Supplementary Information

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

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METHODS

Organotypic slice preparation with implanted microelectrodes

Organotypic slices were prepared using the interface method[1, 2] and maintained on culture inserts with implanted microelectrodes[3]. Seven day old Sprague-Dawley rats were anesthetized with isoflurane and decapitated. The brain was removed and placed in chilled cutting media. Coronal slices (400 µm thick) containing primary auditory or somatosensory cortex were cut using a vibratome. Each slice was placed on a cell culture insert with two attached electrodes (referred to as E1 and E2) and positioned so that the electrodes were under the slice separated by approximately 2 mm, and with the tips located 400-800 µm from the cortical surface.[3] Culture media was changed 1 and 24 hrs after cutting and every 2-3 days thereafter. Cutting media consisted of EMEM (MediaTech cat. #15-010) plus 3 mM $MgCl_2$, 10 mM glucose, 25 mM Hepes, and 10 mM Trisbase. Culture media consisted of EMEM plus 4 mM glutamine, 0.6 mM $CaCl_2$, 1.85 mM $MgSO_4$, 30 mM glucose, 30 mM Hepes, 0.5 mM ascorbic acid, 20% horse serum, 10 units/L penicillin, and 10 µg/L streptomycin. Slices were incubated in 5% CO_2 at 35°C for 10-28 days before beginning stimulation.

Chronic stimulation and training

All electrical stimulation consisted of charge-balanced biphasic current pulses, composed of a 100 µs positive pulse followed by a 100 µs delay and a 100 µs negative pulse[4]. Stimulation intensity was set to 150 µA. Chronic stimulation patterns consisted of a 100 Hz burst of 5 pulses presented either simultaneously via the two microelectrodes (in-phase) or sequentially with a fixed interval (50, 100, 200, or 500 ms; onset-to-onset). Stimulation patterns were generated using a Master-8 (A.M.P. Instruments) or custom written MATLAB software controlling an analog-output board (National Instruments, PCI-6723), and delivered via stimulus isolator units (A.M.P. Instruments).

Both spontaneous and evoked dynamics across slices are highly variable; as can be seen from the raw data some traces (generally from the same cell and slice) do not exhibit any, while other display robust, polysynaptic activity. The source of this variability is not understood (although one factor is probably different developmental time courses), however, experiments in dissociated cell cultures have confirmed that there is significantly less variability between sister cultures (i.e., tissue from the same animal, same sera, incubator, and maintenance schedule).[5] Thus, our experiments were carefully designed to contrast a single manipulation (inter-stimulus of training interval) in 'sister' slices. In our hands given the variability in dynamics between non-sister slices valid comparisons across experiments would require much higher n values.

All training sessions shown here were 2 hours long. The experiments presented in Fig. 1 and 2a,b were trained in the incubator, and the remaining were trained on the rig. Consistent with our previous observations, as a result of the strong input provided by the burst and the relatively short inter-stimulus interval (10s) the training stimuli generally elicited a strong depolarizing response and relatively little polysynaptic activity. However, in the few experiments in which we recorded during training there did seem to be an increase in polysynaptic activity during the course of training (**Fig. S3**).

Electrophysiology

Recordings were made from regular-spiking, supragranular pyramidal neurons[6] located less than 500 μm from the cortical surface using IR-DIC visualization. Two or four neurons were recorded from each slice, half near each of the two stimulating electrodes (N1 and N2 represents neurons near the E1 and E2 electrodes, respectively). Experiments were carried out at 30°C in artificial cerebrospinal fluid (ACSF) adjusted to match the culture media [2]: 125 mM NaCl, 5.1 mM KCl, 2.6 mM $MgSO_4$, 26.1 mM $NaHCO_3$, 1 mM NaH_2PO_4, 25 mM glucose, and 2.6 mM $CaCl_2$. The internal solution for whole-cell recordings contained 100 mM K-gluconate, 20 mM KCl, 4 mM ATP-Mg, 10 mM phospho-creatine, 0.3 mM GTP-Na, 10 mM HEPES, and was adjusted to pH 7.3 and 300 mOsm. Recordings were sampled at 10 kHz, digitized using a CED micro1401 board (Cambridge Electronic Design), and saved for off-line analysis. All analyses were performed using custom-written software in MATLAB.

