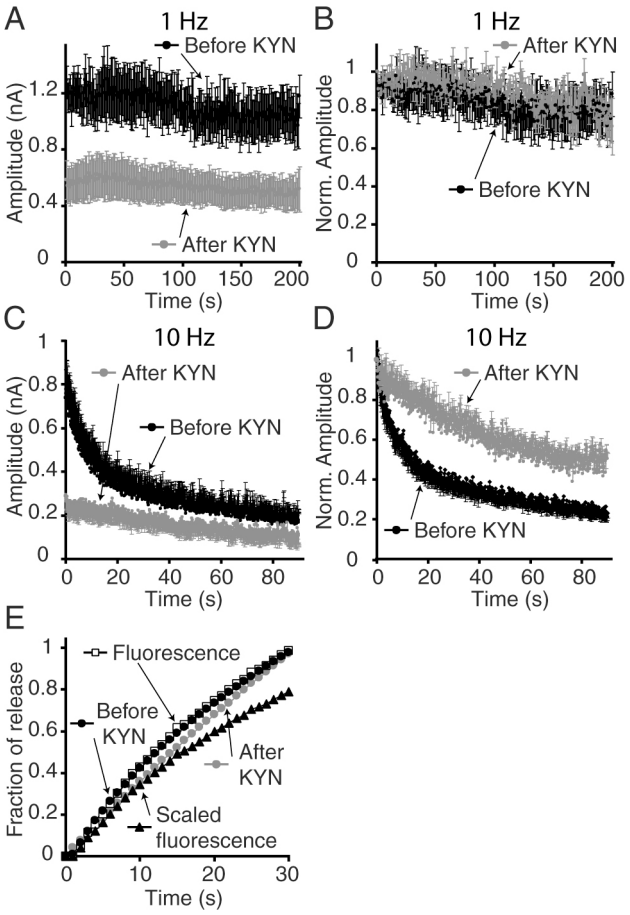

Supplementary Figure 2, Effect of 1 mM kynurenate (KYN) treatment on synaptic depression rate at hippocampal cell cultures at 10 Hz stimulation (for 900 pulses). *A,B,C,D,* normalized and real amplitude plots showing the synaptic depression rate at 1Hz 200s (*A,B*) and 10 Hz 900 pulse trains (*C,D*) recorded before and after KYN perfusion (n=7 cells for each group). *E*, Comparison of FM dye destaining kinetics to neurotransmitter release at hippocampal synapses. Cumulative integral of synaptic current either in control or in the presence of KYN compared to the scaled kinetics of fluorescence loss from FM2-10 loaded hippocampal synapses.

In order test if postsynaptic recordings are indeed linear detectors of presynaptic neurotransmitter release, a basic assumption of these earlier experiments. This concern was motivated by the fact that in dissociated hippocampal cultures electrical stimulation is delivered via two parallel platinum electrodes by application of 1 ms duration current pulses which is necessary to ensure stimulation of all synapses in a field of interest. We surmised that this strong stimulation might increase the occupancy of AMPA receptors towards saturation as stimulation progresses distorting the kinetics of neurotransmitter detection. To address this question we used the rapidly dissociating AMPA receptor antagonist kynurenate (KYN), which competes with the endogenous levels of glutamate (Diamond and Jahr, 1997). As a result of its rapid dissociation the degree of KYN block of AMPA receptors decreases with higher glutamate concentration in the synaptic cleft (Diamond and Jahr, 1997). When we stimulated hippocampal cultures with field electrodes at a frequency of 1 Hz, we did not detect a significant difference in the efficacy of KYN block in the beginning and the end of the train (Supplementary Fig. 2*A, B*). In contrast, stimulation at 10 Hz progressively decreased the relative effectiveness of the KYN block (Supplementary Fig. 2*C, D*). When we compared the integral of the synaptic depression kinetics detected postsynaptically under KYN block to the FM dye release kinetics imaged at 10 Hz, the divergence between the two curves emerged earlier than in previous analysis (Sara et al., 2002; Virmani et al., 2006) (Supplementary Fig. 2*E*). To estimate the time course of synaptic vesicle recycling from the kinetic comparison of dye release and postsynaptic electrical signals we have to assume a linear relationship between the electrical and optical measures. However, this analysis suggests that this assumption may not always be valid. Furthermore, FM dye release kinetics typically has a low time resolution (~ 1 s), which lead to underestimation of rapid alterations in neurotransmitter release kinetics. In contrast to the experiments in dissociated hippocampal cultures, KYN block in hippocampal slices was strictly proportional throughout the stimulation period (data not shown). This is presumably because in the slice recordings only a small number of synapses are activated with weaker stimulation.