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)

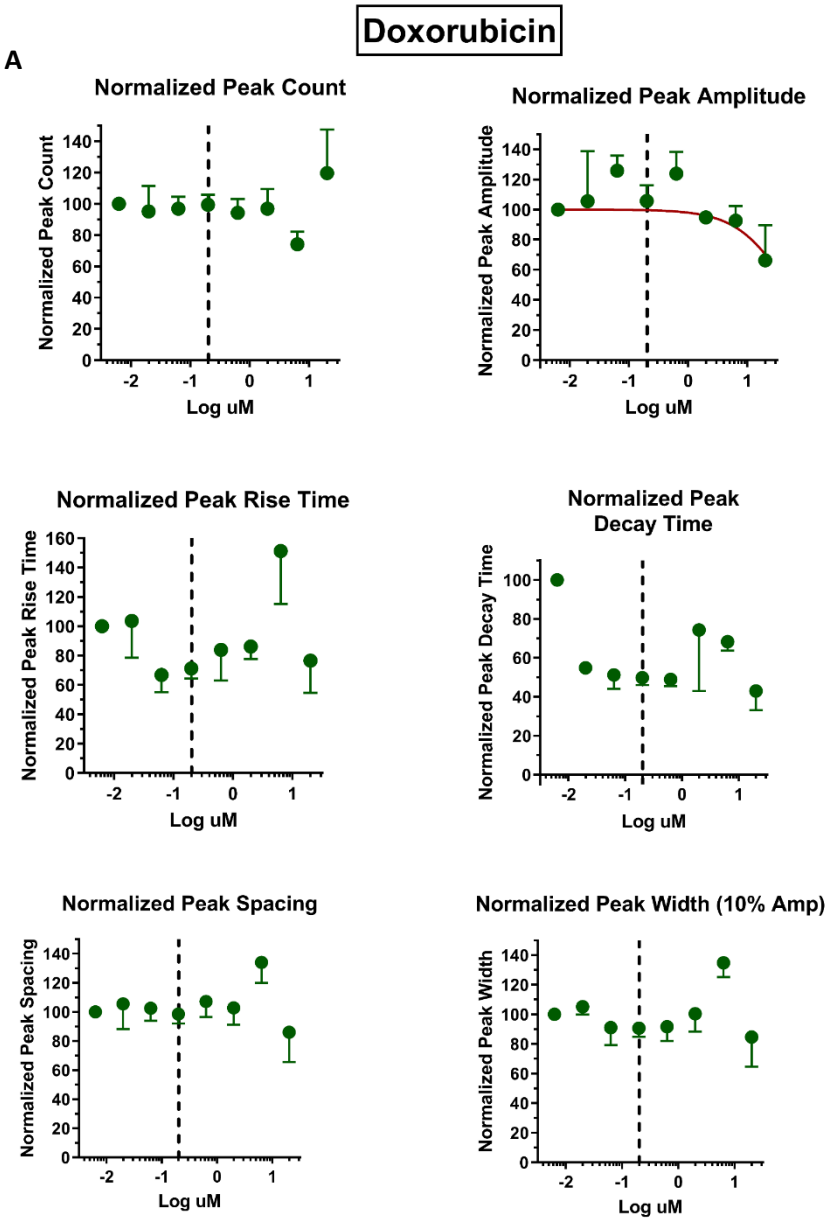
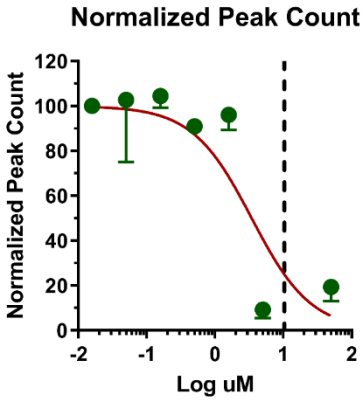


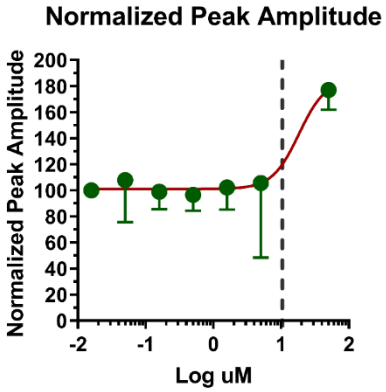
Fig. S2: Exemplary graphs of dose-response curves of the 6 individual endpoints obtained from the calcium oscillation assay for (A) doxorubicin, (B) carboplatin, (C) MPTP, (D) gabapentin, (E) AMPA, (F) acetylcholine, (G) 4-aminopyridine, (H) FPL64176, (I) acetaminophen, and from the cellular ATP assessment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

