Cell and Tissue Research (2019) 375:93–101
https://doi.org/10.1007/s00441-018-2862-6

REVIEW

Neuropeptide signalling in the central nucleus of the amygdala

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Received: 24 April 2018 / Accepted: 17 May 2018 / Published online: 8 June 2018

Abstract

The central amygdala has a rich repertoire of neuropeptides and neuropeptide receptors. The diverse ways in which they modulate neuronal activity and influence synaptic activity are discussed here mostly in the context of fear and anxiety-related behaviour but also with respect to nociception, hunger and satiety and chronic alcohol exposure that often come together with anxiety. It appears that neuropeptides exert rather specific effects on behaviour and physiology that can be quite different from the effects evoked by opto- or chemogenetical stimulation of the central amygdala neurons that synthesise them or express their receptors. Also, neuropeptides might work synergistically or antagonistically to fine-tune the final outcome of sensory processing in the central amygdala and bring about appropriate physiological and behavioural responses to threat. Taken together, we propose that neuropeptide signalling in the central amygdala mainly serves to establish or maintain emotional homeostasis in response to threatening and other sensory stimuli.

Keywords Neuropeptides · Amygdala · Emotion regulation · Emotion homeostasis · Neuromodulation

Introduction

Since the discovery of neuropeptides as modulators of neuronal activity in the 1970s, much research has been devoted to the role neuropeptides play in neural processing, cognition and the control of physiology and behaviour. Neuropeptides and their receptors are found abundantly in the central nucleus of the amygdala, where they have been implicated in a multitude of behaviours and emotional states. The precise roles of some neuropeptides in the central amygdala in emotion regulation and related physiology or behaviour have been unveiled in remarkable detail, whereas the functional significance of the presence of some other neuropeptides is rather understudied. Of the latter group, some abundant neuropeptides are used at times as a marker of amygdala cell types to study the physiology of these cells and the behaviour they induce, without attempting to describe their effects. In the current paper, we aim to give an overview of how neuropeptides and the neurons that produce them in the central amygdala influence emotion regulation, illustrated mainly by examples of neuropeptide action to control fear and anxiety-like behaviour in rodents. The examples presented will serve to construct a more general framework of the role of neuropeptides in the central amygdala that also applies to processing of sensory signals related to pain, hunger and satiety and chronic alcohol exposure, all of which are often linked with anxiety and fear. Before we do this, however, we will present a brief overview of the basic neuroanatomy of the central amygdala on which the neuropeptides exert their modulatory effects.

Neural circuits of the central amygdala

The central amygdala consists of three parts, known as the central lateral (CeL), capsular (CeC) and medial (CeM) subnuclei (Duvarci and Pare 2014). Sometimes the CeL and CeC are considered together (CeL/C). Essentially, all of the neurons in the three subnuclei are GABAergic. In a classical top-down scenario, the CeL receives input from the lateral amygdala and the CeM from the basolateral amygdala (Duvarci and Pare 2014) that carries processed sensory information of negative salience (Namburi et al. 2015). The CeL sends inhibitory projections to the CeM to inhibit the neurons that project to downstream effector regions that bring about fear and anxiety responses (Huber et al. 2005; Duvarci and Pare 2014). These regions include several brainstem nuclei and the

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hypothalamus. The CeM further receives cortical input via the basolateral amygdala, which is important for fear extinction (Duvarci and Pare 2014). In addition, recent research has demonstrated that the CeL receives sensory input not only from the lateral amygdala but also directly from the parabrachial nucleus (nociception; Bernard and Bandler 1998), the insula (interoception; Shi and Cassell 1998; Guzmán-Ramos and Bermúdez-Rattoni 2012), the paraventricular thalamus (fear memory; Do-Monte et al. 2015; Penzo et al. 2015), and the hypothalamic paraventricular nucleus (Knobloch et al. 2012). The central amygdala is hence more than just a passive gateway conveying information from the basolateral amygdala to the brainstem and hypothalamus; it also plays a role in fear learning (Wilensky et al. 2006; Keifer et al. 2015). Furthermore, the central amygdala controls synaptic plasticity underlying learning in the lateral amygdala, by conveying information about the unconditioned stimulus in Pavlovian fear conditioning. However, this seems to concern an indirect control, as direct connections from the central to the lateral amygdala have not been found (Yu et al. 2017).

