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Amygdala, neuropeptides, and chronic pain-related affective behaviors

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Abstract

Neuropeptides play important modulatory roles throughout the nervous system, functioning as direct effectors or as interacting partners with other neuropeptide and neurotransmitter systems. Limbic brain areas involved in learning, memory and emotions are particularly rich in neuropeptides. This review will focus on the amygdala, a limbic region that plays a key role in emotional-affective behaviors and pain modulation. The amygdala is comprised of different nuclei; the basolateral (BLA) and central (CeA) nuclei and in between, the intercalated cells (ITC), have been linked to pain-related functions. A wide range of neuropeptides are found in the amygdala, particularly in the CeA, but this review will discuss those neuropeptides that have been explored for their role in pain modulation. Calcitonin gene-related peptide (CGRP) is a key peptide in the afferent nociceptive pathway from the parabrachial area and mediates excitatory drive of CeA neurons. CeA neurons containing corticotropin releasing factor (CRF) and/or somatostatin (SOM) are a source of long-range projections and serve major output functions, but CRF also acts locally to excite neurons in the CeA and BLA. Neuropeptide S (NPS) is associated with inhibitory ITC neurons that gate amygdala output. Oxytocin and vasopressin exert opposite

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Authors' contributions

All authors contributed various sections of this review article and reviewed the final manuscript.

Competing interests

There are no conflicts of interest.

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(inhibitory and excitatory, respectively) effects on amygdala output. The opioid system of mu, delta and kappa receptors (MOR, DOR, KOR) and their peptide ligands (β-endorphin, enkephalin, dynorphin) have complex and partially opposing effects on amygdala function. Neuropeptides therefore serve as valuable targets to regulate amygdala function in pain conditions.

Keywords

CGPR; CRF; opioids; vasopressin; oxytocin; amygdala; pain

1 Amygdala function in pain

A limbic brain region the amygdala plays an important role in emotional-affective aspects of behaviors, stress integration, and related disorders such as anxiety, depression, and addiction, as well as pain modulation. The amygdala consists of anatomically and functionally distinct nuclei (Pape and Pare, 2010; Janak and Tye, 2015). The circuitry relevant for pain-related functions includes the basolateral complex (BLA), the central nucleus (CeA) and the intercalated cell clusters (ITC) interposed between BLA and CeA (Neugebauer et al., 2004; Thompson and Neugebauer, 2017; Thompson and Neugebauer, 2019) (Fig. 1). The CeA serves major amygdala output functions through projections to effector systems in the brainstem, hypothalamus and basal forebrain regions (Janak and Tye, 2015; Li and Sheets, 2018; LeDoux, 2007; Fadok et al., 2018). The CeA receives pain-related information indirectly from thalamic and cortical areas through the BLA complex and more direct nociceptive inputs through the spino-parabrachio-amygdaloid pathway (Neugebauer et al., 2004; Kato et al., 2018; Bernard et al., 1996; Veinante et al., 2013; Gauriau and Bernard, 2002; Thompson and Neugebauer, 2017; Thompson and Neugebauer, 2019).

The CeA contains neurochemically and functionally distinct populations of neurons, including most notably neurons expressing somatostatin (SOM), protein kinase C delta (PKCδ), and corticotropin releasing factor (CRF), with some CRF neurons co-expressing SOM or dynorphin (McCullough et al., 2018; Fadok et al., 2017; Kim et al., 2017; Haubensak et al., 2010; Pomrenze et al., 2015). These GABAergic neurons are closely interconnected (Fadok et al., 2017; Hunt et al., 2017), but CRF neurons (Marcilhac and Siaud, 1997; Pomrenze et al., 2015; Reyes et al., 2011; McCall et al., 2015) and SOM neurons (Penzo et al., 2014; Ye and Veinante, 2019) also form long range projections to extra-amygdalar targets. These neurons are found in the lateral and capsular divisions of the CeA (CeLC), the target of the nociceptive parabrachial input. CeLC neurons respond exclusively or predominantly to noxious stimuli (Neugebauer and Li, 2002; Bernard et al., 1992), and therefore this division has been termed the “nociceptive amygdala” (Neugebauer et al., 2004). Amygdala output neurons in the CeLC and medial CeA are normally controlled by feedforward inhibition that involves intercalated (ITC) cells; ITC cells are targeted directly or indirectly by (prefrontal) cortical influences important for behavioral control (Royer and Pare, 2002; Duvarci and Pare, 2014).

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Pain-related changes in amygdala activity are now well documented in rodents (for recent reviews see Thompson and Neugebauer, 2017; Thompson and Neugebauer, 2019) and humans (reviewed in Simons et al., 2014). Enhanced excitatory synaptic transmission at the parabrachial and BLA inputs to the CeLC has been recorded in various models of inflammatory and neuropathic pain using brain slice preparations that are disconnected from the site of injury and peripheral and spinal nociceptive centers in different pain models, including formalin test (Adedoyin et al., 2010; Shinohara et al., 2017), knee joint arthritis (Neugebauer et al., 2003), colitis (Han and Neugebauer, 2004), and nerve injury (Ikeda et al., 2007). Therefore, these changes that are preserved in ex vivo preparations are referred to as pain-related synaptic plasticity, which translates into CeA neuronal hyperactivity in acute (Neugebauer and Li, 2003) and chronic (Goncalves and Dickenson, 2012; Ji et al., 2017) pain models. Synaptic plasticity driven hyperactivity of CeA neurons has been linked mechanistically to pain-related behaviors in different models, using interventions that modulate neuronal activity (for reviews see Neugebauer et al., 2004; Veinante et al., 2013; Thompson and Neugebauer, 2017; Thompson and Neugebauer, 2019). However, the amygdala has also been linked to antinociception under certain conditions (Itoga et al., 2016; Oliveira and Prado, 2001; Avegno et al., 2018) and stress-induced or conditioned forms of analgesia involving neuropeptides such as CRF, enkephalin and nociceptin/orphanin FQ peptide (Fox and Sorenson, 1994; Koob, 2008; Veinante et al., 2013; Ortiz et al., 2007; Helmstetter, 1992; Helmstetter and Bellgowan, 1993). A recent study showed cell-type specific differential contributions of CeA neurons to pro- and anti-nociceptive amygdala functions (Wilson et al., 2019).

It should be noted that nociceptive neurons have also been identified in the BLA, and their activation in pain conditions is linked to pain-like behaviors (Corder et al., 2019; Ji et al., 2010). BLA neuronal hyperactivity in pain results in abnormally enhanced feedforward inhibition of medial prefrontal cortical pyramidal cells, resulting in cognitive deficits in a decision-making task (Ji et al., 2010; Ji and Neugebauer, 2014; Kiritoshi et al., 2016). BLA projections to the CeA provide synaptic excitation as well as feedforward inhibition of CeLC neurons, and there is a shift towards excitation in pain conditions (Ren and Neugebauer, 2010; Ren et al., 2013). The medial prefrontal cortex is also a source of feedforward inhibition of CeLC neurons through projections to BLA or ITC neurons, and this inhibitory control is decreased in pain (Kiritoshi and Neugebauer, 2018). The role of feedforward inhibition of individual CeA cell types in different aspects (sensory and affective) pain-related behaviors remains to be determined

Importantly, the amygdala, particularly the CeA, is rich in neuropeptides, which have been identified as important excitatory or inhibitory modulators of pain-related neuroplasticity and pain behaviors. In this review, we focus on the CeA and neuropeptide systems that have been explored for their role in pain modulation (CGRP, CRF, NPS, oxytocin/vasopressin, and opioids). While the amygdala contains other peptides such as SOM, neuropeptide Y, vasoactive intestinal peptide (VIP), hypocretin/orexin, and nociceptin/orphanin FQ peptide, their role in pain modulation remains to be determined.

