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Assessing seizure liability using multi-electrode arrays (MEA)

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ABSTRACT

The purpose of these studies was to develop ex vivo tissue-based and in vitro cell-based assays using multi-electrode array (MEA) technology to predict seizure liability at the early stage of preclinical studies. Embryonic rat hippocampal neurons and adult rat hippocampal slices were used in these studies. Spontaneous activity in cultured neurons and evoked field potentials in hippocampal brain slices were recorded using MEA technology. Six seizurogenic compounds bicuculline, pentylenetetrazole, picrotoxin, gabazine, 4-Aminopyridine and BMS-A increased field potential area and peak number in brain slices and spontaneous spike activity in hippocampal neurons. Physostigmine, another seizurogenic compound, had no effect on brain slices at lower concentrations (0.1, 1, and 10 μM), and mildly increased field potential area at 100 μM. However, physostigmine induced multiple peaks in evoked field potential starting at 10 μM. Physostigmine showed greater potency in the cultured neuron assay, and increased spike rates in the nanomolar range. Two seizurogenic compounds, BMS-B and BMS-C increased the spontaneous activity in hippocampal neurons, but did not increase area and peak number of field potentials in brain slices. These findings suggest that MEA technology and rat hippocampal brain slices or rat embryonic hippocampal neurons, may be useful as early, predictive in vitro assays for seizure liability.

1. Introduction

It has been reported that 6.1% of new onset seizures are drug related and up to 9% of cases of status epileptics presenting to the emergency department may result from drug toxicity (Thundiyil et al., 2011). Not only drugs targeting CNS but those targeting infection, cardiovascular, respiratory, metabolic, gastrointestinal, oncologic and other therapeutic areas can cause seizures (Easter et al., 2009). Drug induced seizures are severe adverse events and can have profound negative impact on drug development. Unfortunately, seizure potential of drug candidates is not typically evaluated until late stage in preclinical discovery, during in vivo toxicology studies, and the timing of this assessment is such that positive findings of seizure liability could result in the need to identify alternate clinical candidates, significantly impacting timelines. Development of a reliable, standardized, in vitro seizurogenicity assay that could be implemented early in discovery would be a valuable tool for the industry.

Recently, several groups developed in vitro (Easter et al., 2007; Markgraf et al., 2014) or in vivo methods (Leiser et al., 2011; Metea et al., 2015) to evaluate the drug induced seizure liability at the early preclinical drug discovery stage. These in vitro systems use conventional electrophysiological recording techniques to measure electrically evoked synaptic activity from rat hippocampal slices. The assays are predictive for seizurogenic effects of a wide range of compounds associated with seizure induction in man. However, such techniques have limitations including being more technically difficult, requiring an experienced electrophysiologist and invasive microelectrodes to record spontaneous or evoked potentials. Also, measurements are highly localized to the regions where single recording electrodes are placed and this can result in misleading or false negative results.

Unlike conventional brain slice recording technique, which utilizes fine extracellular electrodes inserted into the vicinity of a cell or cells to measure field potentials within a tissue, multi-electrode array (MEA) is a technology that allows extracellular recording from many electrodes imbedded in the bottom of the MEA well without the need to penetrate into the tissues. The electrical activity across a brain slice can be measured at multiple points where the imbedded electrodes contact the tissue. Stimulation and recording electrodes can be selected from any of the electrodes based on the position of the brain slice. Therefore, the chance of producing false negatives may be lower than that of the

Abbreviations: 4-AP, 4-Aminopyridine; APAP, Acetaminophen; Bic, Bicuculline; FP, Field Potential; Gab, Gabazine; MEA, Multi-electrode array; Pic, Picrotoxin; PTZ, Pentylenetetrazol

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conventional extracellular recording technique (Steidl et al., 2006). Additionally, in comparison with conventional microelectrode recording techniques, the MEA technique allows for long-term analysis of the spatiotemporal distribution of network-level electrical activity, as well as stable recording that is less sensitive to factors related to tissue impalement with microelectrodes such as mechanical vibration (Liu et al., 2012).

Another way to detect drug-induced seizure liability using MEA technology involves the measurement of spike activity in native neuronal cells that are dispersed and then cultured in the well of the MEA dish (van Vliet et al., 2007). The neurons grow and form synaptic networks during culture, and field potential recordings across the array of electrodes can be used to evaluate seizure liability during application of a test article. This is a particularly useful method of evaluating seizure liability of drugs that are poorly permeable and slow to reach the interior region of tissue preparations. In this study, the utility of MEA technology for assessing drug-induced seizure liability was evaluated in both hippocampal brain slices and in cultured primary hippocampal neurons.

2. Methods

2.1. Rat cultured hippocampal neuron assay

All animal and study related activities were conducted in accordance with applicable Standard Operating Procedures, and current guidelines for animal welfare (National Research Council for the Care and Use of Laboratory Animals, 2011, Animal Welfare Act (AWA), 1966, as amended in 1970, 1976, 1985, and 1990, and the AWA implementing regulations in Title 9, Code of Federal Regulations, Chapter 1, Subchapter A, Parts 1–3). Rat fetal (E18) hippocampi were removed by microdissection and collected in ice cold Hibernate E Dissection Medium (BrainBits LLC). Individual cells were isolated by gentle trituration and passing through a 100 μm filter to remove unprocessed clumps. Cells were cultured at a density of 3×10^5 cells/mL on single or 9-well glass MEA "chips" (MultiChannel systems, www.multichannelsystems.com) pre-coated with poly-D-lysine (100 μg/ml) and laminin (50 μg/ml). Cells were cultured using NbActiv4® (BrainBits LLC) Culture Medium containing Neurobasal/B27, creatine, estrogen and cholesterol plus 25 μM glutamate and Pen-Strep. Glutamate was removed from medium 4 days after culture. Medium was changed every 3–4 days until use. After 18–21 days in culture spontaneous electrical activity was observed from electrodes embedded in the MEA.

