 1 receptor antagonist in women with posttraumatic stress disorder: study protocol for a randomized controlled trial. *Trials*. 2014;15:240.
53. Mercer KB, Dias B, Shafer D, et al. Functional evaluation of a PTSD-associated genetic variant: estradiol regulation and ADCYAP1R1. *Transl Psychiatry*. 2016;6(12):e978.
54. Kirry AJ, Herbst MR, Poirier SE, et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) signaling in the prefrontal cortex modulates cued fear learning, but not spatial working memory, in female rats. *Neuropharmacology*. 2018;133:145–154.
55. Grimm O, Pohlack S, Cacciaglia R, et al. Amygdalar and hippocampal volume: a comparison between manual segmentation, Freesurfer and VBM. *J Neurosci Methods*. 2015;253:254–261.
56. Pohlack ST, Nees F, Ruttorf M, et al. Neural mechanism of a sex-specific risk variant for posttraumatic stress disorder in the type I receptor of the pituitary adenylate cyclase activating polypeptide. *Biol Psychiatry*. 2015;78(12):840–847.
57. Yanos PT, Moos RH. Determinants of functioning and well-being among individuals with schizophrenia: an integrated model. *Clin Psychol Rev*. 2007;27(1):58–77.
58. Owen MJ, O'Donovan MC. Schizophrenia and the neurodevelopmental continuum: evidence from genomics. *World Psychiatry*. 2017;16(3):227–235.
59. Hill JM. Vasoactive intestinal peptide in neurodevelopmental disorders: therapeutic potential. *Curr Pharm Des*. 2007;13(11):1079–1089.
60. Katayama T, Hattori T, Yamada K, Matsuzaki S, Tohyama M. Role of the PACAP-PAC1-DISC1 and PACAP-PAC1-stathmin1 systems in schizophrenia and bipolar disorder: novel treatment mechanisms? *Pharmacogenomics*. 2009;10(12):1967–1978.
61. Yan Y, Zhou X, Pan Z, Ma J, Waschek JA, DiCicco-Bloom E. Pro- and anti-mitogenic actions of pituitary adenylate cyclase-activating polypeptide in developing cerebral cortex: potential mediation by developmental switch of PAC1 receptor mRNA isoforms. *J Neurosci*. 2013;33(9):3865–3878.
62. Reif A, Fritzen S, Finger M, et al. Neural stem cell proliferation is decreased in schizophrenia, but not in depression. *Mol Psychiatry*. 2006;11(5):514–522.
63. Hashimoto R, Hashimoto H, Shintani N, et al. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. *Mol Psychiatry*. 2007;12(11):1026–1032.
64. Hazama K, Hayata-Takano A, Uetsuki K, et al. Increased behavioral and neuronal responses to a hallucinogenic drug in PACAP heterozygous mutant mice. *PLoS One*. 2014;9(2):e89153.
65. Shen S, Gehlert DR, Collier DA. PACAP and PAC1 receptor in brain development and behavior. *Neuropeptides*. 2013;47(6):421–430.
66. Takasaki I, Watanabe A, Yokai M, et al. In silico screening identified novel small-molecule antagonists of PAC1 receptor. *J Pharmacol Exp Ther*. 2018;365(1):1–8.

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