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Plasticity of recurring spatiotemporal activity patterns in cortical networks

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Abstract

How do neurons encode and store information for long periods of time? Recurring patterns of activity have been reported in various cortical structures and were suggested to play a role in information processing and memory. To study the potential role of bursts of action potentials in memory mechanisms, we investigated patterns of spontaneous multi-single-unit activity in dissociated rat cortical cultures *in vitro*. Spontaneous spikes were recorded from networks of approximately 50 000 neurons and glia cultured on a grid of 60 extracellular substrate-embedded electrodes (multi-electrode arrays). These networks expressed spontaneous culture-wide bursting from approximately one week *in vitro*. During bursts, a large portion of the active electrodes showed elevated levels of firing. Spatiotemporal activity patterns within spontaneous bursts were clustered using a correlation-based clustering algorithm, and the occurrences of these burst clusters were tracked over several hours. This analysis revealed spatiotemporally diverse bursts occurring in well-defined patterns, which remained stable for several hours. Activity evoked by strong local tetanic stimulation resulted in significant changes in the occurrences of spontaneous bursts belonging to different clusters, indicating that the dynamical flow of information in the neuronal network had been altered. The diversity of spatiotemporal structure and long-term stability of spontaneous bursts together with their plastic nature strongly suggests that such network patterns could be used as codes for information transfer and the expression of memories stored in cortical networks.

Introduction

Cortical structures produce recurring spatiotemporal activity patterns, which could potentially be involved in cortical information processing and storage. Beggs and Plenz (2004) enumerated the requirements for recurring activity patterns to serve as substrates of memory. In order to represent information, the activity patterns should (1) exhibit long-term *stability* over hours, (2) possess millisecond *temporal precision* and (3) should occur in *diverse varieties* (Beggs and Plenz 2004). They demonstrated that these properties were satisfied by recurring patterns of local field potentials (neuronal avalanches) observed in cultured cortical slices. Induction of synaptic plasticity is widely believed to be necessary for information storage (Martin *et al* 2000). Hence, to ascertain the role of repeated activity patterns in memory mechanisms it is important to study their *functional plasticity*, in addition to the three properties mentioned above.

Several laboratories have demonstrated the presence of recurring spontaneous patterns of action potentials in various brain structures and recommended these patterns as mechanisms for information transmission and storage. *In vivo* recordings from the rat hippocampus and macaque cortex revealed neural ensemble activity patterns which were repeated in a temporally

compressed fashion during slow wave sleep (Nadasdy 2000, Hoffman and McNaughton 2002), possibly to consolidate the information acquired during active behavioral episodes (Wilson and McNaughton 1994, Nadasdy *et al* 1999). Repeating precisely timed ‘motifs’ of Ca^{2+} signals and post-synaptic potentials have been reported in spontaneous activity in cortical networks both *in vivo* and *in vitro* (Cossart *et al* 2005, Ikegaya *et al* 2005), suggesting that these dynamic ensembles are substrates of information storage and flow in cortical networks. Stable spatiotemporal attractors in *sequences* of culture-wide bursts (‘superbursts’) have been found in dissociated cortical cultures, indicating that dissociated networks can exhibit precise spatiotemporal activity patterns previously thought to require a specific network structure (Wagenaar *et al* 2006c). Although these studies provide evidence for repeating patterns in the brain and suggest that they are expressions of memory, the effect of external stimulation on these patterns cannot be directly extrapolated from these results.

In order to investigate the plasticity of spontaneous activity in neuronal networks as a substrate for information storage, we studied the effects of electrical stimulation on spontaneous burst patterns exhibited by dissociated cortical networks. Spontaneously active networks *in vitro* provide a unique model to study and manipulate the network dynamics of intrinsic spontaneous activity, for long periods up to many months (Potter and DeMarse 2001), through electrical recording and stimulation, without the usual *in vivo* confounds of anesthesia and uncontrolled sensory input. Although spontaneous and evoked ensemble activity patterns are now being studied in awake animals *in vivo*, these studies are presently limited to a couple of electrodes (Jackson *et al* 2006) or local field potential recordings (Werk *et al* 2005). We propose that in order to better understand and harness the capabilities of the network, it is necessary to increase the bandwidth of possible inputs and outputs. Multi-electrode arrays (MEAs, figure 1(A)), allowing for simultaneous stimulation and recording of action potentials from thousands of neurons, present the ideal technology to increase input–output bandwidth. We found that tetanic stimulation changed the distribution of spontaneous burst patterns in dissociated cultures and induced the expression of some new patterns, while some other recurring patterns ceased to exist after the tetanus, indicating a change in the intrinsic tendencies of the network to express certain patterns.

Materials and methods

- Cell culture.** A suspension of neocortical cells was prepared by enzymatically and mechanically dissociating brains of day-18 rat embryos. This was plated on MEAs at high density to form a planar network 1–3 cells thick. Timed-pregnant Sasco Sprague-Dawley rats (Charles River) were euthanized with isoflurane according to NIH-approved protocols. Embryos were removed and euthanized by chilling and decapitation. The entire neocortex, excluding the hippocampus, was dissected in the Hanks Balanced Salt solution (HBSS, Invitrogen, Carlsbad, CA) under sterile conditions. After enzymatic digestion in 2.5 U mL^{-1} Papain (Roche Scientific, Indianapolis, IN) in Segal's medium (Banker and Goslin 1998) for 20 min, cells were mechanically dissociated by 6–9 passes through a 1 mL pipette tip (Potter and DeMarse 2001), in the Neurobasal medium (Invitrogen) with B27 (Invitrogen), 0.5 mM glutamax (Invitrogen) and 10% horse serum (Hyclone, Logan, UT). Cells were passed through a $40 \mu\text{m}$ cell strainer (Falcon, Bedford, MA) to remove large debris and then were centrifuged at $150 \times g$ onto 5% bovine serum albumin (BSA) in phosphate buffered saline (PBS). The pellet of cells was resuspended and 50 000 cells were plated in a $20 \mu\text{L}$ drop of Neurobasal on pre-coated MEAs, at a density of ~ 3000 cells per mm^2 , which results in a thin ($15\text{--}30 \mu\text{m}$) but three-dimensional network 1–3 cell bodies thick, with neurites above, below and between somata (determined by multiphoton microscopy, data not shown). MEAs were pre-coated with polyethylene imine (PEI, Sigma, St Louis, MO) and laminin (Invitrogen) as previously described

(Potter and DeMarse 2001). After 30 min of incubation, 1 mL of Neurobasal media was added to each culture dish. After 24 h, the Neurobasal medium was replaced by the feeding medium adapted from Jimbo *et al* (1998) (Dulbecco's modified Eagle's medium (DMEM, Irvine scientific, Santa Ana, CA), 10% horse serum (Hyclone), 0.5 mM glutamax (Invitrogen) and 1% sodium pyruvate (Sigma)). Cultures were maintained in an incubator at 35 °C, 65% RH, 5% CO₂ and 9% O₂. The culture medium was exchanged with a fresh feeding medium every seven days. Cultures were maintained in dishes sealed with a gas-permeable Teflon membrane (Potter and DeMarse 2001) to prevent infection and evaporation. The use of Teflon-sealed dishes allows for the maintenance of the incubator at 65% humidity, making it an electronics-friendly environment. All experiments were performed inside the incubator, ensuring long-term stability of our recordings. All recordings were done on two–four week old cultures.