Activity was recorded using MEA-1060-Inv-BC single-well with MC-rack software from MultiChannel Systems or Maestro multi-well with AxIS software from Axion Biosystems. Each single-well MEA contained 59 recording electrodes (30um diameter with an inter-electrode spacing of 200 μm) and one internal ground electrode. The 48-well MEA plate (M 768 -KAPKAP -48) for the Maestro MEA system has 16 electrodes per well. All MEA recordings were conducted at 37 °C in culture medium without perfusion. Concentration-responses were generated by exposing neurons to cumulative concentrations of test compounds. Spontaneous activity was recorded for 10 min at each concentration. Signals from the amplifier were digitized at a rate of 25 kHz and high-pass filtered (cutoff frequency of 100 Hz). The threshold for spike detection was 3-fold the standard deviation of noise. An active channel was defined as an electrode with at least 6 spikes/min in average. The value of "n" was defined as the number of active electrodes from 2 to 3 different wells. The effects of drugs on spike rates were expressed as percentage, i.e. quantified relative to base line. Data were presented as mean ± SEM. Offlinesorter (Plexon) was used for spike detection. The detailed method for data analysis is shown in Fig. 1.

2.2. Rat hippocampal slice assay

Sprague-Dawley rats (200 – 250 g) were anesthetized and decapitated; and the brain was quickly removed and soaked in ice-cold artificial cerebrospinal fluid (aCF) containing (in mM): 120 NaCl, 3.3 KCl, 1.2 NaH2PO4, 25 NaHCO3, 2.4 MgSO4, 2 CaCl2 (PH 7.4, gassed with 95% O2 and 5% CO2). The hippocampus was carefully isolated and cut into 8–10 slices at 360 μm thickness. The slices were then stabilized at room temperature for at least 1 h in the oxygenated aCF before transferring to the MEA recording chamber for experiments.

Field potentials (FPs) were recorded using MEA-1060-Inv-BC single-well with MC-Rack software from MultiChannel Systems. A single slice was placed onto a 60 electrode MEA chip, viewed under a microscope and perfused continuously with oxygenated aCSF with a reduced concentration (1 mM) of MgSO4 at 33° C. An electrode at the CA3 and CA1 border was disconnected from its amplifier and used to stimulate Schaeffer Collaterals to evoke FPs. FPs included population spikes (PS) and excitatory postsynaptic potentials (EPSP) (Fig. 2). Following 20–30 min recordings with no drug application (baseline), ascending concentrations of the drug were applied for at least 15 min per concentration.

FPs acquired from electrodes in the CA1 area were used for data analysis. FPs recorded during the final 60 s of each test concentration were analyzed. The area of each FP was measured using custom software in Matlab. Seizurogenic drugs can produce multiple peaks, so peak number (PN) was also evaluated. The effects of drugs on FP or PN were expressed as percentage, i.e. quantified relative to base line. The value of "n" was defined as the number of the electrodes in CA1 area from 3 to 6 different hippocampal slices (see Fig. 2). Each seperate slice was considered one experiment. Data were presented as mean ± S.E.M.

One way Anova followed Dunnett's post hoc test was conducted using GraphPad Prism 7.03 software for both cultured hippocampal neuron and hippocampal slice assays to determine if there was a statistical significance. A p-value $< .05$ was considered significant ($^*p < .05$, $^{**}p < .01$ and $^{***}p < .001$).

2.3. Materials

Picrotoxin (Pic), bicuculline(Bic), gabazine (Gab), 4-aminopyridine (4-AP), pentylenetetrazol (PTZ) and acetaminophen (APAP) were purchased from Sigma and internal reference agents BMS-A, BMS-B and BMS-C were provided by BMS Chemistry Department. The stock solution for each compound was prepared by dissolving it in dimethylsulfoxide (DMSO) and stored at −20° C. Each compound was diluted into aCSF to desired concentrations just before the experiments. The final concentrations of DMSO were up to 0.3% for both cultured neuron and brain slice assay.

3. Results

3.1. Vehicle and time control

DMSO was used as vehicle in both rat cultured hippocampal neuron and hippocampal slice assays. In the neuron assay, the spike rates remained stable after cumulative additions of DMSO up to 0.3%, the maximal concentration used, and over a period of time up to 60 min (data not shown, $n = 38$ from 4 slices). Similarly, in the brain slice assay, DMSO, up to 0.3%, did not exhibit any effect on FP area, which remained stable across a period of time up to 75 min (data not shown, $n = 15$, from 3 wells).

3.2. Acetaminophen

Acetaminophen (APAP) has no seizure-liability (Deshpande and Delorenzo, 2011), and was chosen as a negative control agent. In the cultured neuron assay, APAP up to 30 μM had no effect on spike rates (Fig. 5) ($n = 9$, from 2 wells), and in the brain slice assay APAP up to 100 μM had no effect on either the FP area or PN (Fig. 6) ($n = 38$, from 4 slices).

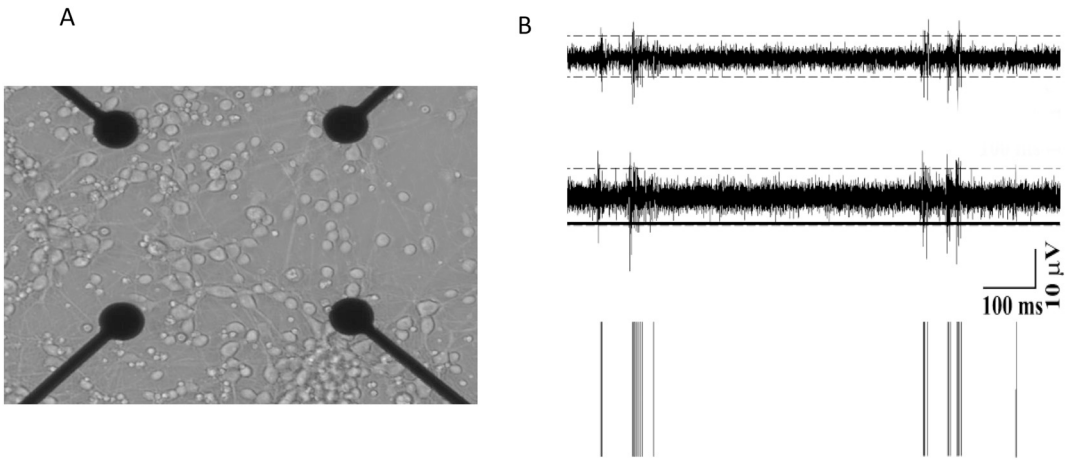

Fig. 1. Spontaneous electrical activity recording in rat cultured brain hippocampal neurons using MEA. A. hippocampal neurons cultured on electrodes. B. A filtered trace recorded from a single electrode. At top panel, the dotted line shows 3 fold the standard deviation of noise with low cut filter at 100 Hz. At middle panel, the solid line indicates the selected threshold for spike detection. At bottom panel, the waveforms crossing the threshold are extracted and counted as spikes.

