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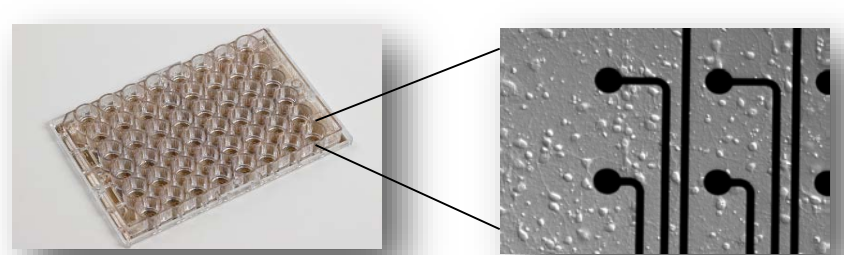
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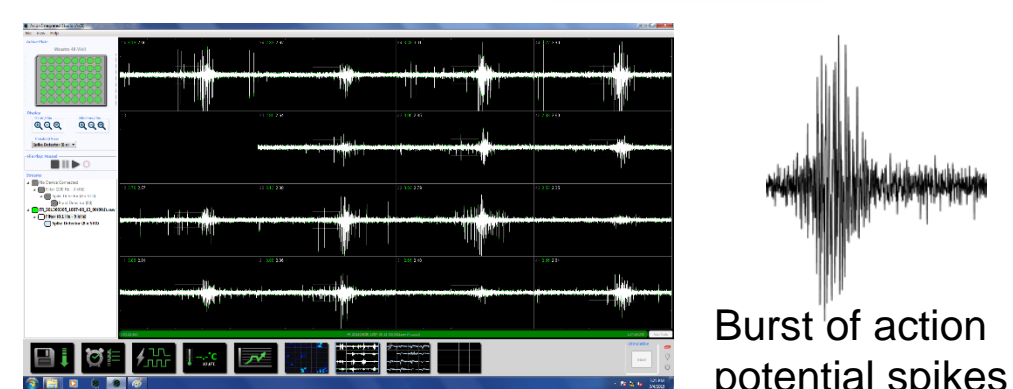
INTRODUCTION

While neurotoxicity screening using neural networks derived from rodent tissue on microelectrode arrays (MEAs) is now routine, data from neural networks derived from human tissue is lacking. In the present study, we compared the activity of neural networks comprised of human neurons made by direct induction and primary human glia to networks from rat primary cortical cells.

METHODS



- Primary cortical neurons and Stanford cells are cultured in 48 well MEA plates and allowed to mature for 21 and 37 days respectively.



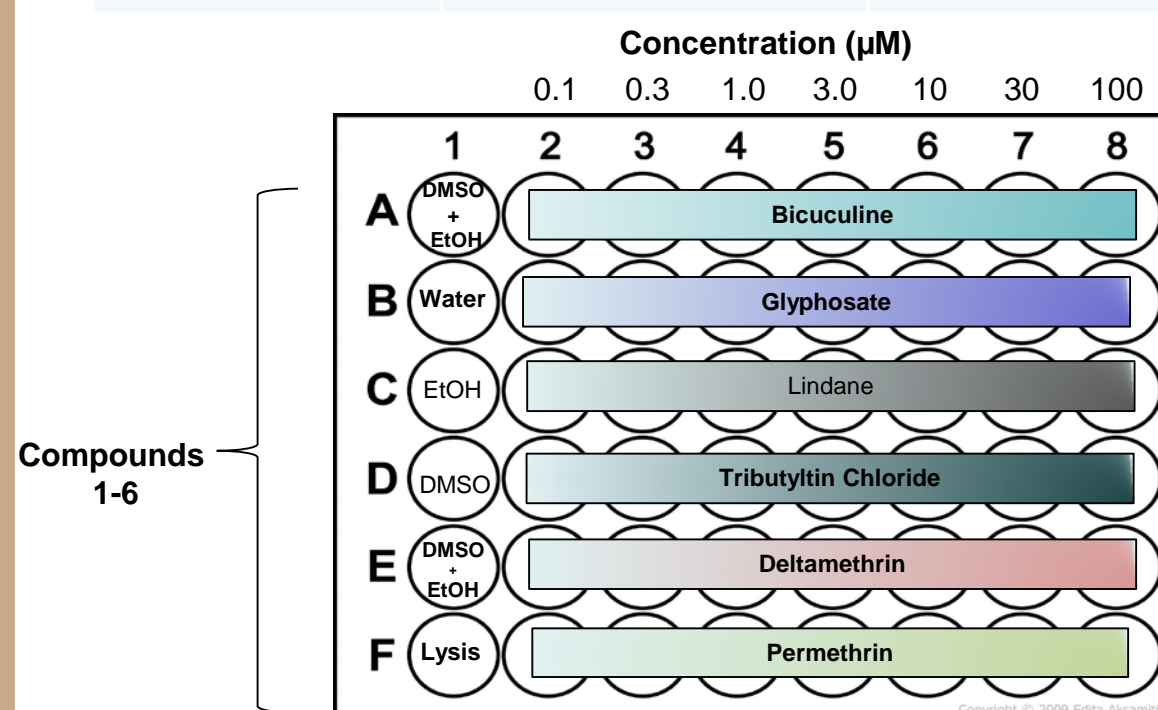
- Determine firing rate in each well for 40 min prior to and 40 min after treatment with compounds
- Plates are placed in the Axion Maestro MEA amplifier