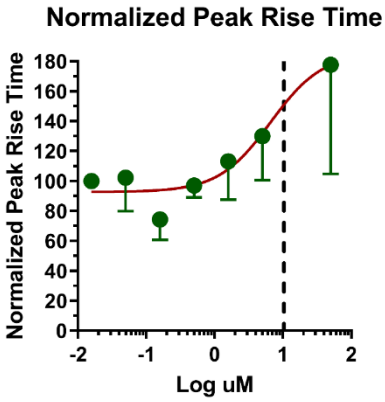
doi:10.14573/altex.2112221s2

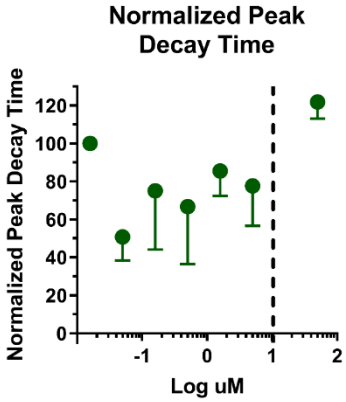
B

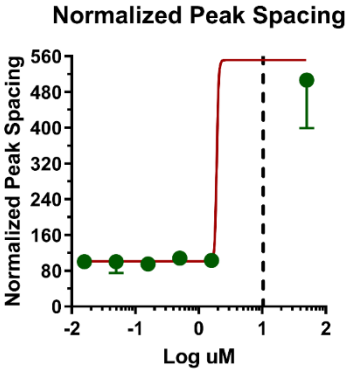
Carboplatin

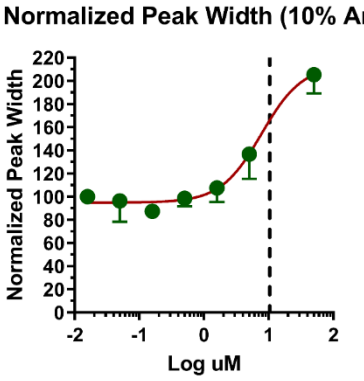












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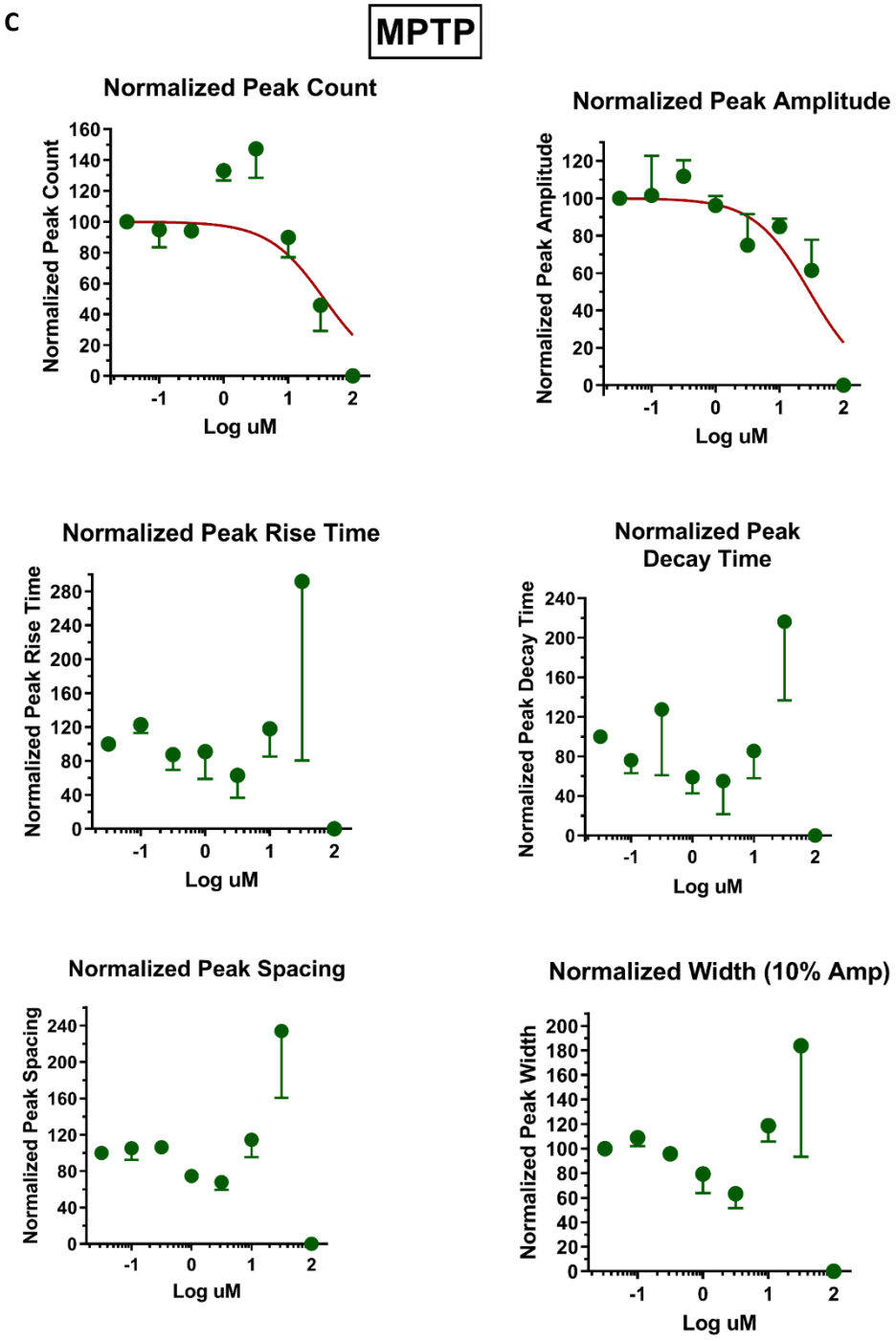
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Normalized Peak Count
