Interpyramid Spike Transmission Stabilizes the Sparseness of Recurrent Network Activity

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Cortical synaptic strengths vary substantially from synapse to synapse and exhibit a skewed distribution with a small fraction of synapses generating extremely large depolarizations. Using multiple whole-cell recordings from rat hippocampal CA3 pyramidal cells, we found that the amplitude of unitary excitatory postsynaptic conductances approximates a lognormal distribution and that in the presence of synaptic background noise, the strongest fraction of synapses could trigger action potentials in postsynaptic neurons even with single presynaptic action potentials, a phenomenon termed interpyramid spike transmission (IpST). The IpST probability reached 80%, depending on the network state. To examine how IpST impacts network dynamics, we simulated a recurrent neural network embedded with a few potent synapses. This network, unlike many classical neural networks, exhibited distinctive behaviors resembling cortical network activity in vivo. These behaviors included the following: 1) infrequent ongoing activity, 2) firing rates of individual neurons approximating a lognormal distribution, 3) asynchronous spikes among neurons, 4) net balance between excitation and inhibition, 5) network activity patterns that was robust against external perturbation, 6) responsiveness even to a single spike of a single excitatory neuron, and 7) precise firing sequences. Thus, IpST captures a surprising number of recent experimental findings in vivo. We propose that an unequally biased distribution with a few select strong synapses helps stabilize sparse neuronal activity, thereby reducing the total spiking cost, enhancing the circuit responsiveness, and ensuring reliable information transfer.

**Keywords:** action potential, hippocampus, lognormal distribution, spike information, synaptic efficacy, synaptic potency, synaptic transmission, neocortex, pyramidal cell

**Introduction**

As cortical excitatory synapses are weak and stochastic, a neuron cannot fire an action potential unless it receives a number of synaptic inputs in a narrow time window. This integrate-and-fire schema naturally leads to the "synfire chain" hypothesis, in which stable spike propagation to downstream network layers requires synchronization among presynaptic neuron groups (Abeles 1991; Diesmann et al. 1999; Reyes 2003); however, this synchrony-based signal propagation is challenged by recent findings.

First, spontaneous spiking by the majority of cortical neurons in vivo is infrequent, usually with a frequency of less than 1 Hz (Margrie et al. 2002; O’Connor et al. 2010; Minamisawa et al. 2011), and highly asynchronous (Ecker et al. 2010; Renart et al. 2010). The sparseness of neuronal activity is practical in terms of signal detection (Ecker et al. 2010) and energy consumption (Lennie 2003), but intuitively, such highly sporadic activity must eventually reach a point of no activity because it cannot depolarize postsynaptic neurons to their spike threshold. Thus, how cortical networks can maintain asynchronous and infrequent activity is enigmatic.

Second, the effects of single neurons on the network dynamics are unexpectedly strong. An early report indicated that single CA3 pyramidal cells (PCs) can initiate synchronized rhythmic burst discharges in disinhibited hippocampal slices (Miles and Wong 1983). We more recently demonstrated that the stimulation of single CA3 PCs can initiate a transition of the network firing state in hippocampal slice cultures (Fujisawa et al. 2006). In vivo studies appear consistent with these findings. Addition of a single extraspike to ongoing cortical activity results in a transient increase in the firing rates of surrounding neurons (London et al. 2010). More surprisingly, single PC stimulation is capable of switching the up/down state of the entire brain in anesthetized rats (Li et al. 2009), and spike trains evoked in a single somatosensory PC can evoke whisker movements or behavioral responses (Brecht et al. 2004; Houweling and Brecht 2008). These results indicate that nonsynchronized PC activity can propagate in noisy cortical network.

These observations cannot be explained by the classic spike propagation model. To reconcile the apparent contradictions, we focused on recent literature concerning the distribution of unitary synaptic weights among neocortical PCs, where a small fraction of PC pairs generate extremely large synaptic potentials, the amplitudes of which occasionally reach nearly 10 mV (roughly corresponding to 2 nS) (Song et al. 2005; Lefort et al. 2009). Because the outliers are 1 or 2 orders of magnitude stronger than common PC synapses, we hypothesized that they can reliably elicit spikes in the postsynaptic PCs and that this phenomenon, termed interpyramid spike transmission (IpST), can thereby stabilize sparse spiking and augment the power of single neurons.

**Materials and Methods**

The experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval numbers: 19–41, 19–43) and according to the University of Tokyo guidelines for the care and use of laboratory animals. Data are reported as the means ± standard deviations (SDs).

