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REVIEW

Zhu et al: NPS pathway in neuropsychiatric disorders

Neuropeptide S pathway in PTSD and neuropsychiatric disorders: A review

Zhi-cheng Zhu[1,2], Xue-jing Han[1,3], Zhen He[1], Meng-yang Liu[1,3], Ning Wu[1], Xiang-min Tong[4]*, Fei Li[1]*

[1]Beijing Institute of Pharmacology and Toxicology, Beijing, China;

[2]Department of Pharmacology, Hangzhou Normal University, Hangzhou, Zhejiang, China;

[3]Department of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning, China;

[4]Department of Hematology, Zhejiang Provincial People's Hospital and People's Hospital of Hangzhou Medical College, Hangzhou, China.

*Correspondence to Xiang-min Tong: tongxiangmin@163.com and Fei Li: lf5335317@163.com

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a multidimensional illness that seldom occurs alone: roughly 80 % of patients also meet criteria for anxiety, depression, chronic pain, substance-use, eating or cognitive disorders. Converging genetic, neurochemical and behavioural findings implicate the neuropeptide S (NPS) system—acting through its G-protein-coupled NPS receptor (NPSR)—as a common regulator of these diverse phenotypes. This narrative review surveys studies published 2000–2024 in PubMed, Embase and Web of Science that examine NPS/NPSR involvement in core PTSD features and typical comorbidities. The functional rs324981 A/T polymorphism, which boosts NPSR surface expression and signalling, consistently associates with greater PTSD risk and symptom severity. In rodent models, exogenous NPS reduces anxiety- and fear-like behaviours, speeds fear-memory extinction, stabilises the hypothalamic-pituitary-adrenal axis, enhances dopaminergic tone and elevates hippocampal brain-derived neurotrophic factor (BDNF)—changes concordant with symptom relief. Additional work shows that NPS lessens pain affect, dampens alcohol and opioid intake, eases withdrawal-induced anxiety and lowers food consumption, hinting at a multimodal therapeutic profile. These effects converge on limbic and mid-brain circuits (amygdala, ventral tegmental area, locus coeruleus, paraventricular nucleus) and engage oxytocinergic, adenosinergic and endocannabinoid pathways. Translation remains limited by NPS's rapid degradation, poor blood–brain-barrier penetration and scarcity of brain-penetrant NPSR ligands, but advances in intranasal delivery, lipid-acylated analogues, biased NPSR agonists and "humanised" NPSR-variant models offer promising solutions. Collectively, current pre-clinical and genetic evidence positions the NPS–NPSR axis as a versatile therapeutic target for both core PTSD symptoms and their disabling comorbidities, warranting rigorous translational studies to refine mechanism, optimise drug-like properties and test clinical efficacy.

Keywords: Post-traumatic stress disorder, related neuropsychiatric disorders, neuropeptide S, neuropeptide S receptor, single nucleotide polymorphism.

INTRODUCTION

Post-traumatic stress disorder (PTSD), also known as delayed psychogenic reactions, refers to the psychological trauma resulting from serious threatening or catastrophic events, leading to delayed-onset and long-lasting mental disorders. PTSD has become a prevalent mental illness with the high incidence rates. The estimated prevalence of PTSD in the general population is approximately 6–9% [1, 2]. However, among individuals who have experienced severe trauma, such as combat veterans, refugees, victims of assault, and those exposed to pandemic-related stressors, the prevalence can increase to approximately 25%. During the COVID-19 pandemic, it has been reported that 15.8%-35.6% of the general population had PTSD in the United States [3], while 10.7%-23.5% frontline healthcare workers developed PTSD [4, 5].

Classic symptoms of PTSD primarily include the re-experiencing trauma, avoidance, negative emotions and thoughts, and hyperarousal. It should be noted that individuals exposed to complex traumatic events are not only at risk of developing PTSD but often present with comorbid conditions such as depression[6], anxiety[7], insomnia[8], cognitive impairment[9], pain[10], substance abuse[11], and eating disorders[12]. Epidemiological studies suggest that approximately 80% of people with PTSD meet criteria for at least one other psychiatric diagnosis, such as depression and substance abuse[13]. As a result, treating PTSD is particularly challenging, especially in the presence of other comorbidities.

In recent years there has been significant attention on the regulatory role of an endogenous substance called neuropeptide S (NPS) in the central nervous system [14]. NPS, a polypeptide made up of 20 amino acids, has a primary structure that is highly conserved throughout vertebrates. It is named after serine due to its presence as the amino-terminal residue in NPS across species[15]. The NPS receptor (NPSR), previously known as GPR154, is a double-coupled receptor of Gs and Gq proteins. NPSR binds to NPS, leading to the intracellular accumulation of cyclic adenosine monophosphate (cAMP) and the release of intracellular Ca^{2+}, thereby activating the downstream mitogen-activated protein kinase signaling pathway[16]. The NPSR gene harbors an A/T single nucleotide polymorphism (SNP rs324981) at the 7P14 locus of chromosome 7, resulting in amino acid exchange (Asn107Ile) [17]. This mutation increases cell-surface expression of the NPSR, making NPSR Ile107 approximately ten-fold more potent effect compared to NPSR Asn107 without altering binding affinity[18, 19]. Haxhibeqiri et al.[20] found that the risk of PTSD was

significantly higher in homozygous T allele carriers compared to homozygous A allele carriers among the population of the Balkan war. In addition, NPSR rs324981 impacts the stress levels[21], incidence of anxiety disorder[22, 23], alcohol use disorders[24], cognition impairment[25], which are the comorbidity symptoms in the PTSD. Moreover, accumulating studies found NPS has the potential for alleviating PTSD-like behaviors, exerting anxiolytic effects[26], diminishing fear-related responses[27], improving learning and memory deficits[28], and regulating substance use disorders[29-31] (Table 1). This evidence suggested NPS-NPSR system may not only involve in the regulation of PTSD, but also its related neuropsychiatric disorders.

This review synthesizes evidence on the role of NPS-NPSR system in: (i) core PTSD symptoms, (ii) PTSD-associated neuropsychiatric syndromes, ultimately evaluating its potential as a multimodal therapeutic agent. This review synthesizes findings from studies published between 2000 and 2024, identified through systematic searches in PubMed, Embase, and Web of Science. The keywords used include “PTSD”, “comorbidity”, “animal models”, “neuropeptide S”, “neuropeptide S receptor”, “single nucleotide polymorphism”, “anxiety”, “substance use disorder”, “pain”, “food intake”, “norepinephrine”, “dopamine”, and “hypothalamic-pituitary-adrenal”. We prioritized English-language original research articles, reviews, and meta-analyses, with a focus on studies exploring the role of NPS in modulating PTSD and related neuropsychiatric disorders. Studies irrelevant to the scope or with significant methodological limitations were excluded.

Role of NPS-NPSR system in PTSD

Anxiety, conditioned fear responses, fear memory disorders, and severe stress reactions are the primary features of PTSD[32, 33]. A growing body of literature emphasizes the role of the NPS system in regulating stress, mood, cognitive function, anxiety, and multiple studies have shown that the amygdala, plays a crucial role in PTSD[34]. Neuroimaging studies on patients with PTSD have identified increased activation in the amygdala[35]. Exposure to predator-scent stress (PSS) induces PTSD-like behaviors in rats. Microinjection of NPS into the basolateral amygdala (BLA) has been shown to alleviate anxiety, diminish the freezing response, and restore the diminished expression of brain-derived neurotrophic factor (BDNF) and NPY-Y1 receptor in hippocampus in this model[36]. Ten days after experiencing acute immobilization stress, the mice displayed a significant increase of PTSD-like behaviors as anxious behavior and fear responsiveness[37]. This could be reduced by injecting NPS into

the lateral amygdala (LA), but was exacerbated by the administration of NPSR antagonists[38]. Jiang et al.[39] observed a significant reduction in NPSR mRNA levels in the hypothalamus of mice exposed to a single prolonged stress model (SPS), while NPS mRNA in this model of PTSD was significantly greater than that in the control group. In addition, the repeated social defeat stress model (R-SDS) serves as an animal model for studying PTSD[40]. In this model, intracerebroventricular (i.c.v.) infusion of NPS (1 nmol) reduced anxiety levels and aggressive behavior in the rats with low anxiety levels and high anxiety levels[41]. Furthermore, in the resident-intruder test, another model for PTSD-like behavior[37], resident mice with NPSR knockout attacked invader mice for longer durations than WT mice. NPS (0.01 nmol - 1 nmol, i.c.v.) reduced the number of attacks and total time spent by resident mice[42]. These findings suggest that the NPS system plays a significant role in modulating the PTSD-like behaviors.

Possible mechanisms of NPS in PTSD

Norepinephrine (NE) is implicated in the body's fear and anxiety response. The hyperawareness and irritability symptoms observed in PTSD patients are associated with increased NE activity[43]. Furthermore, patients with PTSD exhibit elevated cortisol and NE responses to stress[44, 45]. In the clinic, α1 receptor antagonist prazosin or β receptor antagonist propranolol had the potential to treat PTSD[43],[46]. Animal studies indicated that a single injection of isoproterenol, a nonselective β-adrenergic agonist, into the amygdala can enhance the reconsolidation of fear memories, suggesting that increased NE during fear memory may contribute to the persistence of traumatic memories[47]. NE has been shown to regulate the cAMP/PKA and CaMKII/PKC signaling pathways through the activation of β-adrenoceptors, which can lead to PTSD-like memory impairments[47]. Giustino et al.[48] found that stress or locus coeruleus (LC)-NE activation induces deficits in fear memory extinction. This effect is most likely mediated by stress-induced increases in BLA activity, and intra-BLA administration of propranolol blocks the extinction deficits induced by LC-NE activation. NE neurons in the LC have been found to express at least 19 neuropeptide transcripts[49]. NPS can interact with various neuromodulators, including NE[50]. It has been reported that NPS inhibits the NE release. Raiteri et al.[51] discovered that NPS selectively inhibits the release of NE through its action on noradrenergic nerve terminals within the frontal cortex. These findings suggest that NPS may reduce the activity of noradrenergic neurons by suppressing NE release, potentially leading to the alleviation of fear memory reconsolidation and extinction.

