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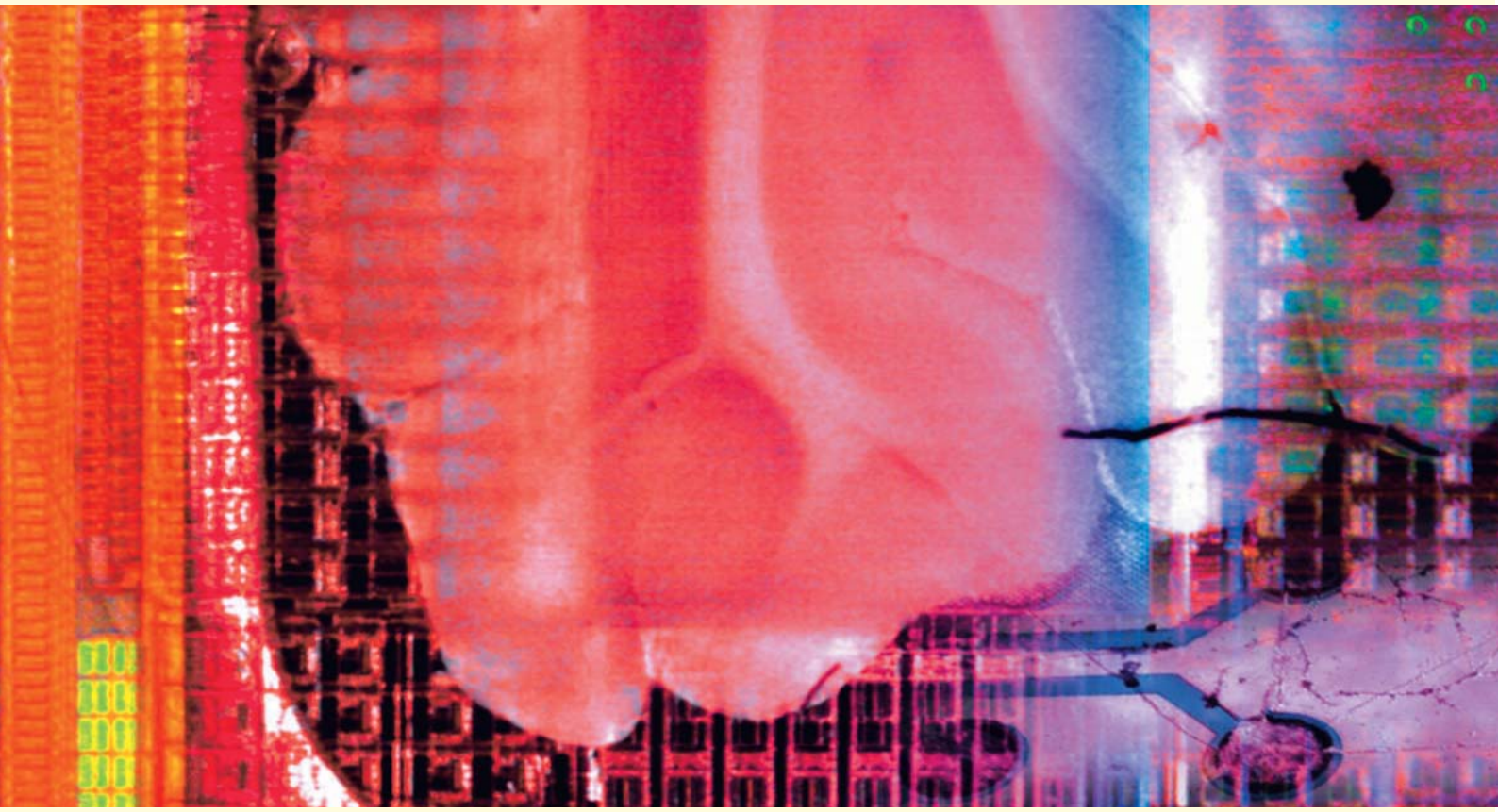

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How Should We Think About Bursts?

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Population bursts of action potentials are the most prominent feature of activity recorded from mammalian neuronal networks grown on MEAs. In the intact brain or spinal cord, they are considered variously as motor patterns, sensory gates, sleep spindles, UP-states, developmental signals, epileptiform activity, or memories. I prefer to remain agnostic about what bursts "mean" in vitro, and to study them in whatever way suits the purpose at hand. In this talk, I summarize MEA work from my lab over the past decade, focusing on our continually changing perspectives on bursts, as new results and methods arrive.

Our overarching goal is to use MEAs and optics to study the basics of learning, memory, and information processing. Cultured networks are more easily accessed, imaged, controlled, and manipulated (physically, chemically, electrically, optically) than are any animal models, but it is important to keep in mind their many limitations and simplifications. To bring them closer to animal models, we developed the idea of embodying cultured networks, so they could interact with the world, express behavior and receive sensory input.[1,2] We developed ways to control bursting, and have recently verified that by doing so, one can more reliably study learning in vitro.

