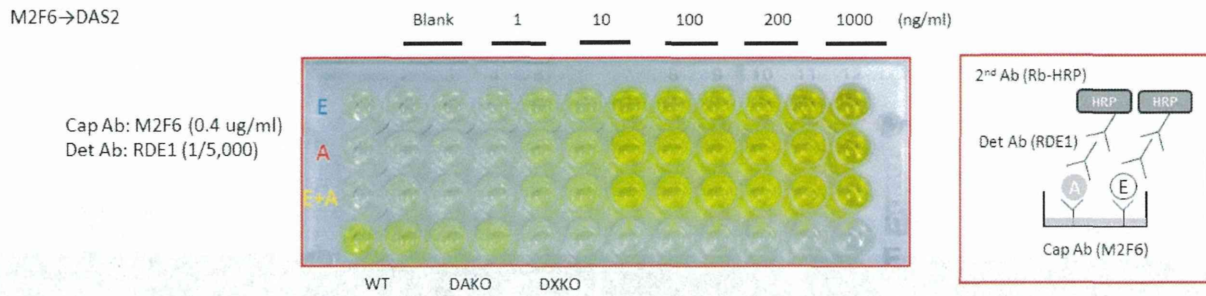
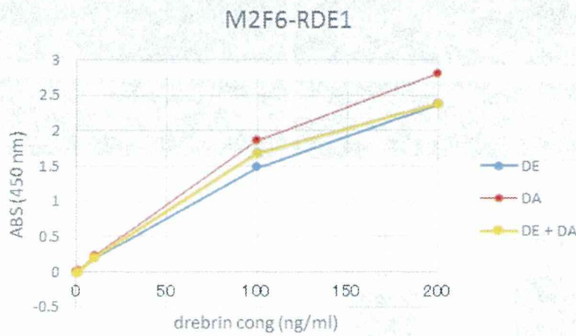
Figure 6

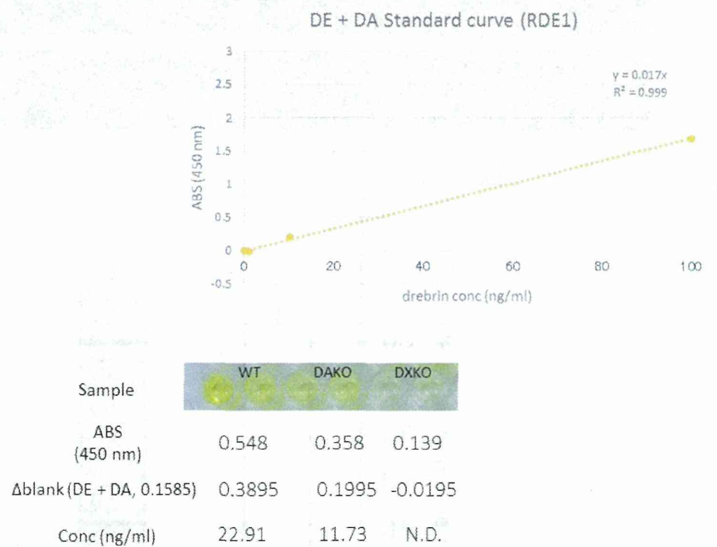
A



B



C

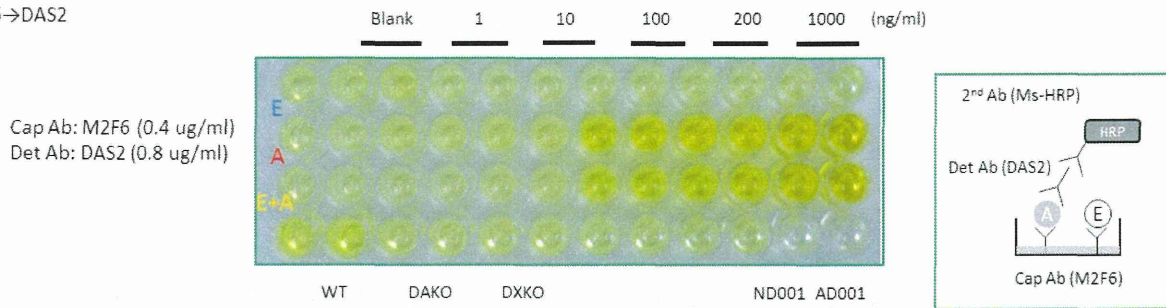


Sample	WT	DAKO	DXKO
ABS (450 nm)	0.548	0.358	0.139
Δblank (DE + DA, 0.1585)	0.3895	0.1995	-0.0195
Conc (ng/ml)	22.91	11.73	N.D.