**Ex Vivo experiments**

Postnatal day 7 Wistar/ST rats of either sex were chilled with ice and decapitated. The brains were removed and cut horizontally using a Dosaka DTK-1500 vibratome into 300-μm thick slices in aerated ice-cold Gey’s balanced salt solution supplemented with 25 mM glucose. Entorhino-hippocampal stumps were cultivated on Omnipore membrane filters (JHP02500, φ25 mm; Millipore) (Koyama et al. 2007).
Cultures were fed with 50% minimal essential medium, 25% Hank's balanced salt solution (Invitrogen, Carlsbad, CA), and 25% horse serum (Cell Culture Laboratory, Cleveland, OH) in a humidified incubator at 37 °C in 5% CO2. The medium was changed every 3.5 days. Experiments were performed at 7–10 days in vitro. Slices were placed in a recording chamber and perfused at a rate of 1.5–2.0 mL/min with artificial cerebrospinal fluid (aCSF) that consisted of (in mM) 123 NaCl, 26 NaHCO3, 2.2 KCl, 1.24 NaH2PO4, 2.0 MgSO4, 2.0 CaCl2, and 10 glucose (32 °C), and they were bubbled with 95% O2/5% CO2. CA3 PCs were visually identified and randomly selected for patch-clamp recordings using infrared differential interference contrast microscopy. Borosilicate glass pipettes (5–9 MΩ) were filled with an internal solution that consisted of (in mM) 135 K-gluconate, 4 KCl, 10 glucose, 0.01% Pluronic F-127 (Invitrogen), and 0.005% Cremophor EL (Invitrogen) in Toto experiments, and fixed to a silicon bottom with 4–6 thin entomological needles so that the AMPA receptor component, and it was fitted by the dual exponential function (\(g_0\) × (\(e^{t/t_1} - e^{t/t_2}\)), where \(t_1\) represents an activation time constant, \(t_2\) is a decay time constant, and \(g_0\) is a scaling factor (\(t_1 < 1\) ms, \(t_2 < 8\) ms for AMPA receptors, \(t_1 = 3\) ms, and \(t_2 = 300\) ms for NMDA receptors). The voltage-dependent release of magnesium blockade in NMDA receptor conductances was modeled as:

\[
1\left(1 + \left(\frac{[Mg^{2+}]_o}{3.57}\right) e^{-0.062 \cdot V(t)}\right),
\]

where \([Mg^{2+}]_o\) is the external magnesium concentration of 1 mM (Jahr and Stevens 1990). After the completion of the physiological measurements, the type of neuron recorded was confirmed by biocytin staining. After overnight fixation with 4% paraformaldehyde, the hippocampus was sliced at a thickness of 100 μm using a vibratome and then incubated at room temperature with 0.3% Triton-X100 for 6 h followed by Alexa Fluor 488-conjugated streptavidin (1:500; S32354, Invitrogen) for 6 h. Biocytin-positive neurons were microscopically identified and visualized with a BioRad MRC-1000 laser scanning confocal system. Only data obtained from PCs were used for analysis.

**In Silico experiments**

All model neurons obey the leaky integrate-and-fire model (Dayan and Abbott 2001). Our recurrent network consisted of 4000 excitatory and 1000 inhibitory neurons, and all excitatory and inhibitory neurons were randomly interlinked. The connectivity between all types of synapses was set to be 10%, unless otherwise specified. Each integrate-and-fire neuron was characterized by leak conductance \(g_{leak} = 4.5\) nS, membrane capacitance \(C = 100\) pF, and resting potential \(V_{rest} = -70\) mV. Whenever the membrane potential \(V(t)\) crossed a spiking threshold of −55 mV, an action potential was generated, and the membrane potential was reset to \(V_{rest}\) where it remained clamped for refractory periods of 25–40 ms for excitatory neurons and 2–4 ms for inhibitory neurons. \(V(t)\) obeyed the following equation:

\[
C \frac{dV(t)}{dt} = -g_{leak} (V(t) - V_{rest}) - I_{syn}(t),
\]

where \(I_{syn}(t)\) is the instantaneous synaptic current and is defined as \(I_{syn}(t) = g(t)(V(t) - V_{rest})\). \(g(t)\) is the reversal potential. The function \(g(t)\) is the synaptic conductance mediated by either AMPA/NMDA receptors or \(\gamma\)-aminobutyric acid type-A (GABA_A) receptors and designed with the double exponential kinetic:

\[
g(t) = g_0 \left( e^{-t/t_1} - e^{-t/t_2} \right),
\]

where \(t_1\) and \(t_2\) are the rise and decay time constants, \(g_0\) is the time when the action potential is generated, and \(D\) is the synaptic delay. For the AMPA receptor component, \(V_{rest} = 0\) mV, \(t_1 = 1\) ms, \(t_2 = 8\) ms, and \(D = 1\) ms; whereas for the NMDA receptor component, \(V_{rest} = 0\) mV, \(t_1 = 1\) ms, \(t_2 = 8\) ms, and \(D = 15\) ms.
$\tau_s = 3 \text{ ms, } \tau_d = 300 \text{ ms, and } D = 1 \text{ ms;} \text{ GABA}_A$ receptor component: $E_{syn} = -80 \text{ mV, } \tau_s = 1 \text{ ms, } \tau_d = 8 \text{ ms, and } D = 2 \text{ ms.}$ The quantal conductance $g_0$ of the AMPA receptors followed a lognormal distribution with the mean $\mu = 10^{-0.33}$ nS and SD $\sigma = 10^{-0.36}$ nS, unless otherwise specified. These $g_0$ values were further subject to trial-to-trial fluctuations (see below). The NMDA/AMPA conductance ratio was fixed at 1:1, and the magnesium block was modeled as in equation (1), where $[\text{Mg}^{2+}]$ was 1 mM (Jahr and Stevens 1990). The conductance of AMPA receptors in the inhibitory neurons was $g_0 = 1$ nS, that of the GABA receptors in excitatory neurons was $g_0 = 4$ nS, and that of the GABA receptors in inhibitory neurons was $g_0 = 5$ nS. Short-term synaptic plasticity was incorporated into all synapses according to previous studies (Markram et al. 1998; Liu and Buonomano 2009), in which the synaptic response generated by the $n$th action potential in a train was defined as:

