Quantifying the Magnitude of Changes in Synaptic Level Parameters With Long-Term Potentiation

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Long-term potentiation (LTP), an experimentally induced increase in synaptic strength, is our best model for how learning and memory work, and consequently, has been widely studied (Bliss and Collingridge 1993; Malenka and Nicoll 1999). Experimental evidence supports a number of mechanisms for the synaptic change that occurs with long-term potentiation (LTP) including insertion of AMPA receptors, an increase in AMPA receptor single channel conductance, unmasking silent synapses, and increases in vesicle release probability. Here we combine experimental and modeling studies to quantify the magnitude of the change needed at the synaptic level to explain LTP with these proposed mechanisms. Whole cell patch recordings were used to measure excitatory postsynaptic potential (EPSP) amplitude in response to minimal afferent stimulation before and after LTP induction in CA1 pyramidal cells. Detailed neuron and synapse level models were constructed to estimate quantitatively the changes needed to explain the experimental results. For cells in normal artificial cerebrospinal fluid (ACSF), we found a 60% average increase in EPSP amplitude with LTP. This was explained in the models by a 63% increase in the number of activated synapses, a 64% increase in the AMPA receptor single channel conductance, or a 73% increase in the number of AMPA receptors per potentiated synapse. When the percentage LTP was above the average, the required increases through the proposed mechanisms became nonlinear, particularly for increases in the number of receptors. Given constraints from other experimental studies, our quantification suggests that neither unmasking silent synapses nor increasing the numbers of AMPA receptors at synapses is sufficient to explain the magnitude of LTP we observed, but increasing AMPA single channel conductance or vesicle release probability can be sufficient. Our results are most compatible with a combination of mechanisms producing LTP.

INTRODUCTION

The magnitude of potentiation with LTP is often quantified over multiple cells from field potentials measured with extracellular electrodes, but this potentiation represents an average over many synapses in many cells. LTP has also been studied in individual cells where extracellular stimulation activates many synapses (Gustafsson et al. 1987; Sastry et al. 1986). The average potentiation measured with these methods is typically 50–100%. In other studies, minimal stimulation techniques have been used in an attempt to assess potentiation at individual synapses. Friedlander et al. (1990) report that unitary excitatory postsynaptic potentials (EPSPs) were rarely potentiated, whereas compound EPSPs could be potentiated 59–84% on average. Debanne et al. (1999) found that, while multiunitary EPSPs were potentiated 75% on average, LTP of unitary EPSPs was quite heterogeneous; some synapses could not be potentiated (Petersen et al. 1998), whereas others could be potentiated >10-fold (mean of 250%). Similarly, Isaac et al. (1996) report individual synapses were potentiated by 145–212%.

These average LTP percentages coupled with synapse LTP heterogeneity do not reveal what actually happens quantitatively at individual synapses. If LTP causes the addition of AMPA receptor channels, how many channels per activated synapse would have to be added to get the observed potentiation? If AMPA single channel conductance increases, how many channels per synapse would have to exhibit a change of a certain magnitude to manifest the observed potentiation? How many “silent” synapses would have to be unmasked or new connections formed? Here we combine experimental and modeling studies to address two specific issues. First, we seek to answer the above questions quantitatively to see if the required numbers make sense physiologically and to see if any of these mechanisms is sufficient by itself to explain LTP. Second, we address how closely the potentiation percentage explains the magnitude of actual changes at the synaptic level given inherent nonlinearities in synaptic and neuron level properties.

METHODS

Experimental procedures

SLICE PREPARATION AND MAINTENANCE. Sprague-Dawley rats (30–60 days old) were sedated by inhalation of a CO2/air mixture and decapitated. The brain was removed, placed into chilled artificial
cerebrospinal fluid (ACSF; equilibrated with 95% O₂, 5% CO₂),
trimmed with a razor blade, glued to the stage of a vibratome, and
sectioned into 400-μm-thick slices. After sectioning, brain slices were
dissected further to free the hippocampus from surrounding structures.
To prevent spontaneous bursting in the presence of the GABA-A
receptor antagonist bicuculline, area CA3 was removed by a small cut.
Hippocampal slices were stored in a holding chamber at the
interface of ACSF and humidified 95% O₂, 5% CO₂ at room temper-
ature. Individual slices were transferred, as needed, to a small volume
(≈200 μl) interface recording chamber (35°C) where they were
perfused at 1–1.5 ml/min with ACSF. The upper surfaces of the slices
were exposed to warmed, humidified atmosphere consisting of 95% O₂, 5% CO₂.

**ELECTROPHYSIOLOGY.** Intracellular recordings were made using the “blind” whole cell recording technique originally described by Blanton et al. (1989; also see Grover 1998; Grover and Chen 1999). The pipette solution was composed of 140 mM potassium gluconate, 10 mM Na-HEPES, and 3 mM MgCl₂, pH 7.2, 280–290 mOsm. Potentials were recorded with an Axoclamp 2B (Axon Instruments). Membrane potentials were low-pass filtered at 1 kHz and signals were fed through an Ithaco 4302 dual filter, where they were amplified (10–100×), filtered (DC–3 kHz), digitized at 7–40 kHz, and stored on a personal computer. Access resistance (compensated using the Axoclamp bridge circuit) and membrane resis-
tance were monitored throughout the recordings. Recordings were
terminated if large or abrupt changes in access or membrane resistance occurred.

Recordings of voltage at the soma were made in response to four
types of stimuli: brief (20 ms) and long (200 ms) −50 pA current
injection through the recording electrode and near minimal stim-
ulation (0.1 Hz) through two stimulating electrodes placed in
mid-stratum radiatum on opposite sides of the recording site.
Bipolar stimulating electrodes were constructed of teflon-insulated stainless steel, and constant voltage stimulation was used (0.1-ms duration, 0.6–7.0 V). One stimulating electrode was used to deliver
low frequency test stimuli and high-frequency tetanic stimulation for LTP induction, and the second electrode was used to deliver test stimuli only to a control pathway. Synaptic stimulation was interleaved with intracellular current stimulation (−50 pA, 20 or 200 ms). Stimulation (synaptic and intracellular) was
delivered every 5 s, so that synaptic stimulation was given once
every 10 s, and intracellular current stimulation was also given
once every 10 s. Recordings were made in the presence of 10 μM
bicuculline to block fast, GABA-A receptor–mediated inhibition.
Test stimulus intensity was initially adjusted so that failures were
observed on some trials. Synaptic potentials were recorded at the
initial resting membrane potential of the cell; any systematic drift
in membrane potential was compensated by DC current injection.
Test stimulation was delivered until a minimum 5-min period of
stable recording was achieved. Responses for each of the four
types of stimuli were averaged over a 5-min period. All membrane potentials were corrected for the calculated liquid junction poten-
tial (−10 mV).