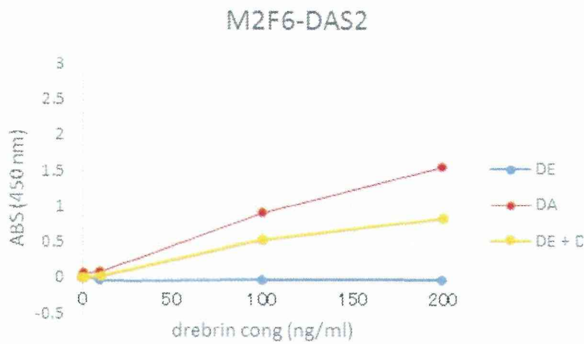
Fig. 6 ELISA for measuring the amount of drebrin E and A by using M2F6 (Capture Ab) and RDE1 (Detection Ab). The actual photo of ELISA plate result and the scheme of this reaction system (A). The absorbance of each drebrin concentration was plotted in graph B. An approximate formula was calculated by the absorbance of samples and drebrin concentration was calculated by the formula (C).

Figure 7

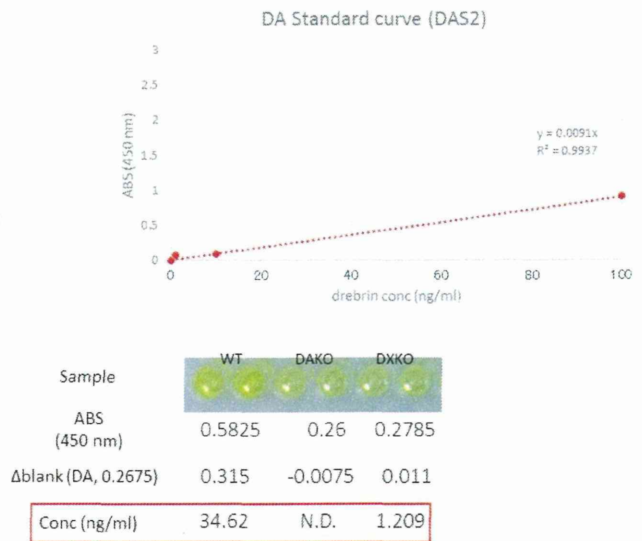
A



B



C



Sample	WT	DAKO	DXKO
ABS (450 nm)	0.5825	0.26	0.2785
Δblank (DA, 0.2675)	0.315	-0.0075	0.011
Conc (ng/ml)	34.62	N.D.	1.209

Fig. 7 ELISA for measuring the amount of drebrin A by using M2F6 (Capture Ab) and DAS2 (Detection Ab). The actual photo of ELISA plate result and the scheme of this reaction system (A). The absorbance of each drebrin concentration was plotted in graph B. An approximate formula was calculated by the absorbance of samples and drebrin concentration was calculated by the formula (C).

Figure 8

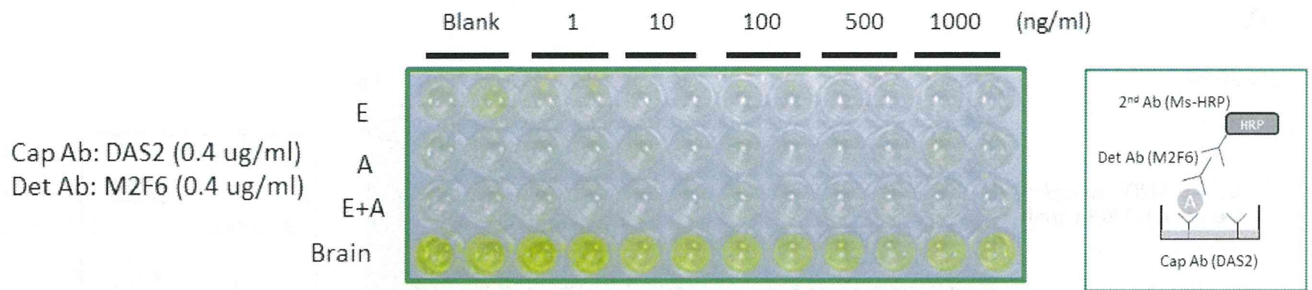


Fig. 8 ELISA for measuring the amount of drebrin A by using DAS2 (Capture Ab) and M2F6 (Detection Ab). The actual photo of ELISA plate result and the scheme of this reaction system.