In Pavlovian fear conditioning experiments, a previously neutral stimulus (the conditioned stimulus, usually a tone or a light) is paired with a noxious stimulus, usually a mild electric foot shock (the unconditioned stimulus), so that the neutral stimulus now becomes predictive of danger. Whenever the experimental animal is exposed to the conditioned stimulus after fear conditioning, it will display fear-related behaviour, mostly assessed by measuring freezing behaviour. This sort of associative learning takes place in the central (Wilensky et al. 2006) and the lateral amygdala by neurons that receive input coding for the conditioned stimulus (CS) as well as the unconditioned stimulus (US) (Rodrigues et al. 2004; Johansen et al. 2011). Lateral amygdala neurons involved in fear learning send glutamatergic projections to different classes of GABAergic neurons in the CeL and indirectly to the CeM via a relay in the basolateral complex (Duvarci and Pare 2014). Synaptic connections between lateral amygdala neurons and GABAergic neurons expressing somatostatin in the CeL become potentiated, making efficient freezing behaviour possible through direct projections from these neurons to the ventrolateral periaqueductal grey (vlPAG) (Li et al. 2013). GABAergic CeM neurons also project to the vlPAG and promote freezing as well (Viviani et al. 2011; Duvarci and Pare 2014). The somatostatin-positive cells in the CeL are known as ON cells, as they switch on freezing behaviour, while cells that inhibit freezing are known as OFF cells (Ciocchi et al. 2010; Haubensak et al. 2010). OFF cells express PKCδ, whereas ON cells do not and hence, PKCδ expression has been used to differentiate between the two cell types. ON and OFF cells mutually inhibit each other, so that the balance of activity between OFF and ON cells in this particular inhibitory microcircuit determines whether an animal will freeze or not (Ciocchi et al. 2010; Haubensak et al. 2010).

Fear acquisition in the central lateral nucleus of the amygdala

Modulation of fear-related behaviour by oxytocin, vasopressin and bombesin-like peptides

Neurons involved in this basic fear circuit within the central amygdala (Fig. 1) express neuropeptide receptors, making modulation of this circuit by neuropeptides possible. Distribution of receptors can be extremely discrete, as evidenced by the exclusive expression of oxytocin receptors in the CeL and that of the closely related vasopressin 1A receptors in the CeM (Veinante and Freund-Mercier 1997; Huber 2005).

Oxytocin receptors are expressed by GABAergic neurons in the CeL that are positive for PKCδ (Haubensak et al. 2010), i.e., the OFF cells (Ciocchi et al. 2010). Activation of these cells in vivo inhibits freezing, which is an indication of anxiolysis. Oxytocin excites these cells by binding to its receptor, thus stimulating GABA release and inhibiting the ON cells to reduce freezing behaviour (Viviani et al. 2011). They inhibit freezing further by virtue of their GABAergic projections to the output cells in the CeM that project to the vlPAG and stimulate freezing (Viviani et al. 2011). The effects of oxytocin are quite powerful. In patch clamp recordings in brain slices containing the amygdala, we found that applying 400 nM of an oxytocin receptor agonist for as little as 30 s in the bath activated the neurons in the CeL robustly, as indicated by an at least fourfold increase of action potential frequency, between 10 and 20 min and hence strongly inhibited the cells in the CeM (Viviani et al. 2011). Furthermore, by optogenetically tagging oxytocin-producing cells in the hypothalamic paraventricular nucleus (PVN), we could show that endogenous oxytocin release from axon terminals in the CeL is sufficient to activate neurons here and induce anxiolysis. This effect was prevented by application of a specific oxytocin receptor blocker, confirming that optogenetic activation did result in oxytocin release (Knobloch et al. 2012). These ex- and in vivo studies clearly show that oxytocin is a powerful anxiolytic, as it can override the activity of the circuits and cells that promote freezing. The anxiolytic activity of oxytocin is potentiated by the action of the neuropeptide angiotensin IV (Beyer et al. 2010). This six-amino acid long cleavage product of angiotensin binds to angiotensin IV receptors, which are constitutively active metalloproteases. Binding inhibits peptidase activity, thus elevating oxytocin levels and increasing anxiolysis (Beyer et al. 2010).