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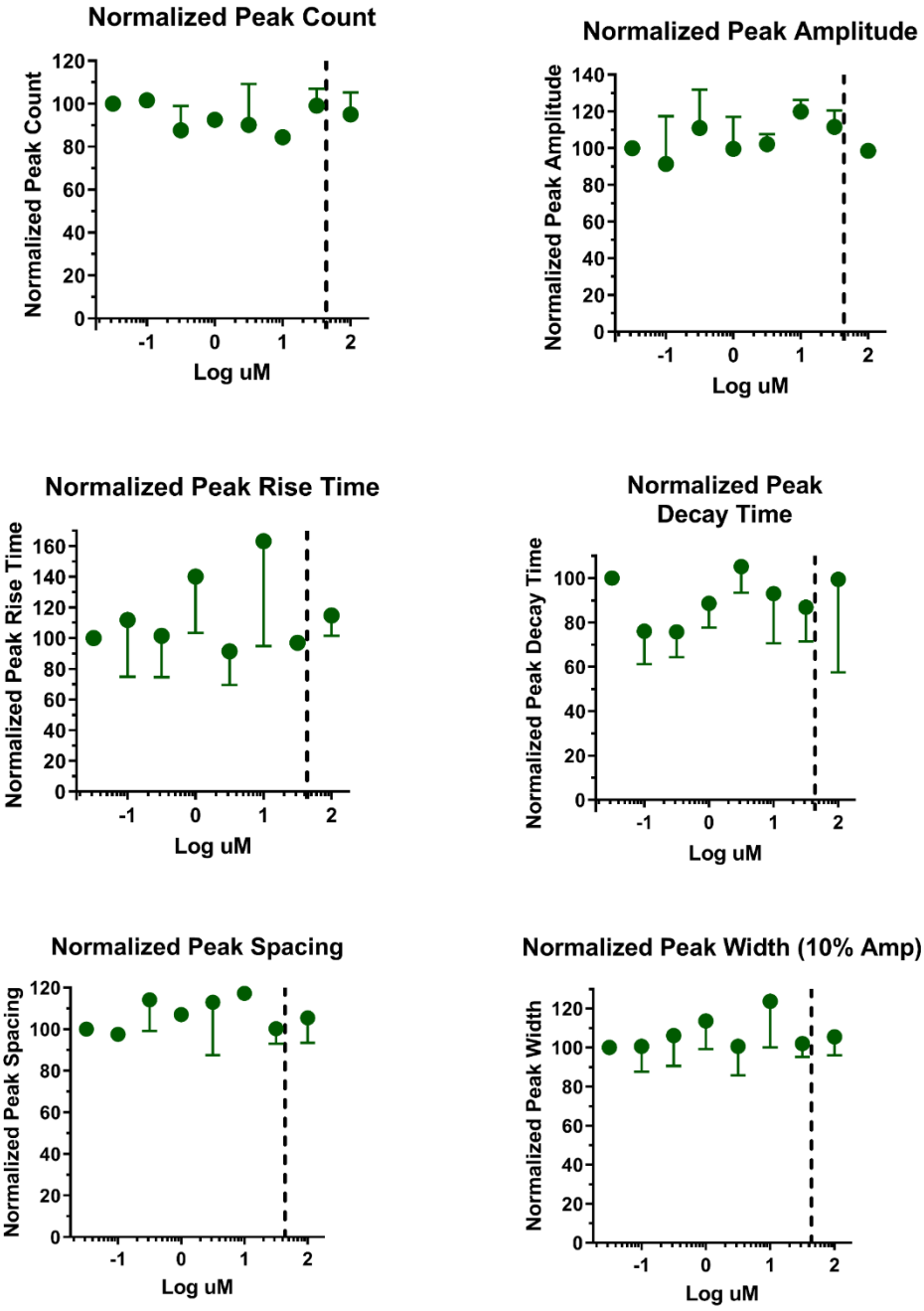
Normalized Peak Amplitude
Normalized Peak Amplitude
Log uM
Normalized Peak Rise Time
Normalized Peak Rise Time
Log uM
Normalized Peak Decay Time
Normalized Peak Decay Time
Log uM
Normalized Peak Spacing
Normalized Peak Spacing
Log uM
Normalized Width (10% Amp)
Normalized Peak Width
Log uM

