Interneuron Diversity series: Rhythm and mood in perisomatic inhibition

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GABAergic interneurons innervating the perisomatic region of pyramidal cells control population discharge patterns, and thereby all cognitive operations in the cerebral cortex. A striking dichotomy in the function of this interneuron population seems to emerge from the synthesis of recent molecular, anatomical and electrophysiological data. Synaptically and electrically coupled networks of parvalbumin-containing basket cells operate as a non-plastic, precision clockwork for gamma and theta oscillations, and are indispensable for basic cortical processing. By contrast, a highly modifiable interneuron syncytium containing cholecystokinin carries information from subcortical pathways about the emotional, motivational and general physiological state of the animal, and appears to be involved in the fine-tuning of network cooperativity. Impairment of this inhibitory mechanism is likely to result in mood disorders such as anxiety.

The question of how morphological diversity translates to functional differences in cortical inhibitory circuits has been extensively investigated in recent decades. High-resolution immunocytochemistry for receptors, neurotransmitters and enzymes involved in synaptic signalling [1] has generated a firm ground of molecular architecture for interpreting functional data, in addition to numerous testable functional predictions. Furthermore, combined anatomical–electrophysiological–pharmacological approaches – capable of such direct tests – have allowed a better insight into the roles played by interneuron types at the network level *in vitro* and *in vivo* [2–4].

The most striking morpho–functional dichotomy in the population of cortical interneurons is the targeting of the dendritic versus the perisomatic domain of principal cells. The functional predictions of this morphological difference were tested by recordings from single cells or connected cell pairs in the hippocampus in several laboratories [3,5–7]. A consensus emerged that dendritic inhibition is likely to control the efficacy and plasticity of excitatory synaptic inputs of principal cells, whereas perisomatic inhibition is ideally suited to control output, synchronizing the action potential firing of large groups of principal cells [8]. The termination strategy of neocortical interneurons appears to follow the same rules, resulting in similar distinct functional roles, but this is less apparent owing to the complex lamination and intrinsic connections in the neocortex [1]. This review focuses on perisomatic inhibitory cells of the hippocampus, with implications for the entire cerebral cortex.

Perisomatic inhibitory cells – involvement in network synchrony

Perisomatic inhibitory cells in the hippocampus innervate the somata, proximal dendrites and axon initial segments of principal (pyramidal and granule) cells, and are ideally suited to control the pattern and timing of their output [5–9]. The action of single perisomatic inhibitory cells can synchronize action potential discharges – at theta and/or gamma frequencies, and/or during hippocampal sharp waves – from all of the 1000–2000 principal cells they innervate. These population oscillations and intermittent synchronous events are thought to be crucially involved in different sleep states, in various cognitive processes including selective attention, and in the encoding and binding of information, for associating features into unified perceived objects at the neocortical level. In addition, coupling of neocortical and hippocampal gamma oscillations might be able to bind representations associated with currently perceived and retrieved information [8–12]. Thus, synchronization does indeed play a fundamental role in cortical functions. Such precise cooperation among principal cells could be brought about by a single perisomatic inhibitory cell class, if principal cells were to be synchronized uniformly in space and time. Otherwise, different perisomatic inhibitory cell types should specialize for different time windows, behavioural states, oscillation frequencies or target cell populations. The fact that at least three distinct cell populations – two basket-cell types and the chandelier or axo–axonic cells – are evolved for perisomatic inhibition suggests that, indeed, the task is more complex. However, no evidence is available to date about profound differences in the spatial or temporal specialization of activity of these three cell types, except for the difference in firing between chandelier and basket cells during hippocampal sharp waves [4]. Common features of perisomatic inhibitory cells underlying their presumed general function in oscillations will be reviewed in the first section of this article. In the second, emphasis will be placed on the heterogeneity in the afferent and efferent connectivity, receptor expression and neurochemical-marker content of the three cell types that led to the hypothesis proposed here for a division of labour among the them.