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2 CGRP

2.1 CGRP and CGRP receptor

Calcitonin gene-related peptide (CGRP), a 37-amino-acid peptide, was discovered in 1982 as an alternative splicing product of the calcitonin gene (Amara et al., 1982). Epigenetic regulation of the CGRP gene (reduced methylation of the CGRP promoter) has been linked to anxiety- and depression like behaviors (Jiao et al., 2013). The CGRP receptor is a G-protein coupled receptor complex of three subunits: the calcitonin receptor-like receptor (CLR) protein, which shares 55% sequence homology with the calcitonin receptor protein; a receptor activity modifying protein (RAMP1), which is important for specificity for CGRP binding and cell surface receptor expression; and the CGRP-receptor component protein (RCP), an intracellular membrane protein that regulates coupling to cAMP signaling (Barwell et al., 2013; Iyengar et al., 2017; Hay et al., 2008; Dickerson, 2013). CGRP receptors couple positively to the cAMP-PKA signaling pathway (Van Rossum et al., 1997).

2.2 CGRP in the amygdala

The main if not exclusive source of CGRP in the amygdala is the afferent input from CGRP-containing neurons in the lateral pontine parabrachial area (PB) (Dobolyi et al., 2005; Kruger et al., 1988; D'Hanis et al., 2007; Palmiter, 2018; Shimada et al., 1989). As part of the spino-parabrachio-amygdaloid pain pathway (Gauriau and Bernard, 2002) PB projections target neurons in the lateral and capsular divisions of the CeA where they form dense basket-like pericellular arborizations around somata and proximal dendrites of CeA neurons (Schwaber et al., 1988; Ye and Veinante, 2019; Honkaniemi et al., 1990), including a substantial proportion (35–42%) of those that express corticotropin releasing factor (CRF) (Harrigan et al., 1994). CGRP terminals also form synaptic contacts with enkephalin containing cells (Shimada et al., 1992), which are distinct from CRF neurons (Veinante et al., 1997) and include PKCδ neurons (Haubensak et al., 2010). In fact, PKCδ neurons (~80%) rather than SOM neurons are innervated by CGRP terminals from PB, but SOM neurons receive non-CGRP input from PB (Ye and Veinante, 2019). CGRP containing thalamic neurons (posterior intralaminar complex) project to the amygdalostriatal area but not to other amygdala nuclei (D'Hanis et al., 2007; but see Yasui et al., 1991). CGRP axon terminals in the CeA costore neurotensin (Honkaniemi et al., 1990), substance P and brain-derived neurotrophic factor (BDNF) (Salio et al., 2007). No CGRP containing neurons have been found in the amygdala except for low density labeling in the posterior medial amygdala (Schwaber et al., 1988; Skofitsch and Jacobowitz, 1985). CGRP receptor binding sites (Van Rossum et al., 1997; Inagaki et al., 1986), CRLR (Oliver et al., 1998) and RCP (Ma et al., 2003) are present at high densities in the amygdala (CeA and BLA). Interestingly, an age-dependent decrease of CGRP immunoreactivity (Andreose et al., 1994) but not binding sites (Guidobono et al., 1989) was found in the amygdala. Genetic labeling strategies detected CGRP receptor expression in the lateral and capsular divisions including predominantly on PKCδ neurons and at a lower level on SOM neurons (Han et al., 2015).

2.3 Neuronal actions in the amygdala

Exogenous CGRP application to amygdala brain slices from normal rats increased excitatory transmission at the parabrachio-amygdaloid synapse and excitability of CeLC neurons. The

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facilitatory effect was due to a postsynaptic action based on electrophysiological analyses; and pharmacological manipulations suggested the involvement of N-methyl-D-aspartate (NMDA) receptors and protein kinase A (PKA) but not protein kinase C (PKC) (Han et al., 2010). More recently, the facilitatory effect of CGRP on NMDA receptor mediated currents at the parabrachio-amygdaloid synapse was shown directly in brain slices from naive mice and was confirmed to be dependent on PKA activation; there was no effect on alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Okutsu et al., 2017). The data suggest that CGRP can strengthen the synaptic drive onto amygdala neurons, which may play an important role in functional pain disorders in the absence of any tissue pathology.

In an arthritis pain model (kaolin/carrageenan-induced knee joint arthritis), single-unit recordings of CeLC neurons in anesthetized rats found that pharmacological blockade of CGRP receptors with selective antagonists ($CGRP_{8–37}$ and BIBN4096BS) in the amygdala inhibited neuronal activity that was increased 6 hours after induction of the knee joint arthritis; the antagonists had little effects under normal conditions (Han et al., 2005). In brain slices from arthritic rats, CGRP receptor antagonists inhibited synaptic plasticity at the parabrachio-amygdaloid synapse through a PKA-dependent postsynaptic mechanism. CGRP receptor blockade also decreased NMDA receptor mediated currents and neuronal excitability. No significant effects were found in brain slices from normal animals (Han et al., 2005). Importantly, potentiation at the parabrachio-amygdaloid synapse in the formalin pain model (6 hours postinduction) was significantly attenuated in CGRP knockout mice (Shinohara et al., 2017).

2.4 Behavioral effects in the amygdala

The electrophysiological data suggest that CGRP in the amygdala could potentially link nociception to emotional processing (Okutsu et al., 2017). Behavioral studies showed that CGRP administered stereotaxically into the CeA of awake rats increased emotional responses (audible and ultrasonic vocalizations) and induced mechanical hypersensitivity (decreased hindlimb withdrawal thresholds) (Han et al., 2010). Behavioral effects of CGRP were blocked by a PKA but not PKC inhibitor, which is consistent with the electrophysiological data implicating NMDA receptors and PKA in the facilitatory effects of CGRP (see 2.3). Interestingly, while these studies targeted the right CeA, administration of CGRP into the left CeA had antinociceptive effects on thermal and mechanical withdrawal thresholds (Xu et al., 2003), which may suggest hemispheric lateralization.

In a rat arthritis pain model, a CGRP receptor antagonist ($CGRP_{8–37}$) administered into the CeA inhibited mechanical hypersensitivity (hindlimb withdrawal reflexes) and emotional responses (vocalizations) 6 hours postinduction (Han et al., 2005). Mechanical sensitivity in the formalin test was significantly decreased 6 hours postinduction in CGRP knockout mice whereas nocifensive behavior was only decreased in the first 20–25 min (Shinohara et al., 2017). The data suggest that certain pain behaviors depend on CGRP-mediated amygdala plasticity.

In conclusion, CGRP in the amygdala is closely aligned with input from the spino-parabrachio-amygdaloid pathway, acts on certain CeA cell types (PKCδ and CRF rather than

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SOM), and plays an important role as a modulator of synaptic plasticity and sensory and affective behaviors in pain conditions.