Figure 9

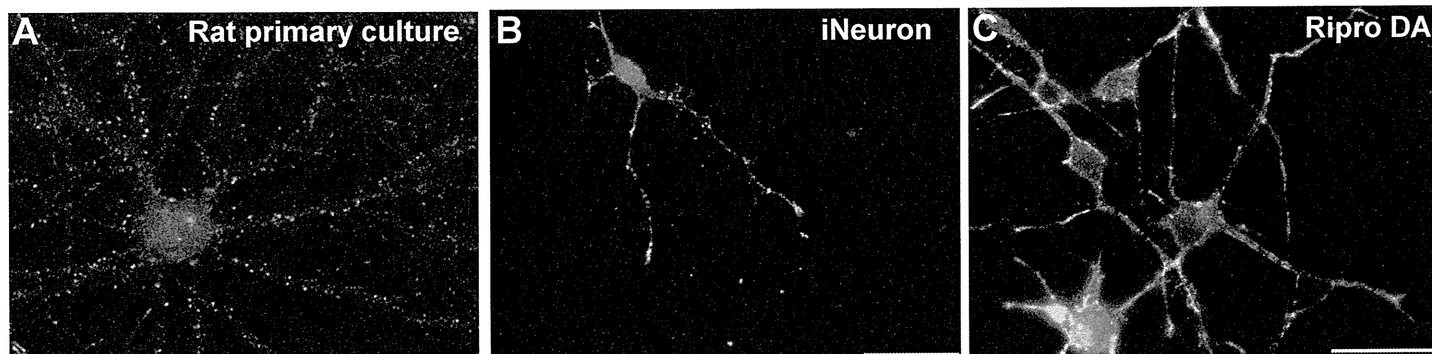


Fig. 9 Immunofluorescence labeling of drebrin in rat hippocampal neuron (A), iNeuron (B), and Ripro DA (C). Neurons were fixed at 21 DIV and immunolabeled with anti-drebrin antibody (M2F6). Scake bars, 30 μm.