Dopamine (DA), the most prevalent catecholamine neurotransmitter in the brain, it contributes to the regulation of a wide range of physiological activities such as reward, motivation, exercise, and emotion. Previous reports indicate that dopaminergic dysfunction may directly contribute to fear extinction, learning and memory impairment in PTSD patients[52]. Animal studies have demonstrated decreased DA levels in the medial prefrontal cortex (mPFC) and BLA of rats following exposure to traumatic stimuli for 18 days using the SPS model[53]. Moreover, D2 receptors in the mPFC and BLA have been implicated in fear extinction, as decreased receptor levels have been observed in rats during the fear extinction process[54]. It has also been observed that the D1 receptor knockout mouse model did not exhibit fear memory in fear-conditioned response experiments, indicating the engagement of the dopaminergic system during fear learning and extinction[55]. Clinical studies have found that the DA receptor agonist kb220z can significantly relieve nightmare symptoms in PTSD patients[56]. Preclinical studies have also demonstrated that DA receptor D2/D3 agonists such as rotigotine and pramipexole can attenuate PTSD-like symptoms in models[57]. NPS has been reported to increase the release of DA. NPS (i.c.v.) enhances DA release in the rat mPFC in a dose-dependent manner[58]. Furthermore, microinjection of NPS into the ventral tegmental area (VTA) of rats has been shown to increase locomotor activity and the metabolites of DA in the nucleus accumbens (NAc)[59]. Although there is no study on the mechanisms of NPS in PTSD so far, the above findings suggest that NPS may target the DA system to potentially exert an anti-PTSD effect by increasing DA levels.

Hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system responsible for regulating the body's stress response[60]. Neuroendocrine hormones involved in the HPA axis include corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol[61]. The HPA axis has been reported to be closely associated with PTSD[62]. Multiple studies have highlighted the dysregulation of the HPA axis in individuals with PTSD. This dysregulation is characterized by an increased sensitivity of the HPA feedback system, lowered cortisol levels, and decreased urinary cortisol excretion, leading to symptoms such as fatigue and mood disturbances[63]. Animal research has shown that on the 18th day post-exposure to stress in the rat PSS model, there is a reduction in the basal corticosterone pulse amplitude and a dampening of the corticosterone response to stressors, suggesting that an impairment in the corticosterone response could potentially act as a susceptibility or risk factor in the development of PTSD[64]. Corticosterone administered to rats one hour after experiencing trauma stimulation diminishes the retrieval of fear memories and facilitate the

extinction of fear memories in PTSD animal model [65, 66]. Elevated in vivo release of NPS in the amygdala has been documented following local depolarization and emotional stress[67]. Administration of NPS triggers activation of the HPA axis, leading to the release of corticotropin-releasing hormone, adrenocorticotropic hormone, and corticosterone[68]. This cascade of events may play a role in the modulation of stress and anxiety by NPS[68, 69].These findings indicate that modulating the function of the HPA axis via NPS could become a potential target for PTSD treatment.

BDNF is the most abundant neurotrophic factor in the body. Upon binding to its specific receptor Tropomyosin-related kinase receptor B (TrkB), BDNF is involved in the modulation of synaptic plasticity, neuronal survival, and apoptosis[70]. Disruptions in the BDNF-TrkB signaling pathway could potentially have a notable impact in PTSD[71]. In the hippocampus, impaired conditional-fear learning and extinction have been associated with the absence of BDNF-TrkB signals in regions such as the ventromedial prefrontal cortex, anterior cingulate cortex, and NAc[72]. Clinical studies have revealed a marked reduction in the levels of BDNF in the blood of PTSD patients compared to healthy individuals, suggesting its potential as a diagnostic biomarker for PTSD[73]. In the SPS rat model, a noteworthy decline in cerebrospinal fluid BDNF levels has been documented[74]. Furthermore, administration of BDNF (1 mmol, 10 mmol, 30 mmol, i.c.v.) in this model has been shown to dose-dependently increase the time spent in the central area relative to the total time in the open field test [74]. Additionally, investigations have revealed that BDNF expression is diminished following PSS exposure but is augmented after NPS microinjection into BLA[36], suggesting that NPS may also regulate neuronal plasticity and other functions through regulation of BDNF.

Role of NPS-NPSR system in the neuropsychiatric disorders related to PTSD

Role of NPS-NPSR system in anxiety disorder and implications for PTSD treatment

PTSD has a lifetime prevalence and shares neurobiological features with anxiety disorders[7]. The 5-year recurrence rate for PTSD was 9.2% among anxiety and depression disorders[75], and comorbidity of PTSD and social anxiety disorder (SAD) is ranging from 14.8% to 46%[76]. Research has found that plasma NPS levels are significantly elevated in individuals with generalized anxiety disorder (GAD) compared to healthy controls. This indicates an association between plasma NPS levels and anxiety symptoms in GAD patients, suggesting

plasma NPS may serve as a candidate biomarker for discriminating GAD[69]. Various experimental paradigms have been employed to examine the impact of NPS on anxiety. NPS (1 nmol, i.c.v.) has been shown to exhibit anxiolytic effects in tests such as the open field test, elevated plus maze, marble-bury test, four-plate test, elevated zero maze, and stress-induced hyperthermia[15, 77]. Notably, bilateral microinjection of NPS (1 nmol, i.c.v.) into the amygdala of mice significantly reduced the anxiety behaviors in both open field test and elevated plus maze experiments, suggesting an anxiolytic effect of NPS[78]. Conversely, NPS receptor antagonist SHA68 by injection into the amygdala of mice significantly reduced the anxiolytic effects of NPS[15, 78]. Xie et al. found that administering NPS (1 nmol, i.c.v.) can alleviate anxiety-like behavior induced by paradoxical sleep deprivation. This treatment significantly increased the expression levels of NPSR mRNA and the number of Fos immunoreactive neurons in the BLA, central amygdala, and medial amygdala (MeA) [79]. In the terms of the anxiolytic mechanisms of NPS, researchers have discovered the effect of NPS is associated with oxytocin (OXT) neurons activity within the paraventricular nucleus (PVN). Grund et al. [80, 81] found NPS activates a specific subset of OXT neurons in the PVN by acting on the NPSR. This activation leads to the local release of oxytocin and a transient increase in intracellular calcium concentration in OXT neuronal subgroups, resulting in an anti-anxiety effect. Pharmacological blockade of the local OXTR, as well as silencing OXT neurons using chemogenetic techniques, hindered the anti-anxiety responses induced by NPS. Furthermore, NPS affects synaptic events in brain regions such as the dorsal raphe nucleus (DRN) and lateral dorsal tegmental (LDT), influencing the excitability of neurons in these regions and contributing to the anxiolytic and arousal-promoting effects of NPS[82].

As the target of NPS, the activity of NPSR is influenced by environmental and physiological states, including stress stimuli. Several studies have reported that the T allele in NPSR rs324981 is associated with enhanced anxiety sensitivity, and enhanced fear response[83-85]. Subjects with the NPSR A allele, especially women, were more sensitive to family relationships and were more likely to develop mood anxiety disorder in adverse family circumstances. This suggests that the effects of NPSR variants are influenced by their environmental interactions and are specific to gender[86-88].

Researchers have used NPSR knockout mice to investigate the biological role of the NPS-NPSR system. Liu et al.[89] used NPS-/- /NPSEGFP double transgenic mice by breeding with C57BL/6J females found that NPSR (-/-) mice scored lower in exploring the central area

of the open field or the open arm region, suggesting that NPSR deletion elevated the anxiety behaviors. DuangDao et al. [90] generated NPSR (-/-) mice based on 129S6/SvEvTac background mice, and found that NPS (0.1 nmol, i.c.v.) significantly increased spontaneous activity in WT mice while had no effect in NPSR (-/-) mice. Furthermore, the NPSR (-/-) mice showed a remarkably prolonged latent period from the dark side to the light box in the dark/light box experiment. Ruzza et al.[91] utilized NPSR (+/+)/NPSR (-/-) with CD-1 genetic background mice and found NPS (1 nmol, i.c.v.) displayed anxiolytic and anticonvulsant effects in NPSR (+/+) mice, but not in NPSR (-/-) mice.

