Methods
Parasagittal cortisol slices (360 µm thick, 30 degree angle) were obtained from Wistar rats (14–18 days old). Within this range, no correlation was found between the likelihood of coupling among FS cells and the animal age. The extracellular solution bathing the slices was kept at 32–33 °C and contained 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaHPO4, 1 mM MgSO4, 2 mM CaCl2, 26 mM NaHCO3, 20 mM glucose, pH 7.4 (315 mosm), and was continuously bubbled with a gas mixture of 95% O2 and 5% CO2.

Cell identification
Cells were selected and identified based on their physiology as well as their immunocytochemistry and morphology. Cells in layer V of the somatosensory and the visual cortices were initially selected according to their morphology using infrared differential interference contrast (IR-DIC) optics. Neurons lacking an apical dendrite with multipolar appearance were selected as FS cell candidates. To distinguish FS cells from other types of non-FS neurons in our field we examined their response to near-threshold current injection. The distinguishing feature that we used to classify FS cells was the generation of high-frequency, non-accommodating discharges of action potentials in response to near-threshold current injection\(^{10,11}\). We found that in FS cells (n = 52 cells), the mean interspike interval (ISI) in these near-threshold discharges was 8.0 ± 0.7 ms (range 2–23.7 ms), the coefficient of variation of the ISI was 0.085 ± 0.007, and the ratio of the second ISI to the first was 1.08 ± 0.02. Nonpyramidal neurons that lacked these characteristic discharges were classified as non-FS nonpyramidal cells. In this group, near-threshold discharges containing >3 spikes had a mean ISI of 62.3 ± 10.8 ms (range 31.6–161.8), a coefficient of variation of the ISI of 0.483 ± 0.096, and a ratio of the second ISI to the first 3.21 ± 1.01 (range 0–10.3). FS cells also had the following characteristic inputs: input resistance 92.4 ± 4.0 MΩ (n = 52), input time constant 8 ± 0.7 ms (measured by fitting the late phase of the response to a small current injection, n = 50), fast afterhyperpolarization amplitude 21.7 ± 0.6 mV (measured from the spike onset, n = 52), and spike width at threshold 0.6 ± 0.02 ms (n = 52). In addition, FS cells exhibited a remarkably high activity in the entorhinal cortex (2.57 ± 0.44 spikes/s for layers II/III). EPSPs. Pyramidal neurons were recognized by their characteristic morphology and pattern of spiking\(^{15}\). Glial cells were identified as having a resting potential more hyperpolarized than −80 mV, low input resistance, absence of synaptic inputs and inability to generate action potentials.

Biomorphology of neurons stained with biocytin was revealed by standard avidin-biotinylated horseradish peroxidase complex (ABC, Vector Laboratories) and 3,3'-diaminobenzidine reaction. Fast-spiking cells successfully recovered were nonpyramidal neurons with aspiny radial dendrites. The axon originated in most cases from the upper part of the soma and branched locally with a predominantly horizontal orientation. Immunofluorescence methods were used to detect the presence of parvalbumin in biocytin-filled cortical and neocortical mouse monosynaptic pathways against SNAP-25 (Swant, #235, 1/5000), and secondary mouse IgG antibodies (Fab specific) conjugated with TRITC (tetramethylrhodamineisothiocyanate) (Sigma, T-7782, 1/400). Biocytin-filled cells were detected using Neuravadin-Alexa 488 (Molecular Probes). Twelve of two (75%) neurons classified as FS cells were immunopositive for parvalbumin. In contrast, none of ten non-FS cells tested for parvalbumin were immunopositive.