Figure 10

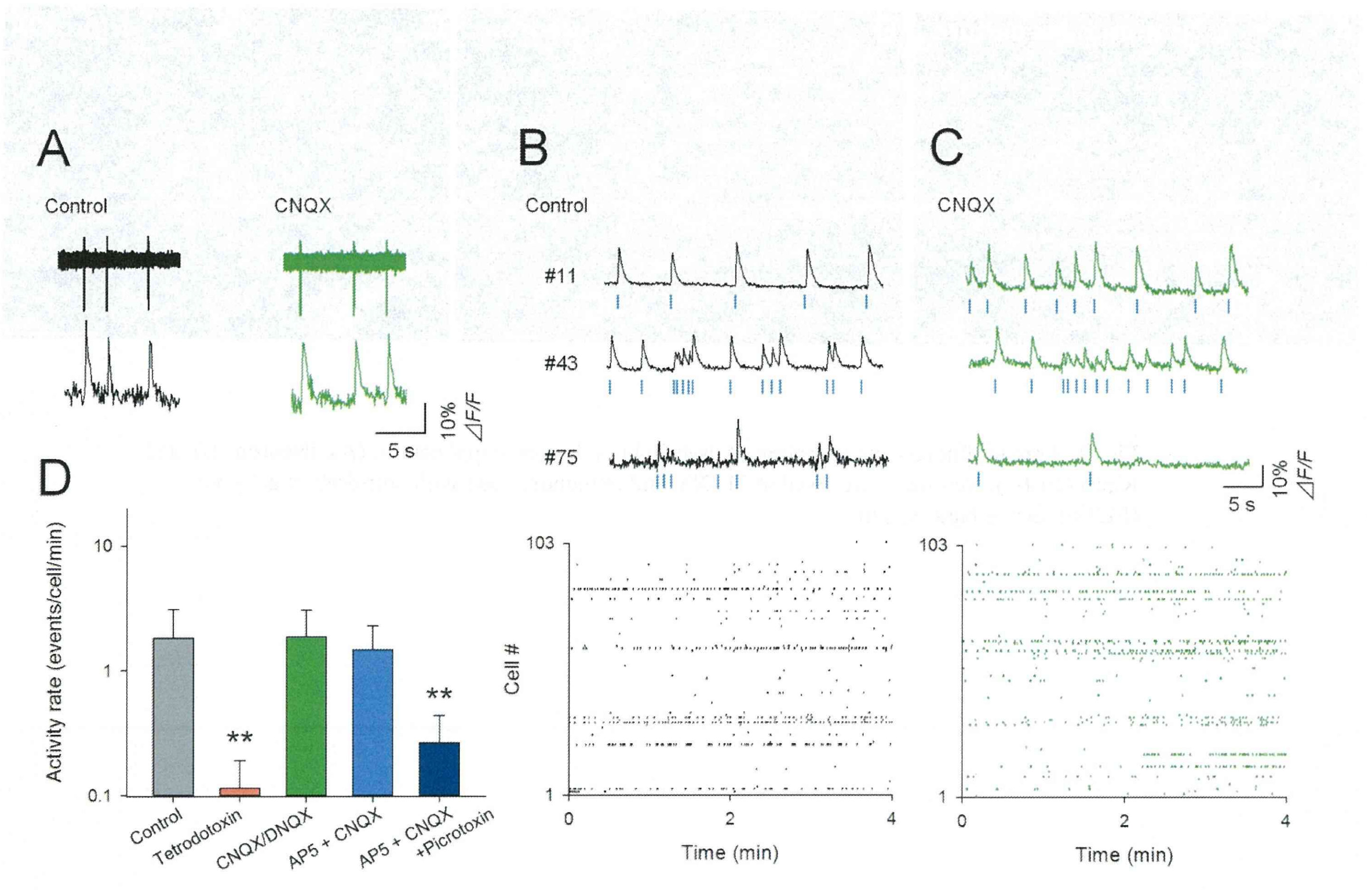


Fig. 10 Non-NMDA receptor antagonists do not affect spontaneous network activity level. (A) Simultaneous cell-attached recording and calcium imaging before (black) and 10 min after (green) perfusion with 50 μM CNQX. Individual calcium transients reflected action potentials of the neuron. (B, C) Calcium traces recorded from 3 representative CA3 neurons (top) and raster plots of spontaneous activity of a total 103 neurons (bottom) before (B) and 10 min after CNQX application (C). Blue dots below the traces indicate the timings of detected spikes. (D) Mean ± SD firing rates of CA3 neurons before (control; n = 11 slices) and after bath application of tetrodotoxin (n = 4 slices), CNQX/DNQX (n = 7 slices), CNQX/DNQX+AP5: (n = 4 slices), CNQX/DNQX+AP5+picrotoxin (n = 4 slices). $^{**}P < 0.01$, Welch's t test after one-way repeated-measure ANOVA ($P = 1.3 \times 10^{-3}$, $F_{4,25} = 6.1$).

