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Development and validation of a high-density MEA pharmacology assay in rat cortical neurons and human iPSC-derived neurons for drug discovery

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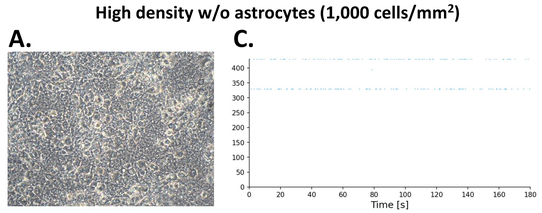
With the partnership of maxwell BIOSYSTEMS

Abstract

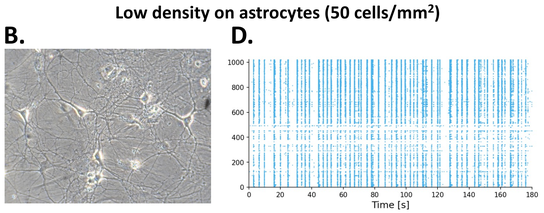
Drug discovery projects rely on neuronal cultures to interrogate the efficacy and potency of novel therapeutic molecules. Functional electrophysiological properties of neurons are currently measured by manual patch clamp recording, which is limited to 1 or 2 neurons at a time. High density microelectrode arrays (HD-MEAs) from MaxWell greatly increases electrophysiological recordings to 100s to 1,000s of neurons at a time. Here we validated a functional high-throughput drug-testing platform for primary rat cortical neurons and human iPSC-derived neurons growing at low density on rat astrocytes. Neurons exhibited reliable spontaneous firing and network activity. Both rat cortical neurons and human iPSC-derived neurons (Fujifilm iCell GlutaNeurons) exhibited more activity as they mature in culture. After maturation, we showed that retigabine reduced spontaneous activity in both rat cortical neurons and human iPSC-derived neurons. NMDA increased spontaneous activity in rat cortical neurons.

Method

High density w/o astrocytes (1,000 cells/mm²)

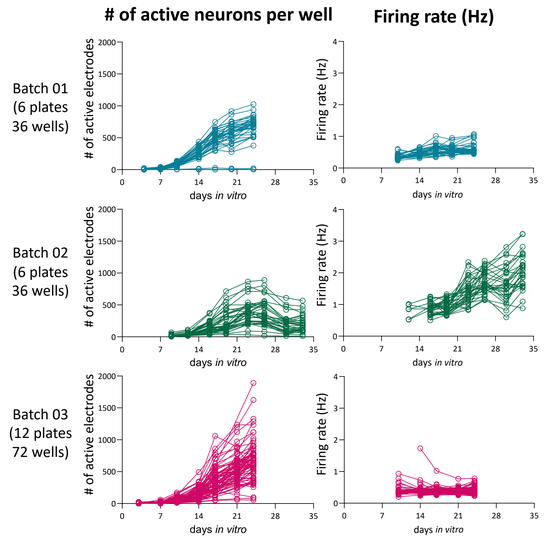


Low density on astrocytes (50 cells/mm²)



A.) Photomicrographs of rat cortical neurons plated at high density without astrocytes and **B.)** plated at low density on a monolayer of astrocytes. **C,D.)** Raster plots of spontaneous activity. Every blue dot represent a spike from one electrode. **C.)** Neurons cultured at high density (1000 cells/mm²) without astrocytes exhibited low activity at 17 days *in vitro*. **D.)** Parallel neuronal cultures at very low density (50–100 cells/mm²) growing on rat astrocytes exhibited high synchronized network activity.