**LTP INDUCTION AND MEASUREMENT.** After achieving a stable
baseline recording, LTP induction was attempted. Afferent stim-
ulation was increased to 25 Hz for 1 s. During LTP induction, each
afferent stimulus was paired with a 1.5-nA, 5-ms current injected
through the whole cell pipette. This protocol resulted in LTP in
>85% of the cells recorded. The same stimulation protocol was
used before and after LTP induction. Responses to short and long
current pulses at the soma and to EPSPs evoked in tetanized and
control pathways were monitored during the posttetanus period for
as long as a stable recording could be maintained. Posttetanus
recording durations ranged from 10 to 30 min or more, over which
time LTP magnitude was typically stable. Potentials were collected
and initially analyzed using the WinWCP software (John Demp-
ster, University of Strathclyde). Further analysis was accomplished
using Origin (OriginLab) and custom software written in the
Python programming language.

**PHARMACOLOGICAL INHIBITION OF Ih.** To eliminate possible ef-
teffects of the hyperpolarization activated cation current I_h (Maccach-
neri et al. 1993) on EPSP amplitude and kinetics, some cells were recorded in the presence of 25–50 μM ZD-7288 (Gasparini and
DiFrancesco 1997; Harris and Constanti 1995). Our intention in
these recordings was to determine if the parameter fitting proce-
sure described below would be easier without this current. In fact,
with I_h blocked, the parameter fitting procedure found excellent fits
to the data very quickly.

**CELL SELECTION CRITERIA.** Cells were accepted for analysis if their
resting membrane potentials were at least −60 mV (after correction
for the liquid junction potential), and their input resistance estimated
from the voltage change at the end of the 200-ms current pulses was
≈±30 MΩ in standard ACSF or ±60 MΩ in ZD-7288 with no large or
abrupt changes during the recording periods before and after LTP
induction. Some cells that satisfied these criteria were rejected later if,
after LTP induction, EPSPs were increased in the control pathway as
well as in the tetanized pathway. The LTP in these latter cells tended
to be larger than in cells where there was no change in EPSP size in
the control pathway, suggesting instability in the slice leading to
nonspecific “run-up” of EPSPs. Finally, cells were excluded if pop-
ulation spikes were observed during EPSPs, which occurred occasion-
ally during posttetanus recording, because this made model calibration
impossible. The criteria were satisfied in eight cells in normal ACSF
and six cells exposed to ZD-7288.

**Modeling procedures**

**CELL RECONSTRUCTIONS.** Models were constructed from recon-
struction data for four CA1 pyramidal cells obtained from public
databases. Specifically, we used cells n123 and n400 from the Duke/
Southampton Archive of Neuronal Morphology (Cannon et al. 1998;
Pyapalli et al. 1998) at http://www.neuro.soton.ac.uk/~jchad/cellAr-
chive/cellArchive.html, cell pc2a from Attila Gulyás’ website
htm, and cell c20465 from the study of Ishizuka et al. (1995)
(morphology obtained directly from the author). Cell n123 was used
in models by Poirazi et al. (2003), and cell n400 was used by Migliore
et al. (1999). CA1 pyramidal cells have been shown to be electrotoni-
cally similar (Mainen et al. 1996), but we used multiple cells to take
into account some of the heterogeneity among cells and their recon-
structions. Cells are pictured in Fig. 1.

**DENDRITIC SPINES.** Dendritic spines were added to these cells in
distributions and numbers consistent with those reported by Megías et
al. (2001). In the models, only a subset of spines, the ones where
synaptic inputs were activated, were modeled explicitly. Other spines
were modeled implicitly by reducing R_m and increasing C_m on each
dendritic segment according to the proportion of area taken up by
spines (Rall et al. 1992). These implicitly modeled spines were
assumed to have a membrane area of 1.0 μm² each, the approximate
average in data from Harris et al. (1992). Three spine shapes (stubby,
mushroom-shaped, and long-thin) were modeled explicitly in the
simulations with dimensions and relative proportions of the three
shapes based on data from Harris et al. (1992). Specifically, spines
were modeled as cylinders with diameter × length measurements as
follows: stubby spines were 0.32 × 0.44 μm, mushroom-shaped

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spines had necks of 0.2 × 0.43 μm and heads of 0.45 × 1.7 μm, and long-thin spines had necks of 0.1 × 0.7 μm and heads of 0.3 × 0.43 μm. The number of spines modeled on each cell was ~30,000 (Megias et al. 2001).

NEURON LEVEL MODELING AND FITTING PROCEDURES. Simulations were done with the NEURON simulator (Hines and Carnevale 1997). NEURON’s multiple run fitter was used to fit parameter values simultaneously to three types of recorded experimental data (20-ms, −50 pA current pulse; 200-ms, −50 pA current pulse; EPSP stimulation data). The error function was the default function in the multiple run fitter, the square norm between data points and simulation results. Differences in parameter scale were handled by using the multiple run fitter option to use the logarithms of the parameter values in the fits. These details are discussed in the APPENDIX of Shen et al. (1999).

Parameter values were fit for each set of experimental data with models that used each of the morphological cells shown in Fig. 1. Each fit was repeated with different starting values to see if a better fit could be obtained. Fits were done separately for data obtained before and after LTP induction. Generally fits were excellent, although in a few cases, there were problems fitting the EPSP time-course. Sometimes the best fits to the EPSP data numerically were not the best fits visually; for example, the best numerical fit sometimes had a larger or smaller peak EPSP amplitude than the data to fit the decay phase more accurately. In these cases, we restricted the fitting domain to make the fitting procedure concentrate on the more relevant parts of the EPSP or else we took the more visually appealing fit even though the error was slightly larger.

