

# Connexon connexions in the thalamocortical system

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**Abstract:** Electrical synapses are composed of gap junction channels that interconnect neurons. They occur throughout the mammalian brain, although this has been appreciated only recently. Gap junction channels, which are made of proteins called connexins, allow ionic current and small organic molecules to pass directly between cells, usually with symmetrical ease. Here we review evidence that electrical synapses are a major feature of the inhibitory circuitry in the thalamocortical system.

In the neocortex, pairs of neighboring inhibitory interneurons are often electrically coupled, and these electrical connections are remarkably specific. To date, there is evidence that five distinct subtypes of inhibitory interneurons in the cortex make electrical interconnections selectively with interneurons of the same subtype. Excitatory neurons (i.e., pyramidal and spiny stellate cells) of the mature cortex do not appear to make electrical synapses. Within the thalamus, electrical coupling is observed in the reticular nucleus, which is composed entirely of GABAergic neurons. Some pairs of inhibitory neurons in the cortex and reticular thalamus have mixed synaptic connections: chemical (GABAergic) inhibitory synapses operating in parallel with electrical synapses. Inhibitory neurons of the thalamus and cortex express the gap junction protein connexin36 (Cx36), and knocking out its gene abolishes nearly all of their electrical synapses.

The electrical synapses of the thalamocortical system are strong enough to mediate robust interactions between inhibitory neurons. When pairs or groups of electrically coupled cells are excited by synaptic input, receptor agonists, or injected current, they typically display strong synchrony of both subthreshold voltage fluctuations and spikes. For example, activating metabotropic glutamate receptors on coupled pairs of cortical interneurons or on thalamic reticular neurons can induce rhythmic action potentials that are synchronized with millisecond precision.

Electrical synapses offer a uniquely fast, bidirectional mechanism for coordinating local neural activity. Their widespread distribution in the thalamocortical system suggests that they serve myriad functions. We are far from a complete understanding of those functions, but recent experiments suggest that electrical synapses help to coordinate the temporal and spatial features of various forms of neural activity.

## Introduction

The most familiar neuronal signaling mechanism is the neurotransmitter-dependent chemical synapse.

Electrical synapses, which are composed of neuronal gap junctions, offer a very different type of signaling that is faster, bidirectional, and simpler in both structure and function (synonyms for electrical synapses include “electrotonic synapses” and “electrical coupling”; for review see [Bennett, 1977, 1997](#); [Connors and Long, 2004](#)). Although there are forms of electrical communication between neurons that do not

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involve gap junctions, such as “ephaptic” interactions (Jefferys, 1995), they are not included in this study.

The idea that neurons communicate through direct electrical connections (Cowan and Kandel, 2001) predates the discovery of electrical synapses in crayfish and shrimp by almost a century (Furshpan and Potter, 1957; Watanabe, 1958). Vertebrate electrical synapses were observed soon after in the brainstem of fish (Bennett et al., 1959), but they proved much harder to demonstrate in mammalian brains. The most convincing way to detect electrical coupling is to record intracellularly and simultaneously from two connected cells, which is exceptionally difficult to do in the intact mammalian brain. The first mammalian evidence — obtained from certain brainstem nuclei in vivo — was necessarily indirect (e.g., Baker and Llinás, 1971; Korn et al., 1973; Wylie, 1973).

Improved microelectrode methods, in vitro slice preparations, and molecular genetic technologies cracked the practical barriers to the study of mammalian electrical synapses. The presence of electrical synapses is now clearly established in the inferior olivary nucleus, locus coeruleus, striatum, cerebellar cortex, pre-Botzinger complex, hippocampus, retina, suprachiasmatic nucleus, olfactory bulb (Connors and Long, 2004), as well as the thalamocortical system. Judging from the distribution of connexin36 (C×36), the neuronal protein most responsible for electrical synapses, it seems likely that they occur in every major region of the central nervous system (Condorelli et al., 2000; Degen et al., 2004). Considering their speed, simplicity, and reciprocity, this should probably come as no surprise.

In this review the presence, properties, and potential functions of the electrical synapses that interconnect many inhibitory neurons of the thalamus and the neocortex have been analyzed. Figure 1 shows a basic circuit diagram of the electrical (and chemical) synaptic connections in the thalamocortical system, based on the present study results (see also Amitai et al., 2002; Gibson and Connors, 2003; Beierlein et al., 2003).

### Electrical synapses in the neocortex

The neocortex is the largest part of the mammalian brain, and it is essential for normal perception, motor

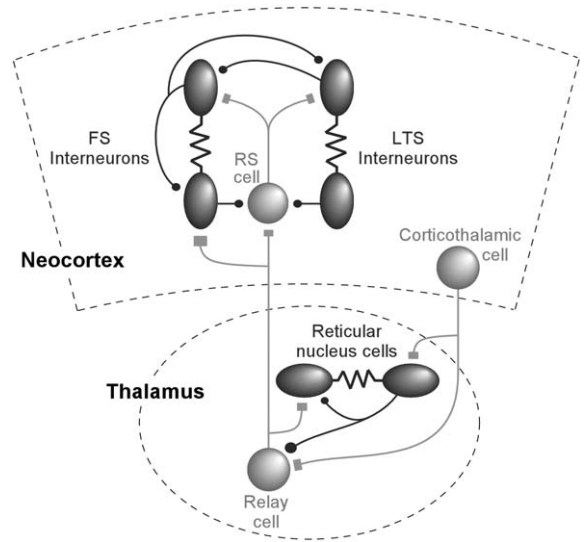


Fig. 1. Electrical synapses in the somatosensory thalamocortical system. Major connections via electrical synapses (zig-zags) and chemical synapses (inhibitory: dots, excitatory: squares); the central neuron in the cortex is a spiny stellate, regular-spiking cell (RS cell), which is excitatory, as is the corticothalamic cell; FS and LTS cells are inhibitory interneurons. In thalamus, relay cells are excitatory, whereas cells of the reticular nucleus are inhibitory. Cortical circuit represents layer 4 only.

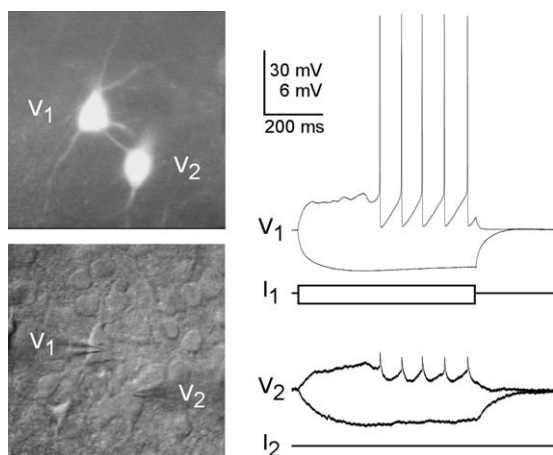
control, cognition, and many forms of memory. Neocortex carries out some of the most complex neural functions anywhere in nature. Electrical synapses appear to be an important component of its complicated circuitry.