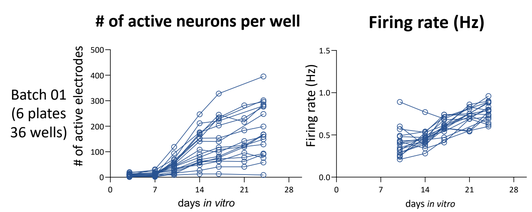
Figure 1. Rat cortical neurons exhibited higher activity as they mature in culture



days *in vitro*	0-7	8-14	15-21	22-28	29-35
# active electrodes	11 ± 1 (212 wells)	104 ± 6 (274 wells)	397 ± 14 (285 wells)	548 ± 22 (177 wells)	202 ± 19 (60 wells)
Firing rate (Hz)	n.a.	0.4 ± 0.0 (168 wells)	0.6 ± 0.0 (273 wells)	0.9 ± 0.1 (172 wells)	1.9 ± 0.1 (55 wells)

Figure 1. MEA recording of spontaneous activity from rat cortical neurons plated at low density (75 cell/mm²) on rat astrocytes. Neurons were considered active if they exhibited a firing rate >0.1 Hz. Number of spontaneously firing neurons (left) and firing rate (right) increased with time in culture. Average ± sem was summarized in the table.

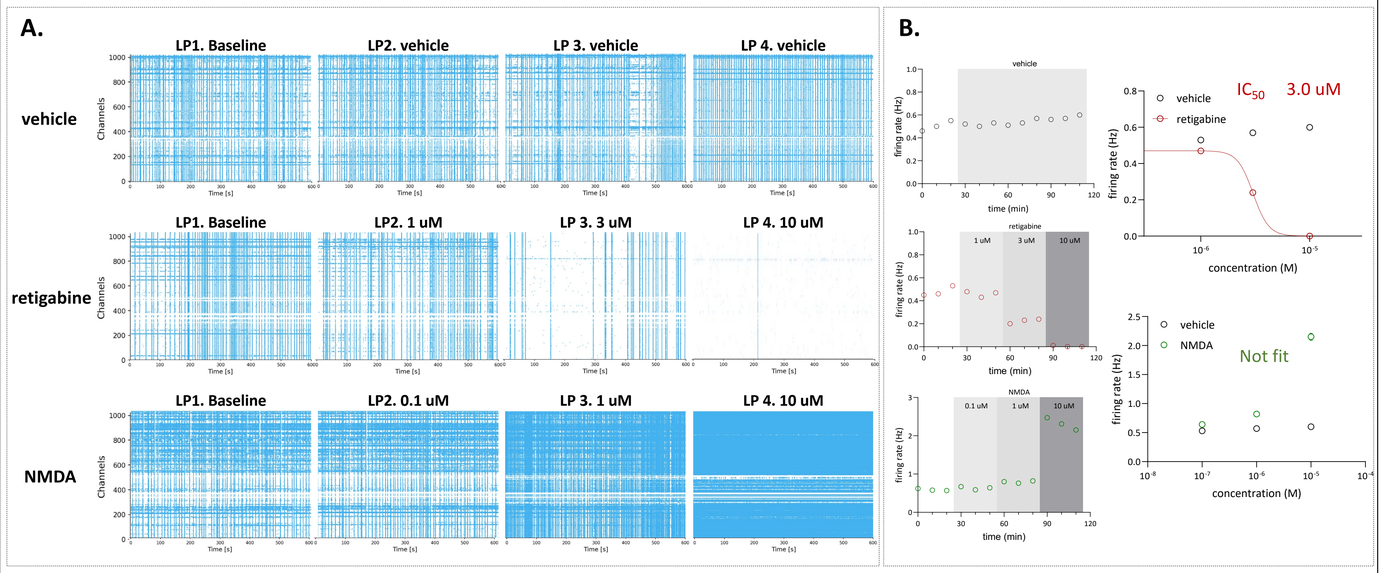
Figure 2. Human iPSC-derived neurons exhibited higher activity as they mature in culture



days *in vitro*	0-7	8-14	15-21	22-28
# active electrodes	11 ± 1 (36 wells)	80 ± 7 (36 wells)	140 ± 9 (30 wells)	188 ± 24 (18 wells)
Firing rate (Hz)	n.a.	0.4 ± 0.2 (33 wells)	0.6 ± 0.3 (29 wells)	0.8 ± 0.0 (17 wells)

Figure 2. MEA recording of spontaneous activity from human iPSC-derived neurons (Fujifilm iCell GlutaNeurons) plated at low density (75 cell/mm²) on rat astrocytes. Neurons were considered active if they exhibited a firing rate >0.1 Hz. Number of spontaneously firing neurons (left) and firing rate (right) increased with time in culture. Average ± sem was summarized in the table.

