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REVIEW

Brain neuropeptide S: via GPCR activation to a powerful neuromodulator of socio-emotional behaviors

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Abstract

Neuropeptide S (NPS) has attracted the attention of the scientific community due to its potent anxiolytic-like and fear-attenuating effects studied in rodents. Therefore, NPS might represent a treatment option for neuropsychiatric disorders, such as anxiety disorders, even more so as single nucleotide polymorphisms in the human NPS receptor gene have been associated with increased anxiety traits that contribute to the pathogenesis of fear- and anxiety-related disorders. However, the signaling mechanisms underlying the behavioral effects of NPS and the interaction with other brain neuropeptides are still rather unknown. To illuminate how NPS modulates the expression of selected emotional and social behaviors, the present review focuses on neuroanatomical and electrophysiological studies, as well as intracellular signaling mechanisms following NPS receptor stimulation in rodents. We will also discuss interactions of the NPS system with two well-described neuropeptides, namely corticotropin-releasing factor and oxytocin, which may contribute to the fear- and anxiety-reducing effects.

Keywords Neuropeptide S · Intracellular signaling · Anxiety · Fear · Neuromodulator

Identification of the brain neuropeptide S system

G protein-coupled receptors (GPCRs) are a central component in the regulation of cellular homeostasis, physiological parameters and emotional behaviors. At least 800 GPCRs participate in diverse physiological and pathological functions (Tang et al. 2012). During the last decades, bioinformatics of DNA sequences gave rise to about 140 GPCRs, whose natural ligands were discovered by "deorphanization," a process based on reverse pharmacology (Pausch 1997; Civelli et al. 2006). GPR154 (also known as GPRA, PGR14, ASRT2 and VRR1) and its ligand were first reported in the patent literature (Sato et al. 2002). Searching public DNA databases, a genomic sequence encoding for the GPR154 ligand was identified, which is highly conserved among various species including humans, rats and mice (Xu et al. 2004). The precursor protein contains a hydrophobic signal peptide that is immediately followed by the initiator methionine. Moreover, a pair lysine/arginine motif might serve for proteolytic cleavage to process the peptide (Reinscheid and Xu 2005a). The mature ligand consists of 20 amino acids (N-SFRNGVGSGVKKTSFRRAKQ-C) with the amino-terminal residue being consistently a serine. Therefore, and according to the nomenclature that has been used earlier (Shimomura et al. 2002), the ligand was named neuropeptide S (NPS). The NPS receptor (NPSR, previously GPR154) is a typical GPCR composed of a seven-transmembrane domain that shares the highest degree of homology with vasopressin and oxytocin (OXT) receptors (Reinscheid and Xu 2005a), which are well described for their neuromodulatory effects on emotionality and social behaviors (Jurek and Neumann 2018).

Localization of NPS neurons within the mammalian brain

NPS is expressed in all vertebrates with the exception of fish (Reinscheid 2007). In rats, the NPS precursor mRNA is expressed in various tissues with the highest levels in the brain, thyroid, mammary, and salivary glands (Xu et al. 2004). In the rat brain NPS-positive cells are located in the locus coeruleus (LC), in the lateral parabrachial nucleus and also in the principle sensory five nucleus (Xu et al. 2004).

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Moderate NPS mRNA expression is also present in the dorsomedial hypothalamic nucleus and the amygdala (Xu et al. 2004)—two brain regions that belong to the limbic system.

In the mouse brain, NPS is one of the least abundant neuropeptides concerning the number of neurons (Clark et al. 2011; Liu et al. 2011). Only about 500 NPS-synthetizing neurons have been detected in the mouse brain stem, which are located more ventrolaterally to the parabrachial region, specifically in an area defined as the Kölliker-Fuse nucleus. The parabrachial region including the Kölliker-Fuse nucleus is a cytoarchitecturally highly organized region that represents essential relay stations for visceral afferents from the brainstem to the mammalian forebrain for higher order processing and emotional behavior.

In the human pons, in situ hybridization of NPS mRNA revealed roughly 22,000 NPS-positive cells. More than 80% of the mRNA signals are located in a cell cluster adjacent to the parabrachial area, specifically in the extension of the medial and lateral parabrachial nuclei and the Kölliker-Fuse nucleus of the pons (Adori et al. 2015a). About 5% of NPS-expressing neurons are located within the LC and 11% in the periventricular area caudally to the fourth ventricle in the human pons.