$$g_{syn} \cdot R_n \cdot u_n$$

$R_1$ after the first action potential is:

$$R_1 = 1 - U$$

$R_n$ for consecutive action potentials in the train is:

$$R_{n+1} = R_n (1 - u_{n+1}) \exp \left( -\frac{At}{\tau_{exc}} \right) + 1 - \exp \left( -\frac{At}{\tau_{exc}} \right)$$

$$u_{n+1} = u_n \exp \left( -\frac{At}{\tau_{facilit}} \right) + U \left( 1 - u_n \exp \left( -\frac{At}{\tau_{facilit}} \right) \right)$$

Specifically, in the glutamatergic synapses $U = 0.5, \tau_{exc} = 500 \text{ ms, } \tau_{facilit} = 10 \text{ ms;} \text{ in GABAergic synapses, } U = 0.25, \tau_{exc} = 700 \text{ ms, } \tau_{facilit} = 20 \text{ ms.}$ The trial-to-trial fluctuation of the synaptic conductance $g_0$ and the release probability were provided with excitatory neurons, based on the parameters obtained with a least-square fit to our experimental data ex vivo (Fig. 1G). The trial-to-trial fluctuation of the synaptic conductance was incorporated into excitatory-to-excitatory synapses. The coefficient of variation (CV) of the synaptic weights was 0.22

$$\frac{\sigma}{\mu} = 0.5 \text{ nS (Fig. 1G, green line). At each arrival of a presynaptic action potential, the peak conductance was chosen according to a Gaussian distribution with } \mu = g_0, \text{ and } \sigma = g_0 \times \text{CV.}$$

No trial-to-trial fluctuations were given to all other types of synapses. The release probability of glutamatergic synapses in excitatory neurons was $1 - e^{-12.3 \times \sigma}$ (Fig. 1G; blue line). At each arrival of a presynaptic action potential, excitatory-to-excitatory synaptic transmission occurred stochastically with this probability. If the transmission failed, no synaptic conductance was added to the postsynaptic neurons. The release probability at all other types of synapses was 1. The periods of 10–120 min were simulated at a time step of 0.1–0.5 ms. In this range, the time step did not affect the results. The simulation was repeated 10–20 times with different random seeds of connectivity and synaptic weights, producing consistent results. The simulation software was written in MATLAB and executed on a Quad-core 3.2 GHz × 2 CPU personal computer.

**Results**

**Distribution of Interpyramid Synaptic Weights**

Using hippocampal slice cultures, whole-cell recordings were performed on 1048 pairs of CA3 PCs to measure synaptic potency, defined as the mean amplitude of uEPSGs across trials with successful synaptic transmission. Depolarizing currents were injected 50–100 times every 10–30 s into presynaptic neurons to induce single action potentials, and the evoked uEPSGs were monitored at a holding voltage of ~70 mV. The distribution of uEPSG potency featured a heavy tail generated by a small fraction of large uEPSGs (Fig. 1A) and approximated a lognormal distribution (Fig. 1B; $R = 0.49, P < 0.0001$). Similar lognormal distributions have been reported in PC-PC synaptic weights in acute neocortical slices (Song et al. 2005; Sarid et al. 2007). In our data, the Gini coefficient was 0.49 in the Lorenz curve, which plots the proportion of the distribution assumed by the weakest proportion of the uEPSG.

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**Figure 1**. Long-tailed distribution of uEPSGs at CA3-to-CA3 synapses in hippocampal slice cultures. (A–C) Normal (A), semilog (B), and Lorenz (C) plots of the distribution in uEPSG potency of 1048 CA3 PC pairs. The uEPSG size was estimated by dividing the peak synaptic current by ~70 mV. The broken line indicates the least-square best fit for the lognormal distribution with $\mu = 10^{-0.33}$ nS, $\sigma = 10^{-0.36}$ nS, $R = 0.49, P < 0.0001$. This distribution gives $\mu = 0.47$ nS and $\sigma = 0.44$ nS in linear scale calculation. The inset in A indicates a presynaptic spike and 30 trials of postsynaptic responses. (D–F) Relationship in uEPSG potency between 2 connections (#1 and #2) in reciprocal 2-cell motifs (D), convergent 3-cell motifs (E), and divergent 3-cell motifs (F). GI The time constant of uEPSG decay (red), the release probability (blue), and the CV of successful trial uEPSG sizes (green) are plotted against the uEPSG potency ($g_0$). Lines are the least-square fits: decay $= 16.7 - 3.7 \times g_0$ (red, $R = 0.32, P < 0.0001$), probability $= 1 - e^{-12.3g_0}$ (blue, $R = 0.71, P < 0.0001$), and CV $= 0.22 \times g_0^{1.00}$ (green, $R = 0.45, P < 0.0001$).
potency (Fig. 1C). Specifically, the weakest half contributed only 17% of the total uEPSGs, whereas the strongest 20% contributed 52%. When postsynaptic PCs were current clamped, the largest fraction of uEPSGs elicited depolarizations of up to 10 mV. In microcircuit motifs, uEPSG potency correlated positively with reciprocal connections made by 2 PCs, but not with 2 convergent or divergent connections made by 3 PCs (Fig. 1D–F).

