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Coupling of synaptic inputs to local cortical activity differs among neurons and adapts after stimulus onset

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Cortical activity contributes significantly to the high variability of sensory responses of interconnected pyramidal neurons, which has crucial implications for sensory coding. Yet, largely because of technical limitations of in vivo intracellular recordings, the coupling of a pyramidal neuron’s synaptic inputs to the local cortical activity has evaded full understanding. Here we obtained excitatory synaptic conductance (g) measurements from putative pyramidal neurons and local field potential (LFP) recordings from adjacent cortical circuits during visual processing in the turtle whole brain ex vivo preparation. We found a range of g-LFP coupling across neurons. Importantly, for a given neuron, g-LFP coupling increased at stimulus onset and then relaxed toward intermediate values during continued visual stimulation. A model network with clustered connectivity and synaptic depression reproduced both the diversity and the dynamics of g-LFP coupling. In conclusion, these results establish a rich dependence of single-neuron responses on anatomical, synaptic, and emergent network properties.

NEW & NOTEWORTHY Cortical neurons are strongly influenced by the networks in which they are embedded. To understand sensory processing, we must identify the nature of this influence and its underlying mechanisms. Here we investigate synaptic inputs to cortical neurons, and the nearby local field potential, during visual processing. We find a range of neuron-to-network coupling across cortical neurons. This coupling is dynamically modulated during visual processing via biophysical and emergent network properties.

coupling; population coupling; synaptic inputs; local field potential; response variability; correlated variability

INTRODUCTION

Cortical neuron sensory responses are remarkably variable across trials (Britten et al. 1993; Carandini 2004; Schölvinck et al. 2015). With advances in recording techniques, it has become increasingly obvious that single-neuron response variability reflects fluctuations that are shared across large regions of cortex (Lin et al. 2015; Okun et al. 2015; Rabinowitz et al. 2015; Schölvinck et al. 2015). That is, sensory input interacts with intrinsic cortical activity, with global cortical fluctuations influencing single-neuron responses. Appropriately, a recent study has introduced the term “population coupling” to describe this relationship (Okun et al. 2015). This and other studies have shown that population coupling in cortex is remarkably diverse across neurons [likely reflecting connectivity (Okun et al. 2015; Pernice et al. 2011)] yet can also change with sensory stimulation (Haider et al. 2016; Tan et al. 2014) and network state (Haider et al. 2016; Lin et al. 2015; Okun et al. 2015). This rich dependence of single-neuron responses on anatomical and emergent network properties appears to represent a fundamental principle of cortical function and is only beginning to be explored. Here we investigate three questions vital to a better understanding of cortical variability and its effects on sensory coding. 1) What is the nature of response variability in cortical microcircuits? 2) How strongly are single-neuron synaptic input fluctuations coupled with those of the local population? 3) To what degree are the dynamics of response variability and population coupling determined by the cortical network, and what are the relevant network parameters?

While the spike-based approach has yielded many important insights, it has two inherent shortcomings. First, it excludes the vast majority of neurons, which are sparse spiking (Henze et al. 2000; O’Connor et al. 2010; Shoham et al. 2006; Thompson and Best 1989) and therefore yield unreliable statistics for the analysis of correlated variability (Cohen and Kohn 2011) (Fig. 1, A and B). Second, it involves sampling populations of neurons that are visible to the experimentalist but may not represent relevant or complete cortical microcircuits. Patch-clamp recordings represent one solution to these two problems (Shoham et al. 2006). First, the subthreshold inputs to a neuron provide a measure of activity that is agnostic to output spike rate. A second perspective, motivated by anatomical connectivity, recognizes the neuron as a network sampling “device” that allows the experimenter to tap into the cortical circuitry itself and infer response properties (e.g., variability) of large populations of neurons (Ikegaya et al. 2004; MacLean et al. 2005; Mokeichev et al. 2007) (Fig. 1, A and B). Yet this approach is relatively rare; it is difficult to obtain stable patch-clamp recordings of cortical sensory responses in vivo, and spatially extended cortical pyramidal neurons confound the interpretation of voltage-clamp data (Armstrong and Gilly 1992; Koch 2004; Spruston et al. 1993). Here we overcome these challenges to address the first two questions above. We recorded subthreshold membrane potential visual responses from cortical putative pyramidal neurons in the turtle eye-
attached whole brain ex vivo preparation (Fig. 1C) and used a recently developed algorithm to estimate the excitatory synaptic conductance \( g \) from membrane potential (Yasar et al. 2016) (Fig. 1C). This inferred conductance provided a membrane potential-independent measure of spiking activity in the pool of excitatory presynaptic neurons. We then quantified the response variability in \( g \) and calculated population coupling, i.e., the correlated variability for \( g \) and the nearby local field potential (LFP). We found that visual stimulation evoked significant increases in \( g \) and LFP variability, which was predominantly additive in nature. Across the population of cells, \( g \)-LFP correlated variability (CC) was highly variable and transiently increased with visual stimulation.

We addressed the third question by implementing a driven network of leaky integrate-and-fire neurons with clustered connectivity. This model qualitatively reproduced the empirical results and suggests a dependence on spatial clustering, network spike rate oscillations, and synaptic adaptation.

Together, our results provide a clearer picture of the subthreshold dynamics underlying suprathreshold response variability and population coupling in cortex. Moreover, they implicate specific anatomical and emergent network properties that shape cortical variability and population coupling during sensory processing.

**MATERIALS AND METHODS**

**Surgery.** All procedures were approved by Washington University’s Institutional Animal Care and Use Committees and conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Fourteen adult red-eared sliders (Trachemys scripta elegans, 150–1,000 g) were used for this study. Turtles were anesthetized with propofol (2 mg/kg) and then decapitated. Dissection proceeded as described previously (Crockett et al. 2015; Saha et al. 2011; Shew et al. 2015). In brief, immediately after decapitation, the brain was excised from the skull with right eye intact and bathed in cold extracellular saline (in mM: 85 NaCl, 2 KCl, 2 MgCl\(_2\)·6H\(_2\)O, 20 dextrose, 3 CaCl\(_2\)·2H\(_2\)O, 45 NaHCO\(_3\)). The dura was removed from the left cortex and right optic nerve and the right eye hemisected to expose the retina. The rostral tip of the olfactory bulb was removed, exposing the ventricle that spans the olfactory bulb and cortex. A cut was made along the midline from the rostral end of the remaining olfactory bulb to the caudal end of the cortex. The preparation was then transferred to a perfusing chamber (Warner RC-27LD recording...
chamber mounted to PM-7D platform) and placed directly on a glass coverslip surrounded by Sylgard. A final cut was made to the cortex (orthogonal to the previous and stopping short of the border between medial and lateral cortex), allowing the cortex to be pinned flat, with ventricular surface exposed. Multiple perfusion lines delivered extracellular saline, adjusted to pH 7.4 at room temperature, to the brain and retina in the recording chamber.