Characterization of the chemical nature of NPS-expressing neurons in rats using double in situ hybridization revealed that NPS mRNA co-localizes predominantly with vesicular glutamate transporter 2 mRNA indicating that these cells are glutamatergic (Xu et al. 2007). A small number of NPS neurons co-express choline acetyltransferase mRNA suggesting the presence of acetylcholine (Xu et al. 2007). These findings highlight a potential co-transmission of NPS with excitatory neurotransmitters that may enhance NPS signaling within the brain. Also, co-localization of NPS mRNA and corticotropin-releasing factor (CRF) mRNA has been identified in the rat lateral parabrachial nucleus. In mice, gene expression profiling of laser-microdissected NPS neurons harvested from the pericoerulear area and the Kölliker-Fuse nucleus revealed co-expression of nicotinic acetylcholine receptors, GABA and glutamate receptors, the CRF receptor 1 and the OXT receptor (Liu et al. 2011), hence indicating possible mechanisms to regulate the activity of NPS neurons. Collectively, analysis of the distribution of NPS mRNA and co-localization with other neurotransmitters and neuromodulators revealed species-specific differences that have to be taken into account with respect to intracerebral NPS circuits.

In addition to the presence of NPS mRNA, immunofluorescent staining using specific NPS antibodies has confirmed the presence of NPS neurons in the regions mentioned above in rats and mice. Beyond that, densitometry analysis of NPS-immunoreactive fibers revealed moderate to high densities of NPS-positive nerve endings in the majority of limbic brain regions (Clark et al. 2011; Adori et al. 2015b). In line, retrograde tracing verified prominent projections of NPS neurons from the brainstem to the rat hypothalamic paraventricular nucleus (PVN) (Grund et al. 2017), which is important for regulating emotional and physiological homeostases. Dense NPS afferents have also been detected within the amygdala further suggesting NPS release within main regulatory centers of fear and anxiety. In line, intra-amygdalar microdialysis revealed increased local NPS release in response to stressor exposure such as forced swimming (Ebner et al. 2011), supporting a functional role of endogenous NPS neurons in stress-related events.

NPSR expression sites in the rodent brain

In contrast to the ligand, the NPSR has a wider distribution in the brain of the species studied so far. Due to the lack of a sensitive and selective NPSR antibody (Slattery et al. 2015), our knowledge about NPSR distribution in the rodent brain completely relies on in situ hybridization studies. NPSR mRNA expression has been identified throughout the rodent central nervous system with low levels within the brainstem but moderate to high levels in the olfactory nuclei, thalamus and hypothalamus, as well as in the cortex, amygdala and hippocampal formation (Xu et al. 2007; Clark et al. 2011). Comparison of NPSR mRNA distribution between rats and mice revealed similar expression patterns with high levels of NPSR mRNA found in the thalamus, the hypothalamic PVN and the anterior and posterior hypothalamus, as well as in the dorso- and ventromedial hypothalamus. With respect to the amygdala, NPSR mRNA was detected in the medial but not the central, amygdala (Xu et al. 2007; Clark et al. 2011). In situ hybridization also revealed areas with species-specific differences in NPSR expression. For example, the basolateral amygdaloid complex shows abundant NPSR mRNA expression in mice but only widely scattered signals in rats. Conversely, an area of high NPSR mRNA signal found in the rat but not in the mouse, is the intercalated nucleus of the amygdala. Prominent NPSR mRNA expression has also been detected in the hypothalamic arcuate nucleus, the supramammillary nucleus and the retrochiasmatic area exclusively in mice (Clark et al. 2011). Furthermore, the hypothalamus represents a remarkable overlap of NPSR mRNA expression and NPS-immunoreactive fibers that have recently been confirmed by retrograde tracing and fluorescence-activated cell sorting, i.e., within the PVN (Grund et al. 2017).

NPSR expression has also been found in regions without detectable NPS afferents such as the cortex, subiculum and anterior olfactory regions (Clark et al. 2011). Such mismatches between local presence of specific neuronal fiber and ligands and receptor expression have already been reported for other neuropeptides, such as OXT (Jurek and Neumann 2018) and CRF (Justice et al. 2008) and can be explained by presynaptic localization of the receptor protein at a significant

