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Neural dynamics of *in vitro* cortical networks reflects experienced temporal patterns

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Learning ultimately relies on changes in the flow of activity in neural microcircuits. The plasticity of neural dynamics is particularly relevant for the processing of temporal information. Chronic stimulation of cultured rat cortical networks revealed experience-dependent plasticity in neural dynamics. We observed changes in the temporal structure of activity that reflected the intervals used during training, suggesting that cortical circuits are inherently capable of temporal processing on short timescales.

Timing and temporal processing in the range of tens and hundreds of milliseconds is critical for many forms of sensory and motor processing, but the neural mechanisms underlying the ability to discriminate or produce short intervals remain unknown[1,2]. Recent studies have lent support to the notion that timing is an inherent computational ability of cortical circuits and may be performed locally[2]. This view implies that the temporal structure of the internal dynamics of cortical networks should be shaped by the temporal patterns that the network experiences. To examine this issue, we studied the effects of the presentation of simple spatiotemporal stimulus patterns on cortical neural dynamics using organotypic slices. As with *in vivo* cortical networks, evoked stimulation in organotypic networks can elicit complex polysynaptic responses that reflect local network dynamics. This preparation is therefore well suited to study the plasticity of neural dynamics.

External stimulation was presented to cortical cultures via two implanted bipolar stimulating electrodes[3]. We first examined whether the temporal pattern of stimulation produced any form of network plasticity, defined as changes in evoked patterns of activity. The implanted electrodes (E1 and E2) were activated with a burst of pulses presented in-phase (synchronously) or with a 100-ms interval (onset to onset), every 10 s for 2 h (see **Fig. 1a** and **Supplementary Fig. 1**). Given the large degree of variability in the presence and structure of polysynaptic activity in naive slices, all of the presented data is derived from paired experiments in which 'sister' slices were trained with one of two protocols and compared[4]. After training in the incubator

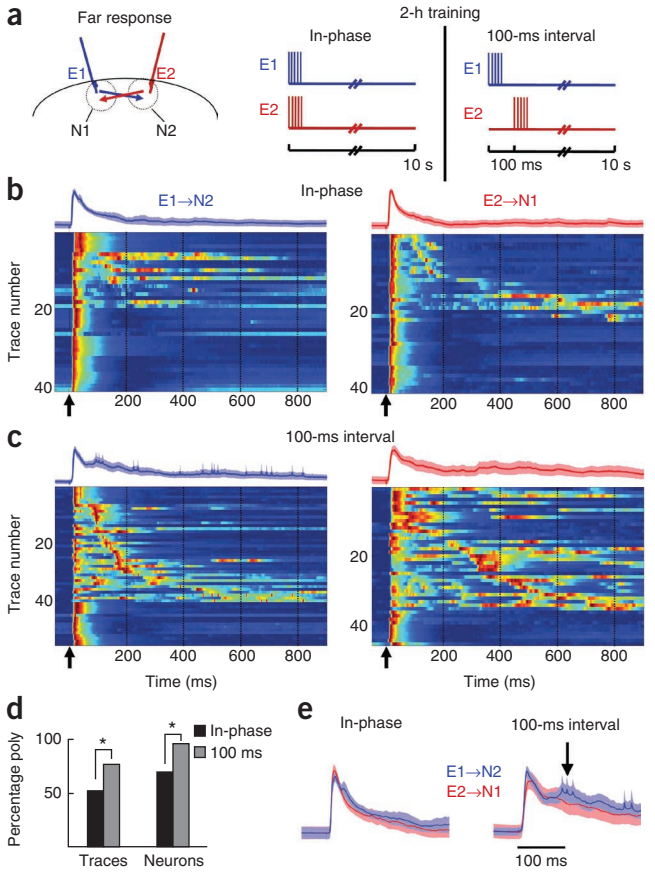

Figure 1 Network dynamics is differentially modified by training. (**a**) After 2 h of training with either an in-phase or 100-ms pattern (right), far pathway responses (E1→N2 in blue, and E2→N1 in red; left panel) were examined. (**b**,**c**) Voltagegrams of E1→N2 (left) and E2→N1 (right) traces in response to a single test pulse (time indicated by arrow) for slices trained with the in-phase ($n = 8$ neurons each pathway, 5 traces per neuron, data from 8 slices, **b**) or 100-ms interval pattern (11 neurons, each from a different slice for E1→N2 pathway, $n = 9$ neurons for E2→N1 pathway, 5 traces per neuron; N1 neurons were not recorded in two of the slices, **c**). Voltagegram traces are normalized and sorted according to latency of the first polysynaptic peak (that is, the first peak after the monosynaptic response). Voltage is represented in color where blue is the minimum and red is the maximum. The traces above each voltagegram are the mean of all traces (shading represents the s.e.m.). Arrows represent the time of the test stimulus and dashed lines are presented for comparison across panels. (**d**) After 100-ms training, 76% of all test traces and 95% of tested neurons exhibited one or more polysynaptic peaks versus 51% of traces and 69% of neurons for the in-phase group (traces, $\chi^2 = 11.98$, $P < 0.001$; neurons, $\chi^2 = 4.41$, $P < 0.05$). (**e**) Mean ± s.e.m. (shading) of waveform in response to far pathways after in-phase or 100-ms interval training. Note the secondary peak in the E1→N2 waveform after 100-ms interval training (arrow), which is lacking in the other traces.