For recordings made in the presence of ZD-7288 to block I_h, model parameters fitted to the data were axial resistivity, R_a, membrane resistivity, R_m, specific membrane capacitance, C_m, and number of synapses (for pre-LTP EPSP data; also for post-LTP EPSP data as appropriate). For recordings of cells in normal ACSF, two types of fits were done that differed in regard to whether the leak conductance was uniform or nonuniform. The parameters fitted were R_m, C_m, number of synapses, and a G_h scaling factor (H-factor) described below.

Cells in normal ACSF were assumed to have an I_h whose maximum conductance density, G_h, increased linearly with distance from the soma (Hoffman et al. 1997; Magee 1998) to the distal end of the s. radius and was constant at more distal locations. The starting value for G_h before scaling by the H-factor was 0.22 mS/cm² at the soma, rising to 1.33 mS/cm² at distal locations. Equations for I_h kinetics were taken from Vasilyev and Barish (2002) and are repeated here

\[ I_h = G_h (V - E_h) \]  

where \( G_h = G_{h0} q_f + G_{h0} q_s \), and \( G_{h0} \) and \( G_{h0} \) are the maximum conductances of the fast and slow components [fast (80%) and slow (20%) of \( G_h \)].

\[ q_f = q_s = 1/[1 + \exp((V + 92)/10.4)] \]

\[ \tau_f = 7.2 \exp((V + 124)/22.5), \text{ for } V \leq -87 mV \]

\[ = 27 + 24 \exp(-(V + 85)/20), \text{ for } V < -87 mV \]

\[ \tau_s = 177 + 67 \exp((V + 159)/35.5), \text{ for } V \leq -83 mV \]

\[ = 267 + 542 \exp(-(V + 85)/20), \text{ for } V > -83 mV \]

We note that the experimental data from Vasilyev and Barish (2002) were recorded at room temperature. However, their equations for \( \tau_s \) given above produce values that are 3–4 times faster than their reported experimental values; the resulting values are similar to those reported by Magee (1998) at 33°C. The reversal potential for I_h was assumed to be −30 mV. Because voltages in the simulations remained in a subthreshold range, we did not add additional voltage-dependent conductances.

Passive conductances were modeled in two different ways. First, (nonuniform leak conductance case) a uniformly distributed passive leak conductance (R_m) reversing at the resting potential was modeled together with a separate passive K-leak current whose density increased with distance from the soma. The K-leak conductance density, \( G_{Kleak} \), was chosen to make \( I_{Kleak} \) balance \( I_h \) in all of the compartments at the resting potential so that there was no net current flow at rest. The reversal potential for \( I_{Kleak} \) was −90 mV. Fits began with initial \( G_h \) and \( G_{Kleak} \) values assigned and computed as just described, and these conductance densities were scaled by a parameter called H-factor in the fitting procedure. This scaling preserved the property of a stable uniform resting potential in all compartments. In a second set of simulations (uniform leak conductance case), a single passive leak conductance (R_m) was uniformly distributed, but the reversal potential was chosen to be between the resting potential and the potassium reversal potential. Instead of fitting the passive conductance

\[ I_h = G_h (V - E_h) \]
reversal potential, \( e_{\text{pas}} \), directly, we initially tried fitting an L-factor (values between 0 and 1) where \( e_{\text{pas}} \) is defined by

\[
e_{\text{pas}} = V_{\text{rest}} + L\text{-factor}(90 - V_{\text{rest}})
\]

and \( V_{\text{rest}} \) is the soma resting potential. For a given L-factor, \( R_m \) and H-factor were constrained by the resting potential and input resistance, and we used this fact to obtain good starting values for these parameters in the fits. However, we found that fitting both \( R_m \) and \( e_{\text{pas}} \) (L-factor) at the same time was problematic; the fitting procedure could not always escape local minima. This was not surprising given that the passive current depends on both \( R_m \) and the reversal potential; fitting both together is likely to be a problem regardless of the fitting method. To circumvent this problem, we fixed L-factor and ran the fits for all four morphologies for a given experiment. We then varied L-factor to find the L-factor value that provided the minimum error for at least three of the four morphologies, and this value was used in the four morphological models. The precision of the L-factor values evaluated enabled \( e_{\text{pas}} \) to be fixed within 0.5 mV. Fits were still quite good with variations in \( e_{\text{pas}} \) of 1–2 mV.

SYNAPtic inputs. Synaptic input locations were chosen from among the synapses explicitly modeled at locations shown in Fig. 1. Synapses were randomly assigned to be long-thin, mushroom-shaped, or stubby according to the proportions given by Harris et al. (1992). The number of open AMPA and N-methyl-D-aspartate (NMDA) receptor channels at each activated synapse as a function of time was precomputed with a synapse level model and read into NEURON from a file. The voltage at individual synapses computed with the neuron level model determined the proportion of open NMDA receptor channels at each synapse that were not blocked by magnesium. Magnesium block kinetics was taken from Ascher and Nowak (1988).

Because this method of modeling synaptic inputs slowed the multiple run fitter considerably (the variable step method could not be used), we used the more traditional two exponential approximation to represent the synaptic conductance in the multiple run fitter. Parameter values for this approximation were obtained from fits to the time-course of the number of open AMPA and NMDA receptor channels computed with the synapse level model. In this representation, the maximum synaptic conductance, \( g_{\text{max}} \), was 187, 496, and 243 pS for the AMPA conductance and 60, 99, and 76.5 pS for the NMDA conductance at long-thin, mushroom-shaped, and stubby spines, respectively. The two time constants were 0.23 and 1.75, 0.34 and 1.65, and 0.24 and 1.75 ms for the AMPA conductance at long-thin, mushroom-shaped, and stubby spines, respectively, and were 3.5 and 40 ms for the NMDA conductance at all spines.

In fits to experimental data, we guessed at the number of activated synapses, but then let the fitting procedure scale the \( g_{\text{max}} \) values at the activated synapses. This scaling factor was used to update our estimated number of activated synapses, and the fits were rerun. This process was repeated iteratively until the fit returned a scaling factor very close to 1.0. We fixed the scaling factor at 1.0 and ran the fit once more. Synapse locations were chosen from a list of synapses by choosing a starting location in the list and selecting every second or third synapse in the list until the desired number was selected. Finally, we went back to using the actual precomputed numbers of open AMPA and NMDA receptor channels at each synapse for final fits. Because the peak of the soma EPSPs differed slightly when precomputed numbers of receptors were modeled instead of the two exponential approximations of the conductances used in the multiple run fitter, we had to modify the number of activated synapses and/or the starting location in our synapse list to match the EPSP peaks better. These modifications were small, and because a high degree of overlap of actual activated synapses was preserved, the EPSP shape did not change noticeably.