Polysynaptic Response Analysis

For each trace recorded in response to a single test pulse from either electrode, polysynaptic events were identified by depolarizing deflections in the PSP with a slope of at least 0.3 mV/ms that was maintained for at least 5 ms, and a peak of at least 5 mV over the membrane potential at slope onset. All the data presented here were from the first two cells – always an N1 and N2 cell collected in a counterbalanced manner. Analysis of all four cells (when collected) revealed a weaker effect, suggesting that network plasticity decays over a 30-60 minute period, or that the effect is being actively 'extinguished' during testing.

To determine if the timing of the polysynaptic events was different between groups we analyzed the distribution of the time of these events. This approach was trial based, and thus reflects the information about how much time has elapsed, since the stimulus, that would be available to a downstream neuron.

The statistical analyses of the changes in the distributions of the timing of the polysynaptic response were based on the E1→N2 pathway between the two groups that comprised a specific experiment (always performed on paired 'sister' slices). For the 50, 100 and 500 ms intervals, there was a significant difference between the distribution of polysynaptic activity between the E1→N2 and E2→N1 pathways (this was not the case for the 200 ms groups). While we believe there is different in the activity elicited from different pathways in trained slices the E2 pathway likely undergoes plasticity also– for example, there was an increase in the proportion of E2→N1 traces with polysynaptic activity between the in-phase and 100 ms groups (E2→N1, $\chi^2 = 3.98$, $P<0.05$). Here we focus primarily on the E1→N2 pathway because the expected result of any potential 'learning' is clear. In contrast, whether plasticity should be induced, and if so, what form it should take is less clear for responses evoked by E2.

The 'voltagegrams' were created by normalizing each trace to its own peak and baseline, and sorting according to the latency of the first polysynaptic event.