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Common features of perisomatic inhibitory cells

Chandelier or axo–axonic cells

Chandelier or axo–axonic cells are specialized to innervate selectively the axon initial segments (AIS) of principal cells, forming multiple, climbing-fibre-like contacts on each target AIS [13], thus having an ideal termination strategy for output control. They contain the Ca^{2+}-binding protein parvalbumin (PV) [14] but no other markers (neuropeptides or Ca^{2+}-binding binding proteins) known so far. In postsynaptic pyramidal cells they evoke large amplitude inhibitory postsynaptic potentials (IPSPs) that are indistinguishable from those evoked by basket cells [3,8], whereas during theta activity recorded *in vivo* they fire counter-phase with pyramidal cells, similar to basket cells [4]. The special functional reason or advantage of terminating on AIS rather than on the soma has been a puzzling question for decades and does not yet have a satisfactory answer.

Basket cells

Basket cells fall into at least two types, based on their neuropeptide or Ca^{2+}-binding-protein content and several other features that correlate with these neurochemical differences (Figs 1–3). The most widely known basket-cell type contains PV [14], whereas the other contains vasoactive intestinal polypeptide (VIP) [15] and/or cholecystokinin (CCK) [16]. Whether there are basket cells that lack all known markers (including PV, CCK and VIP) or others that express CCK alone, in addition to those that contain both CCK and VIP, remains to be established. The present comparison is made between PV-containing and CCK-containing basket cells knowing that, although many of the latter can also express VIP, these two types account for the majority of perisomatic GABAergic terminals. The morphological features of PV- and CCK-containing basket cells are similar in that both types have a bitufted dendritic tree spanning all layers (they are less abundant in the stratum lacunosum-moleculare, indicating a sparse entorhinal input) and an axon terminal cloud largely confined to the stratum pyramidale (or granulosum in the dentate gyrus), where they innervate the somata and proximal dendrites of principal cells [8].

Intracellular recordings from identified cells revealed some differences in firing characteristics and postsynaptic actions. Most PV-containing cells were found to be fast-spiking, able to follow frequencies >100 Hz without accommodation, whereas most CCK cells were regular-spiking (although some fell into the fast spiking category) and their maximum firing rate in neocortex was $\sim 40–50$ Hz, showing considerable accommodation [17–20]. Paired intracellular recordings did not reveal significant differences in the kinetic parameters of postsynaptic responses evoked by PV- or CCK-containing basket cells [19,20]. However, GABA release from axon terminals of indirectly identified CCK-containing cells was shown to be mediated by N-type Ca^{2+} channels, whereas only P/Q-type Ca^{2+} channels were found on boutons of the other basket-cell type [21]. During theta activity recorded *in vivo*, PV-positive basket cells fire counter-phase with pyramidal cells, in the same phase but slightly delayed relative to chandelier cells [4]. Similar data on CCK cells have not been published to date.

Gamma oscillations

Gamma oscillations are known to be associated with phase-locked rhythmic activity of perisomatic inhibitory