SYNAPSE LEVEL MODELING PROCEDURE. The synapse level model was a deterministic model previously described (Holmes 1995), in which a vesicle of 2,000 molecules of glutamate is released into the synaptic cleft, glutamate diffuses in the cleft, binds to AMPA and NMDA receptors on the postsynaptic membrane, and is removed from the cleft by diffusion and uptake mechanisms. AMPA receptor binding kinetics came from Jonas et al. (1993), with rate constants increased 1.5 times to compensate for the higher temperature of our experiments, whereas the NMDA receptor kinetics was based on results from Sather et al. (1992). We presented these kinetic schemes in previous models (Holmes and Levy 1997).

Within the postsynaptic density (PSD), the density of AMPA receptors was 1,000/\( \mu \text{m}^2 \), and the density of NMDA receptors was 300/\( \mu \text{m}^2 \). Taking average PSD areas from Harris et al. (1992) and assuming that PSDs are circular, the PSD radius was 0.125, 0.15, and 0.3 \( \mu \text{m} \) for long-thin, stubby, and mushroom-shaped spines, respectively. With these densities and PSD areas, the numbers of AMPA and NMDA receptors were 49 and 15 at long-thin spines, 71 and 21 at stubby spines, and 283 and 85 at mushroom-shaped spines. These numbers are consistent with estimates from Jonas et al. (1993) for CA3 synapses. Single channel conductance was 8 pS for AMPA receptor channels and 50 pS for NMDA receptor channels. With these parameters, maximum AMPA conductance was 187, 243, and 496 pS for long-thin, stubby, and mushroom-shaped spines, respectively, as mentioned above. Unitary EPSPs at the soma computed with our models were consistent with the range (30–665 \( \mu \text{V} \)) found by Sayer et al. (1990) and ranges (84–197 and 66–275 \( \mu \text{V} \)) reported by Larkman et al. (1991, 1997).

FITTING POST-LTP EXPERIMENTAL RESULTS WITH MODELS. Post-LTP experimental data were fit with models in three different ways. First, parameter values for \( R_m \), \( R_o \), \( C_m \), and H-factor, along with the number of synapses activated, were simultaneously fit to short and long current pulse data and EPSP data, as described above for pre-LTP data. Every synapse included in the pre-LTP fits was included in the post-LTP fits with additional synapses added to fit the post-LTP EPSP. Above we mentioned that, for pre-LTP fits, we chose an arbitrary starting location in our list of synapses and took every second or third synapse in the list until we had the necessary number for the fit. For post-LTP fits, additional synapses were added by going up in the list from the last pre-LTP synapse selected and also down in the list from the first pre-LTP synapse selected, selecting every second or third synapse in the list until we had the necessary number for the fit. Second, given the parameter values for \( R_m \), \( R_o \), \( C_m \), and H-factor computed for the post-LTP data, the number and location of activated synapses were fixed to those in the best pre-LTP fits. Then the AMPA single channel conductance was increased for all channels at all activated synapses to determine the value (within 0.1 pS) that provided the best fit to the peak of the post-LTP EPSP. Third, synapse level models computed new numbers of open AMPA and NMDA receptor channels for synapses at the three types of spines for 5–400% increases in receptor density (in 5% increments) while keeping PSD radius constant. Given the post-LTP parameter values, the pre-LTP synapse number and locations, the fit with increased receptor density that best fit the peak of the post-LTP EPSP was found. Because density increases were computed in 5% increments, there were some cases when one density gave an EPSP that was too small and the next gave an EPSP that was too big. In these cases, the percentage increase in receptor density chosen to match the post-LTP EPSP was estimated by interpolation.

GABA_B input. We showed previously that the hyperpolarization that followed minimal synaptic stimulation was caused by a GABA_B current (Holmes et al. 2006), so we added a GABA_B current at the soma to our synaptic simulations. The functional form was

\[
I_{\text{GABA_B}}(t) = G_{\text{GABA_B}} \text{amp} [1 - \exp(-t/45.2)]^2 \left[0.84 \exp(-t/110.2)
+ 0.16 \exp(-t/516.2)\right]
\]
where GABA\textsubscript{A}-amp is a scaling factor that was fit in the simulations (but does not represent the maximum amplitude of the current). This functional form and parameter values are taken directly from Otis et al. (1993) for data recorded from rat hippocampal slices at 34–35°C. We must emphasize that including this current in the model did not affect other fitted parameter value estimates or estimates of changes with LTP. However, it substantially improved fits to the late afterhyperpolarization after EPSPs.

**RESULTS**

We first briefly outline the sequence of experimental and computational results. Whole cell patch recordings of CA1 pyramidal cells were done to measure the voltage responses to brief (20 ms) and long (200 ms) hyperpolarizing current injection and to a test EPSP evoked by near minimal afferent stimulation before and after LTP was induced in this test input. In some cells, we applied the H channel blocker ZD-7288 to linearize the response and make the subsequent model analysis easier. Characteristics of the cells before and after LTP induction were compared. Morphological reconstructions of four different cells provided the CA1 pyramidal cell structure for use in models. Parameter values were fit in each morphological model to match the data from each experimental cell to ensure that the modeled cells and the experimental cells were similar electrotonically. Fits were done both before and after LTP induction. The characteristics of EPSPs obtained by these models were verified to be similar to typical EPSPs reported in the literature. Fits were done both before and after LTP induction. However, it substantially improved fits to the late afterhyperpolarization after EPSPs.

**Characteristics of cells studied**

Characteristics of cells studied experimentally are shown in Table 1. Resting potential was similar both before and after LTP induction averaging −70.1 mV before and −70.2 mV after LTP induction in cells in normal ACSF and −86.3 mV before and −85.6 mV after LTP in cells treated with ZD-7288.

