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Could electrical coupling contribute to the formation of cell assemblies?

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Abstract: Cell assemblies and central pattern generators (CPGs) are related types of neuronal networks: both consist of interacting groups of neurons whose collective activities lead to defined functional outputs. In the case of a cell assembly, the functional output may be interpreted as a representation of something in the world, external or internal; for a CPG, the output ‘drives’ an observable (i.e. motor) behavior. Electrical coupling, via gap junctions, is critical for the development of CPGs, as well as for their actual operation in the adult animal. Electrical coupling is also known to be important in the development of hippocampal and neocortical principal cell networks. We here argue that electrical coupling – in addition to chemical synapses – may therefore contribute to the formation of at least some cell assemblies in adult animals.

Keywords: axonal properties; cell assembly; central pattern generator; gap junction.

Introduction: cell assemblies vs. central pattern generators (CPGs) – ‘functional networks’

Central pattern generators (CPGs) are defined collections of interconnected neurons that produce programmed rhythmic outputs, generally in the form of signals ultimately directed toward muscles (Marder and Rehm, 2005; Selverston, 2010; Marder et al., 2015; Calabrese et al., 2016). CPGs may contain as few as 10 or so cells and in some invertebrate preparations can be physically isolated

from the rest of the animal – while still retaining many of their network properties. Consequently, the intrinsic properties and pairwise interactions of their constituent neurons can be characterized, including analyses of their communication via gap junctions, action potential-gated synapses or graded synapses. An extensive and elegant literature exists on CPGs, such as those in the stomatogastric ganglion of crustaceans (Daur et al., 2016), in locomotor networks of lamprey (Grillner and Wallén, 2002), amphibians (Wolf et al., 2009; Borodinsky, 2017), zebrafish (Saint-Amant and Drapeau, 2000; Warp et al., 2012; Berg et al., 2018), and mice (Hanson and Landmesser, 2003; Lu et al., 2015), in the brainstem respiratory CPG of rodents (Rekling et al., 2000), and in others (Rybak et al., 2006; Grillner and Jessell, 2009; Garcia-Campmany et al., 2010). The CPG field is one area of study that sets the standard on how basic cellular neurophysiology determines biological function.

In contrast to CPGs, the notion of cell assemblies (Eichenbaum, 2017), while both popular and influential, is rather ill-defined. The Canadian psychologist Donald Hebb (1949) is credited with inventing the concept, which was based on appealing – but speculative – ideas of how excitatory neurons *ought* to be synaptically interconnected, *ought* to have their interconnections modified, and *ought* to function together. These ideas were conceived at a time when cellular neurophysiology, as practiced now, was not available. Even now, however, cell assemblies are difficult or impossible to isolate physically, in contrast to at least some CPGs. It is likely that constituent neurons in a cell assembly can be spread over large territories, intermixed with many other neurons (Wallace and Kerr, 2010). It is thus challenging to identify those cells that belong to a given cell assembly and equally formidable to define criteria on which identification of cell assembly members could be based. The difficulty is that a cell assembly is supposed to consist of interconnected neurons that are functionally related or that represent some feature or event in the world, but on what time scales are the cells to be ‘related’? Is it ~ms synchrony (Gray and Singer, 1989), or is it correlated activity over seconds, as involved in working memory (Miyashita, 1988; Miyashita and Chang, 1988)? And how consistently reproducible must the patterns of activity in a putative assembly be before it can be considered a bona fide assembly? For these reasons, the

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term ‘cell assembly’ has been conceptualized rather differently by different authors.

Our task here is twofold. First is to argue that cell assemblies and CPGs form parts of a single conceptual spectrum, which we denote as ‘functional networks’ – recognizing that CPG outputs repeat stereotypically over time, while cell assembly behavior may be less repetitive, more flexible, and prone to modification. If such a conceptual spectrum is plausible, this approach may lead to greater precision in our thinking, to a clearer experimental basis for ideas surrounding cell assemblies, and perhaps to a meaningful subclassification of different sorts of cell assemblies. Second, we shall argue for the likely importance of synaptic inhibition and especially for electrical synaptic transmission via neuronal gap junctions in the formation and operation of cell assemblies. This second argument, so far as gap junctions go, derives in turn from two strands: (a) the known importance of gap junctions in CPGs, especially (but not exclusively) during development; and (b) the known critical importance of communication via electrical synapses in a variety of collective oscillations in neocortical and hippocampal networks in adult animals. We show how it is possible that some of the requisite gap junctions are to be found on the proximal axons of principal neurons.

The scope of functional networks requires consideration of synaptic inhibition, not just recurrent excitation

Hebb’s original concept of cell assemblies envisioned only excitatory synaptic interactions between constituent neurons. Followers of Hebb, particularly investigators concerned with ‘recurrent excitatory networks’ (such as – to first approximation – those in the hippocampal CA3 area) and with pattern completion, likewise tended to describe cell assemblies only in terms of mutual synaptic excitation, although this is not always made explicit (Palm, 1981; Gerstein et al., 1989; Sakurai, 1998; Huyck and Passmore, 2013; Palm et al., 2014; Holtmaat and Caroni, 2016). As Figure 1 illustrates, however, such a view is not consistent with the known physiology. The reason is that the effects that firing of one pyramidal neuron have on another pyramidal neuron cannot be defined in absolute terms, but many features of the system must be taken into account. Besides the excitatory synapses between pyramidal cells, one such feature includes the activities of GABAergic interneurons and their actions on pyramidal cells (Figure 1, taken from Miles and Wong, 1987).

Figure 1: The behavior of a recurrent excitatory synaptic network is strongly regulated by synaptic inhibition.

Dual intracellular recording from a pair of CA3 hippocampal pyramidal neurons *in vitro*. A burst of action potentials was repeatedly evoked in cell 1 by intracellular current injection; the effects in cell 2 (A2, B, C, D, E2) were observed as the GABA_A receptor blocker picrotoxin (PTX) was gradually washed into the chamber. At first (A2), cell 2 does not ‘see’ an effect of the burst in cell 1; then a slow EPSP appears (B), then a double EPSP (C), then a double EPSP with a large second component (D), and finally a burst of action potentials in cell 2, coincident with a burst in cell 1 (E1,2) and with a field component (Ee) – that is, a population burst is evoked by cell 1 when there is sufficient disinhibition. From Miles and Wong (1987); for further analysis of this phenomenon, see Traub and Miles (1991).