The extremely large uEPSGs can be explained by 3 plausible (not exclusive) mechanisms. First, the large uEPSGs could be generated by structurally large synapses. The size of dendritic spines correlates with synaptic strengths (Matsuzaki et al. 2001) and is lognormally distributed (Loewenstein et al. 2011). Second, the large uEPSGs could occur at dendrites proximal to the soma. Indeed, larger uEPSGs had faster waveform kinetics, consistent with cable theory (Fig. 1G, red line). Finally, large uEPSGs could arise from multiple synaptic sites (Sorra and Harris 1993; Markram et al. 1997). This idea is consistent with the central limit theorem in large-sample statistics in terms of 2 observations: 1) the probability of transmission failure decreased as a single exponential function $e^{-t}$, where $g_0$ is the uEPSG potency and is thus roughly proportional to the number of synaptic release sites (Fig. 1G, blue line) and 2) CV of the trial-to-trial fluctuations in successful-trial uEPSG sizes was inversely proportional to the root square of $g_0$ (Fig. 1G, green line). Therefore, we think that all these 3 mechanisms could contribute to the generation of large uEPSGs, although we cannot determine the ratios of these contributions, which may affect the distribution shape in synaptic weights.

**Interpyramid Spike Transmission (IpST)**

Fluctuating currents (Gaussian noise with $\sigma = 200$ pA) were continuously injected into a current-clamped postsynaptic PC, and the presynaptic PC was made to fire a single action potential. In strongly connected pairs, the postsynaptic PCs responded with single spikes that were time locked to the presynaptic spikes (Fig. 2A), whereas weak synaptic pairs did not exhibit an increase in the firing rate of the postsynaptic neurons (Fig. 2B). Because the increased firing rate persisted for 30 ms after presynaptic stimulation (Fig. 2A), we defined IpST as a postsynaptic spike occurring between 1 and 30 ms after an evoked presynaptic spike and computed the IpST probability as $(N_{IpST} - N_{chance})/(N_{total} - N_{chance})$, where $N_{total}$ is the total trial number, $N_{IpST}$ is the number of trials that showed IpST, and $N_{chance}$ is the false-positive chance number expected by the background firing rate of the postsynaptic PC. Larger uEPSG potencies generated higher IpST probabilities (Fig. 2C, top). We then computed the $P$ value for the IpST probability based on the Z-test for a proportion (Kendall et al. 1994). IpST that occurred with a significantly high probability was evident for uEPSG potencies of more than about 1 nS (Fig. 2C, bottom).

To investigate IpST under conditions of physiological noise, we used in toto hippocampal preparations (Fig. 3A), which are known to self-generate theta oscillations typical of hippocampal networks in vivo (Goutagny et al. 2009). In these preparations, CA3 PCs exhibited spontaneous spikes at frequencies of 0.1–2.0 Hz even without noise current injection. Using the dynamic-clamp technique, we injected a uEPSG-shaped conductance (Fig. 3B). Larger conductance injections more reliably evoked action potentials. The “apparent” IpST was significant at more than about 1 nS (Fig. 3C). CA3 networks alternated spontaneously between theta and nonttheta states, spending $32 \pm 22\%$ of the observation period in the theta state. In the theta state, the resting membrane potential recorded in CA3 PCs was depolared by $2.6 \pm 0.7$ mV ($n = 6$ neurons). Intermittent sharp waves were observed in the nonttheta state. The IpST probabilities were compared between theta and nonttheta states using simultaneous recordings of LFPs from the CA3 PC layer. We found that IpST occurred more reliably in the theta-oscillation state (Fig. 3C inset, 1 nS, $P = 0.01$, $t_c = 3.56$, paired $t$-test). Thus, the IpST efficiency is subject to state-dependent modulation, probably through changes in the intrinsic activity of the CA3 network.

To examine whether IpST could be detected in spontaneous network activity, we returned to slice culture preparations and conducted high-speed fMCI from the CA3 PC layer (Takahashi et al. 2010). Transient calcium increases in the somata, reflecting action potentials, were monitored at 500 Hz from 85 ± 25 neurons per slice ($n = 14$ slices, 53–137 neurons monitored per slice). We defined a delayed event pair (DEP), which may result at least in part from IpST, as any calcium event that occurred in one neuron within 1–30 ms after an event in another neuron. We observed more DEPs in the original data sets than would be expected in random surrogates (Fig. 4A). The surrogates were generated by random shuffling of interevent intervals, in which the time intervals between 2 successive calcium activities were transposed at random within each neuron to collapse the temporal correlation between neurons without changing the activity level of individual neurons (Sasaki et al. 2007). The $P$ value matrix for these DEPs revealed that significant DEPs occurred between a few neuron pairs (Fig. 4B–D). Consistent with the data of Figure 1D, reciprocal $P$ values in pairs were significantly correlated (Fig. 4E, $R = 0.84$, $P < 0.0001$). Indeed,
we encountered bidirectional chains of multiple DEPs during network synchronizations (Fig. 4F), which resemble the forward and reverse replay of hippocampal place cell activity during sharp-wave ripple complexes (Lee and Wilson 2002; Foster and Wilson 2006; Diba and Buzsaki 2007); note that we previously demonstrated that these CA3 synchronizations are associated with sharp-wave ripples in LFPs (Takahashi et al. 2010).

