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Functional neuron-specific endpoints for in vitro Neurotoxicity testing

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Abstract

Statement of the Problem: In accordance with 3Rs, alternative models are required to replace standard neurotoxicity testing. High content, high-throughput tools are needed considering specific features of nervous system (NS) functioning to identify neurotoxic vs. cytotoxic effects. By considering intercellular communication through transmitters and transmitter sensors (receptors), and collective behaviour of neuron network as relevant NS functional features, the purpose of this study is to develop tools providing neuron-specific endpoints. Methodology & Theoretical Orientation: A multi-disciplinary electrophysiological, neurochemical and immune cyto chemical approach, combining electrical activity recording of neuron network (on engineered micro-electrode arrays (MEAs) equipped with 60 electrodes onto which cerebrocortical neurons were cultured; data analysis through a home-made software and measurement of transmitter release was used to assess network maturation and to detect effectiveness of neuroactive/neurotoxic substances. Findings: During network development, maturation of glutamatergic/GABAergic neuron networks, target for relevant neurotoxicity mechanisms (excitotoxicity) and drugs classes, was observed. In mature networks, synaptic connectivity was related to activation of glutamatergic pathways, and the system behaved as a sensitive sensor of glutamatergic transmission functioning. Activation or blockade of NMDA/AMPA receptors, or blockade of glutamate transporters, induced firing and bursting activity variations related to the effects on transmitter release. Also, the network sensed the fine transmission variations involved in synapse plasticity: the collective network behaviour and glutamate release were controlled by NMDA-dependent NO-cGMP pathway, as indicated by its pharmacological manipulation (NO synthase/guanylyl cyclase inhibitors, NO donors/8Br-cGMP). By presenting examples of network activity modulation by neuroactive substances (glutamate/GABA receptor agonists/antagonists) and by known neurotoxic ants (e.g., demonic acid, chlorpyrifos Oxon), and ineffectiveness of molecules not exhibiting acute neurotoxic effects, we report evidence that MEAs-coupled neuron networks can represent an integrated approach for neurotoxicity testing based on functional neuron specific endpoints. They might provide an effective in vitro alternative tool for evaluating substance neurotoxicity, also providing a mechanistic approach. Recent Publications 1. Frega M, Pasquale V, Tedesco M, Marcoli M, Contestabile A, et al. (2012) cortical cultures coupled to Microelectrode arrays: a novel approach to perform in vitro excitotoxicity testing. Neurotoxicol Teratol 34:116???127. 2. Marcoli M, Agnati L F, Benedetti F, Genedani S, Guidolin D, et al. (2015) On the role of the extracellular space on the holistic behaviour of the brain. Rev Neurosci 26(5):489???506. 3. Fuxe J, Agnati L F, Marcoli M and Borroto-Escuela D (2015) Volume transmission in central dopamine and noradrenaline neurons ant its astroglial target. Neurochem Res 40(12):2600???14. 4. Cervetto C, Vergani L, Passalacqua M, Ragazzoni M, Venturini A, et al. (2016) Astrocyte-dependent vulnerability to excitotoxicity in spermine oxidase overexpressing mouse. Neuromolecular Med 18:50???68. 5. Pietropaoli S, Leonetti A, Cervetto C, Venturini A, Mastrantonio R, et al. (2018) Glutamate excitotoxicity linked to spermine oxidase overexpression. Mol Neurobiol. 55(9):7259???7270. Diseases of environmental origin result from exposures to synthetic and naturally occurring chemical toxicants encountered in the environment, ingested with foods, or administered as pharmaceutical agents. They are, by definition, preventable: they can be prevented by eliminating or reducing exposures to toxicants. The fundamental purpose of testing chemical substances for neurotoxicity is to prevent disease by identifying toxic hazards before humans are exposed. That approach to disease prevention is termed "primary prevention." In contrast, "secondary prevention" consists of the early detection of disease or dysfunction in exposed persons and populations followed by prevention of additional exposure. (Secondary prevention of neurotoxic effects in humans. In the most effective approach to primary prevention of neurotoxic disease of environmental origin, a potential hazard is identified through premarket testing of new chemicals before they are released into commerce and the environment. Identifying potential neurotoxicity caused by the use of illicit substances of abuse or by the consumption of foods that contain naturally occurring toxins is less likely. Disease is prevented by restricting or banning the use of chemicals found to be neurotoxic or by instituting engineering controls and imposing the use of protective devices at points of environmental release. Each year, 1,200–1,500 new substances are considered for premarket review by the Environmental Protection Agency (EPA) (Reiter, 1980), and several hundred compounds are added to the 70,000 distinct chemicals and the more than 4 million mixtures, formulations, and blends already in commerce. The proportion of the new chemicals that could be neurotoxic if exposure were sufficient is not known (NRC, 1984) and cannot be estimated on the basis of existing information. However, of the 588 chemicals used in substantial quantities by
American industry in 1982 and judged to be of toxicology.

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