For cells in ACSF, measuring input resistance is problematic because the act of measurement (i.e., by hyperpolarizing pulses) changes input resistance as evidenced by voltage sag. Consequently, we used two measures of input resistance: one based on the maximum voltage displacement and the other based on the steady-state voltage displacement (the traditional measure). Average input resistance was slightly higher after LTP induction, rising from 74.7 to 78.8 MΩ (maximum displacement) or from 66.7 to 71.7 MΩ (steady-state displacement) in cells in normal ACSF and from 128.9 MΩ to 137.1 MΩ in cells exposed to ZD-7288. These differences between pre-LTP and post-LTP measurements were not statistically significant in either cell group. Cells exposed to ZD-7288 responded passively to the hyperpolarizing current pulse with no voltage sag, but the voltage did not always reach a steady state by the end of the 200-ms current pulse. Because input resistance was estimated from the voltage displacement at this point, our values underestimate the actual input resistance.

We did not detect any change in EPSP kinetics after LTP induction in cells in normal ACSF. The time to the peak of the EPSP at the soma averaged 11.4 ms before and 11.3 ms after LTP induction and a scaled pre-LTP EPSP usually matched the post-LTP EPSP time-course well. However, if there was a change in EPSP kinetics, it would have been very difficult to detect. If more synapses contributed to the EPSP after LTP, these might increase or reduce the time to peak observed at the soma depending on whether the added synapses were more distal or more proximal to those activated before LTP induction.

**Table 1. Characteristics of experimental cells**

<table>
<thead>
<tr>
<th>Cell</th>
<th>( V_{m, rest} ) mV</th>
<th>( R_N ) peak/SS MΩ</th>
<th>EPSP-amp, mV</th>
<th>EPSP-tp, ms</th>
<th>( V_{m, rest} ) mV</th>
<th>( R_N ) peak/SS MΩ</th>
<th>EPSP-amp, mV</th>
<th>EPSP-tp, ms</th>
<th>LTP, %</th>
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<td>60.0/53.0</td>
<td>0.76</td>
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<td>54.4/48.6</td>
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<td>73.6/65.2</td>
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<td>1.09</td>
<td>12.7</td>
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<td>082504b</td>
<td>−83.8</td>
<td>77.8/76.4</td>
<td>1.02</td>
<td>6.4</td>
<td>−83.6</td>
<td>84.2/83.2</td>
<td>1.83</td>
<td>8.3</td>
<td>79</td>
</tr>
<tr>
<td>082504c</td>
<td>−73.7</td>
<td>102.5/99.4</td>
<td>1.98</td>
<td>11.9</td>
<td>−73.9</td>
<td>112.3/110</td>
<td>3.12</td>
<td>13.7</td>
<td>57</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>−70.1</td>
<td>74.7/76.7</td>
<td>0.87</td>
<td>11.4</td>
<td>−70.2</td>
<td>78.8/71.7</td>
<td>1.17</td>
<td>13.3</td>
<td>60</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>6.7</td>
<td>15.6/17.5</td>
<td>0.52</td>
<td>2.8</td>
<td>6.6</td>
<td>19/21</td>
<td>0.81</td>
<td>1.9</td>
<td>24</td>
</tr>
</tbody>
</table>

| **ZD-7288 cells** | | | | | | | | | |
| 080304d | −84.1 | 133.0 | 1.46 | 11.4 | −83.0 | 133.2 | 1.67 | 12.8 | 14 |
| 080504c | −87.0 | 122.6 | 1.66 | 10.4 | −86.2 | 136.2 | 2.51 | 10.9 | 51 |
| 081704b | −90.2 | 113.4 | 1.29 | 10.4 | −89.1 | 136.2 | 8.18 | 13.9 | 534 |
| 081804a | −81.0 | 146.2 | 1.96 | 12.7 | −80.5 | 142.1 | 2.89 | 13.7 | 47 |
| 081804c | −85.7 | 110.9 | 1.79 | 15.4 | −84.7 | 125.8 | 3.82 | 17.5 | 113 |
| 082004b | −89.6 | 147.2 | 3.03 | 13.4 | −89.9 | 148.8 | 7.35 | 14.3 | 143 |
| **Mean** | −86.3 | 128.9 | 1.87 | 12.3 | −85.6 | 137.1 | 3.65* | 13.8 | 74* |
| **SD** | 3.5 | 15.8 | 0.62 | 2.2 | 3.6 | 7.8 | 2.2 | 2.4 | 53 |

*These means exclude cell 081704b. LTP, long-term potentiation; EPSP, excitatory postsynaptic potential.
Conversely, the time to peak of the soma EPSP increased after LTP for all of the cells treated with ZD-7288. The time to peak was larger in ZD-7288 treated cells than that for the cells in ACSF, as might be expected with a peak was larger in ZD-7288 treated cells than that for the cells after LTP for all of the cells treated with ZD-7288. The time to peak of the EPSP for cell pc2a (and sometimes for cell n400) often had a faster time-course than the experimental data. This occurred because the current pulse data and the EPSP data pulled parameter values in opposite directions, and the best overall fit usually matched the current pulse data better than the EPSP data. Fits were equally good for cells in ACSF whether we assumed that the leak conductance was uniform or nonuniform.

The calibrated parameter values were generally similar for all experimental data fit to a given cell model, but differed considerably among the four cell models as shown in Table 2. This is consistent with the morphological variation among the four cells (see the cell statistics in Fig. 1), as the calibration made the cells electrotonically similar. Fitted parameter values were also similar before and after LTP induction. There was a trend for $R_m$ values to increase and H-channel density to decrease after LTP, which tracks with the slight increase in input resistance in the experimental cells noted above.

**Characteristics of the synapses used in the models**

After the fits were done, we used the fitted parameter values for each set of experimental data for each cell model to calculate the size and time-course of unitary EPSPs for each synapse activated in our simulations. Our purpose was simply to verify that unitary EPSP amplitudes in the models were similar to those reported from experiments by others. To this end, we lumped results from the four modeled cells in the types of data from each of the 14 experimental cells both before and after LTP induction.

In general, we were able to fit the experimental data quite closely, as shown in Figs. 2 and 3, where the fits with the four morphological models typically overlap each other and the experimental data. The one difficulty we had was that fits to the EPSP for cell pc2a and sometimes for cell n400 often had a faster time-course than the experimental data. This occurred because the current pulse data and the EPSP data pulled parameter values in opposite directions, and the best overall fit usually matched the current pulse data better than the EPSP data. Fits were equally good for cells in ACSF whether we assumed that the leak conductance was uniform or nonuniform.