**Intracellular recordings.** We performed whole cell current-clamp recordings from 39 cells in 14 preparations. In some cases, we recorded simultaneously from pairs of nearby neurons. Patch pipettes (4–8 MΩ) were pulled from borosilicate glass and filled with a standard electrode solution (in mM: 124 KMeSO₄, 2.3 CaCl₂·2H₂O, 1.2 MgCl₂, 10 HEPES, 5 EGTA) adjusted to pH 7.4 at room temperature. The visual cortex was identified as described previously (Shew et al. 2015). Cells were targeted for patching with a differential interference contrast microscope (Olympus). The pipette tip was advanced through the ependymal surface and subcellular layer and into the cellular layer [which is composed primarily of pyramidal neurons (Connors and Kriegstein 1986)]. All recorded cells were located in the cellular layer, within ~150 μm of the subcellular layer. Of the 39 recorded cells, 21 were located within 300 μm of an extracellular recording electrode (also positioned in the cellular layer). Intracellular activity was collected with an Axoclamp 900A amplifier, digitized by a data acquisition panel (National Instruments PClé-63 21), and recorded with a custom LabVIEW program (National Instruments), sampling at 10 kHz. We used stepwise current injection to evoke action potentials in patched neurons and distinguished putative pyramidal cells from putative nonpyramidal cells on the basis of visual inspection of spike width, afterhyperpolarization, spike-height variability, and interspike interval adaptation (Connors and Kriegstein 1986). We present here results for putative pyramidal neurons.

**Extracellular recordings.** We performed extracellular recordings at 12 recording sites, while simultaneously recording the membrane potential from one or more nearby neurons, in seven turtles. We used tungsten microelectrodes (MicroProbes, heat-treated tapered tip), with potential from one or more nearby neurons, in seven turtles. We used visual inspection to determine the quality of the recordings. In general, we excluded recording sites from consideration if voltage traces displayed excessive 60-Hz line noise, low-frequency noise (likely reflecting a damaged electrode), or on average small response amplitudes relative to baseline.

For intracellular recordings, we also excluded some trials and cells. To include a given trial, we required the membrane potential to remain at or above the calculated inhibitory reversal potential from the beginning of the ongoing epoch to the end of the steady-state epoch. The inhibitory reversal potential was calculated with the chloride concentrations in the intracellular and extracellular solutions, but because of partial transfer of intracellular solution to the cell interior, it was possible for the recorded membrane potential to drop below this value. This causes the conductance estimation algorithm (see below) to return a singularity. Rather than reset the inhibitory reversal potential to the minimum membrane potential value for such a trial, we took the more conservative approach of excluding the trial from consideration. We also excluded trials with excessive low-frequency artifacts or membrane potential drift. Finally, we considered only cells with 12 or more retained trials for analysis.

In some cases, an extracellular electrode remained at a single recording site while we performed whole cell recordings either simultaneously or sequentially from multiple nearby cells. To calculate CC for a cell with the nearby LFP (see Correlated variability), we obtained the average LFP response (used to calculate residual traces) from those trials in which both the intracellular and extracellular voltage were recorded and retained.

**Inferred excitatory conductance.** The algorithm for obtaining an estimated excitatory synaptic conductance (g) from membrane potential V for single trials has been described in detail and validated previously (Yaşar et al. 2016). Briefly, the algorithm approximates a solution to the underdetermined equation

\[ 0 = C \frac{dV(t)}{dt} + g_i[V(t) - E_i] + g_e[V(t) - E_e] + g_l(t) \]

where C is the assumed membrane capacitance, V(t) is the measured membrane potential as a function of time, E_i (E_e) is the assumed excitatory (inhibitory) reversal potential, E_l is the assumed leak reversal potential, g_i is the assumed leak conductance, and g_l(t) g_e(t) is the unknown excitatory (inhibitory) synaptic conductance. To estimate g_l(t), we first introduce a mathematical construct \( \eta(t) \), which is defined according to

\[ 0 = C \frac{d\eta(t)}{dt} + g_i[V(t) - E_i] + \eta(t)[V(t) - E_e] \]

For each recording, we solve this equation for \( \eta(t) \). This attributes all membrane potential fluctuations to a single (unrealistic) inhibitory conductance. As such, \( \eta(t) \) contains negative values and rapid downward fluctuations that are due to the influence of excitatory currents on the membrane potential. Because conductance cannot have negative values, we then set the negative values in \( \eta(t) \) equal to zero, resulting in \( \tilde{\eta}(t) \) [previously called “nonnegative \( \eta(t) \)’”]. Next, we use linear interpolation to smooth out the rapid fluctuations in \( \tilde{\eta}(t) \). The output of this smoothing process is \( \xi(t) \), a smoother and therefore more realistic estimation of the inhibitory synaptic conductance. Finally, we substitute \( \xi(t) \) into the equation

\[ 0 = C \frac{dV(t)}{dt} + g_i[V(t) - E_i] + g_e[V(t) - E_e] + \xi(t)[V(t) - E_e] \]

to obtain an estimation of the excitatory synaptic conductance (g). In general, this algorithm sacrifices knowledge about the inhibitory
conductance to gain a better estimation of the excitatory conductance. Furthermore, it capitalizes on the fact that excitatory currents are faster than—and therefore tend to interrupt— inhibitory currents. It should also be noted that this approach does not correct for the low-pass filtering of (primarily thalamocortical) distal synaptic inputs (Smith et al. 1980) or (likely relatively infrequent) dendritic sodium spikes (Larkum et al. 2008).

We have made several improvements to the algorithm since introducing it. The original algorithm reliably estimated excitatory conductances with simulated membrane potentials. A recorded membrane potential, however, will contain high-frequency noise, which can be removed by filtering (with, e.g., a 100-Hz Butterworth low-pass filter). This filtering process also leads to a smoother $\bar{\eta}(t)$. As mentioned above, detecting fast fluctuations in this signal is a critical step in the estimation process, and the algorithm’s performance was thus compromised by the filter (as evidenced by its application to filtered, noisy simulated membrane potentials). We therefore revised the criteria for detecting and replacing rapid fluctuations in $\bar{\eta}(t)$ (see Yasar et al. 2016 for previous criteria). First, after calculating $\bar{\eta}(t)$, we obtained the time series $d[\bar{\eta}(t)]/dt$. We then determined each time $t'$ at which $d[\bar{\eta}(t)]/dt$ crossed a threshold of one negative standard deviation. This threshold optimized the algorithm’s performance when applied to noisy simulated data. Finally, we linearly connected the local maxima of $\bar{\eta}(t)$ immediately prior and posterior to $t'$.

Applying the algorithm to a membrane potential recording also requires estimating the resting membrane potential for that trial. An unrealistic choice will lead to spurious waveforms in the estimated conductance. We estimated the resting membrane potential for each trial by calculating the median membrane potential value during the quiescent period in that trial. To isolate this quiescent period, we first removed a window of activity starting at stimulus onset and ending 6 s after stimulus offset. This resulted in either a 14-s or 24-s trace of “spontaneous” activity that was on average quiescent relative to that in the removed window. We then used an algorithm to detect spontaneous “bursts” of activity lasting at least 1 s within the remaining trace and removed these bursts. Finally, we took the median value (which is more robust to outliers than the mean) of the resulting trace to be the resting membrane potential (or $E_{\text{r}}$) for the corresponding visual stimulation trial.

We used the following values for algorithm parameters: $C = 1 \text{nF}$, $E_{\text{r}} = 90 \text{ mV}$, $E_{\text{s}} = -80 \text{ mV}$, $g_{\text{L}} = 10 \text{nS}$.