Recording and data analysis
Simultaneous somatic whole-cell recordings (Axopatch 200 A/B; Axon Instruments) were made using patch pipettes (2–4 MΩ) filled with a solution containing 130 mM K-methylsulphate, 6.3 mM KCl, 10 mM HEPES, 4 mM MgATP, 20 mM phosphocreatine (Na), 0.3 mM NaGTP, 10 mM EGTA and 0.3% biocytin, (pH 7.3, 295 mosm). The liquid junction potential was corrected in the experiment in the estimation of the conductance change due to the series resistances corrected\(^{10,11}\). Paired pairs of FS cells were obtained in the presence of DNQX (10 µM, RBI) to block AMPA/kainate receptor-mediated synaptic currents. Chemical synaptic transmission was studied by generating action potentials in the presynaptic neuron using brief pulses (3–5 ms) of suprathreshold currents. The reversal potential of the uIPSP was −56 mV (n = 4) and 10–µM bicuculline methiodide (a GABAergic receptor antagonist) blocked the uIPSP generated by FS cells (n = 3). Voltage and current signals were filtered at 10 kHz and digitized at 16-bit resolution (National Instruments). The sampling frequency was 5–10 kHz. To synthesize ‘natural’ stimuli we convoluted a Poisson train (2,000 Hz) with a waveform of an excitatory postsynaptic current (EPSC). The result was convoluted with the convolution of a Poisson train (2,000 Hz) and an IPSC waveform\(^{16}\). Trials were separated by 1 s. Each trial a different randomly generated Poisson train was used. The waveforms of the EPSC and IPSC used in the convolution were previously recorded unitary currents.

Data are given as mean ± s.e.m. Statistical analysis testing two-sample hypothesis was performed using unpaired, two-tailed Student’s t tests. Differences were considered statistically significant if P < 0.05. Peaks in the cross-correlogram were considered significant if individual bins exceeded expected value by 2.5 standard deviations.

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different inhibitory cell types were rare. The electrical synapses were strong enough to synchronize spikes in coupled interneurons. Inhibitory chemical synapses were also common between FS cells, and between FS and LTS cells, but LTS cells rarely inhibited one another. Thalamocortical synapses, which convey sensory information to the cortex, specifically and strongly excited only the FS cell network. The electrical and chemical synaptic connections of different types of inhibitory neurons are specific, and may allow each inhibitory network to function independently.

We recorded data from two common types of inhibitory interneurons of somatosensory neocortex, and tested the possibility that each type participates in a functionally distinct network defined by its synaptic inputs, intrinsic membrane properties, chemical and electrical synaptic interconnections, and output targets. Recordings were performed in layers 4 and 6 of rat barrel cortex using an in vitro slice that preserved connections between thalamus and barrel cortex (Fig. 1a). We targeted neurons with large, usually vertically oriented somata (20–30 µm along the vertical axis, Fig. 1b) because these were most likely to be inhibitory. Intrinsic membrane properties and synaptic inputs distinguished the two interneuron types2–7. Fast-spiking cells had narrow action potentials (a mean of 0.35 ms at half-amplitude), each with a deep, brief after-hyperpolarization, high firing rates (up to 320 Hz) when stimulated, and little or no frequency adaptation (Fig. 1c, mean rates after 600 ms were 97% of initial rates). When stimulated near threshold, FS cells often generated a slowly depolarizing voltage ramp before firing spikes at long latencies (up to 600 ms); stronger stimuli evoked spikes at short latencies. Low-threshold spiking cells had broader spikes (0.55 ms at half-amplitude), pronounced adaptation of firing frequency (mean rates after 600 ms fell to 39% of initial rates) and never generated a long delay before spiking (Fig. 1c). These LTS cells also displayed low-threshold spikes when depolarized from more negative potentials. The two cell types had similar input resistances (FS, 58 ± 26 MΩ, n = 127; LTS, 71 ± 37 MΩ, n = 40; all data are expressed as mean ± s.d.).

Some interneurons in layer 4 were stained with biocytin or neurobiotin (FS, n = 35; LTS, n = 5). All had smooth or sparsely spiny dendrites, usually vertically oriented and bitufted, or multipolar, with dense afferents extending into layers 2/3 and 5. The morphologies of FS and LTS cells were not obviously different. Calcium-binding proteins and neuroactive peptides help to distinguish subtypes of γ-aminobutyric acid (GABA)-mediated neurons.