3 CRF

3.1 CRF and CRF1 and CRF2 receptors

Corticotropin releasing factor (CRF), also referred as corticotropin releasing hormone (CRH), is a 41-amino-acid peptide involved in neuroendocrine, autonomic and behavioral stress responses (Koob, 2009; Schreiber and Gilpin, 2018) that was first isolated and characterized in 1981 (Vale et al., 1981). CRF is secreted from parvocellular neuroendocrine neurons in the paraventricular nucleus of the hypothalamus (PVN) in response to stressors, and triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the secretion of glucocorticoids from the adrenal glands (Dedic et al., 2018a). Outside the hypothalamus, CRF is found at particularly high levels in the central nucleus of amygdala (CeA), and the bed nucleus of the stria terminalis (BNST), playing a neuromodulatory role in synaptic functions (Dabrowska et al., 2013; Morin et al., 1999). CRF exerts its biological roles through G-protein coupled CRF1 and CRF2 receptors, which share high (70%) sequence homology but have different pharmacological profiles and distribution in the CNS and periphery, suggesting that they are involved in different functions (Korosi et al., 2006; Dedic et al., 2018a; Dautzenberg and Hauger, 2002; Chalmers et al., 1995). CRF and the closely related (43% homology) peptide urocortin 1 are the endogenous ligands for CRF1 whereas urocortins 2 and 3 bind selectively to CRF2. Urocortin1 has equal affinity for CRF1 and CRF2 whereas CRF has low affinity for CRF2. Both receptors are expressed in the CNS but CRF2 is predominantly found in peripheral tissues. CRF1 and CRF2 receptors can couple to multiple G-proteins, mainly to the cAMP–PKA signaling pathway, but they typically mediate opposing effects (Koob and Zorrilla, 2012; Blank et al., 2003; Reul and Holsboer, 2002). Availability of CRF and urocortin 1 can be regulated by the CRF binding protein (Seasholtz et al., 2001; Behan et al., 1995), but its role is still not well understood (Dedic et al., 2018a).

3.2 Expression in the amygdala

The amygdala is one of the extra-hypothalamic brain regions with the highest density of CRF cell bodies. CRF containing neurons within the amygdala are mainly localized in the lateral part of the CeA (CeL) (Merchenthaler et al., 1982; Veinante et al., 1997; Gray and Magnuson, 1987) and project to the dorsal and ventral bed nucleus of the stria terminalis (Sakanaka et al., 1986; De et al., 2019), several hypothalamic nuclei (Pomrenze et al., 2015), brainstem regions such as ventral tegmental area (Dedic et al., 2018b; Pomrenze et al., 2015), periaqueductal gray (Pomrenze et al., 2015; Gray and Magnuson, 1992), locus coeruleus (Reyes et al., 2011; van Bockstaele et al., 1998), parabrachial nuclei (Moga and Gray, 1985; Pomrenze et al., 2015),and dorsal nucleus of the vagus nerve (Gray and Magnuson, 1987) as well as locally to the medial CeA (CeM) through direct or indirect (non-CRF) projections (Pomrenze et al., 2015). CRF expressing neurons in CeA are GABAergic and co-localize with other neuropeptides such as dynorphin, somatostatin, and neurotensin, but not with enkephalin or PKCδ (Veinante et al., 1997; Fadok et al., 2017; Pomrenze et al., 2015; Sanford et al., 2017; Marchant et al., 2007; Day et al., 1999). A

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substantial proportion (35–42%) of CRF neurons in the lateral and capsular CeA receive input from CGRP containing PB afferents (Harrigan et al., 1994). CRF mRNA and protein expression in the CeA is increased in neuropathic pain models (Ulrich-Lai et al., 2006; Andreoli et al., 2017; Rouwette et al., 2012).

Expression of CRF1 and CRF2 receptor mRNA and immunoreactivity has been detected in the amygdala. In BLA and CeA, substantial CRF1 expression is found, whereas CRF2 expression is generally low in these nuclei (Chen et al., 2000; Chalmers et al., 1995; Van Pett et al., 2000). In CeA, CRF1 is expressed in GABAergic neurons containing peptides such as enkephalin, somatostatin, dynorphin or CRF as well as in PKCδ neurons (Wolfe et al., 2019), where CRF1 immunoreactivity is largely postsynaptic at non-CRF excitatory synapses but apposing CRF terminals (Treweek et al., 2009). In BLA, CRF1 is expressed on GABAergic parvalbumin and cholecystokinin, but not SOM, interneurons (Calakos et al., 2017); CRF1 expression is also associated with glutamatergic neurons (Savarese and Lasek, 2018; Pliota et al., 2020).

3.3 Neuronal actions

CRF administration into the CeA at low concentrations increased, but at higher concentrations decreased, activity of CeLC neurons recorded in anesthetized rats; the facilitatory and inhibitory effects were blocked with antagonists for CRF1 (NBI27914) and CRF2 (Astressin-2B) receptors, respectively, suggesting opposing functions of the CRF receptor subtypes (Ji and Neugebauer, 2008). Interesting, pharmacological blockade of CRF2 but not CRF1 receptors increased activity of CeLC neurons recorded in vivo (Ji and Neugebauer, 2007), suggesting a CRF2 receptor-mediated inhibitory tone under normal conditions. Patch-clamp recordings of CeLC neurons in brain slices from normal rats showed that exogenous CRF potentiated transmission at the PB-CeLC synapse, activating a latent NMDA component, and this postsynaptic effect was mediated by CRF1 but not CRF2 receptors (Ji et al., 2013). The facilitatory effects of CRF involved PKA but not PKC activation as tested with respective inhibitors (Ji and Neugebauer, 2008; Ji et al., 2013). In medial CeA (CeM) neurons, CRF had differential effects on glutamatergic transmission that were mediated by presynaptic CRF1 receptors, increasing vesicular release but decreasing evoked transmission (Varodayan et al., 2017; Ji et al., 2013). CRF also increased GABAergic inhibitory transmission onto CeM neurons through CRF1 receptors (Nie et al., 2004; Roberto et al., 2010). On the other hand, chemogenetic activation of CRF neurons in the CeA induced FOS, a maker of neuronal activity, in non-CRF CeM and CeL neurons through CRF1 receptors, whereas optogenetic activation of CRF neurons evoked GABAergic responses in non-CRF CeM and CeL neurons (Pomrenze et al., 2015). The data suggest a complex intra-amygdala CRF circuitry of opposing excitatory and inhibitory elements.

In the kaolin/carrageenan-induced knee joint arthritis pain model, a CRF1, but not CRF2, receptor antagonist (NBI27914 and Astressin-2B, respectively) decreased hyperactivity (“sensitization”) of CeLC neurons (Ji and Neugebauer, 2007), suggesting that CRF1 receptors are activated endogenously in this pain condition. Pharmacological blockade of CRF1 receptors in the BLA also reduced neuronal sensitization of BLA (Ji et al., 2010) and

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CeLC (Ji and Neugebauer, 2019) neurons in models of arthritis and neuropathic pain, respectively. Patch-clamp analysis in brain slices found that NBI27914 inhibited increased excitatory transmission at the PB-CeLC and BLA-CeLC synapses in brain slices from arthritic rats through a postsynaptic mechanism that involved an action on PKA-dependent NMDA-mediated synaptic transmission (Fu and Neugebauer, 2008). NBI27914 also inhibited neuronal excitability of CeLC neurons through inhibition of highly tetraethylammonium (TEA)-sensitive ion channels such as Kv3 potassium channels. In contrast, Astressin-2B facilitated synaptic transmission through presynaptic inhibition of GABAergic transmission, resulting in decreased activation of postsynaptic $GABA_A$ receptors and decreased presynaptic $GABA_B$ receptor-mediated inhibition of glutamatergic transmission (disinhibition) (Fu and Neugebauer, 2008). Therefore, endogenous CRF1 receptor activation in pain conditions is an important mechanism of increased drive and output of CeLC neurons.

