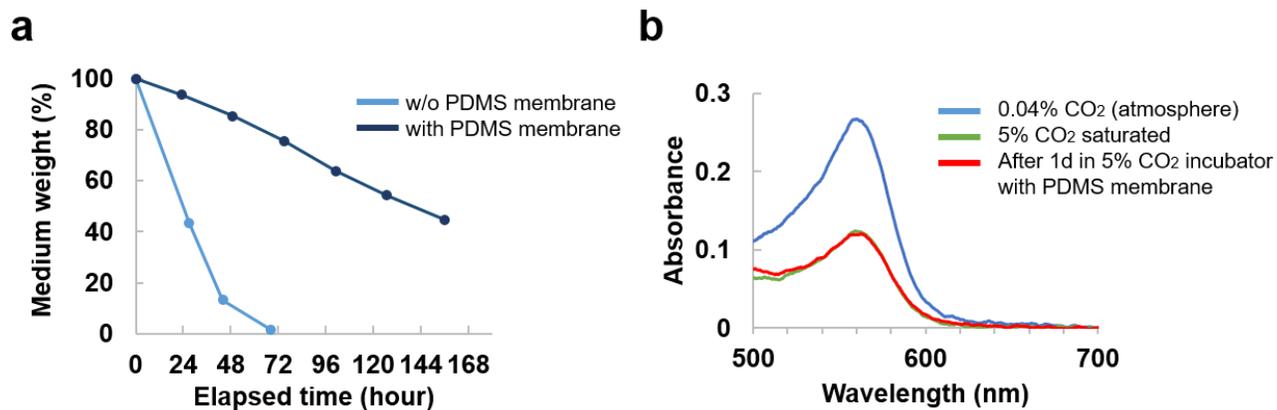


[Electronic Supplementary Information]

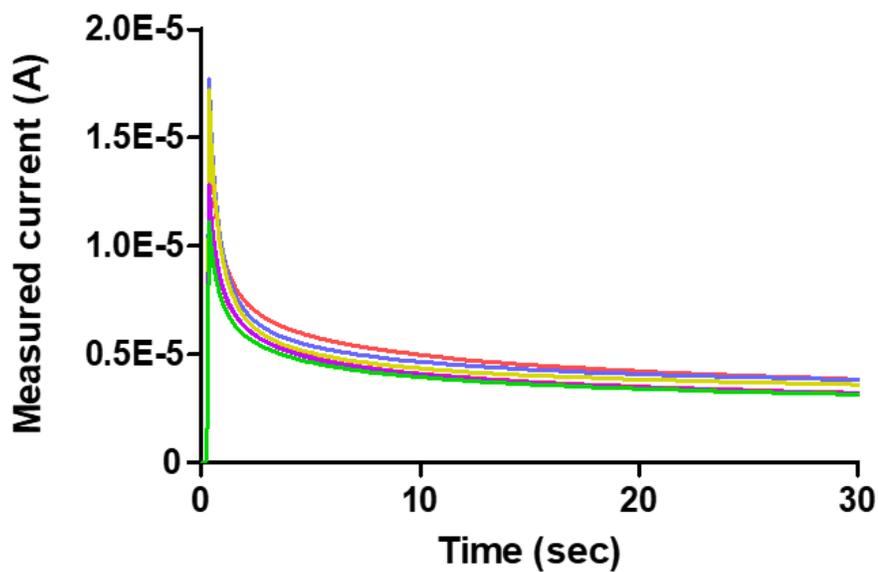
**Compact 256-channel multi-well microelectrode array system  
for *in vitro* neuropharmacology test**