distance from the cell body. Although NPSR localization can be indirectly demonstrated by electrophysiological analysis of NPSR activity, as described in principal neurons in the amygdala of mice (Jüngling et al. 2008), direct evidence for the existence of the NPSR protein is still lacking due to the lack of a sensitive and selective NPSR antibody as mentioned above (Slattery et al. 2015). The design and production of antibodies specifically targeting a GPCR seems challenging but is essential to characterize the cellular identity of a certain cell type expressing the NPSR. Thus, future studies should make use of transgenic NPSR-reporter gene knockin mice, or viral gene transfer approaches allowing the expression of a reporter protein (e.g., Venus or mCherry) selectively under the control of an NPSR promoter fragment. Thereby, these approaches may allow co-expression analyses of cell type-specific markers to examine the characteristics of NPSR-expressing neurons. Moreover, reporter protein labeling of NPSR-positive neurons may permit the application of sensitive and highly specific techniques, i.e., fluorescence-activated cell sorting of NPSR-expressing neurons and, thus, allow co-expression studies in the absence of specific NPSR antibodies. Such approaches seem essential for further functional dissection of NPSR signaling.

Intracellular signaling mechanisms downstream of the NPSR

The pharmacological characteristics of NPSR activation have been examined both in vitro and in vivo (Fig. 1). In vitro, CHO and HEK293T cells stably transfected with an NPSR construct demonstrate reliable increases in intracellular Ca^{2+} and cyclic adenosine monophosphate (cAMP) levels at low nanomolar concentrations of NPS (Reinscheid et al. 2005; Camarda et al. 2009; Liao et al. 2016). This suggests that the NPSR is coupled to both G_q and G_s, and their related effector proteins (Xu et al. 2004; Reinscheid et al. 2005). Whether the two G proteins bind to the same NPSR simultaneously, or on a different NPSR, is still unknown and requires specific G protein/receptor interaction experiments based on fluorescence resonance energy transfer. However, in order to distinguish between the two NPSR-coupled pathways, the human NPS analog NPS (1–10), lacking 10 residues from the C terminus, has been shown to stimulate Ca^{2+} mobilization in a manner comparable with full-length NPS in vitro but failed to induce biological activity in vivo (Liao et al. 2016). Recently, a newly designed biased NPSR agonist called *Compound 4* that predominantly activates the G_q-mediated Ca^{2+} influx in NPSR-expressing HEK293T cells was demonstrated to promote anxiolysis and memory-enhancing effects in mice (Clark et al. 2017). In line, pharmacological inhibition of phospholipase C (activated by G_q signaling) but not adenylyl cyclase (activated by G_s signaling), attenuated NPS-evoked anxiolysis within the rat medial amygdala (Grund and Neumann 2018), a region expressing high levels of NPSR mRNA in rats. Moreover, in amygdala tissue micropunches, NPS induced the synthesis and phosphorylation of Ca^{2+}/calmodulin-dependent kinase II, a major Ca^{2+}-binding protein (Grund and Neumann 2018) that might represent a downstream regulator of NPS-induced anxiolysis. In more detail, NPS evoked a biphasic time course of NPSR-mediated Ca^{2+} influx characterized by a fast and slow component suggesting two major Ca^{2+} routes via intracellular stores and the extracellular space, respectively (Erdmann et al. 2015). In mouse hippocampal NPSR-transfected neurons, selective pharmacological inhibition demonstrated that the NPSR-mediated rise in intracellular Ca^{2+} levels is mediated in an inositol-triphosphate- and ryanodine-receptor-dependent manner representing the fast component of the Ca^{2+} route. Moreover, in Ca^{2+}-free solution, the slow component was drastically reduced in response to NPS suggesting Ca^{2+} influx from the extracellular space. However, it is not known which Ca^{2+} channels mediate the entry of extracellular Ca^{2+} upon NPS administration. It is noteworthy that repeated NPS application failed to evoke repeated Ca^{2+} responses in the same neuron (Jüngling et al. 2008; Meis et al. 2008; Grund et al. 2017) suggesting desensitization of the NPSR following repeated stimulation. In line, both intranasally and centrally administered fluorophore-labeled NPS were localized in cytoplasmic and perinuclear vesicular structures in an NPSR- and time-dependent manner in mice (Ionescu et al. 2012; Dine et al. 2013) suggesting internalization of the ligand-receptor complex.

Taken together, NPS is synthetized in neurons located in defined brainstem nuclei that densely innervate limbic brain regions; after its local release, NPS signaling is mediated by local NPSR. Such functional NPS circuits may provide the neuroanatomical substrate to modulate the expression of distinct emotional behaviors.