- *Recording system.* Electrical signals were recorded through a square array of 60 titanium nitride electrodes (Multichannel systems, Reutlingen, Germany). Fifty nine of the electrodes were 30 μ m in diameter and 200 μ m apart. The signals from these were referenced to those of a larger ground electrode. In all analyses, the ground electrode's signal was included, appearing as a channel with no spikes. After 1200 $\times$ amplification, signals were sampled at 25 kHz using Multichannel Systems data acquisition card (MCCard). Data acquisition, spike detection, artifact suppression (Wagenaar and Potter 2002) and visualization were controlled using our open-source MeaBench software (Potter *et al* 2006) which allows for detecting spikes as early as 2 ms after stimulation (Wagenaar and Potter 2002). Spikes were detected online by thresholding at 5 $\times$ RMS noise.
- *Stimulation system.* Stimulus pulses were delivered using our custom-built 60-channel stimulator (Wagenaar and Potter 2004). Biphasic voltage-controlled rectangular pulses (400 μ s per phase, positive phase first, 600–800 mV) were used, since these were found to be the most effective at eliciting neural responses (Wagenaar *et al* 2004).

Experiment protocols

Before the start of each experiment, each of the 59 recordable electrodes was stimulated ten times with a 600 mV, 400 μ s, biphasic stimulation pulse at 1 Hz. The electrodes that showed the highest network firing rate in the first 200 ms after stimulation were chosen as candidate electrodes. Two out of these candidate electrodes were chosen arbitrarily for delivering electrical stimulation for the duration of one experiment.

Activity-dependent changes in stimulus-evoked responses as a result of rapid-fire focal stimulation (called tetanus) have been successfully used to demonstrate plastic changes both in *in vitro* (Bliss and Lomo 1973, Berry *et al* 1989, Kirkwood and Bear 1994, Castro-Alamancos *et al* 1995, Bi and Poo 1999) and *in vivo* (Mulder *et al* 1997, Werk and Chapman 2003). We used tetanic stimulation to induce functional changes in spontaneously bursting dissociated cultures. Spontaneous activity was recorded for 3 h (period *Pre*). Tetanization consisted of a train of electrical pulses (as described above) at 20 Hz, applied simultaneously on two electrodes for 15 min. Another recording of spontaneous activity for 3 h followed this treatment (period *Post*). The tetanization was unusually long to increase the likelihood of inducing plasticity. This experiment was repeated five times on four cultures. One culture was tested twice with different tetanus electrode pairs. The interval between these two experiments was 4 h.

Analysis

- Burst detection.* Network-wide spontaneous bursts were identified by a burst detector algorithm described elsewhere (Wagenaar *et al* 2005). Briefly, any 100 ms window with more than five spikes on one electrode was considered to be a part of a burst. A burst consisted of multiple single electrode bursts overlapping in time. The onset of a burst was defined as the first time before the peak of the burst when the array-wide spike rate during a burst was 20% of the peak firing rate during the burst (figure 2 (A)). The offset of a burst was defined as the last time after the peak of the burst when the array-wide firing rate was 20% of the peak firing rate during the burst (figure 2 (A)).
- Classification of bursts depending on the burst size.* Bursts with distinct sizes were generally observed. In order to cluster the spatiotemporal patterns of bursts without the bias of size, we classified bursts into two groups, ‘small’ and ‘big’, based on the total number of spikes within each network-wide burst. For each experiment, a histogram of the total number of spikes in every burst in the experiment was generated (figure 1(B)). Two distinct peaks were observed in the histograms in all experiments. The threshold for classifying bursts was determined as the minimum point between the two peaks (figure 1(B)). Bursts having a number of spikes below the threshold were considered ‘small’ and bursts having a number of spikes above the threshold were considered ‘big’.
- Calculation of the burst activity matrix (BAM).* For each burst, a firing rate time histogram was generated for each recording electrode by counting the number of spikes within a 100 ms sliding time bin, moving from the burst onset to the burst offset, with a time step of 10 ms. Histograms of all bursts for a given experiment were made to be of the same length (that of the longest burst) by padding the shorter histograms with zeros at the ends. The burst activity matrix (BAM) for each burst was then constructed by appending (in order of their hardware numbers) the histograms from the 60 recording electrodes, together to form a 1 by 60 N matrix of action potential histograms, where N is the number of time bins in the longest burst (figure 2(A)). The BAM was calculated for big bursts and small bursts separately, and was the same length for all big bursts in a given experiment or all small bursts.

Before settling on 10 ms as the time step to slide the spike histogram window, we tried others. The BAM was calculated with different temporal resolutions by using time steps of 10 ms, 5 ms and 1 ms and clustered using methods described below. No significant difference was found in the number of clusters determined using different temporal resolutions. In order to obtain finer resolution during periods of high firing rates, we also tried variable step sizes that were inversely proportional to the instantaneous firing rates. Again, no significant difference was observed in the number of clusters determined. Therefore, a resolution of 10 ms was selected since this would reduce the dimensions of the BAM and thus lessen the computational time.
- Clustering of BAMs.* The method used for clustering the BAMs was a correlation-based technique with a global contrast function, from Beggs and Plenz (2004), who used the algorithm to cluster local field potentials (LFPs) recorded from cortical slices. It derives from other similarity-based neural data clustering techniques (Fellous *et al* 2004). We applied the same clustering algorithm to investigate the presence of the spatiotemporal structure within big bursts and small bursts separately (see supplementary material available at stacks.iop.org/PhysBio/4/181, figure S1). Bursts with similar BAMs were grouped together, and the optimal number of groups was determined as the peak of a similarity contrast function (see supplementary material).

- *Occurrence and integrated occurrence.* The word ‘occurrence’ is used here to represent when bursts with different spatiotemporal patterns, determined by the clustering results, occur (figure 2(B)). An *integrated occurrence* vector (figure 2(B)) was created by counting the total number of *occurrences* of each cluster in a 15 min time window with a time step of 30 s. One time step of this vector represents the distribution of burst types during a 15 min period. Changes in the network's tendencies to express certain spatiotemporal activity patterns can be quantified by changes in *integrated occurrence* over time.
- *Statistics on integrated occurrence.* The *integrated occurrences* were calculated for both the *Pre* (before the tetanus) and the *Post* (after the tetanus) periods for all experiments. The *Pre* period was split into two periods of equal duration *Pre1* and *Pre2*, separated by a sham interval equal to the duration of the tetanus (15 min). The *Post* period was split into two periods of equal duration *Post1* and *Post2*, also separated by a 15 min sham period. The centroids of *integrated occurrences* in periods *Pre1*, *Pre2* and *Post1* were calculated (figure 2(C)). The mean Euclidean distance of *integrated occurrences* in *Pre2* to the centroid of *integrated occurrences* in *Pre1* (*C*, for change) was compared to the mean Euclidean distance of *integrated occurrences* in *Pre1* to their own centroid (*D*, for drift). The ratio of change to drift, *C/D*, was used to quantify the change from *Pre1* to *Pre2* before the tetanus (no change if this ratio is ~1) (Chao *et al* 2007). Similarly, *C/D* between *Pre2* and *Post1* was used to quantify the change across the tetanus, and *C/D* between *Post1* and *Post2* was used to quantify the stability of tetanus-induced changes. The significance of the tetanus-induced change in *integrated occurrences* was quantified by testing whether *C/D* across the tetanus (*Pre2–Post1*) was significantly greater than *C/D* before the tetanus (*Pre1–Pre2*), by using Wilcoxon's rank sum test. *C/D* after the tetanus (*Post1–Post2*) was also compared to *C/D* across the tetanus (*Pre2–Post1*) to evaluate the stability of induced changes after the tetanus.
- *Shuffling methods.* The BAMs of spontaneous bursts were shuffled in various ways to determine whether their structure was more significant than expected by chance. ‘Frame shuffling’ randomly rearranged the sequence of time bins within each BAM. This method changes the temporal order while preserving the spatial information. ‘Electrode shuffling’ randomly rearranged the electrode order within each BAM. This method changes the spatial order while preserving the temporal information. ‘Matched shuffling’ randomly rearranged the activity on each electrode within each time bin within each BAM. This method changes both the temporal and the spatial structures of the data. Unlike ‘frame shuffling’ and ‘electrode shuffling’, ‘matched shuffling’ does not change the firing rate on each electrode. ‘Spike swapping’ repeatedly exchanged the electrode numbers of a randomly selected pair of spikes. This method retains the same spike times and the same distribution of the total number of spikes at each electrode, but removes the biologically induced correlations between spike times (Rolston *et al* 2007). ‘Spike jittering’ perturbed each spike's time by a Gaussian random noise (with zero mean and 10 ms or 20 ms standard deviation). This method preserved the firing rates at each electrode, but slightly altered the time intervals between spikes. The average correlation values of every pair of BAM clusters were calculated for each shuffled dataset, and compared with results from the original non-shuffled dataset (Rolston *et al* 2007).
- *Quantifying tetanus-induced changes in the information content of cultured networks.* The amount of new information created by the tetanus was defined as the number of new clusters that occurred in the *Post* period but never in the *Pre* period. Note that this measure does not quantify any information that might be represented in the detailed spatiotemporal structure carried within individual bursts, nor the