References

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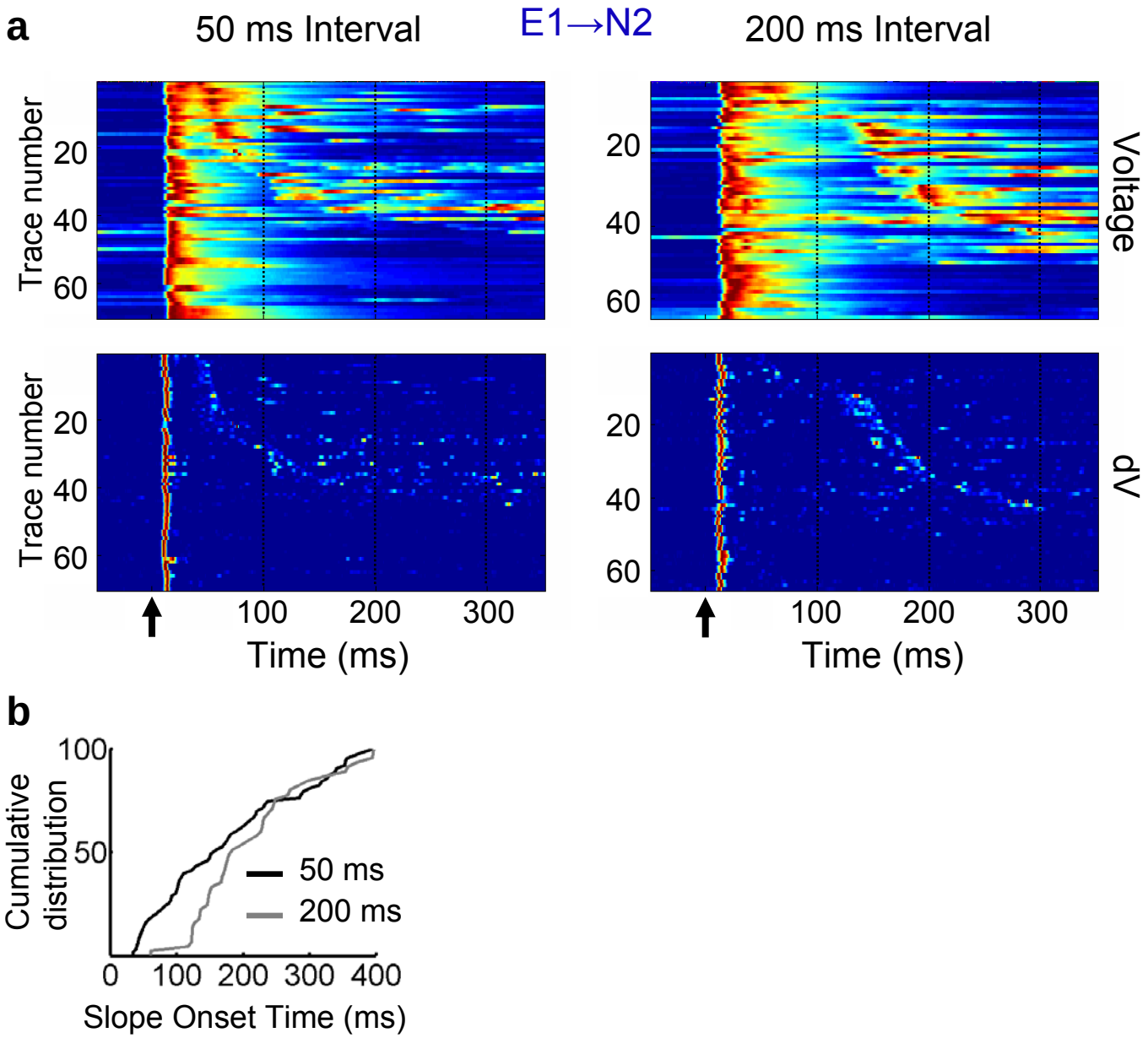
