Properties of Human Stem Cell-derived Neurons in Long-term Cell Culture as a Model for Antiseizure Drug Discovery

Hamed Salmanzadeh and Robert F. Halliwell
Thomas J. Long School of Pharmacy, University of the Pacific, Stockton, CA

Laboratory of NEUROPHARMACOLOGY

Introduction

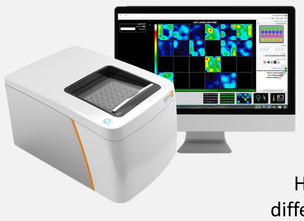
Studies in our lab have addressed the functional properties of receptors and ion channels expressed in neurons derived from human stem cells (e.g., Halliwell *et al.,* 2021). Here we have investigated a pharmacologically diverse selection of antiepileptic agents that act *via* distinct receptors and ions channels on spontaneous spiking and induced epileptiform activity to determine their sensitivity, reliability and validity for drug investigations.

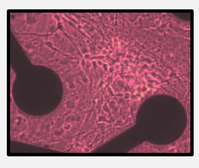
Methods

Cell culture. The human stem cell line TERA2.cl.SP12 was cultured under differentiating conditions towards a neuronal phenotype using retinoic acid (10µM).

Immunocytochemistry. Stem cell-derived neurons were immunolabeled with the neural markers βIII-tubulin, and MAP2.

Multi-Electrode Array Electrophysiology. TERA2.cl.SP12 stem cells were cultured in 6-well MEA plates and allowed to differentiate. Activity of differentiated cells was recorded weekly for 1 year using the Maestro Edge MEA system (Axion Biosystems).





Human stem cell derived neurons differentiated for 120 days in vitro on a 64 electrode MEA well.

Conclusion

2D cultures of human stem cell-derived neurons are now a valuable tool in drug discovery. Here, we addressed the development and maturation of the endogenous electrochemical and pharmacological properties of neurons differentiated over 1 *year in vitro*. Our data show that neurons develop an array of voltage and ligand- ion channels and synaptic neurotransmission is mediated *via* the major inhibitory and excitatory receptors. We also demonstrate that these neural networks are sensitive to established and potentially novel (e.g. MFA) anticonvulsants, supporting their real value in neuropharmacological investigations.

Results

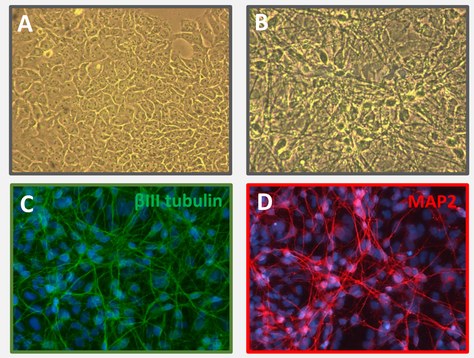


Figure 1. Retinoic acid (RA) promoted neuronal differentiation of TERA2.cl.SP-12. (A) and (B) are phase contrast images showing cells at day 0 (A) and day 80 (B) of differentiation. Immunofluorescence images show fixed stem cell-derived neurons labeled with (C) β-III tubulin (green) and (D) MAP2 (red) at 80 days differentiation.

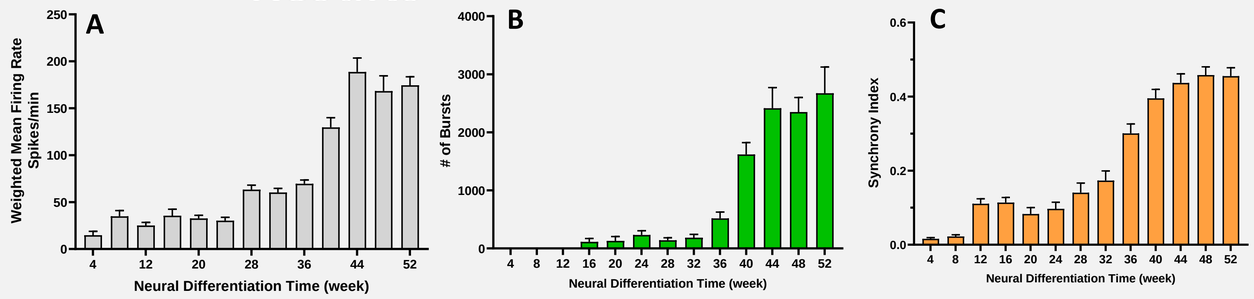


Figure 2. Development and maturation of spontaneous firing of stem cell-derived neurons over one year of differentiation. (A) Time course of the average firing rate from 4 to 52 weeks differentiation and (B) development of spontaneous burst and (C) synchrony firing during long-term culture.