**Coefficient of variation.** The coefficient of variation (CV) is a scaled measure of variability: the standard deviation divided by the mean. Using the set of all cells ($n = 39$), we calculated CV as a function of time [CV($t$)] for the inferred excitatory conductance. First, we applied a 100-ms “box filter” to each g trace: for each time step, we replaced the value of the trace with the average value in a 100-ms window starting at that time step. We then advanced the window 10 ms and repeated the process for the full length of the trace. Then, for each cell, we calculated the across-trial standard deviation and mean of the filtered traces as a function of time. This was done for the entire population, resulting in 39 (mean, standard deviation) ordered pairs for each time step. Next, for each time step we fit the set of means to the set of standard deviations by linear regression. The slope (standard error) of this fit was the CV (SE) for the time step. To determine the significance of a change in CV across epochs, we compared the set of all CV values for one epoch to that from the other (e.g., the 100 values from the ongoing epoch and the 100 values from the transient) with the Wilcoxon signed-rank test. We report the time-averaged CV value in a given epoch as the mean ± 1 standard deviation.

**Additive variability.** To calculate the relative contribution of additive variability to single-trial responses for a given cell, we began by “box-filtering” each trace, as described above for the calculation of CV (except that here we used nonoverlapping 50-ms windows). Then, for each epoch, we regressed the resulting trace onto an across-trial average trace. This average trace was calculated from all trials for that cell, excluding the individual trial in question. This yielded a single-trial $R^2$ value for each epoch, for that trial. We repeated this for all trials and calculated the across-trial median $R^2$ value for each epoch. We repeated this for all cells.

**Correlated variability.** For each single-trial time series $X$, the residual ($X_{\text{r}}$ or deviation from the average activity) was found by subtracting the across-trial average time series from the single-trial time series:

$$X_{\text{r}} = X - \langle X \rangle_{\text{trials}}$$

Residuals were then separated into three epochs: the ongoing epoch (defined to be the 1 s before the onset of visual stimulation), the transient epoch (200–1,200 ms after stimulus onset), and the steady-state epoch (1,400–2,400 ms after stimulus onset). For each gLFP, pair, the Pearson correlation between residuals was then calculated for each epoch and trial. The results were averaged across all trials, resulting in the trial-averaged correlated variability (CC) for each pair and epoch:

$$\text{CC}^{\text{epoch}} = \frac{\text{cov}(g_{\text{L}}^{\text{epoch}}, \text{LFP}^{\text{epoch}}_t)}{\sqrt{\text{var}(g_{\text{L}}^{\text{epoch}}) \text{var}(\text{LFP}^{\text{epoch}}_t)}}$$

Significance tests for each pair and the population of pairs were applied as described in Statistical analysis.

**Power spectral analysis.** For each trial and signal, we extracted a 4.4-s window of activity (with epoch windows and gaps between epochs as described above, plus 500-ms windows on each end to avoid filtering artifacts in the ongoing and steady-state epochs) and calculated the residual time series as described above. For each residual trace, we performed wavelet analysis in MATLAB with software provided by C. Torrence and G. Compo (Torrence and Compo 1998) (available at http://paos.colorado.edu/research/wavelets/). This resulted in a power time series for each cell, for multiple frequencies. For each frequency below 100 Hz, we averaged the time series across each epoch to obtain the average power at each frequency for each epoch. We then averaged across trials to obtain $P^{\text{epoch}}_t$. For each cell, we also obtained the relative power spectrum ($rP^{\text{epoch}}_t$) for the transient and steady-state epochs, defined to be the trial-averaged evoked spectrum divided by the trial-averaged ongoing spectrum:

$$rP^{\text{epoch}}_t = P^{\text{epoch}}_t / P^{\text{ongoing}}$$

For each frequency, we calculated the bootstrap interval for the relative power as described in Statistical analysis.

**Network models.** To investigate the biophysical mechanisms underlaying our experimental results, we implemented a series of model networks composed of 800 excitatory and 200 inhibitory single-compartment leaky integrate-and-fire neurons. In the model that reproduced our principal experimental results, excitatory-excitatory connections had clustered connectivity (Bujan et al. 2015; Litwin-Kumar and Doiron 2012; Watts and Strogatz 1998) (with 3% connection probability), and all other connections were random (with 3% excitatory-inhibitory and 20% inhibitory-excitatory and inhibitory-inhibitory connection probability). To implement the clustered excitatory-excitatory connectivity, we began by constructing a “ring network” of 800 excitatory nodes. Each node in the network was connected to its 24 nearest neighbors (reflecting 3% connection probability). The weight of each of these connections was drawn from a beta distribution with average value 1.0. Finally, 1% of these connections were randomly rewired. That is, for each nonzero connection between a presynaptic and a postsynaptic node a different postsynaptic node was randomly selected from the excitatory network, with a probability of 1%.

The dynamics of the membrane potential (V) of each node evolved according to
We repeated 20 trials for a single model network (defined by $W_{ij}^0$). Each trial was 4.4 s in duration, with stimulus onset at 1.7 s, and the step size was 0.05 ms. The ongoing epoch was defined to be 1,200 ms to 200 ms before stimulus onset, the transient epoch 0 ms to 1,000 ms after stimulus onset, and the steady-state epoch 1,200 ms to 2,200 ms after stimulus onset. The additional 500 ms at the beginning and end of each trial ensured there were no filtering artifacts in the ongoing and steady-state epochs.

We modeled the LFP as the sum of all synaptic currents (similar to Atallah and Scanziani 2009; Destexhe 1998) to 100 neighboring neurons, multiplied by a factor of −1 (to mimic the change in polarity between voltages measured intracellularly and extracellularly). The contribution of each neuron to the LFP was not distance dependent. We then randomly selected 40 neurons from this subpopulation of 100 neurons and used the excitatory synaptic conductances to generate 40 g-LFP pairs for $g_{i-LFP}$, CC analysis. For CV and $R^2$ analysis, we used 40 neurons randomly selected from the full population of 800 excitatory neurons.

**Statistical analysis.** All statistical tests were performed with Python 2.7.

When asking whether a parameter of interest changed significantly across epochs for a population (e.g., whether the population-averaged CC for g-LFP pairs changed significantly from the ongoing to the transient epoch), we applied the Wilcoxon signed-rank test, which returns a $P$ value for the two-sided test that the two related paired samples [representing, e.g., the 21 (CC ongoing, CC transient) paired values] are drawn from the same distribution. This test was implemented with `scipy.stats.wilcoxon` (documentation and references available at https://docs.scipy.org/doc/scipy/reference/generated/scipy.stats.wilcoxon.html). When making multiple comparisons (e.g., ongoing vs. transient, transient vs. steady state, and ongoing vs. steady state), we divided the thresholds for significant $P$ values by the total number of comparisons.

To test whether a trial-averaged parameter of interest for one cell or electrode (e.g., CC, averaged over 15 trials for 1 cell) changed significantly from one epoch to another, we used a bootstrap comparison test. For each epoch of interest, we calculated the ±97.5% confidence intervals for the average value by bootstrapping (that is, resampling with replacement). If the bootstrap intervals for the two epochs did not overlap, we reported that the two sets of values were significantly different ($P < 0.05$).

When calculating correlations between a pair of signals in which at least one is slowly varying, it is possible for broad autocorrelations to introduce spurious cross-correlations. This should be dealt with either by removing the broad autocorrelations (e.g., by “whitening” the signals) or by accounting for their contribution to the cross-correlation. To avoid changing the temporal structure of the visual responses, we chose the latter approach. First, for each epoch and g-LFP pair, we randomly shuffled the trial order for one of the channels. We then calculated the trial-average correlation of residuals (CCshuff) and the bootstrap interval for this shuffled data. The CC value for each pair and epoch was determined to be significant (with $P < 0.05$) if the bootstrap intervals for CC and CCshuff data did not overlap. We indicate a significant CC value with a filled dot in the CC trajectory. Finally, for a given epoch, we compared the sets of CC and CCshuff values for the population of g-LFP pairs with the Wilcoxon signed-rank test (as described above for across-epoch comparisons of CC). The population average for unshuffled data was determined to be significant for $P < 0.05$. We repeated this second test using bootstrap intervals rather than the signed-rank test, with similar results (data not shown).