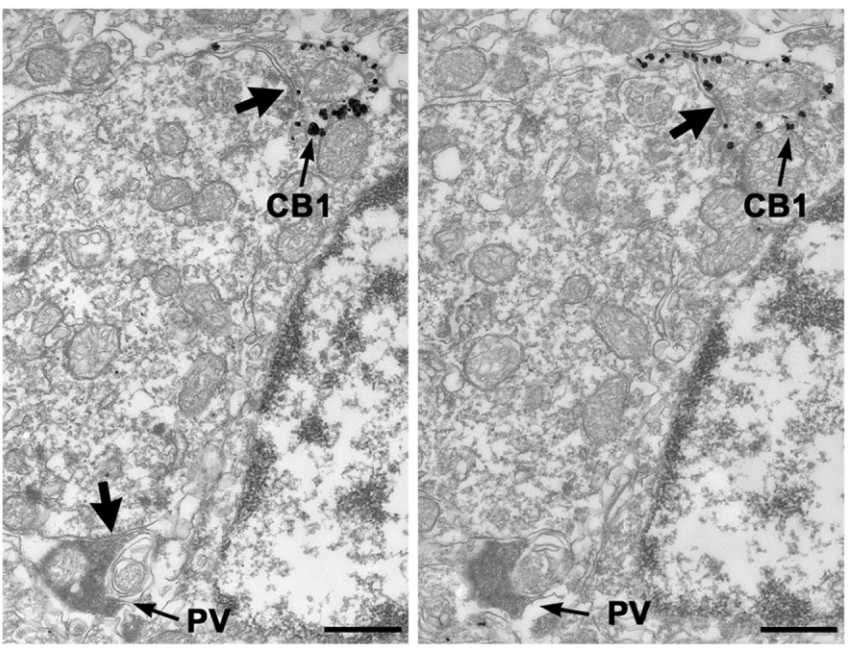

Fig. 1. Serial sections from axon terminals of two different basket-cell types forming symmetrical synapses (thick arrows) on a cell body in the CA3 subfield of the rat hippocampus. The upper bouton is immunoreactive for the cannabinoid receptor CB1 (dense immunogold labelling around the membrane) but not for parvalbumin (PV), whereas the lower terminal is positive for PV [electron-dense, diffuse 3-3′-diaminobenzidine (DAB) precipitate of immunoperoxidase reaction] but negative for CB1. Other double-staining experiments showed that the CB1-positive, PV-negative boutons belong to cholecystokinin (CCK)-containing basket cells [38,41]. Note that silver–gold particles representing CB1 protein cover the axon terminal in addition to the pre-terminal segment, and are restricted to the inner surface of the bouton, where the intracellular C-terminal epitope of CB1 is located. Scale bars, 0.5 μm. Reproduced, with permission, from Ref. [41], © (2000) Blackwell Publishing.

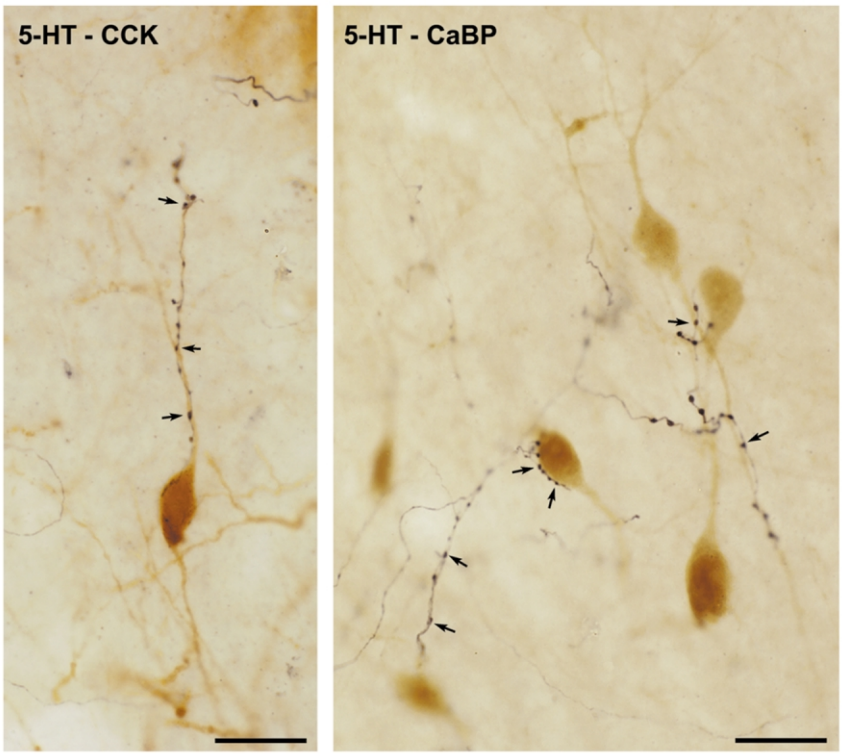

Fig. 2. Serotonergic (5-HT) afferents anterogradely labeled by *Phaseolus vulgaris* leucoagglutinin [black immunoperoxidase reaction product in axons, Ni^{2+}-intensified 3-3′-diaminobenzidine (DAB) used as chromogen] from the median raphe nucleus. These afferents form multiple contacts (arrows) on the somata and dendrites of cholecystokinin (CCK)-containing (a) and calbindin (CaBP)-containing (b) interneurons (brown immunoperoxidase reaction product, DAB used as chromogen) in the stratum radiatum of the rat hippocampus. Scale bars, 20 μm.