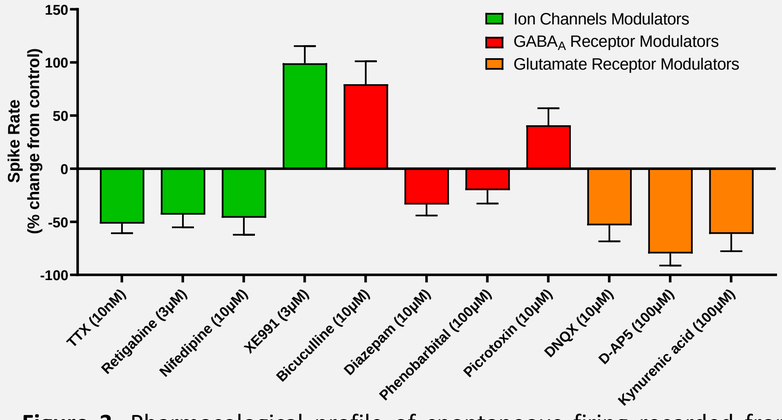


Figure 3. Pharmacological profile of spontaneous firing recorded from stem-cell derived neurons. Addition of ion channel and receptor modulators to the media showed that neurons expressed voltage- and ligand- gated ion channels and that synaptic transmission was mediated *via* glutamate and GABA$_A$ receptors in these neural networks.

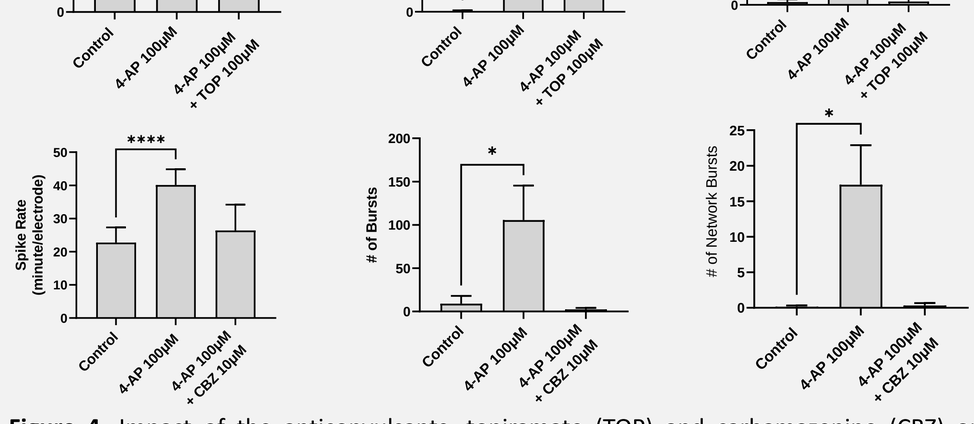


Figure 4. Impact of the anticonvulsants, topiramate (TOP) and carbamazepine (CBZ) on epileptiform activity induced by 4-aminopyridine (4-AP). The histograms show that 4-AP significantly increased spiking, burst firing, network bursts and synchronized epileptiform activity. Topiramate and carbamazepine reduced neural activity to control levels. The cells were 16 weeks differentiation.

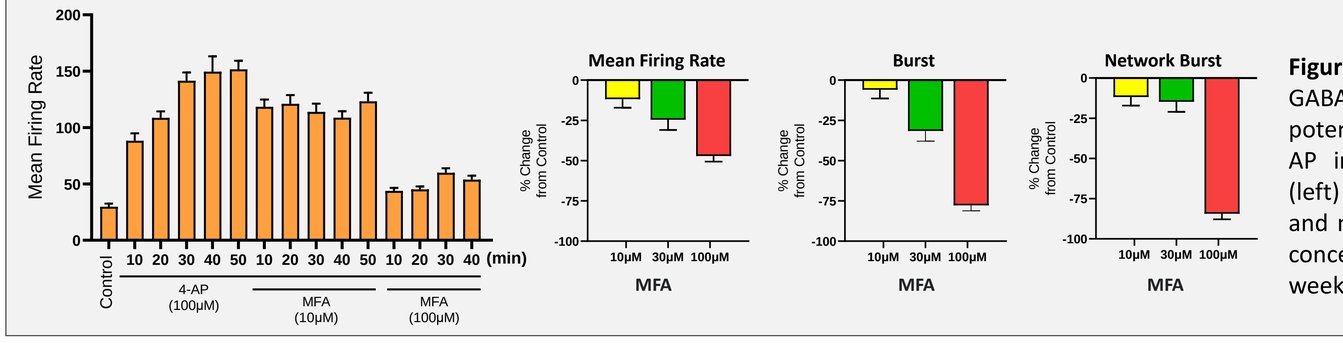


Figure 5. The NSAID, mefenamic acid (MFA) is also a GABA$_A$ receptor modulator. Here, we tested its potential value as a novel anticonvulsant against 4-AP induced epileptiform activity. The histograms (left) show that MFA reduced unit firing, bursting and network burst firing evoked by 4-AP in a highly concentration-dependent manner. The cells were 16 weeks differentiation.