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Machine learning-based prediction of adverse drug effects: An example of seizure-inducing compounds

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Various biological factors have been implicated in convulsive seizures, involving side effects of drugs. For the preclinical safety assessment of drug development, it is difficult to predict seizure-inducing side effects. Here, we introduced a machine learning-based *in vitro* system designed to detect seizure-inducing side effects. We recorded local field potentials from the CA1 alveus in acute mouse neocortico-hippocampal slices, while 14 drugs were bath-perfused at 5 different concentrations each. For each experimental condition, we collected seizure-like neuronal activity and merged their waveforms as one graphic image, which was further converted into a feature vector using Caffe, an open framework for deep learning. In the space of the first two principal components, the support vector machine completely separated the vectors (*i.e.*, doses of individual drugs) that induced seizure-like events and identified diphenhydramine, enoxacin, strychnine and theophylline as "seizure-inducing" drugs, which indeed were reported to induce seizures in clinical situations. Thus, this artificial intelligence-based classification may provide a new platform to detect the seizure-inducing side effects of preclinical drugs.

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1. Introduction

Convulsive seizures involve body shakes that occur rapidly and uncontrollably and are often associated with a loss of consciousness. Seizures generally result from the excessive excitation and synchronization of neurons. Factors that cause hyperactive and synchronous states of neuronal networks involve i) remodeling of synaptic connections (1), ii) changes in extracellular ion concentrations (2), iii) the modification of neurotransmitters and their receptors (3, 4), and iv) medications, *i.e.*, adverse effects (AEs) of drugs (5, 6). In the central nervous system, seizures are one of most severe AEs, leading to a decline in development or withdrawal from the market. In the preclinical safety assessment of drug development, animal experiments are used to screen drugs that potentially exhibit such AEs; however, it is difficult to detect drugs that exert seizure-inducing effects.

Hippocampal slices can be used for *in vitro* epilepsy models and pharmacological validation (7). Epileptiform hyperactivity is induced by proconvulsants (8), electrical stimulation (9), and extracellular ionic changes (10). In these cases, seizure-like events (SLE), as sustained synchronous neuronal discharges, are considered hallmarks (2) and are detectable in local field potentials (LFPs). However, human bias-free and clear-cut criteria to predict the onset of seizure are also required in the analysis of *in vitro* data. The new assessment system based on the recording of LFPs from hippocampal slices may provide a method to identify the seizure-inducing effects of drugs.

In the present study, we treated brain slices, including the hippocampus, with a total of 14 drugs that either do or do not induce seizures in humans. By recording LFPs from the alveus, we automatically detected SLEs in LFP traces using an image recognition technique. Specifically, we used the deep learning network Caffe to extract the features from LFP images and identified SLEs using a linear support vector machine (SVM) in the state space whose dimension was reduced using principal component analysis

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(PCA). The correct rate of prediction obtained using the cross-validation test was 100%. All drugs used here were perfectly assigned as either safe or seizure-inducing drugs.

2. Materials and methods

2.1. Animals

The animal experiments were performed with the approval of the Animal Experiment Ethics Committee at the University of Tokyo (approval number: P24-8) and according to the University of Tokyo guidelines for the Care and Use of Laboratory Animals. Male ICR mice (3- to 4-week-old) were housed under standard laboratory conditions (12-h light/12-h dark cycle, free access to food and water). These experimental protocols were conducted in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 of 2006), the Standards for Breeding and Housing of and Pain Alleviation for Experimental Animals (Ministry of the Environment, Notice No. 88 of 2006), and the Guidelines on the Method of Animal Disposal (Prime Minister's Office, Notice No. 40 of 1995).

2.2. Drugs

Aspirin, cimetidine, dextran, diazepam, diphenhydramine, enoxacin, ibuprofen, imipramine, isoniazid, ketamine, methamphetamine, metoclopramide, oseltamivir, picrotoxin, strychnine, and theophylline were used. Aspirin and theophylline were dissolved at a stock concentration of 50 mM in 33% dimethyl sulfoxide (DMSO), and the final concentrations of these drugs were adjusted to contain 2% DMSO. Dextran was dissolved at a stock concentration of 10% w/v immediately prior to use. Diazepam was dissolved at a stock concentration of 1 mM in a solution comprising 40% propylene glycol and 10% ethanol in double-distilled water. Ibuprofen was dissolved at a stock concentration of 25 mM in 50% DMSO, and its final concentration contained less than 0.6% DMSO. The other drugs were dissolved in double-distilled water at various stock concentrations, stored at 4 °C, and diluted 1:100–100,000 (Table 1) immediately prior to use in artificial cerebrospinal fluid (aCSF) with a low extracellular magnesium ion concentration ($[Mg^{2+}]_e$), comprising (in mM) 127 NaCl, 3.5 KCl, 1.24 KH_2PO_4, 0.1 $MgSO_4$, 2.0 $CaCl_2$, 26 $NaHCO_3$ and 10 D-glucose.