In 1972 John Sloper published a short paper describing electron microscopic observations of gap junctions between neurons of the mature monkey sensorimotor cortex. He followed this in 1978 with a more complete description, coauthored with Thomas Powell, that included many dramatic micrographs of dendrodendritic and dendrosomatic gap junctions interconnecting cells that had the telltale characteristics of inhibitory interneurons. These papers described the first compelling evidence for electrical synapses in the mammalian forebrain, yet they apparently passed with little notice at the time. They accurately predicted much of our current state of understanding about electrical synapses in the neocortex yet, surprisingly, over the ensuing decades each paper has been cited fewer than 100 times.

A key conclusion of [Sloper and Powell \(1978\)](#) was that neuronal gap junctions occur predominantly between cells with the morphological characteristics of interneurons. This has recently been substantiated by the work of [Fukuda and Kosaka \(2003\)](#), who observed dendrodendritic gap junctions between parvalbumin-immunolabeled inhibitory interneurons of primary somatosensory, auditory, and visual areas in mature rats.

Gap junctions are morphological entities, and the presence of neuronal gap junctions alone can provide only indirect evidence for functional electrical synapses. Direct electrophysiological recordings are required for a definitive functional demonstration. In recent years, such recording studies have been conducted ([Fig. 2](#)), confirming that electrical synapses are localized largely to inhibitory interneurons, and that these synapses are strong enough to influence cortical function. The first of these studies were published by [Galarreta and Hestrin \(1999\)](#) and [Gibson et al. \(1999\)](#). Several general properties of interneuron coupling were immediately apparent, and were subsequently confirmed and extended:

- (1) Electrical synapses are very common among closely neighboring pairs of inhibitory



**Fig. 2.** Electrically coupled FS inhibitory interneurons in neocortex. GFP-expressing interneurons from neocortical slice *in vitro* viewed under fluorescence (top left) and infrared-differential interference contrast optics (bottom left). Paired whole-cell recordings from the same interneurons showing electrical coupling (right). (Mancilla, Cruikshank, Huang and Connors, unpublished.)

interneurons (from 60% to as high as 90% in some samples). On the other hand, there are very few reports of electrical coupling among cell pairs that include excitatory neurons ([Venance et al., 2000](#); also discussed on pages 44–46).

- (2) Electrical synapses are cell-type specific; interneurons of a particular subtype tend to be coupled only to cells of the same subtype, and mixed interneuron types are coupled only about 5% of the time ([Gibson et al., 1999, 2004](#)).
- (3) Electrical coupling is relatively strong. On average, the “coupling coefficient” for slow events is nearly 0.1, and can reach as high as 0.4 (coupling coefficient defined here as: the voltage deflection in cell B divided by the voltage deflection in cell A, when the two cells are electrically coupled, and the voltage deflection is initiated in A); the mean cell–cell coupling conductance is about 1.6 nS, with a maximum up to 5.5 nS.
- (4) The average strength of coupling is more than enough to allow neighboring neurons to synchronize their spiking patterns with high precision (about  $\pm 1$  ms; see pages 50–51 and [Figs 4 and 7](#)), and to deliver signals about as strong as chemical EPSPs.
- (5) Some pairs of inhibitory interneurons are connected by both electrical *and* inhibitory (i.e., chemical) synapses.
- (6) Electrical coupling has been observed in several areas of neocortex, among interneurons in all cortical layers ([Gibson et al., 1999](#); [Blatow et al., 2003](#); [Chu et al., 2003](#)), and in fully mature animals ([Galarreta and Hestrin, 2002](#)).

A lot of information about the anatomical/spatial properties of electrical coupling between cortical interneurons is available today ([Gibson et al., 1999, 2004](#); [Amitai et al., 2002](#); [Long et al., 2004](#)). Coupling is strongest and most prevalent between cells  $< 50 \mu\text{m}$  apart, and essentially absent at distances  $> 200 \mu\text{m}$ . The distance-dependence function and measures of interneuron density imply that each interneuron is directly coupled to about 20–50 other interneurons. This information, combined with average unitary conductances and input resistances, allows us to

estimate that the net conductance through all the electrical synapses of an interneuron contribute approximately half of the total resting membrane conductance (Amitai et al., 2002). In other words, networks of interneurons are very densely coupled and this is likely to have a powerful influence on cortical processing.

The neocortex has a variety of distinct types of GABAergic interneurons (Monyer and Markram, 2004). Remarkably, inhibitory interneurons seem to make electrical synapses only with cells of similar type. Our laboratory has studied electrical synapses amongst two interneuron types in particular. The most commonly encountered type is the “fast-spiking” (FS) cell, which generates exceptionally brief action potentials that can fire at unusually high rates without adaptation (McCormick et al., 1985). Most neighboring pairs of FS cells are electrically coupled to each other (Figs. 1–3; Gibson et al., 1999; Galarreta and Hestrin, 1999). Another major type of inhibitory interneuron, characterized in both rats (Gibson et al., 1999) and mice (Deans et al., 2001), expresses the neuroactive peptide somatostatin, generates broader spikes with adapting patterns, and is often called the “low threshold-spiking” (LTS) cell (Kawaguchi and Kubota, 1997). LTS cells are also usually coupled to each other electrically. Pairs of FS and LTS cells are coupled to one another only very occasionally, and such connections are usually weak (Gibson et al., 1999, 2004). In addition, Blatow et al. (2003) described a type of interneuron they named the “multipolar bursting” (MB) cell, which was coupled to cells of the same type but not to FS cells. Chu et al. (2003) characterized a “late-spiking” (LS) type of inhibitory interneuron in layer I of the neocortex that made electrical synapses to other LS cells 83% of the time, but coupled to non-LS interneurons only 2% of the time. Most recently, Galarreta et al. (2004) showed that a fifth type of interneuron — an irregularly spiking, cannabinoid-expressing cell of the upper cortical layers — is also selectively electrically coupled. Thus, the evidence to date is consistent with the hypothesis that, with rare exceptions, neuronal gap junctions in neocortex interconnect inhibitory interneurons of the same type (Fig. 3).