Compound Selection: Six compounds were selected as test compounds for these

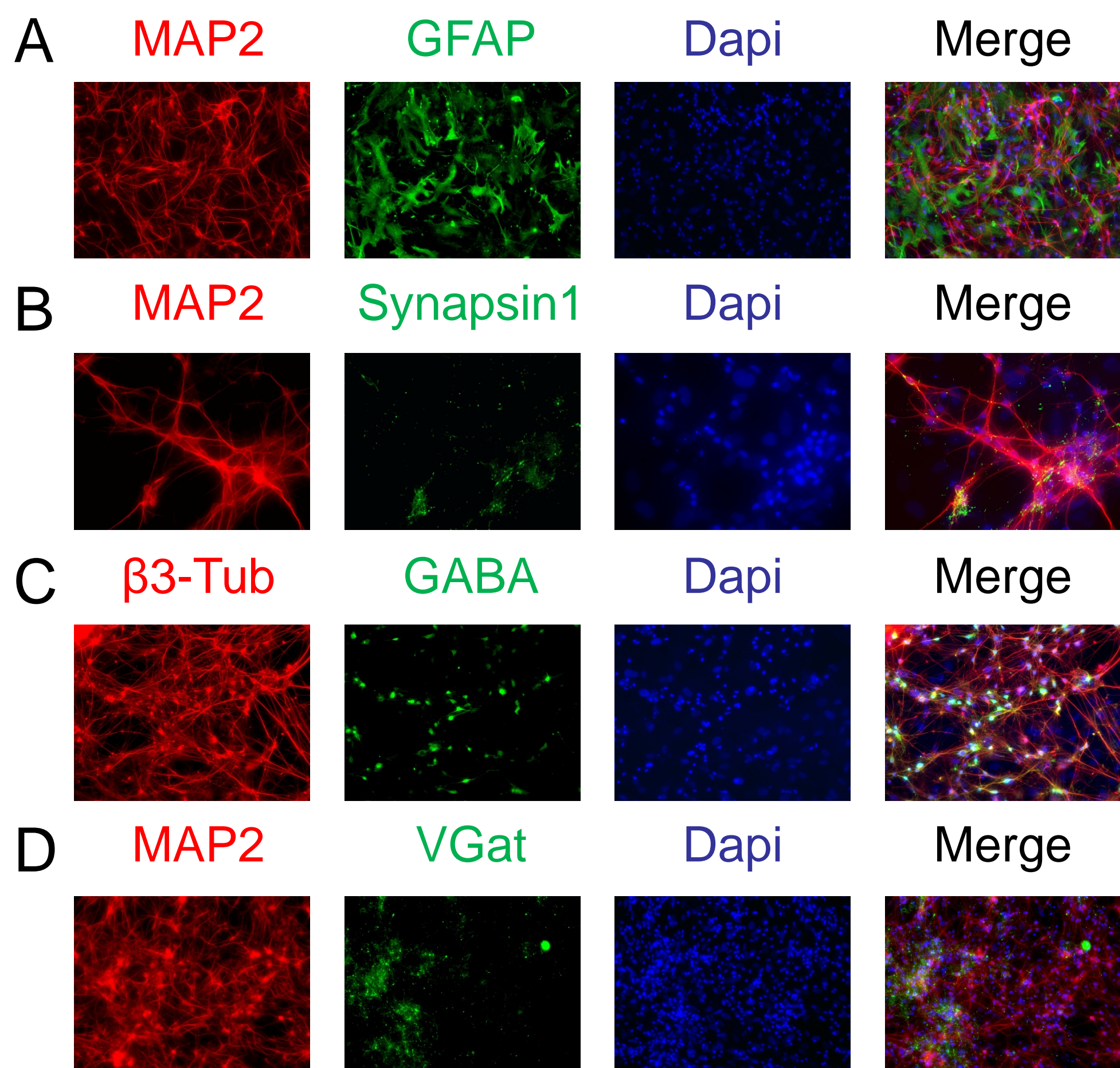
Compound	Effect on Network Activity	Effect on Viability	Reference
Bicuculline	Increase	No Effect	
Glyphosate	No effect	50% reduction	2
Lindane	Increase	No Effect	1, 2
Tributyltin Chloride	Decrease	35% reduction	1
Deltamethrin	Bi-phasic	50% reduction	1
Permethrin	Increase	No Effect	1, 2

