Wang et al.:
Assessment of a 3D Neural Spheroid Model to Detect Pharmaceutical-Induced Neurotoxicity

Supplementary Data

Fig. S1 (video): 3D-perspective video of immunostaining of spheroid with GFAP (green fluorescence) and MAP2 (red fluorescence) in Fig. 1A recorded by Nikon Eclipse Ti2 microscope (see doi:10.14573/altex.2112221s3)

Fig. S2: Exemplary graphs of dose-response curves of the 6 individual endpoints obtained from the calcium oscillation assay for (A) doxorubicin, (B) carboplatin, (C) MPTP, (D) gabapentin, (E) AMPA, (F) acetylcholine, (G) 4-aminopyridine, (H) FPL64176, (I) acetaminophen, and from the cellular ATP assessment for these compounds (J). Dash lines are the clinical reported $t_{pC_{max}}$ for that compound if approved by a regulatory agency. Experiments were conducted in triplicate on three individual neural spheroids for each compound.

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B

**Carboplatin**

**Normalized Peak Count**

**Normalized Peak Amplitude**

**Normalized Peak Rise Time**

**Normalized Peak Decay Time**

**Normalized Peak Spacing**

**Normalized Peak Width (10% Amp)**
C

**Normalized Peak Count**

**Normalized Peak Amplitude**

**Normalized Peak Rise Time**

**Normalized Peak Decay Time**

**Normalized Peak Spacing**

**Normalized Width (10% Amp)**
**Gabapentin**

**Normalized Peak Count**

**Normalized Peak Amplitude**

**Normalized Peak Rise Time**

**Normalized Peak Decay Time**

**Normalized Peak Spacing**

**Normalized Peak Width (10% Amp)**
E

**AMPA**

- **Normalized Peak Count**
  - Graph showing normalized peak count against log uM.

- **Normalized Peak Amplitude**
  - Graph showing normalized peak amplitude against log uM.

- **Normalized Peak Rise Time**
  - Graph showing normalized peak rise time against log uM.

- **Normalized Peak Decay Time**
  - Graph showing normalized peak decay time against log uM.

- **Normalized Peak Spacing**
  - Graph showing normalized peak spacing against log uM.

- **Normalized Peak Width (10% Amp)**
  - Graph showing normalized peak width against log uM.
4-Aminopyridine

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
Acetaminophen

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
Fig. S3: Assay variability for calcium oscillation
Inter-plate variability of different measurements for the vehicle control samples in all the plates, data not normalized. Mean (black squares) is shown for each phenotype overlaid on top of grey circles representing individual well response. Coefficients of variation (%CV) are shown for each phenotype.
Neural spheroids were treated with pharmaceuticals clinically associated with neurodegeneration (doxorubicin (A), paclitaxel (B), colchicine (C)) or control (ibuprofen (D)) for 24, 72 or 144 h. Neural spheroids treated with neurodegenerative pharmaceuticals were associated with a decrease in total cellular ATP in a time and dose-dependent manner, but ibuprofen-treated neural spheroids were not.