Chandelier or axo-axonic cells are the most selective of all cortical GABAergic interneurons, because they exclusively contact axon initial segments of cortical glutamatergic neurons. Owing to their privileged location on initial segments, axo-axonic cells have often been assumed to have the ultimate control of pyramidal cell output. Recently, key molecules expressed at the initial-segment synapses have been identified, and novel in vitro and in vivo electrophysiological studies have revealed unexpectedly versatile functional effects exerted by axo-axonic cells on their postsynaptic targets. In addition, there is also emerging recognition of the mechanistic involvement of these unique cells in several neurological diseases, including epilepsy and schizophrenia.

Introduction
In his pioneering studies of neuroanatomy, Santiago Ramon y Cajal identified many major interneuron subtypes, yet never explicitly described the characteristic cartridges of axo-axonic cells (AACs). Chandelier cells, so named by János Szentágothai because these cartridges (vertical rows of axonal terminals) resemble rows of candles on a chandelier (Figure 1a,b), were discovered only 30 years ago [1,2]. Shortly thereafter, in Golgi-stained tissue from rat cortex, it was recognized that chandelier cells are the source of the previously mysterious axo-axonic boutons [3] and are not dendritically projecting, as previously believed. A few years later, the GABAergic, and therefore inhibitory, nature of these cells was verified [4]. This short but distinguished heritage gave rise to a series of recent breakthroughs that underlie the unique nature of these elusive interneurons.

Location and basic properties
Curiously, nearly all AACs are located in layered cortical areas – the neocortex or allocortex [3,5–7]. However, AAC cartridges have also been found throughout the amygdala, a partly cortical structure that is not laminated [8], and AACs do not exist in the cerebellar cortex, despite its

Figure 1. Chandelier cell morphology and axon initial segment (AIS) labeling. (a) Camera lucida drawing of a Golgi-stained chandelier cell from the CA1 region of monkey hippocampus, illustrating the many characteristic cartridges that give the chandelier cell its name. The asterisks mark representative pyramidal cell somata whose axons are likely to be contacted by this chandelier cell. Scale bar, 50 μm. (b) A Golgi-stained CA1 pyramidal cell, with its AIS contacted by a Golgi-stained axon from an axo-axonic cell. Boutons are represented by small arrows. Scale bar, 10 μm. (c) Light micrographs of the CA3 pyramidal cell layer of rat hippocampus demonstrating colocalization of the K⁺ channel subunit KCNQ2 (green) and ankyrin G (red) to the pyramidal cell AIS. Dark, unstained circles represent pyramidal cell somata. Additional abbreviations: SLM, stratum lacunosum-moleculare; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar, 20 μm. Panel (a) adapted, with permission, from Ref. [15]; (b) adapted, with permission, from Ref. [4]; (c) adapted, with permission, from Ref. [44].
layered nature. Because the home of AACs, the cerebral cortex, is evolutionarily recent, it is perhaps no coincidence that AACs have been identified so far only in mammals, including rats [3], guinea pigs [9], cats [10], rabbits [11], mice [12], ferrets [13], monkeys [14,15] and humans [16].

The first anatomical studies of chandelier cells revealed fusiform somata, and dendrites that are usually clustered in a bitufted manner, parallel to pyramidal cell apical dendrites. However, a recent report revealed a potentially distinct subset of horizontal AACs in CA1 whose dendrites are confined to the stratum oriens [17]; these AACs might receive different inputs to their vertically oriented counterparts. All AAC axons originate from the soma or a proximal dendrite, receive no synapses on their own axon initial segments (AISs) and form a dense network of terminal cartridges 10–50 μm long, each containing 2–12 boutons [14,18,19] (Figure 1a,b). A characteristic, but not functionally understood, feature of these cartridges is that they frequently climb upwards along the AIS — that is, the proximal bouton targets the distal end of the AIS [10,14,18]. Single AACs have been shown to innervate between 250 (in cortex) [14] and 1200 (in hippocampus) [19] pyramidal cells, indicating the potential to synchronize many cells.

Identification, please: markers for AACs
As the field of interneuron diversity developed, biochemical markers were discovered as distinguishing characteristics. The first marker found for AACs was the Ca$^{2+}$-binding protein parvalbumin (PV), expressed in nearly every AAC [5,20]. Unfortunately, PV is not specific to AACs — it is also expressed by some basket cells. AAC cartridges are reliably stained by the high-affinity GABA transporter GAT-1, but identification relies on the presence of characteristic cartridges because GAT-1 labels nearly all GABAergic boutons [21]. Without a specific marker, studies of AACs must rely on unambiguous measurements, such as electron-microscopic verification of synaptic localization. Even within interneuron subtypes, there is variability that has important effects on pyramidal cell output [22]. AACs are no exception to this rule of heterogeneity: their axonal and dendritic fields can vary as already described, and some AAC cartridges express additional proteins, such as corticotropin-releasing factor (CRF) in squirrel monkey neocortex [23,24], polysialated neural cell adhesion molecule (PSA-NCAM) in human temporal cortex and subiculum [25,26] and calbindin in human temporal cortex [27].