specific timing of the occurrences of bursts. The amount of information eliminated by the tetanus was defined as the number of clusters that occurred in the *Pre* period but did not occur in the *Post* period. We define the changes in the information content of a network due to tetanic stimulation as the formation and deletion of the burst patterns, i.e. alterations in the network's repertoire of spontaneous activity patterns. The scale of the tetanus-induced burst pattern formation and/or deletion, ΔI , was defined as the percentage change of the number of newly formed and/or disappeared clusters after the tetanus. Only clusters that occurred at least five times in the 3 h *Pre* and *Post* periods were included in the analysis. We define $C_{Pre} = \{x: x \in \text{cluster indices appearing in } Pre, \text{ occurrences of } x > 5\}$ and $C_{Post} = \{y: y \in \text{cluster indices appearing in } Post, \text{ occurrences of } y > 5\}$; then

$$\Delta I = \left(1 - \frac{|C_{Pre} \cap C_{Post}|}{|C_{Pre} \cup C_{Post}|} \right) \times 100\%,$$

- where $\cap$ and $\cup$ represent the intersection and union of two sets, respectively, and $|\cdot|$ represents the number of elements in the set. Furthermore, the scale of the burst patterns that were retained after the tetanus, $\overline{\Delta I}$, which denotes the amount of information content preserved across the tetanus, is defined as the complement of ΔI :

$$\overline{\Delta I} = \frac{|C_{Pre} \cap C_{Post}|}{|C_{Pre} \cup C_{Post}|} \times 100\% = 100\% - \Delta I.$$

- *Burst initiation site.* Bursts originate from various locations in the network, termed as 'burst initiation sites', and then propagate to the entire network (Maeda *et al* 1998, Eytan and Marom 2006). In order to visualize the changes in network dynamics, in addition to the BAM, we used burst initiation properties as a measure to quantify the tetanus-induced change. We define the burst initiation site as the first electrode that spiked next after the burst onset (burst onset defined earlier, figure 2(A)). For each 15 min time window (time step = 30 s) during an experiment, the burst initiation probability (BIP) was calculated for every electrode. The BIP vector was computed as the number of times that bursts were initiated at each electrode, normalized by the total number of bursts in the time window. This results in a 1 by 60 BIP vector for every time window (summation of these 60 values equals 1). The same statistic (as used to evaluate the *integrated occurrence* of the BAM) was applied on BIP vectors to quantify the changes before and across the tetanus.

Results

General properties of spontaneous network-wide bursts in dissociated cultures

Dissociated cortical networks cultured on multi-electrode arrays (figure 1(A)) (Gross *et al* 1977, Pine 1980) express spontaneous network-wide bursting, persisting for the lifetime of the culture (Murphy *et al* 1992, Gross *et al* 1993, Canepari *et al* 1997, Voigt *et al* 1997, Gross and Kowalski 1999, Corner *et al* 2002, van Pelt *et al* 2005, Wagenaar *et al* 2006a). Spontaneous bursts occur in different sizes and patterns (Wagenaar *et al* 2006a) and can be classified in various ways. A spike raster plot for 5 min of spontaneous recording is shown in figure 1(C). Culture-wide bursts were detected as periods of an increased firing rate across most of the active electrodes (figure 1(C)). Since there was a clear bimodal distribution in the burst size for all tested cultures (figure 1(B)), bursts were classified into two categories, big and small bursts (figure 1(C)), with the threshold set at the minimum in the burst size histogram (figure 1(B)). A burst activity matrix (see Materials and Methods) was generated for each burst by

counting the number of spikes on each electrode within the length of the burst using a 100 ms sliding time window (time step = 10 ms) (figure 2(A)).

Spontaneous bursts express different spatiotemporal structures

To explore the presence of temporal and spatial structures within spontaneous bursts, the correlation between BAMs (see Materials and Methods) of all bursts in *tetanus experiments* was determined (see Materials and Methods) and hierarchical clustering algorithms were used to cluster the BAMs of bursts having the least distance in correlation space into groups (see supplementary material). To visualize these clusters the correlation matrix was sorted (see figure S1, supplementary materials stacks.iop.org/PhysBio/4/181), resulting in regions of high correlation values along the diagonal showing spontaneous bursts that exhibit a high similarity in their spatiotemporal structure (figure 3(A)).

The data were shuffled in various ways to verify that the structure in spontaneous bursts was not an artifact of the clustering algorithm (see Materials and Methods). On shuffling the data, both temporally and spatially (Beggs and Plenz 2004), the correlation values in the shuffled data were significantly lower than those in the original dataset (figure 3(B), $p < 0.001$). Thus, the lack of high correlation values in the shuffled data shows that the original data consisted of various spatiotemporal activity patterns that have a much more specific structure than would be expected by chance. In five experiments on four cultures, we identified an average of 60 ± 8 (mean $\pm$ SEM) clusters of small bursts and 158 ± 23 clusters of big bursts per culture. There were an average of 4128 ± 553 spontaneous bursts per experiment (five experiments on four cultures).

Spontaneous bursts as indicators of tetanus-induced network plasticity

Bursts at the single cell and population level have been suggested as a reliable neural code since they can facilitate neurotransmitter release at a synapse more reliably than a single spike could (Lisman 1997). Since we found a specific structure in spontaneous bursts (figure 3), we sought to use changes in spatiotemporal patterns of spontaneous culture-wide bursts as a measure for detecting stimulus-induced functional plasticity in the network.

Bursts in 3 h periods before and after the tetanus (see Materials and Methods: Experiment Protocols) were classified into big and small bursts depending on their size (figure 1(B)) and clustered into separate types using a correlation-based clustering algorithm. This analysis showed that spontaneous bursts occurred in clusters having different spatiotemporal patterns that remained stable during the 3 h period before the tetanus (figure 4). Stability of occurrence of BAM clusters for spontaneous bursts, in the absence of any stimulation, persisted for at least 9 h (the longest experiment, not shown). The structure within temporally spaced BAM clusters of spontaneous small and big bursts (arrowheads in figure 4) was conserved over time for all five experiments (one representative experiment shown in figure 5). Thus, precisely timed dynamic patterns within spontaneous bursts recurred with high fidelity and remained stable over hours. Wagenaar *et al* (2006b) documented the presence of a recurring spatiotemporal structure in *burst sequences* ('superbursts') in dissociated cortical cultures, which remained stable for hours. The present study was not restricted to 'superbursts'. We found a highly conserved structure for all bursts occurring during the recording period. The stability of burst patterns represents the tendency of the culture to express certain types of bursts spontaneously, and we sought to determine whether a strong (tetanic) stimulus, representing repetitive salient sensory input, would change this tendency.