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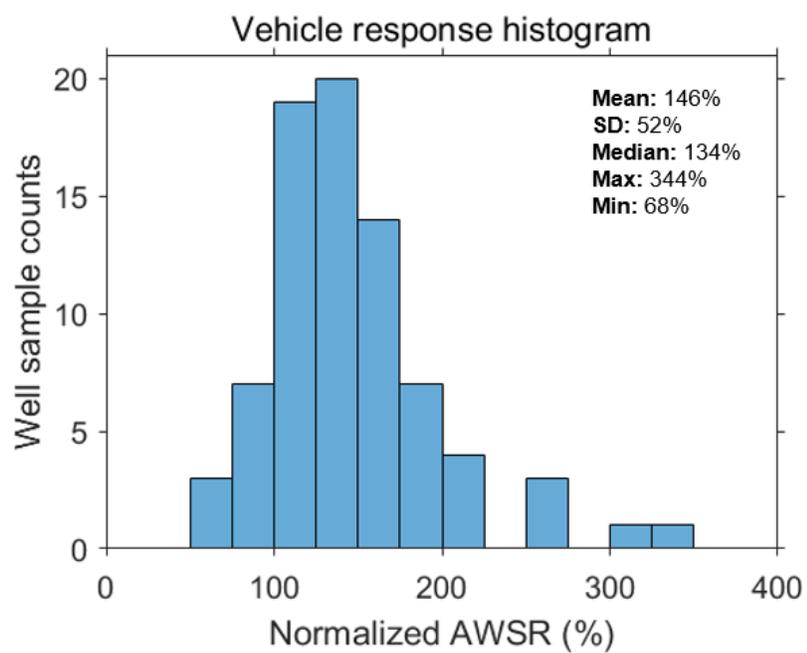
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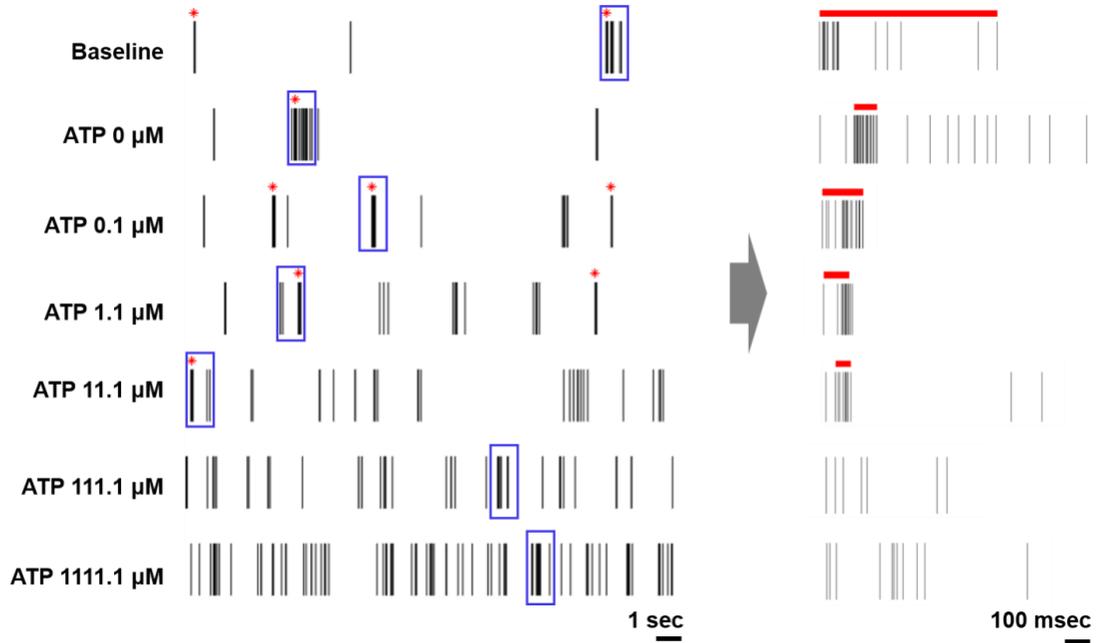
**Fig. S1 Evaporation and gas permeability test of a PDMS multi-well chamber and thin PDMS membrane.** (a) The medium weight decreased less than 20% with PDMS membrane (~0.7mm) compared to 90% reduction when membrane was not used (Dried incubator condition, 37°, 5% CO<sub>2</sub>, no water tank). (b) Spectrophotometer measured absorbance spectra of the culture medium with phenol red in three different conditions; in atmospheric condition (0.04% CO<sub>2</sub>), in 5% CO<sub>2</sub> saturated condition and in 5% CO<sub>2</sub> condition with PDMS membrane. CO<sub>2</sub> permeability of the thin PDMS membrane was tested by identifying reduced absorbance peak (at 560 nm wavelength) from atmospheric conditioned medium to 5% CO<sub>2</sub> saturated culture medium after 1 day in 5% CO<sub>2</sub> incubator with thin PDMS membrane sealed.