Effects of NPS on anxiety-related behavior

In 2004, a robust anxiolytic effect of icv-infused NPS has been demonstrated in mice subjected to the elevated plus-maze, light/dark box and open field tests (Xu et al. 2004). Ever since, the anxiolytic action of NPS has repeatedly been confirmed using various tests for anxiety-related behavior in both mice and rats following icv (Leonard et al. 2008; Rizzi et al. 2008; Vitale et al. 2008; Ruzza et al. 2010) or intranasal application (Ionescu et al. 2012; Lukas and Neumann 2012; Dine et al. 2015). It needs to be mentioned that icv infusion of NPS has been repeatedly but inconsistently, demonstrated to promote arousal and to increase the locomotor activity of rats and mice. However, the stimulatory effects of NPS seem to be dose-dependent and independent of NPS' anxiolytic-like effects.

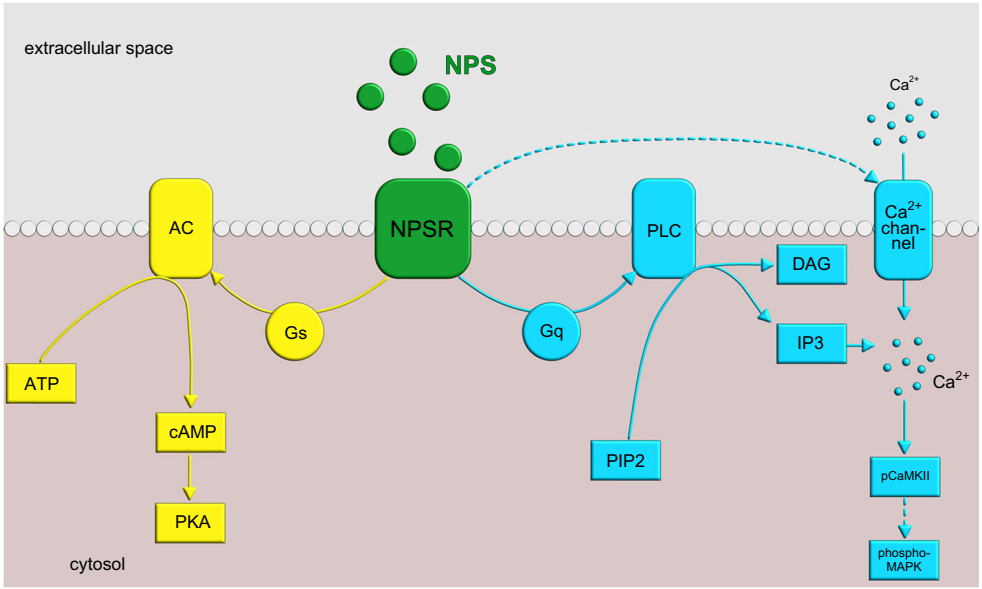

Fig. 1 Representative scheme of neuronal neuropeptide S receptor (NPSR)-coupled signaling cascades summarizing findings from original research articles (Erdmann et al. 2015; Liao et al. 2016; Grund and Neumann 2018). NPS binding to its receptor induces activation of both G_s and G_q proteins and hence activation of the membrane-bound adenylyl cyclase (AC) and phospholipase C (PLC), respectively. Besides intracellular Ca^{2+} stores (e.g., endoplasmic reticulum), influx of extracellular Ca^{2+} via transactivation of membrane-bound Ca^{2+} channels is also a source for NPS-induced elevation of intracellular Ca^{2+} levels. When indirect evidence for NPS-induced activation is available, arrows are in dotted lines. ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PIP2, phosphatidylinositol bisphosphate; DAG, diacylglycerol; IP3, inositol trisphosphate, pCaMKII, phospho-Ca^{2+}/calmodulin-dependent kinase II; phospho-MAPK, phospho-mitogen-activated protein kinase

Thus, NPS-evoked anxiolysis has been confirmed in the rat defensive burying test (Xu et al. 2004; Vitale et al. 2008; Paneda et al. 2009) and stress-induced hyperthermia test (Rizzi et al. 2008), which are not biased by locomotor activity.