Any change in the *distribution* of burst clusters at different intervals was quantified by the change in the *integrated occurrence* of BAM clusters (figure 2(C)). The stability of *integrated occurrence* of BAMs of spontaneous bursts was determined on two 9 h long spontaneous

recordings without any electrical stimulation. The average change in the *integrated occurrence* of BAMs across sequential 3 h periods was not significant ($p = 0.17$, Wilcoxon's rank sum test).

To quantify whether the change in the distribution of burst patterns across the tetanus was more than their drift in the periods of no stimulation, the change in the *integrated occurrence* of BAM clusters across the tetanus was compared to their change across a 'sham' period of equal duration to the tetanus (see Materials and Methods, *Statistics on Occurrence*). The burst patterns changed significantly across the tetanus but not within the spontaneous period before it (figure 4(C)). The average change across the 'sham' period (C/D between *Pre1* and *Pre2* periods) for five *tetanus experiments* was not significantly greater than 1 ($p = 0.063$). $C/D = 1$ indicates that the distribution of burst clusters did not change across the periods (see Materials and Methods). In addition, the average change in the distribution of burst patterns across the tetanus (C/D between *Pre2* and *Post1* periods) for five experiments was significantly greater than C/D before the tetanus ($p < 0.01$, Wilcoxon's rank sum test) (figure 4(C)). This indicates that the tetanus induced significant redistribution in the patterns of bursts expressed by the culture. Following the tetanus, though the cluster distributions varied significantly in a 30 min period immediately after the tetanus, the change across the periods *Post1* and *Post2* was lower than the change across the tetanus ($p < 0.05$, Wilcoxon's rank sum test). This is consistent with the findings in a simulated network of 1000 integrate-and-fire neurons, where strong tetanic stimulation resulted in the network settling down into a new state after a short transition period, during which the network drifted to its new state (Chao *et al* 2005).

Tetanus-induced changes in the information content of cultured networks

As shown above, spontaneous burst patterns possessed high temporal fidelity and a specific spatiotemporal structure, which could be modified by tetanic stimulation (figures 4 and 5). Thus, they satisfy many of the requirements for a memory substrate (Martin *et al* 2000, Martin and Morris 2002, Beggs and Plenz 2004) and can be viewed as containing information. Although not done here, we previously used specific burst patterns to determine the behaviors of simulated animals and robots (DeMarse *et al* 2001, Bakkum *et al* 2004), imbuing them with specific meanings. To quantify the changes in the information content of the network as indicated by the changes in the occurrences of bursts after the tetanus, we measured the information modified or preserved across the tetanus (see Materials and Methods). This simple quantification allows us to estimate the extent of plastic changes caused by the external stimulus. The tetanus caused a change in network dynamics resulting in the creation or loss of an average of $70.91 \pm 15.74\%$ BAM clusters of big bursts (mean $\pm$ SEM) and $55.33 \pm 8.64\%$ BAM clusters of small bursts for $N = 5$ *tetanus experiments*. We take this as a measure of the amount of information in burst tendencies that was modified by the tetanus. Thus, the tetanus induced the creation of new patterns while some other existing patterns were annihilated (figure 4). In the absence of stimulation, an average of 2.9% BAM clusters of big bursts were modified across pre-tetanus periods *Pre1* and *Pre2* and 4.5% BAM clusters of big bursts were modified across the periods *Post1* and *Post2* after the tetanus, indicating that the burst patterns were robust in the absence of external perturbations.

We also quantified the amount of information retained across the tetanus as the number of BAM clusters that occurred in the *Post* period after the tetanus given that they had occurred in the *Pre* period before the tetanus. An average of $29.1 \pm 15.74\%$ BAM clusters of big bursts and $44.67 \pm 8.63\%$ BAM clusters of small bursts were retained across the tetanus in five *tetanus experiments*. This quantity represents the fraction of clusters that were preserved across the tetanus, but does not indicate whether the temporal distribution (*occurrence*) of the 'preserved clusters' was also retained across the tetanus. In order to quantify the stability of the 'preserved clusters' across the tetanus, the *integrated occurrences* of these clusters were compared for the

Pre and *Post* periods. There was no significant change in the *integrated occurrence* of the 'preserved' clusters across the tetanus ($p = 0.55$, Wilcoxon's rank sum test).

Tetanic stimulation caused changes in the locus of burst initiation

In addition to the BAM, we quantified functional changes in the network by tracking tetanus-induced changes in the burst initiation site (see Materials and Methods). Typically, spontaneous bursts originate from various locations in the networks, termed burst initiation sites, and spread to the entire network. Eytan and Marom (2006) identified early-to-fire neurons during the start of the burst, and these initiation areas were found to be characterized by high neuronal density and recurrent excitatory and inhibitory connections (Feinerman *et al* 2007). Hence, changes in the burst initiation site could imply a change in the network topology.

We quantified the probability that neurons around any particular electrode tend to fire at burst onsets, and found that this BIP (see Materials and Methods) was stable if no external stimulation was delivered, but was changed significantly by tetanic stimulation (figure 6(B), $p < 0.05$, Wilcoxon's rank sum test). Hence, the tetanus changed not only the spatiotemporal dynamics of burst patterns (figure 4(C)), but also the locus of the neurons involved in triggering bursts, suggesting that a long, repetitive artificial sensory input substantially altered the functional connectivity in cultured cortical networks.

Discussion

Synchronized or correlated activity within neuronal networks has been proposed as a likely mechanism for encoding information (for a review, see Rieke (1997)). Building on this idea, we demonstrated that spontaneous bursts *in vitro* occurred in diverse but recurring patterns that were stable for hours in the absence of electrical stimulation, and could be altered significantly by a strong tetanus applied simultaneously on two electrodes (figure 4, $p < 0.01$). Furthermore, the loci of burst initiation were changed by the tetanus (figure 6), suggesting that tetanic stimulation caused alterations in the network connectivity. Such changes could entail morphological changes, which are necessary for long-term plastic changes, like long-term potentiation (Matsuzaki 2007). Simultaneous cellular level imaging with extracellular recording might provide further insight into the anatomical changes associated with functional plasticity of spontaneous burst patterns (Potter *et al* 2006). *In vitro* systems are especially useful for such detailed studies since they provide enhanced accessibility to network properties, across time scales of milliseconds to months, compared to animal models. Other multi-electrode array studies on tetanus-induced plasticity in dissociated cultures used responses to single electrode stimulation to quantify functional changes (Jimbo *et al* 1998, Tateno and Jimbo 1999), but we found that these responses vary with time even in the absence of tetanic stimulation (see figure S2, supplementary materials stacks.iop.org/PhysBio/4/181). In contrast, spontaneous bursts occurred in spatially diverse patterns that were comparatively stable until perturbed by strong external stimulation, like a tetanus.

Bursts as information carriers in cortical networks

Single neuron bursts have been proposed to be reliable coding elements (Lisman 1997, Izhikevich *et al* 2003). Recordings from area MT of macaque monkey have shown that the burst rate was on average a more sensitive measure of the visual stimulus direction than the total number of spikes (Bair *et al* 1994). Models of bursting in pyramidal neurons have shown that the spike count of a burst encodes the rate of increase of inputs (Kepecs *et al* 2002). These studies propose that bursts are a special neural code and carry information about input characteristics (Kepecs and Lisman 2004).