Picking partners: postsynaptic targets of AACs
Although other cell types show target specificity, AACs outshine them all with their remarkable precision. In nearly all quantitative studies, regardless of species or brain area, every chandelier cell terminal synapses with the AISs of pyramidal cells, granule cells or mossy cells [3,7,10,14,16,28–30]. In the few studies that could not confirm total specificity, the percentage of synapses onto AISs was never <90% [31–33]. It could be important that these AACs with aberrant targets were all found in rats from a relatively deprived, standard laboratory environment [34], emphasizing the need to investigate effects of enriched environments on interneuron specificity. AACs have never been demonstrated to target other interneurons [14,16] and, although some interneurons receive a few synapses on their AIS, the parent cells remain unidentified [35]. A final layer of discrimination is that AACs project to preferred subsets of pyramidal cells. In visual cortex, AACs target ten times more cortico-cortical pyramidal cells than corticothalamic cells [36]. Most AAC cartridges in human entorhinal cortex are located in layers 2–3, the site of projection neurons to the hippocampus [25]. With such location-specificity of their postsynaptic targets, their presynaptic inputs might also be expected to be precisely tuned. Unfortunately, data on AAC afferents are limited – most studies rely solely on parvalbumin staining and therefore cannot distinguish AACs from basket cells.

Sixteen candles: development of a chandelier
Studying development of specific interneuron subtypes is not trivial because identification relies on Ca$^{2+}$-binding-protein expression and neurite arborization patterns, which often appear relatively late in development. A study in monkeys of AAC cartridges, visualized using Golgi staining, PV-immunoreactivity or GAT-1-immunoreactivity, indicated a biphasic developmental course, increasing from a few stained cartridges at birth to a maximum during adolescence, and then possibly decreasing again to a lower level in adulthood [37]. Although precise details of synaptic arborization remain unclear, AACs do not fully develop until well after birth, and they mature later than other interneuron types. For example, morphologically mature AAC cartridges are not observed in cat cortex until postnatal weeks 4–6, well after the appearance of basket cell terminals [38]. This late developmental appearance of AACs could explain why Cajal never encountered these cells, because he often used tissue from young animals. However, some cells he described as atypical basket cells might be immature AACs [39]. An electron-microscopic time-course study of synapse number on pyramidal cell AISs is needed to quantify AAC synaptic development without relying on the confounding factors of protein expression.

The other side of the synapse: molecular layout of the AIS
The AAC cannot be studied without considering its target the AIS, and the anatomy of the AIS elucidates its function. This axonal structure is precisely defined and exhibits unique structural features on electron micrographs, including microtubule clusters, a membrane undercoating, clusters of ribosomes, and stacks of Ca$^{2+}$-storing cisternae [40,41]. The neuronal cytoskeleton, consisting of interacting spectrins and actins, forms the neuronal structural scaffold and is a spatial delimiter for neuronal membrane proteins [42]; the membrane undercoating is a specialized cytoskeletal element, found only in the AIS. The βIV isoform of spectrin (an actin-binding protein) and ankyrin G (a spectrin-binding protein) mutually confine each other to the AIS [43]. Ankyrin G provides a specific anchor for many AIS-specific proteins, including the K$^{+}$ channel.
subunits KCNQ2 and KCNQ3 [44] (Figure 1c); these channels can assemble heteromerically, underlie the muscarine-sensitive K⁺ current $I_M$, and might also be responsible for the slowly activating current $K_s$. GABA$_A$ receptors are present postsynaptic to AAC boutons, with a particularly high concentration of the $\alpha_2$ subunit [45,46]. AISs of some pyramidal cells in monkey and human temporal neocortex also express 5-HT$_{1A}$ receptors [47]. Molecules localized to the presynaptic terminal include GAT-1, M2 ACh receptors (in hippocampus) [48] and PSA-NCAM (in human neocortex and subiculum) [25,26] (Figure 2, left). Additionally, there are often puncta adherentia, providing more stabilization to the AAC–AIS interface.