3.4 Behavioral effects

The amygdala CRF system plays an important role in the coordination of behavioral and emotional responses to stress (Schreiber and Gilpin, 2018) and has been linked to disorders of stress, fear, anxiety, depression, and alcohol and substance use disorders (Pomrenze et al., 2019; Koob and Zorrilla, 2010; Zobel et al., 2000; Regev and Baram, 2014; Gafford and Ressler, 2015; Pliota et al., 2020). CRF has also emerged as a critical modulator of pain-related neuroplasticity (see 3.3) and behavior (Lariviere and Melzack, 2000; Neugebauer, 2015; Moloney et al., 2016; Greenwood-Van Meerveld et al., 2005; McNally and Akil, 2002).

CRF administered into the CeLC of normal animals increased nocifensive reflexes and vocalizations evoked by innocuous and noxious mechanical stimuli (compression of the knee joint); the facilitatory effects were blocked by NBI27914 or by a PKA, but not PKC, inhibitor (Ji et al., 2013). Increasing availability of CRF in control animals with a CRF-binding protein inhibitor ($CRF_{6–33}$) increased mechanosensitivity (von Frey test) but not averse-affective behaviors (place-avoidance test) (Bourbia et al., 2010). Increased CRF expression in the CeA also produced mechanical and visceral hypersensitivity that was blocked by CRF knockdown in the CeA (Johnson et al., 2015). Interestingly, high doses of CRF in the CeA had antinociceptive effects on thermal and mechanical sensitivity tests (Cui et al., 2004), which is consistent with the dual effects of CRF in electrophysiological studies (see. 3.3).

CRF in the CeA has also been implicated in stress induced analgesia and stress-induced hyperalgesia in different models. Rats exposed to predator odor stress in a conditioned place avoidance test showed thermal hyperalgesia, which was reversed by a CRF1 receptor antagonist (R121919) injected into the CeA and was mimicked by CRF administration into the CeA (Itoga et al., 2016). Knockdown of CRF in the CeA also attenuated visceral hypersensitivity (colorectal distention) and mechanical hypersensitivity (von Frey test) in the repeated water avoidance stress paradigm (Johnson et al., 2015). CRF knockdown or CRF1 receptor blockade (CP376395) in the CeA attenuated visceral hypersensitivity in a model of neonatal early life stress (early life stress) (Prusator and Greenwood-Van Meerveld, 2017).

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Forced swim resulted in stress-induced analgesia measured in the tail flick test in normal animals, which was increased by chemogenetic activation of CRF neurons in the CeA (Andreoli et al., 2017). Administration of CRF1 receptor antagonists (R121919 or MPZP) into the CeA also prevented or decreased nicotine withdrawal abstinence-induced thermal hyperalgesia (Baiamonte et al., 2014) and anxiety-like behavior (elevated plus maze) and mechanical hypersensitivity (von Frey test) (Cohen et al., 2015). Morphine withdrawal-associated thermal hyperalgesia was also reduced by intra-CeA administration a CRF1 receptor antagonist (α-hCRH$_{9–41}$) (McNally and Akil, 2002).

Several lines of evidence suggest that abnormal activation of the CRF system in the amygdala contributes to pain conditions and chronification. Blockade of CRF1 receptors in the CeA with NBI27914 inhibited mechanical hypersensitivity, emotional responses (vocalizations to noxious stimuli) and anxiety-like behaviors (elevated plus maze) in the kaolin/carrageenan model of arthritis pain but had no effect in normal animals (Fu and Neugebauer, 2008; Ji et al., 2007). A CRF2 receptor antagonist (Astressin-2B) had no effect. In a neuropathic pain model, chemogenetic inhibition of CRF neurons decreased mechanical hypersensitivity and restored forced swim stress-induced analgesia (Andreoli et al., 2017). A non-selective CRF receptor antagonist (CRF$_{9–41}$) microinjected into CeA decreased thermal hypersensitivity (tail flick test) associated with morphine withdrawal but had no effect under normal conditions (McNally and Akil, 2002). On the other hand, CRF$_{9–41}$ administrated into the CeA had no effect on mechanical hypersensitivity (von Frey test) and averse-affective behaviors (conditioned place-avoidance test) in the spared nerve injury model of neuropathic pain (Bourbia et al., 2010), but a CRF binding protein inhibitor CRF$_{6–33}$ attenuated aversive behavior while increasing mechanical hypersensitivity.

The neural circuitry in the amygdala mediating CRF signaling related to pain behaviors remains to be determined, but endogenous activation of CRF1 receptors in the CeA contributes to avers-affective and sensory pain behaviors in different conditions.

4 Neuropeptide S (NPS)

4.1 NPS and NPS1 receptor

Neuropeptide S (NPS) consists of 20 amino acids and is named after an N-terminal serine residue that is highly conserved across different species, including humans. The N-terminal sequence Ser-Phe-Arg-Asn-Gly-Val-Gly is identical in all species (Xu et al., 2004). The NPS receptor (NPSR), a typical member of the G-protein-coupled receptor superfamily, was first identified as an asthma susceptibility gene (formerly GPR154) (Gloriam et al., 2005). NPS binds with high affinity to NPSR coupled to either Gq or Gs to increase intracellular calcium and cAMP-PKA signaling (Guerrini et al., 2010; Reinscheid, 2008).

4.2 Expression in the amygdala

NPS and its receptor NPSR are mainly expressed in the brain (Reinscheid, 2008; Xu et al., 2007). NPS precursor mRNA and NPS peptide are strongly expressed in discrete brainstem nuclei, namely the locus coeruleus (LC) area and lateral parabrachial nucleus (PB) in rats and mice and the principal sensory trigeminal nucleus in the rat (Clark et al., 2011; Xu et al.,

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2007). Many of the PB neurons co-express NPS and CRF (Xu et al., 2007). NPS fibers project to limbic and thalamic areas such as amygdala, hypothalamus and paraventricular thalamic nucleus (Clark et al., 2011). Levels of NPS in the amygdala showed a time-dependent decrease over 3 weeks in a neuropathic pain model (SNL) (Yang et al., 2016).

NPSR is more widely expressed than NPS in limbic, thalamic and cortical regions, including the amygdala in rat and mouse (Leonard and Ring, 2011; Xu et al., 2007; Clark et al., 2011). In the rat amygdala, highest level of NPSR mRNA is found in and around ITC cells, while NPSR is absent in other amygdala nuclei in the rat (LA, BLA, and CeA) relevant for pain processing (Xu et al., 2007; Leonard and Ring, 2011). In the mouse amygdala, NPSR appears to be expressed in BLA rather than the adjacent ITC (Clark et al., 2011).

4.3 Neuronal actions

Electrophysiological recordings in anesthetized rats showed that NPS administered nasally or stereotaxically into the ITC area inhibited the activity of CeA neurons in an arthritis pain model (kaolin/carrageenan-induced knee joint arthritis). The inhibitory effects of NPS were blocked by stereotaxic administration of a selective NPR antagonist ([D-Cys(tBu)^5]NPS) (Medina et al., 2014), identifying the ITC as a site of action. GABAergic ITC cells play an important role in the control of amygdala out through feedforward inhibition (Pape and Pare, 2010; Royer and Pare, 2002).

Synaptic mechanism of NPS actions in the amygdala were analyzed in brain slice physiology studies. NPS increased feedforward inhibition of CeA neurons through a presynaptic action on glutamatergic transmission to ITC cells in the mouse amygdala, because NPS increased frequency but not amplitude of mEPSCs and reduced paired pulse facilitation (Jungling et al., 2008). NPS also increased feedforward inhibition of CeA neurons in brain slices from arthritic rats, but this effect involved a direct action on ITC cells based on miniature EPSC analysis, and it was blocked by an inhibitor of PKA (KT5720) but not PKC GF109203x; there was no effect on BLA neurons (Ren et al., 2013). The differential actions in rat and mouse could be explain with different expression patterns (see 4.2).

