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REVIEW

Two ancient neuropeptides, PACAP and AVP, modulate motivated behavior at synapses in the extrahypothalamic brain: a study in contrast

Limei Zhang [1,2] · Lee E. Eiden [1]

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Abstract

We examine evolutionary aspects of two primordial neuropeptides, arginine vasopressin (AVP) and pituitary adenylate cyclase-activating polypeptide (PACAP); the distribution of AVP and PACAP and their receptors in mammals; AVP and PACAP release patterns relevant to their roles in neuroendocrine control in brain and periphery; and finally the intricate interlocking of homeostatic and allostatic regulation created by extrahypothalamic AVP and PACAP projections to brain circuit nodes important in controlling appetitive, avoidance and aggressive motor responses. A cardinal feature of peptide neurotransmission important in regulatory control of organismic responses and emphasized in this review, is that neuropeptides are released from large dense-core vesicles docked not only within axonal varicosities and dendrites but also at presynaptic nerve terminal sites, along with small clear synaptic vesicles, at active zones. Peptide transmitter nerve terminals, from hypothalamic and other projections, are distributed widely to multiple brain areas important in integrative control of behavior. They converge with heterologous inputs that release other transmitters, including other peptides, in the same areas. The concept of a quasi-hormonal effect of peptide neurotransmission through coordinated release at multiple synapses throughout the brain echoes earlier conceptualizations of "action-at-a-distance" by diffusion following peptide release at non-synaptic sites. Yet, it recognizes that peptide delivery occurs with neuroanatomical precision, from discrete peptide-containing brain nuclei, via highly distributed projections to multiple extrahypothalamic nodes, registering multiple homeostatic, hedonistic, aversive and reproductive drives that modulate real-time motor decisions. There is paradigmatic value in the discussion of these two particular ancient neuropeptides, for peptide-centric translational neuroendocrinology and peptide GPCR-based neurotherapeutics.

Keywords Arginine vasopressin · Pituitary adenylate cyclase-activating polypeptide · Magnocellular neurons · Neuropeptide GPCR · Neuropeptide circuits in behavior

Short histories and the ligand-receptor co-evolution, of AVP, PACAP and their receptors

Arginine vasopressin (AVP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are both primordial peptides (Darlinson and Richter 1999; Hoyle 1999; Sherwood et al. 2000).[1] Their existence as active hormones precedes the genomic duplications that spawned later relatives and their structures have been preserved more-or-less intact since these pre-vertebral evolutionary days (Fig. 1). The discovery of the chemical structure of

[1] Referencing throughout this review emphasizes historical developments and translational implications of AVP and PACAP biology. Comprehensive reviews of the basic physiology of vasopressin, in particular, are plentiful and can easily be found within the citations of the literature referenced here.

✉ Limei Zhang
limei@unam.mx

✉ Lee E. Eiden
eidenl@mail.nih.gov

[1] Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, NIMH-IRP, Building 49, Room 5A-32, 9000 Rockville Pike, Bethesda, MD, USA

[2] Department of Physiology, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Av. Universidad 3000, 04510 CDMX, Mexico

AVP and the hypothalamic neurosecretory cells containing the peptide, heralded the age of neuroendocrinology (see Zimmerman and Robinson 1976 and references therein). Neurons were discovered to secrete neurotransmitters at synapses and neuroeffector junctions in the periphery and to exist in the brain, in the early twentieth century (Loewi 1921; Yuste 2015); endocrine cells in the adrenal, pancreatic islets, gut, thyroid, gonads and anterior pituitary, which secreted hormones into the general circulation under the influence of other hormones and neuronal inputs, were also described then (Pearse and Takor 1976). The neurosecretory cell was appreciated to be a unique hybrid between neurons and endocrine cells—a third distinct type of cell. Most neurosecretory neurons have large somata (diameters of 20–35 μm) and are traditionally referred to as magnocellular neurosecretory neurons (MNNs) (Armstrong 2004). The AVP neurosecretory cell possesses synaptic inputs and emits a long axon to join the hypothalamo-neurohypophysial tract reaching the internal medial eminence (MEI) and the neural lobe where AVP is released hormonally. The cell is endocrine-like at these final destinations, with terminals at neurohemal junctions within the internal zone of the median eminence of the hypothalamus and the neural (posterior) lobe of the pituitary. However AVPMNNs also emit ascending collaterals within the brain that project with great anatomical specificity to limbic targets (Zhang and Hernandez 2013; Hernandez et al. 2015, 2016; Zhang et al. 2016).

Release of vasopressin from AVPMNNs directly into the general circulation from the posterior pituitary and from the median eminence into the portal circulatory system bathing the anterior pituitary, was historically studied prior to the neurotransmitter function of AVP at nerve terminals in the brain. Vasopressin released at the MEI was found to be a regulatory factor for the secretion of adrenocorticotropic hormone from the corticotrophs of the anterior pituitary (Zimmerman and Robinson 1976 and references therein). Thus, it was a harbinger of an entire new complement of neurosecretory cells elaborating and releasing hormones into this portal system. These were anticipated by Harris as the hypophysiotropic hormones of the hypothalamus (Harris 1955). They were structurally identified by Guillemin and Schally and their colleagues as TRH, LH-RH and somatostatin (Guillemin 1977; Schally 1977), by Guillemin et al. as GH-RH (1982) and by Vale et al. as CRH (1981).

It was the search for a "missing" hypophysiotropic hormone that, like GH-RH, acts via stimulation of cAMP elevation in pituicytes, that ultimately led to the structural identification of PACAP (Miyata et al. 1989). Despite its discovery as a putative hypophysiotropic hormone, PACAP turned out to have neurosecretory and neurotransmitter roles far beyond the anterior pituitary (Arimura 1998). PACAP became an important paradigm for a type of peptide that is released both from neurosecretory cells to perform hormonally and from neurons to act trans-synaptically. Although PACAP is found in highest concentrations in the hypothalamus, it is not concentrated in the median eminence (Arimura et al. 1991), the neurohemal junction through which hypophysiotropic hormones such as TRH, LH-RH, somatostatin, GH-RH and CRH, act on the pituitary. Rather, PACAP is concentrated at sites within the hypothalamus and extrahypothalamic brain, indicative of frank neurotransmitter actions. Indeed, further investigation showed PACAP to have rather subtle effects on pituitary hormone release in vivo, setting the stage for its emergence as a brain and peripheral autonomic and enteric nervous system neurotransmitter (Christofi and Wood 1993; Portbury et al. 1995; Nagahama et al. 1998; Hamelink et al. 2003; Sherwood et al. 2003; Mustafa and Eiden 2006; Furness 2016).

AVP and PACAP, like all neuropeptides, arise from common prohormone precursors that are processed by proteolytic cleavage within large dense-core secretory vesicles (LDCVs) in neurons and endocrine cells (Millar et al. 1977; Seeburg and Adelman 1984; Chretien 2013). Secretion from LDCVs imparts specific properties to neuropeptide neurotransmission and hormonal action that are well-illustrated by the central and peripheral neuromodulatory and hormonal roles of both AVP and PACAP (Hokfelt et al. 2003). Following the development of methods for cloning of cDNAs based on limited protein/peptide sequence information, the nucleotide sequences of multiple prohormone mRNA coding domains were obtained, replacing previous protein-based methods for this purpose (Wisden 2016). Complementary DNAs encoding the AVP/neurophysin II and PACAP precursors were cloned and sequenced in the 1980s and 1990s (Land et al. 1982; Kimura et al. 1990).

Both AVP and PACAP also interact with more than one G-protein-coupled receptor (GPCR) (see Table 1) and these are also well-identified across the subfamily *Vertebrata*. The genes encoding both the prohormone precursors and the receptors for neuropeptides of many species have been identified and compared. From these comparisons, the concept of neuropeptide ligand-receptor co-evolution has emerged. It sheds considerable light on the establishment of diversity in physiological function of neuropeptide ligand-receptor dyads, in both mammalian and more distantly related vertebrate and even invertebrate species (Beets et al. 2013). Both AVP and PACAP have co-evolved along with companion peptides and with receptors that have some overlap with those of related peptides (Fig. 1). Arginine- and "neutral"-vasopressin peptides (including oxytocin, OT) are ligands for a receptor family that includes three receptors for arginine vasopressin itself (AVP1A, AVP1B and AVP2) and oxytocin (OX1R), while PACAP and the related vasoactive intestinal polypeptide (VIP) are ligands for a receptor family that includes three receptors for PACAP (PAC1, VPAC1 and VPAC2), two of which (VPAC1 and VPAC2) are also receptors for VIP. It is noteworthy that

Table 1 Affinities of PACAP, AVP, VIP and OT for their cognate and related receptors

Receptor	PACAP or AVP	VIP or OT	PACAP EC50	VIP EC50
PAC1	~1 nM	~100 nM	~1 nM	~200 nM
VPAC1	~0.4 nM	~0.4 nM	~1 nM	0.03 nM
VPAC2	~1 nM	~1 nM	~1 nM	0.01 nM
AVPV1a	~1 nM	~120 nM		
AVPV1b	~1 nM	~1800 nM		
AVP2	~1 nM	~90 nM		
OTR	~2 nM	~1 nM		

Values are taken from Harmar et al. (2012), Alexander et al. (2013), Thibonnier et al. (1997) and Manning et al. (2012) (Km's/Ki's for receptor affinity, columns 2–3) and from Emery et al. (2016) and Eiden and Emery, AC, unpublished (EC50s for receptor activation for Gs-coupled signaling, columns 3–4). EC50 values for AVP and OT are not available at this writing. EC50 values are for coupling to cAMP elevation via Gs coupling of human PAC1hop, VPAC1 and VPAC2 receptors in heterologous cell line(s) (HEK293_CBS_GPCRx; Emery et al. 2016, 2017a, b). PAC1 in mammals has four isoforms (hip, hop, hiphop and hiphopless) due to alternative splicing within the third intracellular loop: only one (hop) is reported to couple to Gq (Pisegna and Wank 1993; Spengler et al. 1993; Pisegna and Wank 1996); differences among isoforms for Gs coupling are unremarkable. Gq coupling with PAC1hop is not notably predicted by homology to Gq-specific sequence in the intracellular/extracellular domains of this receptor compared to AVP1a and AVP1b and other Gq-coupled receptors (see for example PRED-COUPLE prediction scores for PAC1hop versus known Gq-coupled GPCRs: http://athina.biol.uoa.gr/bioinformations/PRED-COUPLE2). Gq coupling reported for PAC1hop may be in part a function of extraphysiological stoichiometries between GPCR and Gs/Gq in heterologous-cell experiments, compared to specificity for the endogenous receptor in particular tissues and cells

the ability of AVP and OT to interact with their various receptors and PACAP and VIP with theirs, differ by only two orders of magnitude (see Table 1). Thus the possibilities of VIP acting at PAC1 (Harmar et al. 2012), or OT at AVP1A or 1B (Gimpl and Fahrenholz 2001), at CNS synapses, cannot be dismissed out-of-hand.