The target cells in the CeM of the neurons expressing the oxytocin receptor in the CeL express the vasopressin 1A receptor and are indeed activated by vasopressin (Huber 2005). Vasopressin thus promotes freezing, is anxiogenic and counteracts the anxiolytic effect induced by oxytocin. The balance between vasopressinergic and oxytocinergic activity in the central amygdala therefore determines whether an animal will

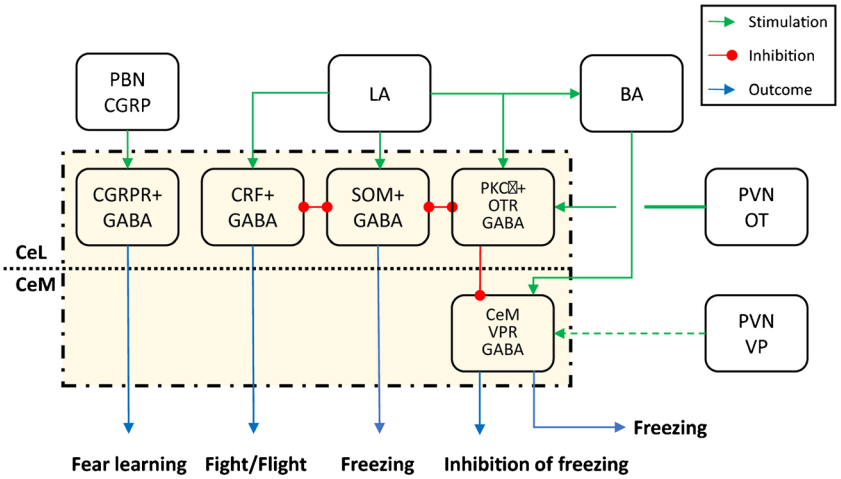

Fig. 1 Proposed circuitry of GABAergic neurons in the CeL/C that controls passive (freezing) or active (fight/flight) defensive behaviour. Four types of neurons are considered; CRF, somatostatin (SOM+), PKCδ and CGRP receptor (CGRPR+)-expressing neurons. CGRPR neurons are contacted by CGRP-positive fibres from the parabrachial nucleus (PBN) and their activity is sufficient for threat learning and, hence, freezing or possibly active defensive responses. Connections to the other GABAergic cell types have currently not been described. CRF and OT cells inhibit SOM cells and vice versa. These three cell types are stimulated by pyramidal neurons from the lateral amygdala (LA). Oxytocin receptor (OTR)-expressing PKCδ cells and vasopressin receptor 1A (VPR)-expressing cells are stimulated by oxytocin (OT) and vasopressin (VP), respectively, from the paraventricular nucleus in the hypothalamus (PVN; although not formally shown for VP, dashed line) to either reduce or promote freezing behaviour. VPR-expressing cells in the CeM are stimulated by input from the basal amygdala (BA) to induce freezing. CRF cells promote active defensive behaviour (fight/flight) and SOM cells promote freezing and inhibit flight. This conceptual model is based on the literature cited in the text. Coloured/dashed box represents the central amygdala with the CeL on top and the CeM at the bottom

express its fear by freezing or not (Huber 2005). This is quite remarkable, considering that oxytocin and vasopressin are homologous, both being well conserved, nine amino acid, cyclic neuropeptides with only two amino acid substitutions to distinguish between them (Donaldson and Young 2008; Stoop et al. 2015). Also, the receptors resemble each other at the molecular level and can bind both neuropeptides with essentially equal affinity (Manning et al. 2008). The specificity of the effects of vasopressin and oxytocin appears to be the consequence of the axonal projections from the PVN where both neuropeptides are synthesised but in distinct, although intermingled, cell populations. Using a viral approach to label oxytocinergic projections throughout the brain, we clearly determined that oxytocin-synthesising cells in the PVN only project to the CeL and not to the CeM (Knobloch et al. 2012).

Of particular interest is that the vasopressin/oxytocin systems in the central amygdala control only one aspect of the physiological expression of fear, which is behaviour as measured by freezing (Viviani et al. 2011). The anxiogenic and anxiolytic effects of vasopressin and oxytocin are mediated by the neurons in the CeM that project to the vlPAG. However, CeM neurons that project to other brainstem nuclei are not influenced by oxytocin. For example, we found no evidence of any modulatory activity of oxytocin on CeM neurons projecting to the dorsal vagal complex, where respiration and blood pressure are controlled. In agreement with this, oxytocin applied in the CeL in vivo did not change heart rate variability (Viviani et al. 2011). Thus, neuropeptides might have a precisely defined role in the regulation of the physiological and behavioural expression of fear: they may act on one aspect but not on another.

