Microelectrode array (MEA) technology is recognized as a robust and reliable tool for assessing the excitotoxic and neurototoxic potential of chemical entities on isolated neuronal models. Lately, neuronal models based on complex brain organization and network characteristics, which made MEA electrophysiological/neurotoxicity prediction challenging. Advances in hNSC neuronal models have addressed these limitations and provided a viable option for assessing hNSC drug-induced damages. We have developed an early screening model for neurotoxicity using hNSC glutamatergic neuronal models. The co-cultures were plated on 48-well MEA plates and maintained for 14-16 days, allowing formation of a robust neuronal network displaying complex burst organization and network synchrony characteristics. When the maturation process was complete, the cultures were exposed to neurotoxic and seizurogenic compounds. The resulting neuronal activity was monitored using the proconvulsants bicuculline and picrotoxin (GABA-A receptor antagonists); SNC80 (δ-aminobutyric acid receptors); pilocarpine (cholinergic and muscarinic receptor agonist); and the neurotoxin domoic acid. Spike train analysis was aligned with respect to the effects of these compounds and confirmed the predictability of the model. Compared to vehicle control 12% DMSO; high-dose aminopyridine caused a complete loss in spike activity; 10 µM pilocarpine caused an increase in burst organization and synchrony. SNC80 caused a unique dose-response pattern with changes in spike density and effects on burst and network activity while SNC80 caused a significant increase in burst activity and changes in burst structure. Picrotoxin caused a complete loss in burst organization and network synchrony with a decrease in firing and burst rate.

In conclusion, hNSC-derived glutamatergic neuronal models plated on hNSC-derived astrocytes are a robust model suitable for the evaluation of neurotoxic and seizurogenic compounds when tested on a multi-well MEA platform.

Results: 4-Aminoglutridine

Bar graph (4A) represents fold difference between baseline and 1 hr post treatment with 4-AG at 0.1, 0.01 and 0.001 µM. 4-AG caused a decrease in burst activity and changes in burst organization. At 0.1 µM, there is an increase in the number of spikes in bursts and percent of isolated spikes. At 0.01 µM, there is an increase in the ISI CV (burstiness) indicating an increase in the organization of spike trains. At 0.001 µM, there is a decrease in the number of spikes in bursts, and increase in the percent of isolated spikes. The synchrony endpoint, mean of ISI, is decrease as a result of the increase in burst rate. There was an increase in burst organization indicated by a decrease in the percent of isolated spikes. SNC80 caused a significant increase in burst activity and changes in burst structure. Picrotoxin caused a complete loss in burst organization and network synchrony with a decrease in firing and burst rate.

Results: SNC80

Bar graph (6A) represents fold difference between baseline and 1 hr post treatment with SNC80 at 20, 10, 5 and 2.5 µM. SNC80 caused a significant increase in burst activity and changes in burst organization. At 20 µM, there is a significant increase in the number of spikes in bursts, and percent of isolated spikes. At 10 µM, there is a decrease in the number of spikes in bursts, and increase in the percent of isolated spikes. The synchrony endpoint, mean of ISI, is decrease as a result of the increase in burst rate. There was an increase in burst organization indicated by a decrease in the percent of isolated spikes. SNC80 caused a significant increase in burst activity and changes in burst structure. Picrotoxin caused a complete loss in burst organization and network synchrony with a decrease in firing and burst rate.

Results: Pilocarpine

Bar graph (8A) represents fold difference between baseline and 1 hr post treatment with pilocarpine at 25, 12.5, 6.25 and 3.125 µM. Pilocarpine caused a significant increase in burst activity and changes in burst organization. At 25 µM, there is a significant increase in the number of spikes in bursts, and percent of isolated spikes. At 12.5 µM, there is a decrease in the number of spikes in bursts, and increase in the percent of isolated spikes. The synchrony endpoint, mean of ISI, is decrease as a result of the increase in burst rate. There was an increase in burst organization indicated by a decrease in the percent of isolated spikes. SNC80 caused a significant increase in burst activity and changes in burst structure. Picrotoxin caused a complete loss in burst organization and network synchrony with a decrease in firing and burst rate.

Conclusions

Early hNSC-derived neuronal models lacked complex burst organization, making MEA-based electrophysiological/neurotoxicity prediction challenging. hNSC-derived neuronal models demonstrate robust and early assortment spike activity with significant increases in burst organization and synchrony over time. This form is complete neural network whose electrophysiological characteristics are easily detected and measured on MEA platforms. There is a distinct pattern of change observed when the cells are at a synchronous level. Additional optimization of the analysis platform and treatment testing may improve the predictability of the model. The synchrony endpoint, mean of ISI, is decrease as a result of the increase in burst rate. There was an increase in burst organization indicated by a decrease in the percent of isolated spikes. SNC80 caused a significant increase in burst activity and changes in burst structure. Picrotoxin caused a complete loss in burst organization and network synchrony with a decrease in firing and burst rate.

References

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