Receptors for peptides in general are found within the first two, A and B, of the five GPCR families (or according to the GRAFS system, the rhodopsin and secretin families) (Fredriksson et al. 2003). Family A ("rhodopsin family") contains four divisions, A-α, β, γ and δ. Peptide receptors are scattered throughout each and have variegated signaling properties (Rashid et al. 2004). Family Aα contains the melanocortin receptors (coupled to Gs). The AVP receptors are contained in family Aβ, with AVPR1a, AVPR1b and OTR (coupling to Gq) and AVPR2 (coupling to Gs). Other Aβ peptide GPCRs are those for tachykinins, orexin, ghrelin and neurotensin (coupled predominantly to Gq), NPY (coupled to Gi), cholecystokinin (coupled to Gs, possibly Gi and Gq) and endothelin (coupled to Gq and Gi). The Aγ family contains the opioid peptide receptors OPRL1, OPRK1, OPRM1 and OPRD1 and somatostatin receptors SSTR1-5 (all Gi-coupled) and melanin-concentrating hormone and chemokine receptors (coupled to Gq and Gi). The TRH receptor (coupled to Gq) and the enkephalin BAM-22P receptor MRGPRX1), among others, are found in the Aδ subfamily.

PACAP receptors are found in family B (secretin family) along with those for glucagon-like and parathyroid hormone-like, calcitonin-like, growth hormone releasing hormone–like and corticotropin receptors and of course the secretin receptor. The secretin family of receptors couple predominantly with Gs (although an isoform of the PAC1 receptor is reported to couple to Gq—see Table 1).

The "punctuated evolution" of both AVP and PACAP and their receptors, speaks to a dynamic co-evolution of these dyads during the process of animal family, genus and species development. This evolutionary history supports a "survival-of-the-future-fittest"—speciation-accelerant and species-stabilizing—role for these neuropeptides and their receptors (Elde et al. 1980).

The evolutionary and functional relationships among peptides and receptors for both vasopressin and PACAP are discussed in greater detail below. The notion that OT mediates prosocial or affiliative behaviors and AVP aggressive behaviors, in rodents and other mammals, including *H. sapiens* has been recently elaborated (Caldwell 2012). PACAP and VIP have both complementary and overlapping roles in both peripheral and central physiology that are being clearly distinguished only now, in part because the VPAC1 and VPAC2 receptors recognize both peptide ligands. The PACAP/PAC1 dyad is involved in stress responding, with VIP actions at the VPAC1 and VPAC2 receptors critical for coordination of circadian rhythm and metabolic interplay between neurons and glia in cerebral cortex and other brain areas (Harmar et al. 2012). In the suprachiasmatic nucleus (SCN), PACAP in retinohypothalamic tract innervation and VIP in intrinsic pacemaker cell synchronization, collaborate to control circadian timing and rhythmicity (Harmar et al. 2002; Colwell et al. 2003, 2004; Aton et al. 2005; Beaule et al. 2009).

Regarding receptor distribution, it is roughly accurate to summarize the receptors for vasopressin as clustering into peripheral (mainly AVP2) and central (mainly AVP1A and 1B) (Young 1992; Terada et al. 1993, Richter 1999). Those for PACAP are fairly widely distributed between the periphery and brain, with PAC1 apparently the dominant receptor for PACAP action in the autonomic nervous system, suprachiasmatic nucleus, hippocampus and extended amygdala

(Hannibal 2002; Macdonald et al. 2005; Ressler et al. 2011; Harmar et al. 2012; Hammack and May 2015). The attribution of AVP action in various brain areas to AVP1a or b receptors, like that of PACAP at PAC1, VPAC1 and VPAC2 receptors, still awaits definitive resolution in many brain areas and circuits (vide infra).

As far as is known, both AVP and PACAP, like other neuropeptides, are found only in large dense-core vesicles (LDCVs; vide supra). They are released upon high-frequency or burst stimulation, unlike classical transmitters that are released in a graded fashion across all stimulation frequencies (Hökfelt et al. 2000, 2003). This has important implications for the roles of these neuropeptides and neuropeptides in general, for modulation of synaptic function, especially when co-released with classical neurotransmitters under conditions in which specific neuronal circuits are activated.

AVP is a remarkably highly conserved (anti) diuretic hormone, across tens of millions of years of evolution and in organisms with and without differentiated kidney-like organs and with and without brains. It is therefore worthy of study considering how neuropeptides in general acquire new physiological roles based on the core function of a ligand-receptor dyad and its co-evolution across gene duplication events. A possible basis for ligand-receptor coevolution is the ability to select for stable expression of both in new tissues and brain areas. If so, it is likely to proceed by modifying a core function with mutation constrained by selection pressure; duplication of ligand and receptor genes; and mutation-selection of duplicated genes at both promoter and protein-coding domains (see Fig. 1). This kind of evolutionary analysis is still not well-developed for peptides and their receptors (Banerjee et al. 2017). Nevertheless, it may be suggested that the AVP/AVPR2 ligand-receptor dyad has supported speciation through differential mutation of duplicated sets of ligand and receptor genes and selection-testing of functional divergence nested within class/family/genus levels of evolution (Porges 1997; Hauser et al. 2006; Banerjee et al. 2017).

Signaling pathways initiated by the GPCRs activated by vasopressin and PACAP

The signaling pathways that are initiated by both AVP and PACAP and lead ultimately to their physiological actions, begin with activation of either Gs or Gq pathways (Table 1). However, the use of alternative G proteins and potentially non-G protein-mediated signaling, as reported for other neuropeptide signaling pathways (Lu et al. 2009), is by no means ruled out.

Vasopressin controls plasma osmolality via activation of AVP2 receptors, which are linked to Gs, causing cyclic AMP elevation (see "Peripheral actions of vasopressin and PACAP"). In the brain, AVP's actions, mainly at V1a and V1b receptors, occur through coupling to Gq, leading to inositol trisphosphate and diacylglycerol elevation and activation of protein kinase C (PKC) as well as calcium mobilization (Birnbaumer 2002). The intracellular signaling effects of activation of these receptors is therefore quite complex. It is not certain that Gq activation from a peptide GPCR leads always to stereotyped cellular responses, predictable by a simple summation of calcium elevation and PKC activation, in specific cells and tissues. Nevertheless, calcium elevation in CNS neurons that respond to AVP via V1a and V1b is a major immediate response. Therefore calcium elevation, along with the anatomical location of V1 receptors in specific brain regions, can still explain much about how AVP modulates brain function and how inhibition of specific receptor subtypes affects post-synaptic responses (Brinton et al. 1994). Further understanding of more subtle differences in V1a/b signaling pathway activation within certain cell types, along with a clearer understanding of pharmacological engagement of each receptor in various brain regions and functions, is desirable. This may yet contribute to development of highly specific therapeutics to enhance or block peptide effects in vivo.

PACAP acts mainly at the PAC1 receptor in the peripheral nervous system. Signaling in the adrenal medulla (i.e., the splanchnicoadrenomedullary synapse) appears to be wholly cAMP-dependent (Smith and Eiden 2012, Stroth et al. 2013). In sympathetic neurons, signaling by PACAP is very complex. Post-synaptic effects of PACAP on sympathetic neurons may involve protein kinase B (a.k.a. Akt) for its neurotrophic effects (May et al. 2010); and cAMP elevation, ERK activation, calcium influx, K channel opening and other mechanisms for its actions on neurotransmitter release and neuronal gene regulation (Braas and May 1999; Beaudet et al. 2000; Girard et al. 2004; Koide et al. 2014). A critical unresolved issue in PACAP signaling is whether or not Gq in addition to Gs coupling to the PAC1 receptor occurs. Original observations of Spengler et al. (Spengler et al. 1993) and Pisegna et al. (Pisegna and Wank 1996) suggest that the PAC1 hop receptor, in particular, has the ability to couple to Gq. However these experiments have been carried out in non-endocrine cells at a likely rather high receptor: G protein ratio. Therefore the possibility exists that Gq activation in this situation is not physiological and that Gs/Gq co-activation via the PAC1 receptor is not a ubiquitous feature of PAC1 signaling in mammalian neurons. This arena is a critical one in the neuropeptide signaling field at this time, as it represents opportunity for profound improvement in our understanding of peptide physiology in general and for neurotherapeutical intervention in particular.

Elevation of calcium plays a major role in PACAP actions on the secretion of other hormones and transmitters from target cells, as it does after V1a,b receptor activation by AVP. The recent discovery of cAMP signaling parcellation in