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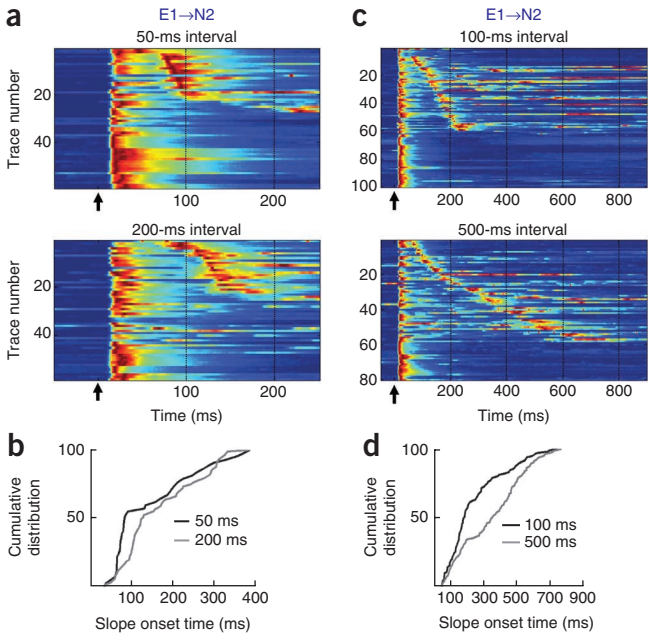

Figure 2 Differential effects of training interval on neural dynamics. (**a**) All raw data plotted as voltagegrams from the 50-ms (top) and 200-ms (bottom) groups ($n = 12$ cells in each group). (**b**) Cumulative distribution (Kolmogorov-Smirnov test, $P < 0.005$) of polysynaptic event onset times for the E1→N2 pathway of the 50-ms (black) and 200-ms (gray) groups. (**c**) Voltagegram data from the 100-ms versus 500-ms experiments. Data are derived from 19 and 15 neurons (one neuron per slice) in the 100-ms (top) and 500-ms (bottom) groups, respectively. (**d**) Cumulative distribution of polysynaptic event onset times for the E1→N2 pathway in experiments trained with a 100-ms (black) or 500-ms (gray) interval (Kolmogorov-Smirnov test, $P < 10^{-4}$).

for 2 h, whole-cell recordings were performed from neurons near each electrode (N1 and N2 refer to neurons close to electrodes E1 and E2, respectively) and the postsynaptic potential (PSP) waveform in response to a single pulse from the 'far' (E2→N1 or E1→N2 responses) pathways was examined. As described previously, in addition to the short-latency monosynaptic PSP, complex polysynaptic PSP waveforms were often observed[5,6]. As these late PSPs provide a measure of the population activity of the neurons that synapse onto the recorded cell, we used the temporal profile of the voltage traces as a measure of the temporal pattern of network activity.

After a 2-h training session, examination of the PSPs evoked by a single test pulse from either electrode revealed that the 100-ms group exhibited a significantly larger number of test traces with one or more polysynaptic events ($\chi^2 = 11.98$, $P < 0.001$, analysis collapsed across pathways; **Fig. 1**, see **Supplementary Methods**). Thus, in response to a single pulse there was a significant difference in the behavior of the network between the in-phase and 100-ms groups. In the in-phase group, as expected, the average of all E1→N2 and E2→N1 traces was very similar. In the 100-ms group, however, there appeared to be a difference between the E1→N2 and E2→N1 traces; specifically, even when collapsed across all cells, the E1→N2 trace exhibited a small secondary peak at approximately 100 ms (**Fig. 1e**). This observation was consistent with the notion that the timing of network activity reflected the interval used during training. Although these patterns are highly variable, any preferential increase in activity around the expected time of the second pulse could be interpreted as a type of pattern completion; that is, after E1 stimulation, the neurons near E2 exhibited increased activity around the interval used during training.

To examine the effect of the training interval on the timing of network dynamics, we performed experiments in which two groups were trained for 2 h with either a 50-ms or 200-ms interval (**Fig. 2a**). An analysis of the distribution of the onset times of the E1→N2 polysynaptic events revealed they were significantly shorter in the 50-ms compared with the 200-ms group (Kolmogorov-Smirnov test, $P < 0.005$; **Fig. 2b**). As shown in the 'voltagegrams' (**Fig. 2a**), the polysynaptic activity tended to be clustered at earlier intervals in the 50-ms group. These results confirm that the temporal structure of neural dynamics evoked by a single stimulus is shaped in an interval-specific manner by the training stimulus.