Role of NPS-NPSR system in pain and implications for PTSD treatment

Pain is commonly defined as an unpleasant sensation associated with actual or potential tissue damage, and individuals experiencing pain may also face negative emotions such as anxiety and fear, often referred to as pain emotion[92]. Patients diagnosed with PTSD frequently struggle with pain-related issues, especially pain-related anxiety. Studies suggest that chronic pain and PTSD can interact, intensifying each other and potentially worsening symptoms of both diseases[93]. In terms of the mechanisms of NPS on pain regulation, it was observed that NPS promoted antinociceptive behavior in rats by activating NPSR and its downstream phosphorylation of ERK1/2 in a subpopulation of pyramidal- neurons located in the medial amygdala, and inhibition of hyperpolarization-activated cyclic nucleotide-gated channel currents in the rat amygdala[94]. The medial amygdala integrates fear memory consolidation (a hallmark of PTSD) and the affective dimension of pain[95]. Thus, NPS-mediated regulation of medial amygdala pyramidal neurons—by modulating pain processing—may contribute to the alleviation of PTSD-related symptoms. Furthermore, NPS can modulate the output of the amygdala and influence pain-related affective behaviors by enhancing the postsynaptic activity of a specific cluster of inhibitory intercalated cells in a protein kinase A (PKA)-dependent manner [96]. In PTSD, the amygdala's inhibitory intercalated cells are functionally impaired[97]. NPS-mediated enhancement of these cells' activity may not only mitigate pain-related anxiety but may also normalize amygdala overactivation in PTSD—suggesting a dual role in alleviating both comorbid symptoms. NPS also promoted the effect of electroacupuncture in suppressing the pain and pain-associated anxiety-like behaviors, and the mechanism of the function could be associated to the enhanced expression of the NPS/NPSR system in the anterior cingulate cortex (ACC)[98]. In the formalin assay of mice, Holanda et al. [99] found that SCH 23390 (selective dopamine D1 antagonist, 0.05 mg/kg, i.p.) slightly blocked the analgesic effects of NPS in the second

phase, while haloperidol (a nonselective dopamine D2-like receptor antagonist, 0.03 mg/kg, i.p.) inhibited the NPS-induced antinociceptive effects in both phases. These findings suggest that the action of NPS in inhibiting formalin-induced nociceptive responses involves D1 and D2 receptors, with a predominant role of D2 receptors. Previous studies have implicated dopamine D2 receptors in the reward deficiency and hypervigilance observed in PTSD[100, 101]. Consequently, NPS-mediated regulation of D2 receptors may coordinate pain relief with the alleviation of PTSD-related symptoms. Moreover, NPS (10 nmol, i.c.v.) produces analgesic effects in the hot plate test, and this effect is mediated by LC- noradrenergic activity, as evidenced by the correlation between NPS-induced hot plate latency (%MPE) and cerebral cortex noradrenaline content (primarily from the LC) [102] . Thus, NPS may interact with noradrenergic neuronal activity in the LC to coordinately modulate pain alleviation. In addition, the adenosine A2A receptor antagonist ZM241385 (0.01 nmol, i.c.v.) blocked the analgesic effects of NPS in the formalin test, while the adenosine A1 receptor antagonist DPCPX (0.001 nmol, i.c.v.) blocked the effects of NPS only in the first phase. The results suggested that central antinociceptive effects evoked by NPS is through activation of A1A and A2A receptors in the first phase of the formalin test and through A2A receptor in the second phase [103]. NPS (0.3 nmol and 1nmol, i.c.v.) induced antinociception in a restraint stress-induced analgesia mouse model. This effect was found to be reversible by intra-vlPAG microinjection of antagonists targeting OX1 receptors (OX1Rs), NK1 receptors (NK1Rs), mGlu5 receptors (mGlu5Rs), and CB1 receptors (CB1Rs), suggesting that NPS exerts analgesic effects in stress-induced analgesia by activating a sequential cascade mediated by OX1R-NK1R-mGlu5R-CB1R during the stress response in PTSD [104]. While direct evidence currently remains lacking to confirm that the NPS-NPSR system modulates PTSD-induced chronic pain, the established role and mechanisms of NPS in pain alleviation suggest that NPSR could serve as a promising therapeutic target for mitigating both chronic pain and associated anxiety symptoms stemming from PTSD.

Role of NPS-NPSR system in substances use disorder and implications for PTSD treatment

There is a close relationship between PTSD and substance use disorders, as PTSD symptoms have been shown to increase the likelihood of alcohol and drug use in the general population [105]. An interesting aspect is that individuals often resort to drinking alcohol to cope with PTSD symptoms, which is associated with their expectations and motivations related to alcohol consumption. It is worth noting that there is a positive correlation between the severity of PTSD and dangerous drinking[106]. It has been demonstrated that the NPS/NPSR

system plays a role in modulating various stages of drug addiction, especially withdrawal and relapse; however, the impact on distinct addictive substances varies significantly. Badia-Elder et al.[107] found that the infusion of NPS（0.075, 0.3, 1.2 nmol, i.c.v.）before the testing reduced 2-hour ethanol intake in the alcohol-preferred rat strain, while having no effect in a non-preferred control strain. Similarly, Cannella et al.[108] also revealed that the injection of NPS (0.1, 0.5, 1.0 or 2.0 nmol/rat, i.c.v.) 5 min prior to the alcohol self-administration session decreased alcohol self-administration in alcohol-preferring rats but not in non alcohol-preferring rats. Notably, the administration of NPS (1.0, 2.0, or 4.0 nmol, i.c.v.) [109] prior to behavioral testing significantly enhanced cue-induced reinstatement of alcohol-seeking behavior, manifesting as increased alcohol recovery, craving, and relapse-like responses. These effects may be mediated by the activation of the Hcrt-1/Ox-A system, as the effect was blocked by hypocretin-1/orexin-A antagonist SB-334867[109, 110]. Laas et al. [24] found the SNP 324981 in NPSR is associated with alcohol use disorder (AUD) and alcohol consumption in a sex, environment, and age -dependent manner. Females carrying the A allele exhibited higher rates of AUD and harmful drinking, while males carrying the T allele showed a higher percentage of alcohol consumption and incidence of AUD at age of 15-18 years.

Opioids such as morphine have potent sedative and analgesic effects but are prone to strong addictive side-effects[29]. In conditioned place preference (CPP) experiment, chronic treatment of morphine developed a significant high score of CPP in the mice. Li et al. [111] found that the administration of NPS (0.3-10 nmol, i.c.v.) alone did not produce a preference or aversive response in the mice, but NPS treatment along with morphine reduced the morphine-induced CPP acquisition and expression. Additionally, NPS (1.0 nmol, i.c.v.) could effectively reduce the anxiety-like behaviors in morphine-abstinent rats. Following acute withdrawal (12 h) and protracted withdrawal (7 days) from morphine, NPSR expression increased in VTA and BLA, while it decreased in the bed nucleus of the stria terminalis (BNST) seven days after morphine withdrawal [29]. Consequently, the researchers postulated that the NPS-NPSR system could play a role in regulating morphine dependence behaviors.

Cocaine is a stimulant drug. In contrary to the effect of NPS on inhibiting morphine dependence, the activation of NPSR by NPS increased cocaine-induced seeking behavior[112] and motor excitability[113]. Chou et al. [114] discovered that NPS produced an increase in restoring cocaine-induced reinstatement of CPP in mice while blocking NPSR reduced restraint stress-induced cocaine CPP. The study also revealed the effect of NPS in cocaine

seeking and relapse is related the activation of orexin neurons in the lateral hypothalamus (LH) and orexins release in the VTA, and the OXRs1 and CBR1-mediated signaling were involved in the function of NPS [114]. Kallupi et al. [112, 115] found that NPS activated Hcrt-1/Ox-A neurons in the LH and the perifornical area (PeF), which may project to the VTA and other mesolimbic regions to promote cocaine-seeking behavior in rats. Intra-LH and intra-PeF administration of NPS increased conditioned reinstatement of cocaine seeking, while NPSR antagonists NPSR-QAA1 and [D-Cys(Tbut)5] NPS significantly reduced cue-induced cocaine-seeking behavior.

Role of NPS-NPSR system in cognitive impairment and implications for PTSD treatment

PTSD is closely associated with cognitive impairment, characterized by attention deficits, executive function impairments and memory deficits. These impairments are linked to fear learning and extinction processes[116]. Numerous studies have demonstrated that individuals with PTSD showed an exaggerated response to fear memory, often accompanied with attention and memory deficits.

The NPS-NPSR system is increasingly recognized as a modulator of cognitive processes. For instance, NPSR or NPS knockout mice displayed reduced prepulse inhibition (PPI) [117]. A phenomenon potentially attributed to attention deficits, a deficit linked to attentional impairments that mirrors the attentional hypervigilance of PTSD[118]. Furthermore, genetic variation in NPSR (T allele of rs324981) in humans had higher total scores of attention deficit hyperactivity disorder (ADHD) symptoms, and homozygous individuals exhibited greater sensitivity to environmental factors such as stress and anxiety compared to heterozygous individuals[88]. The frontal cortex of spontaneously hypertensive rats (SHR), a model of ADHD, exhibited significantly reduced NPS levels and higher activity of dopamine uptake than its control strain [119].

Intraventricular injection of NPS in mice by a dose-dependent manner enhanced long-term memory tested in inhibitory avoidance and novel object recognition[120], and spatial learning and memory in the Morris water maze experiment[121]. Nasal administration of neuropeptide S in rats displayed the improved effect in object memory in object discrimination paradigms [122] and promoted cognitive flexibility in the reversal learning test of T-Maze [123]. Pretreatment of NPSR antagonist SHA68 could reduce the memory-enhancing effect of NPS [89], [120]. Wang et al. [124]. found that endogenous NPS may be a key neural regulator of olfactory spatial memory. By administering of [D-Val5] NPS (20 nmol, i.c.v.) and SHA 68

(10 and 50 mg/kg, i.p.), the NPSR antagonists, a significant reduction in olfactory spatial memory behavior was observed, along with a decrease in the percentage of c-Fos and NPSR immunoreactive neurons in the anterior olfactory nucleus, piriform cortex, subiculum, presubiculum, and parasubiculum [124]. NPS was also proved to reverse the memory impairments induced by MK801, a selective NMDA receptor antagonist, and scopolamine, a muscarinic cholinergic receptor antagonist [125]. Shao et al. [126] revealed that NPS reduced scopolamine and MK801-induced impairment of olfactory spatial memory in a computer-assisted 4-hole-board spatial memory test by selectively activating NPSR-containing neurons in the subiculum. Research indicates that PTSD is associated with dysregulation of the PFC[127]. Neuropeptide modulation of cortical circuits can alter PFC processing of cognitive and affective behaviors, potentially alleviating PTSD-induced cognitive impairments[128]. The cognitive impairment caused by sleep restriction was mitigated by NPS (0.1 nmol, i.c.v.), possibly through its ability to counteract the effects of sleep restriction by enhancing activation in the PFC [129]. NPS (0.1 nmol, i.c.v.) in mice by a dose-dependent manner restored Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced learning and memory reduction in the radial arm maze task, which may be attributable to potentiation of glutamatergic synaptic transmission by NPS[130]. These studies collectively suggest the potential therapeutic effects of the NPS-NPSR system in regulating memory disorder. While direct evidence that the NPS-NPSR system modulates PTSD-induced cognitive impairments remains lacking, the well-established role of NPS in enhancing attentional processes and facilitating learning and memory suggests that NPSR could represent a potential target for mitigating cognitive impairment symptoms linked to PTSD.