So far, the anxiolytic-like effect of NPS has been localized within the mouse basolateral nucleus of the amygdala (Jüngling et al. 2008) and the rat medial nucleus of the amygdala (Grund and Neumann 2018) as well as the rat PVN (Grund et al. 2017). From a translational point of view, it is important that the anxiolytic-like effect of synthetic NPS was also seen in animal models of psychopathology, such as in rats selectively bred for high innate levels of anxiety-related behavior (HAB) (Slattery et al. 2015) and in the Flinders sensitive line—an animal model of depression (Wegener et al. 2011). Also, anxiolytic-like effects of NPS have been described in a rat model of sciatic nerve injury associated with increased anxiety-like behavior, decreased pericoerulear NPS expression and diminished release of NPS within the amygdala (Zhang et al. 2014). As patients suffering from chronic pain have an increased prevalence to develop an anxiety disorder (Hasnie et al. 2007; Kroenke et al. 2009; Newcomer et al. 2010; Reme et al. 2011), it is remarkable that icv NPS was able to reverse pain-induced anxiety, an effect that might be based on NPS-induced increase of GABAergic transmission in amygdalar interneurons (Zhang et al. 2014). In this context, it is worth mentioning that NPS also evoked a dose-dependent antinociceptive effect revealed in the hot-plate and formalin test in mice (Li et al. 2009; Peng et al. 2010), which may contribute to the reduction in pain-induced anxiety-related behavior.

In addition to the effects of synthetic NPS, the question arises whether the endogenous NPS system is involved in the regulation of stress- and anxiety-related behaviors. Consequently, environmental stimuli and endogenous cues that may modulate the activity of NPS neurons and increase endogenous NPS release in brain target regions are of substantial interest. Activation of NPS neurons was found in response to stressor exposure, such as forced swimming, as revealed by microdialysis (Ebner et al. 2011), as well as immobilization stress as indicated by increased NPS expression (Liu et al. 2011; Jüngling et al. 2012). In this context, behavioral phenotyping of NPSR-deficient 129S6/SvEvTac mice (Allen et al. 2006) revealed increased anxiety-related behavior in comparison to their wild-type littermates (Duangdao et al. 2009; Liu et al. 2017). Moreover, intra-amygdalar infusion of the selective NPSR antagonist SHA-68 (Okamura et al. 2008), a bicyclic piperazine, produced a significant anxiogenic response in C57BL/6J mice (Jüngling et al. 2008). This effect has been confirmed in a psychopathological animal model by icv infusion of the NPSR antagonist [D-Cys(tBu)^5]NPS (Camarda et al. 2009) in mice and rats selectively bred for low innate anxiety-related behavior resulting in elevated anxiety levels (Slattery et al. 2015). Together these findings suggest that the endogenous NPS/NPSR system may tonically control

anxiety-related behavior in these particular strains (for details, see Table 1).

In contrast, 129S6/SvEvTac NPSR knockout mice, which have been backcrossed on CD1 strain, are still insensitive to NPS application but demonstrated a superimposable phenotype concerning anxiety-related behavior compared to wild-type littermates (Pulga et al. 2012; Ruzza et al. 2012). This strain-dependent effect has been confirmed in CD1 mice, as the NPSR antagonist [D-Cys(tBu)^5]NPS failed to increase anxiety-related behavior compared to controls (Zoicas et al. 2016). Therefore, the involvement of the endogenous NPS system in the control of anxiety-related behavior seems not to be consistent but rather strain-dependent. To fully understand the contribution of brain NPS to the tonic control of anxiety-related behavior, remote activity control of brainstem NPS neurons using chemo- or optogenetic approaches seems essential.

Effects of NPS on the expression of non-social and social fear

Besides anxiolysis and antinociception, synthetic NPS was found to attenuate the expression of contextual and cued fear in both rats and mice (Jüngling et al. 2008; Chauveau et al. 2012; Slattery et al. 2015; Zoicas et al. 2016) and to block the expression of fear-potentiated startle (Fendt et al. 2010). These fear-attenuating effects of NPS have recently been confirmed in extinction-deficient mice (Sartori et al. 2016) and HAB rats (Slattery et al. 2015), demonstrating the ability of NPS to promote fear extinction also in psychopathological animal models. In confirmation of a significant role of endogenous NPS, infusion of the NPSR antagonist SHA-68 into the basolateral and lateral amygdala increased freezing behavior in fear-conditioned mice, respectively (Jüngling et al. 2008; Chauveau et al. 2012).

In addition to the effects of NPS on non-social anxiety and fear, we studied NPS effects on social fear in a mouse model of social fear conditioning (Toth et al. 2012). In line with its profound fear-reducing effects, icv NPS reliably reduced the expression of social fear indicated by elevated levels of social interaction in social fear-conditioned male mice. Moreover, icv NPS potently reduced social avoidance behavior after social defeat in mice (Zoicas et al. 2016).