**Results**

Visual stimulation evokes significant increases in cortical activity. To quantify the response variability of synaptic inputs and its coupling with that of the local population, we recorded
the membrane potential (V) from 39 putative pyramidal neurons in visual cortex of the turtle ex vivo eye-attached whole brain preparation during visual stimulation of the retina (Fig. 1C; see MATERIALS AND METHODS). V, however, is not a straightforward readout of synaptic activity but rather represents a nonlinear integration of excitatory and inhibitory inputs. We have recently developed and validated an algorithm to estimate the excitatory synaptic conductance (g) from V (Yasar et al. 2016), and here we applied this method to recordings of ongoing and visually evoked activity (Fig. 1C; see MATERIALS AND METHODS). For 21 of these neurons, we also recorded the nearby LFP, which has been shown to be a reliable estimator of local synaptic activity (Haider et al. 2016).

Ongoing activity in turtle visual cortex was relatively quiescent, typically with infrequent postsynaptic potentials at the level of the membrane potential and little baseline LFP activity (Fig. 2). On a minority of trials, this quiescent activity was interrupted by spontaneous “bursts” of activity lasting up to hundreds of milliseconds that were qualitatively similar to visual responses (Fig. 2, A and D). Visual stimulation evoked barrages of postsynaptic potentials and large fluctuations in the nearby LFP (Fig. 1C, Fig. 2A). The power spectra of these evoked membrane potential and LFP fluctuations contained prominent peaks in the 10–100 Hz range (indicating oscillatory cortical activity), with peak location and amplitude varying drastically across trials (Hoseini et al. 2017). This resulted in relatively smooth across-trial average relative power spectra (i.e., power spectra for the transient and steady-state epochs divided by that for the ongoing epoch) for g and LFP (Fig. 2B). On average, power in the 1–100 Hz frequency range increased by orders of magnitude for both g and LFP (population-averaged relative power (rP) = 3,632.7 ± 3,538.0, mean ± SE, Fig. 2, B and C; (rP_LFP) = 1,902.9 ± 1,350.7, data not shown, transient). Response amplitudes (Fig. 2A) and power (Fig. 2, B and C) decreased from transient to steady state, despite continued visual stimulation ((rP) = 1,251.9 ± 962.5, steady state; P = 6.06 × 10^{-8} for transient vs. steady state comparison, Wilcoxon signed-rank test; (rP_LFP) = 557.9 ± 449.1, steady state; P = 1.2 × 10^{-3} for transient vs. steady state comparison, Wilcoxon signed-rank test).

Visual responses are highly variable across trials. For a given cell and nearby LFP, the across-trial average response to a given stimulus displayed clear temporal structure (Fig. 2A). Still, responses were highly variable across stimulus presentations; single-trial fluctuations were large relative to the mean response, with the across-trial variability increasing along with the across-trial average activity (Fig. 2, A and D). To determine the relationship between the variability and the average response of g, we calculated the scaled variability, or coefficient of variation (CV), as a function of time, for the population of all cells (see MATERIALS AND METHODS). While variability of evoked activity was larger than that of ongoing activity (Fig. 2D), CV decreased after stimulus onset and slowly recovered.

Fig. 2. Visual stimulation evokes increases in synaptic activity, and responses are highly variable across trials. A: inferred excitatory synaptic conductance (g, red) and measured LFP (black) for 3 trials (low opacity) and average across 32 trials (high opacity). Colors indicate ongoing (yellow), transient (blue), and steady-state (green) epochs (see RESULTS and MATERIALS AND METHODS). a.u., Arbitrary units. B: relative power spectra (mean ± bootstrap intervals; see MATERIALS AND METHODS) for g (top) and LFP (bottom) for transient (blue) and steady-state (green) epochs for example pair in A. C: total relative power (1–100 Hz) for 39 cells. Each blue (green) dot represents the across-trial mean relative power for 1 cell during the transient (steady state) epoch. High-opacity lines connecting dots indicate significant change across epochs (P < 0.05, bootstrap comparison test; see MATERIALS AND METHODS). Asterisks above line connecting epochs indicate P < 1 × 10^{-3} (Wilcoxon signed-rank test). D: close-up view of ongoing (left) and evoked (right) synaptic activity. E: coefficient of variation (CV) as a function of time, calculated for 39 cells (mean ± SE). Dashed line indicates CV = 1.0.

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(Fig. 2E). Using the windows of activity defined above, we found that this initial decrease was significant (time-averaged coefficient of variation (CV) = 1.83 ± 0.13 ongoing, 0.22 ± 0.04 transient, mean ± SD, P = 1.74 × 10^{-16} for ongoing vs. transient comparison, Wilcoxon signed-rank test). Furthermore, ⟨CV⟩ increased significantly from transient to steady state (⟨CV⟩ = 0.36 ± 0.06 steady state, P = 1.74 × 10^{-16} for transient vs. steady state comparison, Wilcoxon signed-rank test) but remained significantly smaller than during ongoing activity (P = 1.74 × 10^{-16} for ongoing vs. steady state comparison, Wilcoxon signed-rank test).

Additive variability dominates single-trial responses. Having established the presence of significant across-trial response variability, we next sought to determine the relative contribution of “additive variability” to single-trial responses. In the context of response time series, additive variability refers to single-trial deviations from a scaled version of the across-trial average response. These within-trial fluctuations could diminish the ability of cortical neurons to reliably encode sequences of stimuli (e.g., movie frames). In contrast, slower fluctuations in neural excitability (leading to a rescaling of the average response) may be less harmful.

To quantify this additive component, we first binned each single-trial inferred excitatory synaptic conductance (summing over 50-ms bins, resulting in ̃g) and then calculated the across-trial average binned conductance (⟨̃g⟩_trials, Fig. 3A; see MATERIALS AND METHODS). Given the linear relationship between synaptic conductance and presynaptic spiking, this is akin to binning spike counts in the presynaptic population. Finally, we regressed ̃g onto ⟨̃g⟩_trials for each trial and took the across-trial median explained variance (R^2) for each cell and epoch. This measure can be thought of as a proxy for response reliability, with R^2 = 1 indicating a purely scaled version of the average response and R^2 = 0 indicating purely additive variability. By visual inspection, it was evident that additive variability contributed to visual responses (Fig. 3A). For example, while a typical response was somewhat “enveloped” by the average time course, responses also tended to possess small, random fluctuations about the mean, or in some instances larger deviations away from the mean (Fig. 3A, trial 3, steady-state epoch). The response reliability measure (R^2) supported this observation and indicated that for a given cell the across-trial average was a relatively poor predictor of the single-trial response (see example cell in Fig. 3B); across the population, the average response explained only 28.1 ± 13.9% of the variance in individual trials for the transient epoch (across-cell average explained variance (R^2) = 0.28 ± 0.14; Fig. 3C). The explained variance was even lower during the steady state (⟨R^2⟩ = 0.17 ± 0.15; Fig. 3C), decreasing significantly from that of the transient epoch (P = 1.5 × 10^{-3} for transient vs. steady state comparison, Wilcoxon signed-rank test). In sum-