Unfortunately, most other studies of protein localization to the AIS have not looked at cortical pyramidal cells. For instance, ankyrin G is responsible for tethering the Na$^+$ channel subunit Na$_{\alpha,1.6}$ to the cerebellar Purkinje cell AIS [49] and Na$_{\alpha,1.2}$ specifically localizes to the AIS in retinal ganglion cells [50]. The Purkinje cell AIS also expresses the cell adhesion molecules NrCAM and neurofascin, where neurofascin binds to ankyrin G and has an essential role in guiding the basket cell axon to the AIS [51]. Although these data from Purkinje cells and ganglion cells are interesting, these cells are not good models for studying the AAC–AIS interface. Purkinje cells are not only GABAergic but also their AIS is ensheathed with glia and receives no or at most two synapses from surrounding basket cell pinceaux (‘paintbrushes’) [52] (Figure 2, right). Retinal ganglion cell AISs are likewise covered by glia and are completely devoid of synapses. Thus, despite some similarities among all AISs, such as the presence of ankyrin G and $\beta IV$ spectrin, there are significant anatomical differences, and therefore future studies must verify the localization of specific Na$^+$ channel subunits and adhesion molecules on the pyramidal cell AIS, where AAC synapses actually form.

**Chandelier cell function: more than simply inhibitory**

Recently, great strides have been made in understanding physiological roles of AACS. Neocortical and hippocampal AACS, identified by electron microscopy as shown in Figure 3(a), display fast-spiking characteristics and various degrees of spike frequency accommodation, with the decrement in firing rate varying between 2.5% and 81.0% in response to a 500 ms depolarization [13,17,28,30,33,53–55] (Figure 3b). As expected, firing of AACS evokes inhibitory postsynaptic events in pyramidal cells that are mediated by GABA$_A$ receptors and modulated by presynaptic GABA$_B$ receptors [32]. This strong connection exhibits a failure rate of only 0–10%, presumably owing to the multiple boutons between each AAC and the postsynaptic pyramidal cell [17,53].

AACS also contribute to synchrony and oscillations in the hippocampus such as the theta rhythm (4–8 Hz), which occurs during environmental exploration and REM sleep, and fast ripples (120–200 Hz), which occur during slow-wave sleep. In paired recordings, an AAC firing at theta frequency immediately entrains the postsynaptic pyramidal cell to its rhythm, causing it to fire antiphase to the AAC [29]. In vivo, AACS fire consistently at 185° of the naturally occurring extracellularly recorded theta rhythm, antiphase to pyramidal cells (Figure 3c). Additionally, the basal firing rate of AACS increases >100% during theta oscillations [56], suggesting that AACS have more than a simple inhibitory function.
Chandelier cells fire just before the onset of fast ripples and are quiescent during and after this activity [56] (Figure 3d). An interesting question is whether AACs are among the gap-junction-coupled PV-expressing cells [57], because electrical connectivity might add to their oscillatory effectiveness.

For many years, it was presumed that the role of AACs was to gate pyramidal cell output, shutting down the cell to prevent unnecessary firing. This theory was based on the assumption that the AIS is the initiation site of action potentials, owing to a high density of Na+ channels in this segment [58] and to results showing that the action potential initiates at some point in the axon [59]. Along these lines, some data suggest that neocortical AACs dampen excessive excitability. In response to whisker stimulation in vivo, AACs do not receive fast sensory input but, rather, a long-latency sequence consisting of an inhibitory postsynaptic potential (IPSP) followed by an excitatory postsynaptic potential (EPSP), corresponding to the activation of local cortical circuits. In addition, the spontaneous firing rate of AACs is much lower than that of other interneurons in control conditions, but significantly higher without GABA-mediated inhibition. Accordingly, AACs could monitor local GABA activity, and only activate when this activity is insufficient [54]. This mechanism is supported by the nearly linear summation rules detected for interacting axo-axonic inputs, maintaining the impact of the 3–6 individual AACs converging on a single target pyramidal cell [30].

New results suggest that action potential initiation occurs distal to the AIS, at least 30 μm from the soma, and perhaps as far as the first node of Ranvier [60,61]. Therefore, the high density of Na+ channels in the AIS could backpropagate the action potential into the soma [62]. In this case, AACs could effectively decouple suprathereshold operations between the axon and the soma by regulating Na+ channel activation in the AIS. This mechanism might be directionally selective, by blocking axon-to-soma action potential propagation efficiently, owing to the characteristic arrangement of proximal boutons on the distal AIS already described. Because precise measurement of an action potential initiation site can be complex technically, computational modeling could offer a solution. One computational study suggests that action potential threshold in the AIS and axon depends on axonal resistivity, length, diameter and Na+ channel density in this segment [63]. For further predictions from computational models, more precise measurements of Na+ channel subunit specificity and density in the pyramidal cell AIS are needed.