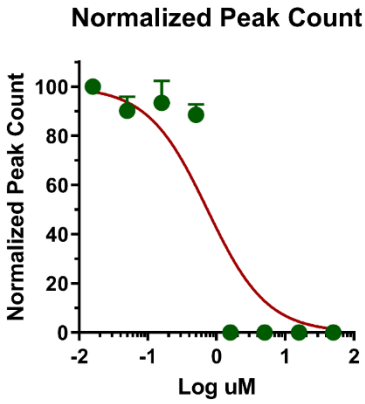
D

Gabapentin



E

AMPA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Normalized Peak Count
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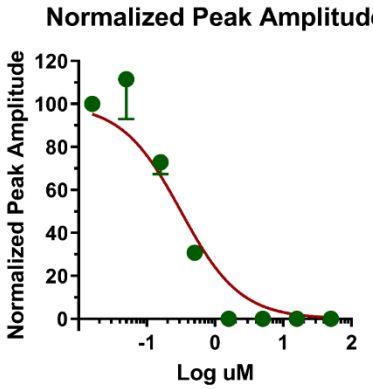
60
40
20
0
-2
-1
0
1
2
Log uM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80
60
40
20
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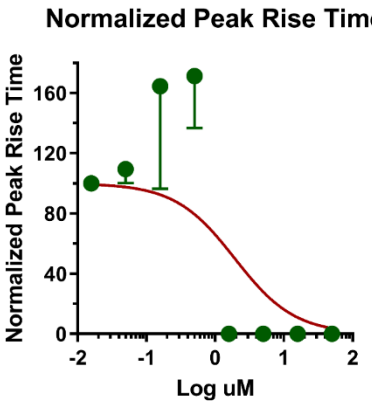
-1
0
1
2
Log uM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Normalized Peak Rise Time
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80
40
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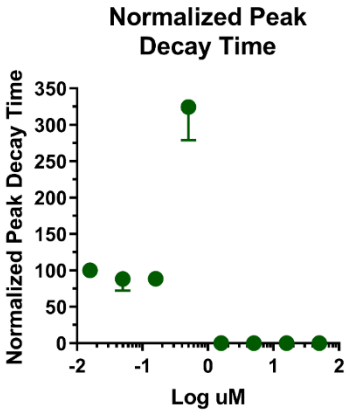
-2
-1
0
1
2
Log uM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Decay Time
Normalized Peak Decay Time
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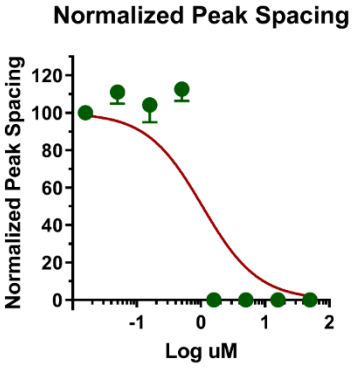
250
200
150
100
50
0
-2
-1
0
1
2
Log uM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Normalized Peak Spacing
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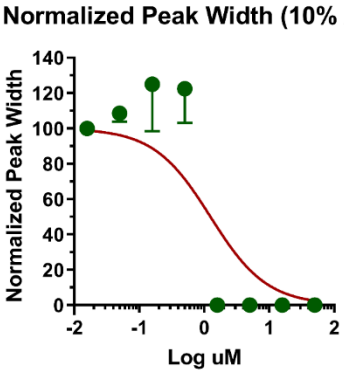
100
80
60
40
20
0
-1
0
1
2
Log uM


Normalized Peak Width (10% Amp)
Normalized Peak Width
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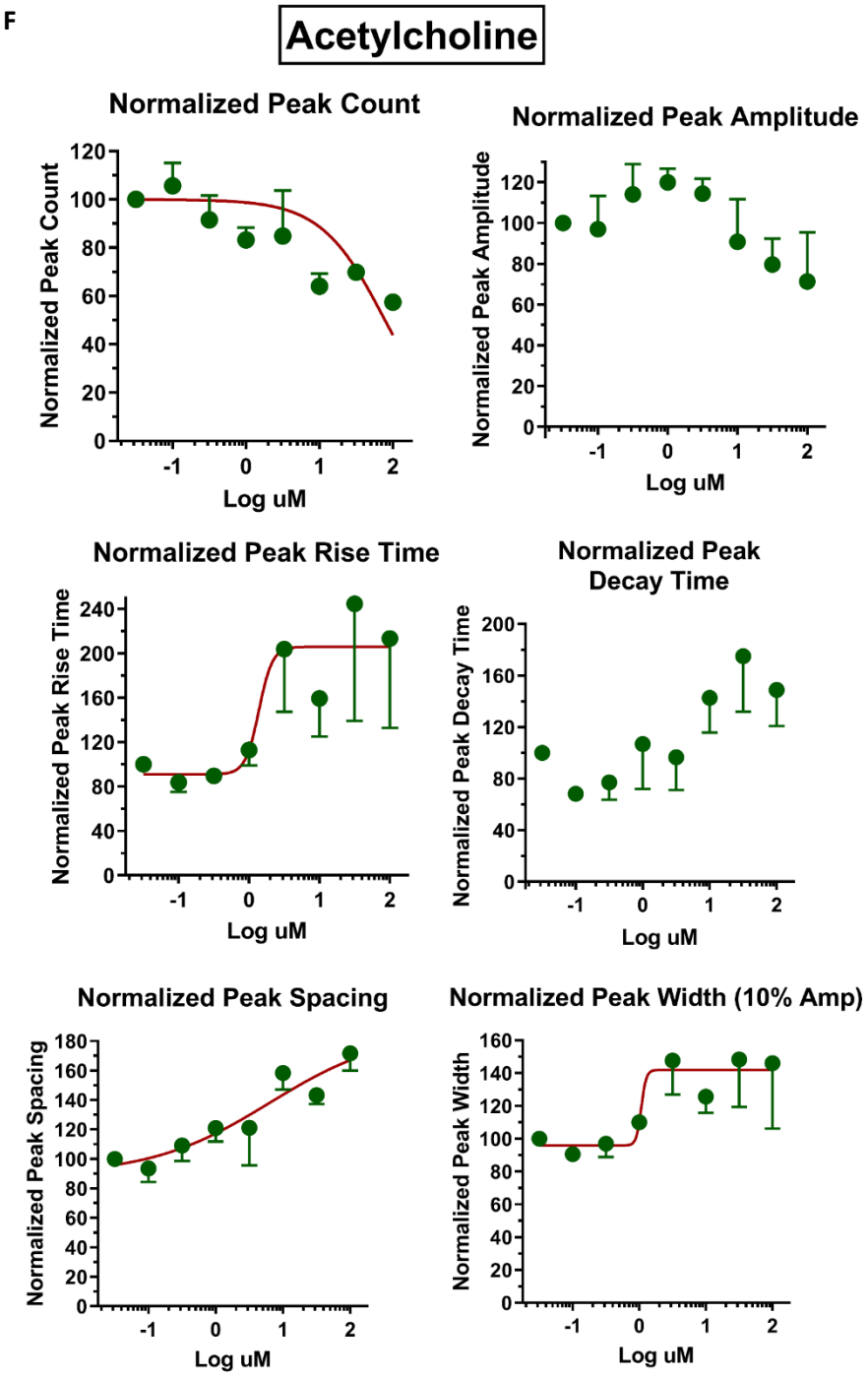
100
80
60
40
20
0
-2
-1
0
1
2
Log uM