Spontaneous bursts observed in cultured dissociated networks could be expressions of stored information; we have shown them to be stable over hours (figure 4), diverse enough to encode different memories (figure 5) and susceptible to stimulus-induced functional plasticity (figure 4). These properties exhibited by spatiotemporal patterns of spontaneous bursting satisfy the requirements of a memory substrate (Beggs and Plenz 2004). We analyzed the *occurrences* of spontaneous bursts in various clusters across the tetanus and showed that while some clusters were preserved across the tetanus others were modified by the tetanus. If spontaneous bursts with different spatiotemporal structures represent different memories, this study suggests that electrical stimulation can drive the expression of new patterns, while retaining some of the old patterns. Since spatiotemporally diverse bursts carry information about the network state, we suggest that a burst with a specific spatiotemporal structure could be used by the brain as an initiator of a specific movement if connected to an efferent system. By artificially re-embodiment cultured networks in robotic or simulated animals, we can attach ensemble activity to real-world events (DeMarse *et al* 2001, Potter *et al* 2006) and quantify the information-carrying capacity of network activity patterns.

For example, we can classify an incoming burst as a new cluster or as one of the existing clusters by the similarity measure, and use *integrated occurrence* to generate a specific motor output in a hybrot (hybrid neural-robotic system) in real time. The sensory inputs from the hybrot's artificial body can be encoded as different stimuli fed back to the network to change the spatiotemporal patterns of bursts (Potter *et al* 2006). The plasticity-inducing artificial sensory input to our cultures in the present experiments was very simple, i.e. repetitive tetani delivered to two electrodes. More complex stimuli could potentially induce specific, predictable changes in burst tendencies. We expect this to be a fruitful approach for studying memory encoding mechanisms at the *network* level.

Bursts as dynamic attractors in cortical networks

Spontaneous bursts with different but stable BAM patterns could represent different intrinsic spatiotemporal tendencies of the network. Our modeling study of network activity in a network of 1000 integrate-and-fire neurons revealed attractors in the network intrinsic synaptic state, represented by network synaptic weights, and a corresponding set of stable spontaneous bursts (Chao *et al* 2005). If the simulated network was driven from one attractor to another (by a tetanus), the spontaneous burst pattern was also changed. In the present experiments using living networks, the change of *occurrence* of spontaneous bursts with different BAMs after tetanus suggests that the network was driven from one attractor in the synaptic weight space to a different one by the tetanus. Precisely timed sequences of a single-unit activity were reactivated with high temporal precision in cortical networks both *in vivo* and *in vitro*, and these repeated sequences have been suggested to represent attractors in the cortex (Ikegaya *et al* 2004, Rolston *et al* 2007).

Here, BAMs of spontaneous bursts consisting of specific sequences of network activity patterns were found to recur over hours. The robust spatiotemporal activity patterns within bursts could represent attractors in dissociated cortical cultures. The steady occurrence of a set of attractors over hours could correspond to a stable underlying intrinsic state of the network. This intrinsic state of the network could be modified by tetanic stimulation (or sensory input *in vivo*) resulting in a new set of attractors, which represents new information in the network.

Comparison with previous work

Spontaneous burst patterns were stable for hours in the absence of external stimulation (maximum recording period was 9 h). This finding is contrary to Sasaki *et al* (2007) who demonstrated that calcium flux patterns in cultured hippocampal slices were 'metastable' over a maximum recording period of 30 min; a pattern was rarely repeated. In addition, weak

electrical stimulation did not perturb the network state more than changes that occurred spontaneously (Sasaki *et al* 2007). Our results provide evidence for the opposite, perhaps due to differences in our preparations or the stimuli delivered. We have the capability of non-invasively recording single action potentials for much longer (up to months, Potter and DeMarse 2001), allowing us to observe the long-term stability of the activity patterns. Our stronger (longer duration) stimulation caused drastic state changes in the network dynamics (figure 4) showing that network attractor-like states, represented by spontaneously repeating patterns, are robust and might need a sufficiently strong stimulation to push the network to a different state (attractor).

The finding that cortical networks express spatiotemporally diverse stable patterns was not unexpected. Other studies on spontaneous activity in cortical slices without tetanic stimulation have shown that spatiotemporal patterns of activity, termed neuronal avalanches, were stable for over 10 h (Beggs and Plenz 2004). These stable recurring patterns in local field potentials (LFPs) lasted tens of milliseconds. The spatiotemporal patterns of bursts in dissociated cortical cultures described here typically last much longer (100 ms–1 s) than avalanches in slices do. Unlike neuronal avalanches, the size distributions of spontaneous bursts (defined here as the number of spikes in a specified time bin) were bimodal and did not follow a power law (supplementary materials, figure S3 stacks.iop.org/PhysBio/4/181). Similar bimodal distribution for burst size (number of electrodes) and burst duration in dissociated cortical cultures has been reported elsewhere (Eytan and Marom 2006). In dissociated cortical cultures, spatiotemporally diverse burst activity motifs have been thought to result from varied connectivity architecture within the network (Volman *et al* 2005). These highly repeatable ensemble patterns could arise from strongly connected neuronal subgroups with different group properties (Izhikevich *et al* 2004). Segev *et al* (2004) have described correlated spontaneous burst patterns in dissociated cortical networks (Segev *et al* 2004), but the study did not attempt to investigate the effects of external electrical stimulation on these patterns. Local chemical stimulation with picrotoxin (GABA antagonist) resulted in the formation of new patterns which persisted for days (Baruchi and Ben-Jacob 2007). We extend the previous findings by demonstrating that the distribution of spontaneous burst clusters changed significantly after electrical stimulation, resulting in the creation of new patterns and elimination of some existing ones.

Responses to single electrode (probe) stimulation pulses have been used to demonstrate functional plasticity in dissociated cultures (Jimbo *et al* 1998, 1999, Tateno and Jimbo 1999, Shahaf and Marom 2001, Ruaro *et al* 2005). However, we found significant variability in the responses to single electrode stimulation, which made it difficult to distinguish between tetanus-induced changes and intrinsic drift in the stimulus-evoked responses (figure S2). Other experiments on dissociated cultures, using several different protocols to induce network-level functional plasticity, failed to demonstrate significant changes unless spontaneous bursting was suppressed by elevated levels of extracellular Mg^{2+} (Wagenaar *et al* 2006b). Considering that cortical networks seem resistant to change, we used an unusually long tetanic train in the present study. The plating densities in our cultures were an order of magnitude higher than those in these studies, i.e. closer to *in vivo* densities if extended in the thickness dimension (Beaulieu 1993). Cultures with higher plating densities show faster development of network activity and more synchronized bursting than cultures with lower plating densities (Wagenaar *et al* 2006a). Possibly due to higher levels of ongoing activity in our densely plated cultures, we observed that stimulus-evoked responses continuously fluctuate with time making it more difficult to detect functional plasticity in evoked responses than in spontaneous bursts (Madhavan *et al* 2006). In simulated networks with high levels of ongoing activity, tetanus-induced changes did not last and the network returned to its pre-tetanus state (Chao *et al* 2005).

Comparison with *in vivo* studies

Spontaneous and evoked ensemble activity patterns are now being studied *in vivo* (Bragin *et al* 2000, Nicolelis 2003). Although acute extracellular recordings are possible *in vivo*, realizing chronic systems that can record single and multi-unit activity over periods of months to years has been limited by reactive tissue encapsulation of the implant (Turner *et al* 1999, Szarowski *et al* 2003) and difficulties in determining the precise location for placement of the electrodes (Brecht *et al* 2004). They have also been limited by not being able to stimulate with complex patterns on many of an array's electrodes, including recording electrodes. We made circuitry to surmount this problem *in vitro* (Wagenaar and Potter 2004), and are now making smaller versions for use *in vivo*. Densely plated cultures on MEAs allow for non-invasive recording and stimulation of neuronal networks with each electrode recording and influencing the activity of multiple neurons in the electrode's vicinity. Recently, some studies have demonstrated functional plasticity *in vivo* using electrical stimulation (Werk *et al* 2005, Jackson *et al* 2006), but these studies involved only a couple of electrodes or local field potential recordings. In contrast, the present study of the spatiotemporally diverse ensemble activity, using recordings of many-single-unit activity coupled with simultaneous stimulation on multiple electrodes over extended periods, can help understand the network-level rules of information storage in cortical networks, without the complexities of recording *in vivo*.