Screening Mode
Test each compound in three replicate multi-well MEA plates from the same culture on the same date

Culture: Primary cortical neurons from Long-Evans rats (PND 0-1) were plated at 150K cells/array on PEI coated 48 well MEA plates and maintained at 37°C in 500 µL of media per well.
MEA system: Comprised of the Maestro 768-channel amplifier, Middle-man data acquisition interface, personal computer with Axion Integrated Studio (AxIS) software, and 48-well plates (Axion M768-KAP-48). Each well contains 16 individual nano-textured gold microelectrodes (~40-50 µm diameter; 350 µm center-to-center spacing) with 4 integrated ground electrodes.
Experimental Recording: Baseline spontaneous neuronal activity was recorded for 1 hr between DIV 12-14. Following baseline recording, test compounds were added to individual well at the concentrations indicated on the plate map (above).
Data Analysis: Mean firing rate (MFR) determined in the presence of compound was expressed as a percentage of its pre-treatment value (% Control) to determine the percent increase or inhibition of MFR. These data were averaged across experiments to produce the concentration-response curves illustrated. CellTiter Blue data are expressed as mean % Control across all replicates. LDH data are expressed as the mean % of total LDH released (% Total) across all replicates.

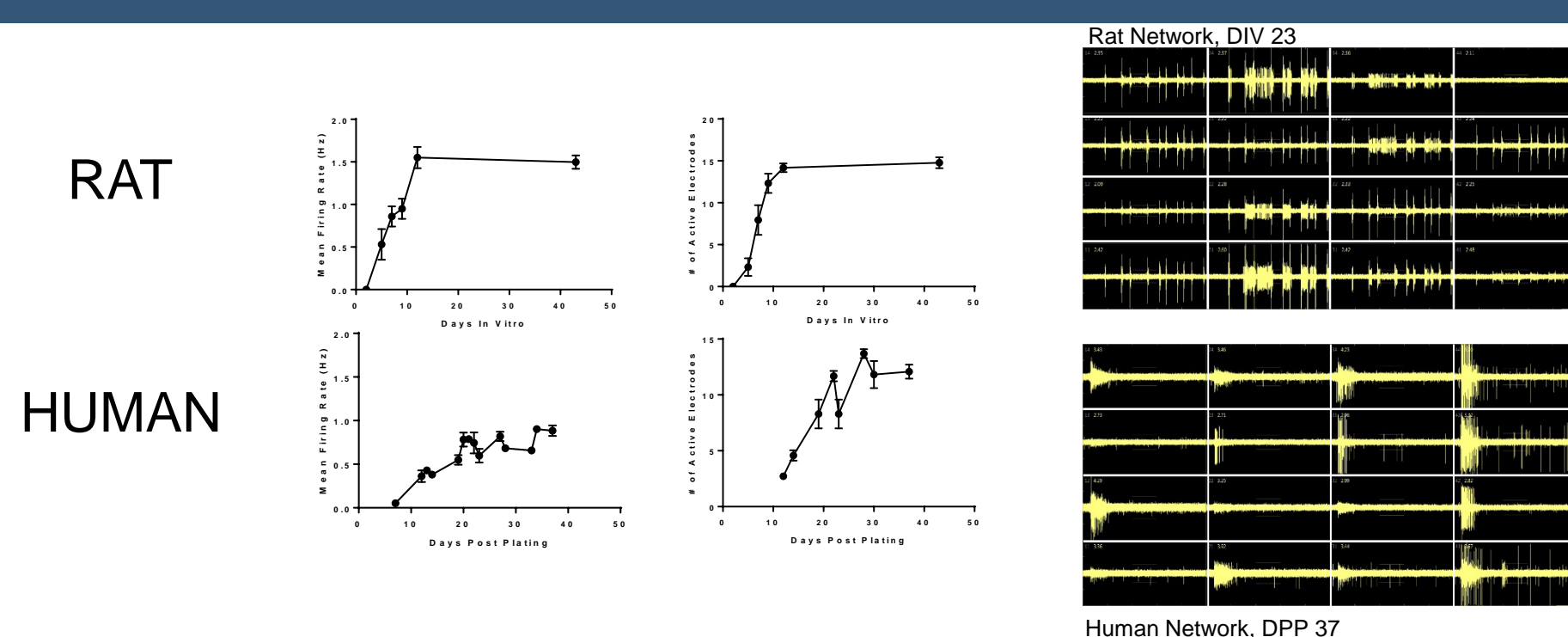


CHARACTERIZATION OF HUMAN NEURONS

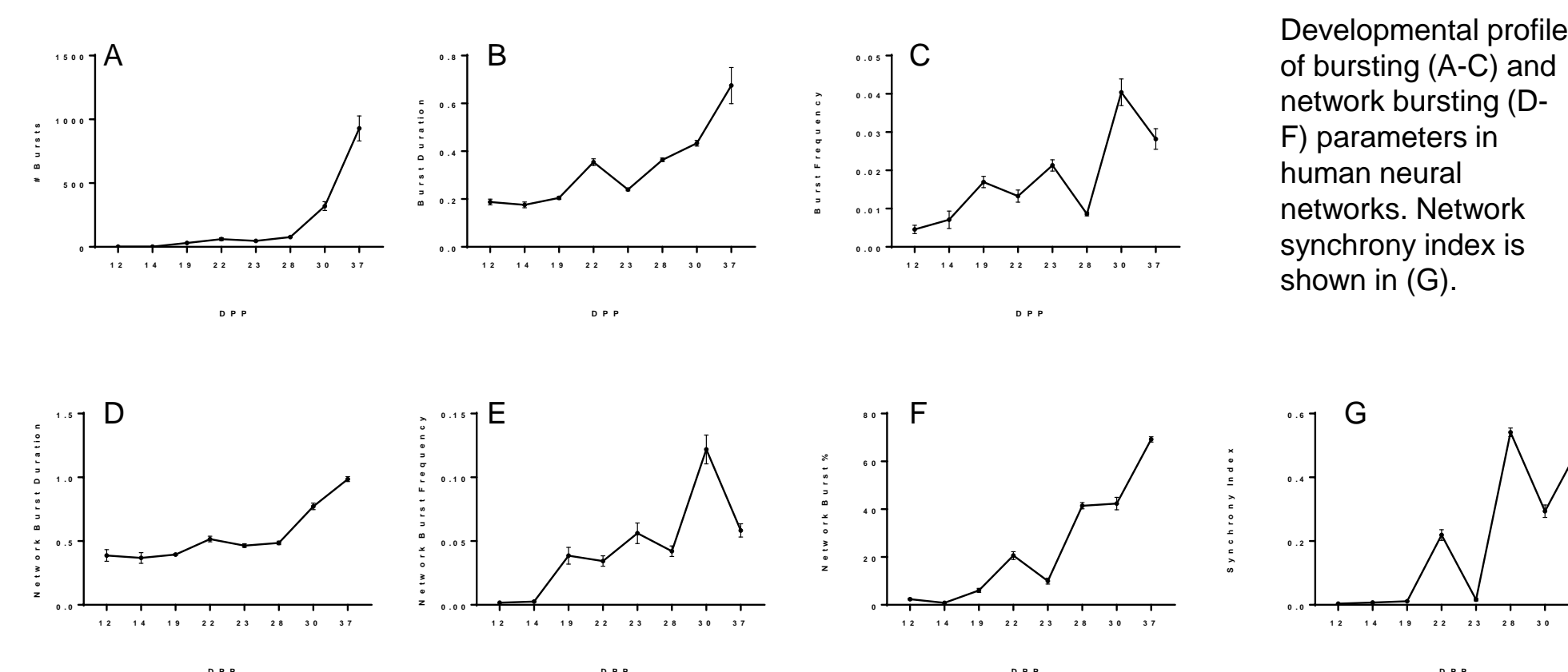


Characterization of human induced neurons by immuno-staining (A) Pan-neuronal marker Map2 / Astroglia marker GFAP / Nuclear staining Dapi. (B) Pan-neuronal marker Map2 / Synaptic marker Synapsin1 / Nuclear staining Dapi. (C) Pan-neuronal marker β3-Tubb (TuJ1) / Inhibitory neuron GABA_A receptor, α1 / Nuclear staining Dapi. (D) Pan-neuronal marker Map2 / Vesicular GABA transporter VGat/ Nuclear staining Dapi.