So far, independent evidence for morphological gap junctions and functional electrical synapses have been discussed. However, the electrical synapses

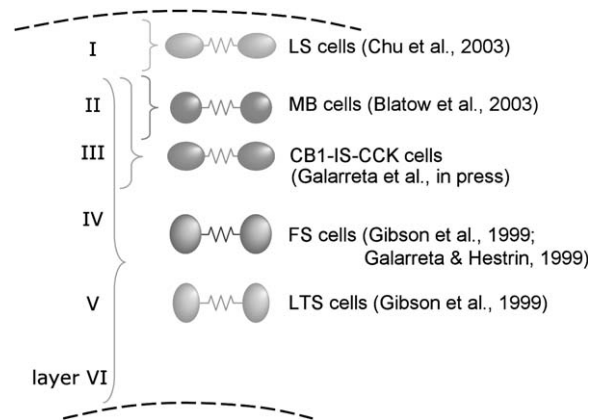


Fig. 3. Five subtypes of inhibitory interneurons in neocortex, each with cell type-specific electrical synapses. Late-spiking cells (LS), multipolar bursting cells (MB), cannabinoid-sensitive, irregularly spiking cells (CB1-IS), fast-spiking (FS), low threshold-spiking cells (LTS).

characterized biophysically in interneurons are almost certainly mediated by gap junctions. Tamás and colleagues have elegantly combined biophysical and ultrastructural methods to definitively demonstrate that electrically coupled FS interneurons (Tamás et al., 2000), as well as electrically coupled non-FS interneurons (so called “regular-spiking non-pyramidal cells” (Szabadics et al., 2001), actually do form dendrodendritic gap junctions. In addition, when the critical neuronal gap junction protein C×36 is knocked out, nearly all electrical synapses between interneurons are abolished (Deans et al., 2001).

There is very little compelling evidence that the excitatory neurons (i.e., pyramidal cells or spiny stellate cells) of the mature neocortex are electrically coupled. Decades of detailed ultrastructural investigations of neocortex have yielded very few convincing micrographs of gap junctions connecting excitatory cells. The only compelling example available was published by Alan Peters in 1980, in a chapter of *Advanced Neurology* about the mechanisms of anticonvulsant drugs. Direct tests of electrical coupling between pairs of excitatory cells, or pairs of excitatory and inhibitory cells, in neocortex have yielded generally negative results (Thomson and Deuchars, 1997; Galarreta and Hestrin, 1999; Gibson et al., 1999). There is at least one report of C×36 mRNA in pyramidal cells, and very occasional

electrical coupling between interneuron–pyramidal cell pairs in immature cortex (Venance et al., 2000; Meyer et al., 2002), but this has not been observed by others (Galarreta and Hestrin, 1999; Gibson et al., 1999). Gutnick and Prince (1981) reported dye-coupling between mature pyramidal neurons, but subsequent studies found that pyramidal cell dye-coupling was prominent during the first post-natal week and very low thereafter (Connors et al., 1983; Peinado et al., 1993; Roerig et al., 1995; Bittman et al., 1997). It is important to note that dye-coupling, particularly when applied to brain slices using sharp microelectrodes, may not be a very reliable measure of gap junctional coupling among some types of neurons (Knowles et al., 1982; Connors et al., 1984; Gutnick et al., 1985).

### Electrical synapses in the thalamic reticular nucleus

The thalamic reticular nucleus (TRN) is a sheet of GABAergic neurons that surround the thalamic relay nuclei (Guillery and Harting, 2003). TRN cells receive excitatory input from both thalamocortical and corticothalamic axons, and send their projections, which are entirely inhibitory, to thalamic relay cells (Figs. 1 and 4). Thus, the TRN is in a position to influence, and be influenced by, the entire thalamocortical system. Consistent with this, the TRN has been implicated in processes as diverse as sensory transformations, generation of sleep-related EEG rhythms, and behavioral attention (Guillery et al., 1998; Pinault, 2004). It had long been assumed that TRN neurons interact with one another exclusively via inhibitory synapses (Ohara, 1988), but our work shows that this is far from true. Using paired recordings from neighboring TRN cells in rats and mice, we found that electrical coupling is common (Landisman et al., 2002; Long et al., 2004). The prevalence, strength, biophysical properties, and C×36-dependence of electrical connections in TRN are very similar to those in the neocortex.

Electrical coupling in TRN may differ from neocortex in its spatial dimensions; TRN cells apparently form much more compact coupled clusters than cortical interneurons (Long et al., 2004). Electrical coupling in TRN seems to be restricted to cells no more than 40  $\mu\text{m}$  apart. In this sense, coupling in

TRN resembles the spatially localized coupling in the inferior olivary nucleus (Devor and Yarom, 2002). Surprisingly, in paired-cell recordings from TRN in vitro, monosynaptic IPSPs were extremely rare ( $\sim 1$  in 100 pairs for closely juxtaposed neurons; Landisman, unpublished).

As in the cortex, electrical synapses can effectively coordinate both the action potentials and slow subthreshold rhythms of local groups of TRN neurons (Fig. 4B–D; see pages 50–51).

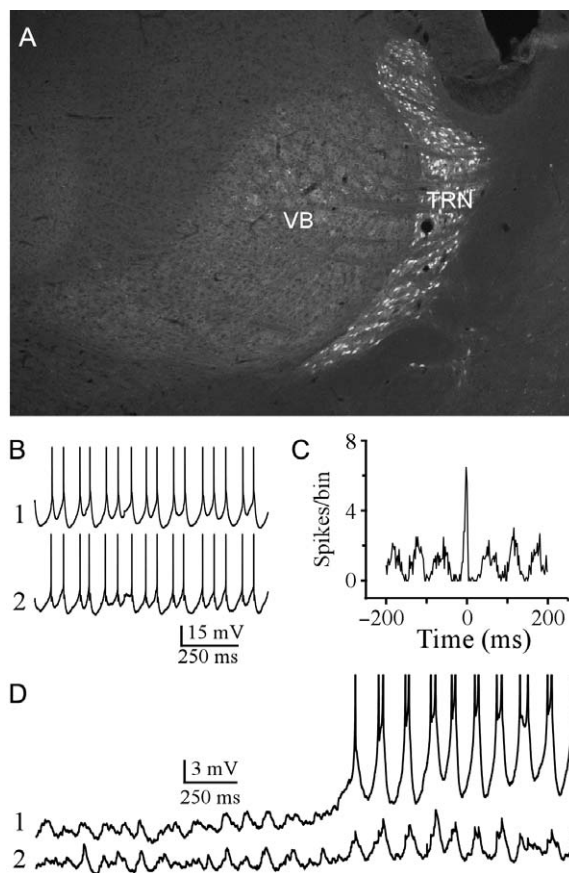


Fig. 4. Electrical synapses in the TRN. A. A fluorescent image of a thalamic slice in which the crescent-shaped TRN is outlined by the presence of GFP-expressing cells (B13 line, Cruikshank, Connors and Huang, unpublished observations). Barreloids in the VB can also be visualized by the clustering of GFP-positive axon terminals (originating from TRN cells). B–D. Activation of mGluRs excites TRN neurons, and induces close synchrony of both action potentials (B–C) and subthreshold membrane fluctuations (D) mediated by electrical synapses. (From Long et al., 2004.)