The data in Figure 1, collected *in vitro*, show simultaneous dual intracellular recordings of two CA3 pyramidal neurons, which are neurons from the ‘archetypical’ excitatory recurrent synaptic network. Illustrated are the effects that a burst of action potentials in one neuron (as in A1 and E1) have on another nearby neuron that is not monosynaptically connected to the bursting neuron. The synaptic excitation of the second cell depends on ‘percolation’ of excitation, along many possible paths from the first cell (Traub and Miles, 1991). As seen in the figure, such excitation is sensitive to the degree of synaptic inhibition (mediated by GABA_A receptors) in the network, because the excitation changes as a GABA_A receptor blocker is increased in concentration. The network behavior in the CA3 situation occurs in part because *disynaptic* inhibition (that is, pyramidal cell to interneuron to pyramidal cell) actually develops faster than *monosynaptic* excitation (pyramidal cell to pyramidal cell; Traub and Miles, 1991). The main conclusion is that recurrent excitatory synaptic connections are not the *sole* determinant of the coincident firing patterns of pyramidal cells. This in turn implies that the consequences of stimulating a subset of pyramidal cells are not determined solely by the strength of recurrent excitatory synaptic connections but also by the state of interneurons and inhibitory synapses. This latter state is in turn regulated by the brain in many ways (e.g. via inhibition of interneurons, extracellular [K⁺], and neuromodulatory state; Miles and Wong, 1987; Miles, 1990, 1991). In other words, putative cell assemblies formed by recurrent excitatory synapses are ‘dynamic’ in ways going beyond excitatory synapses alone.

The behavior of functional networks also requires consideration of axonal activities, which are in general more extensive than somatic activities

Reports concerning cortical and hippocampal cell assemblies, especially *in vivo*, often present complex experimental data (such as in the form of raster plots) that derive from extracellular recordings of (predominantly) somatic action potentials. To some degree, this reflects commonly used recording technologies that allow data collection while awake, behaving animals explore or perform a cognitive task. The emphasis on somatic action potentials may reflect a bias wherein the assumption is made: (a) that this signal determines the backpropagating action

potential into dendrites that will influence synaptic plasticity (Stuart and Sakmann, 1994; Spruston et al., 1995; Magee and Johnston, 1997); and (b) that the somatic action potential also defines whatever signals will be sent to ‘downstream’ neurons along the axon (Stuart et al., 1997a,b). With these assumptions, it would appear that the somatic action potential encompasses the sum total of what is relevant to document for a neuron’s activity and provides the information that would enable conclusions regarding ensemble activity.

The actual situation is more complicated. In particular, there are network behaviors such as hippocampal gamma oscillations (Fisahn et al., 1998; Traub et al., 2000; Cunningham et al., 2003) – behaviors presumed important for cognition (Steriade, 2006) – in which axons fire at considerably higher rates than do somata (Figure 2, taken from Dugladze et al., 2012). Given that somatic potentials during *in vitro* sharp-wave/ripples are antidromic (Papatheodoropoulos, 2008; Bähner et al., 2011) and that somatic spikelets can occur during sharp-wave/ripples or isolated ripples (Draguhn et al., 1998), it is possible that axons are more active than somata during this network behavior as well. Nor do axonal action potentials necessarily lead to more than minimal dendritic backpropagation (Schmitz et al., 2001; Larkum et al., 2008). Remarkably, a plexus of electrically coupled axons can generate very fast oscillations (~100 Hz) on its own, independent of somata (Traub et al., 2003a). While it is, at present, experimentally difficult to record from single axons let alone a large number of them, it may be more appropriate, from a conceptual point of view, to think of cell assemblies in terms of axonal spikes, rather than somatic spikes. This is the case because it is the *output* of cells that determines downstream events and, as we discuss below, somatic spikes only signal a fraction of a cell’s output.

Recent data expanding the actions of axons beyond ‘mere’ transmission lines

How is it that axonal behavior can be – at least under some conditions – either independent of soma/dendritic integration or else capable of modulating somatic spike output? There are several ways this can occur: First, so-called ectopic spikes can originate in axons far from the soma and propagate antidromically (Pinault, 1995). This phenomenon has been described for the cortical terminals of thalamocortical relay neurons in the penicillin cortical epileptic focus (Gutnick and Prince, 1972). It has also

Figure 2: During population behaviors, axons may fire significantly more than somata.

In this case, persistent gamma oscillations were evoked in the CA3 region by bath application of kainate (400 nM). Patch recordings were made from pyramidal cells and recordings were made of the local field potential (LFP) in stratum pyramidale. (A) Cell-attached soma recording with LFP shows gamma oscillations with sparse somatic firing. (B) Cell-attached axonal recording with LFP shows more frequent axonal firing. (C) Summary data show far more frequent firing of the axons than somata. (D) Whole-cell somatic recording and simultaneous cell-attached axonal recording indicate that the same cell was recorded at two different sites. (E) In cell-attached mode for both soma and axon, again the axon fires more than the soma. (F) Summary data of this and similar experiments. From Dugladze et al. (2012; concerning the mechanism by which axons may fire more than somata, see Traub et al., 2000, 2003a).

been observed in a number of experimental situations, including the tetanic stimulation epilepsy model in the hippocampus *in vitro* (Stasheff et al., 1993), where GABA_A receptors have a stimulating effect on ectopic spike generation, during spontaneous hippocampal sharp-wave/ripples, where this is again potentiated by GABA_A receptors (Papatheodoropoulos, 2008; Böhner et al., 2011), and in the 4-aminopyridine (4AP) epilepsy model *in vitro* (Avoli et al., 1998; Traub et al., 2001). In the latter case, block of K⁺ currents in presynaptic terminals may play a role (Geiger and Jonas, 2000), but there is in addition extensive GABA release in this experimental model, with likely excitatory actions on axons as well. These latter actions, so far as is known, do not take place at what can be considered anatomically defined synapses. Of course, GABA_A receptors located on the proximal axonal compartment, often at structurally conventional synapses on the axon initial segment, generally have inhibitory effects (Glickfeld et al., 2009; Dugladze et al., 2012) – although there may be exceptions at certain cortical pyramidal cells (Pinault and Pumain, 1989; Szabadics et al., 2006; Khirug et al., 2008).