ONTOGENY OF HUMAN NETWORKS IS SLOWER COMPARED TO RAT NETWORKS

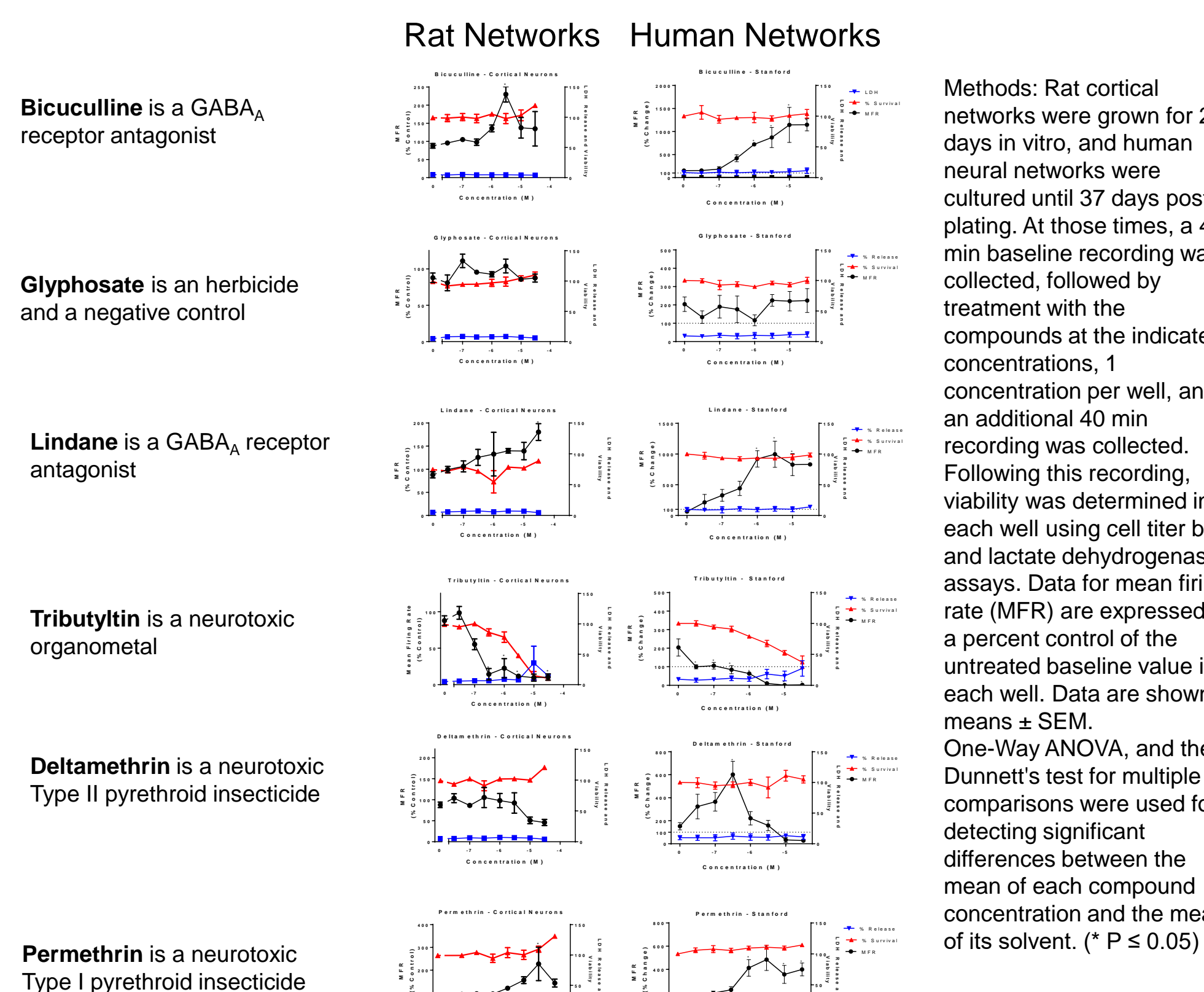


ONTOGENY OF NETWORK PARAMETERS IN HUMAN NEURONAL CULTURES



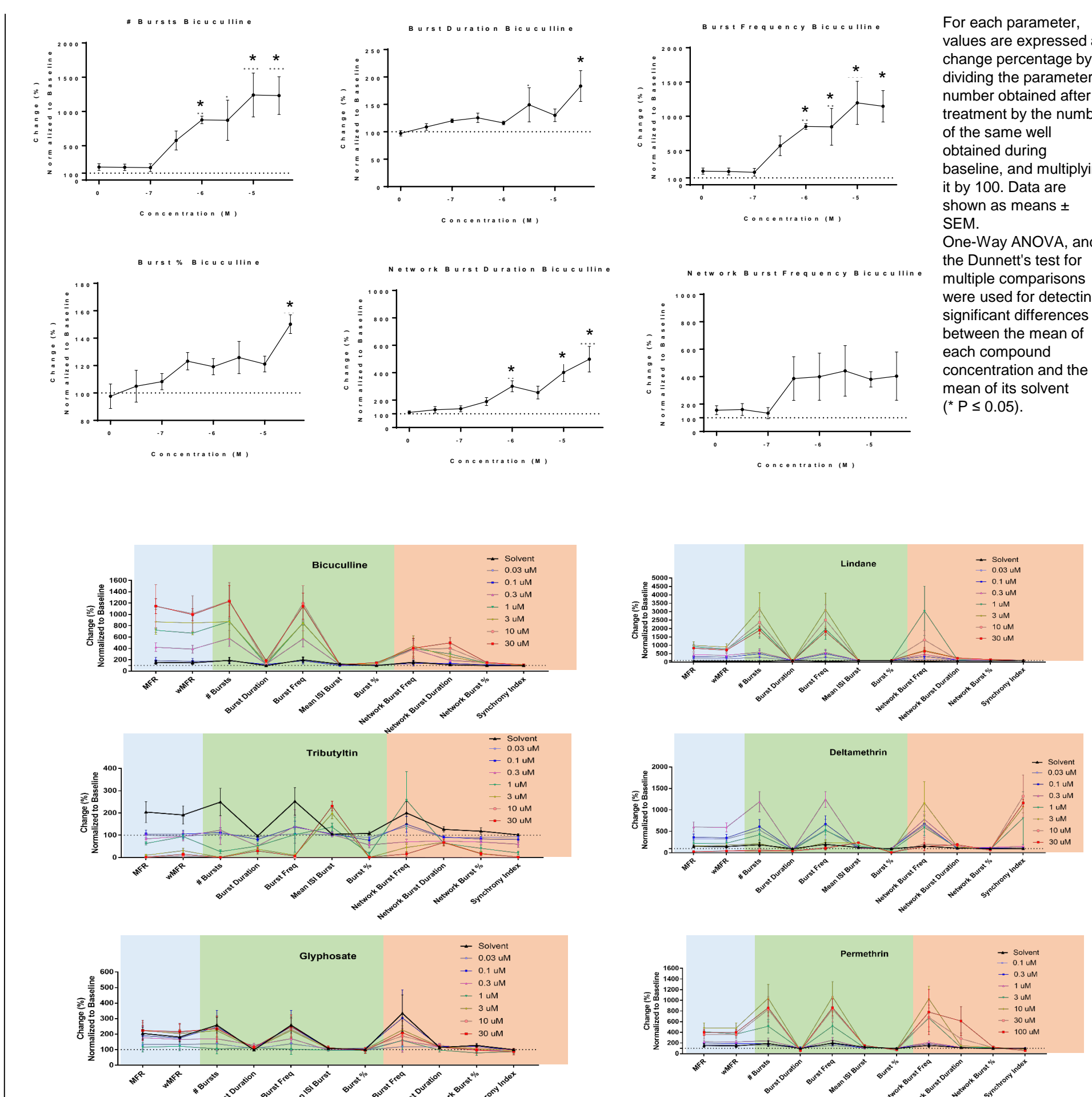
Developmental profile of bursting (A-C) and network bursting (D-F) parameters in human neural networks. Network synchrony index is shown in (G).

RESPONSES TO NEUROTOXICANTS IN RAT AND HUMAN NETWORKS ARE SIMILAR



Bicuculline is a GABA_A receptor antagonist
Glyphosate is an herbicide and a negative control
Lindane is a GABA_A receptor antagonist
Tributyltin is a neurotoxic organometal
Deltamethrin is a neurotoxic Type II pyrethroid insecticide
Permethrin is a neurotoxic Type I pyrethroid insecticide

EFFECTS OF NEUROTOXICANTS ON NETWORK PARAMETERS IN HUMAN NEURONS



For each parameter, values are expressed as change percentage by dividing the parameter number obtained after treatment by the number of the same well obtained during baseline, and multiplying it by 100. Data are shown as means ± SEM. One-Way ANOVA, and the Dunnett's test for multiple comparisons were used for detecting significant differences between the mean of each compound concentration and the mean of its solvent (* P ≤ 0.05).

SUMMARY and CONCLUSIONS

- Human neurons derived by direct induction of iPS cells form functional networks when cultured with primary human astrocytes.
 - These networks show robust spiking, bursting and coordinated activity.
- Human neural networks demonstrate an ontogeny of complex network function that is similar to rat neural networks, but more prolonged in nature.
- Human and rat neural networks respond similarly to a set of neurotoxic compounds
 - GABA-A antagonists increase MFR and other network parameters
 - Tributyltin decreased network activity
 - Only deltamethrin produced a different pattern of effects

Overall, these data demonstrate that human networks exhibit robust spiking, bursting and coordinated activity, and are suitable for neurotoxicity studies.