Figure 11

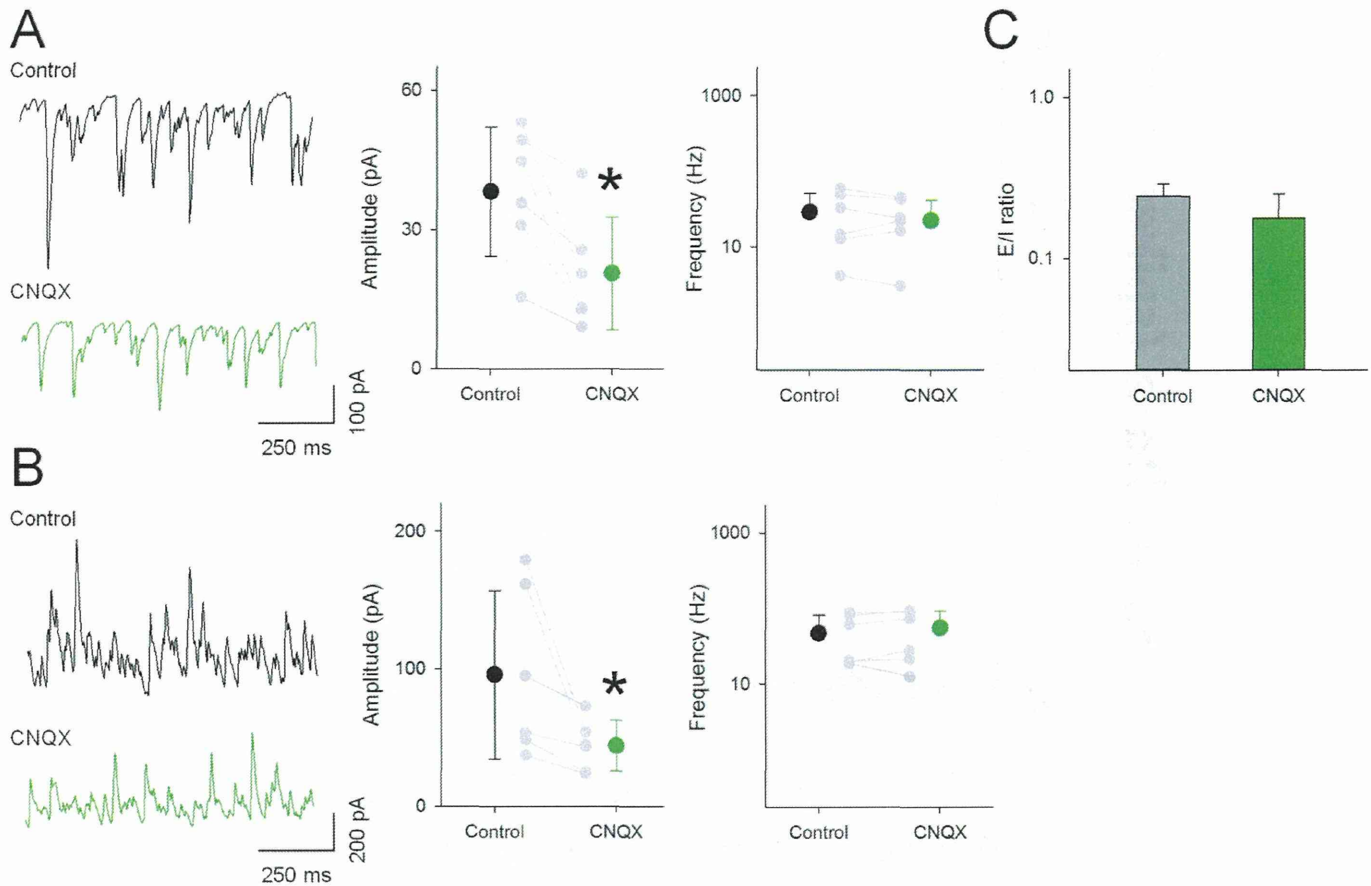


Fig. 11 CNQX reduces both sEPSC and sIPSC amplitudes to a similar level. (A) Representative sEPSC traces recorded from a CA3 pyramidal cell 5 min before and 10 min after CNQX treatment (left). The neuron was clamped at -70 mV. The amplitude (middle) and frequency (right) of sEPSCs were summarized as the means ± SD of 6 neurons, $*P = 0.016$, $W = 21$, Wilcoxon signed-rank test. (B) Same as A, but for IPSCs recorded at 0 mV. $*P = 0.016$. $W = 21$. (C) Mean ± SD E/I ratios of 6 neurons. $P = 0.12$, $t_7 = 1.7$, Welch's t test.