1 Introduction

Our first embodied cultured network consisted of a network of ~50,000 neurons and glia from rat cortex, grown on the 60-electrode MEA from Multichannel Systems, connected to a virtual rat in a virtual world.[20] The spiking activity produced by the cultured network, dominated by population bursts, was classified in real-time using an adaptive nearest-neighbor clustering algorithm.[2] The burst clusters were assigned to control behaviors of the simulated animal, or animat. We created the software and hardware necessary to make a closed-loop system, whereby the sensory input to the neurally-controlled animat was converted to electrical stimuli for the cultured network.[13-16] We observed that certain types of burst patterns, or more generally, spatio-temporal activity patterns (STAPs), tended to recur more often than others, and that sensory feedback created a greater diversity of STAPs expressed by the network.[2] Unfortunately, the neurally–controlled animat showed no lasting changes in behaviour as a result of interaction with its simulated environment.

2 Bursts are Bad!

We noticed early on that when a cultured network receives continuous input, e.g., from the sensory system of its embodiment, its tendency toward dish-wide bursts diminishes. This led to the view of bursts as pathological activity patterns resulting from deafferentation, or sensory deprivation. Probably due to large culture-to-culture differences in the level of spontaneous bursting,[19] we had a difficult time demonstrating reliable learning in cultured networks,[3] and were unable to replicate the in vitro learning results of others.[13] We hypothesized that perhaps bursts were erasing any memories we tried to encode in the networks via electrical stimuli. One can easily reduce or eliminate population bursts by pharmacological manipulations, such as high magnesium or excitatory synaptic blockers. But those produce an "anesthetized" culture, likely to have little capability for learning-related plasticity. Therefore, we developed a more natural means to prevent bursts. Artificial "sensory background" is delivered to the networks using distributed, low-frequency stimulation (~1 Hz/electrode across 10-20 electrodes).[4] This allows the networks to continue to respond to meaningful sensory input, and avoids interference with plasticity mechanisms that would occur with pharmacological manipulation. We have recently demonstrated that quieting bursts aids the induction and detection of lasting functional plasticity (see Madhavan poster). It was necessary to develop analytical tools to deal with the large ongoing drift in functional connectivity of cultured networks, to reveal changes induced by external stimuli.[10] This plasticity can be used to model *goal-directed* learning in embodied cultured networks[18] (see Chao poster). We conclude that *studies of learning in vitro should be carried out under conditions where the networks are not sensory deprived*.

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Even if one takes the view that population bursting is bad, it may still be worth studying as a model for deafferentation syndromes such as chronic pain and epilepsy. This in vitro burst control work has resulted in a project to use closed-loop burst quieting to prevent seizures in epileptic rats, and eventually, humans (see Rolston poster).

3 Bursts are Good!

Although neuronal networks cultured from dissociated brain or spinal cord tissue are much less organized than in vivo, spontaneous bursts in MEA cultures are not random. Clustering the STAPs of many bursts revealed that each culture has fairly distinct patterns