Conclusions and outlook

By following the spontaneous activity patterns in dissociated cortical cultures on MEAs, we found that network-level spatiotemporally diverse patterns of bursting activity are robust in the absence of external perturbations (electrical stimulation), suggesting that such stable patterns could be readouts of memories stored in cortical networks. Application of high frequency tetanic stimulation resulted in the manipulation of the spatiotemporal structure of recurring burst patterns; while some existing patterns were sustained, other new patterns emerged because of the stimulus. We propose that these diverse, recurring patterns of activity involving large ensembles of neurons could potentially carry information in neural circuits, by controlling behaviors.

Brains are essentially information-processing devices. Since electrical stimulation can alter enduring patterns in neuronal networks, it would be interesting to program the neuronal network to express certain patterns based on the structure of the input stimulation. While such studies are still in their infancy, they could potentially reveal crucial parameters for use in the design of closed-loop embodied systems such as hybros and neural prostheses that include both sensory and motor components. Easy manipulation and enhanced accessibility of dissociated cortical networks make MEA cultures the ideal platform to explore the effects of varied input patterns on the structure of output activity patterns. That said, the analyses used here are general measures of spatiotemporal activity patterns, not restricted to *in vitro* studies and could be applied to the analysis of multi-unit activity patterns *in vivo* obtained optically or electrically.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Bair W, Koch C, Newsome W, Britten K. Power spectrum analysis of bursting cells in area MT in the behaving monkey. *J Neurosci* 1994;14:2870–92. [PubMed: 8182445]
- Bakkum, DJ.; Shkolnik, AC.; Ben-Ary, G.; Gamblen, P.; DeMarse, TB.; Potter, SM. Removing some 'A' from AI: embodied cultured networks. In: Iida, F.; Pfeifer, R.; Steels, L.; Kuniyoshi, Y., editors. *Embodied Artificial Intelligence*. New York: Springer; 2004. p. 130–45.
- Banker, G.; Goslin, K. *Culturing Nerve Cells*. 2nd. Cambridge, MA: MIT Press; 1998.
- Baruchi I, Ben-Jacob E. Towards neuro-memory-chip: imprinting multiple memories in cultured neural networks. *Phys Rev* 2007;E 75:050901.
- Beaulieu C. Numerical data on neocortical neurons in adult rat, with special reference to the GABA population. *Brain Res* 1993;609:284–92. [PubMed: 8508310]
- Beggs JM, Plenz D. Neuronal avalanches are diverse and precise activity patterns that are stable for many hours in cortical slice cultures. *J Neurosci* 2004;24:5216–29. [PubMed: 15175392]
- Berry RL, Teyler TJ, Han TZ. Induction of LTP in rat primary visual cortex: tetanus parameters. *Brain Res* 1989;481:221–7. [PubMed: 2720377]
- Bi G, Poo M. Distributed synaptic modification in neural networks induced by patterned stimulation. *Nature* 1999;401:792–6. [PubMed: 10548104]
- Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 1973;232:331–56. [PubMed: 4727084]
- Bragin A, Hetke J, Wilson CL, Anderson DJ, Engel J Jr, Buzsaki G. Multiple site silicon-based probes for chronic recordings in freely moving rats: implantation, recording and histological verification. *J Neurosci Methods* 2000;98:77–82. [PubMed: 10837874]
- Brecht M, Fee MS, Garaschuk O, Helmchen F, Margrie TW, Svoboda K, Osten P. Novel approaches to monitor and manipulate single neurons *in vivo*. *J Neurosci* 2004;24:9223–7. [PubMed: 15496655]
- Canepari M, Bove M, Maeda E, Cappello M, Kawana A. Experimental analysis of neuronal dynamics in cultured cortical networks and transitions between different patterns of activity. *Biol Cybern* 1997;77:153–62. [PubMed: 9323864]
- Castro-Alamancos MA, Donoghue JP, Connors BW. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. *J Neurosci* 1995;15:5324–33. [PubMed: 7623155]
- Chao ZC, Bakkum DJ, Potter SM. Region-specific network plasticity in simulated and living cortical networks: comparison of the center of activity trajectory (CAT) with other statistics. *J Neural Eng* 2007;4:1–15. [PubMed: 17409475]
- Chao ZC, Bakkum DJ, Wagenaar DA, Potter SM. Effects of random external background stimulation on network synaptic stability after tetanization: a modeling study. *Neuroinformatics* 2005;3:263–80. [PubMed: 16077162]
- Corner MA, van Pelt J, Wolters PS, Baker RE, Nuytinck RH. Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks—an inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny. *Neurosci Biobehav Rev* 2002;26:127–85. [PubMed: 11856557]
- Cossart R, Ikegaya Y, Yuste R. Calcium imaging of cortical networks dynamics. *Cell Calcium* 2005;37:451–7. [PubMed: 15820393]
- DeMarse TB, Wagenaar DA, Blau AW, Potter SM. The neurally controlled animat: biological brains acting with simulated bodies. *Auton Robots* 2001;11:305–10. [PubMed: 18584059]
- Eytan D, Marom S. Dynamics and effective topology underlying synchronization in networks of cortical neurons. *J Neurosci* 2006;26:8465–76. [PubMed: 16914671]
- Feinerman O, Segal M, Moses E. Identification and dynamics of spontaneous burst initiation zones in unidimensional neuronal cultures. *J Neurophysiol* 2007;97:2937–48. [PubMed: 17287439]
- Fellous JM, Tiesinga PH, Thomas PJ, Sejnowski TJ. Discovering spike patterns in neuronal responses. *J Neurosci* 2004;24:2989–3001. [PubMed: 15044538]