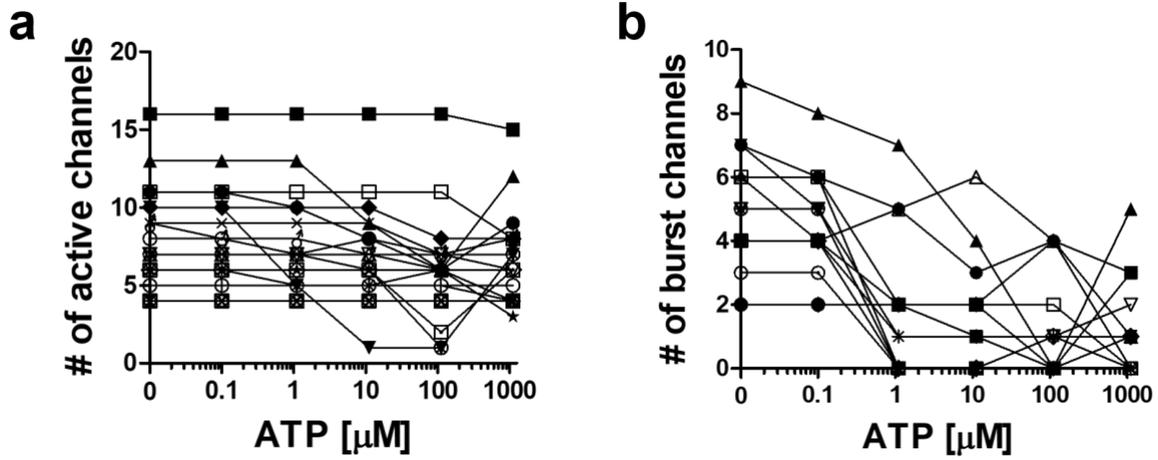
**Fig. S2 Measured current during electropolymerization of PEDOT:PSS on 256 electrodes in array-wide.** PEDOT:PSS conductive polymer was electrodeposited array-wide on 256 gold microelectrodes using potentiostatic 1.2V, 30 second (30  $\mu\text{m}$  electrode, n=6 MEAs). Mean value of measured current from 256 electrodes were plotted for total 6 multiwell MEA chips.



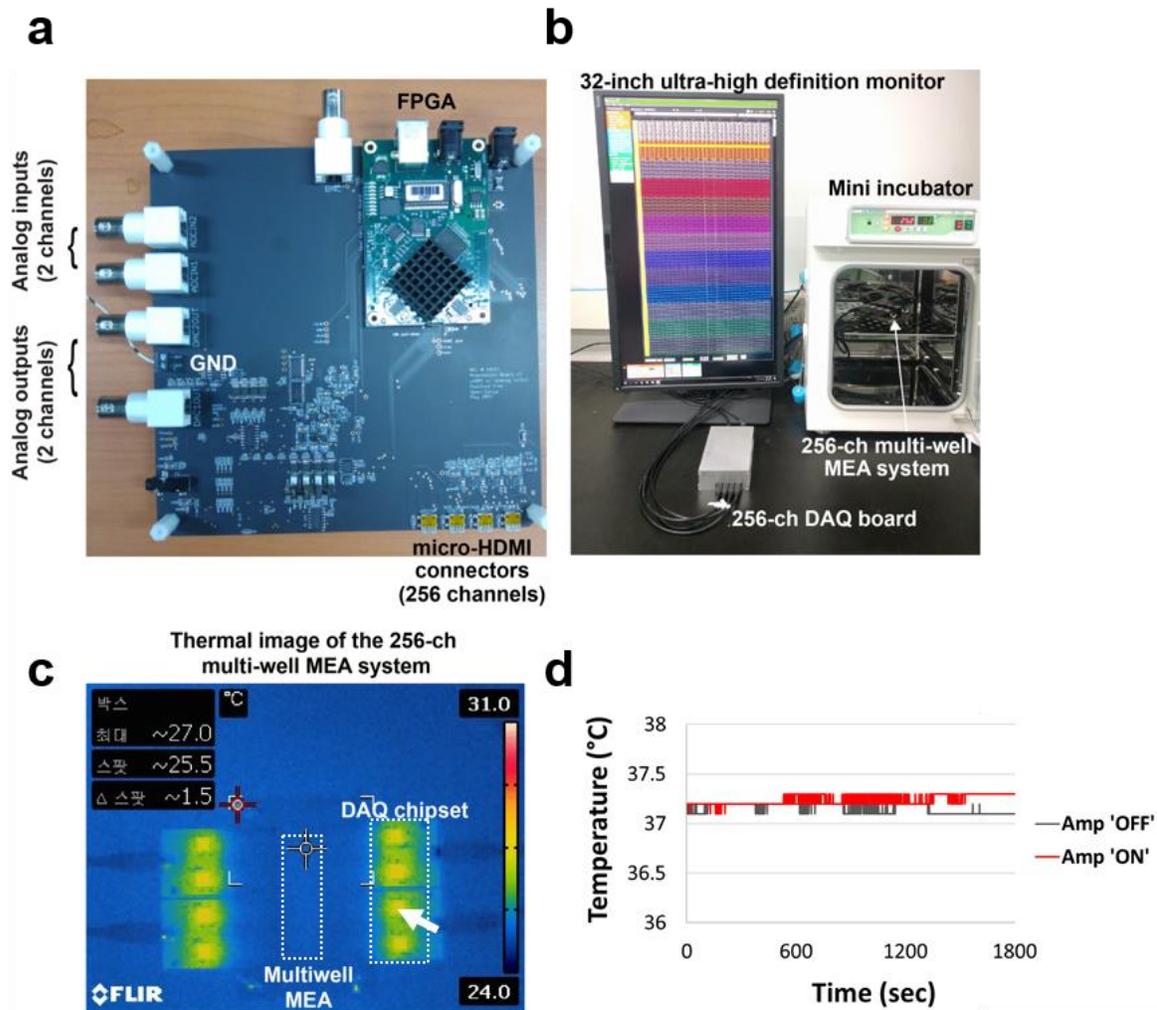
**Fig. S3** Distribution of array-wide spike rate after vehicle treatment. Array-wide spike rate is normalized to the baseline activity.