Second, spikelets can occur in hippocampal pyramidal neurons, not only during sharp-wave/ripples but also during the theta state (where sharp waves do not occur) *in vivo* (Epszstein et al., 2010). Such spikelets are likely the result of axonal action potentials (Schmitz et al., 2001; Larkum et al., 2008). Third, neurotransmitters/neuromodulators in addition to GABA (Xia et al., 2014) can affect the excitability of axons (Sasaki, 2013). Examples of substances affecting axonal excitability include glutamate and adenosine (Sasaki et al., 2011), some though possibly not all of whose actions take place at presynaptic terminals. And fourth, axons do more than conduct propagating action potentials. In some structures, including hippocampal mossy fibers and the axon collaterals of large cortical pyramidal neurons, axons can propagate – decrementally – subthreshold ‘analog’ signals, which still have measurable effects on synaptic transmission despite being subthreshold (Alle and Geiger, 2006; Shu et al., 2006).

From the above, it should be apparent that axons contribute to neuronal operations on their own, so to speak, supplementing the integration taking place as neuronal

somata and dendrites respond to chemical and gap junction-mediated electrical synaptic inputs – responding with their separate repertoire of intrinsic conductances and serving as structures that can independently determine whether and when to initiate spikes in proximal axons.

Development of CPGs in the spinal cord and brainstem

From the above, we conclude that a conceptual framework consisting solely of principal cell somata, interconnected by excitatory chemical synapses, is insufficient for understanding cell assemblies. Where then can we turn in order to enlarge and strengthen the framework? CPGs provide clues as to how the combination of electrical and chemical synapses enables the shaping of network behavior. We turn now to this topic, starting with the development of CPGs. We have argued previously (Traub et al., 2017) that CPGs and their ‘programming’ by neuromodulation are a useful model for thinking about the transition from normal cortical oscillations to epileptiform network behaviors. Here, we contend that CPGs also serve as a suitable model for consideration of cell assemblies, at least local ones, and in particular for a possible role of electrical coupling in the organization of such assemblies. Notwithstanding that CPGs exhibit meaningful, purposeful activities of interconnected collections of neurons, they drive behavior rather than representing features or concepts of the world, as cell assemblies are presumed to do. In general, CPGs are more amenable to experimental analysis than cell assemblies, because their constituent cells are likely to be neighbors and their output is repetitive. This is especially true of CPGs in mammalian spinal cord and brainstem preparations *in vitro* vs. those in cortical structures. We therefore consider some relevant properties of mammalian CPGs in the cord and brainstem as they develop and mature, while noting that many of the important principles seen in the cord/brainstem appear to occur in cortex and hippocampus as well.

Importance of electrical synapses during early development

Two major types of neuronal gap junctions in the brainstem and spinal cord include those between the same or homologous cells types and those at nerve terminals

linking two different cell types and forming mixed synapses. Mixed synapses have the potential for dual chemical and electrical transmission from presynaptic terminal contacts on a soma or dendrite (Pereda et al., 2013; Song et al., 2016; Nagy et al., 2018, 2019). Here, we consider only the first type, which we refer to simply as electrical synapses that mediate electrical coupling between neurons at subcellular locations other than mixed synapses at nerve terminals. Electrical synapses are the earliest structural connection in the developing brainstem and spinal cord, involving motoneurons (Perrins and Roberts, 1995; Reikling and Feldman, 1997; Saint-Amant and Drapeau, 2000) and interneurons (Reikling et al., 2000; Saint-Amant and Drapeau, 2001; Li et al., 2009; Kay et al., 2016), and they are important for normal development (Personius et al., 2007; Montague et al., 2017). Chemical synapses, including motoneuron-to-motoneuron, develop later and persist in the adult, at least in cats (Cullheim et al., 1977). The developing spinal cord produces giant depolarizing potentials, somewhat similar to those in the hippocampus (Czarnecki et al., 2014). As spontaneous activity in the spinal cord depends on both motoneurons and interneurons (Grillner and Wallén, 2002; Hanson and Landmesser, 2003), it is apparent that electrical coupling plays an important role in coordinated network activity across multiple cell types in the immature nervous system.

In addition to this, a considerable degree of electrical coupling in the spinal cord persists into adulthood in lower vertebrates (Grinnell, 1996; Zhang et al., 2009) and to a lesser extent also in mammals (Walton and Navarrete, 1991; Lewis, 1994). Such electrical coupling is enhanced by injury (Belousov and Fontes, 2013), for example, from a toxin (Figure 3, Pastor et al., 2003) or after axotomy (Chang and Balice-Gordon, 2000). [On a historical note: partial spikes observed in axotomized motoneurons were originally attributed to enhanced dendritic excitability (Kuno and Llinás, 1970; Traub and Llinás, 1977), but it now seems possible that enhanced electrical coupling might play a role.]

The principle that electrical coupling is prevalent during development precedes the maturation of chemical synapses and is correlated with organized rhythmic or partially rhythmic collective depolarizations applied equally to both spinal cord and hippocampus (Leinekugel et al., 2002; Dupont et al., 2006; Crépel et al., 2007; Egorov and Draguhn, 2013; Molchanova et al., 2016). Because this principle is so widespread in the nervous system, it appears to be of fundamental significance and therefore raises questions of the possible roles of electrical synapses and their interrelationship with chemical synapses throughout the adult

Figure 3: Dye coupling among tibialis anterior motoneurons, P7 rat, after intramuscular injection of botulinum neurotoxin. Biocytin labeling after injection of one motoneuron. (A) Cluster of dye-coupled cells in apposition to the cell shown in (B; adjacent section). Scale bar, 50 μm (A, B, C). (C) Camera lucida reconstruction of injected cell and coupled cells. Arrows mark axonal collaterals. From Pastor et al. (2003).

CNS (Connors, 2017). Further below, we will address an example of such an interrelationship in our discussion of sharp-wave/ripples.

Electrical coupling between axons

Electrical coupling between axons was first described in the 1950s in an invertebrate preparation wherein a spike in one axon would reliably evoke a spike in the coupled axon (Furshpan and Potter, 1959). The two concepts – axonal coupling and spike transduction – have evolved in parallel, occasionally intertwined. On the one hand, anatomical evidence for gap junctions between axons has been adduced in systems including invertebrates such as leeches (Dykes et al., 2004), in fish (Bennett et al., 1967; Hull et al., 2015) and in the mammalian retina (Bloomfield and Völgyi, 2009). On the other hand, hippocampal and cortical pyramidal neurons can be electrically coupled in such a way that spikes in one cell reliably evoke spikes in another (Mercer et al., 2006; Wang et al., 2010; Figure 4). Spike transduction between axons need not be present under all physiological conditions, because gap junction

conductances are regulated by multiple factors (Orellana et al., 2013; Pereda et al., 2013; Wang et al., 2015; Curti and O'Brien, 2016), including neurotransmitters/neuromodulators such as dopamine (He et al., 2000; Bloomfield and Völgyi, 2009) and adenosine (Li et al., 2014), by activity (Haas et al., 2011), by $[\text{Ca}^{2+}]$ (Spray et al., 1985), and by pH (González-Nieto et al., 2008; Palacios-Prado et al., 2010). [One notion not sufficiently appreciated in the Neuroscience community is that coupling, which allows spike transduction cell-to-cell, can have profound effects on network behavior even if said coupling is extremely rare: the only requirement is that one cell couple to more than one other, on average – not a stringent requirement if there are many potential coupling partners for each neuron.]