**Self-Sustained Spiking Activity in a Network Embedded with a Few Strong Synapses**

Based on our electrophysiological data ex vivo, we simulated a recurrent neural network model. Our network included 5000 leaky integrate-and-fire units consisting of 4000 excitatory and 1000 inhibitory neurons. The 5000 neurons were randomly linked with a connectivity of 10%. The network size and the connection sparsity were nearly identical to those of the hippocampal CA3 recurrent network ex vivo (Takahashi et al. 2010; Kimura et al. 2011). The excitatory-to-excitatory synapses were lognormally distributed in weight and were given stochastic transmission and trial-to-trial fluctuations as shown in Figure 1G. We did not consider dendritic computation because we collected the uEPSG distribution from the cell body (Fig. 1A).

Initially, a small percentage of neurons were randomly selected from the 4000 excitatory neurons, and these neurons were made to fire single action potentials. Surprisingly, these initial spikes were autonomously amplified and maintained at an apparently steady level even without further external inputs or additional spikes (Fig. 5A). This phenomenon is not trivial because neural networks in which synaptic weights conform to Gaussian or homogenous distributions usually require continuous driving noise to maintain spiking activity (data not shown, see also Fig. 6). We therefore analyzed this characteristic self-sustained activity. Consequently, we found that the activity 1) was spatiotemporally sparse, 2) maintained the balance between excitation and inhibition, 3) contained reliable spike transmission between excitatory neurons (expeditiously called IpsT herein) and precise sequences of multineuronal spikes, 4) changed its internal state in response to the stimulation of single neurons, and 5) configured a stable pattern of spiking as a whole (“attractor”). Importantly, these features are similar to those found in the cortex in vivo. We describe these 5 features in the following sections.

**Sparse Spiking**

In the self-sustained state (Fig. 5A), the mean firing rates of excitatory and inhibitory neurons are about 0.3 and 5 Hz, respectively (calculated from a 120-min long data set). This infrequency of spontaneous spiking is consistent with electrophysiological reports obtained using patch-clamp recordings from neocortical layer II/III neurons in vivo (Margrie et al. 2002; O’Connor et al. 2010; Minamisawa et al. 2011) and with...
calcium imaging from CA3 neurons in hippocampal slice cultures (Sasaki et al. 2007; Takahashi et al. 2010). At the single-neuron level, the firing rates varied from neuron to neuron, conforming to a lognormal distribution (Fig. 5B; excitatory: $\mu = 10^{-0.32}$ Hz, $\sigma = 10^{0.70}$ Hz, $R = 0.99$, $P < 0.0001$; inhibitory: $\mu = 10^{0.65}$ Hz, $\sigma = 10^{0.65}$ Hz, $R = 0.98$, $P < 0.0001$). This skewed distribution of the spike rates is also consistent with recent evidence in vivo (Hromadka et al. 2008; O’Connor et al. 2010).

We confirmed the importance of the synaptic weight distribution in maintaining spontaneous activity, using 2 tests, 1) a robustness analysis (Fig. 5C) and 2) replacement with different distributions (Fig. 6).

First, we conducted a robustness analysis in which an increasing portion of the strongest (or weakest) synapses was removed from the network. Removal of the strongest 0.1% of synapses resulted in a lower frequency of spontaneous firing, and removal of more than 0.3% completely abolished the spiking activity (Fig. 5C). Thus, the self-sustained activity is supported by just a small fraction of strong synapses. This result suggests that neural networks can hardly maintain their activity unless embedded with strong synapses. The removal of a small fraction of the weakest synapses did not affect the activity maintenance, but the removal of 30% of these synapses eradicated the activity. Thus, the network activity requires not only infrequent inputs from strong synapses but also massive synaptic bombardment from many weak synapses. This conclusion may not be surprising, as the strongest synapses in our network induce a depolarization of about 10 mV, but the 10-mV depolarization is still smaller than the spike threshold. Therefore, many weak synapses are required to provide depolarizing background noise that helps strong synapse-driven depolarizations to reach the spike threshold. This noise-induced signal enhancement parallels the concept of stochastic resonance (Douglass et al. 1993).

Second, we changed either the mean synaptic weight $\mu$ or the SD $\sigma$ of the lognormal distribution of excitatory-to-excitatory synaptic weights (Fig. 6A) and examined the mean firing rates of self-sustained activity in these networks; note that the experimentally obtained $\mu$ and $\sigma$ were 0.47 and 0.44 nS, respectively (Fig. 1A). Infrequent spontaneous firing (<2 Hz) was maintained in relatively broad ranges of $\mu$ and $\sigma$ values, although the networks failed to maintain spontaneous activity when either $\mu$ or $\sigma$ was too small, and it exploded into an epileptiform state when either $\mu$ or $\sigma$ was too large, respectively. That is, at $\sigma = 0.44$ nS, $\mu$ could range from 0.1 to 0.6 (Fig. 6B) and at $\mu = 0.47$ nS, $\sigma$ could range from 0.4 to 1.5 (Fig. 6C). Incidentally, the mean firing rate in the epileptiform state was about 30 Hz, the maximal rate allowed in our integrate-and-fire neurons, indicating that virtually all neurons fired at the maximal rate.