Role of NPS-NPSR system in food intake and implications for PTSD treatment

The prevalence of comorbid PTSD in patients with eating disorders ranges from 9 to 24%. Research suggests that the presence of comorbid PTSD is associated with more severe symptoms of eating disorders[131, 132]. Specifically, PTSD is strongly correlated with obesity, affecting approximately 5.8% of U.S. veterans who struggle with both conditions[133]. This association leads to significant psychiatric problems and even suicidal tendencies.

Regarding the effect of NPS on food intake, a study conducted by Peng et al. [134] indicated that NPS can serve as a novel anorexic agent by inhibiting food intake in fasting rats without affecting the level of NPY and insulin. Additionally, another study indicated that NPS

decreases the standard food intake in both food-restricted and freely fed rats. Moreover, NPSR antagonists have been shown to block the anorexic effect of NPS[135]. Research suggests the PVN in rats as a brain area where NPS plays a role in inhibiting the food intake[136].

CONCLUSION

NPS-NPSR system plays a potential impact on PTSD and its associated disorder, including anxiety, fear, learning and memory, substances use disorder, eating disorders and pain (Table 1). These effects are mediated by various brain regions (Figure 1) and mechanisms (Figure 2). Based on current preclinical evidence from animal studies, NPSR may serve as a potential target for modulating PTSD responses, offering novel insights into the neural mechanisms underlying PTSD.

However, since the regulatory effects of NPS on neuropsychiatric disorders are exclusively derived from evidence in animal experiments, it remains uncertain whether NPS exerts regulatory effects on PTSD in humans. Therefore, it is necessary to employ animal models with higher validity that can reflect PTSD and its comorbidities to investigate the role of NPS. Concurrently, further research is required to elucidate the mechanisms underlying the actions of NPS and NPSR in PTSD and its comorbidities, such as the molecular and neural circuit mechanisms through which NPSR modulates dopamine, norepinephrine, and the hypothalamic-pituitary-adrenal (HPA) axis in PTSD models.

Limited tools have hindered understanding of the NPS pathway. In recent years, however, techniques such as fluorescent neuromodulator sensors, electrochemical sensors, and in vivo microdialysis coupled with mass spectrometry enable real-time monitoring of fluctuations in neuropeptide release presence. Similarly, tools like photoactivatable neuropeptides and genetically encoded biosensors derived from specific nanobodies provided precise spatial and temporal resolution of GPCR activation and inactivation[128], The development of these technologies is accelerating research progress into targeting the NPS-NPSR system for PTSD therapeutic applications.

In addition, regarding NPS, its clinical application is limited by poor metabolic stability and the inability to cross the blood-brain barrier (BBB). Researchers have explored alternative delivery methods, such as nasal administration instead of ventricular injection[137], and developed lipid acylation-modified NPS-palmitic acid self-assembled coupling formulations,

which demonstrate effective penetration ability and potential pharmacological effects[138]. Thus, further efforts are warranted to develop optimized delivery systems for NPS. Furthermore, it is imperative to conduct further research targeting NPSR to develop NPSR agonists and antagonists capable of crossing the BBB. In recent years, researchers have rationally designed NPSR agonists exhibiting biased signaling properties. RTI-263, a biased NPSR agonist, demonstrates NPS-like anxiogenic-like effects and memory-enhancing effects in preclinical models. Crucially, and distinct from NPS, RTI-263 significantly attenuates cue-induced reinstatement of cocaine seeking in rats[139]. Thus, the functional benefits conferred by biased NPSR agonists provide a direction for targeting NPSR in the therapeutic development for PTSD.

Additionally, SNP rs324981 play a crucial role in the regulatory effects of the NPS-NPSR system. Therefore, utilizing an NPSR mutant "humanized" animal model is essential to explore the biological and pharmacological effects of NPS in PTSD[140, 141]. This approach could provide valuable insights for developing precise therapeutic strategies for PTSD based on the modulation of the NPS-NPSR system.

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REFERENCES

[1]Kilpatrick DG, Ruggiero KJ, Acierno R, Saunders BE, Resnick HS, Best CL. Violence and risk of PTSD, major depression, substance abuse/dependence, and comorbidity: results from the National Survey of Adolescents. J Consult Clin Psychol 2003;71(4):692-700.

[2]Atwoli L, Stein DJ, Koenen KC, McLaughlin KA. Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. Curr Opin Psychiatry 2015;28(4):307-11.

[3]Hong S, Kim H, Park MK. Impact of COVID-19 on post-traumatic stress symptoms in the general population: An integrative review. Int J Ment Health Nurs 2021;30(4):834-46.

[4]Ouyang H, Geng S, Zhou Y, Wang J, Zhan J, Shang Z, et al. The increase of PTSD in front-line health care workers during the COVID-19 pandemic and the mediating role of risk perception: a one-year follow-up study. Transl Psychiatry 2022;12(1):180.

[5]Riaz BK, Islam MZ, Ahmed HU, Akhtar K, Haque A, Amin KMB, et al. Post-traumatic stress disorders and coping strategies of health professionals during COVID-19 pandemic in Bangladesh: findings of a countrywide cross-sectional study. Lancet Reg Health Southeast Asia 2023;11:100131.

[6]Kline AC, Cooper AA, Rytwinski NK, Feeny NC. The Effect of Concurrent Depression on PTSD Outcomes in Trauma-Focused Psychotherapy: A Meta-Analysis of Randomized Controlled Trials. Behav Ther 2021;52(1):250-66.

[7]Williamson JB, Jaffee MS, Jorge RE. Posttraumatic Stress Disorder and Anxiety-Related Conditions. Continuum (Minneap Minn) 2021;27(6):1738-63.

[8]Colvonen PJ, Straus LD, Stepnowsky C, McCarthy MJ, Goldstein LA, Norman SB. Recent Advancements in Treating Sleep Disorders in Co-Occurring PTSD. Curr Psychiatry Rep 2018;20(7):48.

[9]Alves de Araujo Junior D, Sair HI, Peters ME, Carvalho AF, Yedavalli V, Solnes LB, et al. The association between post-traumatic stress disorder (PTSD) and cognitive impairment: A systematic review of neuroimaging findings. J Psychiatr Res 2023;164:259-69.

[10]Brennstuhl MJ, Tarquinio C, Montel S. Chronic Pain and PTSD: Evolving Views on Their Comorbidity. Perspect Psychiatr Care 2015;51(4):295-304.

[11]Subica AM, Claypoole KH, Wylie AM. PTSD'S mediation of the relationships between trauma, depression, substance abuse, mental health, and physical health in individuals with severe mental illness: evaluating a comprehensive model. Schizophr Res 2012;136(1-3):104-9.

[12]Brewerton TD, Perlman MM, Gavidia I, Suro G, Jahraus J. Headache, eating disorders, PTSD, and comorbidity: implications for assessment and treatment. Eat Weight Disord 2022;27(7):2693-700.
[13]Brady KT. Posttraumatic stress disorder and comorbidity: recognizing the many faces of PTSD. J Clin Psychiatry 1997;58 Suppl 9:12-5.
[14]Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ, van IMH. Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. Psychol Bull 2007;133(1):1-24.
[15]Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004;43(4):487-97.
[16]Liao Y, Lu B, Ma Q, Wu G, Lai X, Zang J, et al. Human Neuropeptide S Receptor Is Activated via a Gαq Protein-biased Signaling Cascade by a Human Neuropeptide S Analog Lacking the C-terminal 10 Residues. J Biol Chem 2016;291(14):7505-16.
[17]Tobinski AM, Rappeneau V. Role of the Neuropeptide S System in Emotionality, Stress Responsiveness and Addiction-Like Behaviours in Rodents: Relevance to Stress-Related Disorders. Pharmaceuticals (Basel) 2021;14(8).
[18]Reinscheid RK, Xu YL, Okamura N, Zeng J, Chung S, Pai R, et al. Pharmacological characterization of human and murine neuropeptide s receptor variants. J Pharmacol Exp Ther 2005;315(3):1338-45.
[19]Bernier V, Stocco R, Bogusky MJ, Joyce JG, Parachoniak C, Grenier K, et al. Structure-function relationships in the neuropeptide S receptor: molecular consequences of the asthma-associated mutation N107I. J Biol Chem 2006;281(34):24704-12.
[20]Haxhibeqiri S, Haxhibeqiri V, Agani F, Goci Uka A, Hoxha B, Dzubur Kulenovic A, et al. Association of Neuropeptide S Receptor 1 and Glutamate Decarboxylase 1 Gene Polymorphisms with Posttraumatic Stress Disorder. Psychiatr Danub 2019;31(2):249-55.
[21]Kumsta R, Chen FS, Pape HC, Heinrichs M. Neuropeptide S receptor gene is associated with cortisol responses to social stress in humans. Biol Psychol 2013;93(2):304-7.
[22]Klauke B, Deckert J, Zwanzger P, Baumann C, Arolt V, Pauli P, et al. Neuropeptide S receptor gene (NPSR) and life events: G × E effects on anxiety sensitivity and its subdimensions. World J Biol Psychiatry 2014;15(1):17-25.
[23]He Q, Shen Z, Ren L, Wang X, Qian M, Zhu J, et al. Association of NPSR1 rs324981 polymorphism and treatment response to antidepressants in Chinese Han population with generalized anxiety disorder. Biochem Biophys Res Commun 2018;504(1):137-42.