4.4 Behavioral effects

NPS has been shown to modulate multiple central functions including food intake, alcohol and drug addiction, social behavior, locomotor activity, arousal, wakefulness, memory processes, fear and anxiety (Kallupi et al., 2010; Lukas and Neumann, 2012; Ionescu et al., 2012; Ruzza et al., 2012; Pulga et al., 2012; Zhao et al., 2019; Zhao et al., 2012; Pulga et al., 2012; Slattery et al., 2015; Wegener et al., 2011; Fendt et al., 2010; Donner et al., 2010; Zoicas et al., 2016; Peng et al., 2010a; Reinscheid, 2008; Pape et al., 2010; Smith et al., 2006; Ruggeri et al., 2010). The anxiolytic and fear extinction effects of NPS in particular have been linked to an action in the amygdala based on the results obtained with direct injections of NPS into the amygdala (Fendt et al., 2010; Chauveau et al., 2012)

Antinociceptive effects of NPS were found in mice with intracerebroventricular administration of NPS in the tail-flick, hot-plate and both phases of the formalin tests (Li et al., 2009; Holanda et al., 2015; Holanda et al., 2019; Peng et al., 2010b). Systemic

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application of antagonists for dopamine D1 (SCH 23390) or D2 (sulpiride) (Holanda et al., 2019) and intracerebroventricular injection of antagonists for adenosine A1 (DPCPX) or A_{2A} (ZM241385) receptors blocked the effects of NPS. The amygdala was identified as a critical site of action for inhibitory effects of NPS on pain-like behaviors. Administration of NPS into the ITC, but not CeA, decreased emotional responses (vocalizations to noxious stimuli) and anxiety-like behavior (elevated plus maze) of rats in the kaolin/carrageenan-induced knee joint arthritis pain model but not under normal conditions (Ren et al., 2013). Similar behavioral effects were found with nasal application of NPS (Medina et al., 2014). The inhibitory effects of intra-ITC or nasal application of NPS were blocked by a selective NPSR antagonist ([D-Cys(tBu)^5]NPS) administered stereotaxically into ITC, indicating that activation of NPS receptors in the amygdala mediated the effects of NPS in the pain model. Administration of NPS into the BLA in neuropathic rats (SNL model) attenuated mechanical and thermal hypersensitivity, and these effects were blocked by a selective NPSR antagonist (SHA68). (Yang et al., 2016)

5 Oxytocin (OT) /vasopressin (VP)

5.1 OT and VP and their receptors (OTR, V1aR, V1bR, V2R)

The pituitary neuropeptides OT and VP have an expanding repertoire or putative physiological activity beyond the classical roles in lactation, parturition, and fluid homeostasis, and have been implicated as neuromodulators in a variety of neuropsychiatric disorders such as anxiety and depression (Frank and Landgraf, 2008; Ebstein et al., 2012; Stoop, 2014; Mavani et al., 2015). Data have usually supported a paradigm of mutually opposing VP and OT functions (Neumann and Landgraf, 2012; Carter, 2017; Stoop, 2014). The cyclic (Cys1-Cys6 bridge) nonapeptides OT and VP diverge only in positions 3 and 8, and are thought to have emerged from a gene duplication event early in the bilaterian lineage (Stoop, 2012). VP and OT are produced as part of a precursor protein in magnocellular neuronal populations within the supraoptic (SON), paraventricular (PVN), and accessory nuclei (AN) of the hypothalamus, and also within parvocellular neurons of the suprachiasmatic nucleus (SCN) of the hypothalamus. After posttranslational processing during axonal transport to the posterior pituitary, VP and OT enter the general circulation through synapses of the magnocellular neurons on fenestrated capillaries as demonstrated through retrograde tracing with fluoro-gold (Merchenthaler, 1991), but VP and OT do not generally cross the blood brain barrier from the periphery after release into portal circulation. Outside the hypothalamus, VP is also found in the bed nucleus of the stria terminalis and medial amygdala (Stoop, 2012).

VP shows the highest affinity for VP receptor 1b (V1bR) and lower affinity at V1aR and oxytocin receptor (OTR) in the central nervous system, whereas OT has only been reported to act on central OTR (Manning et al., 2012). V1aR and OTR are abundantly expressed in the brain whereas V2R is expressed primarily in the kidney and there is no convincing evidence for V2R expression in the brain (Dumais and Veenema, 2016; Stoop, 2012). V1aR, V1bR and OTR couple to Gq-proteins to increase intracellular calcium and activate PKC, but OTR can also couple to Gs and Gi/o proteins; V2R couples to Gs to increase adenylyl cyclase (cAMP) levels (Ebstein et al., 2012; Koshimizu et al., 2012; Stoop, 2012).

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Interestingly, expression of VP, V1aR and OT is higher in males than females whereas OT expression is often higher in females (Dumais and Veenema, 2016).

5.2 Expression in the amygdala

Both magnocellular and parvocellular VP and OT neurons have been shown to project to extrahypothalamic sites including the amygdala, and VP neurons have been found in the medial amygdala of rodents but not primates (Johnson and Young, 2017; Albers, 2015). While VP neurons in SCN project strongly to the medial amygdala, VP and OT neurons in PVN project to CeA and BLA (Sofroniew, 1980; Knobloch et al., 2012). OT and VP are released in the CeA in response to forced swim stress (Landgraf and Neumann, 2004; Ebner et al., 2002; Ebner et al., 2005). To date, only PKC-coupled V1aR and OTR have been identified in the amygdala with a distinct expression pattern. OTR is found in the CeLC (on 65% of PKCδ neurons) and in the ITC area, whereas V1aRs exists primarily in CeM neurons (Huber et al., 2005; Haubensak et al., 2010; Veinante and Freund-Mercier, 1997; Stoop et al., 2015).

5.3 Neuronal actions

There is evidence to suggest a mutually opposing functions of VP and OT in the CeA through GABAergic interneurons (Stoop et al., 2015; Huber et al., 2005). Brain slice electrophysiology studies showed that an OTR agonist [Thr^4,Gly^7]-oxytocin (TGOT) activated neurons in the lateral and capsular CeA, which likely include PKCδ (see 5.2), but inhibited projection neurons in the lateral and medial CeA, which include CRF and SOM neurons, respectively; the inhibitory effect involved increased GABAergic transmission (Stoop et al., 2015; Huber et al., 2005). CeA neurons inhibited by OTR activation were activated by VP through an action on V1aR but not V1bR and V2R, because the excitatory effects of VP were blocked by a V1R antagonist $d(CH_2)_5$[$Tyr(Me)^2$,Arg^8]-vasopressin (TMA) but not mimicked by a V1bR agonist [1-deamino-4-cyclohexylalanine]-Argvasopressin (d[Cha]AVP or by a V2R agonist [deamino-Cys^1,Val^4,D-Arg^8]-vasopressin (dVDAVP) (Huber et al., 2005). Optogenetic stimulation of OT-expressing hypothalamic axons in the CeA activated a local GABAergic circuit that inhibited neurons in the CeA output region, which decreased fear-related behaviors in vivo (Knobloch et al., 2012). These findings support an amygdala circuit, in which VP effector neurons of the CeM are under GABAergic control by OTR neurons in the CeLC (Huber et al., 2005; Stoop et al., 2015). Pain-related electrophysiological effects of OT and VP in the amygdala circuitry remain to be determined.