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**Characteristics of the cell models calibrated with experimental data**

Because the experimental cell data and the detailed morphological data used in the models came from different, although presumably representative, cells, models were calibrated to make the experimental cells and the model cells similar electrophysiologically. Such calibration allowed us to use actual CA1 pyramidal cell structures without having to worry about reconstruction errors or the fact that the experimental cells were different. Because of inherent variability among CA1 pyramidal cells, we did not want to rely on the morphology from a different. Because of inherent variability among CA1 pyramidal cell structures without having to worry about recon-

![Graph showing voltage and time relationships](https://via.placeholder.com/150)

**FIG. 2.** Matching the experimental data with models—normal artificial cerebrospinal fluid (ACSF) cell fits. Black lines are the experimental data and red lines are fits with models. Traces are voltage responses to 20 ms, –50 pA current, 200 ms, –50 pA current, and near minimal stimulation (top to bottom). A: pre-long-term potentiation (LTP) induction. Fitted values for $R_m$, $R_m$, $C_m$, H-factor, no. synapses, and $\text{GABA}_A$-amp were 10, 36.6, 1.02, 2.59, 13, and 5.2 for cell c20465, 22.5, 78.7, 0.49, 0.58, 12, and 4.7 for cell n123, 15.7, 61.5, 0.63, 0.64, 11, and 4.8 for cell n400, and 29.4, 44.1, 0.89, 1.86, 11, and 4.5 for cell pc2a. B: post-LTP. Fitted values for $R_m$, $R_m$, $C_m$, H-factor, no. synapses, and $\text{GABA}_A$-amp were 10.3, 32.6, 1.2, 2.86, 29, and 6.4 for cell c20465, 18.6, 73.9, 0.55, 0.59, 21, and 6.4 for cell n123, 16.1, 54.9, 0.74, 0.7, 20, and 6.4 for cell n400, and 28.9, 39.8, 1.04, 2, 22, and 6.3 for cell pc2a. Experimental cell was cell 071204a. The blue lines in the excitatory postsynaptic potential (EPSP) traces are fits for cells n400 and pc2a.
results summarized in Fig. 4. As might be expected, EPSP size at the soma had a tendency to be bimodal with synapses on long-thin spines and stubby spines producing small amplitude EPSPs and synapses on mushroom-shaped spines producing much larger EPSPs (Fig. 4, A and D). Amplitudes ranged from 29 to 251 μV, with a mean of 82 μV in models of cells in ACSF and 94 μV in models of cells treated with ZD-7288. EPSP amplitudes at the location of the synapse (at spine heads) ranged from 0.2 to 14 mV, with most lying between 0.2 and 4 mV (Fig. 4, B and E).

To test the validity of our assumption that our minimal stimulation paradigm would not cause significant activation of voltage-dependent conductances in the dendrites or relieve the Mg$^{2+}$ block of NMDA receptor channels, we computed the peak spine head voltages in each simulation. We found that voltages in the models at spine heads remained in a subthreshold region at the vast majority of synapses. Here again, we lumped the results from all four modeled cells in the figures. In Fig. 4, C and F, we show the peak spine head voltages at all activated synapses in all post-LTP simulations of cells in ACSF and simulations of cells exposed to ZD-7288 when LTP was modeled as an increase in the number of activated synapses. The most depolarized voltages all came from cells that had the largest experimental EPSPs [cells 082504c (ACSF) and 082004a (ZD-7288)]. Even when the voltage at an individual spine was very depolarized, it remained so only for a few milliseconds before the voltage quickly decayed.

**Quantifying the change in the EPSP after LTP**

Model fits were done to quantify the change in synaptic parameters that may occur in LTP. We quantified the number of additional activated synapses, the increase in the AMPA single channel conductance ($\gamma$), and the increase in the number of receptors needed to explain the increased EPSP amplitude observed with LTP. Results are given in Table 3.

While the mean increase in EPSP amplitude observed experimentally with LTP was 60% in cells in normal ACSF and 73% in cells in ZD-7288, we had to increase the number of activated synapses 63% in ACSF cell models with a uniform leak conductance, 74% in ACSF cell models with a nonuniform leak conductance, and 86% in ZD-7288 cell models to match the post-LTP EPSP. To get these numbers, fits were done as shown in Fig. 2 to the pre-LTP and post-LTP data. While $R_v$, $R_m$, $C_m$, and H-factor values were similar in the pre-LTP and post-LTP fits (Table 2), separate calibrations were necessary to compensate for the small differences that did exist and to provide an accurate estimate of the number of additional synapses activated. The actual number of additional synapses in a given simulation depended on whether the additional synapses were disproportionately on long-thin or mushroom-shaped spines. Because the type of spine synapse was assigned randomly in the list of synapses, spine type proportions were not necessarily the same for synapses activated pre-LTP and post-LTP, and this contributed to the variability in the results.

The increase in AMPA single channel conductance necessary to match the post-LTP EPSP amplitude was 64 and 73% in models of cells in normal ACSF with uniform and nonuniform leak conductance, respectively, and 95% in models of cells exposed to ZD-7288. For these fits, the same synapses were activated in pre-LTP and post-LTP models, with the only change being in $\gamma$, the AMPA receptor single channel conductance.

The increase in the number of AMPA receptors at the synapse needed to match the post-LTP EPSP amplitude was the largest for all of the proposed LTP mechanisms, being 73 and 83% in models of cells in normal ACSF with uniform and
nonuniform leak conductance, respectively, and 118% in models of cells exposed to ZD-7288. Again, the same synapses were activated in the pre-LTP and post-LTP models, but here, only the numbers of receptor channels at the synapses were changed. While the numbers of AMPA and NMDA receptors in these simulations were both increased, the voltages achieved were not sufficient for NMDA receptor channels to be open and unblocked to a degree that would affect the EPSP time-course.

We note that the percentage increases in number of activated synapses, AMPA single channel conductance, or number of receptors closely tracked the percentage increase in EPSP amplitude found experimentally after LTP when the amount of LTP was near or below the average (Fig. 5). However, when LTP magnitude was larger, the number of activated synapses, the AMPA single channel conductance, or the number of receptors had to be increased by a greater percentage than the degree of LTP found experimentally because the nonlinear properties of synaptic transmission and synaptic integration began to play a more important role. This was particularly true for increases in the number of receptors.