Starting in the late 1990s (Traub and Whittington, 2010), anatomical and physiological approaches were brought together in a body of work suggesting that pyramidal neuron proximal axons could be electrically coupled and sometimes strongly coupled. This work began with the observation that ~ 200 Hz network oscillations occurred *in vitro* in CA1 hippocampus in a manner that involved multiple neurons, but which did not depend on chemical synaptic transmission (Draguhn et al., 1998). Schmitz et al. (2001) adduced physiological evidence that the requisite

Figure 4: Strong electrical coupling between pairs of layer 5 prefrontal cortex pyramidal neurons, probably regular spiking (RS) cells. (A) An action potential train in one cell induced in another cell either action potentials or spikelets, depending on membrane potential. (*indicates truncated action potentials). Broken line in inset shows apparent threshold for action potential vs. spikelet. Prefrontal cortex (PFC) slice, P18 rat. (B) Action potentials occurred at resting potential -60 mV, but not -70 mV. PFC slice, P32 rat. (C) Summation of spikelets leading to full action potentials in postjunctional neuron. PFC slice, P32 rat. From Wang et al. (2010).

coupling was between axons, which was supported by findings of dye coupling between proximal axons of CA1 pyramidal cells. In addition, modelling approaches using networks of detailed compartmental neurons, together with a highly reduced cellular automaton model, showed that axonal coupling, with spike transduction, could in fact account for network oscillations with the experimentally observed properties (Figure 5, from Traub et al., 1999). This subject is reviewed by Traub and Whittington (2010) and in Traub et al. (2018). Figure 5 gives a sense of how a single oscillation wave can occur after an ectopic spike, but how an oscillation at some particular frequency arises is a more subtle question that we considered earlier (Traub et al., 1999).

We should note that definitive anatomical evidence, including freeze fracture and immunofluorescence labeling for connexins, is still required for establishing the presence of gap junctions between the proximal axons of pyramidal neurons. In the context of this discussion and in light of the above noted electrical coupling in brainstem/spinal cord, it is interesting that immunofluorescence labeling of the gap junction protein connexin-36 (Cx36) has recently been observed in association with the axonal initial segments (AIS) of motoneurons in mouse spinal cord (Figure 6). Although Cx36-puncta at motoneuronal AIS were only rarely encountered, this observation is consistent with previous reports of Cx36 expression in motoneurons (Condorelli et al., 2000; Bautista et al., 2014a,b). However, it remains to be determined whether

Cx36 at sites along AIS represents the formation of bona fide gap junctions and, if so, whether the coupling is truly axo-axonal or whether the axons bearing Cx36 are coupled to some other neuronal subcellular element.

Gap junctions in developing and adult neocortex

Although the expression of various connexins has been reported in some neurons of the cerebral cortex in mammals (e.g. Cx26 and Cx45) at various stages of development (Niculescu and Lohmann, 2014), there is only firm evidence for the mediation of neuronal electrical coupling via gap junctions formed by Cx36. For example, subpopulations of neocortical interneurons are found to be mostly if not exclusively coupled by Cx36 (Venance et al., 2000; Hormuzdi et al., 2001); interneurons in the thalamic reticular nucleus are coupled by Cx36 as well (Zolnik and Connors, 2016). Interneuron electrical coupling is highly specific with respect to which cells connect to which other cells (Shigematsu et al., 2019), and as for the spinal cord and brainstem, electrical coupling is vital for the normal development of interneuronal circuits (Yao et al., 2016).

Gutnick et al. (1985) first noted dye coupling between pyramidal neurons in the cortex. This complemented early anatomical and physiological studies of coupling between principal hippocampal neurons (MacVicar and

Figure 5: One means by which a network of electrically coupled pyramidal cells (through their axons) could generate collective behavior. Collective oscillations can occur if (1) spikes can cross axon to axon (A), with the crossing time small (a fraction of a ms) relative to the oscillation period (several ms); the schematic shows a full action potential in the left axon, inducing across the gap junction an attenuated action potential in the right axon (but possibly still able to propagate orthodromically and across additional gap junctions, even as it induces only a spikelet in the right soma); (2) on average, each axon couples to more than one other (as in B), so that activity can grow (the schematic shows how an axonal spike can induce first two, then four additional spikes, each after some delay); (3) the network is sufficiently large (C). The network illustrated in (C) has random connectivity; within the network the schematic shows how – as in (B) – an action potential in one cell (red) can spread, first to the blue cells, then green ones (further stages not illustrated); (4) there is a background of spontaneous activity (not shown here). From Traub et al. (2018), which has further details. See also Schmitz et al. (2001) and Traub et al. (2018).

Dudek, 1980, 1981, 1982). Peinado et al. (1993a,b) later found extensive dye coupling between cortical pyramidal neurons in rats at postnatal day (PD) 5 to PD12, although there was little coupling after PD16. Yu et al. (2012) reported that sister pyramidal neurons are most likely to be coupled during development and that such coupling in turn regulates specific local chemical synaptic excitatory circuits, which may also occur in a similar sequence during the development of other brain and spinal cord regions. Su et al. (2017) found that Cx26 occurs in superficial layers of the cortex and is important for the development not only of synaptic connections but also of dendrites and for the ability of the more mature cortex to produce network oscillations. However, immunofluorescence labeling of Cx26 is not detectable in mature cortex, and its expression in adult brain appears to be restricted to a minor subpopulation of astrocytes (Nagy et al., 2001, 2011).