Such a specificity, however, may not apply to all neuropeptides in the central amygdala. Others might have a more general role, as we found for bombesin. Bombesin is an amidated, 14-amino acid peptide that was first isolated from the European frog *Bombina bombina* in 1970. Later, two homologous peptides in mammalian brains were found, which are gastrin-releasing peptide (GRP) and neuromedin B (NMB). They bind to their respective receptors that are expressed throughout the brain but especially in those areas that concern themselves with emotional memory. There is still a third receptor, which binds bombesin, GRP and NMB with low affinity only and is to date considered to be an orphan receptor (Roesler et al. 2012). While GRP and NMB bring about many different behavioural and physiological effects, including in the limbic system (Roesler et al. 2014), we found that bombesin application in the central amygdala, where it binds to both GRP and NMB receptors, reduced freezing during fear memory recall (i.e., freezing to the CS 1 day after fear conditioning had taken place) and prevented heart rate variability changes normally seen during presentation of the CS (Viviani et al. 2011). Thus, it appeared that bombesin-like peptides interfere with emotional memory that is stored in the central amygdala and may represent promising pharmaceutical targets for the development of anxiolytics.

Role of CRF and CRF-producing cells in fear behaviour

Corticotropin-releasing factor (CRF) is a neuropeptide of 41 amino acids that is produced locally in the CeL. Innervations from other regions where CRF is synthesised, especially the PVN, is sparse (Sanford et al. 2017). The lion's share of the effects of CRF in the central amygdala on anxiety- and fear-related behaviour can therefore be attributed to CRF of amygdalar origin. CRF cells are PKCδ-negative and do not overlap with somatostatin, indicating that they form a unique cell population within the central amygdala (Sanford et al. 2017). They undergo synaptic plasticity during fear conditioning, as evidenced by increased AMPA/NMDA receptor ratios following electrical stimulation of the lateral amygdala (Sanford et al. 2017). This suggests that CRF-positive cells are engaged during fear processing. Indeed, of all of the peptides expressed in the amygdala, CRF has the most often been associated with fear-related behaviours and, although initial experiments yielded variable results, has anxiogenic properties.

It has recently become clear that these variable results are due to different US intensities in the fear conditioning protocols used. In a recent elegant study, Sanford et al. (Sanford et al. 2017) found that CRF is particularly anxiogenic when low foot shock intensities are used but does not play a role in fear and anxiety responses when the US is strong. Furthermore, CRF is especially important for fear learning but not for the behavioural expression of fear as measured by freezing. CRF mediates fear learning through binding to CRF receptor 1 (CRFR1), which is widely expressed in the three subnuclei of the central amygdala by a number of different cell types. In the rostral central amygdala, the cell types that express CRFR1 have not been identified; in the caudal part, some CRFR1-expressing cells co-express somatostatin. Thus, there is an inhibitory microcircuit, controlled by local release of CRF, within the central amygdala that promotes fear learning but only when US intensity is low to moderate (Sanford et al. 2017). This shows that CRF is important for discriminative fear learning but not for generalised fear learning as occurs when stimulus intensity is high (Sanford et al. 2017). This also nicely illustrates that recruitment of neuropeptides is dynamic, depending on environmental cues.

Interestingly, another study used the expression of CRF to mark the very same cell population and then modulate the activity of the CRF cells by optogenetic means in a fear conditioning paradigm that involved both freezing and escape reactions (Fadok et al. 2017). It turned out that stimulating CRF cells in the CeL with blue light promoted escape behaviour, an attempt to omit the foot shock. Furthermore, this effect was brought about by inhibitory, GABAergic connections from the CRF cells to somatostatin cells that promote freezing (Fadok et al. 2017). The high intensity of the US makes it very unlikely that CRF was involved as well in the escape behaviour. Thus, there seems to be a balance between CRF and somatostatin cell activity that acts as a switch between active (escape) and passive (freezing) fear-related behaviour that, at least for CRF, is highly likely to unroll independently from the action of the neuropeptides.