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**Fig. 3.** Single-trial variability is a mix of additive and multiplicative gain. A: inferred excitatory synaptic conductance, integrated over a 50-ms sliding window (with no overlap) for individual trials (low opacity), and across-trial average (high opacity) (see MATERIALS AND METHODS). B: across-trial distribution of R^2 values for the cell represented in A, calculated via linear regression of single-trial response onto average response for ongoing (left), transient (center), and steady-state (right) epochs. Red vertical lines indicate median values. Black scale bar on right indicates 10%. C: across-trial median R^2 values for 39 cells, for each epoch. Each dot represents the across-trial median R^2 value for 1 cell during the indicated epoch. High-opacity lines connecting dots indicate significant change across epochs (P < 0.05, bootstrap comparison test; see MATERIALS AND METHODS). Asterisks above lines connecting epochs indicate results of comparisons via Wilcoxon signed-rank test (**3.3 × 10^{-4} ≤ P < 3.3 × 10^{-3}, ***P < 3.3 × 10^{-4}).
mary, single-trial responses were in general dominated by additive variability, with the relative contribution increasing over the duration of the response.

Population coupling transiently increases after visual stimulation. Single-neuron response variability of this magnitude has the potential to profoundly influence sensory coding, provided it is significantly coupled across a population of neurons (Abbott and Dayan 1999; Averbeck et al. 2006; Shadlen and Newsome 1998). This is particularly relevant for the steady-state response, which was more variable than the early response (Fig. 3). We quantified this “population coupling” (Haider et al. 2016; Okun et al. 2015) for 21 cells by calculating the single-trial residual responses for the estimated conductance (\( g_r \), the single-trial time series with the across-trial average time series subtracted) and the nearby LFP (resulting in LFP,; Fig. 4A) and calculating the Pearson correlation coefficient for residual pairs for each trial and epoch (similar to Tan et al. 2014; Wright et al. 2017; Yu and Ferster 2010; see MATERIALS AND METHODS).

For a given stimulus condition, the trial-averaged correlation coefficient (CC) was broadly distributed across the population (Fig. 4B). During ongoing activity, CC was significantly nonzero for 7 of 21 pairs (\( P < 0.05 \), comparison to shuffled data by Wilcoxon signed-rank test; see MATERIALS AND METHODS). With visual stimulation, the population of pairs became more anticorrelated (Fig. 4B); CC amplitudes increased significantly for 10 pairs (\( P < 0.017 \), across-epoch bootstrap comparison), and the population average decreased significantly (such that the amplitude increased; \( \text{CC} = 0.009 \pm 0.04 \) ongoing, \( P = 0.50 \) for comparison to shuffled data; \( \text{CC} = -0.07 \pm 0.04 \) transient, \( P = 1.1 \times 10^{-4} \) for comparison to shuffled data; \( P = 1.9 \times 10^{-4} \) for ongoing vs. transient comparison, Wilcoxon signed-rank test; Fig. 4, B and C, top). During this transient epoch, CC was significantly nonzero for 14 pairs (\( P < 0.05 \), comparison to shuffled data). This elevated level of coordination soon relaxed: from transient to steady state, CC amplitudes significantly decreased for five pairs (\( P < 0.05 \), across-epoch bootstrap comparison), such that CC was significantly nonzero for seven pairs (\( P < 0.05 \), comparison to shuffled data) and the population average increased significantly toward zero (\( \text{CC} = -0.02 \pm 0.05 \) steady state, \( P = 0.005 \) for transient vs. steady state comparison, Wilcoxon signed-rank test) to values that were not significant across the population (\( P = 0.11 \) for comparison to shuffled data, Fig. 4, B and C, bottom). Finally, as suggested by the crossing of lines in Fig. 4B, CC values in one epoch were in general not strongly predictive of those in any other epoch (\( R^2 = 0.01 \), \( P = 0.66 \), ongoing vs. transient comparison; \( R^2 = 0.01 \), \( P = 0.65 \), ongoing vs. steady state comparison; \( R^2 = 0.33 \), \( P = 0.007 \), transient vs. steady state comparison; linear regression).

These results suggest that the across-trial variability in evoked synaptic inputs to an individual neuron is, on average, coupled to that of other neurons in a nearby population in the early response phase. Moreover, the strength of this coupling is highly variable across cells. Coupling is not static, however; while response reliability decreases from the early to the late phase of the visual response (Fig. 3), the coupling strength does as well (Fig. 4, B and C), suggesting that large single-trial fluctuations in the late response are more effectively “averaged out” across a large population. Finally, the fact that the coupling of a neuron in one epoch was not in general predictive of

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**Fig. 4.** Correlated variability of synaptic input and LFP transiently increases with visual stimulation. A: residual traces for \( g \) (red) and LFP (black) for multiple trials. B: across-trial average Pearson correlation coefficient for \( g \) and LFP residual traces for 21 \( g \)-LFP pairs. Each dot indicates the across-trial average CC value for a given \( g \)-LFP pair. Filled dots indicate significant average values (\( P < 0.05 \), bootstrap comparison to shuffled data; see MATERIALS AND METHODS). High-opacity lines connecting dots indicate significant change across epochs (\( P < 0.05 \), bootstrap comparison test; see MATERIALS AND METHODS). Asterisks above lines connecting epochs indicate results of comparisons via Wilcoxon signed-rank test (**3.3 \times 10^{-3} \leq P \leq 1.7 \times 10^{-2} ***, \( P < 3.3 \times 10^{-4} \)). C: top: distribution of change in across-trial average CC values (multiplied by \(-1\)) from ongoing to transient, for 21 \( g \)-LFP pairs (with results for shuffled data shown in gray). Black scale bar indicates 10%. Bottom: same, but for transient to steady state.
that in another epoch suggests that variables other than connectivity determined population coupling during ongoing and visually evoked activity.

Network properties shape response variability and g-LFP correlated variability. Cortical response variability and population coupling are likely shaped by three general sources: bottom-up sensory drive, recurrent (intracortical) activity, and top-down (“brain state”) modulation (Rabinowitz et al. 2015). We sought to determine the relative contribution of feedforward drive and recurrent activity to the phenomena we observed in experiment [i.e., the dynamics of scaled variability (Fig. 2E), response reliability (Fig. 3), and population coupling (Fig. 4)] and identify the biophysical mechanisms involved. To this end, we implemented a simple model cortical network subject to external drive and analyzed the resulting excitatory synaptic conductances and “local field potentials” (Fig. 5).