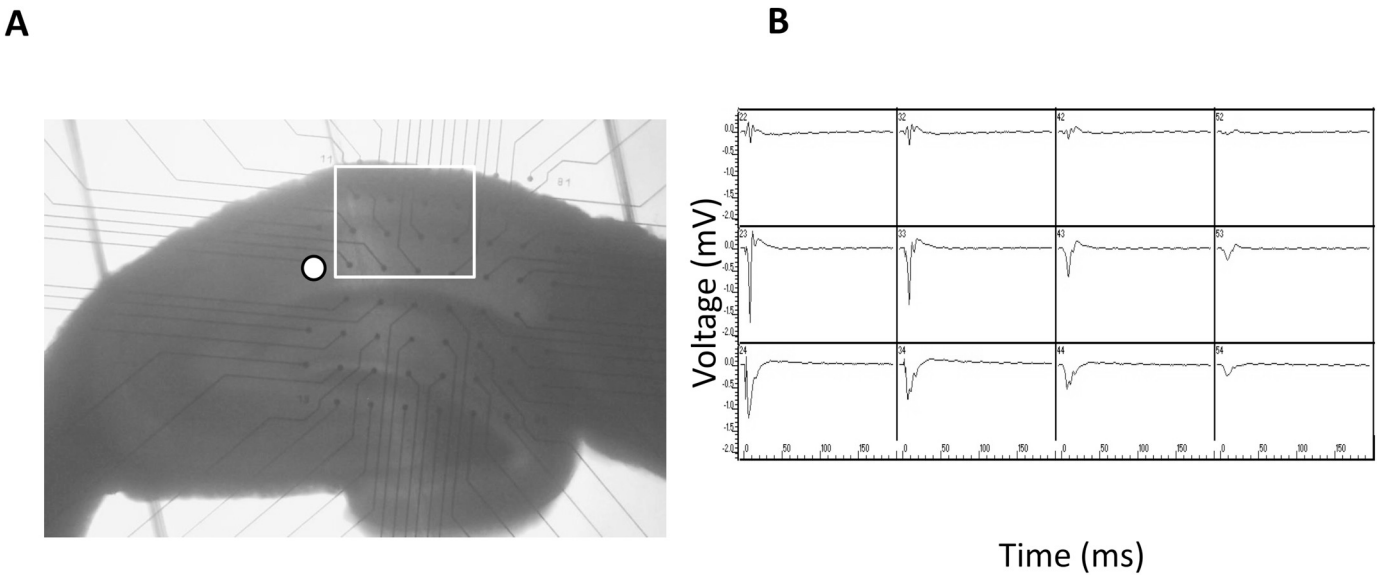

Fig. 2. Field potential recording in rat brain hippocampal slice using MEA. A. A hippocampal slice submerged in the MEA recording chamber. The stimulating electrode is indicated by the white filled circle. The recording electrodes are surrounded by a white line. B. The field potential traces which were recorded by the corresponding electrodes in the white line.

3.3. Positive controls

Five commercially available seizurogenic drugs were examined in both the cultured neuron assay and the brain slice assay: picrotoxin (Davidson and Barbeau, 1975), bicuculline (Rodrigues et al., 2004; Sokal et al., 2000), gabazine (Savrasova et al., 2010; Sokal et al., 2000), 4-aminopyridine (4-AP) (Fueta and Avoli, 1992) and pentylenetetrazole (PTZ) (Leweke et al., 1990).

Picrotoxin, a non-competitive antagonist of GABA receptors, increased spike rates by 387.8 ± 85.8%, 930.6 ± 189.4%, 814.3 ± 179.3%, 801.5 ± 191.9% and 830.1 ± 179.8% at 0.3, 1, 3, 10 and 30 μM, respectively (n = 12, from 2 wells) in the cultured neuron assay (Fig. 3). Picrotoxin increased the FP area by 80.2 ± 11.9%, 99.4 ± 11.0% and 121.2 ± 12.3% and PN by 114.3 ± 11.7%, 170.0 ± 14.5% and 198.6 ± 15.4% at 10, 30 and 100 μM, respectively (n = 35 from 4 slices), in the brain slice assay (Fig. 4),

Results for the other 4 positive reference agents tested in rat cultured hippocampal neuron and rat hippocampal slices are shown in Fig. 5 and Fig. 6, respectively. Bicuculline is a competitive antagonist of GABA receptors (Ueno et al., 1997) which exclusively couples to the chloride channel. In the cultured neuron assay, bicuculline increased spike rates by 45.6 ± 5.7%, 107.7 ± 15.2%, 280.0 ± 55.7%, 296.2 ± 87.8% and 296.5 ± 93.1% at 0.3, 1, 3, 10 and 30 μM (n = 13, from 2 wells). In the brain slice assay, bicuculline increased the FP area by 70.6 ± 10.9%, 259.2 ± 64.2% and 297.2 ± 68.6% at 3, 10 and 30 μM, respectively (n = 34 from 4 slices). Bicuculline also produced the multiple peaks. The PNs were increased by 119.1 ± 15.8%, 213.2 ± 19.0% and 221 ± 26.2% at 3, 10 and 30 μM, respectively.