Figure 12

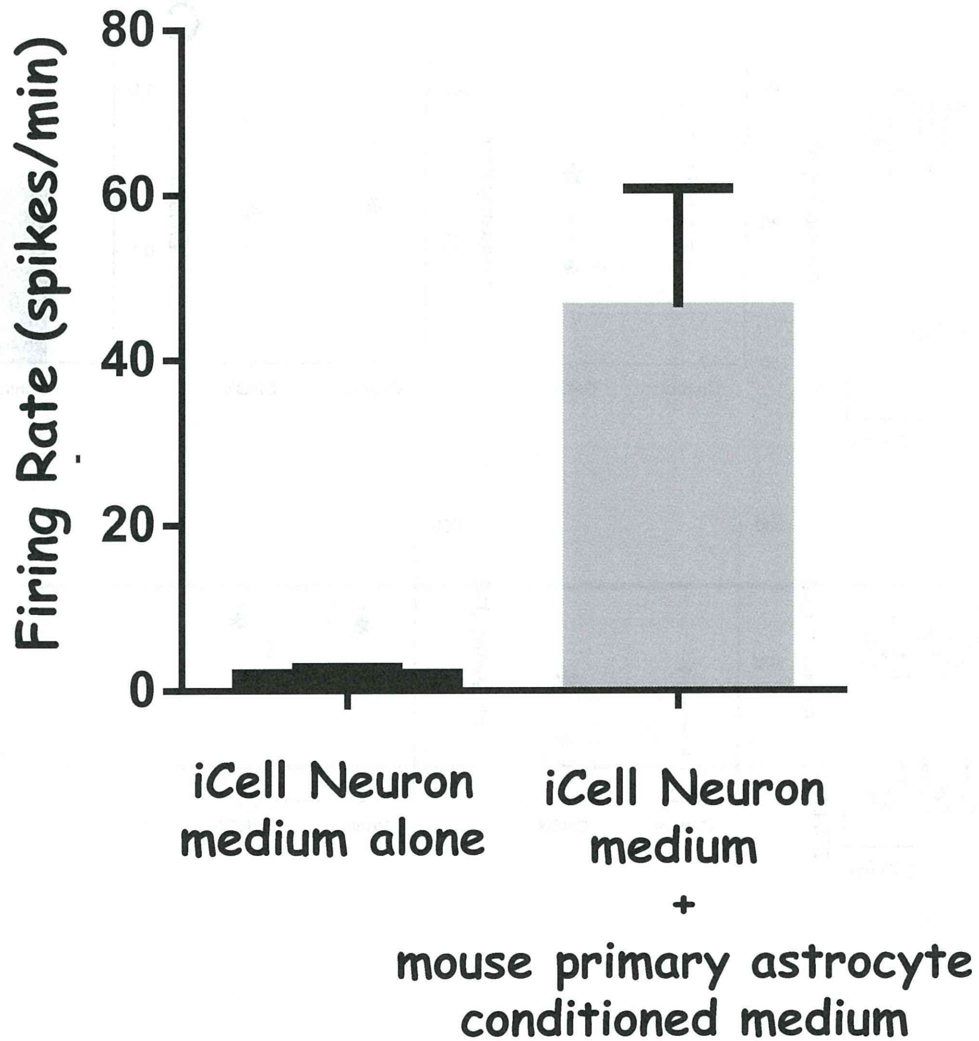


Fig. 12 Astrocyte-derived extrinsic stimuli generate spontaneous spikes in iCell Neurons
Baseline spontaneous electrophysiological activity readout by MEA from iCell neurons cultured with iCell Neurons maintenance medium alone or iCell Neurons maintenance medium with mouse astrocyte-conditioned medium for 63 days. Baseline spike numbers were determined by using 100 nM TTX treated background value correlated with RSM value at 550. Probes over 1 Hz of recorded electrically evoked signals were selected as neuron positive probes.

Figure 13

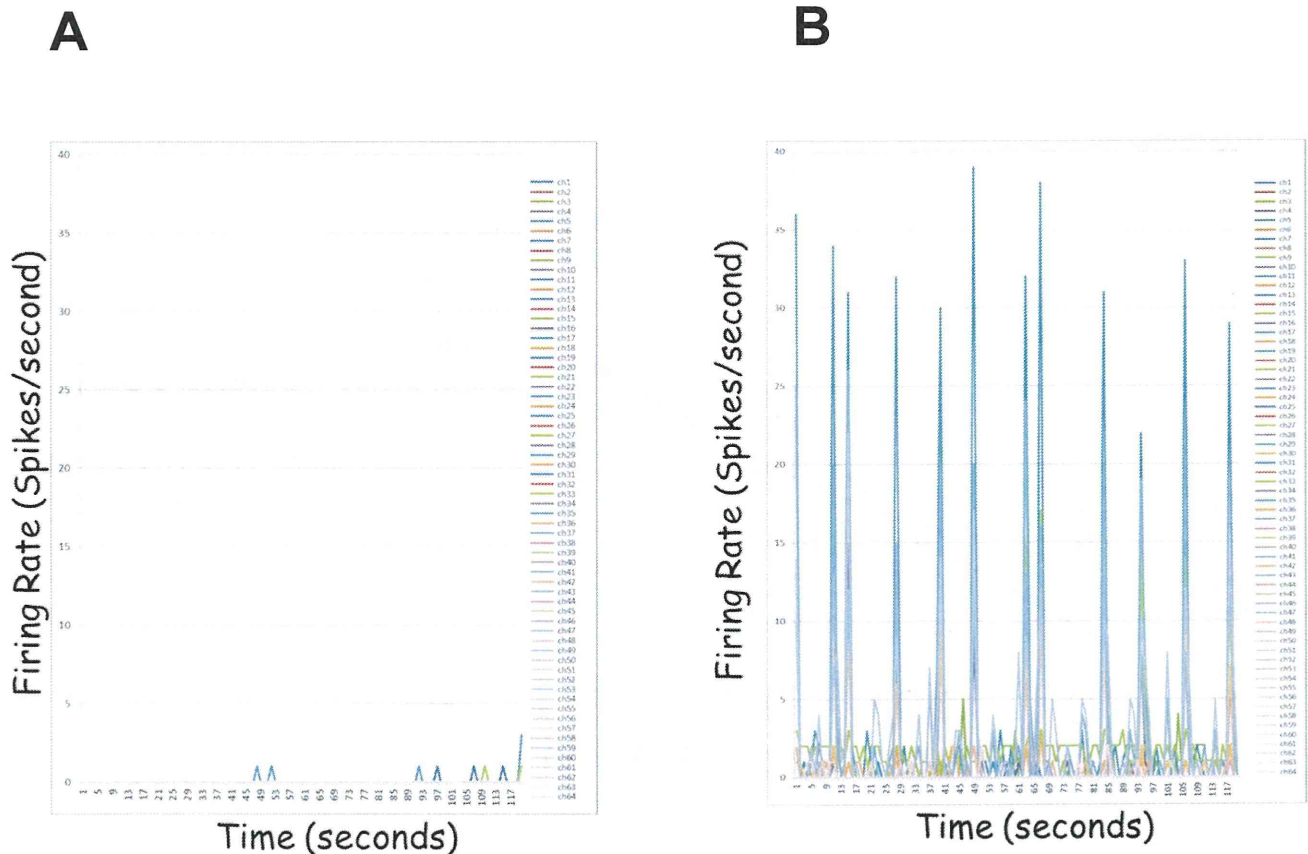


Fig. 13 The astrocyte-derived extrinsic stimuli induced periodic spontaneous synchronized burst spikes in iCell Neurons