NPS effects on aggression

In contrast to the well-described and robust effects of NPS on social and non-social anxiety, very little is known about its capacity to modulate aggression. So far, studies in rats have shown that icv NPS reduced intermale aggression during the resident-intruder test—specifically in rats bred for low levels of anxiety, which display elevated and abnormal aggression (Beiderbeck et al. 2014). However, this effect could not be localized so far. These antiaggressive effects of NPS have been confirmed in mice also subjected to the resident-intruder test (Ruzza et al. 2015) and were abolished in the presence of the non-peptidergic NPSR antagonist SHA-68.

Effects of NPS on memory

The NPSR is prominently expressed in brain structures involved in learning and memory, e.g., the hippocampal formation, which opens the question, whether NPS affects social and non-social memory. Using the inhibitory avoidance paradigm, central NPS administration dose-dependently enhanced memory retention in mice, indicating that NPS may act during the consolidation phase to enhance long-term memory (Okamura et al. 2011). Moreover, NPS has been demonstrated to enhance hippocampal-dependent, non-aversive memory in the novel object recognition task (Okamura et al. 2011), which has been confirmed in rats (Lukas and Neumann 2012). In contrast to the reduction of social avoidance behavior after social defeat in mice (Zoicas et al. 2016), icv as well as intranasally applied NPS failed to alter naturally occurring social preference behavior and failed to prolong social memory in a social discrimination paradigm in male rats (Lukas and Neumann 2012), emphasizing context-dependent and species-specific effects of NPS.

Regulation of NPS neuron activity by CRF and GABA

As any other neuropeptide of the brain, the NPS system interacts with various other neurotransmitter and neuropeptide systems. For example, it has been shown that CRF fibers, which have their origin predominantly within the PVN and central amygdala, are located in proximity to NPS-synthesizing neurons within the LC (Reyes et al. 2005; Reyes et al. 2008; Jüngling et al. 2012; Dimitrov et al. 2013). These NPS neurons co-express the CRF receptor 1 (Liu et al. 2011), which suggests a stress-induced activation of NPS neurons. Indeed, various stressors including forced swimming and acute immobilization were found to increase the expression of the immediate early gene cFos in NPS-immunoreactive neurons of the LC and Kölliker-Fuse nucleus in mice (Liu et al. 2011; Jüngling et al. 2012). In line, electrophysiological analysis of pericoerulear NPS neurons revealed that bath application of CRF induced an inwardly directed membrane current resulting in depolarization, i.e., activation of NPS neurons (Jüngling et al. 2012). As a result of stress-induced activation of NPS neurons, NPS is likely to be released within brain regions involved in the processing of fear and anxiety, such

Table 1 Species- and strain-dependent effects of synthetic NPS and NPSR antagonists (NPSR-A, SHA-68, [D-Cys(tBu)^5]NPS) on anxiety-related behavior and the expression of fear in conditioned animals (*n.d.*, not determined; ↓, decrease; ↑, increase; -, no change).

Species/strain	General anxiety		Fear		Reference
	NPS	NPSR-A	NPS	NPSR-A	
Mouse					
C57BL/6J	↓	↑	↓	↑	Xu et al. 2004, Jüngling et al. 2008
129S1/SvImJ	n.d.	n.d.	↓	n.d.	Sartori et al. 2016
CD1	↓	-	↓	-	Zoicas et al. 2016
Swiss	↓	-	n.d.	n.d.	Ruzza et al. 2010
HAB	↓	-	↓	n.d.	Slattery et al. 2015
LAB	n.d.	↑	n.d.	-	Slattery et al. 2015
Rat					
Wistar	↓	-	n.d.	n.d.	Ruzza et al. 2010, Grund et al. 2017
Flinders sensitive	↓	n.d.	n.d.	n.d.	Wegener et al. 2011
HAB	↓	n.d.	↓	n.d.	Slattery et al. 2015
LAB	-	↑	-	-	Beiderbeck et al. 2014, Slattery et al. 2015

as the amygdala (Roozendaal et al. 2009; Ebner et al. 2011). NPS pericoerulear neurons also receive monosynaptic, GABAergic afferents, which originate in the central amygdala (Jüngling et al. 2015). In detail, these GABAergic neurons have been identified as so-called fear$_{on}$ neurons and co-express dynorphin and somatostatin. Once activated, e.g., during fear memory retrieval (Jüngling et al. 2015), dynorphin hyperpolarizes NPS neurons in a *kappa*-opioid receptor-dependent manner (Jüngling et al. 2016), which may consequently regulate fear- and anxiety-related behavior.