cells [6,12] but whether the two basket-cell types and the chandelier cells contribute to a similar extent is still unknown. In PV-knockout animals, the power of kainate-induced gamma oscillations is approximately twice that in wild-type animals, suggesting a causal involvement of cells that normally contain PV [22]. A common feature of both PV- and CCK-containing basket cells is the formation of ensembles coupled by both electrical and chemical junctions, which are required for an efficient entrainment of principal cells at gamma frequency [23,24]. PV-containing (fast-spiking) basket cells innervate each other, and are also extensively connected via gap junctions [25–28]. PV-containing cells appear to form a large electrically coupled syncytium in various cortical areas, each PV-containing cell being connected (directly or indirectly) to 20–50 others [28]. Whether chandelier cells are part of this syncytium is unknown, but they are definitely not connected to each other synaptically. CCK-containing cells do innervate each other [16], and in addition apparently form gap junctions [29], although the extent of electrically coupled CCK-containing cell syncytia might be smaller than those of PV-containing cells because the incidence of connected cell pairs in paired recording experiments was lower (compare data in Refs [27,29] and Ref. [28]). These data suggest that, although theoretically each of the three perisomatic inhibitory cell types could be involved in gamma oscillations, the most likely candidates – owing to the extensive synaptic and electrical coupling, and the ability to fire at high frequency without accommodation – are the PV-containing basket cells. Chandelier cells are less likely to be involved as they are not connected to each other synaptically, but they might be entrained by input from basket cells. CCK-positive cells are also unlikely to play a role, because most of them are unable to fire at high frequencies without accommodation. Thus, a hypothesis is proposed for an alternative function of CCK-containing interneurons, which might complement that of PV-containing baskets.

Hypothesis

The electrically and synaptically coupled, and mostly locally driven, ensembles of PV-containing basket cells are indispensable components of the oscillating cortical hardware; they represent the rigid (non-plastic) precision clockwork without which no cortical operations are possible. The activity of a similar syncytium of CCK-containing basket cells is superimposed on the PV-containing basket-cell-entrained network, conveying the emotional and motivational effects carried by serotonergic and cholinergic pathways. In addition, actions of the CCK-containing cell ensemble are highly modifiable by local neuromodulators and retrograde signalling molecules, which might allow further fine tuning of principal cell cooperation. Impairment of this tuning system is likely to result in mood disorders such as anxiety.

Evidence for the hypothesis – implications for anxiety

This hypothesis is supported by several lines of evidence: differences in postsynaptic actions, differences in presynaptic receptors, and differences in