[24]Laas K, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, et al. Neuropeptide S receptor gene variant and environment: contribution to alcohol use disorders and alcohol consumption. Addict Biol 2015;20(3):605-16.
[25]Reinscheid RK. Phylogenetic appearance of neuropeptide S precursor proteins in tetrapods. Peptides 2007;28(4):830-7.
[26]Meis S, Bergado-Acosta JR, Yanagawa Y, Obata K, Stork O, Munsch T. Identification of a neuropeptide S responsive circuitry shaping amygdala activity via the endopiriform nucleus. PLoS One 2008;3(7):e2695.
[27]Ghazal P. The Physio-Pharmacological Role of the NPS/NPSR System in Psychiatric Disorders: A Translational Overview. Curr Protein Pept Sci 2016;17(4):380-97.
[28]Zhang ZR, Tao YX. Physiology, pharmacology, and pathophysiology of neuropeptide S receptor. Prog Mol Biol Transl Sci 2019;161:125-48.
[29]Ghazal P, Ciccocioppo R, Ubaldi M. Morphine dependence is associated with changes in neuropeptide S receptor expression and function in rat brain. Peptides 2013;46:6-12.
[30]Ruggeri B, Braconi S, Cannella N, Kallupi M, Soverchia L, Ciccocioppo R, et al. Neuropeptide S receptor gene expression in alcohol withdrawal and protracted abstinence in postdependent rats. Alcohol Clin Exp Res 2010;34(1):90-7.
[31]Lage R, Diéguez C, López M. Caffeine treatment regulates neuropeptide S system expression in the rat brain. Neurosci Lett 2006;410(1):47-51.
[32]Compean E, Hamner M. Posttraumatic stress disorder with secondary psychotic features (PTSD-SP): Diagnostic and treatment challenges. Prog Neuropsychopharmacol Biol Psychiatry 2019;88:265-75.
[33]Norrholm SD, Jovanovic T. Fear Processing, Psychophysiology, and PTSD. Harv Rev Psychiatry 2018;26(3):129-41.
[34]Rainnie DG, Bergeron R, Sajdyk TJ, Patil M, Gehlert DR, Shekhar A. Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. J Neurosci 2004;24(14):3471-9.
[35]Hinojosa CA, George GC, Ben-Zion Z. Neuroimaging of posttraumatic stress disorder in adults and youth: progress over the last decade on three leading questions of the field. Mol Psychiatry 2024;29(10):3223-44.
[36]Cohen H, Vainer E, Zeev K, Zohar J, Mathé AA. Neuropeptide S in the basolateral amygdala mediates an adaptive behavioral stress response in a rat model of posttraumatic stress disorder by increasing the expression of BDNF and the neuropeptide YY1 receptor. Eur Neuropsychopharmacol 2018;28(1):159-70.

[37]Verbitsky A, Dopfel D, Zhang N. Rodent models of post-traumatic stress disorder: behavioral assessment. Transl Psychiatry 2020;10(1):132.
[38]Chauveau F, Lange MD, Jüngling K, Lesting J, Seidenbecher T, Pape HC. Prevention of stress-impaired fear extinction through neuropeptide s action in the lateral amygdala. Neuropsychopharmacology 2012;37(7):1588-99.
[39]Jiang JH, Peng YL, Zhang PJ, Xue HX, He Z, Liang XY, et al. The ventromedial hypothalamic nucleus plays an important role in anxiolytic-like effect of neuropeptide S. Neuropeptides 2018;67:36-44.
[40]Hammamieh R, Chakraborty N, De Lima TC, Meyerhoff J, Gautam A, Muhie S, et al. Murine model of repeated exposures to conspecific trained aggressors simulates features of post-traumatic stress disorder. Behav Brain Res 2012;235(1):55-66.
[41]Beiderbeck DI, Lukas M, Neumann ID. Anti-aggressive effects of neuropeptide S independent of anxiolysis in male rats. Front Behav Neurosci 2014;8:185.
[42]Ruzza C, Asth L, Guerrini R, Trapella C, Gavioli EC. Neuropeptide S reduces mouse aggressiveness in the resident/intruder test through selective activation of the neuropeptide S receptor. Neuropharmacology 2015;97:1-6.
[43]Strawn JR, Geracioti TD, Jr. Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. Depress Anxiety 2008;25(3):260-71.
[44]Bremner JD. Traumatic stress: effects on the brain. Dialogues Clin Neurosci 2006;8(4):445-61.
[45]McCall A, Forouhandehpour R, Celebi S, Richard-Malenfant C, Hamati R, Guimond S, et al. Evidence for Locus Coeruleus-Norepinephrine System Abnormality in Military Posttraumatic Stress Disorder Revealed by Neuromelanin-Sensitive Magnetic Resonance Imaging. Biol Psychiatry 2024;96(4):268-77.
[46]Young C, Butcher R. CADTH Rapid Response Reports. Propranolol for Post-Traumatic Stress Disorder: A Review of Clinical Effectiveness. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health

[47]Liu XH, Zhu RT, Hao B, Shi YW, Wang XG, Xue L, et al. Norepinephrine Induces PTSD-Like Memory Impairments via Regulation of the β-Adrenoceptor-cAMP/PKA and CaMK II/PKC Systems in the Basolateral Amygdala. Front Behav Neurosci 2019;13:43.
[48]Giustino TF, Ramanathan KR, Totty MS, Miles OW, Maren S. Locus Coeruleus Norepinephrine Drives Stress-Induced Increases in Basolateral Amygdala Firing and Impairs Extinction Learning. J Neurosci 2020;40(4):907-16.

[49]Caramia M, Romanov RA, Sideromenos S, Hevesi Z, Zhao M, Krasniakova M, et al. Neuronal diversity of neuropeptide signaling, including galanin, in the mouse locus coeruleus. Proc Natl Acad Sci U S A 2023;120(31):e2222095120.
[50]Shirsath KR, Patil VK, Awathale SN, Goyal SN, Nakhate KT. Pathophysiological and therapeutic implications of neuropeptide S system in neurological disorders. Peptides 2024;175:171167.
[51]Raiteri L, Luccini E, Romei C, Salvadori S, Calò G. Neuropeptide S selectively inhibits the release of 5-HT and noradrenaline from mouse frontal cortex nerve endings. Br J Pharmacol 2009;157(3):474-81.
[52]Torrisi SA, Leggio GM, Drago F, Salomone S. Therapeutic Challenges of Post-traumatic Stress Disorder: Focus on the Dopaminergic System. Front Pharmacol 2019;10:404.
[53]Lin CC, Tung CS, Lin PH, Huang CL, Liu YP. Traumatic stress causes distinctive effects on fear circuit catecholamines and the fear extinction profile in a rodent model of posttraumatic stress disorder. Eur Neuropsychopharmacol 2016;26(9):1484-95.
[54]Madsen HB, Guerin AA, Kim JH. Investigating the role of dopamine receptor- and parvalbumin-expressing cells in extinction of conditioned fear. Neurobiol Learn Mem 2017;145:7-17.
[55]Zweifel LS, Fadok JP, Argilli E, Garelick MG, Jones GL, Dickerson TM, et al. Activation of dopamine neurons is critical for aversive conditioning and prevention of generalized anxiety. Nat Neurosci 2011;14(5):620-6.
[56]McLaughlin T, Blum K, Oscar-Berman M, Febo M, Agan G, Fratantonio JL, et al. Putative dopamine agonist (KB220Z) attenuates lucid nightmares in PTSD patients: role of enhanced brain reward functional connectivity and homeostasis redeeming joy. J Behav Addict 2015;4(2):106-15.
[57]Malikowska-Racia N, Sałat K, Nowaczyk A, Fijałkowski Ł, Popik P. Dopamine D2/D3 receptor agonists attenuate PTSD-like symptoms in mice exposed to single prolonged stress. Neuropharmacology 2019;155:1-9.
[58]Si W, Aluisio L, Okamura N, Clark SD, Fraser I, Sutton SW, et al. Neuropeptide S stimulates dopaminergic neurotransmission in the medial prefrontal cortex. J Neurochem 2010;115(2):475-82.
[59]Mochizuki T, Kim J, Sasaki K. Microinjection of neuropeptide S into the rat ventral tegmental area induces hyperactivity and increases extracellular levels of dopamine metabolites in the nucleus accumbens shell. Peptides 2010;31(5):926-31.