**Chandelier cells in disease states**

Given the specificity and functional roles of AACs, a disturbance of these cells would be expected to cause major problems for normal function. In fact, changes in AACs have been reported in both epilepsy and schizophrenia. Many studies of both diseases have shown various amounts of decreased PV-immunoreactivity or mRNA expression [64–68], but this measure is not specific for AACs, nor does it necessarily signal cell death of PV-positive cells. However, some specific changes suggest
a role for chandelier cell dysregulation in the pathology of epilepsy and schizophrenia.

Various synaptic rearrangements of AACs have been observed in epilepsy, from losses of synapses on AISs to increases in their number (Figure 4). Synapses can be lost from the pyramidal cell AIS in animal models of epilepsy, such as in the somatosensory cortex of monkeys with alumina-gel-induced epilepsy [69] and in CA1 of rats with pilocarpine-induced seizures [70]. However, electron-microscopic studies on human tissue from temporal lobe epilepsy patients indicate no change in synapse number on surviving CA1 pyramidal cells, and even show an increase in synapse number on the AISs of dentate granule cells [71,72]. Finally, human epileptic tissue displays de novo expression of calbindin in hippocampal AAC cartridges [26]. These seemingly contradictory changes indicate that, although reorganization of AACs might contribute to epilepsy, we do not fully understand their precise relevance.

The prefrontal cortex is essential to normal working memory, which is disrupted in schizophrenia. Schizophrenia is a neurodevelopmental disorder that presents in late adolescence, when the density of AAC cartridges is changing. Loss of AAC function in schizophrenic prefrontal cortex would be significant because no pyramidal cell loss occurs [73]. No counts have been done of AIS synapse number in schizophrenic tissue, to measure AAC cartridge loss directly. However, there are many protein and mRNA expression changes that are consistent with AAC dysfunction (Figure 4). Specifically, the number of GAT-1-immunoreactive cartridges decreases [74], and the number of GABA A 2-receptor-positive AISs increases [75]. Finally, levels of mRNA for PV, GAT-1 and the GABA synthetic enzyme GAD67 have been reported to decrease [73,75]. Functionally, this could correspond to decreased GABA production, which spurs a decrease in presynaptic GABA reuptake to allow GABA to remain in

**Figure 4.** Axo-axonic cells (AACs) in disease states. Normal AACs express the following mRNA and proteins in their cell bodies: parvalbumin (PV), glutamic acid decarboxylase (GAD)67 and the high-affinity GABA transporter GAT-1. They also express GAT-1 protein in their cartridges. The normal postsynaptic (granule or pyramidal cell) axon initial segment (AIS) can be visualized using GABA A 2-immunoreactivity. In epilepsy, three different fates have been observed for AAC cartridges, depending on brain area, species and cell type. In the neocortex, numbers of synapses onto pyramidal cell AISs were reduced in the somatosensory cortex (SS) of monkeys with alumina-gel-induced epilepsy [69]. In the hippocampus, numbers of synapses onto CA1 pyramidal cells were unchanged in CA1 tissue from humans with temporal lobe epilepsy [72], but numbers onto CA1 pyramidal cells were decreased in rats with pilocarpine-induced epilepsy [70] and were increased in dentate granule cells (DG) in tissue from humans with temporal lobe epilepsy [71]. In schizophrenia, there are many reported changes in mRNA expression at the cellular level of the AAC, such as reduced expression of PV mRNA [65], GAD67 mRNA and GAT-1 mRNA [73,75]. Additionally, the number of GAT-1-immunoreactive (GAT-1-IR) cartridges is decreased [74], and the number of GABA A 2-immunoreactive synapses is increased [75], possibly indicating an increase in overall expression of this receptor.
the synapse, and an increase in postsynaptic GABA receptors so that the lower level of GABA can be detected by the pyramidal cell [75].

Future directions
A wealth of recent information about anatomy and physiology of AACs and the AIS indicates that these associated structures play an important part in cortical network activity. However, more work is needed to understand fully the role of AACs. For example, synaptic innervation of the pyramidal cell AIS needs to be studied using electron microscopy over the course of postnatal development, and in both control and schizophrenic tissues, to verify changes seen in protein expression studies. Given the inherent limitations of the Purkinje cell AIS model system, the pyramidal cell AIS will need to be examined further for the presence of specific Na+ channel isoforms and cell adhesion molecules, and for its role in action potential generation or backpropagation. Detailed neurochemical and electrophysiological analysis of intracortical and subcortical afferents will be essential for better understanding of AAC function. Finally, paired recordings combined with imaging will be crucial in determining the effect of AACs on distinct subcellular domains of pyramidal cells, and on populations of pyramidal cells in a variety of brain states and areas.

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