Physiological evidence for strong electrical coupling between cortical and between hippocampal pyramidal cells has been reported, as has dye coupling, illustrated in Figure 4 (MacVicar and Dudek, 1981; Mercer et al., 2006; Wang et al., 2010). Additionally, indirect evidence

for electrical coupling in cortical structures, specifically between principal neurons, derives from observations on sharp-wave/ripples (Draguhn et al., 1998; Maier et al., 2003), from pharmacologically induced gamma oscillations that are suppressed by gap junction blockers, and from the occurrence of spikelets in pyramidal neurons (Fisahn, 1999; Traub et al., 2000; Cunningham et al., 2003, 2004). Hippocampal sharp-wave/ripples recorded *in vitro*, putatively dependent on axonal electrical coupling (Traub and Bibbig, 2000), are also suppressed by gap junction blockade (Maier et al., 2003; Nimmrich et al., 2005). [We would, however, like to emphasize that no gap junction blocker is truly specific, possibly as a consequence of the fact that putative blocking molecules in the extracellular fluid do not have physical access to the gap junction channel. Each known gap junction blocker has effects on processes other than gap junction conductances. Investigators sometimes try to compensate for this by using more than one blocker, hoping that the nonspecific actions of the various blockers are distinct, with the common action between them being gap junction blockade. This

Figure 6: Evidence for Cx36 localization in axon initial segments (AIS). Triple immunofluorescence labeling for motoneuron marker peripherin (blue), AIS marker sodium channel (Na_v , green) and gap junction marker Cx36 (red) in lumbar spinal cord of postnatal day 10 rat. (A1) Overlay of three colors in a maximum intensity projection shows localization of several Cx36-puncta (red) at a peripherin-positive motoneuron AIS labeled for Na_v (green). (A2, A3) Same image as in A1, with 3-D rendering of red/green overlay and 70° rotation around the horizontal axis in A3 vs. A2 to show Cx36-puncta surrounding the axon. J. I. Nagy (unpublished data).

is not completely satisfactory. Even the use of gap junction protein knockout mice can be problematic, given the possibilities of enhanced expression of other compensating gap junction proteins. The gap junction field requires mutually reinforcing data from multiple experimental approaches.]

Despite the wealth of literature suggesting the occurrence of electrical synapses between contacting axon initial segments and despite the formulation of concepts surrounding fundamental mechanisms whereby integration and information processing in the CNS occur based crucially on the existence of these axonal electrical synapses, morphological evidence for gap junctions between contacting axon initial segments of neocortical or hippocampal pyramidal cells, as noted above, is still at a rudimentary stage of investigation. Although we have found no evidence for the presence of Cx36 at the AIS of at least hippocampal pyramidal cells, our survey of several connexins for possible AIS localization revealed a striking distribution of immunolabeling with an antibody generated against Cx45. This antibody produced punctate labeling at the AIS of CA1 hippocampal pyramidal cells (Figure 7) and cerebral cortical neurons (Figure 8A), where each AIS was identified by its strong labeling for ankyrinG. It is compelling that this punctate labeling with an anticonnexin antibody was observed in precisely the brain regions and localized precisely to the particular cell types, and at the exact subcellular location (i.e. AIS) where gap junctions have been hypothesized or predicted to exist. Moreover, based on modeling and dye coupling studies (Traub et al., 1999; Schmitz et al., 2001) in the hippocampus, the hypothesized axonal gap junctions were proposed to be no more than 150 μm from the axon hillock of pyramidal cells, precisely where we find Cx45 antibody labeling associated with the AIS plexus of hippocampal pyramidal cells.

Notwithstanding the above points and despite previous characterization of Cx45 detection by the anti-Cx45 antibody (Invitrogen; Cat No. 41-1700) that produced labeling at AIS giving the typical pattern of puncta seen with most connexin antibodies (Li et al., 2008), the authenticity of Cx45 recognition at AIS remains to be determined to exclude the possibility of cross-reaction with some other AIS protein component; hence, we refer to this labeling

Figure 7: Immunofluorescence labeling for the AIS marker ankyrinG (A1, green) shows punctate Cx45-like immunoreactivity (A2, red) along CA1 pyramidal cell AIS in the stratum oriens of adult rat hippocampus, as seen in overlay (A3) and magnified in inset. J.I. Nagy, unpublished data.

Figure 8: Confocal immunofluorescence images of ankyrinG-positive pyramidal cell AIS in rat cerebral cortex (A) and CA1 hippocampus CA1 (B), showing Cx45-like immunoreactivity at or near AIS intersections (A, B, C1, arrows) and near AIS branch points (C1, arrowheads). Surface rendering of image in C1 is shown in C2. The red puncta suggest the existence of gap junctions on proximal axons, and perhaps even between axons (arrows in A–C), but additional studies are required to confirm that the puncta represent bona fide gap junctions that interconnect axons. J. I. Nagy, unpublished data.

as Cx45-like immunoreactivity. In this regard, we note that Cx45 expression in the hippocampal CA1 pyramidal cells has been detected by some (Weickert et al., 2005; Cembrowski et al., 2016) but not others (Maxeiner et al., 2003). Further, some of the above-noted reports on dye coupling between hippocampal pyramidal cells was routinely obtained using Lucifer Yellow or carboxyfluorescein (Andrew et al., 1982; Knowles et al., 1982; Dudek et al., 1983; Núñez et al., 1990; Baimbridge et al., 1991; Church and Baimbridge, 1991; MacVicar et al., 2006), neither of which passes through Cx36-containing gap junctions (Mills et al., 2001), but both of which can pass through Cx45-containing gap junctions (Rackauskas et al., 2007; Kanaporis et al., 2010).

Electrical synapses, collective bursts, and sharp waves in developing and mature hippocampus: sharp waves as candidate cell assemblies

The main theme of this review is that coupling via electrical synapses contributes to the formation of cortical and hippocampal cell assemblies, in cooperation with chemical synapses. That such cooperation is to be expected early in development comes from extensive literature (some of which is cited above) on the early appearance of electrical coupling, which is then supplemented by chemical synapses whose organization is determined, at

least in part, by earlier intercellular relationships between these two forms of transmission (Allène et al., 2008; Egorov and Draguhn, 2013; Molchanova et al., 2016). To further develop ‘plausibility arguments’ for the contribution of electrical coupling in the cell assembly formation in mature CNS, it is of benefit to review a well-studied (but incompletely understood) collective behavior in the brain, namely hippocampal sharp wave-ripple complexes (SPW-R), in which both electrical coupling and chemical synapses participate in important ways. We shall begin with consideration of spontaneous SPW-R in *in vitro* slices of ventral hippocampus, a preparation that most readily allows study of cellular mechanisms (Maier et al., 2003, 2011; Nimrich et al., 2005; Bähner et al., 2011). This will be followed by consideration of the structure and possible significance of cell assemblies that appear to be formed during SPW-R *in vivo*.