Figure 2 Chemical synaptic connections of inhibitory interneurons. a, inhibitory synapses interconnecting FS and LTS neurons. During paired recordings, spikes in an FS cell evoked inhibitory postsynaptic potentials (IPSPs) in a neighbouring LTS cell (left); in a different pair, spikes in an LTS cell evoked IPSPs in an FS cell (right). In each case, 12 trials are illustrated from the postsynaptic cell, each aligned on the presynaptic spike that evoked it, and a single presynaptic spike is shown for each pair. b, Thalamocortical EPSPs evoked in an FS and an LTS cell with threshold stimuli. Multiple trials are overlaid. c, Summary of mean single-axon thalamocortical EPSP amplitudes, showing that FS cells responded more strongly than both LTS and regular-spiking (RS) cells. Boxes delineate the 25th and 75th percentiles, the line within the box is the sample mean, and lines outside the boxes plot the rest of the range (n = 38 FS, 8 LTS, 26 RS cells). d, Upper trace, thalamocortical (TC) inputs to FS cells strongly depress (average of 20 trials of suprathreshold stimuli applied at 40 Hz). Lower trace, intracortical excitatory inputs to LTS cells strongly facilitate (LTS cell response from presynaptic RS cell spikes evoked at 40 Hz; average of 20 trials).
in neocortex. When immunocytochemistry was combined with intracellular biocytin staining, 6 out of 14 FS cells were parvalbumin immunoreactive, and 3 out of 6 LTS cells were somatostatin immunoreactive. No LTS cells were positive for parvalbumin (n = 4), and no FS cells were positive for somatostatin (n = 8). This labelling specificity is consistent with previous electrophysiological studies of these cell types.

Paired recordings confirmed that these interneurons were inhibitory. Spikes in presynaptic FS and LTS cells evoked inhibitory postsynaptic potentials (IPSPs) in postsynaptic neurons of all types (Fig. 2a; Table 1); however, inhibitory interconnections were much more prevalent between pairs of FS cells (62%) than between pairs of LTS cells (15%). Both FS cells and LTS cells also inhibited regular-spiking (RS) cells, which are excitatory neurons. Regular-spiking neurons occasionally made excitatory chemical synapses with each other (11%), but RS cells more frequently excited the interneurons (41%).

Excitatory inputs to the two interneuron networks differed in several ways. Thalamocortical axons convey sensory information to the neocortex by excitatory synapses in layers 4 and 5/6 (ref. 1). They also evoke rapid and powerful feedforward inhibition, and physiological and anatomical evidence suggests that FS cells may be involved. Single thalamocortical axons were stimulated, and excitatory postsynaptic potentials (EPSPs) were recorded (Fig. 2b). FS cells had, on average, the largest thalamocortical EPSPs (4.3 ± 3.7 mV, n = 38), whereas LTS cells had the smallest (0.8 ± 0.5 mV, n = 8; FS versus LTS, p < 0.001, t-test) (Fig. 2c). Thalamocortical responses were impossible to evoke in most LTS cells, even when neighbouring FS cells showed strong responses. The results indicate that LTS cells may receive weaker and far fewer thalamocortical inputs than FS cells. A less likely possibility is that afferents were differentially separated during slicing. Regular-spiking cells, the excitatory neurons, have thalamocortical responses of intermediate size (1.9 ± 1.5 mV, n = 26) (ref. 12). Thus, FS cells are very likely to be mediators of fast, feedforward thalamocortical inhibition.

Repetitive stimulation of thalamic axons at frequencies of 1–40 Hz produced strong depression of EPSPs in FS cells (Fig. 2d; mean EPSP amplitude fell by 73% after 8 stimuli at 10 Hz). Thalamocortical inputs to RS cells also depressed13, but not as strongly as those to FS cells. Thalamic responses of LTS cells also clearly depressed, but they were too weak to measure. Both types of inhibitory cells received excitatory inputs from local RS cells (Table 1), and each had distinctive dynamics. The FS–FS synapses depressed at all frequencies (mean EPSP amplitude fell by 46% after 8 stimuli at 10 Hz), whereas the synapses from RS to LTS cells markedly facilitated at frequencies above about 30 Hz (Fig. 2d; mean EPSP amplitude rose by 900% after 8 stimuli at 40 Hz).