**DISCUSSION**

**Changes at the synapse with LTP are larger than the naïve prediction when potentiation is high**

In our experiments, the increase in EPSP amplitude after LTP averaged 60% for cells in normal ACSF and 74% for cells in ZD-7288. The naïve prediction is that LTP could be explained by identical percentage increases in the number of activated synapses, the AMPA single channel conductance, or the number of receptors at a synapse. We tested this with detailed neuron and synapse level models. While this naïve prediction was close to what the models found when the degree of potentiation was average or less than the average, changes at the synaptic level had to be larger than the percentage increase in the EPSP after LTP when potentiation was large. This occurred because of nonlinear properties of synaptic transmission and synaptic integration.

First, we modeled the mechanism of LTP as an increase in the number of activated synapses, as could happen with an increase in the probability of vesicle release or through unsilencing postsynaptically silent synapses. If more synapses are activated, the voltage change in the dendrites is larger, and this reduces the
driving force for each synapse. Spatial summation should be less than linear requiring a greater than linear increase in the number of activated synapses to achieve the observed percentage increase in EPSP size. We saw this effect in our models when potentiation was large, but not when potentiation was average or less. In our models, activated synapses were separated by \( \frac{20}{\mu m} \) and a larger increase in the number of activated synapses would have been required if activated synapses had been more clustered.

Second, we modeled the mechanism of LTP as an increase in the AMPA single channel conductance. Because the increased conductance occurred at the individual synapses, voltage changes at the synapses were larger causing the driving force to be reduced directly. This should make the increment in EPSP size sublinear, requiring a greater than linear increase in single channel conductance to match the observed EPSP increase with LTP. However, the voltage change at individual synapses in our models was typically small (Fig. 4), making the effect of the reduced driving force a factor only when potentiation, and hence the increase in single channel conductance, was large.

Third, we modeled the mechanism of LTP as an increase in the number of receptors at the synapse. The increase in EPSP amplitude was sublinear in this case for two reasons. An increase in receptor number had the same effect on driving force as the increase in the AMPA single channel conductance, but in addition, because the number of glutamate molecules in a vesicle was fixed in the models, a smaller percentage of the additional receptors were activated. Receptors were not saturated by the release of 2,000 glutamate molecules in our models, and with more receptors, there was more competition for the neurotransmitter. Consequently, the percentage increases in the number of receptors at each synapse had to be larger than the percentage increases in single channel conductance or number of activated synapses to match the post-LTP EPSP (73% ACSF cells; 118% ZD-7288 cells). We note that the use of 3,000 glutamate molecules per vesicle in the models would have reduced this additional sublinear effect significantly.

**Implications of the quantification for mechanisms of LTP**

The numbers found by the models show that the change with LTP is quite large regardless of the mechanism. What are the implications of these numbers for proposed mechanisms of LTP?

**LTP AS AN INCREASE IN VESICLE RELEASE PROBABILITY.** If LTP is caused by an increase in vesicle release probability, the increase must be of such magnitude that, for our ACSF cells, the
number of activated synapses would increase from 11 to 18 (assuming a maximum of one vesicle released per synapse). We saw considerable variability in the experimental EPSP size, which could be caused by a low probability of release at individual synapses. If the probability of release averaged 0.25 before LTP, it would have had to average 0.41 after LTP. If the probability of release averaged 0.10 before LTP, this would have had to increase to 0.16 after LTP. Although most investigators do not favor this hypothesis for LTP currently, there are still reasons to consider a presynaptic component for LTP (Voronin and Cherubini 2004), and our quantitative analysis does not reject this hypothesis because these changes in the probability of release do not seem implausible.

LTP AS AN INCREASE IN AMPA SINGLE CHANNEL CONDUCTANCE. If LTP is caused by an increase in the AMPA single channel conductance (γ), our numbers suggest that an increase from 8 to 13 pS would explain our LTP data. Benke et al. (1998) reported a similar percentage change in γ, but their initial and final values were low (4.8–7.2 pS). Derkach et al. (1999) expressed homomeric GluR1 AMPA channels in HEK-293 cells and found a mean increase in γ from 11.5 to 19.9 pS on exposure to calcium-calcmodulin–dependent protein kinase II (CaMKII). This increase was the result of a higher probability of channels conducting at the 21- and 28-pS states. Poncer et al. (2002) also observed an increase in γ with α-CaMKII exposure from a mean of 11 pS in control to 16.9 pS. These observed changes are entirely consistent with our model prediction of the changes needed to explain LTP. However, these studies were done with young or juvenile animals, and it is not clear that such changes in γ occur in the adult. Andrasfalvy and Magee (2004) saw increases in the number of AMPA receptors with CaMKII activity, but no change in γ in the adult.

LTP AS UNSILENCING SILENT SYNAPSES. It has been proposed that a large percentage of synapses are silent in that they have no AMPA receptors but do have NMDA receptors and that LTP induction can cause AMPA receptors to be inserted into these synapses in minutes, particularly early in development (Isaac et al. 1995; Liao et al. 1995, 2001; but see Friedman et al. 2000; Groc et al. 2002). Estimating the percentage of silent synapses is inherently problematic, but even accepting the estimates reported, there is a limit to the number of silent synapses that can be unsilenced. The percentage of silent synapses...
LTP AS AN INCREASE IN AMPA RECEPTOR NUMBER. Increasing the number of AMPA receptors at a synapse would seem to be the least efficient method for expressing potentiation. First, this reduces the driving force, and second, a smaller proportion of the additional receptors will be activated (because of the lack of receptor saturation, unless there are also presynaptic changes as well, such as a concomitant increase in the number of neurotransmitter molecules in a vesicle or the number of vesicles released). Nevertheless, it has been convincingly shown that receptors are transported to synaptic membrane during LTP (Hayashi et al. 2000; Shi et al. 1999).

What is not clear from the experiments is how large of an increase in AMPA receptor number occurs with LTP. Our quantification shows that the percentage increase in number of receptors has to be larger than the percentage of observed LTP and considerably larger when the degree of potentiation is large. We found that, if the EPSP amplitude were increased 60% with LTP, this could be accounted for an increase in the number of receptors on synapses on long-thin, stubby, and mushroom-shaped spines from 49, 71, and 490, respectively, in our ACSF cells. In other words, 36, 52, and 207 AMPA receptors would have to be added to the three types of spine synapses in our ACSF cells. In other words, 36, 52, and 207 AMPA receptors would have to be added to the three types of spine synapses. Matsuzaki et al. (2004) suggested that LTP occurs preferentially on small synapses on long-thin and stubby spines and not on the large synapses on mushroom-shaped spines. If the increase in receptor number was to occur only on long-thin or stubby spines, the number of additional receptors per synapse predicted by our models would have to be much greater, perhaps reflecting the conversion of a long-thin or stubby spine into a mushroom-shaped one. There are reports of an increase in spine size after LTP (Harris et al. 2003; Yuste and Bonhoeffer 2001) that may reflect this type of conversion.

