**Synapses undergo rapid modifications during learning and development that can introduce instability into neural circuits.  Homeostatic plasticity encompasses a set of mechanisms that stabilize neural circuits by placing constraints on cellular excitability and synaptic strength.  The most widely-studied form of homeostatic plasticity is synaptic scaling, a phenomenon in which the quantal amplitudes of all synaptic inputs onto a neuron are strengthened (upscaling) or weakened (downscaling) by a multiplicative scaling factor to compensate for chronic changes in activity levels (Turrigiano, 2012).  While synaptic scaling has been observed across many different systems (Davis, 2006), the activity perturbations that directly trigger scaling remain unclear.  Here we use a combination of chronic multisite electrophysiology, closed-loop optogenetic stimulation, and pharmacology to show that prolonged reductions in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamatergic transmission are critical for triggering synaptic upscaling.  We demonstrate that upscaling does not require changes in spiking activity, and that upscaling that occurs following a spiking blockade is significantly attenuated by partial restoration of AMPA receptor (AMPAR) activation.  These findings suggest that the role of homeostatic synaptic scaling may be to stabilize synaptic activity, rather than neuronal spiking or spike-dependent processes.  This model of scaling raises questions about how homeostatic synaptic modifications are coordinated within cells and circuits in order to preserve relative synaptic weights, which are widely hypothesized to be the physical locus of memory in the brain..**

Spiking is the most salient electrophysiological feature of a neuron and forms the basis of rapid signaling and information transmission in the nervous system.  Prolonged changes in neuronal spiking are also thought to drive mechanisms of homeostatic plasticity, such as synaptic scaling.  Despite its importance in homeostatic plasticity, spiking activity is rarely monitored during experimental perturbations that lead to synaptic scaling.  For example, chronic blockade of voltage-gated Na+ channels with tetrodotoxin (TTX) or chronic blockade of AMPARs with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) both lead to upscaling, but the longterm profile of spiking activity elicited by these perturbations is unknown.

We sought to continuously measure spiking activity in many cells throughout a neural circuit during perturbations that lead to upscaling.  To this end, we used planar microelectrode arrays (MEAs; Fig. 1a-b) to record extracellular spiking activity from hundreds of neurons embedded in dissociated cortical cultures before and during 24-hour TTX or CNQX application.  We used the MEA recordings to detect network-wide bursts of action potentials occurring synchronously across many electrodes (hereafter referred to as bursts) and to assess a culture’s overall firing rate by summing spiking levels throughout the array (Fig. 1c, Wagenaar et al., 2006).  By measuring spiking activity from many electrodes over many hours, we could quantitatively evaluate changes in spiking and bursting that accompany synaptic upscaling.

Consistent with our expectations, TTX application eliminated spiking activity for the entire 24-hour treatment (Fig. 1d-e).  In contrast, CNQX only partially reduced spiking activity compared to pre-drug levels (Fig. 1d-e).  The CNQX-induced reduction in firing rate was primarily due to a reduction in burst frequency (Fig. 1f), while spiking between bursts was not significantly affected (Fig. 1f).  In most CNQX-treated cultures, bursting was significantly reduced during the first few hours, but began to recover by the end of 24 hours (Fig. 1d), likely due to NMDAergic transmission (Suppl. Fig 1). While the CNQX-induced reduction in spiking was variable across cultures, some degree of spiking and bursting always persisted, unlike cultures treated with TTX.