The same parameter analysis was conducted for Gaussian distributions of excitatory synaptic weights (Fig. 6D). The Gaussian network at the default values of $\mu = 0.47$ nS and $\sigma = 0.44$ nS failed to maintain spontaneous activity (Fig. 6E,F). Therefore, we increased the $\mu$ value until the network could maintain the persistent activity, by fixing $\sigma = 0.44$ nS (Fig. 6G). The activity persisted at $\mu >$ about 0.5 nS; however, at $\mu >$ 0.8 nS, the mean firing rate jumped to about 30 Hz. Thus, the Gaussian distribution of synaptic weights could maintain infrequent network activity only in a narrow range of $\mu$. Similarly, at $\mu = 0.47$ nS, infrequent network activity was observed at $\sigma$ values between 0.5 and 1 nS (Fig. 6F).

Why is the lognormal distribution less sensitive to changes in the parameters in terms of stabilizing infrequent network...
activity? A simple possibility is an extremely inhomogeneous distribution of synaptic weights with a small fraction of synapses generating very large EPSGs. To examine whether the presence of a few strong synapses and many other weak synapses, rather than a lognormal distribution of synaptic weights, is enough to maintain infrequently persistent activity, we tested an extreme case in which 5% of excitatory synapses were at 3 nS and the remaining 95% were 0.2 nS (Fig. 6G). We found that this simplified network could maintain infrequent spikes (Fig. 6H). Therefore, a key feature required to maintain infrequent activity seems to be a highly inhomogeneous distribution of synaptic weights.

The maintenance of cortical recurrent network activity is known to require NMDA receptors (Metherate and Cruikshank 1999; Buonomano 2003). To investigate the role of NMDA receptors in our network activity, we reduced NMDA receptor-mediated conductance at excitatory synapses, as if the network was treated with a competitive NMDA receptor antagonist at increasing concentrations (Fig. 5D). The level of spontaneous activity decreased with the level of NMDA receptor blockade and eventually reached no activity at 30% blockade.

Another distinctive characteristic of the self-sustained activity is asynchronicity, which is also a feature of neocortical activity in vivo (Ecker et al. 2010; Renart et al. 2010). We computed the correlation coefficients between the spike trains of all possible pairs of the 4000 excitatory neurons \((n = 7,998,000\) pairs, \(bin = 10\) ms). The distribution of the correlation coefficients peaked at zero (Fig. 5E), indicating that spontaneous activity is largely asynchronous among individual neurons. To further evaluate the degree of asynchronicity, we calculated the asynchrony index, which is the Shannon entropy normalized to a range between 0 and 1, with higher values representing states that are more asynchronous (Usami et al. 2008; Ujita et al. 2011). The asynchrony index of the spontaneous activity in our network was 0.98, confirming that the activity is nearly completely asynchronous.

**Excitation-Inhibition Balance**

While the network remained in a steady state, the sets of active neurons at a given moment varied as a function of time, which indicates that the internal state of the network fluctuated dynamically. To depict these microscopic dynamics, we obtained a time series of vectors that represented a set of active neurons during a period of 2 s and compressed the vector dimension using a principal component analysis (Sasaki et al. 2007). The time evolution of the vectors in the principal component space revealed that the network state drifted gradually (Fig. 5F), which indicates that active neuron populations vary over time. Interestingly, during these microscopic fluctuations, the entire network maintained the instantaneous balance between excitation and inhibition; the total excitatory and inhibitory synaptic conductances in the network were proportional to each other, whereas the total conductance per se fluctuated as a function of time (Fig. 5G). Interestingly, similar balanced fluctuations were observed in excitation and inhibition of infrequent activity that was...
maintained in a Gaussian network with $\mu = 0.5$ nS and $\sigma = 0.5$ nS (Fig. 6F “inset”). Therefore, the excitation and inhibition balance is not a feature of lognormal networks per se, but it may be rooted in self-sustained network activity.

With respect to the balanced excitation and inhibition, we next examined the effect of network architectures on infrequent network activity. We changed the number of inhibitory neurons and the network connectivity. Our default network contained 4000 excitatory and 1000 inhibitory neurons and had a 10% connection probability between pairs of 5000 neurons. Thus, we changed the number of inhibitory neurons in a range between 100 and 2000 without changing the number of excitatory neurons. We also changed the connection probability in a range between 1% and 100%. The mean firing rates are plotted as a dual function of these parameters, that is, 200 neurons and a wide range of these parameters, for example, the weights of the 3 synapses responsible for 4-spike PFSs in Figure 8D are shown by arrows in Figure 8B. The total number of PFSs in a 1-min data set obtained from 20 networks that were executed with different random seeds was compared with the number found in surrogates in which spikes were randomly exchanged among neurons (Ikegaya et al. 2004; Sasaki et al. 2007). For each data set, 100 surrogates were generated. In all 20 data sets, the PFSs found in the original raster plot were more frequent than the chance level estimated for the surrogates (Fig. 8E).