[60]Juruena MF, Eror F, Cleare AJ, Young AH. The Role of Early Life Stress in HPA Axis and Anxiety. Adv Exp Med Biol 2020;1191:141-53.
[61]Dallman MF. Stress update Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. Trends Endocrinol Metab 1993;4(2):62-9.
[62]Almeida FB, Pinna G, Barros HMT. The Role of HPA Axis and Allopregnanolone on the Neurobiology of Major Depressive Disorders and PTSD. Int J Mol Sci 2021;22(11).
[63]Yehuda R, Giller EL, Southwick SM, Lowy MT, Mason JW. Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. Biol Psychiatry 1991;30(10):1031-48.
[64]Danan D, Matar MA, Kaplan Z, Zohar J, Cohen H. Blunted basal corticosterone pulsatility predicts post-exposure susceptibility to PTSD phenotype in rats. Psychoneuroendocrinology 2018;87:35-42.
[65]Vafaei AA, Nasrollahi N, Kashefi A, Raise-Abdullahi P, Rashidy-Pour A. Corticosterone injection into the dorsal and ventral hippocampus impairs fear memory reconsolidation in a time-dependent manner in rats. Neurosci Lett 2023;808:137302.
[66]Ali Vafaei A, Nazari M, Omoumi S, Rashidy-Pour A, Raise-Abdullahi P. Corticosterone injection into the basolateral amygdala before and after memory reactivation impairs the subsequent expression of fear memory in rats: An interaction of glucocorticoids and β-adrenoceptors. Neurobiol Learn Mem 2023;205:107829.
[67]Ebner K, Rjabokon A, Pape HC, Singewald N. Increased in vivo release of neuropeptide S in the amygdala of freely moving rats after local depolarisation and emotional stress. Amino Acids 2011;41(4):991-6.
[68]Smith KL, Patterson M, Dhillo WS, Patel SR, Semjonous NM, Gardiner JV, et al. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology 2006;147(7):3510-8.
[69]Baykan H, Baykan Ö, Durmaz O, Kara H, Hişmioğullari AA, Karlidere T. Plasma Neuropeptide-S Levels in Populations Diagnosed with Generalized Anxiety Disorder: A Controlled Study. Noro Psikiyatr Ars 2019;56(1):52-6.
[70]Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol 2014;220:223-50.
[71]Chen L, Zhang Y, Wang Z, Zhang Z, Wang J, Zhu G, et al. Activation of GPER1 by G1 prevents PTSD-like behaviors in mice: Illustrating the mechanisms from BDNF/TrkB to mitochondria and synaptic connection. CNS Neurosci Ther 2024;30(7):e14855.
[72]Green CR, Corsi-Travali S, Neumeister A. The Role of BDNF-TrkB Signaling in the Pathogenesis of PTSD. J Depress Anxiety 2013;2013(S4).

[73]Zhang L, Li XX, Hu XZ. Post-traumatic stress disorder risk and brain-derived neurotrophic factor Val66Met. World J Psychiatry 2016;6(1):1-6.
[74]Yin JB, Liu HX, Shi W, Ding T, Hu HQ, Guo HW, et al. Various BDNF administrations attenuate SPS-induced anxiety-like behaviors. Neurosci Lett 2022;788:136851.
[75]Spinhoven P, Penninx BW, van Hemert AM, de Rooij M, Elzinga BM. Comorbidity of PTSD in anxiety and depressive disorders: prevalence and shared risk factors. Child Abuse Negl 2014;38(8):1320-30.
[76]McMillan KA, Sareen J, Asmundson GJ. Social anxiety disorder is associated with PTSD symptom presentation: an exploratory study within a nationally representative sample. J Trauma Stress 2014;27(5):602-9.
[77]Tillmann S, Skibdal HE, Christiansen SH, Gøtzsche CR, Hassan M, Mathé AA, et al. Sustained overexpression of neuropeptide S in the amygdala reduces anxiety-like behavior in rats. Behav Brain Res 2019;367:28-34.
[78]Jüngling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, et al. Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. Neuron 2008;59(2):298-310.
[79]Xie JF, Shao YF, Wang HL, Wang C, Cui GF, Kong XP, et al. Neuropeptide S Counteracts Paradoxical Sleep Deprivation-Induced Anxiety-Like Behavior and Sleep Disturbances. Front Cell Neurosci 2018;12:64.
[80]Grund T, Neumann ID. Brain neuropeptide S: via GPCR activation to a powerful neuromodulator of socio-emotional behaviors. Cell Tissue Res 2019;375(1):123-32.
[81]Grund T, Goyon S, Li Y, Eliava M, Liu H, Charlet A, et al. Neuropeptide S Activates Paraventricular Oxytocin Neurons to Induce Anxiolysis. J Neurosci 2017;37(50):12214-25.
[82]Roncacè V, Polli FS, Zojicic M, Kohlmeier KA. Neuropeptide S (NPS) is a neuropeptide with cellular actions in arousal and anxiety-related nuclei: Functional implications for effects of NPS on wakefulness and mood. Neuropharmacology 2017;126:292-317.
[83]Donner J, Haapakoski R, Ezer S, Melén E, Pirkola S, Gratacòs M, et al. Assessment of the neuropeptide S system in anxiety disorders. Biol Psychiatry 2010;68(5):474-83.
[84]Domschke K, Reif A, Weber H, Richter J, Hohoff C, Ohrmann P, et al. Neuropeptide S receptor gene -- converging evidence for a role in panic disorder. Mol Psychiatry 2011;16(9):938-48.
[85]Beste C, Konrad C, Uhlmann C, Arolt V, Zwanzger P, Domschke K. Neuropeptide S receptor (NPSR1) gene variation modulates response inhibition and error monitoring. Neuroimage 2013;71:1-9.

[86]Laas K, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, et al. Interaction of the neuropeptide S receptor gene Asn^{107}Ile variant and environment: contribution to affective and anxiety disorders, and suicidal behaviour. Int J Neuropsychopharmacol 2014;17(4):541-52.
[87]Laas K, Eensoo D, Paaver M, Lesch KP, Reif A, Harro J. Further evidence for the association of the NPSR1 gene A/T polymorphism (Asn107Ile) with impulsivity and hyperactivity. J Psychopharmacol 2015;29(8):878-83.
[88]Laas K, Reif A, Kiive E, Domschke K, Lesch KP, Veidebaum T, et al. A functional NPSR1 gene variant and environment shape personality and impulsive action: a longitudinal study. J Psychopharmacol 2014;28(3):227-36.
[89]Liu X, Si W, Garau C, Jüngling K, Pape HC, Schulz S, et al. Neuropeptide S precursor knockout mice display memory and arousal deficits. Eur J Neurosci 2017;46(1):1689-700.
[90]Duangdao DM, Clark SD, Okamura N, Reinscheid RK. Behavioral phenotyping of neuropeptide S receptor knockout mice. Behav Brain Res 2009;205(1):1-9.
[91]Ruzza C, Pulga A, Rizzi A, Marzola G, Guerrini R, Calo G. Behavioural phenotypic characterization of CD-1 mice lacking the neuropeptide S receptor. Neuropharmacology 2012;62(5-6):1999-2009.
[92]Williams ACC, Craig KD. Updating the definition of pain. Pain 2016;157(11):2420-3.
[93]Sharp TJ, Harvey AG. Chronic pain and posttraumatic stress disorder: mutual maintenance? Clin Psychol Rev 2001;21(6):857-77.
[94]Zhang S, You Z, Wang S, Yang J, Yang L, Sun Y, et al. Neuropeptide S modulates the amygdaloidal HCN activities (Ih) in rats: Implication in chronic pain. Neuropharmacology 2016;105:420-33.
[95]Li J, Li ZA, Tian HM, Tao SS, Zhang Q, Li F, et al. Corticotropin-releasing hormone neurons in the central Amygdala: An integrative hub for pain, emotion, and addiction neurobiology. Brain Res 2025;1863:149753.
[96]Ren W, Kiritoshi T, Grégoire S, Ji G, Guerrini R, Calo G, et al. Neuropeptide S: a novel regulator of pain-related amygdala plasticity and behaviors. J Neurophysiol 2013;110(8):1765-81.
[97]Kwon OB, Lee JH, Kim HJ, Lee S, Lee S, Jeong MJ, et al. Dopamine Regulation of Amygdala Inhibitory Circuits for Expression of Learned Fear. Neuron 2015;88(2):378-89.
[98]Du J, Fang J, Xu Z, Xiang X, Wang S, Sun H, et al. Electroacupuncture suppresses the pain and pain-related anxiety of chronic inflammation in rats by increasing the expression of the NPS/NPSR system in the ACC. Brain Res 2020;1733:146719.

[99]Holanda VAD, Oliveira MC, Souza LS, Lobão-Soares B, André E, Da Silva Junior ED, et al. Dopamine D(1) and D(2) receptors mediate neuropeptide S-induced antinociception in the mouse formalin test. Eur J Pharmacol 2019;859:172557.
[100]Enman NM, Arthur K, Ward SJ, Perrine SA, Unterwald EM. Anhedonia, Reduced Cocaine Reward, and Dopamine Dysfunction in a Rat Model of Posttraumatic Stress Disorder. Biol Psychiatry 2015;78(12):871-9.
[101]Montag C, Buckholtz JW, Hartmann P, Merz M, Burk C, Hennig J, et al. COMT genetic variation affects fear processing: psychophysiological evidence. Behav Neurosci 2008;122(4):901-9.
[102]Jinushi K, Kushikata T, Kudo T, Calo G, Guerrini R, Hirota K. Central noradrenergic activity affects analgesic effect of Neuropeptide S. J Anesth 2018;32(1):48-53.
[103]Holanda AD, Asth L, Santos AR, Guerrini R, de PS-RV, Calo G, et al. Central adenosine A1 and A2A receptors mediate the antinociceptive effects of neuropeptide S in the mouse formalin test. Life Sci 2015;120:8-12.
[104]Lee MT, Chiu YT, Chiu YC, Hor CC, Lee HJ, Guerrini R, et al. Neuropeptide S-initiated sequential cascade mediated by OX(1), NK(1), mGlu(5) and CB(1) receptors: a pivotal role in stress-induced analgesia. J Biomed Sci 2020;27(1):7.
[105]Bonumwezi JL, Tramutola D, Lawrence J, Kobezak HM, Lowe SR. Posttraumatic stress disorder symptoms, work-related trauma exposure, and substance use in first responders. Drug Alcohol Depend 2022;237:109439.
[106]McDevitt-Murphy ME, Luciano MT, Tripp JC, Eddinger JE. Drinking motives and PTSD-related alcohol expectancies among combat veterans. Addict Behav 2017;64:217-22.
[107]Badia-Elder NE, Henderson AN, Bertholomey ML, Dodge NC, Stewart RB. The effects of neuropeptide S on ethanol drinking and other related behaviors in alcohol-preferring and -nonpreferring rats. Alcohol Clin Exp Res 2008;32(8):1380-7.
[108]Cannella N, Kallupi M, Li HW, Stopponi S, Cifani C, Ciccocioppo R, et al. Neuropeptide S differently modulates alcohol-related behaviors in alcohol-preferring and non-preferring rats. Psychopharmacology (Berl) 2016;233(15-16):2915-24.
[109]Cannella N, Economidou D, Kallupi M, Stopponi S, Heilig M, Massi M, et al. Persistent increase of alcohol-seeking evoked by neuropeptide S: an effect mediated by the hypothalamic hypocretin system. Neuropsychopharmacology 2009;34(9):2125-34.
[110]Cannella N, Kallupi M, Ruggeri B, Ciccocioppo R, Ubaldi M. The role of the neuropeptide S system in addiction: focus on its interaction with the CRF and hypocretin/orexin neurotransmission. Prog Neurobiol 2013;100:48-59.