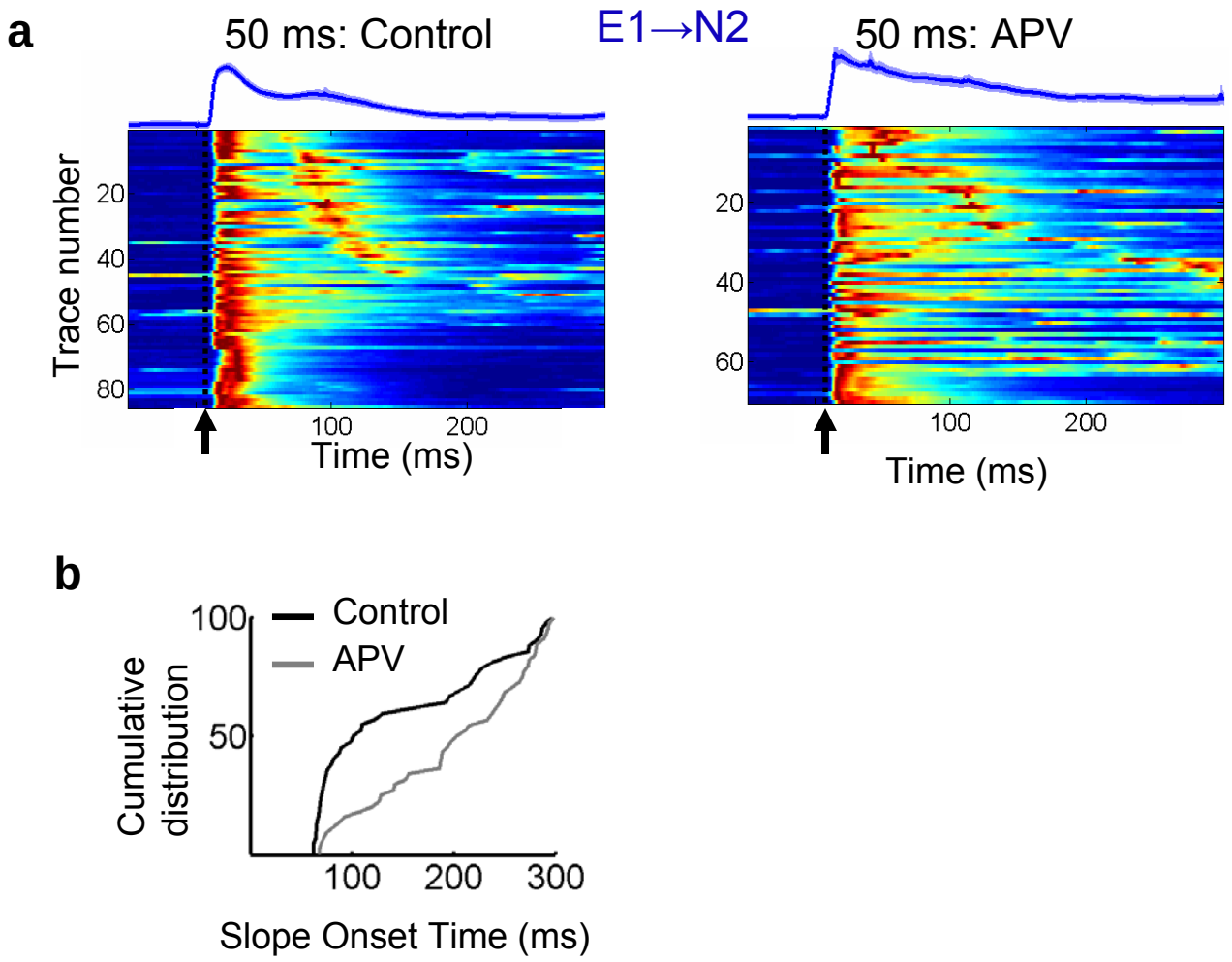
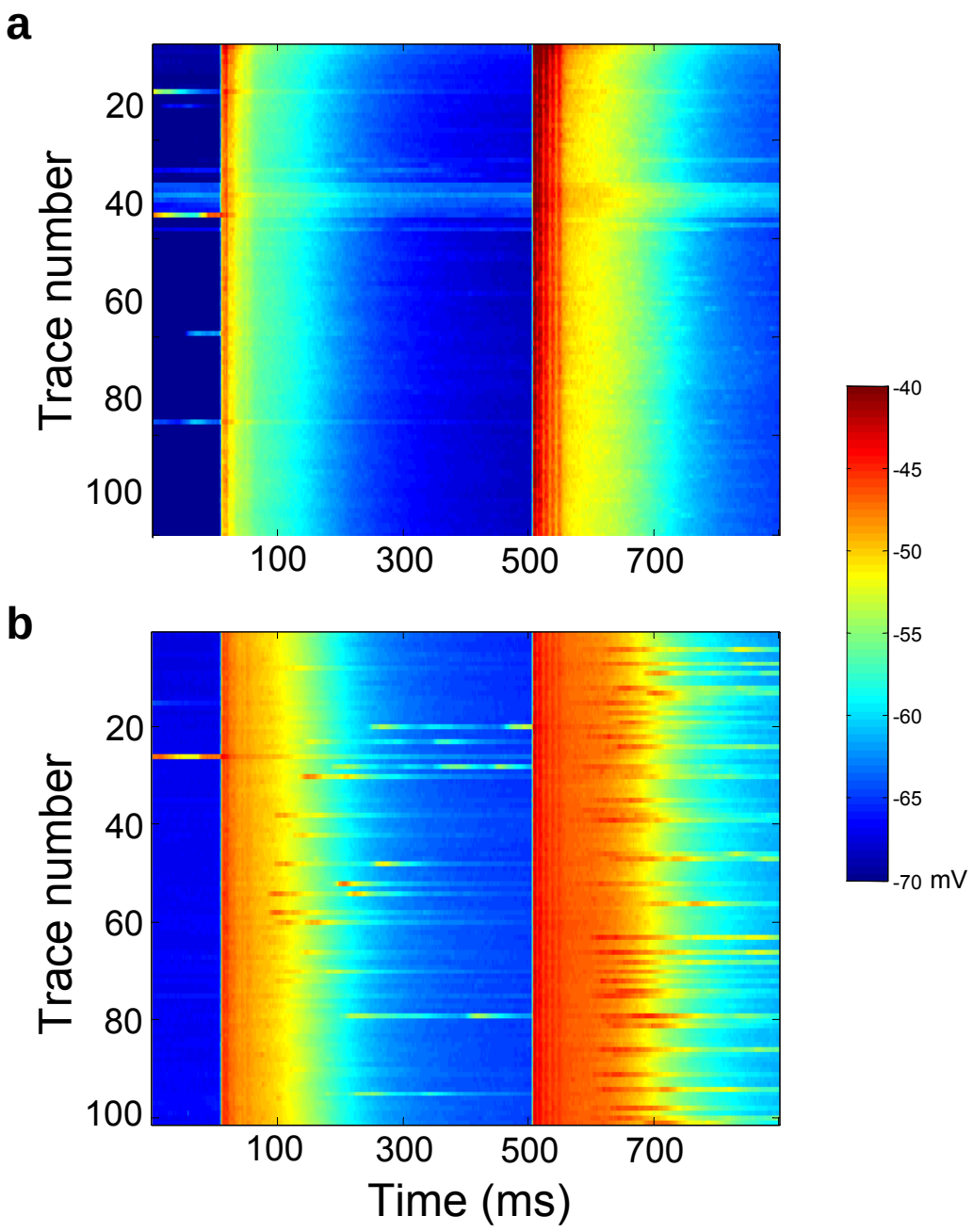


