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Development and application of mathematical models to examine neurotoxicity in aggregating brain cell cultures.

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In vitro aggregating brain cell cultures containing all types of brain cells have been shown to be useful for neurotoxicological investigations. These cultures have been used for the detection and study of compound effects on the nervous system by regarding multiple endpoints. Concentration-dependent neurotoxicity has been determined through enzyme activity and gene expression measurements at several time points during exposure to eight selected compounds.

The aim of the thesis is to develop biologically-based mathematical time-concentration-response models that offer a mechanistic and quantitative description of the neurotoxicity of compounds by a detailed description of the impact on the different brain cell populations. In total three novel types of stochastic models for enzyme activity and gene expression measurements based on a Markov model for brain cell populations are developed. In addition, the expected, deterministic behaviors of all the stochastic models are derived and used as the response functions in nonlinear regression models. Moreover, two multiresponse regression models for a joint evaluation of enzyme activity and gene expression data are set up.

First, a Markov process that models the survival of brain cells undergoing a chemical attack is developed. Brain cells are assumed to be either in a healthy or stressed state and only stressed cells are susceptible to cellular death. Cells may switch between these states or die with transition rates as functions of the exposure concentration. Since cell numbers are not directly measurable, enzyme activities and fold changes in gene expression are used as a surrogate. It is assumed that changes in cell numbers are proportional to changes in lactate dehydrogenase (LDH) activity. Therefore, two stochastic activity models accounting for two different normalization procedures of activity data are derived: a time activity model, in which data are normalized with respect to activities at time zero and a time-concentration activity model, in which data are normalized with respect to activities of untreated control samples at the same time point. Assuming that the enzyme levels may differ between cells in the healthy and in the stressed state, a generalized time-concentration activity model is set up. Since it is also assumed that gene expression differs between cells in the healthy and in the stressed state, and that the fold change in gene expression is proportional to the cell number in each of the states, the generalized time-concentration activity model can additionally be considered for the evaluation of gene expression data. LDH activity is the only measured endpoint that is proportional to the number of living cells, but which does not allow for cell type-specific conclusions, whereas the remaining measured endpoints deliver brain cell type-specific information. However, generally enzyme activities and fold changes in gene expression may not necessarily be proportional to the number of living cells in the corresponding cell population. Therefore, two types of multiresponse regression models are set up to analyze LDH activities and cell type-specific enzyme activities jointly or LDH activities and fold changes in gene expression jointly.

All the models developed in this thesis contain biologically-based parameters, usually represented by the transition rates of the Markov process. Maximum likelihood and nonlinear least squares regression techniques are applied for estimation of the model parameters. Likelihood ratio tests and extra sums of squares analyses are performed to test hypotheses about the model parameters. In order to investigate the accuracy of parameter estimation, the stochastic activity models and the two multiresponse regression models are applied in computer simulation studies.

The time-concentration activity model is applied to real LDH activity assay data taken during exposure to eight compounds. Compound-specific transition rates are estimated, interpreted and allow for a mechanistic and quantitative comparison of neurotoxicity between the compounds. However, LDH activity assays have been performed only at two measurement time points. In order to examine whether data at late measurement time points can be correctly extrapolated using data from early time points, two compounds are selected and investigated in further experiments with more measurement time points. The modeling results of the evaluation of LDH activity assays during exposure to the two compounds are used to propose two new experimental enzyme activity assay designs. For the two compounds, LDH activities from early time points can be used to correctly extrapolate data to late time points, such that long-term experiments with the two compounds can be reduced. Moreover, in order to generate qualitative hypotheses about brain cell type-specific toxicity of compounds, the multiresponse regression models are applied to real enzyme activity assays and gene expression measurements.

The models developed in this thesis can be used to discriminate between different biological hypotheses regarding the effect of a compound on the transition rates of the Markov process. The effects of different compounds on the transition rate estimates can be mechanistically and quantitatively compared. Data can be extrapolated to late measurement time points to investigate whether cost- and time-consuming long-term experiments could possibly be reduced. The activity models can be used to propose reasonable experimental designs for neurotoxicity testing of compounds. Biologically-based hypotheses about brain cell type-specific toxicity of compounds can be generated by applying the multiresponse regression models to several endpoints that are measured.