Our model was similar to that described previously (Wright et al. 2017). The network consisted of 800 excitatory and 200 inhibitory leaky integrate-and-fire neurons (see MATERIALS AND METHODS). Excitatory-to-excitatory connections had clustered connectivity (3%), and all other connections were random (3% excitatory-to-inhibitory, 20% inhibitory-to-excitatory and inhibitory-to-inhibitory). The clustered connectivity not only approximates the space-dependent connectivity of the cortex (Perin et al. 2011; Song et al. 2005) but has been shown to influence spatiotemporal network activity patterns (Litwin-Kumar and Doiron 2012; Wright et al. 2017), response variability (Litwin-Kumar and Doiron 2012), and correlated variability (Litwin-Kumar and Doiron 2012; Wright et al. 2017). Nonzero synaptic weights were drawn from a beta distribution with mean value 1.0, which approximated the heterogeneous nature of synaptic strengths across cortex (Cossell et al. 2015). All neurons received Poisson process external inputs, and the stimulus was modeled as an increase in external input rate. The external drive was unique across neurons and trials during the ongoing epoch. After stimulus onset, the external drive was a mix of two components: one that was unique across neurons, but identical across trials (with proportionality constant 0.75), and one that was unique across both neurons and trials (with proportionality constant 0.25). Because visual stimulation reliably evoked strong LFP oscillations in experiment (Fig. 2A; see Hoseini et al. 2017), we selected a set of synaptic rise and decay times that were consistent with network spike rate oscillations in response to strong external drive (Fig. 5B). Each synapse depressed and slowly recovered in response to a presynaptic spike. We modeled the LFP as the sum of all synaptic currents (similar to Atallah and Scanziani 2009; Destexhe 1998) to a subpopulation of 100 neighboring excitatory neurons. We selected 40 neurons from the geometric center of this population for analysis of excitatory conductances (single region and single trial in black below). We selected neurons near the geometric center of this subset and analyzed the excitatory synaptic conductances (single trial in red below, corresponding to neuron indicated by red arrow).

We first asked how the dynamics of scaled variability were shaped by feedforward and recurrent inputs. As in experiment, g and LFP varied considerably across trials (Fig. 6A), despite the stimulus being primarily the same across trials (see MATERIALS AND METHODS). We calculated CV for excitatory synaptic conductances and found that the CV dynamics were determined by both the statistics of the external drive and network properties. When external drive during the ongoing epoch was sufficiently strong to cause sparse network spiking, the CV for the total excitatory synaptic conductance to network neurons hovered near 1.0 (time-averaged coefficient of variation ⟨CV⟩ = 0.95 ± 0.25, mean ± SD for the ongoing epoch; Fig. 6B). This value greatly exceeded that of the external inputs alone (⟨CV⟩ = 0.15 ± 0.09; Fig. 6B). The difference arises from the highly variable distribution of nonzero synaptic weights (Fig. 6C, top). With stimulus onset, the CV for external inputs decreased by design (to ⟨CV⟩ = 0.004 ± 0.01 for the transient epoch), and CV for total excitatory conductance initially did as well (⟨CV⟩ = 0.40 ± 0.16 for the transient...
This decrease in CV was due in part to the increase in external drive (concerted across neurons) and in part to the stimulus possessing a component that was identical across trials (Fig. 6C, middle). Over the course of hundreds of milliseconds, the CV for total excitatory conductance recovered to nearly that of the ongoing epoch ((CV) = 0.81 ± 0.17 for steady-state epoch, \( P = 1.80 \times 10^{-16} \)).
for transient vs. steady state comparison, \( P = 1.1 \times 10^{-4} \) for ongoing vs. steady state comparison, Wilcoxon signed-rank test), which was an exaggeration of the empirical scaled variability dynamic observed here (Fig. 2E) and elsewhere (Churchland et al. 2010). Synaptic depression mediated this recovery (Fig. 6C, bottom). Thus CV values and dynamics depended on the distribution of synaptic weights, the across-trial variability of external inputs, and synaptic adaptation.

We next investigated the relative contributions of feedforward drive and intracortical activity to the dynamics of additive variability we observed in experiment (Fig. 3A) and what mechanisms might be involved. Specifically, we controlled “feedforward” variability by setting \( R^2 = 0.75 \) for the external drive during the transient and steady-state epochs (see MATERIALS AND METHODS). Despite this static feedforward variability, the model qualitatively reproduced the dynamics of additive variability in transient and steady-state activity. As in experiment, individual trials contained large additive fluctuations (Fig. 6D, compare with Fig. 3A) and response reliability significantly decreased from transient to steady state \((R^2) = 0.50 \pm 0.13\) transient, \((R^2) = 0.37 \pm 0.13\) steady state, \( P = 7.6 \times 10^{-6}\) for transient vs. steady state comparison, Wilcoxon signed-rank test; Fig. 6E, compare with Fig. 3C). This decrease was not related to synaptic depression (data not shown), suggesting single-trial “errors” compounded over the duration of the response. Notably, the percentage of single-trial variance explained by the average response in either epoch was smaller than the 75% predicted by the stimulus. This surplus variability was therefore due to the only other source of randomness in the model: the state of the intracortical synapses at stimulus onset (due to the variable external drive and intracortical synaptic depression during the ongoing epoch; see MATERIALS AND METHODS). Together, these results suggest that recurrent cortical activity—and its sensitivity to conditions at stimulus onset—contributed significantly to the additive variability dynamics we observed in experiment.

Next, we investigated the intracortical mechanisms that shaped cortical population coupling distributions. As in experiment, we calculated correlated variability for g-LFP pairs (that is, \( g_r \)-LFP, CC, the Pearson correlation coefficient of residuals; Fig. 6F). The synaptic weight distribution strongly influenced \( g_r \)-LFP, CC distributions. For each epoch, CC was broad across the population (Fig. 6G). While some variability is to be expected from such a sparsely connected network, CC distributions were far less variance in a network with binary synapses (but the same average synaptic weight; Fig. 6H, left).

Finally, we asked whether intracortical mechanisms could explain the changes in population coupling across epochs (Fig. 4B). We found that the dynamics of \( g_r \)-LFP, CC did indeed depend on a variety of network parameters and emergent network properties, such as oscillations. We recently used a similar network to demonstrate the effects of coordinated spiking on membrane potential correlated variability (Wright et al. 2017). Briefly, when the external drive triggers network spike rate oscillations, high-frequency (20–100 Hz) membrane potential fluctuations become more correlated. Synaptic adaptation subsequently reduces these correlations by modulating the network oscillations. Here, we find that this coordination dynamic is also manifested as an increase in \( g_r \)-LFP correlated variability from the ongoing to the transient epoch \((CC) = -0.12 \pm 0.03\) ongoing; \((CC) = -0.27 \pm 0.05\) transient; \( P = 3.57 \times 10^{-8}\) for ongoing vs. transient comparison, Wilcoxon signed-rank test; Fig. 6G). Synaptic depression with slow recovery (see MATERIALS AND METHODS) diminished network activity levels and, crucially, abolished large-scale coordinated spiking (Fig. 5B). This had the effect of drastically reducing \( g_r \)-LFP, CC amplitudes from transient to steady state \((CC) = -0.22 \pm 0.03\) steady state; \( P = 1.1 \times 10^{-7}\) for transient vs. steady state comparison, Wilcoxon signed-rank test; Fig. 6G), despite continued network activity (Fig. 5B, Fig. 6A). When either synaptic depression was removed (Fig. 6H, center) or the network was tuned to remain asynchronous (Fig. 6H, right; see MATERIALS AND METHODS), changes in (CC) were much smaller across epochs and did not qualitatively match the experimental results. As such, these results implicate emergent network oscillations—and the corresponding relevant anatomical network properties (i.e., synaptic time constants and synaptic depression)—in the determination of population coupling dynamics.

Taken together, these model results suggest that 1) cortical properties are sufficient to qualitatively reproduce the experimentally observed response variability and population coupling of synaptic inputs; 2) the salient cortical properties include synaptic clustering, time constants, and depression; and 3) these properties modulate \( g_r \)-LFP, CC distributions and dynamics in large part via their roles in generating and modulating emergent cortical phenomena.