The presence of dye coupling15–17 and gap junctions18,17 suggests that neocortical interneurons may be electrically coupled. To assess the electrical strength, specificity and functional consequences of coupling, we made paired recordings from neighbouring interneurons (<50 μm between somata). Among FS cell pairs, 62% were coupled (Table 1). Current steps applied to Cell 1 evoked voltage changes in Cell 2, and vice versa (Fig. 3a). The average attenuation of low-frequency voltages, or “coupling coefficient”11,18, between FS cells was 0.07 ± 0.06 (n = 24). Action potentials attenuated much more than low-frequency voltages: the coupling coefficient for FS action potentials, when measurable, was 0.010 ± 0.006 (n = 14). We estimate that the coupling conductance between FS cells was 1.6 ± 1.3 nS (range of 0.4–5.5 nS, n = 24; Fig. 3b). Coupling strength was roughly equal in both directions, and showed no dependence on junctional voltage over ±40 mV. The coupling conductance accounted for up to one-third of the input conductance for the most strongly coupled FS cell pairs. The filtering characteristics of FS–FS cell coupling were tested with subthreshold current steps or sine waves of varying frequencies; coupling was strongest for steady voltages, and rolled off (decreased steeply) at high frequencies with a corner frequency of about 10 Hz. Among
LTS cell pairs, 85% were electrically coupled (Table 1), with an average coupling coefficient at low frequencies of 0.10 ± 0.09 (n = 11), and 0.015 ± 0.01 (n = 7) for action potentials. The average junctional conductance between LTS cells was 2.1 ± 2.3 nS (range 0.3–6.9 nS, n = 11; Fig. 3b). Strong coupling was observed at every age tested, including one pair of FS cells at postnatal day 26 and one pair of LTS cells at day 29. There was no correlation between junctional conductance and age. Although we demonstrated coupling only between pairs of cells, the prevalence of coupled pairs together with the spatial density of interneurons makes it likely that larger clusters of interneurons are electrically coupled. Electrical synapses were common only between interneurons of the same type (Table 1). Only 3 out of 32 mixed-type (FS–LTS) cell pairs were coupled, and 2 of those had very low junctional conductances (Fig. 3b). Furthermore, we never observed RS cells to be coupled to other RS cells or to interneurons.

Electrical coupling between neurons is usually mediated by gap junctions, which are clusters of intercellular channels formed from connexin proteins19. Alternative explanations, such as ephaptic or electrical field interactions20, are very unlikely in this case because signal polarity was preserved and no transient capacitive coupling was observed. When electrically coupled interneurons were exposed to the gap junction blocker octanol21 (1–2 mM), coupling was abolished in all cases (Fig. 3c; four FS–FS pairs, one LTS–LTS pair), and coupling recovered after washout of octanol in three of these. Octanol also nonspecifically suppressed action potentials, increased voltage noise and changed cell input resistance, but these effects did not account for the abolition of coupling.

Gap junctions often mediate intercellular chemical signalling19, and dye coupling (the intercellular passage of a dye of low molecular weight) is often used as an indicator of gap junctions. We filled 24 interneurons with the dye neurobiotin. Although the dye-injected cells were all darkly stained, only one (an FS cell) had a clearly stained interneuron dye coupled to it. Dissociation between dye coupling and electrical coupling may have occurred because the diffusion rate of the dye through small, distally located gap junctions was too slow to allow the coupled cells to be seen. Alternatively, interneurons may be coupled through subtypes of connexins that form channels impermeable to the commonly used dyes22. A variety of connexins exist in the neocortex23, but the connexin expression patterns of specific neuron types are not known. It is unlikely that the electrical coupling of interneurons is related to the developmental decline in dye coupling, primarily in excitatory cells, that has been observed24,25.