The above experiments were performed by training in the incubator and testing on the electrophysiology rig. To determine the robustness of the phenomenon and sensitivity to training conditions (training in culture media versus artificial cerebrospinal fluid, and 35 °C incubator versus 30 °C on-rig), we repeated these experiments while training for 2 h on the recording rig. Again, we observed a significant difference in the timing of the E1→N2 polysynaptic events between the 50- and 200-ms groups, as can be observed in the voltage traces and in the derivative of the voltage traces, which highlights the upward deflections in the voltage as a result of synaptic input (Kolmogorov-Smirnov test, $P < 0.005$; **Supplementary Fig. 2**). To ensure that the timing effects were not somehow specific to 50- and 200-ms intervals and to examine the range over which interval-selective effects are observed, we also trained two groups of slices with intervals of 100 and 500 ms. Again, the results revealed that the distribution of the polysynaptic events was significantly shorter in the 100-ms as compared with the 500-ms group (Kolmogorov-Smirnov tests, $P < 10^{-4}$; **Fig. 2c,d**). We emphasize that, although these data establish that the timing of polysynaptic activity is influenced by the interval used during training, it is not the case that all of the cells in the network learn to time at the trained interval or that the timing is highly accurate (in some experiments, but not in others, the difference in temporal structure was detectable as a secondary peak of the averaged traces). Indeed, because activity during any time window T presumably relies on activity at time window $T - 1$, mechanistic considerations (see below) are consistent with the notion of a fairly broad distribution of timed responses and that network plasticity is best understood as changes in the distribution of polysynaptic responses.

It has previously been reported that evoked stimulation in naive slices can induce propagation of activity characterized by a time-varying pattern of neurons firing at different points in time[5,6]. Thus, one hypothesis is that the first pulse (E1) engages a time-varying pattern of activity and the second pulse (E2) functions as a reinforcer, potentiating the synapses that are active at the time of the second pulse through conventional associative synaptic plasticity. Consistent with this hypothesis, the timing of the PSPs was significantly different ($P < 0.005$) in slices trained with a 50-ms interval in the presence or absence of the NMDA receptor antagonist D(−)-2-amino-5-phosphonovaleric acid (AP5; **Supplementary Fig. 3**). This result suggests a role for the NMDA receptor, but the interpretation is limited by the fact that AP5 itself can alter neural dynamics during training or have induced homeostatic forms of plasticity. Furthermore, the amplification of pre-existing responses at the time of the second pulse does not explain the qualitative changes in the distribution of responses (for example, note the slope of the diagonal band in **Fig. 2c**). We hypothesize that plasticity of neural dynamics may be an emergent property that relies on orchestrated changes at the multiple synaptic and cellular loci that ultimately govern the propagation of activity through recurrent neural networks. For example, theoretical studies have shown that some forms of homeostatic plasticity can lead

to increases in the propagation of activity throughout networks[7,8]; these patterns might then be further shaped by associative forms of synaptic plasticity. Nevertheless, the types of synaptic and cellular changes underlying the network plasticity that we found remain to be elucidated.

It is well established that cortical function and network dynamics are sculpted by sensory experience[9]. Our results indicate that the behavior of cultured cortical networks is also shaped by the stimulus history of the network in a manner that suggests that cortical networks are capable of learning or adapting to the timing of the stimuli. Specifically, the dynamics of the network activity was altered in a manner that reflected the temporal patterns of stimuli used during training. Previous studies have reported stimulation-dependent changes in the levels of activity or in the correlation of activity after repeated stimulation of *in vitro* networks[10–13]. In addition, studies in dissociated cultures[14] and in the *Xenopus* optic tectum[15] have revealed that specific temporal patterns of stimulation can alter the timing of neural responses in recurrent circuits. For example, in dissociated cultures, the interval of paired-pulse stimulation resulted in the emergence (or disappearance) of polysynaptic PSPs and changes in their timing. However, the timing of these events was primarily a product of the propagation delays of the circuitry and not directly predictable from the training interval *per se*[14]. Our results extend previous studies in two ways. First, we found that neural dynamics in a complex circuit can be modified in a computationally functional fashion as a result of experience; that is, these circuits would be better able to 'tell time' around the stimulated interval. Second, the temporal structure of neural dynamics reflects the temporal interval used during training. In this sense, our results represent an example of stimulus-specific modifications of network dynamics and an *in vitro* analog of learning. Although further work is required to dissect the mechanisms, our findings support the view that, on short timescales, cortical circuits are inherently capable of telling time, suggesting that temporal and spatial processing are inextricably linked in cortical networks and that specialized mechanisms and circuits are not necessary for temporal processing[1,2].

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

H.A.J. and A.G. conducted all of the experiments. All authors participated in the data and statistical analyses. H.A.J. and D.V.B. conceived and designed the experiments and were responsible for preparing the manuscript.

COMPETING FINANCIAL INTERESTS

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