[111]Li W, Gao YH, Chang M, Peng YL, Yao J, Han RW, et al. Neuropeptide S inhibits the acquisition and the expression of conditioned place preference to morphine in mice. Peptides 2009;30(2):234-40.
[112]Kallupi M, de Guglielmo G, Cannella N, Li HW, Caló G, Guerrini R, et al. Hypothalamic neuropeptide S receptor blockade decreases discriminative cue-induced reinstatement of cocaine seeking in the rat. Psychopharmacology (Berl) 2013;226(2):347-55.
[113]Pañeda C, Huitron-Resendiz S, Frago LM, Chowen JA, Picetti R, de Lecea L, et al. Neuropeptide S reinstates cocaine-seeking behavior and increases locomotor activity through corticotropin-releasing factor receptor 1 in mice. J Neurosci 2009;29(13):4155-61.
[114]Chou YH, Hor CC, Lee MT, Lee HJ, Guerrini R, Calo G, et al. Stress induces reinstatement of extinguished cocaine conditioned place preference by a sequential signaling via neuropeptide S, orexin, and endocannabinoid. Addict Biol 2021;26(3):e12971.
[115]Kallupi M, Cannella N, Economidou D, Ubaldi M, Ruggeri B, Weiss F, et al. Neuropeptide S facilitates cue-induced relapse to cocaine seeking through activation of the hypothalamic hypocretin system. Proc Natl Acad Sci U S A 2010;107(45):19567-72.
[116]Quinones MM, Gallegos AM, Lin FV, Heffner K. Dysregulation of inflammation, neurobiology, and cognitive function in PTSD: an integrative review. Cogn Affect Behav Neurosci 2020;20(3):455-80.
[117]Si W, Liu X, Pape HC, Reinscheid RK. Neuropeptide S-Mediated Modulation of Prepulse Inhibition Depends on Age, Gender, Stimulus-Timing, and Attention. Pharmaceuticals (Basel) 2021;14(5).
[118]Punski-Hoogervorst JL, Engel-Yeger B, Avital A. Attention deficits as a key player in the symptomatology of posttraumatic stress disorder: A review. J Neurosci Res 2023;101(7):1068-85.
[119]de Santana Souza L, de Siqueira PA, Fernandes A, Silva Martins R, Cussa Kubrusly RC, Paes-de-Carvalho R, et al. Role of Neuropeptide S on Behavioural and Neurochemical Changes of an Animal Model of Attention-Deficit/Hyperactivity Disorder. Neuroscience 2020;448:140-8.
[120]Okamura N, Garau C, Duangdao DM, Clark SD, Jüngling K, Pape HC, et al. Neuropeptide S enhances memory during the consolidation phase and interacts with noradrenergic systems in the brain. Neuropsychopharmacology 2011;36(4):744-52.
[121]Han RW, Yin XQ, Chang M, Peng YL, Li W, Wang R. Neuropeptide S facilitates spatial memory and mitigates spatial memory impairment induced by N-methyl-D-aspartate receptor antagonist in mice. Neurosci Lett 2009;455(1):74-7.

[122]Lukas M, Neumann ID. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology 2012;62(1):398-405.
[123]Bicakci AO, Tsai PL, Kahl E, Mayer D, Fendt M. Dissociative Effects of Neuropeptide S Receptor Deficiency and Nasal Neuropeptide S Administration on T-Maze Discrimination and Reversal Learning. Pharmaceuticals (Basel) 2021;14(7).
[124]Wang C, Xin L, Cai CC, Cong CY, Xie JF, Kong XP, et al. Neuropeptide S Displays as a Key Neuromodulator in Olfactory Spatial Memory. Chem Senses 2020;45(3):195-202.
[125]Han RW, Zhang RS, Xu HJ, Chang M, Peng YL, Wang R. Neuropeptide S enhances memory and mitigates memory impairment induced by MK801, scopolamine or $A\beta_{1-42}$ in mice novel object and object location recognition tasks. Neuropharmacology 2013;70:261-7.
[126]Shao YF, Wang C, Xie JF, Kong XP, Xin L, Dong CY, et al. Neuropeptide S ameliorates olfactory spatial memory impairment induced by scopolamine and MK801 through activation of cognate receptor-expressing neurons in the subiculum complex. Brain Struct Funct 2016;221(6):3327-36.
[127]Koenigs M, Grafman J. Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. Neuroscientist 2009;15(5):540-8.
[128]Casello SM, Flores RJ, Yarur HE, Wang H, Awanyai M, Arenivar MA, et al. Neuropeptide System Regulation of Prefrontal Cortex Circuitry: Implications for Neuropsychiatric Disorders. Front Neural Circuits 2022;16:796443.
[129]Thomasson J, Canini F, Poly-Thomasson B, Trousselard M, Granon S, Chauveau F. Neuropeptide S overcomes short term memory deficit induced by sleep restriction by increasing prefrontal cortex activity. Eur Neuropsychopharmacol 2017;27(12):1308-18.
[130]Özkan A, Bülbül M, Derin N, Sinen O, Akçay G, Parlak H, et al. Neuropeptide-S affects cognitive impairment and depression-like behavior on MPTP induced experimental mouse model of Parkinson's disease. Turk J Med Sci 2021;51(6):3126-35.
[131]Rijkers C, Schoorl M, van Hoeken D, Hoek HW. Eating disorders and posttraumatic stress disorder. Curr Opin Psychiatry 2019;32(6):510-7.
[132]Mitchell KS, Aiello AE, Galea S, Uddin M, Wildman D, Koenen KC. PTSD and obesity in the Detroit neighborhood health study. Gen Hosp Psychiatry 2013;35(6):671-3.
[133]Stefanovics EA, Potenza MN, Pietrzak RH. PTSD and obesity in U.S. military veterans: Prevalence, health burden, and suicidality. Psychiatry Res 2020;291:113242.

[134]Peng YL, Han RW, Chang M, Zhang L, Zhang RS, Li W, et al. Central Neuropeptide S inhibits food intake in mice through activation of Neuropeptide S receptor. Peptides 2010;31(12):2259-63.
[135]Cifani C, Micioni Di Bonaventura MV, Cannella N, Fedeli A, Guerrini R, Calo G, et al. Effect of neuropeptide S receptor antagonists and partial agonists on palatable food consumption in the rat. Peptides 2011;32(1):44-50.
[136]Fedeli A, Braconi S, Economidou D, Cannella N, Kallupi M, Guerrini R, et al. The paraventricular nucleus of the hypothalamus is a neuroanatomical substrate for the inhibition of palatable food intake by neuropeptide S. Eur J Neurosci 2009;30(8):1594-602.
[137]Medina G, Ji G, Grégoire S, Neugebauer V. Nasal application of neuropeptide S inhibits arthritis pain-related behaviors through an action in the amygdala. Mol Pain 2014;10:32.
[138]Li S, Guo C, Zhang X, Liu X, Mu J, Liu C, et al. Self-assembling modified neuropeptide S enhances nose-to-brain penetration and exerts a prolonged anxiolytic-like effect. Biomater Sci 2021;9(13):4765-77.
[139]Huang Y, Wojciechowski A, Feldman K, Ettaro R, Veros K, Ritter M, et al. RTI-263, a biased neuropeptide S receptor agonist that retains an anxiolytic effect, attenuates cocaine-seeking behavior in rats. Neuropharmacology 2023;241:109743.
[140]Song C, Zhu ZC, Liu CC, Yun WX, Wang ZY, Lu GY, et al. Neuropeptide S Receptor 1 variant (I107N) regulates behavioral characteristics and NPS effect in mice in a sex-dependent manner. Neuropharmacology 2024;242:109771.
[141]Rappeneau V, Tobinski AM, Guevara LMC, Meyer N, Jüngling K, Touma C. Role of the neuropeptide S receptor 1 rs324981 polymorphism in modulating emotionality and cognitive flexibility: Insights from a gene-edited mouse model. Behav Brain Res 2025;485:115530.