- Gross GW, Kowalski JM. Origins of activity patterns in self-organizing neuronal networks *in vitro*. *J Intell Mater Syst Struct* 1999;10:558–64.
- Gross, GW.; Rhoades, BK.; Kowalski, JK. Dynamics of burst patterns generated by monolayer networks in culture Neurobionics: An Interdisciplinary Approach to Substitute. In: Bothe, HW.; Samii, M.; Eckmiller, R., editors. *Impaired Functions of the Human Nervous System*. Amsterdam: North-Holland; 1993. p. 89-121.
- Gross GW, Rieske E, Kreutzberg GW, Meyer A. A new fixed-array multi-microelectrode system designed for long-term monitoring of extracellular single unit neuronal activity *in vitro*. *Neurosci Lett* 1977;6:101–5.
- Hoffman KL, McNaughton BL. Coordinated reactivation of distributed memory traces in primate neocortex. *Science* 2002;297:2070–3. [PubMed: 12242447]
- Ikegaya Y, Aaron G, Cossart R, Aronov D, Lampl I, Ferster D, Yuste R. Synfire chains and cortical songs: temporal modules of cortical activity. *Science* 2004;304:559–64. [PubMed: 15105494]
- Ikegaya Y, Le Bon-Jego M, Yuste R. Large-scale imaging of cortical network activity with calcium indicators. *Neurosci Res* 2005;52:132–8. [PubMed: 15893573]
- Izhikevich EM, Desai NS, Walcott EC, Hoppensteadt FC. Bursts as a unit of neural information: selective communication via resonance. *Trends Neurosci* 2003;26:161–7. [PubMed: 12591219]
- Izhikevich EM, Gally JA, Edelman GM. Spike-timing dynamics of neuronal groups. *Cereb Cortex* 2004;14:933–44. [PubMed: 15142958]
- Jackson A, Mavoori J, Fetz EE. Long-term motor cortex plasticity induced by an electronic neural implant. *Nature* 2006;444:56–60. [PubMed: 17057705]
- Jimbo Y, Robinson HP, Kawana A. Strengthening of synchronized activity by tetanic stimulation in cortical cultures: application of planar electrode arrays. *IEEE Trans Biomed Eng* 1998;45:1297–304. [PubMed: 9805828]
- Jimbo Y, Tateno T, Robinson HP. Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons. *Biophys J* 1999;76:670–8. [PubMed: 9929472]
- Kepecs A, Lisman J. How to read a burst duration code. *Neurocomputing* 2004;58–60. 1–6.
- Kepecs A, Wang XJ, Lisman J. Bursting neurons signal input slope. *J Neurosci* 2002;22:9053–62. [PubMed: 12388612]
- Kirkwood A, Bear MF. Hebbian synapses in visual cortex. *J Neurosci* 1994;14:1634–45. [PubMed: 8126560]
- Lisman JE. Bursts as a unit of neural information: making unreliable synapses reliable. *Trends Neurosci* 1997;20:38–43. [PubMed: 9004418]
- Madhavan, R.; Chao, C.; Wagenaar, DA.; Bakkum, DJ.; Potter, SM. Multi-site stimulation quiets network-wide spontaneous bursts and enhances functional plasticity in cultured cortical networks. *Proc. of the 28th Int. Conf. of the EMBS* 2006; New York, NY. 2006.
- Maeda E, Kuroda Y, Robinson HPC, Kawana A. Modification of parallel activity elicited by propagating bursts in developing networks of rat cortical neurones. *Eur J Neurosci* 1998;10:488–96. [PubMed: 9749711]
- Martin SJ, Grimwood PD, Morris RGM. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* 2000;23:649–711. [PubMed: 10845078]
- Martin SJ, Morris RGM. New life in an old idea: the synaptic plasticity and memory hypothesis revisited. *Hippocampus* 2002;12:609–36. [PubMed: 12440577]
- Matsuzaki M. Factors critical for the plasticity of dendritic spines and memory storage. *Neurosci Res* 2007;57:1–9. [PubMed: 17070951]
- Mulder AB, Arts MP, Lopes da Silva FH. Short- and long-term plasticity of the hippocampus to nucleus accumbens and prefrontal cortex pathways in the rat, *in vivo*. *Eur J Neurosci* 1997;9:1603–11. [PubMed: 9283815]
- Murphy TH, Blatter LA, Wier WG, Baraban JM. Spontaneous synchronous synaptic calcium transients in cultured cortical neurons. *J Neurosci* 1992;12:4834–45. [PubMed: 1361198]
- Nadasdy Z. Spike sequences and their consequences. *J Physiol Paris* 2000;94:505–24. [PubMed: 11165916]

- Nadasdy Z, Hirase H, Czurko A, Csicsvari J, Buzsaki G. Replay and time compression of recurring spike sequences in the hippocampus. *J Neurosci* 1999;19:9497–507. [PubMed: 10531452]
- Nicolelis MA. Brain-machine interfaces to restore motor function and probe neural circuits. *Nat Rev Neurosci* 2003;4:417–22. [PubMed: 12728268]
- Pine J. Recording action potentials from cultured neurons with extracellular microcircuit electrodes. *J Neurosci Methods* 1980;2:19–31. [PubMed: 7329089]
- Potter SM, DeMarse TB. A new approach to neural cell culture for long-term studies. *J Neurosci Methods* 2001;110:17–24. [PubMed: 11564520]
- Potter, SM.; Wagenaar, DA.; DeMarse, TB. Closing the loop: stimulation feedback systems for embodied MEA cultures. In: Taketani, M.; Baudry, M., editors. *Advances in Network Electrophysiology Using Multi-Electrode Arrays*. New York: Springer; 2006. p. 215-42.
- Rieke, F. *Spikes: Exploring the Neural Code*. Cambridge, MA: MIT Press; 1997.
- Rolston JD, Wagenaar DA, Potter SM. Precisely-timed spatiotemporal patterns of neural activity in dissociated cortical cultures. *Neuroscience* 2007;148:294–303. [PubMed: 17614210]
- Ruaro ME, Bonifazi P, Torre V. Toward the neurocomputer: image processing and pattern recognition with neuronal cultures. *IEEE Trans Biomed Eng* 2005;52:371–83. [PubMed: 15759567]
- Sasaki T, Matsuki N, Ikegaya Y. Metastability of active CA3 networks. *J Neurosci* 2007;27:517–28. [PubMed: 17234584]
- Segev R, Baruchi I, Hulata E, Ben-Jacob E. Hidden neuronal correlations in cultured networks. *Phys Rev Lett* 2004;92:118102. [PubMed: 15089177]
- Shahaf G, Marom S. Learning in networks of cortical neurons. *J Neurosci* 2001;21:8782–8. [PubMed: 11698590]
- Szarowski DH, Andersen MD, Retterer S, Spence AJ, Isaacson M, Craighead HG, Turner JN, Shain W. Brain responses to micro-machined silicon devices. *Brain Res* 2003;983:23–35. [PubMed: 12914963]
- Tateno T, Jimbo Y. Activity-dependent enhancement in the reliability of correlated spike timings in cultured cortical neurons. *Biol Cybern* 1999;80:45–55. [PubMed: 9951397]
- Turner JN, Shain W, Szarowski DH, Andersen M, Martins S, Isaacson M, Craighead H. Cerebral astrocyte response to micromachined silicon implants. *Exp Neurol* 1999;156:33–49. [PubMed: 10192775]
- van Pelt J, Vajda I, Wolters PS, Corner MA, Ramakers GJ. Dynamics and plasticity in developing neuronal networks *in vitro*. *Prog Brain Res* 2005;147:173–88. [PubMed: 15581705]
- Voigt T, Baier H, Delima AD. Synchronization of neuronal-activity promotes survival of individual rat neocortical neurons in early development. *Eur J Neurosci* 1997;9:990–9. [PubMed: 9182951]
- Volman V, Baruchi I, Ben-Jacob E. Manifestation of function-follow-form in cultured neuronal networks. *Phys Biol* 2005;2:98–110. [PubMed: 16204862]
- Wagenaar, DA.; DeMarse, TB.; Potter, SM. MEABench: a toolset for multi-electrode data acquisition and on-line analysis. 2nd Int. IEEE EMBS Conf. Neural Engineering; Arlington, VA. 2005. p. 518-21.
- Wagenaar DA, Pine J, Potter SM. Effective parameters for stimulation of dissociated cultures using multi-electrode arrays. *J Neurosci Methods* 2004;138:27–37. [PubMed: 15325108]
- Wagenaar DA, Potter SM. Real-time multi-channel stimulus artifact suppression by local curve fitting. *J Neurosci Methods* 2002;120:113–20. [PubMed: 12385761]
- Wagenaar DA, Potter SM. A versatile all-channel stimulator for electrode arrays, with real-time control. *J Neural Eng* 2004;1:39–45. [PubMed: 15876621]
- Wagenaar DA, Pine J, Potter SM. An extremely rich repertoire of bursting patterns during the development of cortical cultures. *BMC Neurosci* 2006a;7:11. [PubMed: 16464257]
- Wagenaar DA, Pine J, Potter SM. Searching for plasticity in dissociated cortical cultures on multi-electrode arrays. *J Negat Results Biomed* 2006b;5:16. [PubMed: 17067395]
- Wagenaar DA, Nadasdy Z, Potter SM. Persistent dynamic attractors in activity patterns of cultured neuronal networks. *Phys Rev* 2006c;E 73:519071–8.
- Werk CM, Chapman CA. Long-term potentiation of polysynaptic responses in layer V of the sensorimotor cortex induced by theta-patterned tetanization in the awake rat. *Cereb Cortex* 2003;13:500–7. [PubMed: 12679296]