F


Acetylcholine
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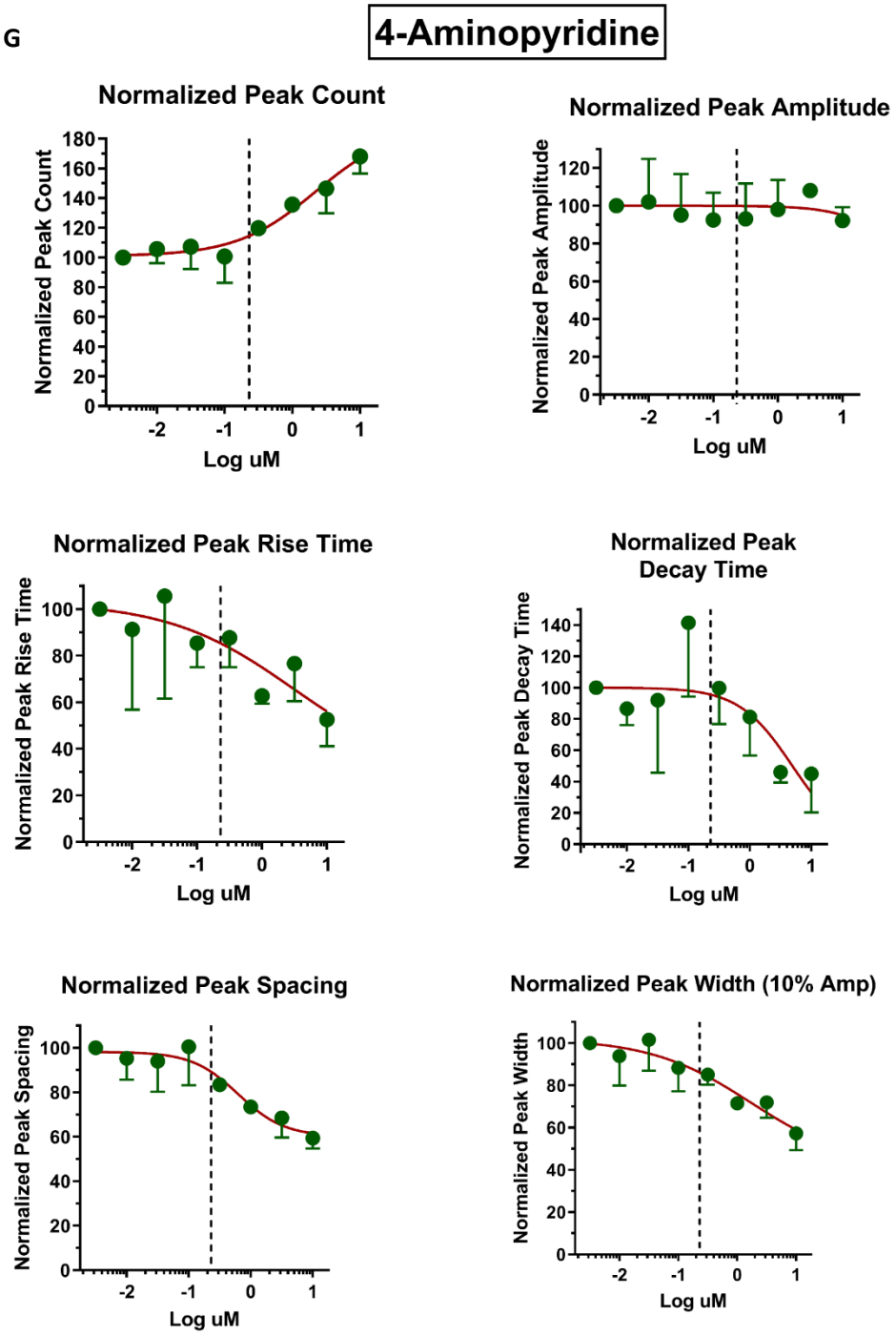
Normalized Peak Count
Log uM
Normalized Peak Amplitude
Normalized Peak Amplitude
Log uM
Normalized Peak Rise Time
Normalized Peak Rise Time
Log uM
Normalized Peak Decay Time
Normalized Peak Decay Time
Log uM
Normalized Peak Spacing
Normalized Peak Spacing
Log uM
Normalized Peak Width (10% Amp)
Normalized Peak Width
Log uM