TABLES AND FIGURES WITH LEGENDS

Table 1. Effects of NPS on the animal behaviors associated with PTSD and its related neuropsychiatric disorders

Animal	Active doses and route of administration	Symptoms	Experiments	Effects	Ref
SD rats	NPS (1.0 nmol/0.5 µl, intra-BLA)	PTSD-like behaviors	Predator scent stress, elevated plus maze, cut-off behavioral criteria model, acoustic startle response, freezing behavior	Alleviate the PTSD-like behaviors, including anxiety, freezing response, and hyperarousal	36
C57BL/6J	NPS (10 µM/0.5µl, intra-LA)	PTSD-like behaviors	Immobilization Stress, elevated plus maze, fear conditioning	Alleviate anxiety, reduce conditioned fear responses	38
Wistar rats	NPS (1 nmol, i.c.v.)	Aggressive and anxiety behaviors	Resident-intruder test, elevated plus maze	Alleviate anxiety, reduce aggressive behavior	41
Swiss mice, $NPSR^{(+/+)}$ and $NPSR^{(-/-)}$ with CD-1 strain	NPS (0.01 - 1 nmol, i.c.v.)	Aggressive behaviors	Resident/intruder test	Reduce aggressive behavior	42
Wistar rats	NPS (0.05 and 0.5 nmol/side,	_	Locomotion	Increase locomotor	59

	intra-VTA)			activity	
SD rats	NPS (1 nmol, i.c.v.)	Sleep deprivation and anxiety	Paradoxical sleep deprivation, Open field test, light-dark box	Reduce paradoxical sleep deprivation induced anxiety-like behavior	79
$NPSR^{(+/+)}$/ $NPSR^{(-/-)}$ mice with 129S6 genetic background	NPS (0.1 nmol, i.c.v.)	Anxiety	Locomotor	Increase locomotor activity in $NPSR^{(+/+)}$ mice but not in $NPSR^{(-/-)}$ mice	90
$NPSR^{(+/+)}$/$NPSR^{(-/-)}$ with CD-1 genetic background	NPS (1 nmol, i.c.v.)	Anxiety	elevated plus maze, open field test	alleviate anxiety in $NPSR^{(+/+)}$ mice but not in $NPSR^{(-/-)}$ mice	91
SD rats	NPS (10 nmol, i.c.v.)	Pain	Hot plate, tail flick	Prolong the hot plate latency but not tail flick latency	102
Swiss mice	NPS (0.1 nmol, i.c.v.)	Pain	Formalin test	Reduced formalin-induced nociception	103
C57BL/6J	NPS (0.3 and 1 nmol, i.c.v.)	Pain	Hot-plate test	Prolong the hot plate latency	104
Alcohol-preferring and non-	NPS (0.075, 0.3, and 1.2	Substances use disorder	Alcohol preferences, Elevated plus-	Reduced alcohol consumption in the alcohol-	107

preferring rats	nmol, i.c.v.)		maze	preferring rat	
Alcohol-preferring and nonpreferring rats	NPS (0.1, 0.5, 1.0, and 2.0 nmol, i.c.v.)	Substances use disorder	Alcohol self-administration	Reduced alcohol self-administration	108
Wistar rats	NPS (1.0, 2.0, 4.0 nmol, i.c.v.)	Substances use disorder	Alcohol self-administration	Increase ethanol seeking elicited by ethanol-associated cues	109
Kunming strain mice	NPS (1, 3, 6 and 10 nmol, i.c.v.)	Substances use disorder	Conditioned place preference	Reduce the morphine-induced CPP acquisition and expression	111
C57BL/6	NPS (1 nmol, i.c.v.)	Cognition deficit	Inhibitory avoidance, novel object recognition	Enhance memory retention, but not acquisition or recall	120
Kunming strain mice	NPS (1 nmol, i.c.v.)	Cognition deficit	Morris water maze	Facilitate spatial memory, mitigate spatial memory impairment	121
Adult male Wistar rats	NPS (1 nmol, i.c.v.)/Nasal NPS Administration	Cognition deficit	Elevated plus-maze, object discrimination, social	Reduce non-social anxiety, facilitate object discrimination but not social	122

	(40 nmol)		discrimination	discrimination	
C57BL/6	Nasal NPS Administration (10 μL of 1 mM solution)	Cognition deficit	T-Maze	Facilitate reversal learning without affecting the acquisition	123
Kunming strain mice	NPS (0.1 nmol, i.c.v.)	Food intake in fasted mice	Food intake in fasted mice	Inhibit food intake in fasted mice	134

The doses of NPS presented in the table are statistically effective for alleviating the corresponding symptoms (P<0.05). i.c.v.: intracerebroventricular infusion; intra-LA: intra-lateral amygdala; intra-BLA: intra- basolateral amygdala; intra-VTA: intra- ventral tegmental area.

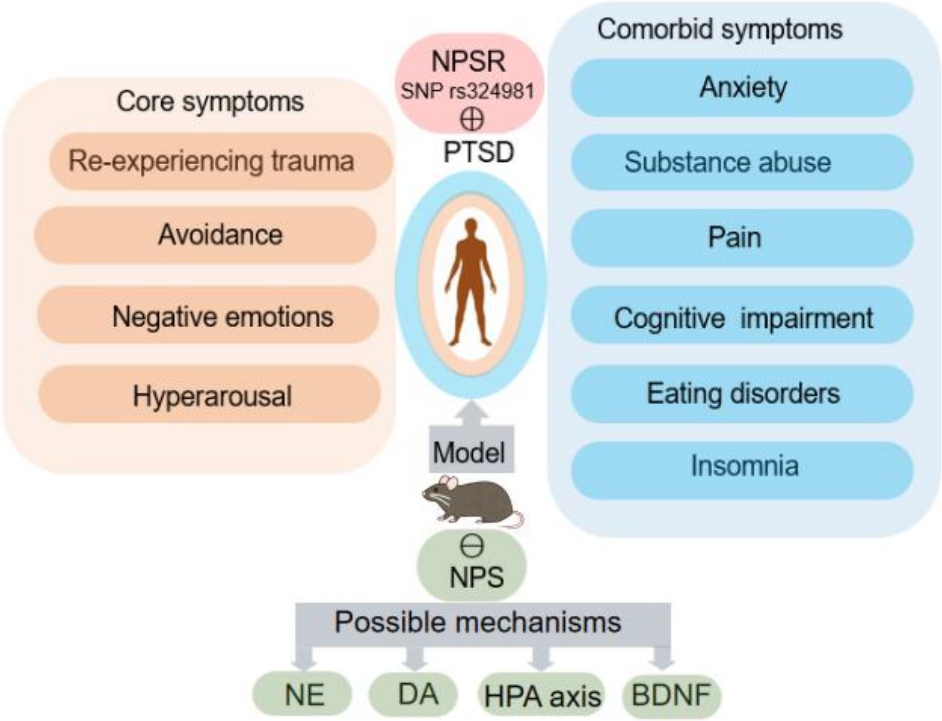

Figure 1. Role of NPS-NPSR system in PTSD and related neuropsychiatric disorders. The orange and blue boxes describe the core symptoms of PTSD and related neuropsychiatric disorders, respectively. A variation in the NPSR gene (SNP rs324981) increases the incidence of PTSD in human. Animal models can mimic the core symptoms of PTSD and related neuropsychiatric disorders. Administration of NPS inhibits PTSD through mechanisms involving norepinephrine (NE), dopamine (DA), the hypothalamic-pituitary-adrenal (HPA) axis, and brain-derived neurotrophic factor (BDNF).

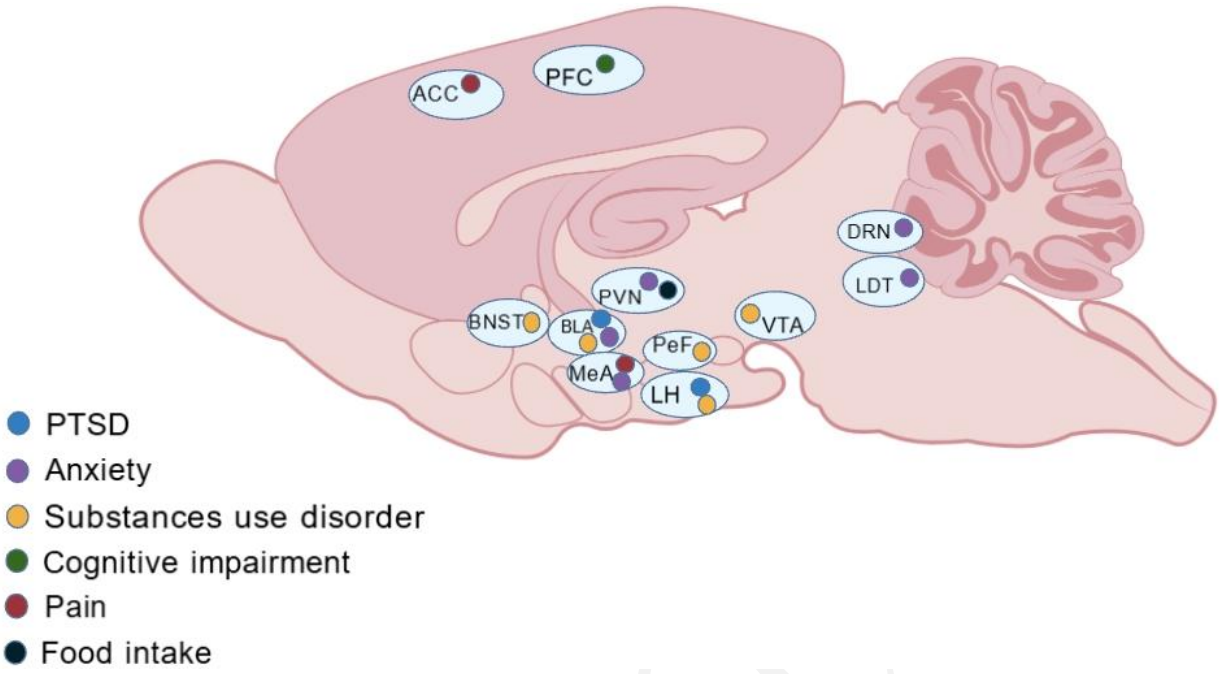

Figure 2. Brain regions mediate the alleviating effects of NPS on the animal behaviors associated with PTSD and its related neuropsychiatric disorders. The various colored solid circles displayed in the different brain regions represent the various symptoms that can be relieved by NPS. ACC: Anterior Cingulate Cortex. PFC: Prefrontal Cortex. BNST: Bed Nucleus of the Stria Terminalis. BLA: Basolateral Amygdala. PVN: Paraventricular Nucleus of the Hypothalamus. LH: Lateral Hypothalamus. PeF: Perifornical Area. MeA: Medial Amygdala. VTA: Ventral Tegmental Area. DRN: Dorsal Raphe Nucleus. LDT: Laterodorsal Tegmental Nucleus. The Generic Diagramming Platform (GDP), accessible at http://biogdp.com, was employed to create the schematic diagram.