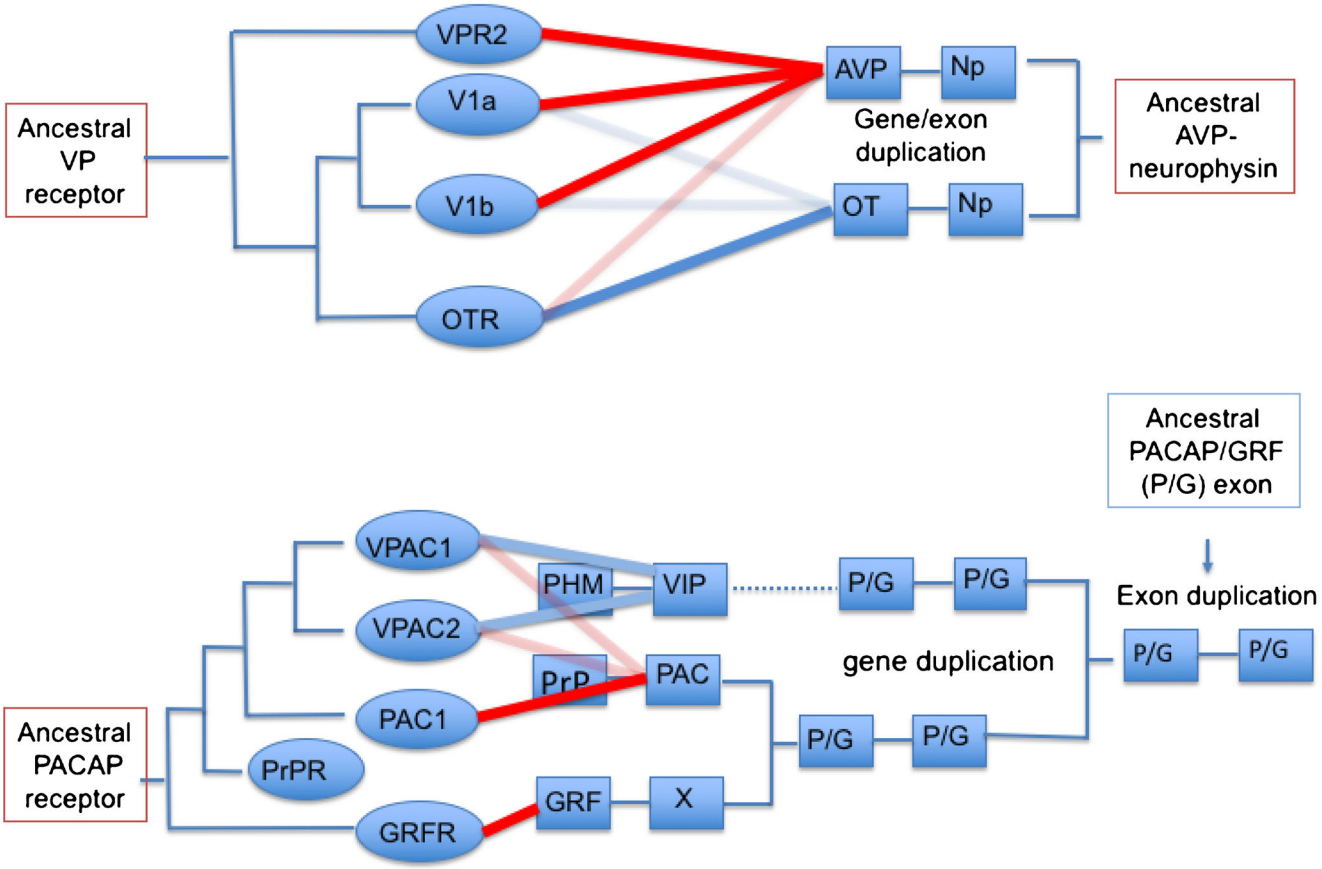


Fig. 1 Co-evolution of two ancient neuropeptides and their receptors. Evolutionary development of diversity within the secretin/family B and vasopressin/oxytocin receptor families and their cognate ligands. Adapted from Sherwood et al. (2000), Hoyle (1999), Tam et al. (2007) and references therein. Intensity of lines connecting ligands and receptors approximate the relative potency of the ligand at the indicated receptor

chromaffin cells and chromaffin-like cell lines, in culture has created a new pharmacology for cyclic AMP signaling that allows further understanding of how PAC1 activation might lead to both catecholamine secretion and activation of biosynthetic enzyme and peptide precursor genes (Emery et al. 2014). This signaling may be important in long-term adaptation in various target organs/tissues in response to prolonged stress (Eiden et al. 2018). Understanding how cAMP elevation affects calcium entry leading to cellular depolarization in endocrine cells, in post-ganglionic sympathetic and parasympathetic neurons and in the brain, should benefit greatly from the new optogenetics of peptide signaling (Knobloch et al. 2012). This is an area of research just beginning for AVP (Cui et al. 2013; Pagani et al. 2014) and yet to begin for PACAP. In fact, how PACAP alters calcium metabolism through PAC1 is a genuine mystery unsolved either centrally or peripherally (Costa et al. 2008; Tompkins and Parsons 2008; Smith and Eiden 2012; Merriam et al. 2013; May et al. 2014a, b and see Fig. 2). PAC1 causes calcium influx rather than calcium mobilization in HEK cells (May et al. 2014a, b), consistent with reports that PAC1-induced catecholamine secretion is cAMP-dependent, i.e., that PAC1 calcium effects are mediated through Gs rather than Gq (Kuri et al. 2009; Hill et al. 2011). Yet many important aspects of PACAP signaling to neuronal cells, both centrally and peripherally, depend on either calcium influx, altered neuronal excitability, or both. The important question of how PACAP-PAC1 signaling causes these cellular changes and whether Gs, Gq, or a combination of both are required for them in a given PACAP-responsive neuronal cell type, is still an open one.

AVP and PACAP are likely to be co-secreted with classical neurotransmitters at most or all sites of release from nerve terminals or neurohemal junctions. PACAP and acetylcholine are co-released from the splanchnic nerve innervating the adrenal medulla (Hamelink et al. 2003 and references therein; (Goto et al. 1989; Kuri et al. 2009; Smith and Eiden 2012; Stroth et al. 2013). PACAP and glutamate are co-released from nerve terminals of the retinohypothalamic tract innervating the suprachiasmatic nucleus (Colwell and Waschek 2001; Colwell et al. 2004; Lindberg et al. 2004; Michel et al. 2006; Beaule et al. 2009; and see Zhang et al., submitted for publication). AVP is released from both neurosecretory and synaptic terminals emanating from magnocellular neurons that also synthesize, store and release glutamate (Hrabovszky et al. 2006; Zhang and Hernandez 2013; Zhang et al. 2018) (Fig. 3). The co-release of either peptide with classical

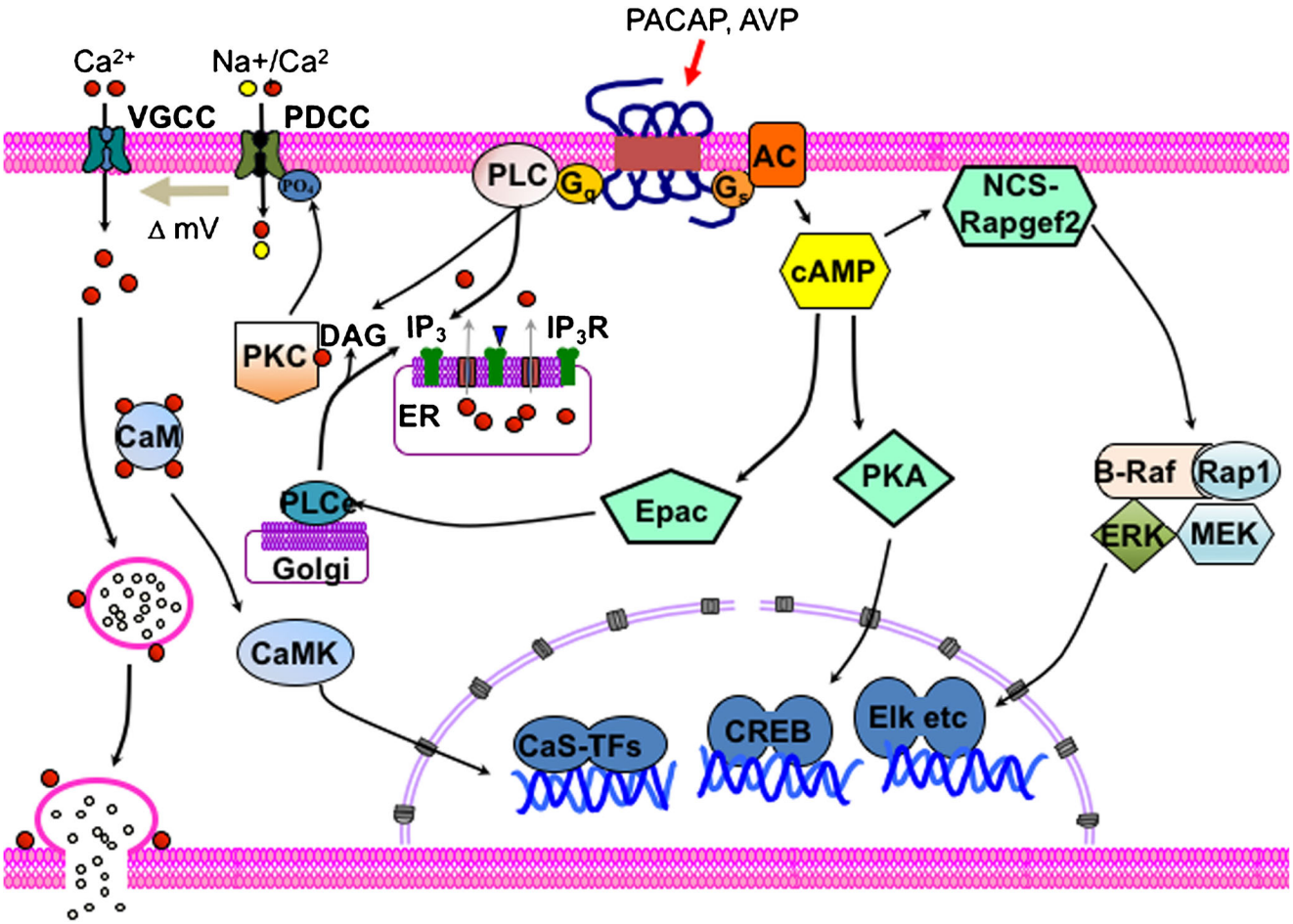


Fig. 2 Signaling pathways for Gs-coupled (PAC1, VPAC1/2 and VP2) and Gq-coupled (VP1a/b) PACAP and AVP, respectively signaling. Depicted are the second- and third-messenger systems activated by Gs and Gq-coupled activation of adenylate cyclase and phospholipase C, respectively, after GPCR activation by vasopressin or PACAP. See relevant citations to the literature in the text