Fig. 6. A model network qualitatively reproduces the experimental results. A: excitatory synaptic conductance for 1 model neuron (\( g_r \) red) and nearby LFP (black) for 3 trials (low opacity) and average across 20 trials (high opacity). Colors indicate ongoing (yellow), transient (blue), and steady-state (green) epochs. Example cell is located at the geometric center of the pool defining the LFP (see RESULTS and MATERIALS AND METHODS). B: coefficient of variation (CV) as a function of time (mean \pm SE), calculated using 40 cells randomly selected from the network, for total excitatory synaptic conductance (red) and for external excitatory conductance (green). Dashed line indicates CV = 1.0. C: CV for alternate model versions. Top: network with binary synaptic weights. Middle: network subject to unique stimulus on each trial. Bottom: network without synaptic adaptation. Scale bar and dashed line same as in B. D: excitatory synaptic conductance for 1 model neuron, integrated over a 50-ms sliding window (with no overlap) for individual trials (low opacity), and across-trial average (high opacity; see MATERIALS AND METHODS). E: across-trial median \( R^2 \) value for 40 cells randomly-selected from network, for each epoch. Each dot represents the across-trial median \( R^2 \) value for 1 cell during the indicated epoch. High opacity lines connecting dots indicate significant change across epochs (\( P < 0.05\), bootstrap comparison test; see MATERIALS AND METHODS). Asterisks above lines connecting epochs indicate results of comparisons via Wilcoxon signed-rank test (***\( P < 3 \times 10^{-4}\), F: residual traces for \( g_r \) (red) for 1 model neuron and nearby LFP (black) for multiple trials. G: across-trial average Pearson correlation coefficient for \( g_r \) and LFP residual traces for 40 g-LFP pairs (where 40 cells were selected from geometric center of pool defining LFP). Each dot indicates the across-trial average CC value for a given g-LFP pair. Filled dots indicate significant average values (\( P \leq 0.05\), bootstrap comparison to shuffled data; see MATERIALS AND METHODS). Filled dots indicate significant average values. High-opacity lines connecting dots indicate significant change across epochs (\( P < 0.05\), bootstrap comparison test; see MATERIALS AND METHODS). Asterisks above lines connecting epochs indicate results of comparisons via Wilcoxon signed-rank test (***\( P < 3 \times 10^{-4}\), H: same as in G, for alternate model versions. Left: network with binary synapses (i.e., synaptic weights either 1 or 0). Center: network without synaptic depression. Right: asynchronous network (\( 3.3 \times 10^{-3} \leq P < 1.7 \times 10^{-2}\), ***\( 3.3 \times 10^{-3} \leq P < 3.3 \times 10^{-3}\)).
DISCUSSION

To obtain a spike rate-independent measure of single-neuron across-trial response variability, and to measure the neuron’s coupling with local population activity, we simultaneously recorded the membrane potential from putative pyramidal neurons and the nearby LFP in the turtle visual cortex during ongoing and stimulus-modulated activity (Fig. 1). We estimated the excitatory synaptic conductance ($g$) from the membrane potential and quantified the across-trial variability in $g$ and correlated variability with the LFP. We discovered that visual responses were highly variable across trials and that both additive and multiplicative gain contributed to the response variability. Importantly, we found a range of neuron-to-network coupling across cortical neurons. The results of a model investigation suggest that this coupling is dynamically modulated during visual processing via biophysical and emergent network properties.

Studies spanning several decades have described a remarkable degree of variability in the sensory-evoked spiking responses of cortical neurons (Britten et al. 1993; Carandini 2004; Schölvinck et al. 2015). Recent work suggests that this variability is shaped by the cortex itself. First, cortical variability surpasses that of the inputs from thalamus (Schölvinck et al. 2015). Second, scaled variability decreases with stimulus onset across a variety of cortical areas and behavioral states, suggesting that the phenomenon is a property of large, recurrent networks (Churchland et al. 2010). Third, single-neuron response variability can be modeled as a mix of multiplicative and additive gain due to global cortical activity (Goris et al. 2014; Lin et al. 2015; Rabinowitz et al. 2015). Our work, which incorporates a novel conductance estimation method, generally strengthens this “cortico-centric” view of response variability, and our network model identifies specific cortical properties that likely shape cortical response variability.

For instance, we found that individual neurons received excitatory synaptic inputs that were extremely variable across stimulus presentations (Fig. 2, A and D, Fig. 3). This variability was predominantly additive in nature (i.e., the fluctuations occurred on short timescales relative to the duration of the stimulus), and the contribution from this additive variability increased from transient to steady state (Fig. 3). What determined the level of variability and its dynamics during the response? There are at least three possible candidates: bottom-up inputs (i.e., sensory drive), recurrent intracortical activity, and fluctuations in brain state (Rabinowitz et al. 2015). To identify the potential contribution from feedforward and recurrent inputs, we implemented a simple model network with known feedforward variability (that was constant across the transient and steady-state epochs). This model qualitatively reproduced the empirical results (Fig. 6, D and E) and suggests that small deviations in initial conditions (e.g., intracortical synaptic strengths) lead to a modest initial amplification of thalamic response variability, with single-trial error compounding over the duration of the response. In other words, recurrent activity likely contributed significantly to the $R^2$ dynamics we observed in experiment. Such chaotic dynamics are a hallmark of balanced networks (Shadlen and Newsome 1998; van Vreeswijk and Sompolinsky 1996). While at first glance this seems extremely disadvantageous to sensory coding, the balanced regime has other advantages, including fast responses to changes in external stimuli (van Vreeswijk and Sompolinsky 1996), effective signal propagation (Vogels and Abbott 2005), and maximized information capacity (Shew et al. 2011).

Previous work has shown that (spike based) population coupling is broadly distributed across cells, which may reflect the degree to which a given neuron samples the local population (Okun et al. 2015) and the structure of that connectivity (Pernice et al. 2011). In agreement with this, we found that g-LFP correlated variability was broadly distributed across cells for a given stimulus condition (Fig. 4B). Our model results reinforce the hypothesis that this across-cell variability is related to the underlying connectivity; CC values were broadly distributed for relatively realistic, heavy-tailed synaptic weight distributions (Fig. 6G) but narrowly distributed for binary synapses (Fig. 6H, left). These weight distributions also shaped the dynamics of scaled variability (Fig. 6, B and C, top). These results suggest that cortical connectivity patterns are manifested in the response variability and coordinated variability of synaptic activity. Given the relevance of synaptic activity to spiking (Doiron et al. 2016; Litwin-Kumar et al. 2011; Lyamzin et al. 2015), this likely reflects the corresponding response properties of population spiking observed elsewhere (Okun et al. 2015).

Anatomical connectivity was not the only relevant variable; we found that g-LFP correlated variability amplitudes significantly increased with visual stimulation (Fig. 4, B and C, top), in agreement with previous work (Haider et al. 2016). Interestingly, CC amplitudes decreased after the early response phase (Fig. 4, B and C, bottom), despite persistent synaptic and local population activity (Fig. 2, A–C, Fig. 4A). Was this dynamic imposed by the external inputs, or was the cortical network itself capable of exhibiting multiple population coupling “states”? Because of the limitations of our experimental approach, we cannot rule out the former explanation. Still, our model results support the latter hypothesis. Specifically, we constrained the model network to reproduce two aspects of empirical visual responses: LFP oscillations (Fig. 5B, bottom, Fig. 1B, bottom) and evoked activity that was more coordinated across the network in the early response and more locally coordinated in the later response (Fig. 5B, bottom; see Shew et al. 2015). These response properties are not themselves trivially related to local population coupling, yet imposing these constraints recovered the $g_r$-LFP, CC dynamic (Fig. 6G). That is, the coupling dynamics at one scale may be concomitant with networkwide state fluctuations. Such a relationship would be consistent with the observations that spontaneous fluctuations in cortical state can influence $g_r$-LFP (Haider et al. 2016) and spike-spike (Okun et al. 2015; Schölvinck et al. 2015) population coupling and further suggests that variations in the cortical state (i.e., the excitatory-inhibitory balance) across recording sessions and experiments could be responsible for the general reordering of CC values across epochs (Fig. 4B). Taken together, our results advance a “cortico-centric” view of population coupling by identifying specific features of cortex (e.g., synaptic time constants and synaptic adaptation) capable of influencing population coupling dynamics via emergent network phenomena.