Another competitive antagonist of GABA receptors, gabazine (Ueno et al., 1997), was tested as a positive control. Gabazine increased spike rates by 95.9 ± 47.4%, 125.7 ± 20.5% and 301.8 ± 86.8% at 0.3, 1 and 3 μM (n = 5, from 2 wells) in the cultured neuron assay. Gabazine increased the FP area by 70.3 ± 10.0%, 82.0 ± 9.5% and 101.7 ± 10.8% and PNs by 91.7 ± 16.3%, 138.9 ± 17.4% and 147.2 ± 14.8% at 0.3, 1 and 3 μM (n = 18 from 6 slices), respectively, in the brain slice assay.

4-aminopyridine (4-AP) is a seizurogenic voltage-gated potassium channel inhibitor (Pena and Tapia, 2000). In the cultured neuron assay,

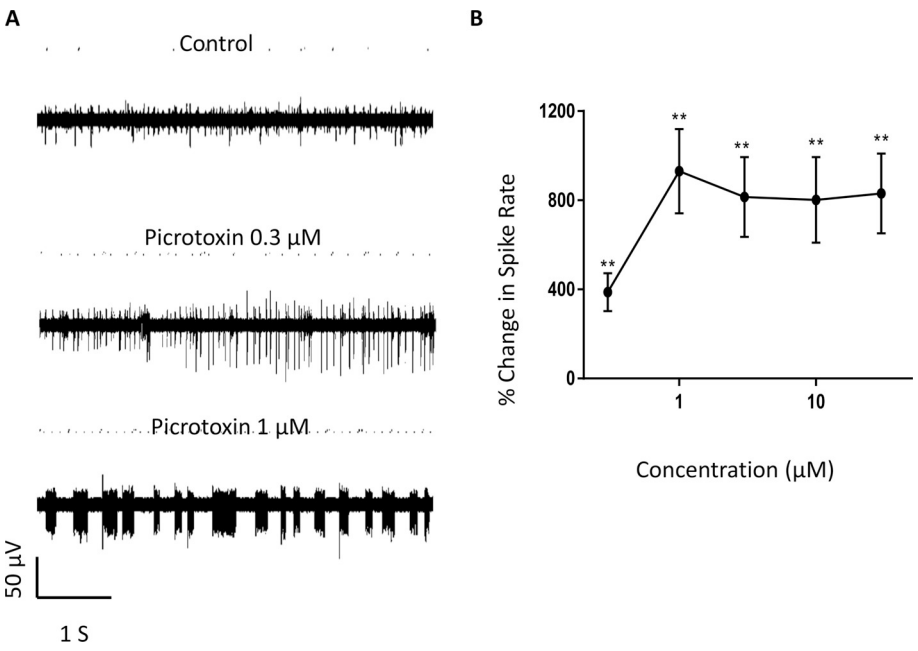

Fig. 3. The effect of picrotoxin on spike rate in rat cultured hippocampal neurons. A. Typical spontaneous activity traces recorded before (top panel) and after addition of 0.3 µM (middle panel) and 1 µM (bottom panel) of picrotoxin. B. Concentration-response curve for the effect of picrotoxin on spike rate. All data are presented as mean ± S.E.M, $n = 12$, from 2 wells, $^{**}p < .01$.

1, 3, 10, 30 and 100 µM of 4-AP increased spike rates by 11.7 ± 8.6%, 71.4 ± 23.3%, 86.7 ± 16.9%, 151.9 ± 24.1% and 196.2 ± 27.0%, respectively ($n = 12$, from 2 wells). In the brain slice assay, 4-AP increased the FP area by 98.1 ± 10.7%, 196.6.5 ± 22.2% and 405.8 ± 38.5% and PN by 142.0 ± 12.6%, 206.8 ± 16.6% and 219.3 ± 14.7% at 10, 30 and 100 µM, respectively ($n = 44$ from 4 slices).

Pentylenetetrazol (PTZ) can cause convulsions at high concentrations (Ramzan & Levy, 1985) by blocking GABA receptors (Squries et al., 1984) and increasing influx of Ca and Na into neurons (Papp et al., 1987). In the cultured neuron assay, 1, 3, 10, 30 and 100 µM of PTZ increased spike rates by 74.9 ± 16.0%, 115.5 ± 21.0%, 201.7 ± 51.5%, 201.5 ± 53.1% and 228.0 ± 55.4%, respectively ($n = 17$, from 3 wells). In the brain slice assay, PTZ increased the FP area by −3.6 ± 4.8%, 41.1 ± 8.6% and 60.4 ± 11.7% and PN by 36.1 ± 11.3%, 119.4 ± 21.8% and 150.0 ± 17.6% at 1, 3 and 10 mM, respectively ($n = 18$ from 3 slices).

Physostigmine is a reversible inhibitor of acetylcholinesterase (AchE) which at nM concentrations can lower seizure threshold and initiate and exacerbate seizure activity (Hermann & van Amsterdam, 2015). However, in in vitro studies, no seizure liability was observed at low concentrations (Easter et al., 2007). Consistent with those findings, FP area did not significantly change at 0.1, 1, and 10 µM, and only at 100 µM a mild but significant change was seen in this study (10.6 ± 3.7%, $p < .05$). However, physostigmine caused multiple peaks at lower concentrations. PN significantly increased by 36.1 ± 8.4% ($p < .001$) and 54.2 ± 10.5% ($p < .001$) at 10 and 100 µM, respectively ($n = 36$ from 4 slices), although no significant