**IpST and Spike Sequences**

To evaluate the IpST, we plotted peri-presynaptic spike time histograms for postsynaptic spikes. In the case of a strong synapse, such as that shown in Figure 8A (1.45 nS), the histogram showed a sharp peak in the firing rate, which is a sign of IpST. We thus computed the IpST probability for all 1 600 000 synaptic pairs and plotted their $P$ values against their synaptic weights (Fig. 8B). In accordance with our experimental data (Figs 2C and 3C), significant IpST was evident at synapses stronger than about 1 nS. We next computed the mutual information between presynaptic and postsynaptic spike trains. The amount of information carried by individual synapses increased with synaptic potency (Fig. 8C); the Gini coefficient was 0.83 in the Lorenz curve, and the strongest 10% synapses accounted for about 77% of the total network information (Fig. 8C inset).

Consistent with the existence of significantly reliable IpST, we often observed sequences of spikes that appeared repetitively across multiple neurons (Fig. 8D). We defined a precise firing sequence (PFS) as a chain of spikes of at least 3 different excitatory neurons with fixed delays that repeated at least 3 times (10-ms jitters allowed). PFSs seemed to occur through specific circuit pathways defined by strong synapses; for example, the weights of the 3 synapses responsible for 4-spike PFSs in Figure 8D are shown by arrows in Figure 8B. The total number of PFSs in a 1-min data set obtained from 20 networks that were executed with different random seeds was compared with the number found in surrogates in which spikes were randomly exchanged among neurons (Ikegaya et al. 2004; Sasaki et al. 2007). For each data set, 100 surrogates were generated. In all 20 data sets, the PFSs found in the original raster plot were more frequent than the chance level estimated for the surrogates (Fig. 8E).

**Responsiveness to Small Perturbations**

The existence of IpST suggests that the network can respond to even a single spike from a single neuron. We examined whether the network activity patterns changed after the addition of an extraspoke. In a representative raster plot (Fig. 9A), an extraspoke (arrow) was found to cause a different spiking pattern compared with the nonperturbed control. The effect of the extraspoke was rapidly propagated through the entire network, resulting in bifurcation of the principal component orbit between control and extraspoke-added patterns (Fig. 9B; bin = 50 ms).

We quantified this rapid effect of the extraspoke by computing the Euclidian distance between 2 activity vectors in the original and extraspoke-added raster plots (bin = 5 ms). The Euclidian distance increased immediately after the spike addition and stabilized within 100 ms (Fig. 9C). We repeated the same extraspoke simulation for all excitatory neurons at various timings of network activity. In all >20 000 trials, single spikes induced rapid and persistent increases in the Euclidian distance. The average responses of all 4000 excitatory neurons demonstrate that the network is sensitive enough to largely change its spiking pattern in response to a small external perturbation (Fig. 9D); note that the mean maximal Euclidian distance was about 5, due to the bin size for the Euclidian distance and the network activity frequency.

**Macroscopic Stability Against Perturbations**

In the above extraspoke simulations, we noticed that the level of the entire network activity was maintained, even though the spiking pattern was fully changed after the spike addition. To examine the stability of the self-sustained activity, we applied stronger perturbations by adding or removing spikes for multiple neurons. The altered levels of network activity were found to spontaneously revert to the steady state of infrequent and asynchronous spiking (Fig. 10A).

We thus sought to elucidate the robustness of the network activity more comprehensively. We forced the network activity pattern into various configurations and monitored its spontaneous recovery. This network state was defined by the mean firing rate and the asynchrony index (Fig. 10B). The state-space
analysis revealed that the network state was robust against a wide range of external perturbations; the altered network states were drawn into a stable point (attractor) in which the firing rate and the asynchronous index were about 0.3 and >0.9 Hz, respectively. We also found that a network given extremely synchronized spikes was attracted into another “epileptiform” stable point in which the mean firing rate was about 30 Hz. Thus, the state space contained a boundary separating 2 stable points toward which the network was eventually attracted (Fig. 10B, green line).

**Discussion**

We demonstrated that a recurrent network with a long-tailed distribution of synaptic weights exhibited stability and flexibility of activity patterns, both of which are important for brain function. The sparseness and sensitivity of this network activity are in good accordance with recent experimental evidence showing sparse cortical activity in vivo (Margrie et al. 2002; Ecker et al. 2010; O’Connor et al. 2010; Renart et al. 2010; Minamisawa et al. 2011) and high responsiveness to single PCs (Miles and Wong 1983; Brecht et al. 2004; Fujisawa et al. 2006; Houweling and Brecht 2008; Li et al. 2009).

**Long-Tailed Distribution of Synaptic Weights**

A computational study has shown that spontaneous self-organization of a recurrent neural network through spike timing-dependent plasticity leads to many weak synapses (median synaptic potential amplitude <0.1 mV) and a few synapses that produce >5 mV depolarization (Izhikevich et al. 2004). Moreover, the lognormal distribution of spine sizes is reported to emerge spontaneously when the magnitude of the changes in spine size during synaptic plasticity is proportional to the size of the spine (Loewenstein et al. 2011). Therefore, the long-tailed distribution of synaptic weights is likely a natural consequence of neuronal networks with synaptic plasticity. Consistent with this notion, the long-tailed distribution prevails among synapses in various brain regions that exhibit synaptic plasticity, including the neocortex (Feldmeyer et al. 1999; Song et al. 2005; Lefort et al. 2009), hippocampus (Sayer et al. 1990), and cerebellum (Brunel et al. 2004).