Werk CM, Harbour VL, Chapman CA. Induction of long-term potentiation leads to increased reliability of evoked neocortical spindles *in vivo*. *Neuroscience* 2005;131:793–800. [PubMed: 15749334]
 Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. *Science* 1994;265:676–9. [PubMed: 8036517]

Abbreviation list

BAM	Burst activity matrix
BIP	Burst initiation probability
MEA	Multi-electrode array
<i>Pre</i>	Periods before the tetanus
<i>Post</i>	Periods post-tetanus
<i>C/D</i>	Ratio of network functional change to drift

Glossary

<i>Tetanus</i>	high frequency (e.g., 20 Hz) electrical stimulation used to induce functional plasticity
<i>Spike</i>	a recording of a neuron's action potential
<i>Bursts</i>	short periods (tens to hundreds of milliseconds) of intense spiking occurring simultaneously on a large fraction of active electrodes
<i>Hybrot</i>	a hybrid of living neurons and robotic components

Figure 1.

Multi-electrode recording of spontaneous activity in a dissociated cortical culture. (A) Typical three week old culture on a multi-electrode array (MEA). (B) Bursts were classified into two categories: small and big bursts depending on the threshold number of spikes within a burst. The figure shows a histogram of the number of spikes in each burst from three of the cultures used. The red line indicates the threshold for each culture. Note that the axis scales are different for each culture. (C) Spike raster plot and array-wide spike rate histograms of spontaneous activity for 5 min of spontaneous recording. The inset shows typical examples of bursts classified as 'big' and 'small' bursts. Recording was taken at 24 days *in vitro*.

Figure 2.

Pictorial description of the analysis of spatiotemporal patterns within spontaneous bursts and statistics on the change of distribution of burst patterns across the tetanus. (A) Raster plot and corresponding histogram of spontaneous spiking activity on 60 electrodes. Small and big bursts were detected, the onset and offset times of bursting activity were calculated and the difference between the offset and onset times was determined as the length of the burst. The *burst activity matrix* (BAM) for each burst was generated by counting the number of spikes within the length of the burst on each of the 60 electrodes using a 100 ms moving time bin (time step = 10 ms). The right panel shows frames of a BAM for different time bins for 60 electrodes (arranged in the original coordinates of the multi-electrode array). The size of the circle represents the number of spikes on that particular electrode for a particular time bin with the colored circle representing the value at electrode k , and the firing rate time histogram for electrode k is shown below. The BAM for each burst was then constructed by appending the histograms from the 60 recording electrodes, together to form a 1 by $60 \times N$ matrix, where N is the number of time

bins in the longest burst. (B) A two-dimensional cross-section of a BAM plotted in 60 by N dimensional space is shown. BAMs of all bursts were compared against each other and clustered using a paired clustering algorithm (dendrogram, see supplementary materials stacks.iop.org/PhysBio/4/181). *Occurrence* represents when a burst occurs within a specific BAM cluster (figures 3 and 4). *Integrated occurrence* was defined as the total number of *occurrences* of bursts in a 15 min time window moved by a time step of 30 s. *Integrated occurrences* were calculated across three periods of the same length equally spaced in time, *Pre1*, *Pre2*, *Post1*, and *Post2*. Periods *Pre1* and *Pre2* were before the tetanus stimulation and periods *Post1* and *Post2* were after the tetanus. (C) A two-dimensional cross-section of *integrated occurrences* of all BAM clusters (S by R dimensional matrix, S = number of BAM clusters, R = number of 15 min moving time windows) is shown. The color bar represents the time elapsed during the experiment and each point represents the *integrated occurrence* of the BAM clusters. The centroids of *integrated occurrences* in periods *Pre1*, *Pre2* and *Post1* were calculated. The change across periods was calculated as the Euclidean distance of each point in a period from the centroid of the previous period (d_{12} , d_{23} and d_{34} in the figure) normalized by the Euclidean distance of each point in the previous period from its centroid (d_{11} , d_{22} and d_{33} in the figure). This calculation yielded three quantities: C/D before the tetanus (*Pre1–Pre2*), C/D across the tetanus (*Pre2–Post1*) and C/D after the tetanus (*Post1–Post2*). The difference between two C/D s from consecutive periods was tested (Wilcoxon's rank sum test) to evaluate changes in the burst pattern across periods.

Figure 3.

Shuffled data do not show a significant structure in the BAM of spontaneous bursts. (A) Sorted correlation matrix of actual unshuffled data for a representative *tetanus experiment*. There were high correlations indicating a high similarity among the BAM of spontaneous bursts. (B) Average correlation values between the BAM clusters from $N = 5$ *tetanus experiments* after different shuffling methods: ‘electrode shuffle’, ‘temporal shuffle’, ‘matched shuffle’, ‘spike swapping’ and ‘spike jittering’ with 10 and 20 ms standard deviations. The average correlation of the original data showed significantly higher correlation values compared to shuffled data (the asterisk indicates $p < 0.001$, Wilcoxon's rank sum test for equal medians).

Figure 4.

Occurrence of spontaneous bursts was stable before the tetanus and changed across the tetanus. (A) Each stroke represents one occurrence of a BAM cluster of small spontaneous bursts. (B) Each stroke represents one occurrence of a BAM cluster of big spontaneous bursts. This is a representative example from one experiment. The gray bar indicates the period of tetanization. The arrowheads indicate the timing of the different BAM clusters shown in figure 5. (C) In five experiments, the average change in integrated occurrences across the tetanus (C/D between *Pre2* and *Post1*) was significantly greater than the average drift before the tetanus (C/D between *Pre1* and *Pre2*) ($p < 0.01$). $C/D = 1$ indicates that there was no change in the occurrence between periods. C/D before the tetanus (*Pre1–Pre2*) was close to 1, indicating

that there was very little drift in the *Pre* period before the tetanus and the *integrated occurrence* of the BAM of spontaneous bursts was stable for this period. **indicates $p < 0.01$ and * indicates $p < 0.05$ (Wilcoxon's rank sum test for equal medians).

Figure 5.

Examples of clusters for the BAM of spontaneous bursts for the experiment of figures 4(A) and (B). The sampled times are marked by arrows in figure 4. A given cluster shows similarity in the spatiotemporal structure over hours, but there was an easily visible difference between the patterns of any two clusters. (A) Frames from two representative BAM clusters for small bursts are shown, in the original coordinates of the multi-electrode array, at 20 ms intervals. (B) Frames from two representative BAM clusters for big bursts are shown, in the original coordinates of the multi-electrode array. Frames are shown at 100 ms intervals since the big bursts were much longer than the small bursts shown in (A). The sizes of the circles represent the number of spikes in that particular electrode for a particular time bin.

Figure 6.

Burst initiation sites were significantly changed by a strong tetanus. (A) The probability (burst initiation probability, BIP) of a recorded neuron having initiated a burst is shown by the different sizes of the circles for a representative experiment. Although this probability did not change much in the spontaneous recording periods before the tetanus (*Pre1* and *Pre2*), it changed significantly across the tetanus. (B) The average change in the probability of burst initiation for all experiments changed significantly after ($p < 0.05$) but not before the tetanus ($N = 5$ experiments). The asterisk indicates $p < 0.05$, Wilcoxon's rank sum test. The error bars represent SEM.