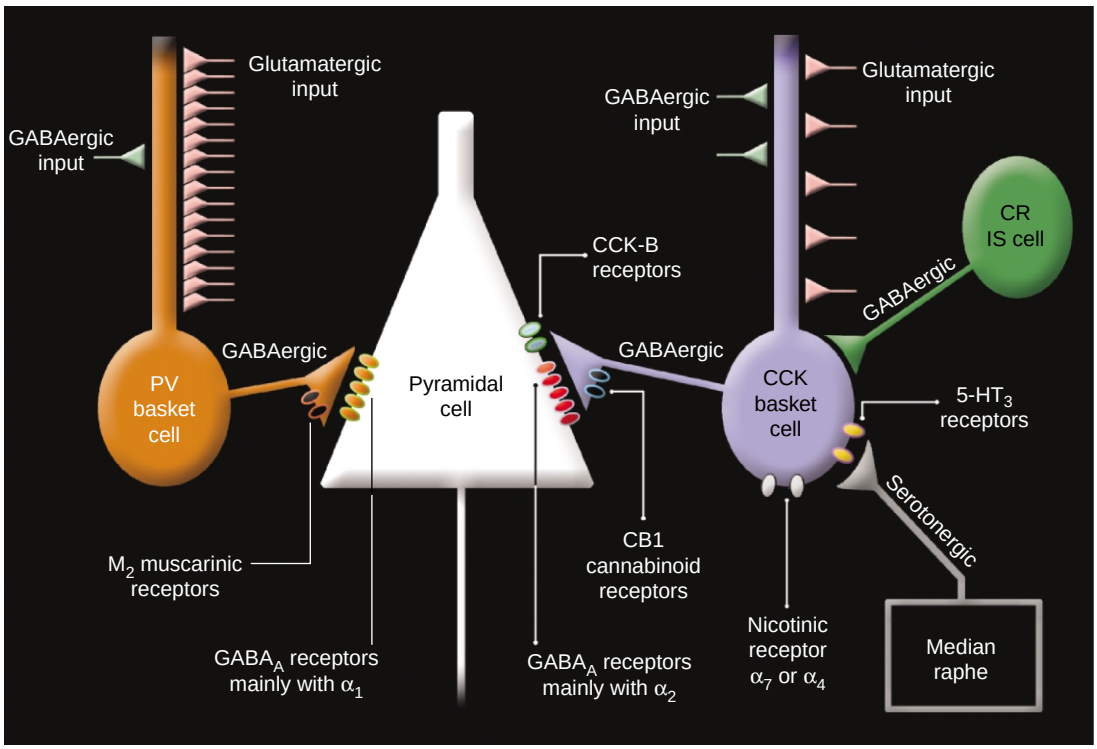

Fig. 3. Major differences between parvalbumin (PV)- and cholecystokinin (CCK)-containing basket cells in their connectivity features and receptor expression patterns. Each axon terminal synapsing on the interneurons [glutamatergic (pink) or GABAergic (light blue)] corresponds to ~1000 synapses, reflecting true differences in the relative weight of excitatory and inhibitory inputs. CCK-containing cells express presynaptic CB1 cannabinoid receptors and postsynaptic 5-HT_3 5-hydroxytryptamine (serotonin) receptors and nicotinic α7 (or α4) ACh receptors. They receive input from serotonergic median raphe afferents and local interneuron-selective inhibitory cells expressing calretinin (CR IS cell; green). All of these features are absent in PV-containing cells, but these cells express presynaptic M_2 muscarinic receptors [59]. Knowing that anxiolytic effects of benzodiazepines are mediated solely by α2-subunit-containing $GABA_A$ receptors, it is important to note that synapses formed by CCK-positive basket cells on pyramidal cells operate mostly via α2-subunit-containing $GABA_A$ receptors, whereas PV-positive basket-cell synapses contain largely α1 subunits.

subcortical and local synaptic input with associated postsynaptic receptors (Fig. 3).

Postsynaptic actions

(1) Benzodiazepines are the most commonly used anxiolytics, and their anxiolytic effects are selectively mediated by α-2-subunit-containing $GABA_A$ receptors [30]. Synapses formed by CCK-containing basket cells on hippocampal pyramidal cells are selectively enriched in α-2 subunits [31], in contrast to PV-containing basket cells, which form synapses predominantly via α-1-subunit-containing $GABA_A$ receptors [18,32]. Thus, benzodiazepines achieve anxiolysis via potentiating inhibition evoked by CCK-containing, but not PV-containing, basket cells.

(2) CCK-B-receptor antagonists are potent anxiolytics [33]. CCK-containing interneurons represent the major source of CCK released in cortical areas in a Ca^{2+}-dependent fashion [34].

Presynaptic receptors

(3) Cannabinoid ligands in specific doses are known to have anxiolytic effects [35–37]. CB1 cannabinoid receptors are expressed predominantly by axons of CCK-containing interneurons (Fig. 1) in the hippocampus [38], amygdala [39] and neocortex [40], and they reduce GABA [38,41] and CCK release [42] when activated. Depolarization-induced suppression of inhibition [43], mediated by endocannabinoids [44], affects only CCK-containing cells – not PV-containing cells, because these do not express CB1 receptors [38].

(4) $GABA_B$ receptors are synthesized in large numbers in the somata of CCK-containing cells, but not in PV-containing cells [45]. These receptors are likely to be transported down to the axon terminals in a similar way to CB1 receptors because presynaptic $GABA_B$-mediated inhibition of GABA release has been extensively documented [46]. However, its existence in CCK-positive cells and absence in PV-positive cells has not been demonstrated yet by direct electrophysiological methods.

Subcortical input

(5) Selective 5-HT_3 5-hydroxytryptamine (serotonin)-receptor antagonists have strong anxiolytic effects [47,48]. In cortical areas, 5-HT_3 receptors are selectively expressed by a subset of GABAergic neurons, which includes predominantly the CCK-containing cells, in addition to some (probably overlapping) sets of VIP-, calbindin- and calretinin-containing cells [49–51]. The very same interneuron populations are selectively innervated by serotonergic afferents from the median raphe nucleus (Fig. 2), whereas PV-containing interneurons and pyramidal cells are devoid of 5-HT_3 receptors and serotonergic synaptic input [50,52,53].

(6) Low doses of nicotinic ACh-receptor agonists and nicotine itself have anxiolytic effects [54,55]. GABAergic interneurons, most notably those containing CCK, selectively express α-7 nicotinic-receptor subunits in the

hippocampus [56,57] and α-4 subunits in the neocortex [58]. PV-positive cells do not contain nicotinic-receptor subunits but do express presynaptic M_2 muscarinic receptors [59]. Thus, action of ACh on the two basket-cell types appears to be opposite: it excites the CCK-containing cells via nicotinic receptors but reduces GABA release from the PV-containing cells via presynaptic M_2 receptors.