G


4-Aminopyridine
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Normalized Peak Count
Log uM
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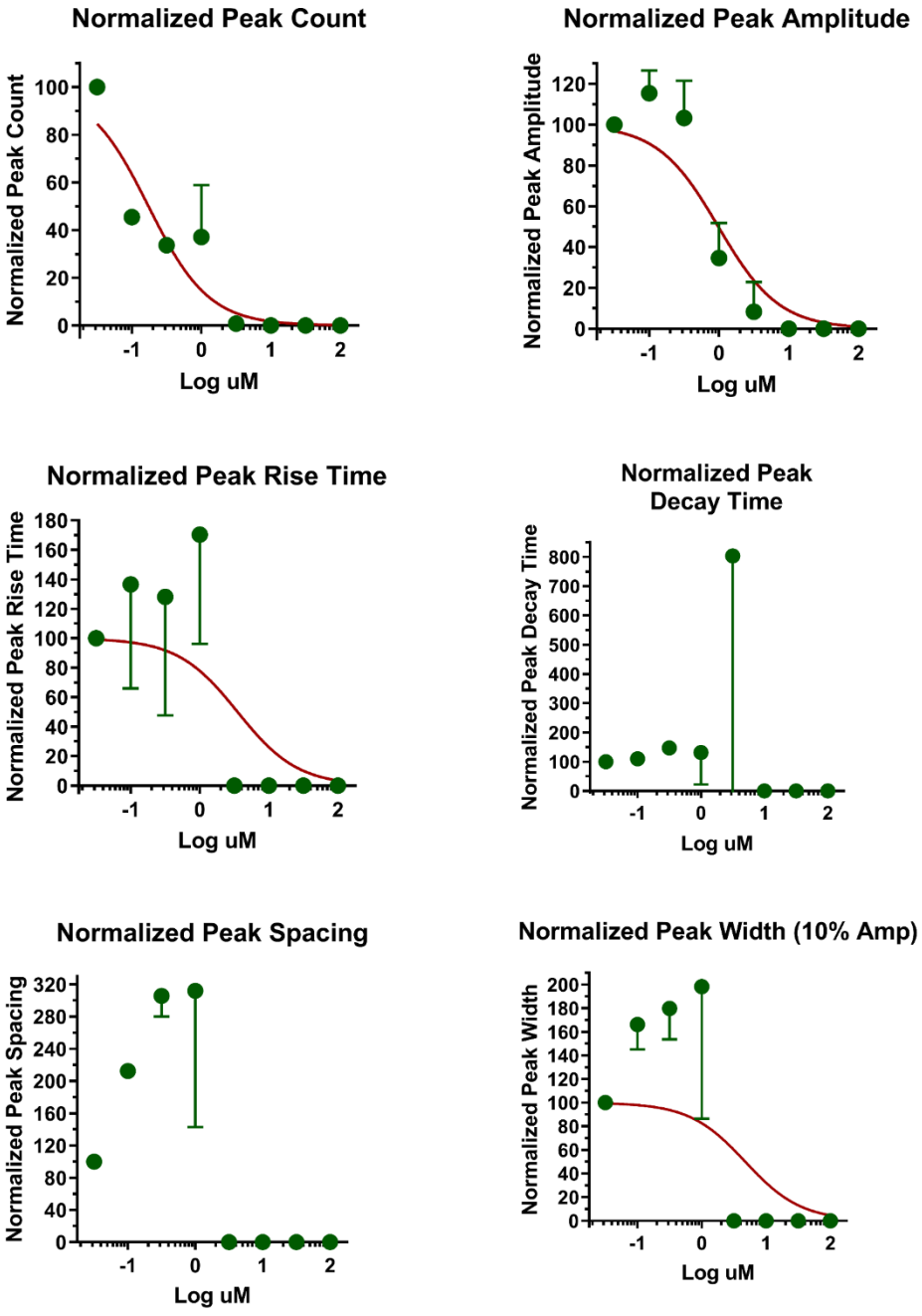
Normalized Peak Amplitude
Log uM
Normalized Peak Rise Time
Normalized Peak Rise Time
Log uM
Normalized Peak Decay Time
Normalized Peak Decay Time
Log uM
Normalized Peak Spacing
Normalized Peak Spacing
Log uM
Normalized Peak Width (10% Amp)
Normalized Peak Width
Log uM

H

FPL64176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Log uM
Normalized Peak Amplitude
Normalized Peak Amplitude
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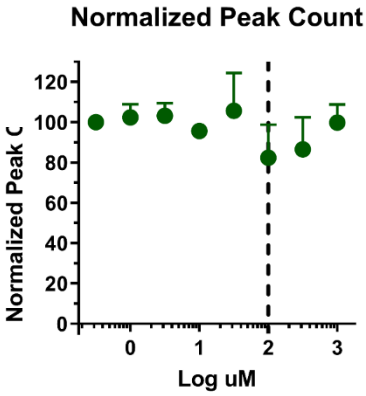
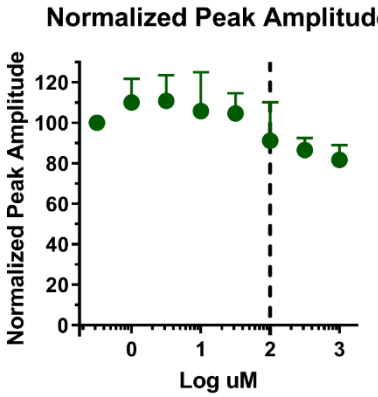
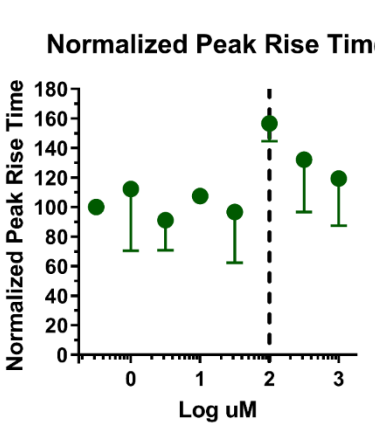
Normalized Peak Rise Time
Log uM
Normalized Peak Decay Time
Normalized Peak Decay Time
Log uM
Normalized Peak Spacing
Normalized Peak Spacing
Log uM
Normalized Peak Width (10% Amp)
Normalized Peak Width
Log uM