The problem with increases in the number of AMPA receptors as a mechanism for LTP is similar to that for the silent synapse hypothesis. Do synapses have such large numbers of AMPA receptors available for insertion? Again the labeling studies do not show available AMPA receptors in the required numbers, either intracellularly or extrasynaptically, to explain the observed LTP (Baude et al. 1995; Nusser et al. 1998; Racca et al. 2000), particularly near the smaller spines. Lu et al. (2001) found that the cell surface percentage of AMPA receptors in cultured CA1 pyramidal neurons rose from 58 to 73% after LTP, but unless there is recruitment of receptors from other synapses, these numbers are again too small to explain the observed LTP.

Recently, Andrasfalvy and Magee (2004) found that patches pulled from CA1 pyramidal dendrites after LTP contained 70–75% more AMPA receptors than control patches. These numbers are closer to what might be required to explain the observed LTP but would still be too small if potentiation was limited to the smaller synapses. The increase in AMPA receptors found by Andrasfalvy and Magee (2004) was in the number of extrasynaptic receptors and was restricted to the region of the cell where LTP was induced. Interestingly, given the size of the patches, the density of AMPA receptors could have been on the order of 200–300/µm². The starting density of AMPA receptors in our models was only 4–5 times larger than this. Given that there is a delay before the new AMPA receptors seen at the synapses after LTP induction (Hayashi et al. 2000; Shi et al. 1999) and that the number of available receptors before LTP induction seems insufficient, one explanation could be that receptors are synthesized locally. If this occurs, the required number of receptors could be obtained.

To summarize, our quantification suggests that unsilencing silent synapses or increasing the numbers of AMPA receptors at synapses may not be sufficient mechanisms to explain the LTP we observed. While an increase in y or an increase in probability of release is consistent with our results, experimental work has cast doubt on these mechanisms as explanations for LTP in adult animals. Our analysis does not rule out multiple mechanisms for LTP expression or mechanisms acting at different times, and in fact suggests that some combination of mechanisms is most probable. In particular, the very large values of potentiation that are observed on occasion (e.g., our cell 081704b, see Table 3; Debanne et al. 1999) are inconsistent with a single mechanism. It has also been shown that different mechanisms are used in different proportions in different stages of development (Palmer et al. 2004).

Notes on the models

The results of this study and its implications for the various proposed hypotheses about the mechanism of LTP depend on a number of modeling assumptions that deserve comment.

RECEPTOR NUMBERS. The number of receptors we chose for the three types of synapses is based on reported PSD size and an assumed density of AMPA receptors. These numbers of receptors produce EPSPs at the soma in our calibrated models of actual morphology that are comparable in size with exper-
Results of number estimates (Andersen 1982, 1990; Jonas et al. 1993; Larkman et al. 1991, 1997; Sayer et al. 1989, 1990). The peak number of open AMPA receptor channels is 23, 30, and 62 for long-thin, stubby, and mushroom-shaped spine synapses, respectively. Others have proposed higher estimates for the synaptic conductance than we used in the models (Forti et al. 1997; Markram et al. 1997; Stricker et al. 1996). In these studies, it seems likely that either the methods used are biased toward selection of particularly large synapses or there is some uncertainty about estimates of the number of synapses that may have contributed to a unitary EPSP. Conversely, Nimchinsky et al. (2004) suggested that <10 AMPA channels are open at the peak, which is much smaller than in the models. This number would produce EPSPs in our calibrated models that would generally be 5–30 µV at the soma if AMPA single channel conductance is 8 pS. AMPA receptor number has been shown to be quite variable among synapses, whereas NMDA receptor number seems more stable, so it is possible that we biased our synapse types to those that would produce EPSPs detectable experimentally. However, the relatively small reported percentages of silent synapses in the adult suggest that our numbers are plausible. Our NMDA channel numbers produce one to two open NMDA receptor channels at the peak, in agreement with Nimchinsky et al. (2004), but these are blocked by magnesium at the potentials generated in the models.

Calibrated models. Typically, modeling studies use the morphology of a single cell. We used morphological data from four cells in this study. One reason is that we do not have reconstructions for the specific cells that were used in the physiological experiments, and we felt that four different cells might represent some of the heterogeneity in CA1 cells. However, second, even if we had morphological information for each of our experimental cells, measurements in morphological reconstructions are subject to a number of difficulties that can affect model results (Ambros-Ingerson and Holmes 2005). These difficulties are manifested in the large variability in cell statistics listed in Fig. 1. For this reason, we believe that for modeling results to be robust, simulations should be done with several reconstructions of the same cell type, as we have done here. We argue elsewhere (Holmes et al. 2006) that model calibration can overcome inherent problems with cell heterogeneity and reconstructions to make modeled cells similar to experimental cells electronically while preserving the basic structure of the cell type modeled. While such calibration can lead to model parameter values that are not traditional, as in Table 2, we believe this is the correct approach to use.

Uniqueness. Whenever one does fits of the type done here, it is always a concern whether or not fitted parameter values provide a unique solution. Here we presented two ways to represent the leak conductance and could not distinguish between them in the fits. However, once we settled on a means to represent the leak conductance, assigned $R_a$ and $C_m$ to be constant and fixed the functional form for the $H$ conductance distribution, our final results were very consistent with a wide range of starting values. In no case did we find highly different sets of parameter values that provided equally good (best) fits to the data. Very different parameter values can give fits that may not look so bad visually, but these fits had an error several-fold larger than the best fit and were never the final fit returned by the fitting procedure. Our fits were done with averaged data and one might argue that perhaps averaged data are not truly representative or that it includes features not characteristic of the actual system. If true, the very good fits we obtained might be fitting these noncharacteristic features. We cannot guarantee that our fits are unique or that slight changes in the experimental data points would not cause very different parameter values in the fits. However, our fitted parameter values were quite consistent within a morphological model across experimental cells with the relatively few parameters we included in the fits, suggesting that a possible lack of uniqueness was not an issue for our results.

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