Total spontaneous firing rate (spike numbers per second) from each electrode probe in a recorded 64-electrode probe dish are shown. Baseline spontaneous electrophysiological activity was recorded by MEA from iCell Neurons cultured in iCell neuron maintenance medium alone (A) or iCell neuron maintenance medium with mouse astrocyte-conditioned medium (B) for 63 days. Periodic increased numbers of spikes were observed only in B. The periodic burst spikes were synchronized among multiple electrode probes. Baseline spike numbers were determined by using 100 nM TTX treated background value correlated with RSM value at 550..

Figure 14

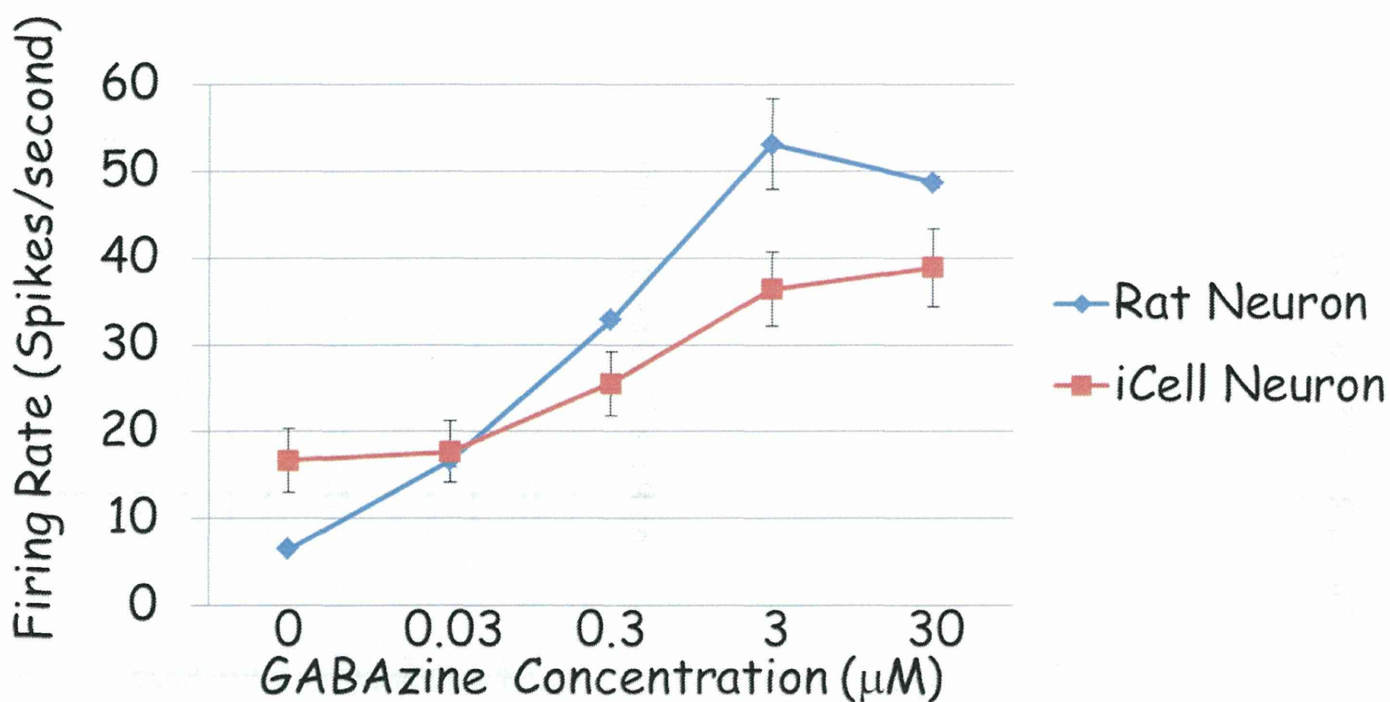


Fig. 14 GABAzine enhances spontaneous spike activity dose-dependent manners in rat primary cortical neurons and iCell Neurons

GABAzine was applied to the rat cortical primary neurons and iCell Neurons. iCell Neurons were supplied with astrocyte-conditioned medium for 2 months. The baseline activity was determined by subtraction of background value which was determined after 100 nM TTX treatment. Probes over 1 Hz of spike numbers were selected to data analysis. Dose-dependent mean spike responses are shown at the concentration of 0, 0.03, 0.3, 3 and 30 μM of GABAzine.