### Electrical synapses in the thalamocortical system require C×36

Gap junctions are structurally distinct, electron-dense, intercellular connections that bridge the narrow gap of extracellular fluid (about 2–3 nm thick) separating the membranes of two cells. Gap junctions are composed of clusters of transcellular channels. Each channel is created by the union of two hemichannels, one from each cell. Each of these hemichannels, also known as ‘‘connexons’’, is each made of six connexin subunits. Gap junction channels are permeable to all biologically interesting inorganic ions, and also to some small organic molecules. There are about 20 connexin isoforms distributed over nearly all tissues in the body (Willecke et al., 2002). The most common nomenclature uses their predicted molecular weights (e.g., C×36 is about 36 kDa). Most cells can express multiple connexins, and connexons can be homomeric or heteromeric. Only some combinations of connexons can form functional heterotypic channels; current evidence is that C×36 channels only function homotypically (Al-Ubaidi et al., 2000; Teubner et al., 2000).

About half of the connexins are abundant in the central nervous system, but there are good reasons to believe that C×36 is the primary neuronal connexin. Several connexin types are strongly expressed in astrocytes and oligodendrocytes, but these types are almost never observed in neurons (Nagy and Rash, 2000; Nagy et al., 2001). C×36 mRNA is found widely in the mammalian brain (Condorelli et al., 2000), and C×36-like immunoreactivity consistently appears in neuron–neuron gap junctions but not gap junctions between glia (Rash et al., 2001). Single-cell RT-PCR shows that C×36 message is often present in interneurons of hippocampus and neocortex (Venance et al., 2000), and a histochemical reporter enzyme driven by the C×36 promoter labeled a variety of interneurons that included both parvalbumin- and somatostatin-expressing cells (Deans et al., 2001).

The most telling evidence that C×36 forms most electrical synapses comes from work on knockout mice. It has been found that electrical coupling between interneurons of two types in the neocortex (Deans et al., 2001), and between neurons in the

TRN (Landisman et al., 2002), inferior olive (Long et al., 2002), and suprachiasmatic n. (Long et al., 2005) is virtually abolished in C×36 knockout mice. In addition, electrical coupling between MB interneurons of neocortex (Blatow et al., 2003), and neurons in hippocampus (Hormuzdi et al., 2001) and retina (Deans et al., 2002) is also C×36-dependent.

Within the thalamus, several lines of evidence indicate the presence of C×36 mediated electrical coupling in the TRN, but not in relay nuclei. This includes *in situ* hybridization for C×36 mRNA (Condorelli et al., 2000), expression of C×36 reporter gene (Deans et al., 2001), C×36-like immunoreactivity (Liu and Jones, 2003), and paired-cell recording in C×36 knockout mice (Landisman et al., 2002). However, there is some indirect electrophysiological evidence for electrical coupling in relay cells as well (Hughes et al., 2002, 2004). It remains possible that if electrical synapses occur in relay cells, they depend on a gap junction protein other than C×36. Interestingly, even in the TRN, where there is general agreement about the role of C×36 in electrical synaptic transmission, electron microscopic studies have not revealed neuronal gap junctions *per se* (Liu and Jones, 2003). It is possible that connexin mediated channels are not clustered together in large gap junctional plaques in the TRN.

As prevalent as C×36 seems to be, it is probably not the only connexin involved in mammalian electrical synapses. Neurons thought to be electrically coupled, but which may not express C×36, include those of locus coeruleus (Alvarez et al., 2002), the horizontal cells of the retina (Deans and Paul, 2001), and perhaps some pyramidal cells in the hippocampus (MacVicar and Dudek, 1981; Schmitz et al., 2001). C×45 is apparently expressed in neurons of olfactory epithelium and bulb (Zhang and Restrepo, 2002), in horizontal cells (David Paul, personal communication), and in other brain regions (Maxeiner et al., 2003), and is a candidate neuronal gap junction protein. In addition, a newly described family of invertebrate-like gap junction proteins — the ‘‘pannexins’’ — has been discovered in the mammalian genome, and two of them are expressed in the brain (Bruzzone et al., 2003).

## Biophysical properties of electrical synapses

Gap junction channels have the unique ability to interconnect the cytoplasmic compartments of two adjacent cells. The number and permeation properties of these channels have profound effects on the characteristics of individual electrical synapses. Most connexin channels have large single-channel conductances (up to 300 pS; Harris, 2001), but C×36, the central nervous system-specific connexin, has the smallest conductance of any connexin tested, about 10–15 pS (Srinivas et al., 1999). It is likely that only a small fraction of the channels in a gap junction is open at any moment (Lin and Faber, 1988; Pereda et al., 2003). A rough estimate can be done so as to understand the state of a prototypical electrical synapse in the neocortex: First, we assume its channels are comprised of C×36 (Deans et al., 2001), with a unitary channel conductance of 14 pS (Teubner et al., 2000). Second, the typical gap junction interconnecting two mature interneurons has about 150–380 connexin channels (Fukuda and Kosaka, 2003). Third, we assume one of these gap junctions per electrical synapse. Fourth, as Galarreta and Hestrin (2002) reported, the mean conductance of electrical junctions between mature interneurons is about 0.2 nS. These data imply that only 4–9% of junctional channels are open in a mature electrical synapse at any moment; the fraction would be even lower if there were more than one gap junctional contact per electrical synapse (Fukuda and Kosaka, 2003). Since immature neocortical interneurons tend to be more strongly coupled (mean strengths of 0.7–1.6 nS (Galarreta and Hestrin, 1999; Gibson et al., 1999), the gating properties of their channels or the size of their gap junctions — or both — may be different compared to mature neurons. Nevertheless, if only a small proportion of channels tend to be open, there is an interesting opportunity to modulate the strength of an electrical synapse by either decreasing or increasing the open probability of its channels.