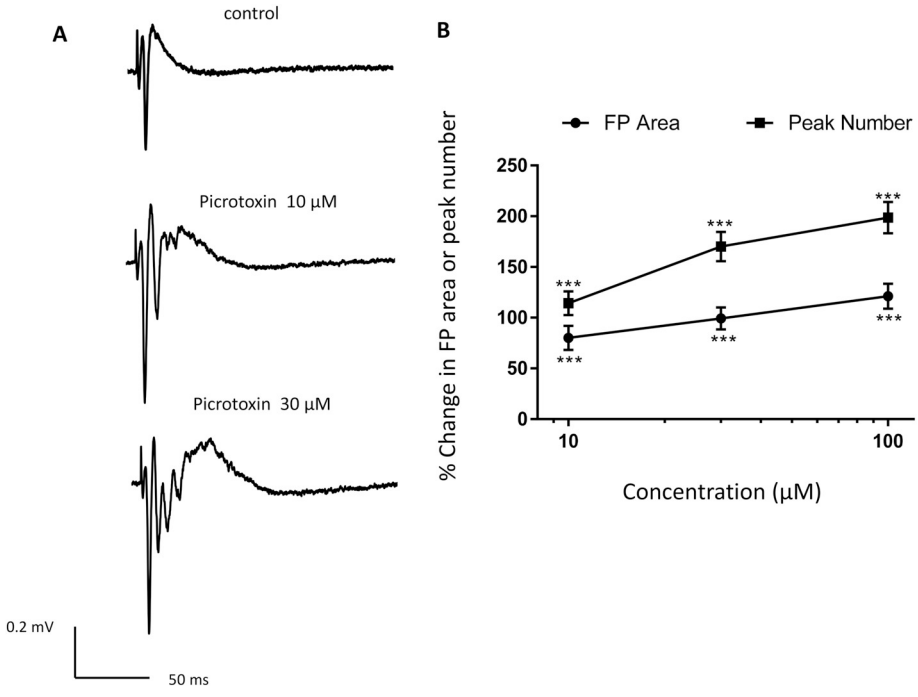

Fig. 4. The effect of picrotoxin on field potential in rat hippocampal slices. A. Typical field potential traces before (top panel) and after perfusion of 10 µM (middle panel) and 30 µM of picrotoxin (bottom panel). B. Concentration-dependent increase in field potential area (filled black circle) and peak number (filled black square) by picrotoxin. All data are presented as mean ± S.E.M, $n = 35$ from 4 slices, $^{***}p < .001$.

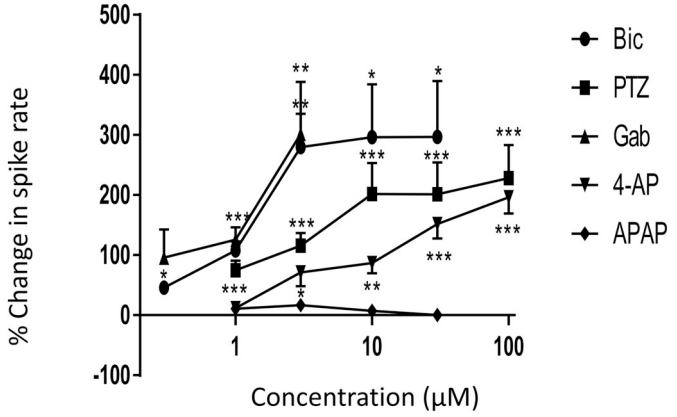

Fig. 5. Mean percentage change in spike rate against the increasing concentrations in rat cultured hippocampal neurons. Bicuculline, PTZ, Gabazine and 4-AP concentration-dependently increased the spike rate. Acetaminophen had no effect on spike rate. All data are presented as mean ± S.E.M, n = 5–17 from 2 to 3 wells for each compound, $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$.

change was seen at 0.1, and 1 μM (Fig. 7).

In contrast, the cultured neuron assay showed greater sensitivity to physostigmine which increased the spike rates by 68.9 ± 14.5%, 116.0 ± 28.9%, 127.9 ± 35.9% and 93.9 ± 28.3% at 0.1, 0.3, 1 and 3 μM (n = 9, from 3 wells), respectively (Fig. 8).

3.4. Seizurogenic compounds from BMS programs

After testing the negative control APAP and six positive reference agents, we investigated the effects of three in-house compounds (BMS-A, BMS-B and BMS−C) that caused seizures in internal in vivo animal studies. The effects of these agents on the spike rate in cultured hippocampal neurons and the FP in hippocampal slices are shown in Figs. 9 and 10, respectively.

BMS-A, from a cardiovascular program, was considered a clinical candidate before producing seizures in mice and rabbits. BMS-A increased spike rate in the cultured neuron assay by 92.2 ± 17.7%, 158.2. ± 30.4% and 160.6 ± 35.1% at 0.3, 1 and 3 μM, respectively, but a decrease in spike rate was seen at 10 μM (29.4 ± 40.3%) (n = 13, from 3 wells). In the brain slice assay, BMS-A dose-dependently increased the FP area by 26.3 ± 5.7%, 37.0 ± 6.3% and 73.6 ± 11.7% and PN by 0 ± 0%, 41.7 ± 10.3% and 45.8 ± 10.4% at 3, 10 and 30 μM, respectively (n = 18 from 3 slices).

The two other proprietary compounds that were tested, BMS−B, from an oncology program, and BMS−C, from an anti-obesity program, also caused seizures in early in vivo efficacy studies. However, in the brain slice assay, neither BMS-B (up to 100 μM, n = 17 from 3 slices) nor BMS-C (up to 10 μM, n = 12 from 3 slices), showed any significant changes in FP areas or PN. However, in the neuron assay, both compounds had significantly increased spike rate. BMS-B dose-dependently increased the spike rate by 38.4 ± 24.0%, 104.8 ± 35.5% and 220.6 ± 70.0% at 10, 30, and 100 μM, respectively (n = 20, from 3 wells), and BMS-C increased spike rate by 74.5 ± 24.5%, 179.0 ± 49.3%, 302.8 ± 80.5% and 362.8 ± 102.8% at 0.3, 1, 3, and 10 μM, respectively (n = 16 from 3 wells).

4. Discussion

In the present study, 9 seizurogenic compounds and one non-seizurogenic compound were tested in vitro for seizurogenic activity using hippocampal primary neurons and brain slices and MEA. Both assays showed good correlation for most compounds, although the cultured neuron assay showed higher sensitivity to a few of compounds.

Standard extracellular microelectrode recording of electrical activity in brain slices has been used to understand seizure mechanisms and for risk assessment (Easter et al., 2007; Markgraf et al., 2014). Advantages of the MEA technique used in this study are that it is non-invasive in that it does not require extracellular microelectrodes, offers multi-point measurement of neuronal networks and brain slice activity and recordings are stable and can be made for longer periods.

Concentrations of test agents that produced seizurogenic signals in MEA recordings of brain slices in this study compare well to traditional microeletrode recordings published by other groups (Table 1). The active concentration ranges for both methods are very close or overlap. The data suggests that MEA could be a suitable and more facile alternative to standard extracellular recording of hippocampal brain slice for detecting drug-induced seizure liability.