Cellular NPS effects within the amygdala and the PVN

Indeed, an NPS-sensitive pathway has been identified in the amygdaloid complex, which is principally involved in the control of fear responses. NPS was found to promote both the excitatory and inhibitory drive onto the basolateral amygdala, thus regulating a subpopulation of projection neurons (Meis et al. 2008; Meis et al. 2011). Such balancing of inhibitory and excitatory influences seems essential to control contextual fear expression (Meis et al. 2008). In detail, NPS was found to increase glutamatergic transmission to intercalated GABAergic neurons via presynaptic signaling predominantly involving principal neurons of the lateral amygdala. In turn, intercalated neurons evoke increased GABAergic input to pyramidal neurons of the central amygdala, thus inhibiting stress-induced amygdala output (Jüngling et al. 2008; Pape et al. 2010). Accordingly, infusion of NPS into the basolateral amygdaloid complex prevented the expression of fear- and anxiety-related behaviors, whereas local NPSR antagonism aggravated freezing in fear-conditioned mice (Jüngling et al. 2008; Chauveau et al. 2012).

In addition to the amygdala, NPSR expression is also highly abundant in the hypothalamic PVN of rats and mice (Xu et al. 2007; Clark et al. 2011)—a brain region densely innervated by NPS-immunoreactive fibers (Clark et al. 2011; Adori et al. 2015b). Recently, fluorescence-activated cell sorting on Venus-labeled OXT neurons in combination with quantitative real-time PCR identified OXT neurons as the major expression site of NPSR mRNA in rat PVN (Grund et al. 2017). Using complementary approaches, we could reveal that NPS activates OXT neurons of the PVN (Fig. 2). For example, based on ultrasensitive fluorescent proteins introduced into paraventricular OXT neurons by viral gene transfer, we found that NPS induced a transient Ca^{2+} influx in a subpopulation of OXT neurons. This stimulatory effect of NPS on OXT neurons is mediated by NPSR since NPS failed to increase intracellular Ca^{2+} levels in the presence of the selective NPSR antagonist SHA-68. Moreover, NPS evoked OXT release within the PVN as revealed by intracerebral microdialysis. As not only NPS but also OXT exerts robust anxiolytic effects directly within the PVN (Blume et al. 2008; van den Burg et al. 2015), we tested the possibility that OXT mediates the anxiolytic effect of NPS. Indeed, chemogenetic silencing of PVN OXT neurons using an inhibitory DREADD (designer receptors exclusively activated by designer drugs) prevented the anxiolytic effects of NPS (Grund et al. 2017). Although the question remains which OXT projections and target regions are specifically essential for the anxiolytic effect of NPS, these data demonstrate that NPS modulates the activity of hypothalamic OXT neurons and that OXT at least partly mediates the robust anxiolytic-like effect of NPS. In this context, it is noteworthy that parvocellular OXT neurons of the

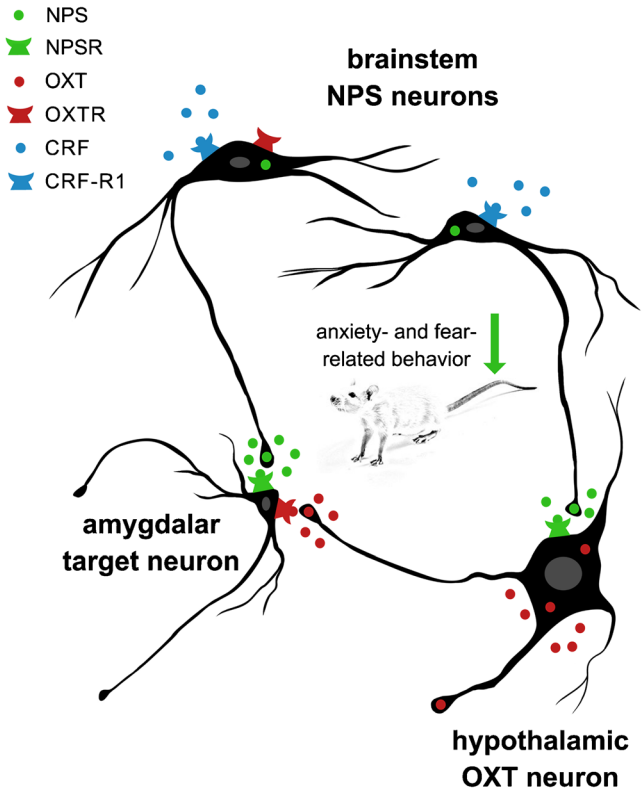

Fig. 2 Working hypothesis on interactions between the NPS and OXT systems in the context of anxiety. Enhanced activity of brainstem NPS neurons evokes NPS release within the amygdala, e.g., during stressful events, in a CRH/CRHR1-dependent manner, thereby modulating amygdala responsiveness. Moreover, NPS activates OXT neurons within the hypothalamic PVN in an NPSR-dependent manner and consequently OXT release within the PVN and within limbic brain areas, such as the amygdala, from OXT projecting neurons. Altogether, these routes of NPS beneficially modulate fear- and anxiety-related behavior in rodent models