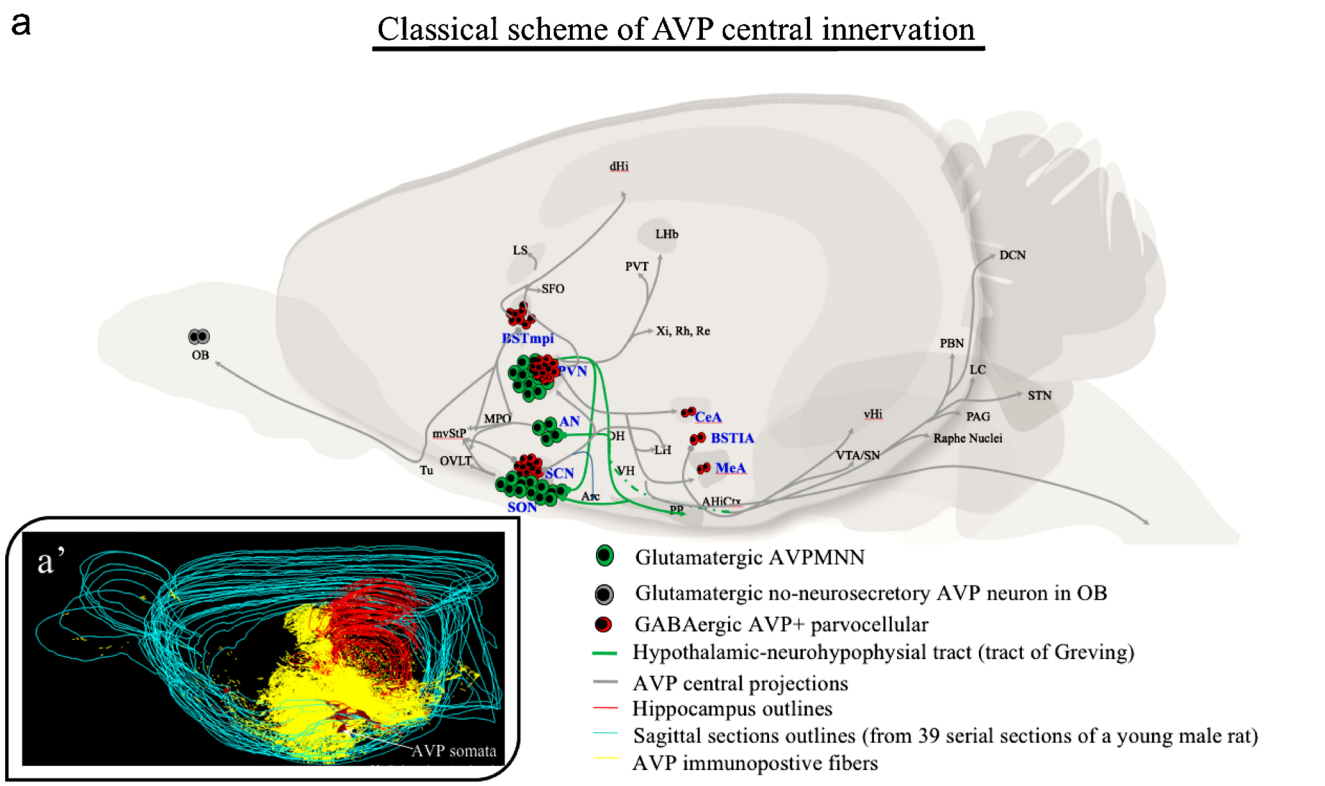
excitatory neurotransmitters including ACh and glutamate, may be a key to how both affect neuronal excitation, and excitatory potential, in the nervous system under physiological conditions (Hernandez et al. 2015, 2016; Zhang et al. 2016, 2018).

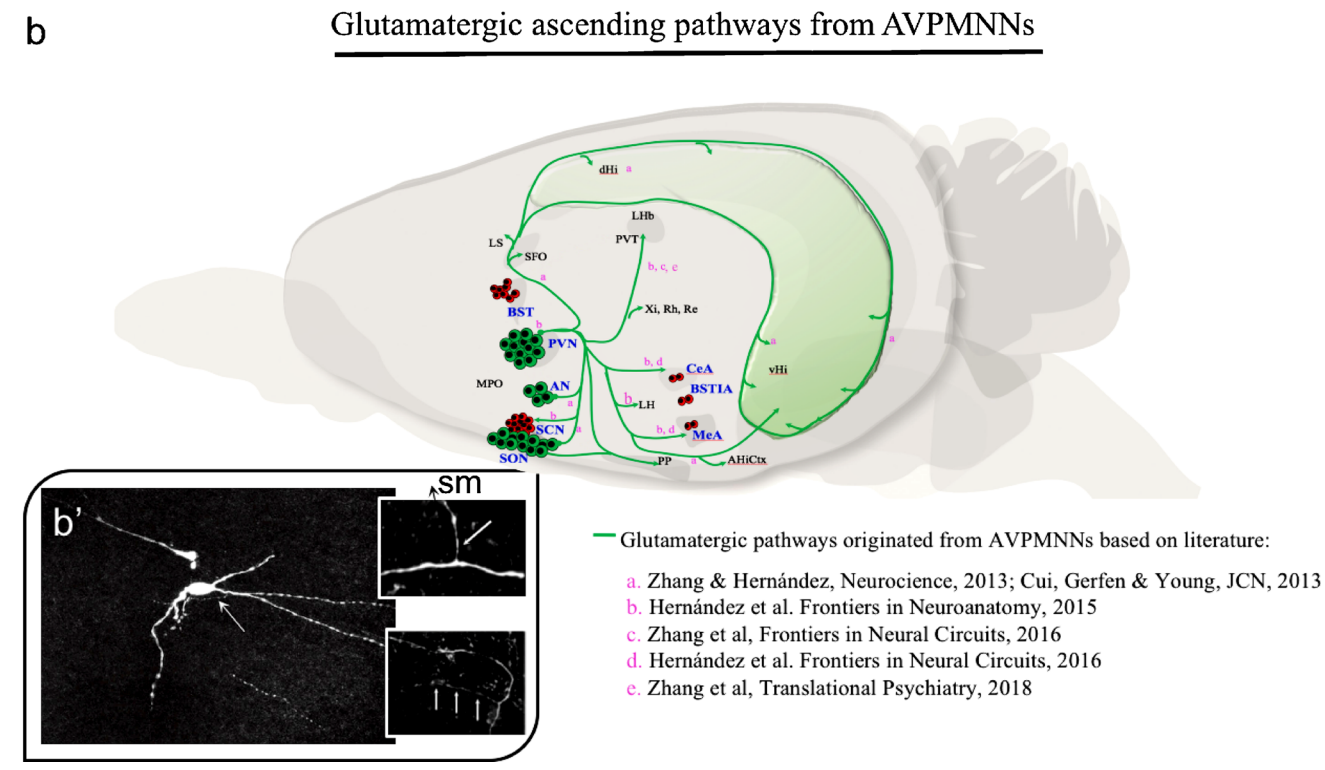
Peripheral actions of vasopressin and PACAP

Vasopressin issues from the posterior pituitary and acts non-synaptically; PACAP issues from the autonomic nervous system and acts synaptically but also from mobile and non-mobile cells within the immune system, from which it acts non-synaptically (Armstrong et al. 2003, 2008; Abad et al. 2006; Tan et al. 2009; Waschek 2013).

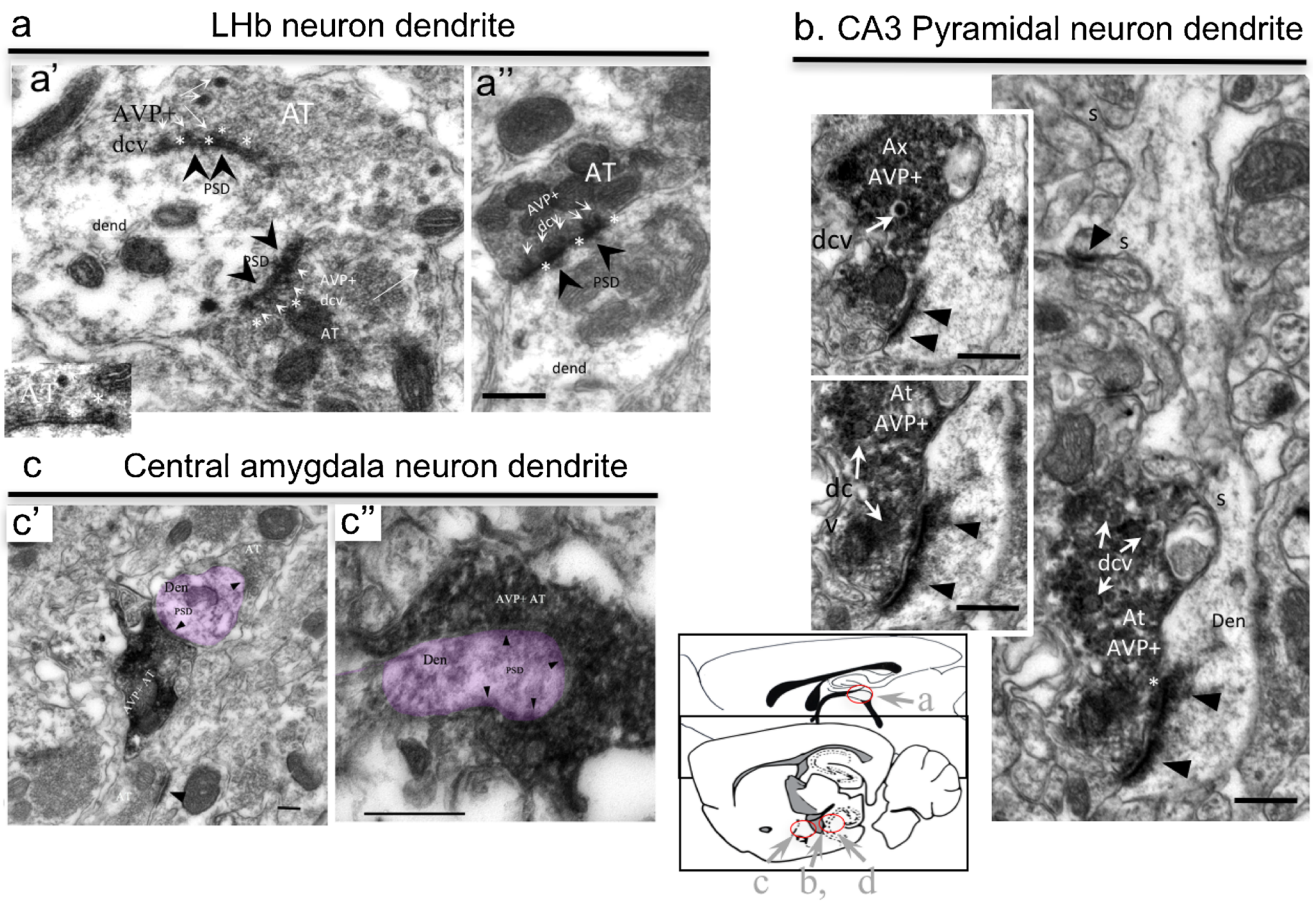
The peripheral actions of AVP ensue after its release into the general circulation from neurosecretory nerve terminals in the posterior pituitary and into the pituitary portal circulation from the median eminence of the hypothalamus. Although a weak secretogogue on its own, AVP acts on anterior pituitary corticotrophs to enhance ACTH release elicited by CRH