2.3. Slice preparations

Acute slices were prepared from the ventral part of the hippocampal formation in 3- to 4-week-old ICR male mice (SLC, Shizuoka, Japan). The mice were anesthetized with isoflurane, followed by decapitation, and the brain was horizontally sliced at a thickness of 400 μm in the fronto-occipital direction using a vibratome in ice-cold oxygenated modified aCSF comprising (in mM) 222.1 sucrose, 27 $NaHCO_3$, 1.4 NaH_2PO_4, 2.5 KCl, 0.5 ascorbic acid, 1 $CaCl_2$, and 7 $MgSO_4$. Subsequently, the slices were maintained in normal ACSF comprising (in mM) 127 NaCl, 3.5 KCl, 1.24 KH_2PO_4, 1.2 $MgSO_4$, 2.0 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose and bubbled with 95% O_2 and 5% CO_2 at 37 °C for at least 1 h. One to three slices per mouse were used in the following experiments.

2.4. Local field potential recording

A brain slice, which included the hippocampus and the temporal neocortex, was placed on an 8 × 8 planar multi-electrode array (MED-P515A, Alpha MED Scientific; electrode size: 50 × 50 μm; inter-polar interval: 150 μm) and maintained in the interface condition at 35 °C. Low $[Mg^{2+}]_e$ aCSF was bubbled with 95% O_2 and 5% CO_2 at room temperature. aCSF was perfusion at a flow rate of 1 ml/min using a peristaltic pump. As the bath volume of the chamber was 0.8 ml, aCSF was thought to be replaced with new aCSF within about 1 min. Based on the total volume of the inlet tube, the onset time at which the solution reached the chamber was defined as the time at which the solution was switched. LFPs were simultaneously acquired from the 64 recording electrodes of the multi-electrode arrays and digitalized at 10 kHz using an Alpha MED Scientific system.

2.5. Data analysis

The data were exported at 1 kHz and processed using custom-made routines in Matlab (The MathWorks, Natick, MA, USA). The raw data were hamming-filtered with a time window of 30 ms (11) and differentiated at an interval of 50 ms. The peaks were automatically detected when their absolute values exceeded 0.1 μV/ms. To ensure that the drug concentration in the chamber reached to objective values during the occurrence of the peaks, for each 10-min trace of a certain concentration, the first and last 1 min were cut out from analysis. For each peak, the LFP trace form −200 to 2070 ms relative to the peak was transformed into a 227 × 227-pixel binary picture, and the horizontal and vertical axis showed

Table 1
Concentrations of the drugs tested. Each drug was bath-applied at increasing concentrations as shown in the table. The values in the gray-shaded cells represent the clinical blood concentrations of the corresponding drugs in healthy persons.

	Stock concentration	Bath concentrations					
Aspirin	50 mM	30	100	300	1000	3000	μM
Cimetidine	10 mM	1	3	10	30	100	μM
Dextran	10 w/v %	0.003	0.01	0.03	0.1	0.3	w/v %
Diazepam	1 mM	0.1	0.3	1	3	10	μM
Diphenhydramine	50 mM	1	3	10	30	100	μM
Enoxacin	25 mM	1	3	10	30	100	μM
Ibuprofen	25 mM	3	10	30	100	300	μM
Imipramine	10 mM	0.1	0.3	1	3	10	μM
Isoniazid	100 mM	10	30	100	300	1000	μM
Ketamine	50 mM	1	3	10	30	100	μM
Methamphetamine	50 mM	1	3	10	30	100	μM
Metoclopramide	10 mM	0.1	0.3	1	3	10	μM
Oseltamivir	10 mM	1	3	10	30	100	μM
Picrotoxin	5 mM	1	3	10	30	100	μM
Strychnine	25 mM	0.3	1	3	10	30	μM
Theophylline	50 mM	30	100	300	1000	3000	μM

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