The population coupling values we observed were in general small, even during the early response phase (Fig. 4B), which is roughly in line with previous observations in mouse (Haider et
al. 2016) and monkey (Tan et al. 2014) V1. In other words, most of the response variability in synaptic inputs to a given neuron was not explained by fluctuations in the local network defined by the reach of the nearby extracellular electrode. This likely reflects the “constellation-like” connectivity of cortex, in which a given neuron is driven by a sparse subset of the local population (as well as more distant neurons). In line with this, we have recently shown that during visual responses prespike depolarizations (Wright and Wessel 2017) and high-frequency subthreshold oscillations (Wright et al. 2017) are only weakly shared between pairs of nearby neurons. This suggests that the LFP is unlikely to isolate individual cortical microcircuits, making the population-averaged g-LFP CC value in a given epoch difficult to interpret on its own. Nevertheless, changes in g-LFP CC across stimulus conditions likely track shifting network coordination levels. Thus the CC dynamics we observed here suggest that networkwide coordination is stronger during the early response and returns to baseline within 1 s of stimulus onset. This is supported by our network modeling results (Fig. 6) and our previous studies of large-scale cortical activity in this same preparation (Clawson et al. 2017; Shew et al. 2015). According to one view of population coding, this decrease in coupling in the late response may benefit cortical function: while steady-state activity is less reliable than that in the early response (Fig. 2E, Fig. 3), these later fluctuations are more private and therefore tend to average out across a neural ensemble (Zohary et al. 1994). Our model results suggest that this transition is mediated by adaptation (i.e., synaptic depression), which is consistent with the emerging view that cortical adaptation serves as much more than a modulator of activity levels but is in addition a “knob” for fine-tuning a variety of functionalities (Benucci et al. 2013; Gutnisky and Dragoi 2008; Ollenereshaw et al. 2014; Shew et al. 2015; Whitmire and Stanley 2016; Wright et al. 2017; Zheng et al. 2015). In other words, adaptation modulates both the overall level (i.e., total spike rate) and the regime (i.e., spatiotemporal neuronal correlations patterns) of cortical activity.

There exist several useful algorithms for estimating properties of synaptic activity from membrane potential recordings (Bédard et al. 2012; Berg and Ditlevsen 2013; Borg-Graham et al. 1998; Lankarany et al. 2013; Paninski et al. 2012; Puggioni et al. 2017; Rudolph and Destexhe 2003; Wehr and Zador 2003). The analysis performed here requires knowledge of the synaptic conductance time series on individual trials, which a few cleverly crafted algorithms can provide (Bédard et al. 2012; Berg and Ditlevsen 2013; Lankarany et al. 2013; Paninski et al. 2012). Each of these algorithms has a particular set of strengths, weaknesses, and underlying assumptions. For instance, one that uses a Bayesian approach requires prior knowledge of the statistics of presynaptic firing (Paninski et al. 2012), and several do not appear to resolve fluctuations that occur at timescales on the order of 10 ms (Berg and Ditlevsen 2013; Lankarany et al. 2013; Paninski et al. 2012). Another far outperforms our algorithm in the estimation of inhibitory conductances (Bédard et al. 2012), but excitatory conductances are less reliable (compare Fig. 4 in Yaşar et al. 2016 and Fig. 8 in Bédard et al. 2012). Here we apply an algorithm that complements these approaches and is particularly well suited for this study; it makes no assumptions about the statistics of network activity and allows us to calculate population coupling for excitatory conductances with relatively high temporal resolution.

One major limitation of our experimental work is the lack of direct measurements of inhibitory synaptic conductances, which are a key component of single-neuron and networkwide response properties. Inhibition represents a significant proportion of the total synaptic input to a given neuron (Haider et al. 2013) and tends to be correlated across pairs of neurons (Hasenstaub et al. 2005), and the relative timing of excitatory and inhibitory currents may determine precise spike timing (Haider and McCormick 2009; Hasenstaub et al. 2005; Nowak et al. 1997) and feature selectivity (Wilen and Contreras 2005). Furthermore, the inhibitory population is known to play a vital role in such emergent network phenomena as spike rate oscillations (Brunel and Wang 2003), and the excitation-inhibition balance may represent a fundamental aspect of the cortical code (Denève and Machens 2016). In future studies, it will be straightforward to modify our experimental approach and conductance estimation algorithm to investigate inhibition. More importantly, this approach can be combined with multi-whole cell recording to simultaneously infer excitatory conductances in one cell, and inhibitory in another, similar to studies of evoked activity in rat somatosensory cortex (Okun and Lampl 2008) and spontaneous activity in rat hippocampus (Atallah and Scanziani 2009) and mouse thalamocortical slice (Graupner and Reyes 2013). This would be particularly useful in areas such as visual cortex, where responses can be complex and highly variable across trials (thus limiting the utility of recording excitation and inhibition from one cell on alternating trials).

The turtle ex vivo preparation is well suited to obtaining whole cell recordings of cortical visual responses (Crockett et al. 2015; Wright et al. 2017; Wright and Wessel 2017), but there are important differences between turtle and mammalian visual cortex that should be taken into account when seeking to generalize these results. Most notably, the turtle cortex has six rather than six layers, and the single “cellular layer” may be comparable to L5 and L6 of mammalian visual cortex (Reiner 1993). In fact, the cytoarchitecture of turtle visual cortex is perhaps more reminiscent of mammalian olfactory cortex or hippocampus (Fournier et al. 2015). Nevertheless, the turtle visual thalamocortical system possesses anatomical and phenomenological properties that make it useful for investigating cortical function generally (Shepherd 2011) [e.g., diverse morphological and electrophysiological cortical cell types (Connors and Kriegstein 1986; Crockett et al. 2015; Mancilla and Ulinski 2001), feedforward inhibition (Mancilla and Ulinski 2001), and cortical oscillations (Clawson et al. 2017; Hoseini et al. 2017; Shew et al. 2015)]. Thus we anticipate that cortical response variability and population coupling in mammalian visual cortex are in general shaped by the variables identified here and that specific results (e.g., population coupling distributions) may vary considerably across cortical layers (as would be predicted from the strong dependence on connectivity demonstrated by our model, Fig. 6).

Taken together, our results demonstrate the highly variable nature of visually evoked synaptic inputs (and therefore spatiotemporal spike patterns) in cortical microcircuits. Furthermore, these results suggest that several properties of this variability are largely determined intracortically and identify specific, highly relevant cortical parameters. Importantly, these
cortical properties together lead to an adapted network state that is in many ways ideal for sensory processing. As such, this work contributes to a clearer picture of the effects of anatomical and emergent network properties on single-neuron sensory responses and networkwide function.

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REFERENCES


VARIABILITY AND ADAPTATION OF SYNAPTIC INPUT COUPLING