The effects of CRF are antagonised by at least two another neuropeptides, i.e., NPY and nociceptin. While CRF is considered a pro-stress, anxiogenic neuropeptide in the central amygdala, NPY is an anti-stress, anxiolytic peptide (Sajdyk et al. 2004; Gilpin and Roberto 2012). Interactions between the two neuropeptides have especially been investigated in stressed animals and in alcohol dependence and withdrawal. Withdrawal induces anxiety and excessive drinking, likely via modulation of the GABAergic microcircuits in the central amygdala by CRF and CRFR1 (Nie 2004; Roberto et al. 2010). In contrast, withdrawal-induced anxiety is reduced by NPY through binding to presynaptic NPY receptor 2 (Gilpin et al. 2011). Although there are clear interactions between CRF and NPY signalling in the central amygdala and elsewhere in the extended amygdala (Pleil et al. 2015), the cells involved in this have not been characterised. However, it has been shown that CRFR1 is expressed in neurons that express PKCε, which appears to be downstream of, and activated by, CRFR1. The CRFR1-PKCε intracellular signalling pathway mediates synaptic GABA release and regulates anxiety and alcohol intake (Bajo et al. 2008). NPY has been associated with PKCδ signalling in the brain (although this has not been specifically demonstrated in the central amygdala) (Kuo et al. 2010) and might thus involve the PKCδ-positive neurons that are targeted by oxytocin to induce anxiolysis. Nociceptin has been shown to block and even reverse CRF-induced GABA release within the central amygdala, a process that depends on PKA signalling and is amplified following chronic alcohol exposure (Cruz et al. 2012).

Consistent with its role in stress and anxiety, CRF in the central amygdala evokes an increase in mean arterial pressure as well blood catecholamine concentrations (Brown and Gray 1988; Ku et al. 1998). Microinjection of angiotensin-II, the active form of angiotensin obtained following cleaving of the propeptide by angiotensin converting enzyme, likewise augments blood pressure (Brown and Gray 1988). These hemodynamic effects are mediated through angiotensin 1 and CRF 1 receptors and depend on $GABA_A$ receptor activation (Watanabe et al. 2010). Thus, both angiotensin and CRF increase blood pressure through their actions within the central amygdala, which is a typical physiological expression of fear and stress.

Somatostatin and somatostatin-producing cells and the control of fear behaviour

A third class of GABAergic interneurons in the CeL, in addition to PKCδ- and CRF-positive cells, expresses the neuropeptide somatostatin. These cells promote freezing and

undergo synaptic plasticity changes following fear conditioning (Li et al. 2013). They are reciprocally connected with CRF cells (Fadok et al. 2017) and probably also with PKCδ cells that express the oxytocin receptor (Haubensak et al. 2010). Somatostatin cells also project outside the CeL, i.e., to the vlPAG, to bring about freezing behaviour (Penzo et al. 2014). While activation of somatostatin cells leads to an anxiogenic phenotype, the neuropeptide itself seems to be anxiolytic in the central amygdala. It binds to the somatostatin 2 receptor to induce anxiolysis but it is not known which of the cell types in the central amygdala express the receptor (Yeung and Treit 2012).

Threat memory in the central amygdala

Threat memory in the central amygdala and CGRP

The CeL receives strong input from the parabrachial nucleus, as part of the spino-parabrachio-amygdaloid pathway that originates in lamina I (Todd 2010). This pathway is thought to contribute to the emotional component of pain (Neugebauer et al. 2009), which is brought about by the 37 amino acid neuropeptide calcitonin gene-related peptide (CGRP). Electrophysiological studies have shown that CGRP increases long-term synaptic plasticity at the parabrachio-amygdaloid synapse in the CeL/C by a postsynaptic mechanism that depends on PKA and glutamatergic NMDA receptors (Han 2010).

Further studies on the projections of CGRP-producing cells in the parabrachial nucleus have revealed that they terminate on CGRP receptor 1-expressing cells in the CeL. These cells are characterised on the basis of the synaptic input they receive and not on the basis of molecular markers (PKCδ, CRF, somatostatin). CGRP receptor 1-expressing cells are in large majority negative for somatostatin, and some of the cells located in the caudal part of the CeL express PKCδ (Han et al. 2015). Their activation, either during threat learning or artificially with optogenetic means, is sufficient for the induction of fear memory, as indicated by increased freezing behaviour to the CS 24 h after fear conditioning or photostimulation (Han et al. 2015). This also applies to activation of their upstream CGRP-positive neurons in the parabrachial nucleus: they are activated during fear learning (Han et al. 2015) and fear memory retrieval (Campos et al. 2018) and drive the formation of a threat memory by virtue of contacting their target cells in the CeL/C (Han et al. 2015). Thus, there is a precise role for CGRP-producing cells in the parabrachial nucleus and CGRP-receiving cells in the CeL in the acquisition and recall of fear memory, although somatostatin and CRF cells might contribute to this as well, considering the plastic changes they undergo during fear conditioning (Li et al. 2013; Fadok et al. 2017). However, a role for CGRP and its receptor in threat memory have not formally been addressed by applying a receptor antagonist, for example. Threat memory formation might be due to other factors that are released by CGRP cells in the parabrachial nucleus. Ultrastructural analyses reveal symmetric axosomatic and asymmetric axodendritic synapses between CGRP terminals and GABAergic cells in the CeL, indicating GABAergic signalling on the soma and glutamatergic signalling on the dendrites (Lu et al. 2015). Thus, the precise mechanism by which CGRP cells in the parabrachial nucleus and their target cells in the CeL bring about fear memory has not been elucidated as yet, although it is very likely that CGRP plays an important role in this.