Most connexin channels are moderately permeable to organic molecules (including tracers such as Lucifer yellow and neurobiotin, and endogenous substances such as cAMP, cGMP, IP<sub>3</sub>, glucose, and Ca<sup>2+</sup>), but C×36 channels may be relatively less

permeable (Teubner et al., 2000). It is important to point out that, in principle at least, neuronal gap junctions may be more important for conveying chemical signals than electrical signals. There is only the most indirect of evidence for this sort of function in central neurons, but it remains feasible (Hatton, 1998; Roerig and Feller, 2000). The apparently poor permeability of C×36 channels for dyes is unfortunate, because the phenomenon of “dye-coupling” — when it works well — can be exploited to reveal complex spatial patterns of gap junction connections between neurons (e.g., Vaney, 2002). Some dye-coupling between neocortical interneurons has been reported (Connors et al., 1983; Benardo, 1997), but it has generally been difficult to observe in neocortical interneurons and TRN under the same conditions where electrophysiological demonstrations of electrical coupling are undeniable (e.g., Gibson et al., 1999; Landisman et al., 2002). Dye-coupling across C×36-dependent gap junctions is easier to see where neurons are coupled unusually strongly, as the AII amacrine cells of the retina seem to be (Deans et al., 2002). Recently, by using relatively high neurobiotin concentrations and long diffusion times, it has been possible to show dye-coupling between electrical coupled neocortical interneurons (Cruikshank and Connors, unpublished observations).

Connexin channels are slowly gated by their transjunctional voltage ( $V_j$ ; the potential difference of the cytoplasm of two coupled cells), with maximal conductance centered on  $V_j=0$ . However, C×36 subunits form the most poorly voltage-dependent of connexin channels. Even when  $V_j$  varies by  $\pm 100$  mV, C×36 channel conductance falls by less than half (Srinivas et al., 1999; Al-Ubaidi et al., 2000). This is consistent with our measurements from neocortical interneurons, where no measurable voltage dependence was apparent over a range of  $\pm 40$  mV (Gibson et al., 2004). Thus, C×36 channels behave as linear, non-rectifying conductors under physiological conditions.

Electrical signals passing between neurons via electrical synapses are, of course, also a subject to influence the non-junctional membranes of the neurons involved (Bennett, 1977). Gap junctions in the neocortex are typically located at soma-dendritic or dendro-dendritic sites (Sloper and Powell, 1978;

Tamás et al., 2000; Fukuda and Kosaka, 2003). Signals originating in one soma must typically traverse a dendrite, the gap junction itself, and another dendrite before arriving at the soma of the second cell. Because of membrane time constants and dendritic cable effects associated with this arrangement, electrical synapses behave as first-order, low-pass electrical filters. Typically for cell pairs within the thalamocortical system, the corner frequencies are about 10 Hz (Galarreta and Hestrin, 1999; Landisman et al., 2002; Gibson et al., 2004). Thus, relatively small signals that are slow, such as after-hyperpolarizations, burst envelopes, or subthreshold oscillations, are communicated more effectively than action potentials, which are much larger but briefer (Fig. 6; also discussed in pages 50–51).

Despite the lack of transjunctional voltage dependence of C×36 channels, strong voltage-dependent effects of gap junction signals have been observed in TRN (Landisman and Connors, unpublished). Depolarization of the resting post-synaptic membrane can effectively increase the spike-evoked PSP transmitted via gap junctions by as much as threefold, due to the activation of persistent sodium currents in the post-synaptic membrane. This effect does not depend on transjunctional voltage differences, and the voltage amplification can be completely blocked by the application of low concentrations of tetrodotoxin. Similar voltage-dependent modulation of gap junction signals has been observed in neocortical interneurons (Gibson et al., 2004) and at the Mauthner cell synapse of goldfish (Curti and Pereda, 2004).

### **Modulation, regulation, and pharmacology of electrical synapses**

Chemical synapses are famously plastic, and enormous effort has been spent trying to understand how, when, why, and by what mechanisms they are regulated. Electrical synapses can also be modified, by activity and chemicals, but little is known about these processes in the mammalian brain. Understanding the regulation of electrical synapses in the thalamocortical system is an important line of research, but efforts (and progress) have so far been modest; and hence, this section is short.

The conductances of gap junction channels are often affected by changes in intracellular  $[H^+]$  or  $[Ca^{2+}]$  (Rose and Rick, 1978; Rozental et al., 2001), and these effects may constitute physiological mechanisms of electrical synapse regulation. Neural activity can induce significant changes of intracellular pH ( $pH_i$ ) in central mammalian neurons (Chesler, 2003), and channels made from the neuronal connexin, C×36, can be closed by strong acidification in expression systems (Teubner et al., 2000). More direct evidence for activity-dependent regulation of mammalian electrical synapses does not yet exist, but the prospects for finding it are good. Experiments in goldfish show that electrical synapses can either increase or decrease their junctional conductance for hours as a function of prior neural activity (Yang et al., 1990). Enhancement seems to depend on a close interaction between C×35 channels and neighboring glutamatergic receptor channels (Smith and Pereda, 2003; Pereda et al., 2003), and depends on NMDA receptor activation, post-synaptic  $[Ca^{2+}]_i$ , and  $Ca^{2+}$ /calmodulin-dependent protein kinase II (Pereda et al., 1998). (Shades of hippocampal long-term potentiation!) This has very interesting implications for the mammalian case, because fish C×35 and mammalian C×36 are orthologues, and include several shared phosphorylation consensus sites that modulate their conductance (Mitropoulou and Bruzzone, 2003).

As with  $pH_i$  and neural activity, little research has been done on the chemical modulation of electrical synapses in the mammalian brain (Roerig and Feller, 2000). The best data come from the studies on retina, where dopamine-induced activation of adenylyl cyclase, increased cAMP concentrations, and activated cyclic AMP-dependent protein kinase (PKA) leads to reduced electrical synapse strength and altered visual receptive fields (McMahon et al., 1989). Preliminary experiments in the thalamocortical system suggest that the strength of electrical synapses between cortical interneurons is reduced during application of serotonin (Cruikshank and Connors, 2002), and coupling between TRN neurons decreases for long periods after transient activation of metabotropic glutamate receptors (Landisman and Connors, 2004). The mechanisms responsible for these effects are under investigation.