Figure 3. Retigabine reduced and NMDA increased the firing rate in rat cortical neurons

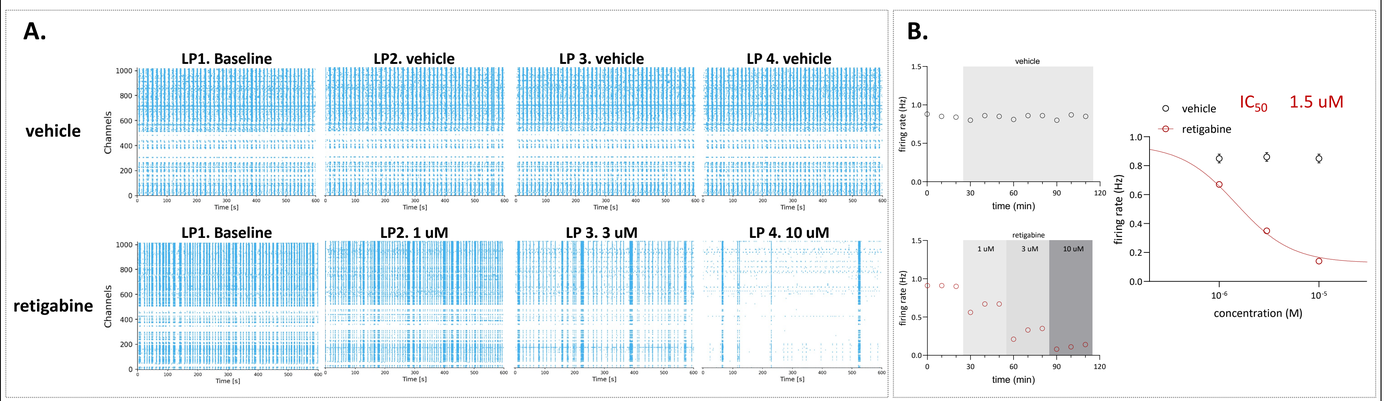


C.

liquid period (LP)	LP 1	LP 2	LP 3	LP 4
vehicle	0.55 ± 0.01 Hz (5219)	0.53 ± 0.01 Hz (5219)	0.57 ± 0.01 Hz (5219)	0.60 ± 0.01 Hz (5219)
retigabine	0.53 ± 0.01 Hz (3793)	0.47 ± 0.01 Hz (3793)	0.24 ± 0.00 Hz (3793)	0.00 ± 0.00 Hz (3793)
NMDA	0.57 ± 0.01 Hz (4834)	0.64 ± 0.01 Hz (4834)	0.82 ± 0.02 Hz (4834)	2.15 ± 0.06 Hz (4834)

Figure 3. **A)** Raster plots of spontaneous activity in rat cortical neurons. **B.)** Retigabine reduced activity and NMDA increased activity. **C.)** Average ± sem firing rate (Hz) was summarized in the table (end of each LP). Number of electrodes (neurons) measured were shown in parenthesis.

Figure 4. Retigabine reduced the firing rate in human iPSC-derived neurons



C.

Liquid period (LP)	LP 1	LP 2	LP 3	LP 4
vehicle	0.84 ± 0.03 Hz (1484)	0.85 ± 0.03 Hz (1484)	0.86 ± 0.03 Hz (1484)	0.85 ± 0.03 Hz (1484)
retigabine	0.90 ± 0.02 Hz (1477)	0.67 ± 0.02 Hz (1477)	0.35 ± 0.01 Hz (1477)	0.14 ± 0.01 Hz (1477)

Figure 4. **A)** Raster plots of spontaneous activity in human iPSC-derived neurons (Fujifilm iCell GlutaNeurons). **B.)** Retigabine reduced activity. **C.)** Average ± sem firing rate (Hz) was summarized in the table (end of each LP). Number of electrodes (neurons) measured were shown in parenthesis.