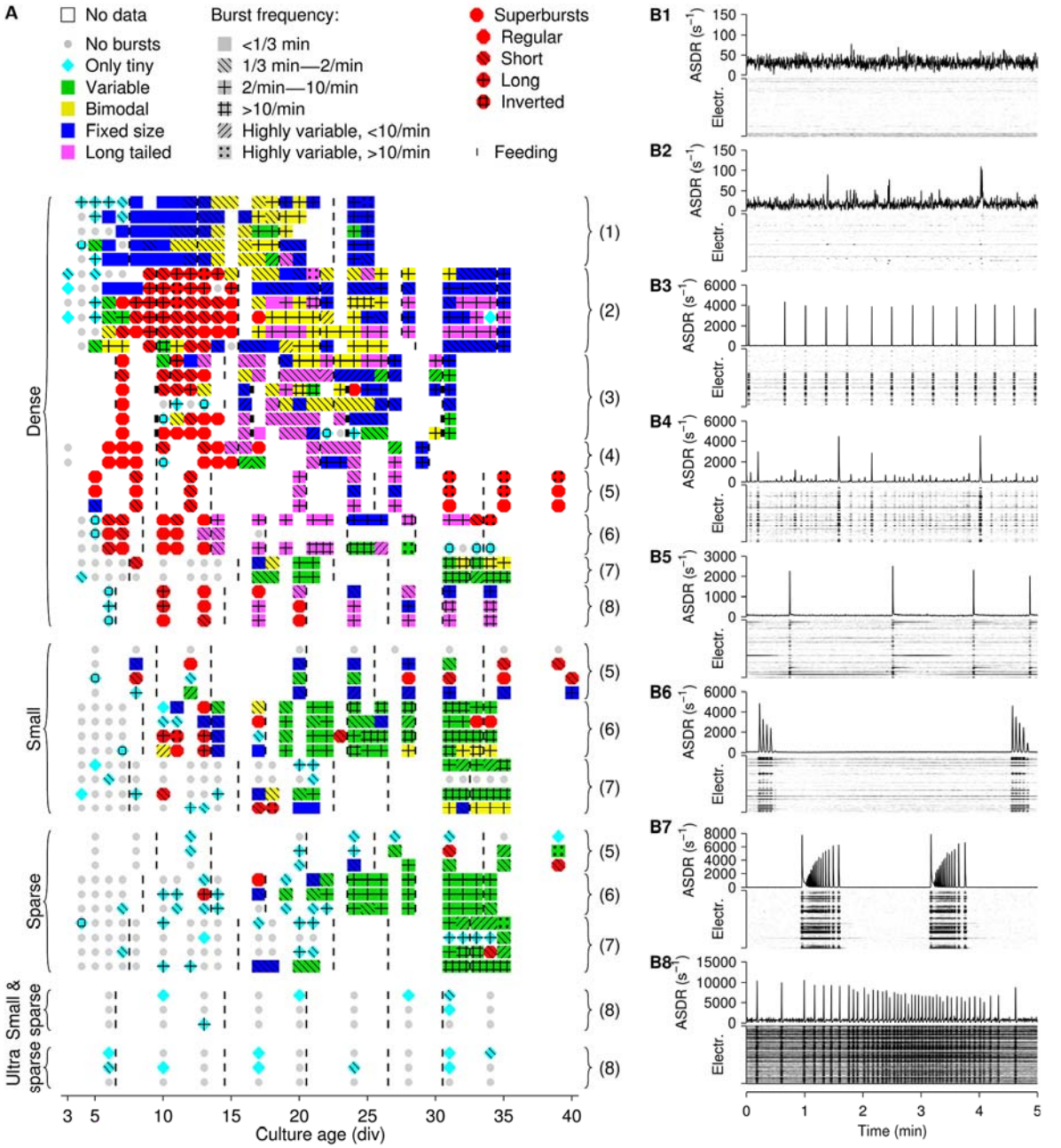

Figure 1. Classification of observed bursting behaviors. (A) Overview of the different classes of bursting behavior observed in our cultures. Numbers in parentheses indicate cell preparation number. Vertical bars indicate partial medium replacement times. Hash patterns indicate burst frequency for all types of burst patterns except superbursts. (B) Examples of burst pattern classes, with array-wide spike detection rates and gray-scale rasters for all electrodes, all taken from dense cultures. (B1) No bursting. (B2) Tiny bursts. (B3) Fixed size bursts. (B4) Variably sized bursts. (B5) Long-tailed bursts. (B6) Regular superbursts. (B7) Inverted superbursts. (B8) Dramatic burst rate variation.

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that get repeated at irregular intervals.[5-8] We found both gross patterns, involving the entire network of thousands of neurons, as well as detailed sequences of action potentials propagating through a few neurons.[9] In this respect, MEA cultures replicated observations of recurring bursts or "avalanches" in brain slices,[8,22] although they do not usually exhibit a power-law distribution of burst sizes, but either involve a few electrodes or all active electrodes.[5] Since any given network expresses a diversity of bursts with non-random spatio-temporal structure, it is reasonable to think of bursts as information carriers, or the expressions of stored memories. We created new metrics to quantify these patterns, such as the Center of Activity Trajectory,[10] and have used them as reporters of changes induced in a network's input-output function by electrical (or chemical) stimuli. These functional changes can be thought of as learning. [5,11,12,21]

Bursts may serve as signals that the developing nervous system uses to form proper connections. Even in vitro, we found that certain types of bursts such as superbursts tend to occur most commonly at specific stages in a culture's development (Fig. 1).[19] We collected a large set of spontaneous and evoked activity from many cultures across the first month in vitro. These data[19] are available for others to study using their own burst analysis tools or other techniques.[24]

4 Bursting—What is next?

Along with the ability to control bursting by electrical stimulation comes the ability to evoke bursts at will. It is likely that the barrage of activity during a population burst engages a variety of plasticity mechanisms. Therefore, directed learning in cultured networks may be best effected by taking advantage of bursts by inducing them, rather than eliminating them completely. This may explain earlier successes at inducing functional plasticity in vitro using tetanic stimuli that repeatedly evoke bursts.[23] Through the use of a variety of electrodes and stimulus patterns to evoke different classes of bursts, perhaps different types of learning can be demonstrated.

The whole concept of "spontaneous activity" loses its meaning in vivo, where all neural events are initiated or greatly influenced by the continuous barrage of sensory inputs delivered by the millions of sensory axons leading to the brain. We often forget the unusual situation provided by cultured networks, of having zero input from the outside world — they are in "sensory deprivation". In cases where the network is interfaced using stimulating MEAs, this is no longer the case, and we have complete control of its inputs. We suggest that by delivering artificial sensory input continuously, cultured networks can serve as a more realistic model of brain processes in vivo. Whether bursts are bad or good, they provide a rich set of phenomena for studying learning, memory, and pathology in vitro and in vivo.

Acknowledgements

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(Our papers can be found online at neuro.gatech.edu)

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