Figure 15

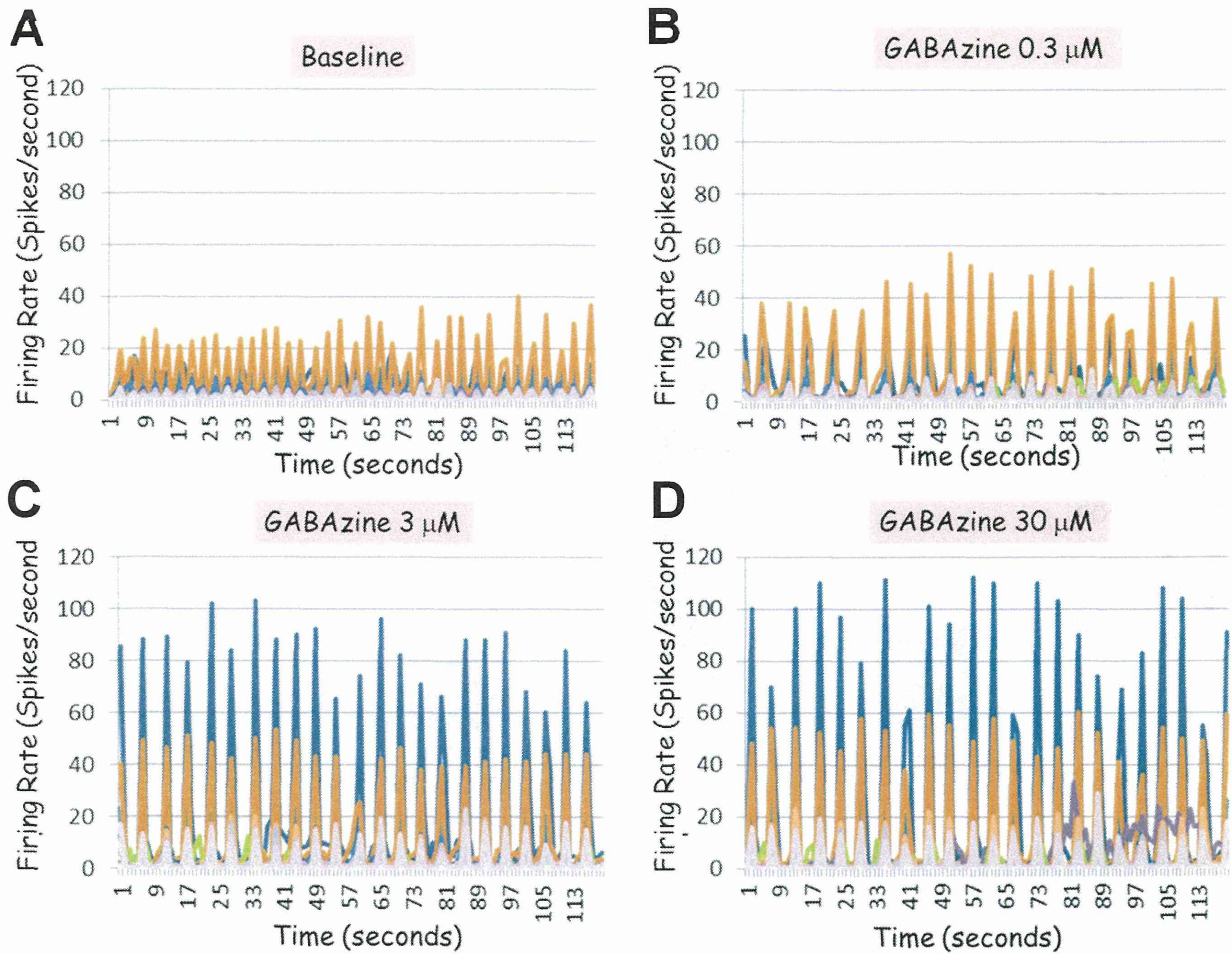


Fig. 15 GABAzine enhanced periodic synchronized burst spikes dose-dependent manner in iCell Neurons
The spontaneous spikes were monitored by MEA from iCell Neurons cultured in iCell neuron maintenance medium with mouse astrocyte-conditioned medium for 2 months. Baseline spike numbers were determined by using 100 nM TTX treated background value (A). Altered baseline spike numbers are shown after 3 min treatment with 0.3 μM (B), 3 μM (C) and 30 μM (D) of GABAzine.