A number of differing models have been offered for the cellular mechanisms of SPW-R or of the ripple component alone. These may be divided into two general classes: those in which electrical coupling plays little or no role and where ripples are generated primarily by interneurons (e.g. English et al., 2014; Schlingloff et al., 2014); and those in which electrical coupling between pyramidal neurons is critical and the interneurons – while still essential – follow the activities of pyramidal axons (Traub and Bibbig, 2000, here denoted as the ‘T-B’ model). The subject is controversial, but here we confine ourselves to the latter type of model, which made an experimental prediction that was both surprising and experimentally confirmed. We refer the reader to the primary papers cited below for discussion of ‘pure-synaptic’ models (English et al., 2014; Schlingloff et al., 2014).

In T-B, the ripple and the sharp wave can be considered as separable entities. The sharp wave is presumed to arise by recurrent synaptic excitation among a local population of pyramidal neurons, as originally proposed by Buzsáki (1986) and later studied by others (Traub and Wong, 1982, 1983; Miles and Wong, 1987). In contrast, Draguhn et al. (1998) showed that ripples at ~200 Hz could occur spontaneously *in vitro* in the absence of chemical synaptic transmission, but in a manner dependent on electrical synapses. Importantly, these ‘pure ripples’ did not involve interneurons and must, therefore, be generated by electrically coupled pyramidal cells. Using the occurrence of pyramidal cell spikelets as a clue, it was then shown with modeling that axonal coupling could account for population ripples in networks of pyramidal neurons, if the coupling allowed spikes to cross cell to cell (Traub et al., 1999).

The separation of sharp wave and ripple components is also consistent with developmental data, where spontaneous synchronized bursts lasting 0.5 to 3 s occur in the neonatal hippocampus and where they are dependent upon GABA_A and glutamate receptors (Leinekugel et al., 2002). Ripples were originally reported to occur only later, at the end of the second postnatal week (Buhl and Buzsáki, 2005); however, more recent data indicate that ripples occur in CA1 during the first two postnatal weeks (see Figure S3C of Brockmann et al., 2011). Additionally, Palva et al. (2000) have recorded 100–400 Hz field oscillations in the neonatal rat hippocampus that were locked to local gamma oscillations.

The model of T-B (see also Traub et al., 2003a) synthesizes the chemical and electrical synaptic interactions described above, with the addition of synaptic excitation of interneurons and pyramidal cells, and synaptic inhibition of pyramidal cells and interneurons. Of particular importance in that model were the relatively large-conductance rapid-kinetic excitatory conductances onto fast-spiking interneurons, such as basket cells (Miles, 1990; Gulyás et al., 1993; Geiger et al., 1997). In this way, ripple-frequency network oscillations in the plexus of pyramidal cell axons could reliably evoke ripple-frequency firing in fast-spiking interneurons, as observed experimentally *in vivo* (Ylinen et al., 1995). At the same time, pyramidal neurons tended to hyperpolarize during the simulated SPW-R, as is typically the case *in vitro* (Maier et al., 2003; Bähner et al., 2011) – although not *in vivo* (Ylinen et al., 1995). Additionally, T-B predicted the occurrence of ripple-frequency excitatory synaptic currents in both pyramidal cells and in interneurons, as actually occur *in vitro* (Maier et al., 2011; Schlingloff et al., 2014).

The most compelling prediction of T-B was that action potentials in hyperpolarized pyramidal cells, during

SPW-R, were antidromic – that is, originated in axons. This prediction has been confirmed *in vitro* (Figure 9; Papatheodoropoulos, 2008; Bähner et al., 2011). In our opinion, the matter remains to be settled definitively *in vivo*. The observation of spikelets in CA3 pyramidal neurons, occurring spontaneously *in vivo* (Spencer and Kandel, 1961), suggests that antidromic firing could be taking place *in vivo*. However, the interpretation of Spencer and Kandel of their data was different, involving postulated dendritic action potential initiation. An analogous coincidence of pyramidal cell hyperpolarization with antidromic action potentials and spikelets has been demonstrated in an *in vitro* 4AP epilepsy model (Avoli et al., 1998; Traub et al., 2001), and it is possible here that 4AP-induced GABA release excites axons via GABA_A receptors (Stasheff et al., 1993; Bähner et al., 2011). Whatever the mechanism, the *in vitro* data indicate again that axonal activities must be taken seriously in consideration of those cell assemblies that are associated with SPW-R.

An interesting question for the *in vitro* SPW-R model is this: if axons are generating the majority of the axon potentials, what determines if and when a particular pyramidal cell *soma* fires? Two considerations are relevant here. First, Dugladze et al. (2012) have shown that antidromic invasion to the soma is tightly regulated by axoaxonic interneurons (see also Figure 1). However, there is evidence that these interneurons are silenced by sharp waves *in vivo*, while being active before the sharp wave (Viney et al., 2013). Second, as Vladimirov et al. (2013) have proposed, if axonal plexus rippling is generated in electrically

Figure 9: Antidromic (axonally generated) action potential during an *in vitro* sharp-wave/ripple.

Spontaneous sharp-wave ripple in CA1 region of mouse middle hippocampal slice. Pyramidal cell somatic intracellular recording above, stratum pyramidale field potential below. Note how the intracellular action potential arises from a hyperpolarization. From Bähner et al. (2011).

coupled axonal collaterals, then synaptic excitation can regulate collateral/proximal axon invasion (by modulating the potential at axonal branch points), and hence somatic firing. The situation *in vivo* is, of course, far more complicated because pyramidal cells can be depolarized during the SPW-R, raising the opportunity for a complex interplay between antidromic and orthodromic activations.

What might be the functional significance of antidromic firing during SPW-R? Bukalo et al. (2013) showed *in vitro* that SPW-R led to a reduction of synaptic strengths of afferents to CA1 pyramidal neurons, which appeared to depend on electrical coupling and axonal excitability. Furthermore, direct electrical stimulation of axons produced long-term depression. The actions on synaptic depression required L-type Ca^{2+} channels, but – interestingly – not glutamate receptors. Conversely, synaptic stimulation after antidromic firing produced synaptic potentiation. Thus, the behavior of somatodendritic regions of a pyramidal neuron is influenced by axonal spikes in complex ways. Norimoto et al. (2018), working *in vivo*, also found that SPW-R downregulated evoked EPSPs, but in this case glutamate receptors (specifically, NMDA receptors) were needed. Both studies indicate that SPW-R might allow for synaptic ‘rescaling’ of afferents to CA1 pyramidal cells, in addition to what other effects they might have on downstream neurons (Girardeau et al., 2014). An analogous function of synaptic rescaling has been proposed for sleep rhythms, particularly in the delta range (Tononi and Cirelli, 2006).