The reference seizurogenic compounds evaluated in this study also showed good correlation in the cultured primary hippocampal neuron

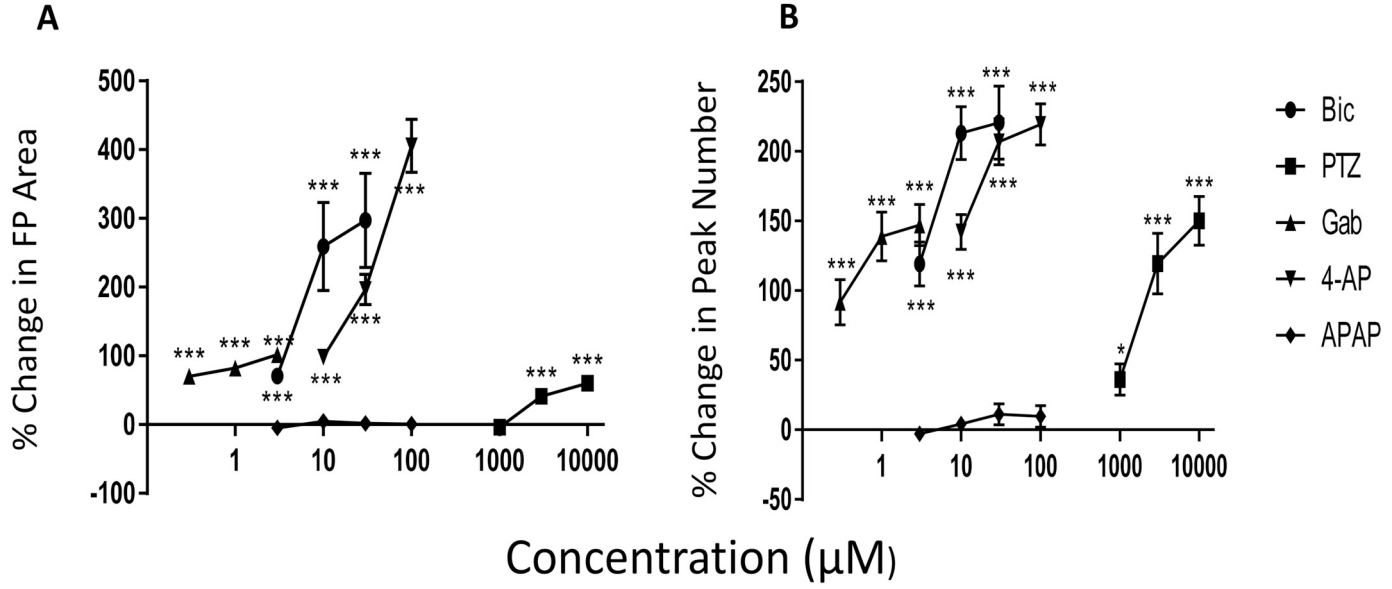

Fig. 6. Mean percentage change in field potential against the increasing concentrations in rat hippocampal slice. Bicuculline, PTZ, Gabazine and 4-AP concentration-dependently increased the field potential area (A) and the peak number (B). Acetaminophen had no effect on either the field potential area (A) or the peak number (B). All data are presented as mean ± S.E.M, n = 18–44 from 3 to 6 slices for each compound, $^{*}p < .05$, $^{***}p < .001$.

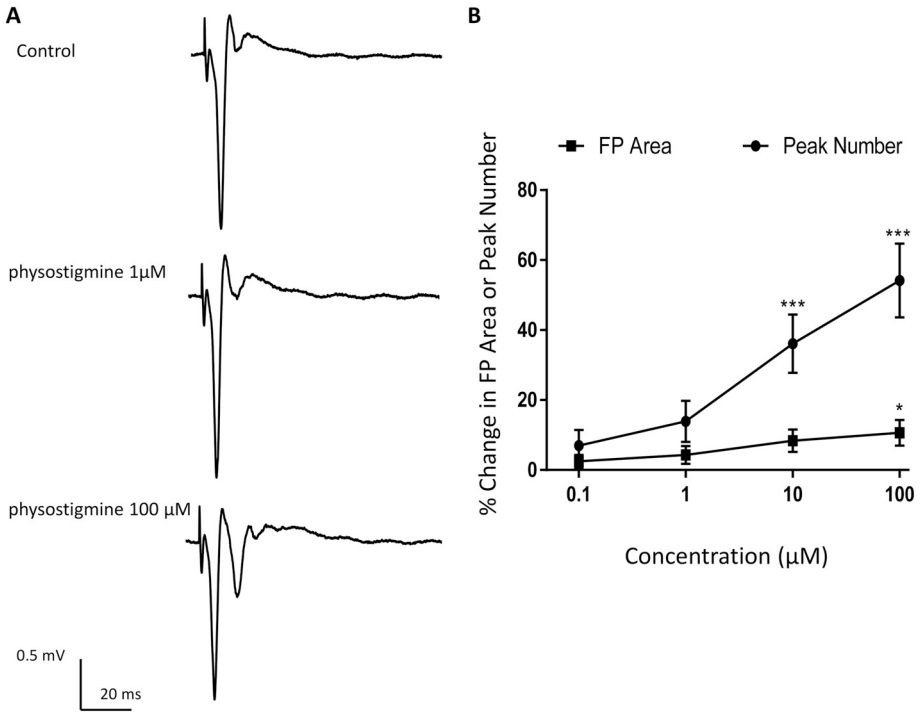

Fig. 7. The effect of physostigmine on field potential in rat hippocampal slices. A. Typical field potential traces before (top panel) and after perfusion of 1 μM (middle panel) and 100 μM of physostigmine (bottom panel). B. Concentration-dependent increase in field potential area (filled black square) and peak number (filled black circle) by physostigmine. All data are presented as mean ± S.E.M, $n = 36$ from 4 slices, *p < .05, ***p < .001.