Finally, CGRP has been shown to potentiate synapses between basolateral but not lateral amygdala and central amygdala neurons (Wu et al. 2015). Exogenous CGRP injected into the central amygdala facilitated fear extinction learning, rather than promoting fear acquisition as described above in the CeL and depended on PKA and NMDA receptors (Wu et al. 2015). Although not indicated precisely, these results suggest that the effects of CGRP on fear extinction are brought about in the central medial amygdala (CeM), as inputs from the basolateral to central medial amygdala play a role in fear extinction (Duvarci and Pare 2014). Thus, it seems likely that CGRP, released in the CeL when experiencing pain, is sufficient to induce fear learning and on the other hand is able to promote fear extinction when released in the CeM. It is currently not known whether CGRP is of the same source as the CGRP that is involved in fear learning.

The many faces of PACAP, opposite to those of galanin

Pituitary adenylate cyclase-activating polypeptide (PACAP) exerts effects on pain processing in the central lateral/capsular amygdala that are quite similar to those exerted by CGRP. PACAP-positive fibre terminals in the CeL/C originate from the lateral nucleus of the parabrachial nucleus, where PACAP is at times co-expressed with CGRP (Missig et al. 2014). PACAP in the CeL/C alters nociception as indicated by decreased latency in thermal sensitivity tests and threshold for pain responses are lower in mechanical sensitivity tests (Missig et al. 2014). PACAP immunoreactivity in the CeL/C increases with pain and the neuropeptide itself seems to contribute to the emotional component of pain through activation of its cognate PAC1 receptor and downstream intracellular MAP kinase pathways (Missig et al. 2017).

The emotional component of pain brings about fear- and anxiety-related behaviour and PACAP is therefore considered to be an anxiogenic. This is in line with the more general reported role of PACAP in stress responses, as well as in fear and anxiety. Indeed, bilateral infusion of PACAP in the central amygdala reduced the time rats spend on the open arm of the elevated plus maze and stimulates the activity of the

hypothalamo-pituitary-adrenal axis to increase the release of the stress hormone corticosterone into the blood (Iemolo et al. 2016). Furthermore, PACAP seems to recruit melanocortin 4 receptors (MC4R) in the central amygdala to bring about these effects (Iemolo et al. 2016). The precise mechanisms underlying the interaction between PACAP and α-MSH, the ligand of the MC4R, still await clarification.

Finally, PACAP induces anorexia when administered in the central amygdala (Iemolo et al. 2015). The central amygdala harbours an inhibitory network that regulates feeding and overlaps with the one that controls fear. Cai et al. (Cai et al. 2014) demonstrated that PKCδ-positive neurons process anorexic signals as diverse as satiety and sickness signals (Cai et al., 2014). These neurons are probably at least partially overlapping with PKCδ-positive neurons that have been described as OFF cells or that express the oxytocin receptor (Ciocchi et al. 2010; Haubensak et al. 2010). A population of PKCδ-negative neurons but positive for the serotonin receptor 2a, promotes feeding through long-range inhibition of cells within the parabrachial nucleus (Douglass et al. 2017). It is currently unknown which cell types in the CeL/C express the PAC1 receptor. However, like for fear and anxiety, PACAP mobilises the MC4R to inhibit feeding and does so independently from CRF (Iemolo et al. 2015).

The effects of PACAP are opposite to those exerted by a neuropeptide of 30 amino acids long, galanin. Galanin is expressed in the PVN in the hypothalamus as well as in the medial and central amygdala (Davidson et al. 2011). Galanin infusions into the central amygdala increase withdrawal latency to thermal and mechanical noxious stimulations to the hind paws, demonstrating that galanin is antinociceptic. Antinociception is blocked by a galanin receptor 1 antagonist or a PKC inhibitor (Li et al. 2017) and involves opioid signalling through mu- and delta-opioid receptors (Jin et al. 2010). Although galanin has anxiolytic activity, this has not formally been demonstrated in the central amygdala but rather, amongst others, in the medial paracapsular intercalated nuclei of the amygdala, due to interactions of galanin receptor 2 and NPY receptor Y1 (Narváez et al. 2015).