In vivo SPW-R involve cell assemblies in two related ways. First, disruption of SPW-R by an experimental stimulating circuit or with molecular techniques interferes with memory consolidation (Girardeau et al., 2009; Nakashiba et al., 2009). It follows from this that assemblies constructed during SPW-R have functional significance, either for rescaling of excitatory synaptic strengths on hippocampal neurons (see above) or for communicating specific information to brain regions outside the hippocampus itself. Second is the replay/preplay phenomenon (Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Nádasdy et al., 1999; Diba and Buzsáki, 2007; Pastalkova et al., 2008; Girardeau et al., 2009; reviewed by Carr et al., 2011; Figure 10), where there is a relation between firing sequences of hippocampal place cells that are active during locomotion and of those same cells firing (somatically) during sharp waves – a relation that spans time-scales, the several-second timescale of the motor activity vs. the 100–200 ms timescale of the SPW-R.

To expand somewhat on replay/preplay, there is a correlate in the external world of a place field, as exhibited by hippocampal pyramidal cell (somatic) firing in certain areas of the environment and not other areas,

during locomotion and the associated theta rhythm (see, e.g. the central raster plot of Figure 10, where place fields are delineated as a rat runs along a track). Nevertheless, the correlate of a place field with the external world is not fixed in time: the correlate changes in complex ways with, for example, environmental rotation or the movement of visual cues, a phenomenon called ‘remapping’ (Muller and Kubie, 1987; Cressant et al., 2002). Thus, the functional significance of the SPW-R cell assembly – the aggregate of cells somatically firing at some location – is ‘relative’, that is, defined in terms of the present state of things.

The tying of a particular hippocampal pyramidal cell to a particular place field cannot be completely arbitrary. The reason behind this assertion is that cells with overlapping place fields – in some particular environment – have their (somatic) action potentials temporally linked together, either before running through the environment (‘preplay’, left of Figure 10) or afterward (‘replay’, right of Figure 10). Strangely, the linking occurs on a faster time-scale than the timescale of the running or the exploration. Instead, as Figure 10 shows, preplay and replay occur during SPW-R, which last in the order of 100 ms, while the exploration shown in Figure 10 occurs instead over seconds – and the exploration is associated with theta waves, not SPW-R. In addition, during the SPW-R, somatic potentials are phase-locked to the ripple oscillation, although the functional significance of this is not known.

How to make sense of these dual behaviors, SPW-R vs. theta? At first glance, it appears that the ensemble of somatically firing cells during the SPW-R – the cell assembly associated with the SPW-R – encodes a path through the environment, either a path that will be taken or a path that has recently been taken. Information about the path is then ‘forwarded’ downstream – this, in addition to rescaling afferent synapses onto hippocampal neurons that were active along the path. In order, however, to be more confident in this interpretation, it would be helpful to know which *axons* of pyramidal cells are active during the SPW-R – not just the somata, which one can record *in vivo* with existing technology, for it is the axons that define the messages sent downstream, not the somata.

In order to make further sense of the SPW-R cell assembly, it would also help to have more information on how place cell activity is generated, but mechanisms that govern this activity are beyond the scope of this review. We note only that important factors are likely to include grid cell activity in the entorhinal cortex (Rowland et al., 2016; Poulter et al., 2018), dendritic plateaus in CA1 pyramidal neurons that cause, or at least signal, the formation of a place field (Bittner et al., 2017), and synaptic input from the CA3 region (Davoudi and Foster, 2019).

Figure 10: Demonstration of CA1 pyramidal cell assembly behavior at two timescales – of the sharp wave-ripple complex (tens of milliseconds) and of the theta oscillation (frequency $\sim 4\text{--}8$ Hz, lasting several seconds).

Forward and reverse preplay and replay of place cell firing sequences are shown. Awake behaving rat that runs on a linear track with a water reward at each end and with position tracked with an LED device. Silicon probes were implanted in the hippocampus to record multiple units (with pyramidal cells extracted by spike-sorting techniques) and local field potential (LFP, shown at the top of each panel). Pyramidal cell action potentials are shown below the LFPs, sorted by place field locations during the run. The topmost LFP trace in the upper panel shows a sharp wave-ripple complex during near immobility (red box), the running phase with associated theta rhythm and place cell firing, and then another sharp wave-ripple complex during immobility (blue box). Details of the sharp wave-ripple complexes are shown below in time-expanded traces, preplay in the red box, and replay in the blue box. In these expanded traces, the ripples are apparent in the LFP, as is the similarity in firing sequences for the preplay/replay vis-à-vis place-field firing. The cellular mechanisms by which these particular cells ‘assemble’ remains to be determined (but see Bähner et al., 2011). From Diba and Buzsáki (2007).

Electrical coupling itself can lead to collective behavior – the nature of which depends on cellular intrinsic properties

There are special cases in which networks of nerve cells, interacting exclusively or nearly so through gap junctional electrical coupling, can generate collective behaviors and oscillations in particular. This can happen in nonphysiological conditions, in which synaptic transmission is suppressed experimentally (Draguhn et al., 1998; Traub

et al., 2003a; Nimrich et al., 2005; Roopun et al., 2006), but it can also happen under relatively normal conditions. Examples of the latter include the medullary pacemaker nucleus of weakly electric fish (Elekes and Szabo, 1985; Dye and Heiligenberg, 1987; Dye, 1991; Moortgat et al., 2000), motor networks in cnidarians (Takaku et al., 2014), the small cell network of the decapod heart CPG (Calabrese et al., 2016), and the mammalian inferior olive, in which the size of synchronously oscillating assemblies is regulated by GABAergic afferents (Leznik et al., 2002). Interestingly, the *in vivo* examples are all networks that are part of a motor or electromotor system, and intrinsic cellular pacemaker properties are important in most or all

of the *in vivo* cases. We are not aware, however, of *in vivo* neuronal networks that act as cell assemblies, in which electrical coupling alone underlies the sole type of interaction between cells.