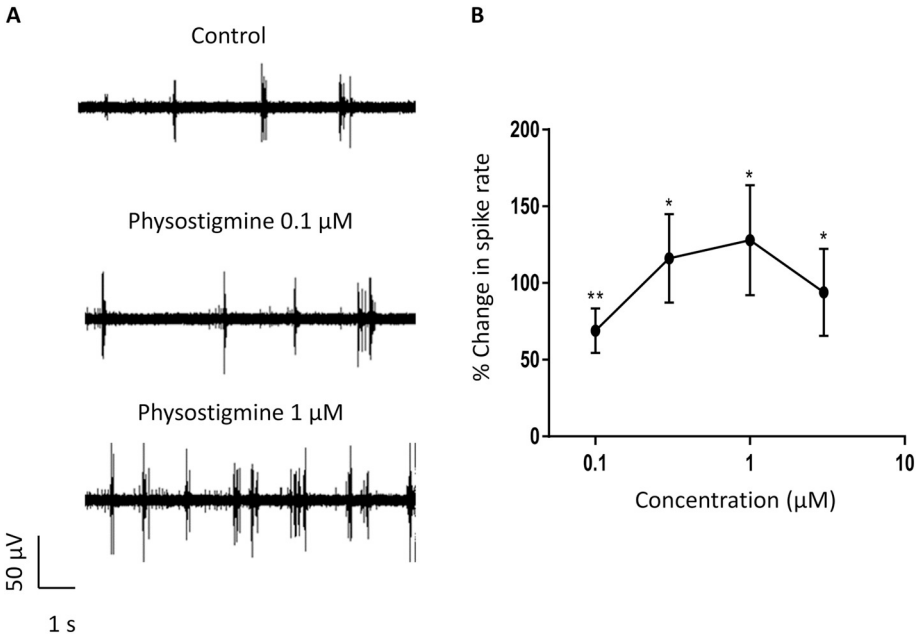

Fig. 8. The effect of physostigmine on spike rate in rat cultured hippocampal neurons. A. Typical spontaneous activity traces recorded before (top panel) and after addition of 0.1 μM (middle panel) and 1 μM (bottom panel) of physostigmine. B. Concentration-response curve for the effect of physostigmine on spike rate. All data are presented as mean ± S.E.M, $n = 9$ from 3 wells, *p < .05, **p < .01.

and brain slice assays, although spontaneous spiking activity in the neurons was more sensitive than evoked potentials in brain slices to a few of the agents tested (Table 2). The seizure liability induced by all 6 known seizurogenic drugs bicuculline, 4-AP, picrotoxin, gabazine, physostigmine and pentylenetetrazole (PTZ) were detected by both assays, although physostigmine and PTZ showed effects at much lower concentrations in hippocampal neuron assay than in hippocampal slice assay.

Three BMS compounds from different discovery programs which caused seizures in in vivo studies were also examined. In the cultured neuron cell assay, spontaneous spiking activity increased with all three compounds. In the brain slice assay, only BMS-A increased evoked FP area and peak number significantly, though the effective concentrations were much higher than in the cultured hippocampal neuron assay (3 μM versus 0.3 μM). Overall, in this study the cultured neuron assay was more sensitive to drug induced seizure liability than the brain slice assay.

It is unclear why the sensitivity of these two assays is different. One possibility is that some drugs are not easily penetrable into the interior region of the brain slice tissue, causing right-shifted potency or false negative results. Another possibility is that the morphologic changes in evoked field potentials in brain slices are a less sensitive parameter than spontaneous spiking frequency in cultured neurons. For instance, physostigmine can induce seizures at nanomolar concentrations but it affected evoked potentials in hippocampal slices in this study only at ≥10 μM. In contrast, the spontaneous spike activity in the hippocampal brain slices was increased at nanomolar concentrations in a published study (Hermann and van Amsterdam, 2015). It will be interesting in

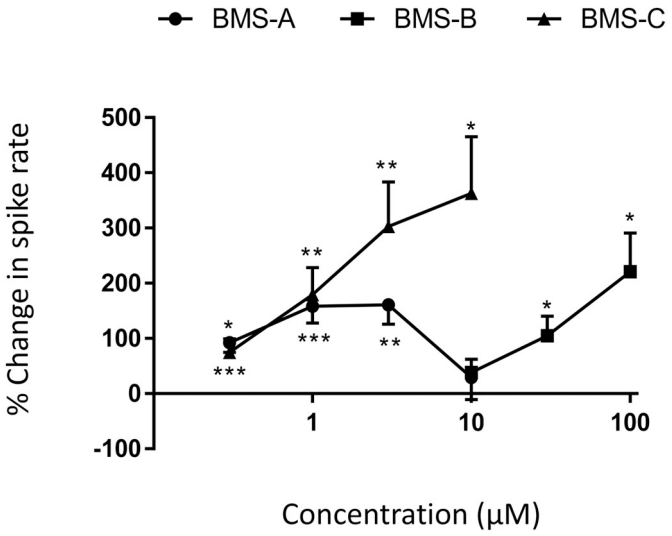

Fig. 9. . Mean percentage change in spike rate against the increasing concentrations in rat cultured hippocampal neurons. BMS-B and BMS-C concentration-dependently increased the spike rate. BMS-A at 0.3 and 1 μM increased spike rate in concentration-dependent manner and the effect plateaued at 3 μM. BMS-A decreased spike rate at 10 μM. All data are presented as mean ± S.E.M, $n = 13–20$ from 3 wells for each compound, *p < .05, **p < .01 and ***p < .001.

follow-up studies to explore the effects of these drugs on spontaneous spike activity in brain slice assay.

In addition to better prediction of drug-induced seizure liability, advantages of the cultured neuron assay over brain slice assay are its potential for higher throughput and more chronic studies. The cultured neuron assay can be performed in up to 96 well MEA systems (Wallace et al., 2015). A large number of compounds can be tested in a short period of time, which significantly reduces the cost and shortens the time for drug development.