Neurokinin B and neurokinin 3 receptor modulate fear memory consolidation in the CeM

Neurokinin B (NKB), closely related to substance P and neurokinin A and its receptor are strongly expressed in the CeM, while their presence in the CeL is very low (Andero et al. 2014). The gene encoding NKB, Tac2, is upregulated as soon as 30 min following fear conditioning, before returning to baseline after 120 min. Antagonising the neurokinin 3 receptor (NK3B, specific for NKB) did not impair fear acquisition but rather inhibited fear memory consolidation (Andero et al. 2014). Interestingly, NK3R antagonism reduced enhanced fear memory consolidation in a mouse model of posttraumatic stress disorder (PTSD), indicating that the NK3R is an interesting pharmacological target for the treatment of fear-related disorders (Andero et al. 2014).

Neuropeptides and central amygdala function

As we illustrated above with a few examples, neuropeptides can and do, modulate behaviour and physiology by acting in the central amygdala in a variety of ways. Despite this variability, there seems to be a unifying theme in the role neuropeptides play in signal processing and that is that of emotional homeostasis. Indeed, many neuropeptides can regulate fear responses, CGRP and PACAP modulate the emotional component of pain and CGRP is important for threat memory in the central amygdala (cited above). These functions are conceptually not unlike homeostatic processes in the hypothalamus, which is generally considered as a homeostatic centre for a variety of physiological parameters and behaviours, including feeding, temperature regulation, reproduction, growth and development, metabolism and others. When homeostasis is threatened, the paraventricular nucleus in the hypothalamus (PVN) triggers stress responses to restore it and the central amygdala may do the same for emotional homeostasis. Indeed, the PVN and amygdala are interconnected (Knobloch et al. 2012), although the CRF neurons in the PVN that initiate stress responses are only indirectly contacted via relays in the bed nucleus of the stria terminalis, the brainstem and other hypothalamic nuclei (Herman et al. 2005). Furthermore, both the PVN and the central amygdala integrate internal and external sensory input and pair this with memory signals from thalamic and cortical regions, as well as the hippocampus. Thus, while the hypothalamus is mainly concerned with endocrine and behavioural responses, the (central) amygdala is responsible for emotional and behavioural responses and influences the brainstem to modulate autonomic responses.

In this context, it is perhaps not surprising that many sensory and interoceptive signalling pathways share the neural network within the central amygdala that controls fear and anxiety-like behaviour. In this way, the central amygdala can attribute emotional salience to threats and physiological signals, for instance, to the ones that we have discussed here, i.e., pain, satiety and hunger and alcohol withdrawal. Indeed, pain is known to have a negative emotional component to it (Neugebauer et al. 2009) and alcohol withdrawal increases CRF signalling and causes a state of anxiety (Nie 2004; Roberto et al. 2010). Feeding behaviour might be adjusted, for instance, following taste aversion to direct the choice of food (Schiff et al. 2018) or during neophobia as exemplified by CGRP neuron activity in the parabrachial nucleus (Campos et al. 2018).

However, the precise roles of neuropeptides in the central amygdala in the regulation of emotional responses are still quite difficult to discern. For instance, it has been demonstrated many times, including by ourselves, that oxytocin in the central amygdala reduces freezing in rodents and is therefore considered to be an anxiolytic (Viviani et al. 2011). However, as stated earlier, oxytocin does not influence any other fear-related parameters tested and inhibition of freezing may also be interpreted as reduced passive coping with a treat. In support of this, oxytocin promotes aggressive behaviour and inhibits freezing in the central amygdala of lactating rat dams to protect the pups against intruders in a maternal defence test or when exposed to predator odour (Bosch 2005; Rickenbacher et al. 2017). While it might be that the dams become more courageous, or less anxious, it is also possible (and perhaps more likely) that the mother has switched from a passive to active coping style now that this is required by the presence of another rat or of a predator. Thus, this example of oxytocin signalling in the central amygdala illustrates that neuropeptides can have specific functions, depending on requirements that the context imposes for appropriate behaviour.