Drugs are important tools in biological research, and the study of gap junctions is no exception. A wide

variety of chemicals reduce gap junction function, but unfortunately most of them tend to be low in potency, only partially effective, and poorly selective. In the brain, most gap junction blockers affect glial connexins as well as those in neurons, and they are non-specific, affecting non-connexin ion channels, receptors, and enzymes (Rozental et al., 2001). Octanol, halothane, and carbenoxolone in particular have been widely used in neurophysiology, and their positive effects are often the primary evidence for implicating electrical synapses in the phenomena under study. Considering the well documented side effects of these drugs, it becomes obvious that such results should be interpreted with caution.

A few other gap junction blockers have recently shown more promise. All-trans-retinoic acid potently reduces electrical coupling between horizontal cells of fish retina (Zhang and McMahon, 2000), and other gap junction-coupled systems. Quinine, the antimalarial drug, selectively blocks Cx36 and Cx50, and has little effect on other connexins (Srinivas et al., 2001). However, quinine has a variety of non-junctional effects on neural systems. Mefloquine, a quinine derivative, is 100-fold more potent than quinine in blocking Cx36, and seems much more specific (Fig. 5; Cruikshank et al., 2004). Unlike quinine, mefloquine causes little change in action potentials. Furthermore, at concentrations sufficient to block electrical coupling between cortical interneurons, mefloquine has virtually no effect on evoked chemical synaptic transmission or resting membrane potential (Fig. 5B). Mefloquine is not the perfect drug, however, since it induces an increase in miniature chemical synaptic events in interneurons. Like quinine, mefloquine has a high degree of potency for the main neural connexin (Cx36), with only moderate effects on glial connexins (e.g., Cx26, Cx32, Cx43; Fig. 5C), so it should prove useful as an experimental tool.

### Functions of electrical synapses in the thalamocortical system

What role do electrical synapses play in thalamocortical function? Perhaps this is an unfair question at this early stage (electrical synapses were discovered in neocortical interneurons five years ago, and in TRN

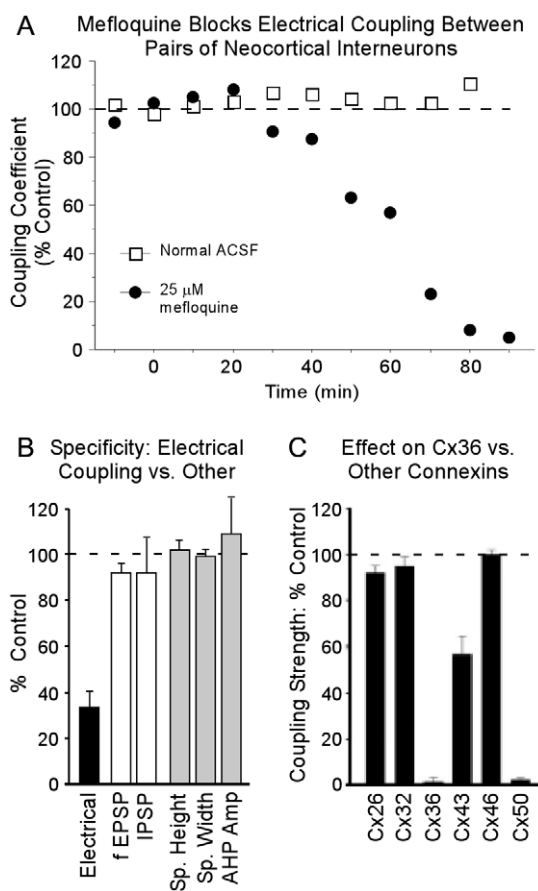


Fig. 5. Specific block of electrical coupling by mefloquine. A. Effect of mefloquine on electrical coupling between a pair of FS interneurons in somatosensory cortex of acute slices. Coupling is nearly eliminated after 90 min of mefloquine perfusion whereas no change occurs for the pair recorded in normal ACSF. B. Group effects of mefloquine on electrical coupling and other properties after approximately 1 h of 25–30  $\mu\text{M}$  mefloquine, in acute slices. The only major effect is a reduction in electrical coupling. C. Effects of 10  $\mu\text{M}$  mefloquine on coupling between N2A cells transfected with selected connexin cDNAs. Each bar represents 4–10 pairs. While pairs expressing the neural gap junction protein (Cx36) are completely uncoupled by mefloquine, those expressing most other connexins are only modestly affected; the exception being Cx50, a lens connexin. (From Cruikshank et al., 2004.)

cells only three years ago). However the classical properties of gap junctions and recent studies of the thalamocortical system offer clues.

Although electrical synapses are faster than chemical synapses (Bennett, 1977, 1997; Jaslove and Brink,

1987), the difference in speed fades to irrelevance at mammalian body temperatures, where chemical synaptic delays can be as low as 150  $\mu$ sec (Sabatini and Regehr, 1996). The lack of short-term plasticity observed with electrical synapses does set them apart, however. Chemical synapses have interesting and widely variable short-term dynamics (Zucker and Regehr, 2002), whereas conductance across electrical synapses is extremely reliable from one event to the next, with no apparent dependence on inter-event interval.

Another potentially important feature of electrical synapses is their ability to communicate subthreshold information from one cell to the next, including hyperpolarizing potentials. In fact, because subthreshold potentials generally have slower kinetics than action potentials, they tend to be transmitted more effectively, due to the low pass filtering characteristics of electrical synapses.

The low pass behavior produces clear differences in signaling between different types of cortical interneurons. For example, it yields a distinctly biphasic electrical PSP for FS cells. This is because the high amplitude positive-going phase of FS spikes is extremely fast, and therefore strongly attenuated, while the lower amplitude negative-going AHP phase has slower kinetics, and is therefore less attenuated. In contrast, LTS cells have longer duration positive phases and weaker AHPs, which produces fairly monophasic electrical PSPs (Fig. 6A). Low pass filtering also has functional implications in the thalamus, given the classic ability of thalamic neurons to generate state-dependent firing in either tonic or bursting modes (Jahnsen and Llinás, 1984). The depolarizing envelope that underlies a burst in TRN cells is rather slow compared to sodium spikes, and such bursts generate electrical PSPs that are about 5 times the amplitude and 25 times the area of the electrical PSPs evoked by single spikes (Fig. 6B). Furthermore, when coupling is weak, individual spike-evoked electrical PSPs ('spikelets') are imperceptible, but burst-evoked PSPs ('burstlets') are still  $\sim 1$  mV in amplitude (Long et al., 2004).