5.4 Behavioral effects

Both VP and OT have well documented antinociceptive effects in tail-flick, hot-plate and formalin tests after systemic, peripheral, intrathecal or intracerebroventricular administration (Koshimizu and Tsujimoto, 2009; Poisbeau et al., 2018), which is in contrast to their generally opposing roles in the regulation of stress responses, fear and anxiety (Neumann and Landgraf, 2012). However, little is known about pain modulation by VP and OT actions in the amygdala. Microinjection of VP into the CeA suppressed an electromyographic nociceptive jaw opening reflex evoked by electrical stimulation (Ahn et al., 2001). The antinociceptive effect was blocked by a V1R antagonist ($d(CH_2)_5$Tyr(Me)AVP) but not a

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V2R antagonist (d(CH_2)$_5$[Ile^2, Ile^4]AVP). Stereotaxic administration of VP into CeA had no effect on mechanosensitivity (nocifensive reflexes) but increased emotional responses (vocalizations to noxious stimuli) and anxiety-like behavior (elevated plus maze), and these effects were blocked by a selective V1aR antagonist (SR 49059, Relcovaptan) but not by an OTR antagonist (L-371,257). L-371,257, but not SR 49059, increased vocalizations, suggesting an OTR-mediated inhibitory tone (Cragg et al., 2016). In the kaolin/carrageenan-induced knee joint pain model, SR 49059 inhibited vocalizations and anxiety-like behavior but had no effect on mechanosensitivity, suggesting that endogenous V1aR activation contributes to emotional-affective rather than sensory aspects of pain conditions. VP by itself had no effect in the pain model but was anxiolytic in the presence of SR49059, implicating OTR in the inhibitory effects of VP (Cragg et al., 2016). There may be a shift from from OTR-mediated inhibition to V1A-mediated facilitation in the pain condition.

6 Opioids

6.1 Opioids and Mu, delta and kappa receptors

Opioid receptors are found throughout the brain and spinal cord in circuit distributions relevant to the modulation of pain. Three highly homologous opioid receptors termed mu (MOR), delta (DOR) and kappa (KOR) have been identified and are encoded by three genes, OPRM1, OPRD1 and OPRK1 (Darcq and Kieffer, 2018). A fourth, structurally related receptor is the ORL-1 receptor that is sometimes considered as a member of the opioid receptor family (Corder et al., 2018). Active ligands for the opioid receptors result from processing of precursors including pro-opiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN). Endogenous opioid receptor ligands all have an N-terminal tyrosine that is essential for binding and a five amino acid motif of Tyr-Gly-Gly-Phe-Leu or Tyr-Gly-Gly-Phe-Met. The endogenous opioid peptides that have received the most study include β-endorphin, Leu- and Met-enkephalin and dynorphin. With the exception of dynorphin, these peptides are relatively non-selective in binding at the MOR and the DOR; the enkephalins have somewhat higher affinity for DOR while β-endorphin has relatively higher affinity for the MOR. Dynorphin was named for its high affinity at the KOR and, while it has significant but lower binding affinity to the MOR, physiological actions of dynorphin appear to be largely mediated at the KOR (Bruchas et al., 2010). Significant separation in affinity is seen with peptides that bind to MOR and DOR from their affinities at KOR. Opioid receptors are generally coupled to inhibitory Gi/o-proteins and produce inhibitory effects on cellular signaling including inhibition of cAMP and N-type calcium channels and opening of potassium channels (Standifer and Pasternak, 1997).

6.2 Expression in the amygdala

Opioid neuropeptides Leu- and Met-enkephalins and dynorphin can be synthetized and released locally by neurons within the amygdala nuclei. These endogenous peptides can also be released from terminals of neurons located in brain regions that innervate the amygdala. Very low levels of POMC mRNA have been detected in the amygdala, but β-endorphin terminals, originating from the arcuate nucleus of the hypothalamus, are found in the CeA (Gray et al., 1984). Enkephalins are therefore more likely the peptides that activate the MOR and DOR in the amygdala promoting physiological effects. *In situ* hybridization studies

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found many proenkephalin (PENK) mRNA expressing neurons in the CeA and intercalated cells (ITC), while in the BLA only few neurons appear to synthetize enkephalins. In the CeA, a subset of enkephalin expressing neurons overlaps with PKC-δ positive cells (Poulin et al., 2008). In the ITC, Met-enkephalin immunoreactivity has been found to be concentrated in dense core vesicles of axons that form synapses onto dendrites or other axon terminals, suggesting both post-synaptic and pre-synaptic effects (Winters et al., 2017). The KOR-preferring ligand dynorphin is synthesized primarily in neurons in the lateral subdivision of the CeA (Marchant et al., 2007). Dynorphin immunolabeling is localized in dendrites, perikarya and rarely in axons of CeA neurons (Kravets et al., 2015). About one-third of the prodynorphin positive neurons co-express CRF (Marchant et al., 2007). Many dynorphin-containing dendrites, including double labeled dynorphin and CRF positive dendrites, receive direct contacts from noradrenergic (NE) afferents (Kravets et al., 2015), providing the anatomical basis for interactions of the NE, CRF and dynorphin systems in stress-related responses.

Opioid peptides act at MOR, DOR and KOR that are all expressed at various levels in the amygdala. Similar to enkephalins, MOR is highly expressed on the ITC cells and by neurons in the CeA, with fewer neurons in the BLA. In contrast, DOR positive neurons are mainly found in the BLA. KOR expressing cells are located in both the BLA and the CeA. MOR is found in some pyramidal neurons and some interneurons in the BLA. Electron microscopic immunolabeling in this region of the amygdala showed a primary location of MOR on dendritic shafts and spines often receiving asymmetric (i.e., excitatory) synapses. Some MORs in the BLA were also identified on axons forming asymmetric synapses on spines. This structural localization suggests that MOR inhibits excitatory inputs to pyramidal neurons (Zhang et al., 2015). In the CeA, MOR is found on neuronal somata, dendrites and axons (Jaferi and Pickel, 2009). MOR containing dendrites and spines in the CeA receive excitatory type synapses, while MOR labeled terminals form symmetric (i.e., inhibitory) synapses, although electrophysiological studies (see 6.3) suggest that MOR activation can inhibit glutamatergic transmission presynaptically (Zhu and Pan, 2005). Some of the MOR neurons, but not axon terminals, in the CeA co-express CRF receptors, consistent with opposing roles of CRF and MOR signaling in pain. Ultrastructural electron microscopic analysis found DOR immunoreactivity on dendritic processes as well as on axon terminals in the BLA and CeA (Reyes et al., 2017). Importantly, in the CeA two thirds of CRF neurons contain DOR, and co-localization of DOR with CRF is found in neuronal profiles in close proximity to noradrenergic afferents, supporting the role of DOR in the inhibition of anxiety-like behavior (Reyes et al., 2017). Although lateralized distribution pattern of opioid peptides and receptors have not been systematically investigated, *in situ* hybridization images in the Allen Brain Atlas (https://mouse.brain-map.org) do not support differential mRNA expression between left and right amygdala nuclei. Additionally, stress increases expression of dynorphin and phosphorylation of KOR in both the right and left CeA even though functional relationship to pain is localized to the right (see below, Xie et al., 2017).