Fig. 3 Vasopressin containing nuclei and central pathways in rodent brain. **a** Conventional scheme of AVP central innervation according to current literatures. Chemical nature of AVP cellular groups is symbolized by green, gray and red coloring of symbols for cell soma (Zhang and Eiden, in preparation); **a'** computerized 3D "one-to-one" mapping to visualize the AVP immunopositive fiber distribution and cell bodies of a young male rat. **b** Central projections of AVP magnocellular neurosecretory neurons. Recent additions to the literature on AVP neurosecretory system central projections, reports on each pathway's discovery are indicated with small purple letters. **b'** An in vivo juxtacellularly labeled AVPMNN, with white arrows indicating the central branches of the main axons. PVN: hypothalamic paraventricular nucleus; SON: supraoptic nucleus; SCN: suprachiasmatic nucleus; AN: accessory nuclei (which include nucleus circularis and the posterior fornical nucleus); BSTmpi: bed nucleus of stria terminalis, medial poterior internal division; BSTIA: BST, intra-amygdala division; CeA: central amygdala; MeA: Medial Amygdala; LS: lateral septum nuclei; dHi: dorsal hippocampus; vHi: ventral hippocampus; LHb: lateral habenula; PVT: paraventricular thalamic nucleus; OB: olfactory bulb; Tu: olfactory tubercle; OVLT: organum vasculosum of lamina terminalis; mvStP: medial ventral striatal-pallidum region; MPO: medial preoptic nuclei; SFO: subfornical organ; Xi, Rh, Re: thalamic xiphoid, rhomboid and reuniens nuclei, respectively; AHi: amygdalohippocampal area; VTA/SN: ventral tegmental area/substantia nigra; PAG: periaqueductal gray; STN: solitarii tractus nucleus; LC: locus coeruleus; PBN: parabrachial nuclei





(Bilezikjian et al. 1987). This effect is mediated by V1b receptors (Thibonnier et al. 1997; Streefkerk and van Zwieten 2006). AVP acts at AVP2 receptors to control organismic osmotic balance by modulating water permeability at cells of the collecting duct of the kidney and may serve the same function in extrarenal peripheral locations (Juul et al. 2014).


AVP+ ATs establishing Gray type I synapse onto
a
LHb neuron dendrite
a'
AVP+
dcv
AT
PSD
dend
PSD
AVP+
dcv
AT
AT
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dcv
PSD
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b. CA3 Pyramidal neuron dendrite
Ax
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Central amygdala neuron dendrite
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d
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◀ **Fig. 4** Ultrastructural features of AVPMNN innervation of limbic regions. AVP immunoperoxidase labeled axon terminals (ATs) at three limbic regions relevant for emotional reactivity and motivational behavior are shown at the ultrastructural level. **a** Electron microscopy photomicrographs showing the AVP+ dense core vesicles (dcv, thin white arrows) inside the ATs of an established Gray type I synapse (post-synaptic densities, PSD, were indicated with black arrowheads) onto dendrites (dend) of a habenular neuron. Asterisks adjacent to AVP+ dcv show their docking onto presynaptic membranes. Scale bars: 500 nm. (Modified with permission and retained right for re-usage from Zhang et al. 2016.) **b** A postsynaptic dendritic shaft from CA3 pyramidal neuron of ventral hippocampus receives a presynaptic innervation from a large diameter AT containing dense-core vesicles (dcv) that are AVP+. It also emits several spines (s), one of them receives an unlabelled type I small synapse (arrowhead), which is a typical feature of pyramidal neurons in the hippocampus. Insets show the same synapse in adjacent sections (modified with permission and retained right of re-usage from Zhang and Hernandez 2013). **c** Sample taken from the central amygdala (modified with permission and retained right of re-usage from Hernandez et al. 2016). **d** Serial sections of an AVP+ axon (Ax) forming a type I synapse with a large spine (Ax-s) originating from an axon initial segment (AIS) in the ventral CA3. The electron-opaque membrane undercoating, characteristic of the AIS, is indicated by a white arrowhead. The axonal spine contains membranous intracellular organelles and receives a type II synapse from an unlabeled terminal (axon-axonic GABAergic interneuron terminals) (modified with permission and retained right of re-usage from Zhang and Hernandez 2013)

The key role of vasopressin in water homeostasis is dramatically illustrated in the Brattleboro rat. These animals lack detectable peripheral vasopressin due to a failure to process/release vasopressin from the posterior pituitary and as a consequence exhibit severe diabetes insipidus (Kim and Schrier 1998). Pressor effects of AVP throughout the vasculature are mediated through V1 receptors. New roles for vasopressin in peripheral (subcutaneously initiated) pain perception have been ascribed to V1b and, intriguingly, OT receptors (Manzano-Garcia et al. 2018).

In contrast to AVP, there was no prominent peripheral physiological role for PACAP postulated at the time of its discovery. Rather, PACAP was discovered via a search for a candidate hypophysiotropic hormone: side fractions of hypothalamic peptide extracts were screened in a pituitary function assay (cyclic AMP elevation in hemi-perfused pituitary (Miyata et al. 1989). However, PACAP was soon found to be present in the peripheral autonomic nervous system (Arimura 1998) and to have prominent effects pharmacologically on plasma epinephrine elevation (Hamelink et al. 2003 and references therein). These effects were later shown (vide infra) to reflect the role of PACAP as the major splanchnicoadrenomedullary transmitter in regulating stress-induced catecholamine secretion in vivo (Hamelink et al. 2002, 2003; Smith and Eiden 2012; Stroth et al. 2013). The role of PACAP in autonomic regulation following release from cholinergic preganglionic neurons of both sympathetic and parasympathetic nervous system is an area of active investigation (Eiden et al. 2018 and references therein).

Central actions of vasopressin and PACAP in the hypothalamus, the endocrine control center of the brain

Vasopressin synthesis and release in hypothalamus are dynamically regulated in response to altered water and salt. As a result, correlations between altered vasopressin levels and behaviors not related to drinking per se, gave early clues to the interplay between homeostatic and allostatic regulation by vasopressin. In the Brattleboro, correlations between lack of AVP and behavior set the stage for the hypothesis that vasopressin might act via extrahypothalamic neural control of higher brain function. The discovery that vasopressinergic projections not only descend to the posterior lobe and to the median eminence but also ascend into the limbic system, has provided a neuroanatomical basis for this control (Zhang and Hernandez 2013; Hernandez et al. 2015, 2016; Zhang et al. 2016; see Fig. 3).

AVP is found in both magnocellular and parvocellular neurons of the brain. The parvocellular is sparser populations, with some found alongside magnocellular neurons in the paraventricular nucleus of the hypothalamus and in the amygdala as well as the bed nucleus of the stria terminalis (BNST) (Armstrong and Hatton 1980, Buijs et al. 1983; Caffe et al. 1987). Parvocellular vasopressinergic neurons are of considerable physiological importance in their own right (vide infra). However, we focus here on the magnocellular neurons, found exclusively in the hypothalamus, because they illustrate an important evolutionary feature of vasopressin function in the brain. The arginine vasopressin magnocellular neurosecretory neurons (AVPMNNs) are now shown to dually convey hormonal (via posterior pituitary and median eminence release of vasopressin) and synaptic (via Gray type 1 excitatory synapses in amygdala, hippocampus, habenula and possibly elsewhere) signals (Figs. 3 and 4). The development from an endocrine homeostatic role for vasopressin, to a dual endocrine-neurotransmitter one, parallels the emergence of allostatic (anticipatory) control mechanisms throughout the vertebrata and most extensively studied in mammalia.

An important group of PACAPergic neurons are contained within the hypothalamus, and PACAP does in fact regulate the HPA axis via effects on anterior pituitary release of ACTH (Hannibal 2002; Tsukiyama et al. 2011). In contrast to AVP however, PACAP does not appear to act directly on corticotrophs upon release into the hypophyseal portal circulation at the median eminence. Rather, PACAP acts indirectly, through enhancing the biosynthesis of CRH which is itself secreted into the hypophyseal portal circulation to act upon anterior pituitary corticotrophs. This mechanism for PACAP action on the HPA axis is supported chemoanatomically by histochemical evidence of PACAPergic terminals on or near CRH neurons of the PVN, as well as pharmacological actions of PACAP on CRH gene expression in this hypothalamic nucleus

(Grinevich et al. 1997; Legradi et al. 1998; Agarwal et al. 2005). Furthermore, PACAP is required to maintain CORT elevation after chronic psychogenic but not systemic, stress (i.e., restraint and social defeat but not inflammation, cold, or hypoglycemia) (Hamelink et al. 2002; Tsukiyama et al. 2011; Lehmann et al. 2013). The secretion arm of signaling shown in Fig. 2 does not appear to be activated on PVN neurons stimulated with PACAP. PACAP activates stimulus-secretion coupling in CRH neurons in vivo, since elevation of CRH mRNA by psychogenic stress is abrogated in PACAP-deficient animals (Stroth and Eiden 2010). However, short-term activation of CORT elevation by restraint stress occurs equally in wild-type and PACAP-deficient mice, suggesting that CRH secretion in response to stress is not PACAP-regulated (Jiang and Eiden, 2016a). These observations have allowed the partial deconstruction of the hypothesis that the behavioral effects of chronic stress are secondary to the central effects of CORT on the brain after stress-induced activation of the HPA axis (Chrousos and Gold 1992). Rather, it appears that HPA axis activation (CORT) elevation and behavior effects of stress, are mediated via separate and parallel endocrine and behavioral pathways (Jiang and Eiden 2016a, b). These coordinated but separate signaling actions of PACAP are reminiscent of AVP's related but separate hormonal and neurotransmitter actions in the periphery and brain, respectively.

Functional neuroanatomy of AVP and PACAP circuits of the brain

In the 1970s, brain synapses that contained neuropeptides in addition to classical small-molecule transmitters such as glutamate or GABA were identified, raising the question of whether neuropeptides modify excitatory or inhibitory neurotransmitter actions upon co-release (Kandel and Squire 2000). However, this possibility has not been particularly well-developed in the intervening time, despite the intense morphological examination of vasopressinergic neurons in the context of exocytotic secretion initiated in the 1980s (Morris and Pow 1988). In fact, it was originally considered that the actions of AVP in extrahypothalamic regions of the brain were due to non-synaptic mechanisms. AVP/neurophysin II can be visualized in nerve terminals at the median eminence of the hypothalamus, in the posterior pituitary and at sites adjacent to tanycytes making up the lining of the cerebrospinal fluid compartment (Zimmerman and Robinson 1976). Furthermore, it has been suggested that AVP release from dendrites of AVPMNNs and AVP transport via extracellular fluid (i.e., quasi-hormonal delivery) could explain distant brain effects of AVP released from AVPMNNs (Landgraf and Neumann 2004; Leng and Ludwig 2008).