Local afferents

(7) PV-containing cells on average receive a total of ~15 000 asymmetrical (mostly glutamatergic) and 1000 symmetrical (mostly GABAergic and monoaminergic) synapses [60], whereas CCK-containing basket cells receive only 5000 asymmetrical, but >2500 symmetrical, synapses [61]. This suggests that local glutamatergic input is the major drive of PV-positive cells, whereas the relative weight of local inhibition and subcortical input is much larger for CCK-positive cells.

(8) NMDA-receptor expression in glutamatergic synapses terminating on PV-positive cells is very sparse, often absent, whereas similar synapses on other interneurons (probably including CCK-positive cells) contain many more NMDA receptors [62]. This again suggests that the PV-containing-cell clockwork is not plastic; rather, it is hardly modifiable even at the level of its glutamatergic afferent input.

(9) CCK-containing cells are innervated by calretinin-containing interneuron-selective interneurons, whereas PV-containing cells are not [63], suggesting that the former are influenced by an additional modulatory system that might induce synchrony independent of the PV-positive cell syncytium.

In addition to these characteristic differences converging onto the proposed hypothesis, we also know that the brain regions playing central roles in anxiety are the amygdala (particularly the basolateral nucleus) and the hippocampus [64–66]. These are among the brain regions that contain the highest density of CCK-positive neurons and axon terminals [67], and of CB1 and 5-HT_3 receptors associated with them [38,39,50,68]. Anxiety-related effects of the various transmitter systems and modulators mentioned in this review have been extensively documented without a satisfactory explanation for the well-known interactions among them. The unique molecular and connectivity features of CCK-containing GABAergic interneurons of cortical areas (including, in particular, the hippocampus and basolateral nucleus of the amygdala) might provide an explanation and common ground for these interactions, because cells of only this type (1) use GABA and CCK as transmitters, (2) selectively express nicotinic α-7 receptors, nicotinic α-4 receptors, serotonergic 5-HT_3 receptors and cannabinoid CB1 receptors, (3) employ α2-subunit-containing $GABA_A$ receptors in their efferent synapses, and (4) receive multiple synaptic innervation from serotonergic afferents (Fig. 3). It is too early to speculate about the directions of modulation of CCK-containing cell actions by any one or combination of these modulators, because the contribution of receptor desensitization, the precise timing of action relative to ongoing network activity, and the molecular mechanisms of interactions among these or any other signalling systems, are still unknown.

It should also be noted that, in addition to the perisomatic region, CCK-containing axon terminals can be found in large numbers in the dendritic layers of the hippocampus. Thus, CCK-positive cells appear to form a continuum from typical basket cells, through wide-axonal basket cells, all the way to bistratified cells [18–20,69], and apparently the characteristic input and receptor expression patterns already described hold for all varieties. By contrast, PV-containing axon terminals are found exclusively within or immediately adjacent to the cell-body layers; thus, PV-positive basket cells represent a rather conservative group of perisomatic interneurons. This suggests that CCK-containing cells can best fulfill their mission by terminating largely in the perisomatic, but also in the dendritic, region of principal cells – another argument in favour of their involvement more in 'mood' than in rhythm.

Concluding remarks

The numerous features distinguishing the CCK- and PV-containing interneurons discussed in this review (Fig. 3) lead to the conclusion that the locally driven syncytium of PV-positive basket cells represents a non-plastic clockwork that operates the machinery of principal-cell ensembles in the cerebral cortex, synchronizing their activity intermittently, or rhythmically, at gamma and theta frequencies [70]. By contrast, CCK-positive cells appear to be highly sensitive fine-tuning devices, which extend several antennae to pick up information related to 'mood' (i.e. to the emotional, motivational and general physiological state of the animal). They receive a far less efficient local excitatory drive but are exposed to modulatory effects of extrinsic inputs and signalling molecules deriving from their own axons or targets. Corollary to this reasoning, CCK-containing GABAergic interneurons of cortical structures are likely to play a central role in some mood disorders (most notably in anxiety) and to mediate the anxiety-related effects of nicotinic, 5-HT_3 and cannabinoid receptors, representing the structural node for their physiological interactions.

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