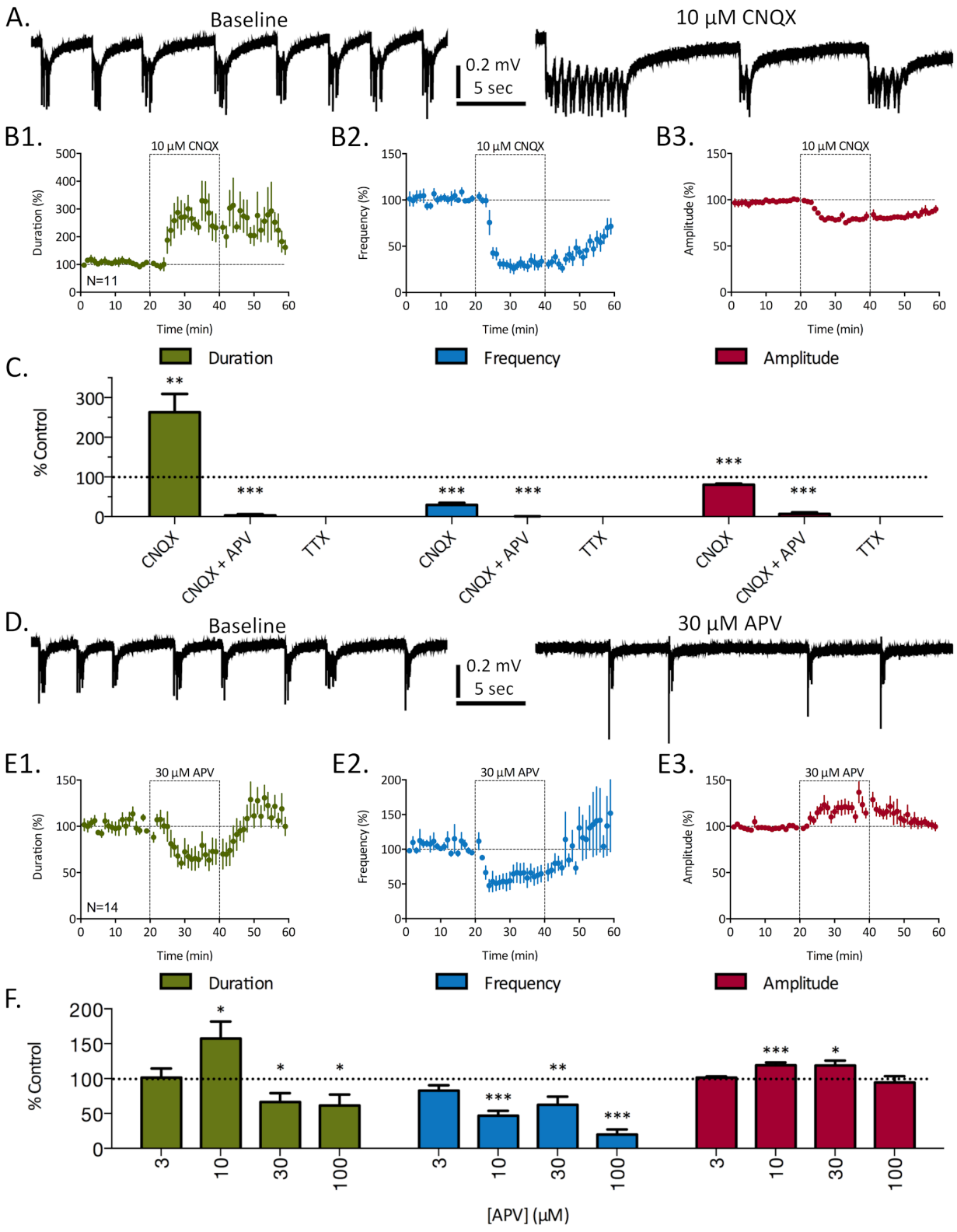

Figure S2: Glutamate receptor antagonists fail to fully inhibit REDs. A) Representative traces of REDs recorded before (baseline) and in the presence of 10 µM CNQX. B) Time courses for 10 µM CNQX's effects of RED duration (B1, green), frequency (B2, blue), and amplitude (B3, red).

Dashed-line boxes from 20 to 40 minutes represent the duration of CNQX exposure. Data points represent mean ± SEM for RED parameters in 1-minute bins. C) Bar graph representing the effects of CNQX (10 μM), CNQX (10 μM) + APV (50 μM), and TTX (1 μM) on RED duration (green), frequency (blue), and amplitude (red). D) Representative traces of REDs recorded before (baseline) and in the presence of 30 μM APV. E) Time courses for 30 μM APV's effects of RED duration (E1, green), frequency (E2, blue), and amplitude (E3, red). Dashed-line boxes from 20 to 40 minutes represent the duration of APV exposure. Data points represent mean ± SEM for RED parameters in 1-minute bins. F) Concentration-dependent effects of APV on RED duration (green), frequency (blue), and amplitude (red). In all bar graphs, error bars represent SEM and * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

3 Results

3.1 AMPA/Kainate and NMDA receptor antagonists differentially affect REDs

First, we examined the effects of 10 μM CNQX on RED duration, frequency, and amplitude. As illustrated in Figure S2 panels A-B, 10 μM CNQX failed to completely block REDs in these slices. Instead, in 11 slices, CNQX significantly increased burst duration (263.0 ± 46.4% of baseline, $p=0.0056$) and decreased burst frequency and amplitude (29.3 ± 5.5% of baseline, $p<0.0001$, and 80.2 ± 3.0% of baseline, $p<0.0001$, respectively). Since these data indicated that REDs may be additionally mediated by NMDA receptors under our experimental conditions, we assessed a combination of 10 μM CNQX and 50 μM APV. A 20-minute bath exchange with this combination of antagonists effectively eliminated REDs (Figure S2 panel C: duration: 3.3 ± 3.0%, $p<0.0001$; frequency: 0.6 ± 0.4 %, $p<0.0001$; amplitude: 6.5 ± 4.3%, $p<0.0001$, with respect to baseline, N=9). Finally, a 20-minute bath exchange with 1 μM TTX completely, and irreversibly, eliminated all REDs (Figure S2 panel C: all parameters: 0 ± 0% of baseline, N=3).

The role(s) of NMDA receptor mediated synaptic transmission in mediating REDs were further examined by evaluating the concentration-dependent effects of the NMDA receptor selective antagonist, APV, alone on REDs (Figure S2 panels D-F). Unlike a previous report showing a complete block of REDs in a low-Mg^{2+} based model using brain slices from naïve rats at 20-30 μM[8], APV (3-100 μM) failed to completely block REDs in mEC slices obtained from our KA-rats. At the highest concentration tested here (100 μM, N=9), APV significantly, but incompletely, attenuated both RED duration and frequency (61.4 ± 15.7%, $p=0.0489$ and 19.8 ± 7.3%, $p<0.0001$, respectively). Interestingly, APV's concentration-dependent effects on RED duration were biphasic; at 10 μM, APV significantly increased burst duration in a manner similar to 10 μM CNQX (157.5 ± 24.2% of baseline, $p=0.0323$, N=15).