**Fig. S4 Visual inspection of bursts with applied ATP concentration.** Bursts were detected by surprise based method with minimum 10 surprise. Burst starting points are indicated as red asterisk above rasters and magnified raster plots of blue box regions are drawn on the right with red bar indicating burst duration.



**Fig. S5 ATP concentration dependent variation on the number of active channels and burst channels.** (a) The number of active channels depending on ATP concentrations (n=20 wells, 3 multiwell MEA chips). (b) The number of burst channels depending on ATP concentrations (n=17 wells, 3 multiwell MEA chips). Each data set represents a well sample.



**Fig. S6 256-ch multi-well MEA system configuration.** (a) 256-channel acquisition board with BNC connectors for analog input and output signals (two channels for each). (b) Overall neural recording setup. The entire 256-channel multi-well MEA system (i.e. multi-well MEA chip with 256-ch digital amplifiers) is placed in a mini incubator. (c) Thermal image (Infrared camera FLIR E6, Estonia) of the 256-ch MEA system after 30 minutes of operation in room temperature. Surface temperature of ASIC chip increased by 3 °C (pointed by arrow) compared to PCB background. (d) Thermocouple measured medium solution temperature inside 37 °C incubator for 30 minutes with amplifier powered on and off. To confirm that generated heat from DAQ chips have little impact on the recording environment, we measured the medium solution temperature by thermocouple (K type hyperdermic Needle Probes, Omega, USA) connected to DAQ (USB-TC01, National Instrument, USA) for 30 minutes with incubator temperature set to 37°C (incubator sensor readout fluctuated between 37.0°C and 37.1°C). The result showed that medium temperature within a well changed in between 37.1°C and 37.3°C. When the amplifier power was off, the medium temperature maintained between 37.1°C and 37.2°C.

**Table. S1 Comparison of 256 channel multi-well MEA slide and other commercial multi-well MEA plates**

| <i>Company/ Group</i> | <i>Total electrodes</i> | <i>Well #</i> | <i>Electrodes/well</i> | <i>Electrode size <math>\phi</math> (<math>\mu\text{m}</math>)</i> | <i>Chip size, L (mm) <math>\times</math> W (mm)</i> | <i>Well density (well count/ chip area(<math>\text{cm}^2</math>))</i> | <i>Compact factor (electrode #/ chip area(<math>\text{cm}^2</math>))</i> |
|-----------------------|-------------------------|---------------|------------------------|--|---|---|--|
| NEL<br>(in this work) | 256                     | 16            | 16                     | 10, 30 or 50   | 75 $\times$ 25                                      | 0.85  | 13.65  |
| Multichannel systems  | 288                     | 24            | 12                     | 100  | 127.76 $\times$ 85.48                               | 0.22  | 2.64   |
| Multichannel systems  | 288                     | 24            | 12                     | 30   | 127.76 $\times$ 85.48                               | 0.22  | 2.64   |
| Multichannel systems  | 288                     | 96            | 3                      | 100  | 127.76 $\times$ 85.48                               | 0.88  | 2.64   |
| Multichannel systems  | 1152                    | 96            | 12                     | 100  | 127.76 $\times$ 85.48                               | 0.88  | 10.55  |
| Axion BioSystems      | 768                     | 12            | 64                     | 150  | 127.76 $\times$ 85.48                               | 0.11  | 7.03   |
| Axion BioSystems      | 768                     | 48            | 16                     | 150  | 127.76 $\times$ 85.48                               | 0.44  | 7.03   |
| Axion BioSystems      | 768                     | 96            | 8                      | 150  | 127.76 $\times$ 85.48                               | 0.88  | 7.03   |
| Alpha MED Scientific  | 384                     | 24            | 16                     | Square, 50 $\times$ 50   | 105 $\times$ 140                                    | 0.16  | 2.61   |
| Alpha MED Scientific  | 384                     | 48            | 8                      | Square, 50 $\times$ 50   | 105 $\times$ 140                                    | 0.33  | 2.61   |
| Alpha MED Scientific  | 384                     | 96            | 4                      | Square, 50 $\times$ 50   | 105 $\times$ 140                                    | 0.65  | 2.61   |
| Alpha MED Scientific  | 64                      | 4             | 16                     | 20 or 50   | 100 $\times$ 35                                     | 0.11  | 1.83   |
| Alpha MED Scientific  | 64                      | 8             | 8                      | 20 or 50   | 100 $\times$ 35                                     | 0.23  | 1.83   |