Rat hippocampal slice assays have been used in drug safety assessment to predict the drug induced seizure liability. Most of studies use the traditional extracellular recording technique. MEA method shares the same mechanisms as traditional extracellular technique except that MEA is non-invasive and multiple electrodes are used. In this study, active concentrations between these two assays are very close (Table 1), which confirmed that the MEA hippocampal slice assay can be used instead of the traditional method in drug safety assessment. However, in hippocampal slice assays, drug induced seizure liability is not always detected. For instance, in this study, only at very high concentrations, physostigmine induced seizure liability was detected although it causes seizure in nM range in in vivo studies. Also, for three BMS compounds which were identified as seizurogenic drugs in in vivo studies, we could identify only one compound with seizure liability. Therefore, we developed hippocampal neuron assay in hope to improve the sensitivity. Table 2 shows that nine seizurogenic drugs produced seizurogenic signals in the neuron assay. So, a combination of hippocampal slice and primary neuron assays may precisely predict the drug induced seizure liability and may be used in drug safety assessment.

Table 1
Comparison of active concentration range between this MEA study and published standard extracellular recording results in rat hippocampal slice.

	Active concentration range (μM)		
Compound	This Study	Published	References
Bicuculline	3–30	1–100	Post-Munson et al. (1994)
PTZ	3000–10,000	3000–10,000	Easter et al. (2007)
Picrotoxin	10–100	10–300	Easter et al. (2007)
Gabazine	0.3–3	5	Wulfert and Margineanu (1998)
4-AP	10–300	10–100	Easter et al. (2007)
Physostigmine	10–100	10–300	Easter et al. (2007)

5. Limitations

These studies are limited by the relatively small number of compounds tested in these two assays. Further studies on additional seizurogenic and negative control compounds will enhance our understanding of how best to use hippocampal brain slice or cultured primary

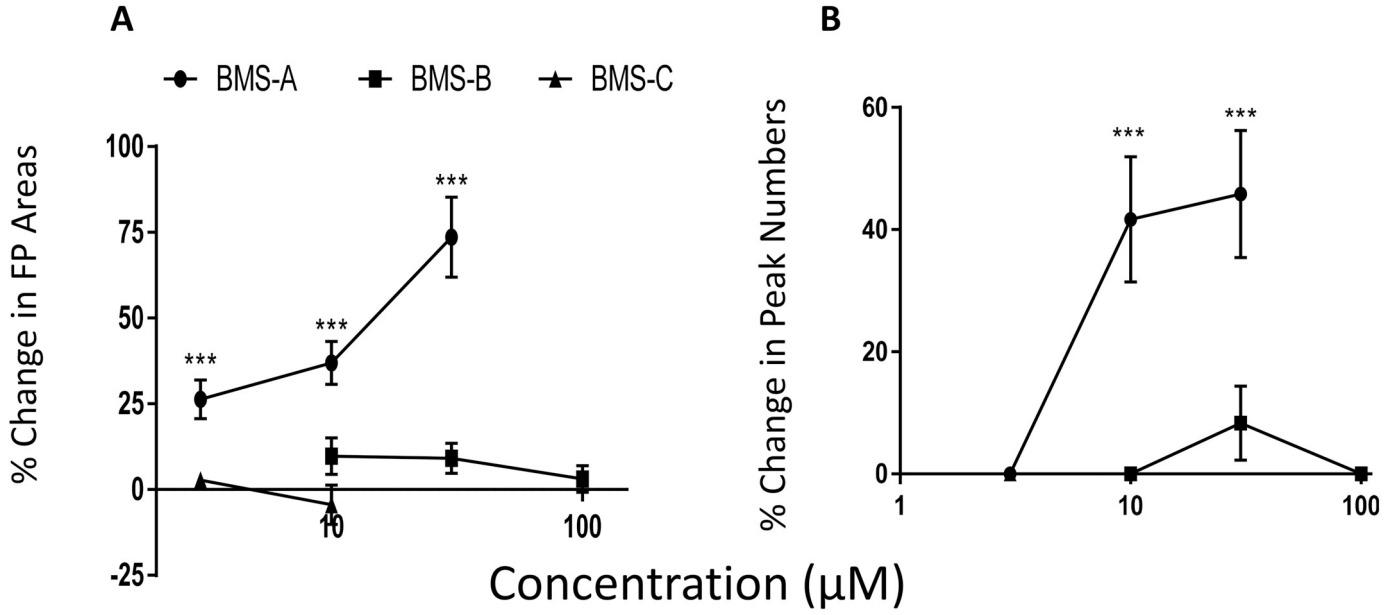

Fig. 10. Mean percentage change in field potential against the increasing concentrations in rat hippocampal slice. BMS-A evoked a concentration-dependent increase in both field potential area (A) and peak number (B). Neither of BMS-B nor BMS-C evoked any significant change in either of the field potential area (A) or peak number (B). All data are presented as mean ± S.E.M, $n = 12–18$ from 3 slices for each compound, ***p < .001.

Table 2
Comparison between rat brain slice and neuronal cell assays in MEA.

Compound	Active concentration range in MEA studies (μM)	
	Slices	Neuronal cells
Bicuculline	3–30	0.3–30
PTZ	3000–10,000	1–100
Picrotoxin	10–100	0.3–100
Gabazine	0.3–3	0.3–3
4-AP	10–300	3–100
Physostigmine	10–100	0.1–3
BMS-A	1–30	0.3–3
BMS-B	–	30–100
BMS-C	–	0.3–10

neurons in seizure risk assessment. Another limitation is that these assays focused on hippocampus and seizures may be elicited via a number of different brain regions (Foldvary-Schaefer and Unnwongse, 2011). It will be interesting to compare these results with those from other studies using cortical neurons for example. Lastly, these studies used rat as a source of hippocampal brain slices and primary neurons, and the seizure data for reference agents used for comparison is largely from human, and it's possible that there are differences in species sensitivity to effects of some of these reference agents. It will be interesting to compare the hippocampal neuron data reported in this study to neurons derived from human induced pluripotent stem cells.

6. Conclusion

These findings demonstrate the utility of MEA technology for seizure liability assessment using either rat hippocampal brain slice or rat embryonic hippocampal neurons. The cultured embryonic hippocampal neurons were more sensitive to some known seizurogenic substances than brain slices, and further studies will investigate possible reasons.

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