Perhaps the most remarkable property of electrical synapses is bidirectionality; the vast majority of chemical synapses are emphatically unidirectional, or at best highly asymmetric. When gap junctions interconnect neurons with similar biophysical

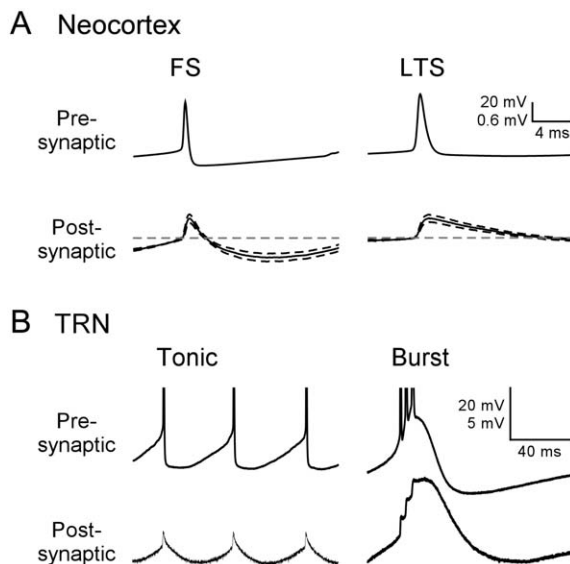


Fig. 6. Electrical post-synaptic potentials (PSPs) differ according to cell type and state. A. recordings from pairs of neocortical interneurons. Average presynaptic action potentials (top) and PSPs with standard error lines (bottom) from FS ( $n=9$ ) and LTS ( $n=16$ ) cell pairs are plotted. The action potentials are from steady state firing during a long (600 ms) current step. The FS-PSP has a biphasic nature, which is lacking in the LTS-PSP. (From Gibson et al., 2004.) B. Recordings from pairs of TRN cells in tonic mode (left) and burst mode (right). Note the difference in electrical PSP size between the two spike modes. (From Long et al., 2004.)

properties, as they very often do, the resulting electrical synapses work equally well in both directions. "Presynaptic" and "post-synaptic" often cease to have meaning. Electrically synapses spread the word, regardless of the message.

One consequence of rapid, reliable, bidirectional signaling tends to be closely coordinated activity. When two or more electrically coupled neurons are active, the most consistent and robust outcome is synchronization. Differences in membrane potential, even small ones, between coupled neurons lead to the flow of small current through their electrical synapses. Thus, an action potential in one neuron causes a small positive deflection in the membrane potential of its coupled neighbors; this tends to bring them more quickly to spike threshold. A hyperpolarizing event (such as an IPSP or afterhyperpolarization) will transiently hyperpolarize coupled neighbors, often phase-delaying their spikes by a small amount.

In electrically coupled neuronal systems of mammals, both action potentials (Galarreta and Hestrin, 1999; Gibson et al., 1999; Mann-Metzer and Yarom, 1999; Landisman et al., 2002) and subthreshold fluctuations (Benardo and Foster, 1986; Christie et al., 1989; Beierlein et al., 2000; Long et al., 2002) can robustly synchronize (Figs. 4 and 7). Computational models of coupled neurons predict that weak coupling can sometimes lead to antiphase or asynchronous spike firing, and the stability of the synchronous and antisynchronous states may depend strongly on the frequency of firing and the detailed properties of the neurons (e.g., Sherman and Rinzel, 1992; Chow and Kopell, 2000; Lewis and Rinzel, 2003; Pfeuty et al., 2003).

The situation becomes more interesting and complex in neuronal systems with both electrical and chemical synapses operating in parallel. An example is the network of FS interneurons of neocortex, which are densely interconnected by both gap junctions and GABAergic synapses (Gibson et al., 1999). Experiments on pairs of FS cells show that inhibitory synapses alone tend to promote antisynchronous (out-of-phase) spiking, especially at low firing frequencies (Gibson et al., 2004). However, if weak electrical coupling is added to the inhibitory network, synchronous states tend to be more prevalent, and under some conditions the FS pairs are bistable, existing in either synchronous or antisynchronous states (Mancilla et al., 2004). The implications of these complex dynamics are not yet clear, although there is increasing evidence that networks of FS interneurons are important in the generation of certain cortical rhythms of the electroencephalogram (EEG; Whittington and Traub, 2003).

The effects of electrical synapses have been studied in larger groups of neurons by activating them with receptor agonists. When mGluRs or muscarinic cholinergic receptors are activated in neocortex, the LTS class of interneurons fires in irregular patterns that are closely synchronized and driven by well correlated subthreshold membrane fluctuations (Beierlein et al., 2000). Muscarinic receptors also drive synchronous rhythms in MB interneurons, and in this case both electrical and GABAergic inhibitory synapses participate (Blatow et al., 2003). In the thalamus, when mGluRs are activated by endogenous or exogenous agonists, local neighborhoods of

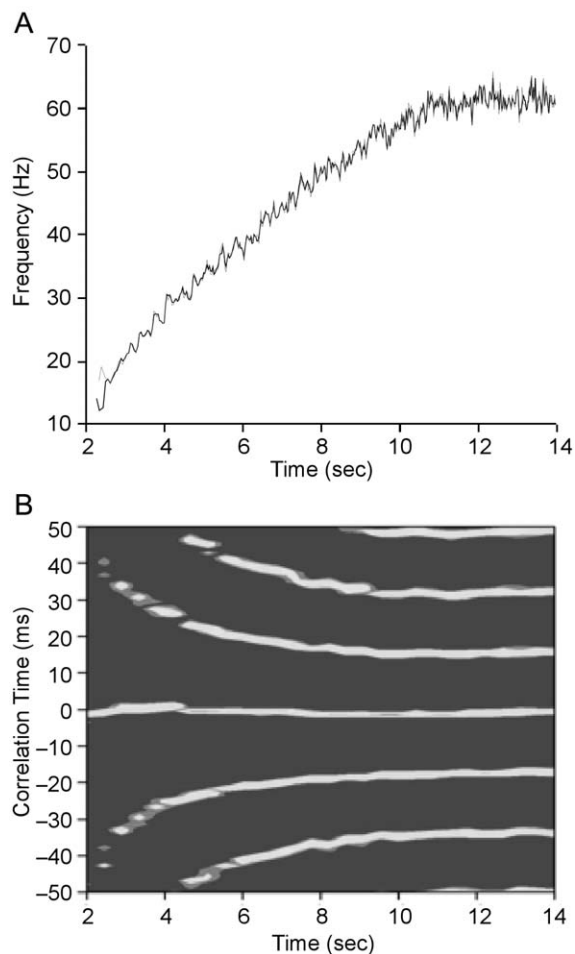


Fig. 7. Electrical coupling between cortical interneurons can induce robust spike synchrony. Recordings are from two coupled FS cells in which linear current ramps (>10 sec duration) are injected into both cells to progressively increase firing rates. The current levels for the ramps were selected to roughly match the spike rates for the two cells. Panel A plots the frequencies for each cell (shown separately in gray and black) throughout the ramps. Notice the high degree of frequency locking, even during the very small fluctuations in rate. Such frequency locking could not be achieved in cells that were uncoupled. Panel B plots data from the same experiment to show the phase relation between the cells during the ramp stimuli. The spike cross-correlation (indicated by gray levels in the z-axis; lighter intensities correspond to higher correlation values) is highest at zero phase lag (y-axis). There are additional smaller peaks 1, 2, and 3 cycles out (cycle width depends on rate, which gets faster throughout the ramp, as shown in panel A). Notice that the peaks are very narrow (<3 msec), indicating a high degree of precision in the synchrony. Also note that the synchrony persists across a wide range of spike rates.