Neocortical and hippocampal networks generate behaviors dependent on cooperation of chemical and electrical synapses: gamma oscillations, interictal spikes, and delta oscillations

Many other types of collective behavior can be shown to depend on both chemical and electrical synapses. An excellent example is the lobster stomatogastric ganglion (Elson and Selverston, 1992). In addition, however, a number of examples are known from experimental *in vitro* studies (including with human tissue) combined with network modeling. Besides the SPW-R discussed above (and see Figure 11 for summary), examples include persistent gamma oscillations (Fisahn et al., 1998; Traub et al., 2000), neocortical delta oscillations (Carracedo et al., 2013), and epileptiform synchronized bursts (Traub and Wong, 1982; Roopun et al., 2010). The *in vitro* behaviors have the experimental and conceptual advantages of occurring in small volumes of tissue, so that correlated but widely separated neuronal subpopulations are not an issue (unlike *in vivo*). These systems have the property that the activities of a given cell type – say, neocortical layer 5 intrinsic bursting pyramidal cells – tend to be stereotyped, so that the activities of many cells can be reasonably inferred from observing only a few cells. For *in vitro* gamma oscillations and synchronized bursts, electrical coupling between pyramidal cells is of dominant importance, but coupling between interneurons contributes as well (Hormuzdi et al., 2001; Traub et al. 2003b).

While the *in vitro* behaviors described above are important model systems, in themselves and for understanding *in vivo* oscillations and seizures, these behaviors have limitations for understanding *in vivo* cell assemblies. Most importantly, the behaviors are autonomous, resembling in that sense a CPG; one cannot attach meaning, relative to the external world, to the activities of this or that group of neurons. It may still be true, however, that the *in vitro* models provide a substrate for the type of *in vivo* activities during which meaningful cell assemblies operate.

Figure 11: Summary figure showing how chemical synapses and pyramidal cell gap junctions could interact to produce sharp wave-ripple complexes in hippocampal CA1 (and perhaps other cell assembly behavior).

Components of the diagram include (A) the collection of pyramidal cell axons (upper), which putatively interact with each other via gap junctions and so generate ripples and which also form glutamatergic synapses on pyramidal cell dendrites and on interneurons; (B) the collection of pyramidal cell somata and dendrites, which we conceptualize as a separate entity from the axons, even though the somata and axons are physically contiguous; (C) interneurons, which chemically inhibit each other, pyramidal cell somata and dendrites, and pyramidal cell axons; (D) excitatory afferents (e.g. from CA3 and entorhinal cortex), which can trigger sharp waves. Interneuron gap junctions not shown (but see, e.g. Traub et al., 2003b); inhibitory afferents also not shown. This scheme helps to illustrate how two forms of assembly behavior can be generated separately, even as they are coupled together. From Traub and Bibbig (2000).

Conclusion: could electrical synaptic interactions form the ‘core’ of cell assemblies, with chemical interactions superimposed?

The considerations above suggest that there is unlikely to be a definition of cell assembly that is simultaneously useful, precise, and applicable to all biological situations. Comparing cell assemblies in different situations (e.g. during an SPW-R vs. a response to visual stimulation), there are too many differences in function, anatomy, dynamic cell properties, and the role of time (e.g. whether rhythmical activities occur or not). Perhaps, then, it is best to consider each type of cell assembly case by case. We view SPW-R to be a particularly interesting type of cell assembly behavior, but of course there are many others.

We have argued for taking into account the contributions of electrical synapses and of axons in the formation of cell assemblies. Our argument rests on a number of pillars, including the known contributions of electrical coupling and axons in CPGs, as well as experimental demonstrations that electrical synapses and axons are involved in the generation of SPW-R and persistent gamma oscillations, among other behaviors. Insofar as cell assemblies are formed by learning and experience, this matter is of vital importance, because electrical and chemical synapses each have their own fashion of plastic changes (Magee and Johnston, 1997; Markram et al., 1997; Bi and Poo, 1998; Pereda et al., 2013; Coulon and Landisman, 2017; Sevetson et al., 2017), and axons themselves are modulated by neurotransmitters (such as GABA and adenosine), as well as exhibiting other, so far poorly understood, forms of plasticity (Sheffield et al., 2011).

Perhaps most importantly, another distinction applies in how electrical coupling and excitatory chemical synapses organize cell assemblies. Homotypic gap junctions (in which the same connexin proteins reside at each side of the junction) do not rectify, so that coupling is bidirectional and allows a symmetry to exist. In contrast, chemical synapses transmit from a presynaptic to a postsynaptic cell, and are not symmetrical. This notion suggests that electrical synapses could indeed be used to form a ‘core assembly’, with excitatory chemical synapses serving to link one assembly – in directed fashion – to another assembly. Also relevant here are the different spatial scales over which electrical coupling vs. chemical synapses can interconnect neurons. Electrical synaptic transmission typically occurs among neighboring cells, while chemical synapses are not so constrained, resulting from the possibility of lengthy intervening axon from neuron to neuron. If a cell assembly does indeed have a ‘core’ and the core neurons are neighbors, then it might make sense to interconnect them with gap junctions; that strategy clearly will not work for cell assembly constituents that are far apart.

The developmental history of neocortex suggests that a ‘core’ of gap junctionally coupled neurons exists during early development, which is subsequently superimposed with chemical synapses at later developmental points. A key question concerns the means by which such an idea could be tested experimentally? Blocking gap junctions pharmacologically is a blunt instrument, with nonspecific and nonlocalized drug actions, and there are difficulties in crossing the blood brain barrier (Leshchenko et al., 2006). The invention and discovery of more precise and specific methods to manipulate electrical synapses, as well as to observe the activities of pools of axons, will form a major

experimental challenge, necessary however for a deeper understanding of cell assemblies.

Outlook for the future

Physiological measurements are, and always will be, essential for the characterization of assembly behavior, *in vitro* and *in vivo*, with behavioral correlations in the latter case whenever feasible. Nevertheless, so far as gap junctions and cell assemblies are concerned, it is our opinion that *anatomical* data are the most urgent necessity. Such data are needed for a number of reasons, including the following: (a) to localize in which cellular compartments gap junctions occur – a critical matter, as the functional consequences of axonal electrical coupling are quite different than soma/dendritic coupling; (b) to determine which gap junction proteins are involved, as these will determine how the channels are affected by pH, modulators, and other like parameters; and knowing the protein identities will allow application of molecular/genetic experimental techniques; (c) discovering how the above gap junction features are altered during development and perhaps by learning. Acquisition of this anatomical data is difficult, however, as gap junctions in pyramidal cells may be sparse, both in the number of gap junction plaques, and in the number of channels in each plaque. We look forward to the application of high-resolution electron microscopic approaches to address these critical issues.

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