To obtain the uEPSP distribution, we used slice culture preparations rather than acute slice preparations because of the high probability of finding synaptically connected pairs (Takahashi et al. 2010; Sasaki et al. 2011). In acute slices in which more than 80% of the excitatory axons seem to be amputated during slicing (Stepanyants et al. 2009), not only the connection probability but also the true synaptic weights may...
be underestimated because of partial axon lesions; note that synaptic contacts between a single PC pair are often multiple and distributed over postsynaptic dendrites (Markram et al. 1997) and may be partially removed by slicing. Nonetheless, it is noteworthy that the uEPSPG potency conformed to a long-tailed distribution with an upper bound of roughly 2 nS in both acute slices (Song et al. 2005; Lefort et al. 2009) and our slice cultures. Therefore, we believe that the IpST seen in our

Figure 9. Single spike-induced alternation in the internal state of network activity: simulation. (A) Comparison of spontaneous spiking activity between control conditions (top) and conditions under which one extraspike (red) was added at time 0 (bottom). Blue dots indicate spikes different from the control (i.e., perturbed spikes). (B) Time evolution in the 3 principal components (PCs) of network activity. Single-spike addition (red arrow) differentiates trajectories between control (black) and extraspike (blue). A period of 800 ms is shown. (C) Euclidean distance between spike patterns under conditions with and without an extraspike. Bin = 5 ms. The arrow indicates the time of spike addition. (D) Extraspike addition was repeated for all 4000 excitatory neurons, and the mean ± SD of the Euclidean distance was plotted as a function of time.

Figure 10. Stability of recurrent network activity: simulation. (A) Representative responses of excitatory neurons to spike addition (top, stimulation) and spike removal (left, suppression). Spikes were added or removed so that the mean firing rate became 3 or 0.03 Hz for a period of 250 ms. (B) Responses of the network to various levels of 250-ms perturbations (stimulation or suppression) were evaluated in light of the mean firing rate and the asynchrony index. The parameters at a given time were calculated in a time window of 250 ms. The average response changes across 500 trials are indicated by arrows. Arrows are not shown at data points where the network behavior varied among trials with CV of the arrow angle > 0.5.
Interpyramid Spike Transmission (IpST)

In contrast to the theoretical demand of the conventional spike propagation model, the spiking of cortical neurons in vivo is highly asynchronous (Ecker et al. 2010; Renart et al. 2010) and infrequent (Margrie et al. 2002; O’Connor et al. 2010; Minamisawa et al. 2011). Our model study showed that a network provided with large EPSGs can maintain a low network activity level, which implies a critical role for IpST in vivo-like network activity.

IpST-like phenomena have been suggested by several previous studies. First, in vivo whole-cell patch-clamp studies have demonstrated that the addition of a single extraspike to ongoing activity in a rat barrel cortex induces a transient increase in the firing rates of nearby neurons, which is a sign that a single spike in a PC is propagated to the surrounding network (London et al. 2010). Second, studies using behaving animals have indicated that a small but significant fraction of putative PC pairs exhibits a sharp correlogram peak with a monosynaptic delay (Ts’o et al. 1986; Fujisawa et al. 2008; Quilichini et al. 2010; Takeuchi et al. 2011).

In vitro studies have also suggested the presence of IpST. For example, background synaptic activity enhances the probability that subthreshold inputs elicit spikes (Stacey and Durand 2001; Destexhe et al. 2003; Shu et al. 2003). These studies, however, did not consider the long-tailed uEPSG distribution, and it was unclear whether IpST occurs specifically in a few selected synaptic pathways. We found that IpST was only significant at synapses with amplitudes of more than about 1 nS. Such strong connections composed a very small portion of the total connections. Therefore, in a web of weak PC networks, a few strong connections define stable trajectories through which the majority of neural information flows. Given that a single PC innervates thousands of other PCs in vivo, each PC is expected to have tens of strongly connected postsynaptic targets, which may underlie the unexpectedly potent influence of single PC spiking. In this scheme, weak synapses are thought to provide background noise that modulates the efficiency of IpST by changing the overall activity level and thereby define the network state. Another possibility is that these synapses could offer neuronal networks opportunities for learning and memory because weaker synapses exhibit more capacity for synaptic plasticity (Bi and Poo 1998; Matsuzaki et al. 2004).

The PFSs were rich in spontaneous activity. Because PFSs are likely mediated by IpST, they are not necessarily accompanied by synchronized network activity. Therefore, our PFSs are different from PFSs found in synfire chains, which are theoretically predicted to occur through a feedforward excitatory network (Abeles 1991; Diesmann et al. 1999).

Finally, we speculate that IpST could settle the long-standing debate regarding the dichotomy between 2 neural code hypotheses: the temporal code and the rate code. Large uEPSGs act as “instructive” inputs, and the resultant IpST has a high degree of spike fidelity that favors temporal coding. Small uEPSGs provide “ambient” inputs, and their mass activity level gates IpST, which favors rate coding. Therefore, the temporal and rate code hypotheses may be different perspectives on the same phenomenon.

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Notes

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References