coupled TRN cells generate synchronized rhythms at about 10 Hz (Fig 4B–D; Long et al., 2004). Electrical synapses may serve to coordinate local groups of TRN neurons, given their tight spatial clustering, whereas more distant interactions within TRN may occur via inhibitory connections or common input from cortex and relay thalamus.

A classical way to test the function of something is to eliminate it. Electrical synapses can be reduced or abolished in most central mammalian neurons by knocking out the gene for Cx36, and this has been done by several research groups (Deans et al., 2001; Guldenagel et al., 2001; Hormuzdi et al., 2001). The cellular phenotype of the Cx36 knockout mouse is decisive, since electrical coupling is almost entirely absent among neurons of the neocortex, thalamus, hippocampus, inferior olive, suprachiasmatic nucleus, and retina. The absence of coupling between interneurons strongly reduces the synchronized activity induced by metabotropic receptor activation in both LTS and MB interneurons of the neocortex (Beierlein et al., 2000; Deans et al., 2001; Blatow et al., 2003).

The role of electrical synapses in systems-level functions of the thalamocortical system has not been well explored. This may prove difficult using the knockout approach, or even systemically applied blockers of gap junctions, given the widespread distribution of connexins throughout the brain. For example, the pathways from rod photoreceptors to ganglion cells are virtually absent in the knockouts due to the loss of critical electrical synapses (Deans et al., 2002), making sensory studies of visual thalamocortical properties complicated. Knockout animals also have deficits in their sleep–wake cycles that may be related to a loss of electrical synapses in the suprachiasmatic nuclei (Long et al., 2005). In addition, these mice have a measurable decrease in the power of their EEG in the gamma frequency range, compared to wild type controls (Buhl et al., 2003), which may, or may not, be due to the loss of thalamocortical gap junctions. Furthermore, some compensatory developmental changes have also been reported in Cx36 knockouts (De Zeeuw et al., 2003), and this may be part of the reason why predicted deficits in behavior following Cx36 deletion are

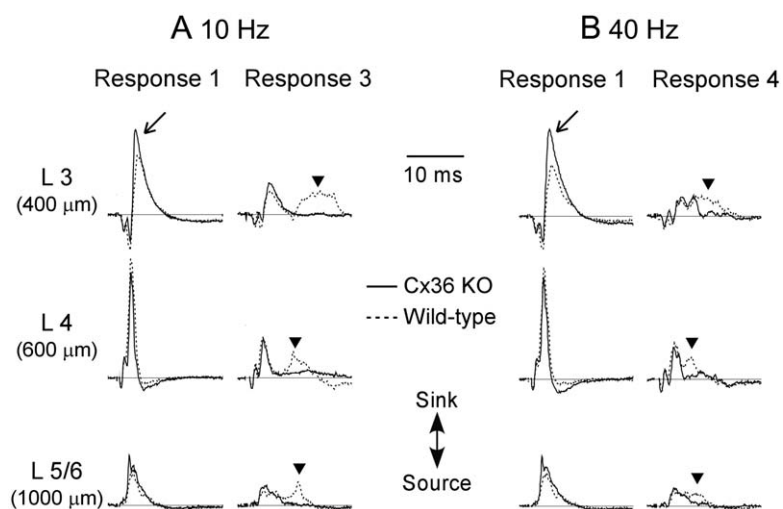


Fig. 8. Effect of knocking out Cx36 on cortical responses to thalamic input. A. Current source density (CSD) responses to the 1st and 3d stimuli in a 10 Hz thalamic train. Responses were recorded at 9 cortical depths, separated by 200  $\mu\text{m}$  each, beginning at the pia. However, only three depths are shown, which correspond to the major sinks in layers 3, 4, and 5/6. For the 1st response in the train, the layer 2/3 sink is larger for the knockout (arrow). By the 3d response in the train, the sinks across all the layers are longer lasting for the WT but not for the KO (arrowheads). B. Similar effects were observed for 40 Hz stimulation, but extension of sink durations was most pronounced for the 4th stimulus in 40 Hz trains. Data represent averages across slices from 7 KO and 8 WT mice. Stimulus intensities were 3X threshold.

subtle (Kistler et al., 2002; Long et al., 2002; Placantonakis et al., 2004).

Despite the difficulties, it is obviously important to determine the roles of thalamic and cortical electrical synapses in sensation and other functions mediated by these systems. The prevalence of electrical synapses in cortical interneurons, and the importance of these inhibitory interneurons in controlling cortical responses to thalamic input (Swadlow, 2003), suggests that electrical synapses play a major role in thalamo-cortical transformations. In an attempt to address this, we have examined cortical responses to thalamic stimulation using laminar current source density (CSD) in isolated brain slices from Cx36 knockout and wild-type mice. Preliminary findings are depicted in Fig. 8, where CSD sinks indicate excitatory synaptic events. The initial layer 4 responses are virtually identical for the two genotypes. However, there do appear to be two subtle differences at later time points. First, the sinks in layer 2/3 are larger for the knockout animal. This is consistent with disruption of the inhibitory network in layer 4, allowing greater excitatory throughput to the upper layers. Second, the late sinks in all layers, produced by repetitive stimulation, are reduced in the knockouts. This suggests that electrical coupling between cortical interneurons, or perhaps even TRN cells, may be involved in responding to the sort of rhythmic repetitive input that occurs during active sensory exploration. Obviously these experiments are in their infancy and much work needs to be done. It will be extremely interesting, as more systems-level results come in, to find out whether or not electrical synapses do play the types of functional roles that are indirectly implicated for them by the large array of cellular data reviewed here.

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