AVP neurons have been classified as magnocellular AVP neurosecretory neurons (AVPMNNs) and parvocellular AVP neurons, which are distinct in size and are reported to be located in the SCN, the bed nucleus of stria terminalis (BST), medial posterior internal division (BSTmpi), the central and medial amygdala (CeA and MeA) and the BST intra-amygdaloid division (BSTIA) (Fig. 3, panels A and A′) (Buijs et al. 1978; Buijs 1978; Armstrong et al. 1980; Caffe and van Leeuwen 1983; Sofroniew 1983; Castel and Morris 1988; Rood and de Vries 2011; Rood et al. 2013; Otero-Garcia et al. 2014; Hernandez et al. 2016). In addition, vasopressinergic neurons may be neurosecretory per se (releasing AVP into the circulation) and neuronal (possessing synaptic relationships with other neurons). They may also co-release classical neurotransmitters including glutamate and GABA. The role of AVP in homeostatic versus allostatic regulation may therefore vary depending on its location and mode of secretion/release, making the current understanding of vasopressin's central effects as primarily "anxiogenic" somewhat problematic (see following section). In this connection, the glutamatergic nature of AVPMNNs has been unequivocally demonstrated (Hrabovszky et al. 2007), whereas the parvocellular AVP neurons in limbic regions express the GABAergic neuron marker, VGAT (Zhang and Eiden unpublished) (Fig. 3b and b' and see references of figure legend).

These differences may be relevant to the apparent duality of AVP's role in anxiety and in motivational behavioral effects in the brain. Thus, the effects of infused AVP on enhancement of anxiety-related behavior and of endogenous AVP action and expression in linkage with anxiety traits, have been extensively documented and linked to vasopressin involvement in human anxious behavior (Bielsky et al. 2004; Bunck et al. 2009; Meyer-Lindenberg et al. 2011; Neumann and Landgraf 2012 and references therein). However, recent studies have found a profound pro-motivational effect of a vasopressin-glutamatergic pathway to lateral habenula in rats undergoing long-term (48 h) water deprivation (Zhang et al. 2016; and see following Section). Furthermore, activation of AVPMNN projections to GABAergic interneurons in the LHB suppresses the functional output of the LHb (Zhang et al. 2018). Both types of AVP neuron regulation, GABAergic and glutamatergic, may have adaptive value. For example, adult rats previously deprived of maternal care have a potentiated AVP system (Zhang et al. 2012) promoting caution (enhanced avoidance) and better preparation for dealing with a threatening environment when the animal's survival is not severely threatened. In contrast, when under severe physiological stress, such as prolonged food and water deprivation, animals may be prepared to take greater risks in balancing external versus homeostatic threats to survival. Thus, both external threats and internal homeostatic demands can exert powerful yet integrated modulation of emotion, motivation, and motor circuits. That both glutamatergic and GABAergic vasopressinergic neurons are intrinsically regulated by sex

steroids and gonadal function adds a further level of integration encoded within the evolutionarily conserved vasopressinergic neuronal system(s) (Rood et al. 2013; Otero-Garcia et al. 2014; Zhang et al. 2018).

Demonstration of AVP in nerve terminals of extrahypothalamic projections originating in AVPMNNs is technically challenging. It has been attempted, however, largely because of pharmacological evidence for the presence of functional AVP receptors in several brain regions, with strong effects of locally infused vasopressin suggestive of physiological relevance (Veenema and Neumann 2008) and references therein). There is also a clear association between AVP biosynthesis in PVN neurons in response to homeostatic demand and AVP-dependent behaviors, suggesting a synaptic relationship between AVPMNNs of PVN and these brain areas (Zhang et al. 2012; Hernandez et al. 2016; Zhang et al. 2016, 2018). Neurochemical and ultrastructural demonstration of vasopressinergic synapses of magnocellular origin in amygdala of the rat is illustrated in Fig. 4 (Hernandez et al. 2016). Connection between cell bodies of PVN and nerve terminals in other brain areas (the AVPMNN system) has been unambiguously demonstrated, through the employment of techniques such as juxtacellular labeling, optogenetics and ultrastructural analysis (Cui et al. 2013; Zhang and Hernandez 2013; Hernandez et al. 2016; Zhang et al. 2016). The demonstration of *dual* projections from AVPMNNs of the paraventricular nucleus (PVN) to both the posterior pituitary (hormonal) and to the amygdala, hippocampus, and habenula (neurotransmitter) provides a neuroanatomical basis for understanding how vasopressinergic cells integrate homeostatic and allostatic regulation. Reflexive endocrine control of the internal milieu (homeostasis) and neuronal control of drives that promote homeostasis (e.g., thirst) occur at the level of the hypothalamus and hypophysis. At the same time, through projections to extrahypothalamic regions, these responses are linked to appetitive/rewarding aspects of thirst and allostatic regulation of complex behaviors such as escape and fear responses (Zhang et al. 2018). Developmental environmental inputs such as maternal deprivation, in fact, can have lifelong effects on stress responding and anxious behavior through long-term plasticity of AVPMNNs (Zhang et al. 2012).

In summary, AVP is released from MNNs at both neurohemal neurosecretory terminals and at the synaptic terminal throughout the brain, especially limbic regions influencing emotional responses during stress coping and complexly motivated behaviors (Zhang et al. 2012, 2018; Zhang and Hernandez 2013). The actions of AVP in brain may also be non-synaptically derived from generalized release from MNN neurosecretory cells; and from vasopressinergic projections arising from non-magnocellular AVP cell bodies in BNST, amygdala and elsewhere (Buijs 1978; Buijs et al. 1978). The extent to which vasopressinergic control of amygdalar, hippocampal and habenular outputs is hormonal (through AVP release into CSF and diffusion to these nuclei) versus synaptic is not clear. Hybrid effects of synaptic and quasi-hormonal release of AVP may, finally, underlie the totality of AVP actions in extrahypothalamic brain and on behavior (Landgraf and Neumann 2004; Leng and Ludwig 2008). Knowing when either volume transmission or neurotransmission is dominant within a given behavioral circuit may affect therapeutic strategies for pharmacological intervention in disorders linked to overexpression or deficiency of amine and peptide first messengers (Bunin and Wightman 1999). An important test of these alternative hypotheses will come from more detailed analysis of the relative distribution of AVP receptors immediately post-synaptic to AVP terminals, or more widely, in specific brain areas.

The functional neuroanatomy of brain PACAP (chemically identified in 1989), is much less well-understood than that of AVP (chemically identified in 1955). Nevertheless, one can ask (i) where are the cell bodies, (ii) where are the terminals and (iii) how are they linked in circuit projections, i.e., a connected group of cell bodies and nerve terminals. The classic investigation of PACAP terminal and cell body distribution in the rat was published in 2002 (Hannibal 2002), using immunohistochemistry for PACAP-38 and in situ hybridization histochemistry for proPACAP mRNA detection, PACAP mRNA was visualized in cell bodies of all layers of cortex except for layer IV; in hippocampus and overlying cortex and subiculum; throughout central, medial and basolateral amygdala and nucleus of lateral olfactory tract; throughout hypothalamus (except for supraoptic, suprachiasmatic, arcuate and paraventricular nuclei) and especially ventromedial, premammillary and mammillary nuclei; in habenula, pretectal and precommissural nuclei; predominantly in parabrachial nucleus and several sensory and motor nuclei of brain stem; in cerebellar Purkinje cells; in two of the circumventricular organs (subfornical and OVLT); and in the intermediolateral column. PACAPergic neurons were notably absent from olfactory bulb, basal ganglia and thalamus. PACAPergic nerve terminals were most abundant in central and medial amygdala and BNST; in all nuclei of hypothalamus; in ventral pallidum; in interpeduncular and parabrachial as well as sensory and motor nuclei of brain stem; and prominently in circumventricular organs as well as layers I–II of spinal medulla, consistent with the known PACAPergic phenotype of peripheral sensory neurons conveying pain (Moller et al. 1993; Dickinson and Fleetwood-Walker 1999). The existence of PACAP expression in the retinohypothalamic innervation of suprachiasmatic nuclei of the hypothalamus is well-established (Hannibal et al. 2002; Hannibal 2006); and it is likely that PACAPergic innervation of the remainder of the hypothalamus is relatively local. The source of hippocampal PACAPergic innervation is not yet known. Central, amygdalar and bed nucleus of the stria terminalis innervation

and possibly also that to the ventral pallidum, is likely to arise from PACAPergic/CGRP-positive neurons of the lateral parabrachial nucleus (Missig et al. 2014).

Clearly establishing the anatomical relationships between PACAP cell bodies and terminals, in both rat and mouse, is a critical next step in the field. Determining the role(s) of putative brainstem PACAPergic projections to extended amygdala and ventral pallidum in long-term PACAP-dependent behaviors, such as stress-dependent relapse to cocaine self-administration and stress-induced depressive and anxious behaviors (Jiang and Eiden 2016a, b; Miles et al. 2017) is critical. It will establish a connection between aversive sensory input and long-term behavior, with clear implications for understanding and treatment of PTSD, depression and generalized anxiety syndromes (Hammack and May 2015). Finally, defining the role(s) of PACAPergic neurons of the cortex (Crestani et al. 2013), associational and projection, will help to integrate the "top down" and "bottom up" aspects of PACAPergic regulation of stress responding (Fig. 5).

Endocrine and behavioral control by vasopressinergic circuits of the brain appear to be anatomically linked via dual projection neurons to pituitary/median eminence and to social/behavioral brain nuclei regulating behavior, such as the hippocampus, amygdala and habenula (Zhang et al. 2016, 2018). Endocrine and behavioral control by PACAPergic circuits of the brain appear to be arranged in parallel, with hypothalamic neurons regulating endocrine stress responding and projections from brain stem and possibly cortex modulating and controlling both acute and chronic stress-responsive behaviors.

Global actions of AVP and PACAP in homeostatic versus allostatic regulation: vasopressin as a "master modulator of need satisfaction" and PACAP as a "master regulator of the stress response"

The Brattleboro rat provided an early genetic model for the homeostatic functions of AVP release from the posterior pituitary and also for the important pivot towards appreciating the role of AVP in allostatic regulation. These studies complemented early pharmacological demonstrations of the behavioral effects of infused vasopressin (references vida supra), implying an actual behavioral as well as a physiological role for endogenous vasopressin in the brain. Initially, the Brattleboro rat was discovered by forward genetic analysis to be defective in the regulation of water retention due to lack of production of AVP secondary to a propressophysin processing defect (Kim and Schrier 1998). Thus, the Brattleboro rat performs less well than normal counterparts in adaptation to novelty and during development, suggesting a role for brain AVP consistent with that discovered by infusion of AVP into normal rat brain (Veenema and Neumann 2008; Csikota et al. 2016; Paul et al. 2016). Appreciation that the behavioral effects of AVP infused into the brain are not merely pharmacological was in part due to the discovery of cognate behavioral deficits in the Brattleboro rat.

