Electrophysiological Effects of Ionising Radiation on Cortical Rat Neurons in vitro*

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Motivation

Previous studies in mice and rats have shown that the developing central nervous system (CNS) is particularly sensitive against ionising radiation [3], yet there exists hardly any information if ionising radiation affects cellular communication of neuronal cells. Therefore we for the first time investigated the effects of X-ray on neuronal embryonic network using the method of microelectrode arrays (MEA). The cells, in this case primary cortex neurons from 18 to 19 days old rat embryos, were plated onto microelectrode arrays and reaggregated into an spontaneously, electrically active network. This network typically develops in three phases. First, electrical activity can be detected as random single spikes. Second, it starts to express a train-like spiking activity that further develops into burst-like activity. These bursts represent the mature signalling activity of the network [1]. With this method possible radiation induced effects can be extracellularly recorded.

Material and Methods

The experiments were performed with commercially available cortical rat neurons, isolated from 18 to 19 days old rat embryos. The cells were seeded onto the centre of the MEA chips and cultivated over a period of 4 weeks (Fig.1). This method can be used to assess the electrical activity of neuronal tissue and allows non-invasive, simultaneous recordings from 60 electrodes. In order to examine the effects of ionizing radiation on the neuronal network formation, the cells were irradiated after 16 days of cultivation with X-ray doses of 1 Gy and 2 Gy (90 kV, 33,7 mA) at the Technische Universität Darmstadt. Electrical signals could be recorded for about two weeks after irradiation and were analysed with regard to the number of spikes and bursts, the number of spikes per burst, the height of the amplitude, the duration of the bursts and other parameters.

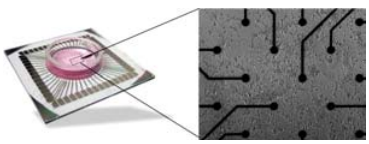

Figure 1: Cortical neurons from rat embryos on a MEA chip, cultured for 20 days in vitro.

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Results

The first experiments showed that the neuronal network functionality is very robust and not affected significantly by the applied doses. None of the examined parameters provided clear evidence of radiation-induced changes in the electrophysiological response of the networks. As an example the number of network spikes per minute is shown in figure 2 as a function of dose. This parameter is very important since preceding studies have shown that the application of several drugs, such as carbamazepine (data not shown) and neurotoxins, such as methyl mercury chloride [2], leads to a reduction of the number of spikes within a defined period of time.

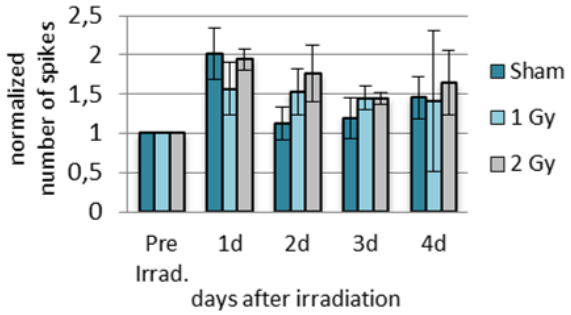

Figure 2: Number of spikes per minute of the neuronal network after X-ray exposure. The values are normalized to the measurement before irradiation (Pre Irrad.). Each bar represents at least three samples.

Conclusion

Our preliminary results suggest that the irradiation does affect neither the neuronal network nor the cell communication. It is planned to repeat this experiment applying higher doses. In addition, immunohistochemical techniques will be used to study radiation induced cell death.

References

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