Supplementary Figure 1. Timing of polysynaptic activity also reflects training interval in "on-rig" experiments. Networks were trained for 2 hours on the recording rig with sequential activation of the E1 and E2 electrode pathways using either a 50 ms or 200 ms interval (see Figure 2a,b). (**a**) Normalized voltagegrams (upper panels) and derivatives of the raw voltage response (lower panels) of the N2 neurons in response to a single pulse from the E1 electrode (indicated by arrow); for the derivatives range shown is 0 in blue, to 1 in red, and negative values are represented as 0; n = 14 and 15 for 50 ms and 200 ms interval trained, respectively). (b) Cumulative distribution (K-S test, $P < 0.001$) of polysynaptic event onset times for the E1→N2 pathway in networks trained with a 50 (black) or 200 ms (grey) interval.



Supplementary Figure 2. Effects of APV on training with a 50 ms interval. (**a**) Raw data expressed in voltagegrams from groups trained with a 50 ms interval in the absence (Control) or presence of 50 μM APV. Drug was washed out prior to recordings (n = 17 and 14 for control and APV groups, respectively). Traces above the voltagegrams represent the average of all traces. (**b**) Cumulative distribution of polysynaptic events occurring between 30 and 400 ms after the test pulse in response to the E1→N2 pathway (control in black, APV in gray; K-S test, $P < 0.005$). Note that here, because of the low N, we have included up to four cells from each slice (two N1 and two N2 cells). While the difference in neural dynamics between the control and APV groups suggests a role for NMDA receptors in the training-induced changes in temporal structure interpretation of this result is complicated by the fact that APV altered the dynamics during training, or induced homeostatic forms of plasticity that interfered with the induction network plasticity[7].



Supplementary Figure 3. Voltage responses during training. During training each of the input bursts generally elicited a strong depolarizing response and relatively little polysynaptic activity. In a few of the 100 x 500 ms experiments we recorded during training. Shown is an example from a recording that lasted the two hour training period. (a) The first 100 training traces (0-16 minutes, 1 trace every 10 sec). (b) The last 100 training traces (1:44 to 2:00 hours).