Considerable effort has been devoted to understanding how AVP neurosecretory cells might control both water balance and behavior and whether these dual functions were physiologically and anatomically related. To illustrate the integration of homeostatic with allostatic regulation by AVP dual ascending and descending projections we focus here on three projections from AVPMNNs of PVN, to amygdala, to habenula and to the hippocampus. Initially, despite the strong expression of the AVP1 receptor(s) in the hippocampus, it was deemed that vasopressin's actions at this locus were due to a hormone-like effect of vasopressin released initially from AVPMNN dendrites, or a frank hormonal action of AVP released from posterior pituitary (Landgraf and Neumann 2004). Direct anatomical evidence for AVPMNN projections to hippocampus was adduced by Zhang et al., in the rat, using juxtacellular labeling in conjunction with immunohistochemistry for AVP/neurophysin (Zhang and Hernandez 2013). In 2013, Cui et al. showed in the mouse that AVP action in CA2 of hippocampus was most likely due to direct projections from PVN, based on the V1a antagonist-blockable effects of optogenetic stimulation of PVN AVPMNNs in vivo (Cui et al. 2013).

Juxtacellular labeling to identify vasopressinergic projections to the amygdala and habenula were followed by functional investigations, in which it was shown that the behavioral importance of these projections waxed or waned as a function of physiological manipulations of AVPMNNs by water deprivation or salt loading. These experiments strengthened the linkage between the AVPMNN system and stress-coping and aversive behaviors and also suggested that homeostatic drives like thirst might function as contingencies for behaviors via AVPMNN projections. The further dependence on the gonadal status of patent vasospressinergic projections to habenula, local generation of estrogen via aromat6ase expression in AVPMNNs and estrogen receptors on post-synaptic elements ("GERN", GABAergic-estrogen receptive neurons) in LHb link thirst/water balance, gonadal status and behavior. Figure 6 depicts the hub-like arrangement, reminiscent of those identified in the invertebrate nervous system (Macosko et al. 2009) in which habenular function is modulated by AVP and other inputs. Whether the AVPMNN projections to habenula, hippocampus and amygdala are in parallel or in series from the PVN and whether the dramatic loss of vasopressin delivery to magnocellular projections to habenula upon castration (Zhang et al. 2018) is reflected in parallel loss of amygdalar and hippocampal projections, are two critical questions that must be answered before the full circuit logic of the AVPMNN

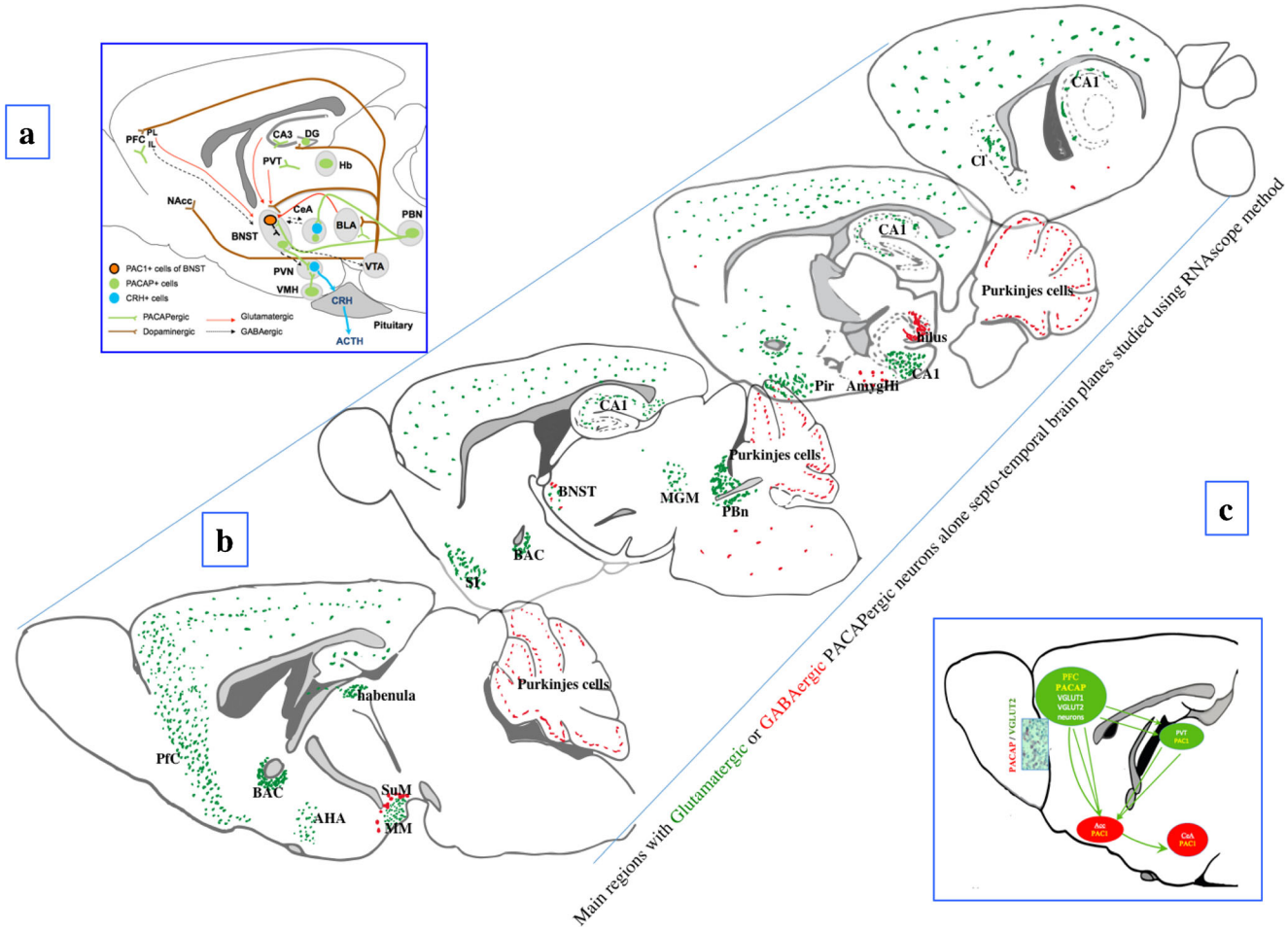

Fig. 5 PACAPergic projections in the CNS. Diverse chemical nature and wide brain distribution of PACAPergic neurons and proposed circuitry. **a** Schematic of current understanding of PACAP circuitry in the rodent brain. **b** Charting along septo-temporal brain planes showing the wide distribution and diverse molecular signatures of PACAPergic neurons revealed by the highly sensitive RNAscope multichannel in situ hybridization method. Glutamate-PACAPergic neurons are symbolized in green and GABA-PACAPergic neurons are red. The newly discovered region/cell type, which have not been properly studied, include the bed nucleus of anterior commissure (BAC) in which more than 90% of the neurons are both VGluT1/VGluT2 and PACAP positive. **c** The recently discovered cingulate-PrL-IL pathways to accumbens, VTA and CeA may use PACAP as a co-transmitter; and accumbens, VTA and CeA strongly express PAC1 (unpublished data). Pathways using PACAP signaling may be involved in emotion-based decision-making and motor coordination, with particular relevance to stress coping, anxiety, depression, PTSD and sex-steroid/neurosteroid-mediated mood disorders

modulation of behavior can be apprehended. The role of altered dynorphin expression in magnocellular neurons, which may be decisive in determining preference for fluid imbibition, also has relevance both for how these circuits operate and how they arose in evolution (Brown et al. 2007; Greenwood et al. 2015).

It would be useful to recapitulate in the mouse the fascinating features of extrahypothalamic projections and their functions described in rat. Optogenetic manipulation at the level of the vasopressinergic and orexinergic inputs to GERN could then be used to establish the relevance of these inputs to specific behaviors. Determining the extent of aromatase-mediated neurosteroid circuit modulation in nuclei besides LHb and potential presence of GERN in other nuclei besides LHb, will establish the generality of this circuit architecture in the brain. Finally, characterizing further the remarkable gonadal status-driven changes in supply of vasopressinergic terminals to the LHb, will likewise facilitate extension to other peptidergic behavioral circuits, perhaps including the PACAPergic projections governing stress responding (King et al. 2017).

PACAP circuitry controlling behavioral responses, as mentioned previously, differs from that of AVP in that endocrine and behavioral circuits appear to function in parallel, rather than as single dual-projecting neurons (see Fig. 7). Nevertheless, considerable additional effort will be required to learn the circuit architecture that governs PACAP modulation of both endocrine and behavioral stress responding. While these appear to be parallel circuits, a functional

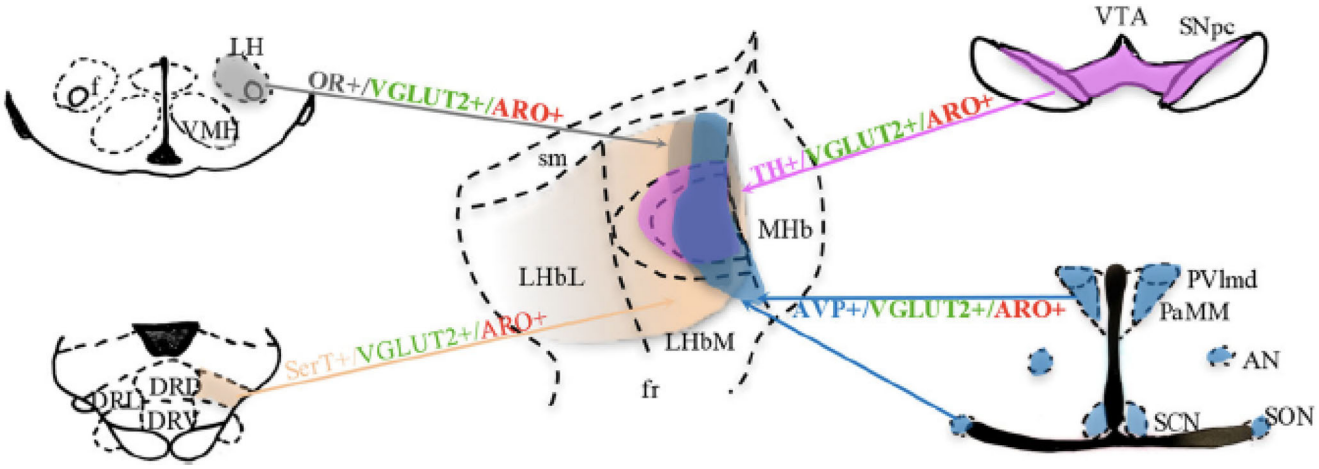

Fig. 6 Convergence of peptide/amine inputs onto an AVP/neurosteroid control point in lateral habenula. Vasopressinergic and orexinergic projections from hypothalamus converge with aminergic projections onto GERN cells of lateral habenula orexinergic allowing aversive behavioral decisions to be modulated by gonadal and fluid (osmotic) status. Adapted from Zhang et al. (2018)

connection between the two, equivalent to the dual projection mode of AVP action on endocrine and behavioral limbs of homeostatic/allostatic responses, may yet be uncovered.