6.3 Neuronal actions

In the BLA, opioid analgesics would be expected to inhibit neuronal activity because of hyperactivity of BLA neurons in pain conditions (Ji et al., 2010; Corder et al., 2019). Brain

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slice electrophysiology studies showed MOR activation hyperpolarized neurons in the lateral amygdala (LA) that were identified as non-pyramidal cells (Sugita et al., 1993). MOR agonists also inhibited presynaptic GABA release onto LA neurons (Sugita and North, 1993), suggesting MOR modulation of local GABAergic interneurons. Another study used retrograde tracing to determine MOR effects on specific CeA-projecting neurons in the BLA. MOR activation was found to attenuate presynaptic GABA release onto neurons that project to CeA (Finnegan et al., 2006). These studies suggest that MOR activity in the LA-BLA network may produce analgesic effects through disinhibition of CeA-projecting neurons. However, other studies have found an inhibitory effect of MOR activation on principal neurons in the BLA. For example, an MOR selective agonist (DAMGO) decreased neuronal activity in LA pyramidal cells by modulating a Kv1.2 containing voltage-dependent potassium channel (Faber and Sah, 2004). MOR action on BLA pyramidal cells would be consistent with inhibitory effects of MOR on glutamatergic transmission onto ITC (Winters et al., 2017) and CeM neurons (Zhu and Pan, 2005), although negative results with MOR agonists (DAMGO) at these synapses have also been reported (Blaesse et al., 2015). A KOR agonist (U-69,593) increased inhibitory synaptic transmission in BLA pyramidal cells from adolescent rats (postnatal day 30–45) but had no effect in adult rats (postnatal day >60); conversely, U-69,593 increased glutamatergic transmission at the adult but not adolescent age (Przybysz et al., 2017). The observed diversity of physiological responses to opioids in distinct populations of BLA neurons may be necessary for encoding of a wide range of behavioral outcomes. Recently, a neural ensemble in the BLA has been identified that encodes the negative affective valence of pain (Corder et al., 2019), but it remains to be determined if and how this ensemble is engaged in opioid analgesia.

ITC cells modulate the flow of information within amygdala microcircuits, can regulate CeA output neurons, and are driven by direct or indirect (prefrontal) cortical influences (see 1.). ITC cells express high levels of opioid peptides and receptors. Brain slice electrophysiology studies found that MOR activation by DAMGO hyperpolarized neurons in medially located ITC by activating a potassium conductance (Blaesse et al., 2015; Winters et al., 2017). MOR activation also inhibited glutamatergic transmission onto ITC cells (Winters et al., 2017). GABAergic ITC-mediated feedforward inhibition of CeM neurons was attenuated by DAMGO, while excitatory transmission was unaffected (Blaesse et al., 2015). Opioid activity within the ITC regions may therefore have a major impact on the contribution of cortical and BLA inputs to pain processing in the CeA.

In the CeM, MOR but not DOR or KOR agonists have been found to inhibit glutamate release from presynaptic glutamatergic terminals (Zhu and Pan, 2005). MOR agonists also reduced GABAergic synaptic inputs to PAG-projecting CeA neurons through a presynaptic mechanism (Finnegan et al., 2005). Similarly, dynorphin or a selective KOR agonist (U69593) decreased inhibitory GABAergic synaptic transmission in CeM neurons (Kang-Park et al., 2013; Gilpin et al., 2014; Kang-Park et al., 2015), and this effect was not age dependent (Przybysz et al., 2017). Although activation of MOR and KOR elicits similar cellular effects, physiologically distinct populations of CeA neurons are inhibited by MOR and KOR agonists (Chieng et al., 2006). Differential expression of these receptors on CeA neurons and synapses suggests potentially opposing behavioral effects of MOR and KOR agonists.

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6.4 Behavioral effects

Most studies on opioid modulation of pain in the CeA have emphasized the role of the MOR. Some studies report inhibition of the rat tail-flick response following CeA microinjection of morphine (Rodgers, 1978; Pavlovic et al., 1996) or β-endorphin (Pavlovic et al., 1996). On the other hand, minimal effects in the modulation of acute pain were observed with administration of morphine into CeA but more prominent effects following administration into BLA (McGaraughty and Heinricher, 2002). Notably, following microinjection into the CeA, morphine and β-endorphin were significantly more effective in the jump test than in the tail-flick test, suggesting preferential effects in modulating affective components of acute pain (Pavlovic et al., 1996). The analgesic effects of MOR activation in the CeA were inhibited by a general opioid antagonist (naltrexone) and antagonists for MOR (beta-funaltrexamine) and DOR (naltrindole isothiocyanate) in the vlPAG, implicating inhibition of CeA output to a key descending modulatory region (Pavlovic et al., 1996). Additional evidence implicates MOR in the vlPAG in antinociceptive (Oliveira and Prado, 2001) and pronociceptive effects of CeA stimulation (Avegno et al., 2018). Studies using models of pain that are associated with tissue injury, including formalin, reported contributions of CeA MOR to systemic morphine analgesia that were primarily observed as inhibition of the second (tonic) phase of the formalin assay, consistent with contributions to ongoing pain (Manning and Mayer, 1995).

Information about consequences of DOR activation in the CeA on acute pain is very limited, and this receptor has been primarily associated with circuits relevant to anxiety (Welsch et al., 2020). In contrast, KOR in the CeA has been linked to negative affective states associated with stress (Smith et al., 2012; Tejeda et al., 2017) and with ongoing pain (Liu et al., 2019; Navratilova et al., 2019a). In the absence of injury or sensitization, KOR antagonists do not produce analgesia or hyperalgesia (Xie et al., 2017; Phelps et al., 2019). Strong evidence suggests that stress can upregulate dynorphin in the brain. Following repeated stress, there is increased phosphorylation of the KOR in both the right and left CeA (Xie et al., 2017) that likely results from increased dynorphin release secondary to CRF (Bruchas et al., 2009). In uninjured animals, stress produces allodynia that is prevented or reversed by microinjections of KOR antagonists into the CeA (Xie et al., 2017). Interestingly, in spite of phosphorylation of KOR in both the right and left CeA, blockade of stress-induced allodynia is observed following administration of KOR antagonists in the right, but not left, CeA, suggesting a lateralized pronociceptive effect of KOR signaling (Xie, De Felice et al. 2017). Hemispheric lateralization of CeA-mediated pain modulation has been reported previously in some pain conditions (Carrasquillo and Gereau, 2008; Goncalves and Dickenson, 2012; Ji and Neugebauer, 2009) but there is evidence for a different role of the left CeA that remains to be determined (Sadler et al., 2017; Cooper et al., 2018). Additionally, stress elicits a priming effect that promotes vulnerability to subsequent provocative stimuli including additional episodes of stress. The consequences of repeated stress priming may play a role in promoting the transition of episodic to chronic conditions including functional pain syndromes such as migraine (Xie et al., 2017). As KOR antagonists in the CeA block the priming actions of repeated stress, these compounds have been suggested to be useful as preventative treatments for stress-related functional pain syndromes (Xie et al., 2017).

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In humans, KOR agonists are both aversive (Freeman et al., 2014) and analgesic (Coffeen and Pellicer, 2019) possibly reflecting actions in supraspinal and spinal circuits, respectively (Pande et al., 1996). Recent studies suggest that the negative affective, but not sensory, aspects of experimental ongoing pain are due to KOR signaling in the CeA (Phelps et al., 2019) and other brain regions (Liu et al., 2019; Massaly et al., 2019). Following peripheral nerve injury, antagonism of KOR in the right, but not left, CeA prevented the motivation to seek relief from gabapentin in the conditioned place preference assay without affecting allodynia (Bannister et al., 2017). A physiological basis for these effects was provided by the observation that KOR antagonism decreased synaptically evoked spiking of CeA neurons in brain slices, suggesting restoration of feedforward inhibition of CeA output neurons (Navratilova et al., 2019a).