PVN project towards the parabrachial nucleus and the LC in rats (Swanson and Sawchenko 1983). Also, OXT receptor expression was found in pericoerulear NPS neurons (Liu et al. 2011). Thus, it is likely that locally released OXT—in turn—activates NPS neurons.

The NPS system as a pivotal marker for emotional dysfunctions in humans

Previous studies identified a possible panic disorder susceptibility locus on human chromosome 7p15 (Knowles et al. 1998; Logue et al. 2003), which has later been identified as single nucleotide polymorphism (SNP) in the human NPSR gene located on chromosome 7p14-15 (Laitinen et al. 2004). Analysis of genomic DNA from blood samples of a Japanese cohort revealed that the functional NPSR A/T SNP (SNP database accession number rs324981), which leads to an amino acid exchange of asparagine into isoleucine (Ile) at position 107, is associated with panic disorders in male patients (Okamura et al. 2007). A second population-based study of NPSR SNPs in a Swedish cohort confirmed a correlation between NPSR polymorphisms and anxiety disorders (Donner et al. 2010). Moreover, a multilevel approach was applied to further elucidate the role of the NPS/NPSR system in the etiology of human anxiety (Domschke et al. 2011). Herein, the A/T polymorphism was also found to be associated with panic disorders with converging evidence for a female-dominant role of NPSR gene variation (Domschke et al. 2011). Regarding the T risk allele, a significant gene-environment interaction has been observed explaining increased anxiety sensitivity (Klauke et al. 2014) and altered amygdala responsiveness to aversive stimuli in healthy European participants free from any lifetime history of psychiatric disorders (Dannlowski et al. 2011).

Paradoxically, this particular SNP leads to a gain-of-function in NPSR signaling by increasing its sensitivity to NPS about tenfold (Reinscheid and Xu 2005b). This functional alteration might be explained by increased expression of the Ile107 allele and the related increased cell surface expression of the NPSR (Bernier et al. 2006). This hypothesis has recently been confirmed in a psychopathological animal model, in which a synonymous SNP in the coding region caused a higher cAMP response to NPS stimulation in HAB rats and HAB mice (Slattery et al. 2015). Thus, it is likely that the Ile107 allele, characterized by increased agonist sensitivity, might lead to an overstimulation of the previously described NPS-sensitive circuits (Okamura et al. 2007; Raczka et al. 2010) and, hence, contribute to the development of anxiety- and stress-related disorders. Conserved across rodent models and humans, this particular SNP has been found to be associated with hyperanxiety in HAB rodents, impaired cued-fear extinction in HAB rats, enhanced fear expression in HAB mice, increased anxiety sensitivity and altered amygdala responsiveness to aversive stimuli in humans, respectively.

Concluding remarks and future perspectives

Neuropeptides have been recognized to play a crucial role in the etiology of anxiety- and fear-related disorder (Mathew et al. 2008; Neumann and Landgraf 2012). Although from the evolutionary point of view, anxiety, fear and arousal are beneficial for survival, they need to be in perfect balance and the NPS system has been identified as an essential factor for regulating these responses but also to contribute to dysregulation and psychopathologies.

As described above, NPS potently promotes anxiolytic and fear-attenuating responses. Thus, the brain NPS system represents a potential target system for the development of new therapeutic approaches (e.g., specific blood-brain-barrier-permeable NPSR agonists/antagonists). As a prerequisite, detailed mechanisms regarding NPSR-mediated interneuronal

signaling pathways and interactions with other neurotransmitters and neuromodulators underlying the physiological and more importantly behavioral responses are essentially needed. Future studies will, therefore, need to address the functional connectivity of NPS-synthetizing neurons and the release patterns of NPS within distinct brain regions under various physiological and stress conditions and demonstrate local NPSR and their related intracellular signaling pathways in order to draw a complete picture of neuromodulatory processes mediated by NPS that encode a balanced emotional state.

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