The role of intercellular and intracellular signaling networks for peptide therapeutics: how do AVP and PACAP actually act on post-synaptic neurons to alter behavior?

A fundamental set of questions in understanding how neuropeptides modulate organismic behavior at the level of the central nervous system involves the post-synaptic actions of peptides, through activation of their G protein coupled receptors, on both short-term (ionotropic) and long-term (metabotropic) post-synaptic cellular behavior.

The intriguing hypothesis has been put forward that the effects of vasopressin on CA2 neurons of hippocampus underlies the function of these neurons as "social cells" akin to "place cells" in the hippocampus and involves the induction of glutamate-dependent long-term potentiation (LTP) in these cells (Pagani et al. 2014). The intracellular mechanisms by which LTP is induced in these cells through vasopressin-initiated signaling, presumably in a Gq-coupled fashion, have not yet been elucidated.

PACAP signaling through PAC1 is predominantly Gs-coupled and occurs through activation of at least three cAMP sensors in neurons, following elevation of cyclic AMP. Cyclic AMP arming of each sensor, in neuroendocrine cells in culture, leads to activation of CREB (via PKA), p38 (via Epac) and ERK (via NCS-Rapgef2) (Emery and Eiden 2012; Emery et al. 2013, 2014, 2017a, b). The correlation for each of these pathways in mediating both endocrine and behavioral stress responding is yet to be determined. For example, PACAP acts in the PVN to couple stress responding to CRH gene transcription, and elsewhere in the brain to mediate more acute behavioral effects of stress that may depend on PACAP coupling to secretion as well as gene transcription (Jiang and Eiden 2016a, b). Different post-synaptic responses to PACAP neurotransmission may require separate cAMP effectors, or all three may act in concert to exert PACAP's post-synaptic effects. The two limbs of the PACAP-dependent stress response, endocrine and behavioral, probably reside in anatomically distinct PACAPergic cell groups/nuclei (Jiang and Eiden 2016a, b). In contrast, it is clear that AVPMNN neuronal projections to limbic targets, including hippocampus, amygdala and lateral habenula, do arise from neurons that also project to the posterior pituitary (Zhang and Hernandez 2013, Hernandez et al. 2016; Zhang et al. 2016). What is not yet known is whether these projections represent in themselves distinct PVN subpopulations that project separately to each of the three limbic areas. Further investigation of these possibilities for AVPMNNS, as well as the delineation of the PACAPergic projections subserving endocrine and behavioral stress responses, should yield fascinating results important for understanding neuropeptide synaptic signaling and its role in physiological and psychological functioning.

A broader question is whether neuropeptides like AVP and PACAP act on their own post-synaptically, or modulate the post-synaptic actions of classical neurotransmitters such as GABA and glutamate. Both AVP and PACAP alter the voltage-activation parameters of neurons expressing their respective receptors, suggesting modulation of excitatory and inhibitory neurotransmission. This principle of action has been suggested for neuromodulatory neuropeptides in non-mammalian organisms (Stawicki et al. 2013). PACAP is modulatory to glutamate on phase-shifting of electrical activity in neurons of the suprachiasmatic nucleus in the rat (Gillette et al., in review, Frontiers in Neuroscience). On the other hand, PACAP causes secretion from chromaffin cells of the adrenal medulla

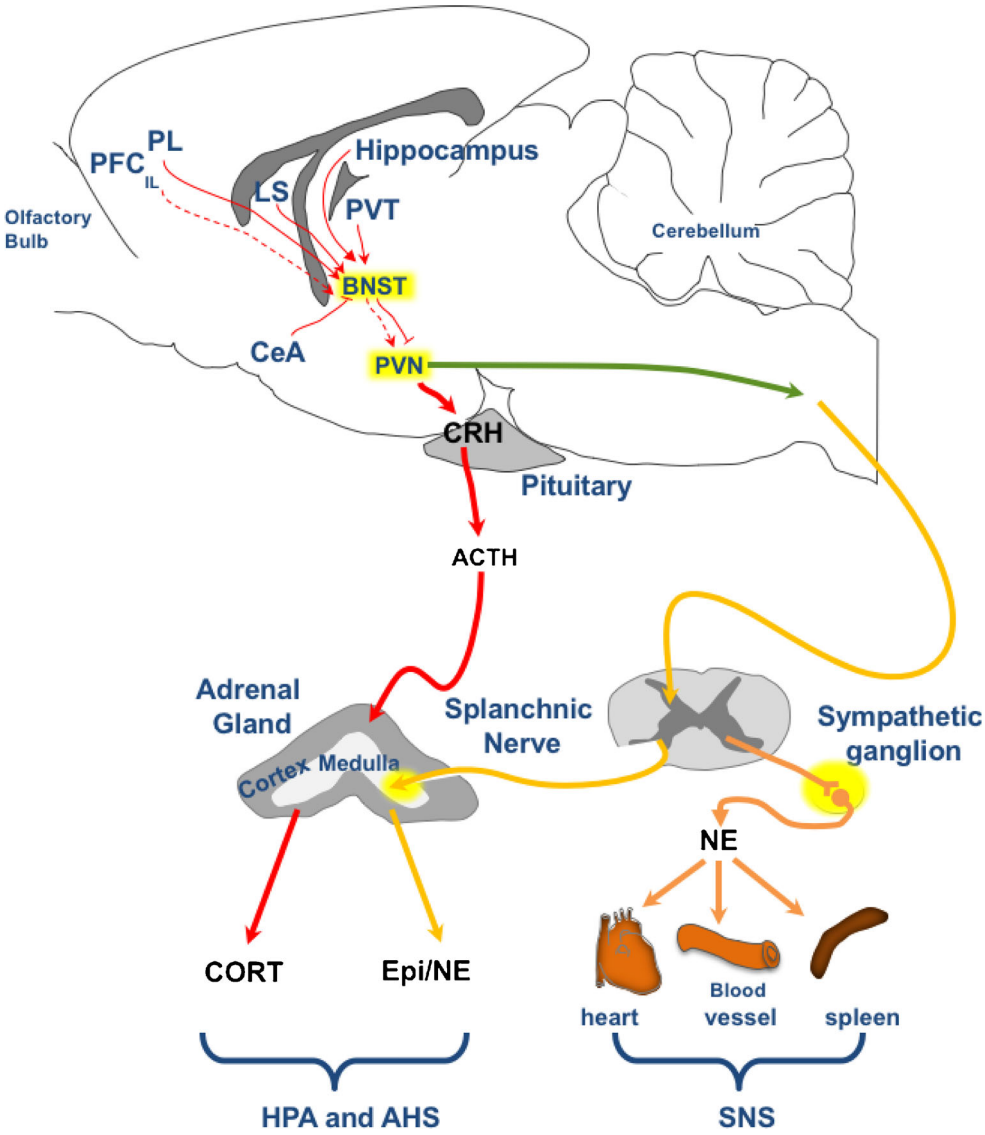

Fig. 7 PACAP as a master regulator of stress signaling. Peripheral and presumptive central anatomical loci of PACAP action related to stress-responding is shown. Adapted from Jiang and Eiden (2016a, b)

in a way that appears to be wholly independent of co-released acetylcholine at that synapse (Smith and Eiden 2012). Elucidation of these questions will be important in focusing on translational efforts for both peptides, in a variety of pathophysiological and physiological contexts.

Conclusions: the lessons of AVP and PACAP for general themes of regulatory peptide modulation in the brain

AVP has made the journey (rather, biologists have transported it) from physiologically necessary antidiuretic hormone in virtually all tissue-diversified animals who must conserve and regulate body water osmolality, to brain modulator of thirst, and prioritization of thirst along with other drives, via its synaptic actions throughout the brain. The homeostatic actions of AVP as a hormone controlling solute-free water resorption via activation of a single kidney tubule-expressed receptor seem relatively straightforward. Its allostatic functions in the brain, including anticipation of thirst, stress coping, aversion and reward are more complex, involving the projection of organismic and even species drives to insure reproduction and self-preservation through behaviors motivated by aversion and reward. Vasopressin's actions are contingent at the synaptic level on co-secretion of glutamate, at the hormonal level on gonadal status and at the physiological level on hydroelectrolyte balance. These represent a hierarchy of functions whose integration should be understood for rational application to peptide-centric therapeutics.

PACAP has made a similar journey, from hypothalamic hormone/hypophyseal factor to master regulator of stress responding throughout the nervous system. The peripheral actions of PACAP, both in the immune and sensory and

autonomic systems, however, clearly demonstrate that PACAP circuitry has evolved in a fundamentally different way than that for AVP. The co-evolution of neuropeptides and their receptors, along with the nervous circuits and structures that contain them, remains a fascinating area of inquiry with profound implications for neurobiology, as well as for translational application to human disorders in which neuropeptide function may be a decisive factor.

Post-script

This review is an occasional one: the event celebrated being the life of molecular neurobiologist Peter Seeburg. Peter began his independent career characterizing at the molecular level the hormones of the pituitary gland, moved to the peptide hormones elaborated by the neurosecretory cells of the hypothalamus and finally, shifted his research focus from the molecular basis of hormone action, to the molecular bases of neurotransmitter action. Interestingly, the peptide hormones with which Seeburg originally concerned himself have made a similar journey, at least in the minds of the scientists who study them: from initial consideration solely as hormones to characterization and examination as neurotransmitters. Exploring this new world that he did so much to help create is perhaps Peter Seeburg's most important legacy.

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