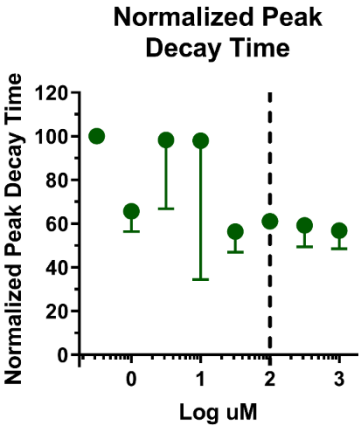
I

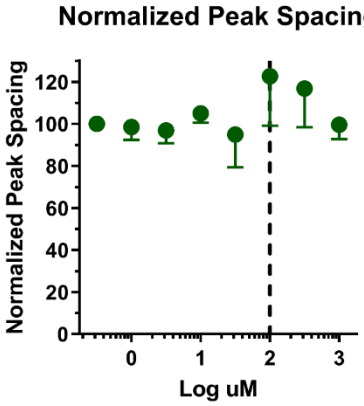
Acetaminophen

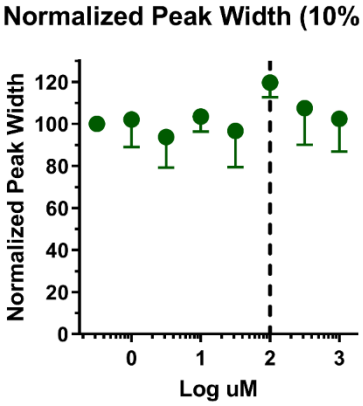


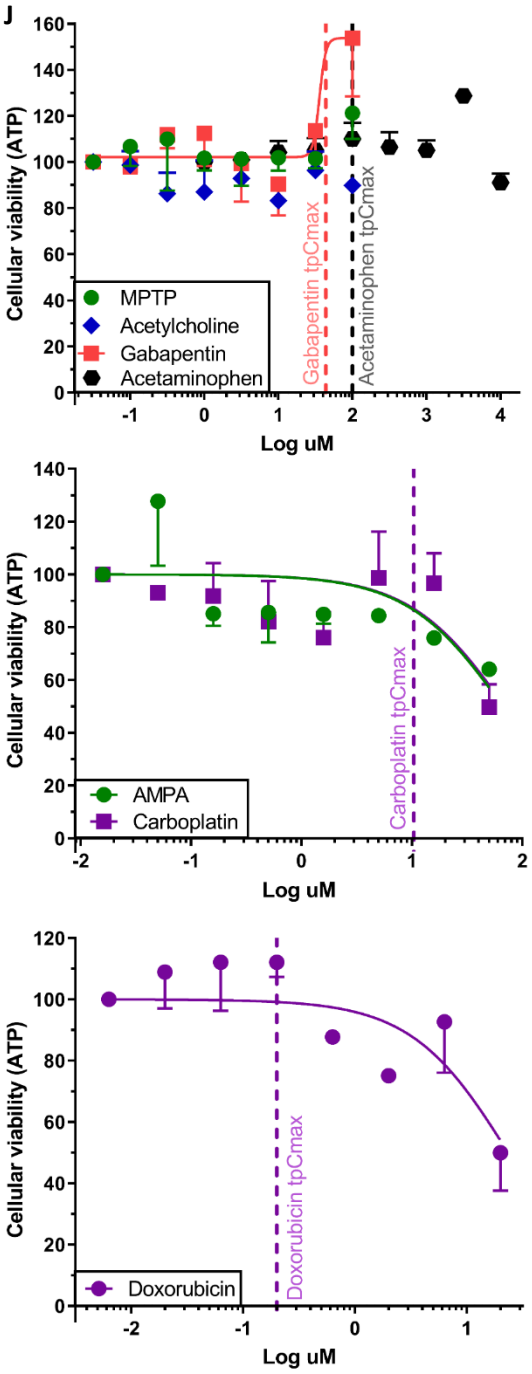











J
Cellular viability (ATP)
Log uM
MPTP
Acetylcholine
Gabapentin
Acetaminophen
Gabapentin tpCmax
Acetaminophen tpCmax
AMPA
Carboplatin
Carboplatin tpCmax
Doxorubicin
Doxorubicin tpCmax