Another caveat in interpreting the roles of neuropeptides in the central amygdala concerns the difference between the actions of the neuropeptides themselves and the activity of the neurons that synthesise them. We gave the example of somatostatin, which reduces freezing and somatostatin-producing cells, which, when activated by optogenetic means, promote freezing (Yeung and Treit 2012; Li et al. 2013). How can such a difference be explained? A possible answer may be sought in earlier observations that neuropeptides are only released during high-frequency stimulation, whereas other neurotransmitters (GABA, glutamate, catecholamines, serotonin) are already released under mild stimulation protocols (Hökfelt 1991). Indeed, different neurotransmitters can be stored in different microdomains within the same axon terminal, making it possible that each neurotransmitter can be released independently from the other (Zhang et al. 2015). Also, the differentiation between cells on the basis of the neuropeptide they express, although greatly facilitating the study of these cells with the aid of modern CRE-based opto- and chemogenetic technologies, does not necessarily mean that these cells form a homogeneous cell population. They might share the expression of one neuropeptide but not the neurons they synapse on, or the upstream neurons with their neurotransmitters and neuropeptides. For instance, a combined tracing and genetic labelling study on agouti-related protein (AGPR)-expressing neurons in the hypothalamus revealed that specific groups of AGRP neurons each project to one of several brain regions involved in feeding behaviour (Betley et al. 2013). Optogenetic stimulation of AGRP neurons projecting to the central amygdala had no effect on food intake, whereas stimulation of subpopulations of AGRP neurons projecting to other brain regions increased food intake (Betley et al. 2013). Thus, neurons expressing the same neuropeptide might have different targets to induce different behavioural effects.

Finally, the molecular and cellular mechanisms underlying neuropeptide action in the central amygdala are only partially known and it is therefore not clear how neuropeptides bring about their specific effects. For instance and as mentioned earlier, PKCδ-positive neurons mediate the influence of multiple anorexigenic signals, without increasing anxiety (Cai et al. 2014). This is quite remarkable, considering that PKCδ-positive cells are known as OFF cells, reducing freezing behaviour and a large majority of them (65%) express the oxytocin receptor (Ciocchi et al. 2010; Haubensak et al. 2010). Hence, it seems that mere activation of these cells by anorexigenic signals, or by opto- and chemogenetic means (Cai et al. 2014), is unrelated to fear and anxiety. Rather, anxiolytic responses are only brought about upon stimulation of the oxytocin receptor by oxytocin (or by any other anxiolytic neuropeptide or signalling molecule). Neuropeptides are coupled to specific intracellular pathways through G protein activation and their intracellular effectors are compartmentalised within the cell to form signalling entities that control the response of a cell to receptor activation (McCormick and Baillie 2014). Although the identity of G proteins for most neuropeptide receptors is known, the downstream intracellular pathways in central amygdala neurons are not, with a few exceptions on PKA, PKC and MAPK signalling (cited above). For oxytocin receptor signalling, the intracellular effectors are known in more detail in the PVN. Here, oxytocin exerts its anxiolytic effects through opening of TRPV2 channels and influx of extracellular Ca^{2+} and subsequent activation of MAPK signalling (Van den Burg et al. 2015). Whether the same intracellular effectors are recruited by the oxytocin receptor in the central amygdala is not known.

Conclusions and perspectives

The examples of neuropeptide action in the central amygdala that we presented here show that these neuromodulators are not generic activators or deactivators of cellular activity. Instead, they modulate neuronal activity in a specific manner, depending on context and intracellular signalling pathways that their receptors recruit. Their effects do therefore not always overlap with those evoked by opto- or chemogenetical stimulation of activity of neurons that either synthesise the neuropeptide or express a neuropeptide receptor.

Another important aspect of neuropeptide signalling in the central amygdala that we illustrated is that neuropeptides can antagonise each other's action (CRF vs nociception and NPY; vasopressin vs oxytocin), or at least have opposing effects (PACAP and CGRP vs galanin), work synergistically (oxytocin and angiotensin IV), or have at least similar effects

(PACAP and CGRP, CRF and angiotensin-II). This makes neuropeptide signalling in the central amygdala highly dynamic, which is further amplified by their ability to interfere with synaptic plasticity as seen for CGRP in threat learning (Han et al. 2010). Taken together, the rich diversity of neuropeptides and their receptors in the central amygdala, second only to the hypothalamus, makes it possible to fine-tune amygdala activity and resulting output to maintain emotional homeostasis.

Acknowledgements EvdB is supported by a Swiss Federal grant from the Commission of Technology and Innovation.

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