While MOR agonists have produced variable analgesic responses in uninjured animals, recent studies demonstrated that microinjection of morphine into the CeA of animals with experimental neuropathic pain can modulate the affective, but not sensory, dimensions of ongoing pain (Navratilova et al., 2019b). Like the effects of KOR signaling, this effect was lateralized to the right, but not left, CeA so that morphine microinjection was able to elicit conditioned place preference in neuropathic animals with spinal nerve injury. A MOR agonist in the right or left CeA had no effect on nerve-injury induced allodynia or mechanical hyperalgesia in the neuropathic pain condition (Navratilova et al., 2019b). Whether MOR activation in the left CeA could modulate affective or sensory qualities of pain in animals with injuries of the right spinal nerves remains unclear. One possibility is that KOR activation decreases feedforward inhibition of pronociceptive output cells from the CeA that may express MOR (Navratilova et al., 2019a).

The experience of pain is thought to reflect the consequences of ascending sensory information that reach the brain following modulation by descending circuits that either inhibit or facilitate nociceptive transmission depending on the context in which nociceptors are activated and on assessment of the degree of threat (Navratilova and Porreca, 2014). Consequently, a possible imbalance between facilitation and inhibition may contribute to pain syndromes (Ossipov et al., 2014) as demonstrated by dynamic assessment strategies in human and animal studies that measure the efficiency of the diffuse noxious inhibitory controls (DNIC) (Bannister et al., 2015; Yarnitsky, 2015) or conditioned pain modulation (CPM) response (Nir and Yarnitsky, 2015). DNIC/CPM is lost or diminished in patients with functional (Perrotta et al., 2013) and other pain syndromes (Nasri-Heir et al., 2015). A consequence of stress-related priming is an apparent loss of the DNIC phenomenon that can be restored by KOR blockade in the right, but not left, CeA (Nation et al., 2018), suggesting enhanced descending facilitation that arises from the right, but not the left, amygdala. Experimental neuropathic pain also resulted in the loss of the DNIC response measured in the hindpaw ipsilateral to the nerve injury that was restored by KOR antagonists administered into the right, but not left, CeA (Phelps et al., 2019). Collectively, these data suggest that enhanced descending facilitation arises from KOR signaling in the right, but not left, CeA in both stress-related functional pain syndromes as well as in chronic pain conditions. Consistent with these observations, microinjection of morphine into the right CeA of animals with neuropathic pain injury on the left restored DNIC measured in the left hindpaw (Navratilova et al., 2019b).

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7 Summary and conclusions

A brain area rich in neuropeptides the amygdala plays an important role in affective aspects of pain and pain modulation (Fig. 1). Neuropeptides and their receptors in the central nucleus (CeA) are attractive targets because they can modulate amygdala output neurons either directly (CGRP-R, CRF1-R, MOR, DOR, V1aR) or indirectly on excitatory (MOR, DOR) and inhibitory (CGRP-R, CRF1-R, NPS-R, OT-R) synaptic drives. The peptide systems are not significantly engaged under normal conditions, except perhaps for OT-R, making them useful targets for pain and other conditions, possibly because neuropeptides require special conditions for release. In general, evdicne suggests that activation of NPS-R, MOR, but inhibition of CGRP-R, CRF1-R, KOR, and V1a-R, can exert beneficial effects in pain conditions.

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- The amygdala plays an important role in affective aspects of pain and pain modulation.
- The amygdala is a particularly rich in neuropeptides.
- Neuropeptides can modulate amygdala output neurons either directly or indirectly on excitatory and inhibitory synaptic drives.
- Neuropeptides and their receptors in the central nucleus of the amygdala are attractive therapeutic targets.

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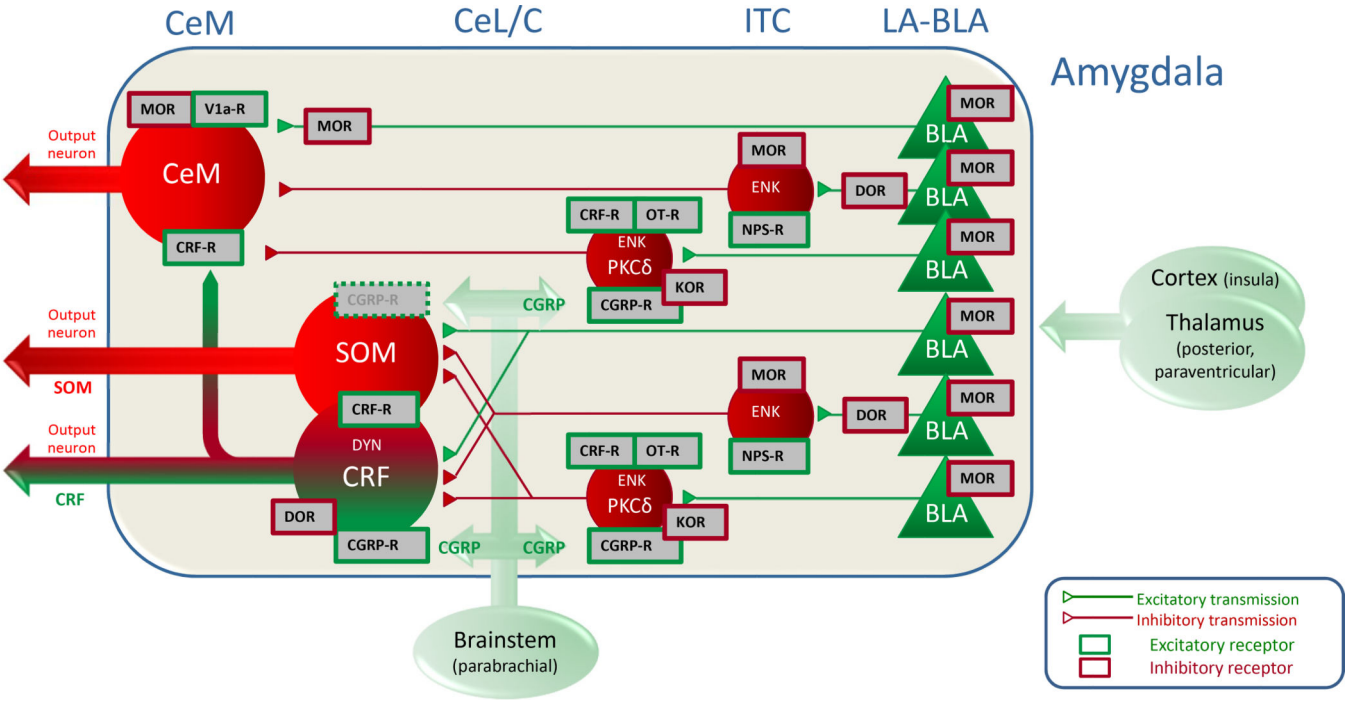

Figure 1. Site of action of neuropeptides in the amygdala circuitry related to pain processing.

The amygdala regions relevant to pain processing include basolateral nucleus (BLA), intercalated cells (ITC), and central nucleus (CeA) with its medial (CeM) and lateral and capsular (CeLC) divisions. Nociceptive input reaches the amygdala from the external lateral parabrachial area (PB) in the brainstem, targeting PKCδ, SOM and CRF neurons, but CGRP containing PB afferents contact mainly PKCδ and CRF rather than SOM neurons; SOM neurons also express only low levels of CGRP receptors (indicated by the dashed line). Polymodal including nociceptive information reaches the amygdala from sensory cortical and thalamic areas. Different cell types containing excitatory and inhibitory neuropeptides and/or receptors are shown. Output neurons with known projections to brain/brainstem regions outside the amygdala include SOM and CRF neurons mainly in the lateral CeA as well as CeM neurons. CeA neurons are mostly GABAergic (in red) and many co-express neuropeptides that can be excitatory (green) or inhibitory (red).