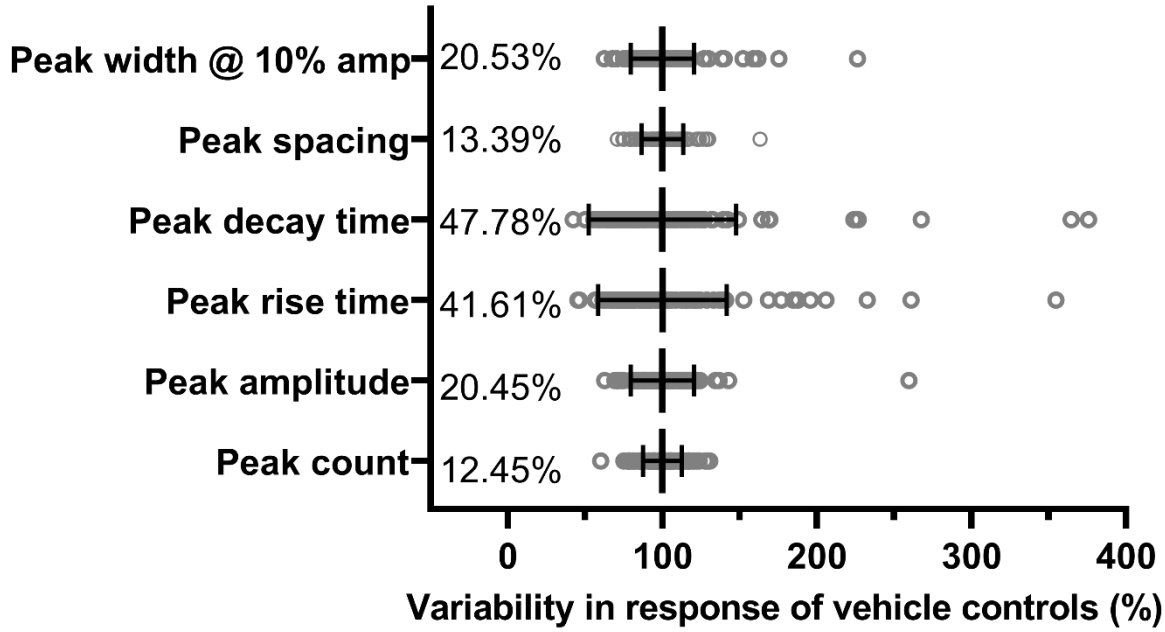


Fig. S3: Assay variability for calcium oscillation
Inter-plate variability of different measurements for the vehicle control samples in all the plates, data not normalized. Mean (black squares) is shown for each phenotype overlaid on top of grey circles representing individual well response. Coefficients of variation (%CV) are shown for each phenotype.

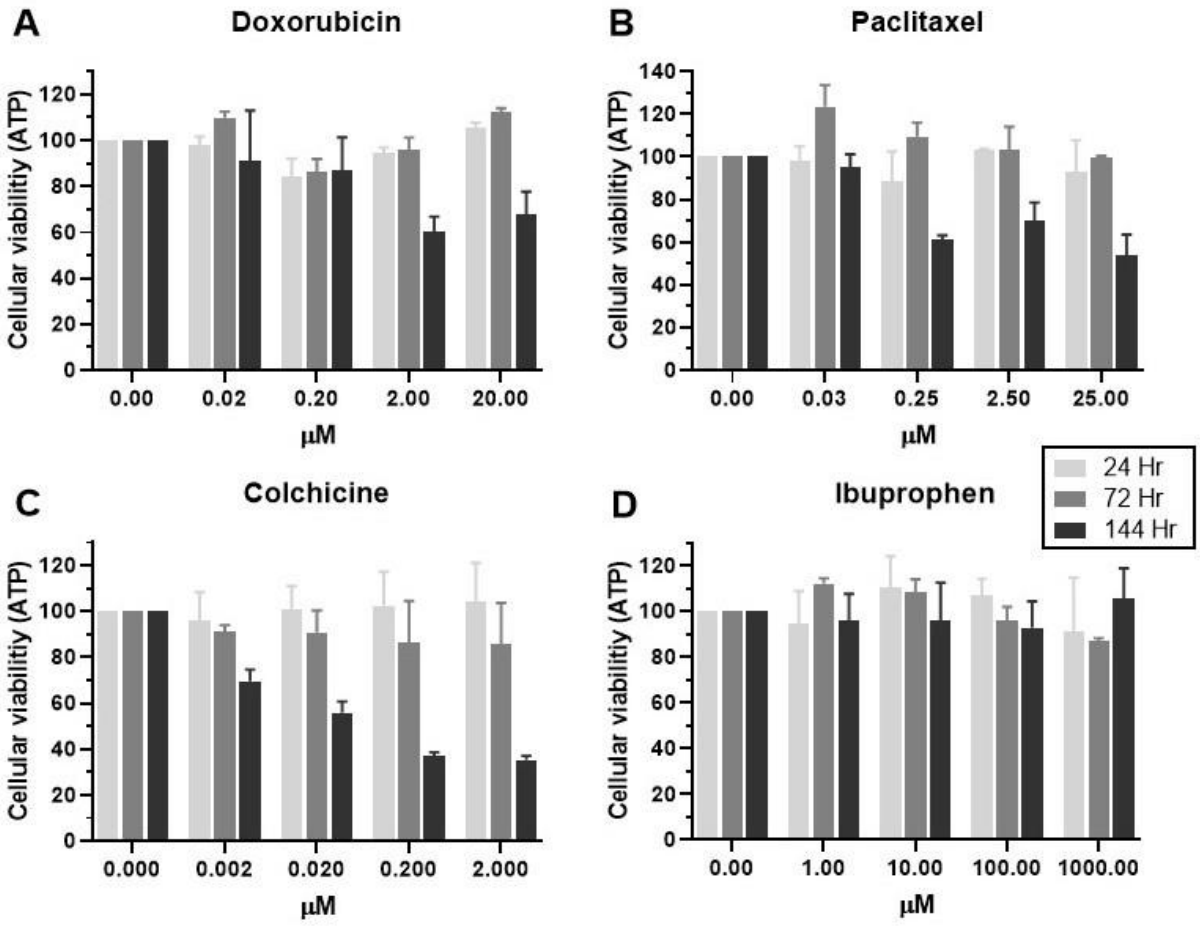


Fig. S4: Neural spheroids were treated with pharmaceuticals clinically associated with neurodegeneration (doxorubicin (A), paclitaxel (B), colchicine (C)) or control (ibuprofen (D)) for 24, 72 or 144 h

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.