Many FS cell pairs displayed both an electrical synapse and a chemical inhibitory synapse in at least one direction (Table 1). In such pairs, a spike in one cell evoked a biphasic voltage change in the coupled cell: a brief, short-latency, reliable depolarization communicated by the gap junction, and a slower, longer-latency, fluctuating IPSP triggered by the GABA-mediated synapse (Fig. 3d). Because the inhibitory synapses showed short-term depression and electrical connections did not, spike trains (0.5–40 Hz) in the first cell generated relatively constant depolarizations, but diminishing IPSPs, in the coupled cell (for example, 10 Hz in Fig. 3e). Thus, interneurons can communicate through both electrical and chemical synapses that display distinct polarities, timing and short-term dynamics.

Electrical coupling may serve to synchronize the electrical activity of neurons, as it does in the locus coeruleus26 and inferior olive27. Intercellular communication in the neocortex display various modes of synchronous and rhythmic activity, and both chemical and electrical synapses have been proposed as mediators of their synchrony28,27. We tested the synchronizing ability of electrical coupling by injecting subthreshold sine wave currents (0.5–100 Hz) into one neuron while holding a neighbouring coupled cell at a membrane potential just below spike threshold (n = 9, FS–FS pairs). Spiking in the second cell was easily entrained by the depolarizing peaks of the current applied to the first cell at frequencies up to 40 Hz (Fig. 4a). Alternatively, two coupled cells were induced to fire action potentials repetitively (50–100 Hz) with constant current injections. Chemical synapses were either blocked with drugs, or did not exist between the recorded neurons. In all cases (four FS–FS pairs, one LTS–LTS pair) sharply synchronous firing occurred (Fig. 4b).

Electrical coupling may contribute to the markedly sharp (±1 ms) spiking synchrony observed between pairs of FS cells in vivo29. Close synchrony among electrically coupled LTS cells is also predicted. The paucity of electrical connections between networks, however, should ensure that the fine patterns of FS and LTS spiking are independent. Some coordination between the two networks may occur through their reciprocal inhibitory connections. Several studies suggest that interneurons generate a variety of synchronous inhibitory rhythms in the neocortex25,27,28. Some of this variety may arise from the independent actions of electrically coupled LTS and FS networks, which are regulated by their differential sensitivity to neuromodulators26,29.

**Methods**

**Slice preparation and recording**

Thalamocortical slices25–45 μm thick were obtained from Sprague–Dawley rats aged P14–P21 (unless otherwise noted). Slices were incubated at 32 °C for 1 hr, and then held at 32 °C during recordings. The bathing solution contained 126 mM NaCl, 3 mM KCl,
Complex lipid determines tissue-specific replication of Mycobacterium tuberculosis in mice

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Tuberculosis is the leading cause of death in the world resulting from a single bacterial infection1. Despite its enormous burden on world health, little is known about the molecular mechanisms of pathogenesis of Mycobacterium tuberculosis. Bacterial multiplication and concomitant tissue damage within an infected host, including experimentally infected mice, occurs primarily in the lungs—the favored niche of M. tuberculosis2. Although it has been proposed that the distinctive cell wall of M. tuberculosis is important for virulence, rigorous genetic proof has been lacking. Here, using signature-tagged mutagenesis, we isolated three attenuated M. tuberculosis mutants that cannot synthesize or transport a complex, cell wall-associated lipid called phthiocerol dimycocerosate (PDIM) which is found only in pathogenic mycobacteria3,4. Two mutants have transposon insertions affecting genes implicated in PDIM synthesis; the third has a disruption in a gene encoding a large transmembrane protein required for proper subcellular localization of PDIM. Synthesis and transport of this complex lipid is only required for growth in the lung; all mutants are unaffected for growth in the liver and spleen. This clearly shows that a lipid is required for M. tuberculosis virulence.

To identify M. tuberculosis genes that are required for growth in vivo, we adopted the signature-tag mutagenesis (STM)5 scheme to screen multiple ’inoculum’ pools of 48 random transposon mutants

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